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

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

MANUSCRIPTS: The diversity of interests and fields of endeavor represented by contributors and readership of the Proceedings precludes strict conformance as to style. Authors should refer to recent issues of Mosquito News for general guidance. Authors of technical papers should follow the basic recommendations as presented in the Council of Biology Editors Style Manual. Authors should submit an original on white bond paper, with one additional copy. All parts of manuscripts (text, tables, references and legends) must be typed, double-spaced with ample margins. Avoid footnotes in text. Author should indicate with pencil, in the margins, the approximate positions desired for illustrations and tables.

The complete scientific name of an organism must be given the first time it is used. Terms commonly abbreviated in specific fields should be given in full the first time used, followed with the abbreviation. The abbreviation alone is acceptable in further usage in the paper. Common latin abbreviations (et al., e.g., i.e., etc.) are not italicized. Use of the metric system is encouraged. The bi-letter system of generic abbreviations is used for Culicidae.

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As Program Chairman I would like to extend my appreciation to all that participated in the presentation of papers and information at the Annual Conference in Anaheim.

I also thank all those that helped me develop the final program and also the moderators who kept the program on time for the many sessions necessary for a good program.

As in all programs there are problems fitting people into certain time slots, so if in the future, anyone desires a certain time to participate in the program, I would suggest that you contact the Program Chairman early.

Thank you all again.

Donald A. Merritt
Program Chairman
Consolidated Mosquito Abatement District

California Mosquito and Vector Control Association, Inc.

Volume 48 January 20 – 23, 1980

PRESIDENTIAL ADDRESS[1]

Gilbert L. Challet

Orange County Vector Control District

Post Office Box 87, Santa Ana, California 92702

The California Mosquito and Vector Control Association is in a state of transition. We are moving away from our stated and unstated objectives and becoming more politically conscious and active. Our objectives and concerns are moving away from solving our technical problems to solving the problem of operating on limited resources. Our nonprofit association should be providing services to its members, and I believe that the C.M.V.C.A. will be measured in the future by how well we meet our members' needs and help solve their problems. We are getting to the position of our members paying dues not because they are loyal but because they expect results.

To provide these services there is a need for an Executive Director/Administrator of the California Mosquito and Vector Control Association whose duty and responsibility will be to provide coordination, continuity, direction, and control of association activities. At present, there is no one person that is providing that service to the Association. There is no officer of the C.M.V.C.A. that has time to coordinate and have a thorough knowledge of the C.M.V.C.A. With the fragmentation of duties between widely geographically dispersed officers, a degree of coordination is required that is not being met. Each of the officers takes time out from his District job to perform duties of the Association, which an Executive Director could be performing. Therefore, the C.M.V.C.A. is costing each of our Districts a considerable amount beyond dues each year considering the amount of committee work, etc. done by each District. This will provide an operational plan for the C.M.V.C.A. similar to the operational plans we have for our Districts.

The Joint Powers Self-Insurance Program has an administrator. He provides coordination and direction or a plan for the V.C.J.P.A. This administrator and consultants are paid out of the premiums paid by the self-insurance program participants.

It is suggested here that the Executive Director for the C.M.V.C.A. and the Administrator for the Joint Powers Self-Insurance Program be the same person. This person's salary can be paid jointly by the J.P.A. and C.M.V.C.A. This would insure an ongoing funding for the Exeucitve Director/Administrator. With this combination of duties and the funding possibilities, the C.M.V.C.A. could be more cost effective, reduce the amount of dues paid and provide more services to the Association and individual members.

If we do get one person to administer the C.M.V.C.A. and the V.C.J.P.A., then we should have a permanent central office and it should be in Sacramento. The Sacramento location offers an easy access central location that is accessible to state government. The legislative happenings in Sacramento will influence us more in the future than any other aspect of our operation.

Along these lines, there are three areas which I would like to see developed as a service to member agencies:

1. Education Seminars on all phases of management.
2. Association funding of specific applied research.
3. A statewide coordinated data processing system for vector control.

I feel that these are projects that will strengthen our Association and individual agencies.

What are the prospects of doing some of these projects? Unfortunately, not good. Without taking away from the next speaker, Dr. Lyman and the panel to follow, things do not look good in the immediate future. Our revenues are down by 50 percent due to Proposition 13 and the little state aid received by most of the Districts will be eliminated next year in all probability. Our individual agencies will survive but we won't be doing the job that we can and should be doing.

There is hope, however, in the resourcefulness and determination of the people that govern/manage and work for our agencies. Most of our programs started on a shoestring with meager amounts of money and surplus equipment. Through prudent management and fiscally responsible boards, these programs were built up to be efficient, effective, well documented programs that the world has used as a model.

[1]Presented at the 48th Annual Conference of the California Mosquito and Vector Control Association at the Quality Inn Hotel, Anaheim, California, January 20-23, 1980.

I have appreciated serving as President this past year. Unfortunately, I have not done many of the projects that I would have liked to have started. My excuse is that we expended our entire efforts on fighting for survival at the local and state level. The area we can be most proud of in accomplishment is the formation of the Vector Control Joint Powers Agency. It was the sheer determination of Warren Cook that forced this project through a maze of difficulties. This one area alone will have more affect on our Association and agencies in the future than we all realize at this time. I view the J.P.A. as a means to accomplish many cost saving methods as well as a vehicle in which to solve other problems. It can be used by the Association as a means to accomplish many of its goals.

Another area in which we accomplished a great deal is the legislative area in which Bill Hazeltine spent hundreds of hours. Because of his efforts, Senate Bill 382 made it as far as it did and the legislators became very aware of mosquito and vector control in the state. This is evidenced by the task force report the following speaker will comment on. Also, he spent many hours screening and lobbying on legislation that would affect our Districts.

The last area in which we accomplished something is in the State Vector Biology and Control Section cut backs. While we didn't save many jobs and areas of technical expertise, we awoke the legislature and some of the Bureaucrats to the broad support this vital group of people has in the state. In all this mess of Proposition 13 and the V.B.C.S. cuts, Don Womeldorf supported our Districts and efforts. I thank Don for this dedicated effort this past year.

Another area which made great headway, but in which we had little influence, is the coordination and communication of University of California research. Russ Fontaine has done an outstanding job this year as evidenced by the "Mosquito Research Annual Report" and "Mosquito Research Hi-lites". Thanks also to Bill Hazeleur who helped promote and expedite these efforts.

In conclusion, I thank the rest of the Association for their support and wish the incoming officers good luck.

ASSESSMENT OF THE RELATIVE VALUE OF ALTERNATIVE APPROACHES FOR SURVEILLANCE AND PREDICTION OF ARBOVIRAL ACTIVITY[1]

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On June 6, 1978 Proposition 13 was passed into law in California and resulted in a 53% decrease in the local tax funding base for mosquito control. Official and public concerns were expressed that mosquito-borne disease epidemics would develop. We now have records of mosquito populations and the activity of mosquito-borne disease organisms for the ensuing 2 years. Vector populations and encephalitis viral activity have resurged over much of California but an epidemic has not occurred. This presentation will assess the above developments and the need to maintain a continued surveillance and predictive program as such activities are essential to the success of vector and disease control.

Information Routinely Used for Arboviral Surveillance.–As background, 5 types of information are used routinely in arboviral surveillance (Table 1). Indices of important environmental factors are provided by 2 sources. The U. S. Weather Bureau compiles precipitation and temperature data. The Department of Water Resources, Division of Flood Management provides information concerning flood forecasts and the volume of water that is or probably will be available in the major water resources of the state. A knowledge of flooding and the availability of excess or cheap water for irrigation, industrial and domestic use can be used to predict the development of large populations of disease vectors. Vector control agencies and persons concerned with disease prediction closely follow records on water availability in the winter and spring. The relationships of excess water to high vector populations and high encephalitis rates in past years have been well documented (Reeves 1970). High temperatures are essential for rapid growth of vector populations and rapid maturation of viruses to infective levels in the vector. Again, these relationships are well documented.

Table 1.–Categories of information routinely utilized in arboviral surveillance in California.

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

The third variable is the economic resources that are available for vector control. There has always been a concern of how the annual increased costs of control would be met. However, when economic resources were drastically reduced 2 years ago this factor became even more important. State tax sources may be further reduced by future legislative and public mandate such as Proposition 9 on the June, 1980 ballot. Such action would compound the decrease in funding for vector control. It is obvious that the availability of economic resources for vector control has become as important a factor influencing the occurrence of vector-borne diseases as were other recent events such as the development of insecticide resistance in the vectors or legal restrictions on the use of insecticides for vector control.

The fourth factor that is monitored is the level of vector populations. The relationship of vector populations to viral activity has been well documented (Olson et al. 1979). If there are few or no vectors there will be reduced viral activity; and if vectors are abundant there may be an increased viral activity and disease risk to man and equines. The most feasible index of vector populations that has emerged over decades of research and control evaluation is to measure the number of female vectors collected per light trap night. This index is used as a weekly moving indicator of population changes through the summer.

The preceding 4 items are more or less indirect but are readily available predictor measures. The most direct approaches are to determine viral activity, and this requires special laboratory resources and technical staff. The indicators most commonly used are:

1. Isolation and identification of virus from mosquito pools.
2. Detection of antibodies as they develop in sentinel flocks that are subject to infection by mosquito vectors.
3. Diagnosis of clinical cases in humans, equines or other vertebrate hosts.

The remainder of this paper will focus on the measures of these variables that have accumulated in the past 3 years. The specificity, sensitivity and feasibility of each of these surveillance measures will be discussed. The purpose of the discussion is to stress that surveillance is a basic part of programs for vector control and health maintenance.

Developments Post Proposition 13.–Water resources and temperature are relatively or completely unaffected by Proposition 13. Water availability and temperatures in the past several years have been at or above normal and thus favored increased viral activity.

As was stated earlier, there was a 53% reduction in the economic resources from local tax revenues that support mosquito/pest abatement districts in California. Supplemental state funds were awarded for an interim period, but the future for further supplementation is uncertain. The specifics

[1]These studies were supported in part by Research Grant AI-03028 from the National Institute of Allergy and Infectious Diseases and General Research Support Grant I-SO1-FR-05441 from the National Institutes of Health, and special State funds for mosquito control research appropriated annually by the California Legislature.

of these developments are documented in a number of reports and in much more detail than can be done in this brief presentation. It must be expected that a major reduction in resources will be reflected in a further decreased level of mosquito control.

Records show that *Culex tarsalis* populations have increased each of the past 2 years in the majority of mosquito control jurisdictions in the Central Valley of California (Table 2). Vector populations in many districts are now at or above the levels previously associated with high levels of viral transmission in basic cycles (Table 3). In 1979, in some areas, indices approached levels that were associated with the occurrence of clinical cases in man in earlier years (Olson et al. 1979; Reeves 1968, 1970).

In 1979, the levels of viral activity increased along with increases in vector populations. Dr. Emmons reported on the recoveries of Western equine encephalomyelitis (WEE) virus from 113 pools of mosquitoes, principally *Cx. tarsalis*, collected throughout the state (Emmons et al. 1980). St. Louis encephalitis (SLE) virus was recovered from 29 pools of *Cx. tarsalis* collected in Southern California. Infection rates for WEE virus in Kern County exceeded 1:1000 vectors at several sites that were sampled each summer month and reached a peak rate of almost 1:50 at 1 site by early fall. Infection rates of 1:1000 vectors tested occurred in other regions of the state.

In 1979, 31 sentinel chicken flocks were placed in representative areas of the state from Shasta County in the north to Imperial County in the south (Table 4 and 5). Monthly blood samples were collected and tested for antibody development to WEE and SLE viruses at the Department of Health Services and our laboratories. Sixteen of the 31 flocks were located at the same sites as in 1977 and 1978 and significant increases in the WEE viral infection rates were observed in 1979 (Table 6). There had been no evidence of viral activity in 1977. In 1978, 26% of the birds became infected. There was a further overall increase to 47% in 1979. The tests indicated that high rates of infection had occurred with WEE virus in all regions sampled and that the highest rates were in the Central Valley region. The only region with any significant evidence of SLE infection was in Imperial County where 58% of the birds became infected. However, an occasional bird developed what appeared to be SLE antibody in the San Joaquin and Sacrament Valley. Such findings may indicate there was a very low level of SLE viral activity in the most northern region. The SLE findings are of sufficient importance that they are the subject of ongoing research to clarify their specificity.

There was a statistically significant correlation between the levels of *Cx. tarsalis* populations and the levels of viral transmission to sentinel chickens (Table 4). The sites at which virus transmission took place all had seasonal light trap indices in the range that was previously associated with maintenance of WEE viral transmission cycles (Reeves 1968, 1970). One site where no birds became infected had a light trap index below 1 per trap night.

We have an extensive surveillance program in California to identify suspect clinical cases and to subject them to extensive diagnostic tests. Dr. Emmons has reported on the details of this program and its findings each year (Emmons et al. 1980). We think it is an important service and as thorough as is practical. The predictive value of this activity is that if clinical cases are identified early in the summer, it is probable that more will develop.

Table 2. Seasonal light trap indices of *Culex tarsalis* females from 22 mosquito control agencies in the Central Valley, 1977, 1978 and 1979.

Agency and trap record	1977	1978	1979
Sacramento Valley			
Butte (Rural)	30.3[w]	44.6[c,s,w]	33.8[w]
Colusa (Rural)	2.4[w]	15.9[w]	35.3[w]
Glenn (Rural)	11.9[w]	45.6[c,s,w]	61.1[c,s,w]
Los Molinas (Rural)	0.6	5.9[w]	6.9[w]
Sacramento-Yolo (Urban)	0.3	4.1[w]	8.5[w]
Shasta (Urban)	4.0[w]	2.3[w]	5.1[w]
Sutter-Yuba (Urban)	0.8	3.8[w]	3.9[w]
Tehama (Urban)	10.7[s,w]	9.5[w]	8.8[w]
Northern San Joaquin Valley			
Eastside (Urban)	0.1	0.1	0.3
Merced (Rural)	2.2	4.0	6.0[w]
San Joaquin (Urban)	0.2	0.3	0.4
Turlock (Urban)	0.1	0.1	0.1
Southern San Joaquin Valley			
Consolidated (Urban)	0.0	0.1	0.1
Delano (Rural)	0.5	5.2[w]	8.6[w]
Delta (Urban)	0.1	0.2	0.1
Fresno (Urban)	0.0	0.1	0.0
Fresno Westside (Urban)	0.6	2.8[w]	1.9[w]
Kern (Rural)	7.2[w]	7.3[w]	10.5[w]
Kings (Urban)	0.4	1.2[w]	1.0[w]
Madera (Urban)	0.4	0.3	0.8
Tulare (Urban)	0.8	1.7[w]	3.9[w]
West Side (Urban)	1.6[w]	1.6[w]	4.2[w]

w = Index high enough for WEE viral activity in basic cycles
s = Index high enough for SLE viral activity in basic cycles
c = Index high enough for human encephalitis to occur

Table 3.—Expected viral activity based on 1979 seasonal light trap indices for *Culex tarsalis* females, 22 Central Valley mosquito control agencies.

Expectation	No. of agencies
WEE virus active	15
SLE virus active	1
Human cases	1

In earlier publications we determined the levels of *Cx. tarsalis* populations that were associated with the occurrence of human and horse cases. Such indices were reached in a few regions of the Central Valley in 1979. Fortunately, only 1 human case of WEE was identified. There was an increase of proven WEE in horses. Eighteen cases were diagnosed in 1979 as compared with 12 in 1978 and 1 in 1977. The horse cases were predominantly from regions in the Sacramento or Northern San Joaquin Valleys where *Cx. tarsalis* seasonal indices were the highest in the state.

Unfortunately, it has not proven feasible to obtain current data on the susceptibility of human populations in the age and occupational groups most likely to develop WEE or SLE. We

Table 4.--Correlations[a] of seasonal female *Culex tarsalis* light trap indices and proportions of chickens infected with WEE virus, Central Valley 1979.

Seasonal Light Trap Index		Chicken Infection		County
Rank	*Cx. tarsalis* per trap night	Rank	%WEE positive[b]	
1	150	2	88	Buttc
2	122	11	50	Colusa
3	87	14	43	Sutter
4	67	15	39	Kern
5	52	12	46	Glenn
6	51	16	36	Yuba
7	40	13	44	Fresno
8	26	5.5	67	Yuba
9.5	17	1	92	Kern
9.5	17	8.5	57	Colusa
11	16	7	63	Kern
12.5	12	17	35	Butte
12.5	12	26	14	Kern
14.5	10	20.5	24	Placer
14.5	10	4	74	Kern
16	9	22.5	21	Kern
17	8	25	17	Yuba
18	4	22.5	21	Yuba
20.5	3	20.5	24	Butte
20.5	3	19	29	Kern
20.5	3	3	86	Kern
20.5	3	5.5	67	Kern
24	2	18	32	Imperial
24	2	28.5	0	Tulare
24	2	8.5	57	Shasta
27	1	10	52	Butte
27	1	24	18	Shasta
27	1	27	4	Riverside
29	<1	28.5	0	Kern
	?		5	Riverside
	?		0	San Diego

[a]Rank correlation coefficient = .45 ($p < 05$)

[b]Positive by both immunoflorescent and plaque reduction neutralization tests

have reason to believe that there are very few immunes in the general population of California today. If true, there are many people who would develop encephalitis if infected and we have projected estimates of 1,000 or more cases if the attack rates that prevailed in the 1950's recurred today (Reeves and Milby 1979). To carry out an annual survey of immunity in representative samples of the residents of the state would be costly but a useful surveillance procedure for predictive purposes.

Comments on the Comparative Values of the Surveillance Methods.--Some brief comments should be made on the comparative value of the above methods. The measures of water availability and temperature provide the cheapest surveillance data as large and separately funded agencies collect the information for other purposes. The data are the least specifically related to vectors and disease prevalence but are available at the earliest date. These measures are available before the development of vector populations or disease transmission. Thus, they are indirect predictors but valuable.

Indices of a vector population are tedious to collect and analyze. The light traps have the advantage that they measure both disease vector and pest populations of mosquitoes. Thus, resultant data are valuable for disease prediction and are also used to evaluate the effectiveness of the control of most species. The data identify areas most in need of intensified control. Thus, the cost aspects of such programs can be divided among the several purposes. The indices of *Cx. tarsalis* populations allow the prediction of probable levels of encephalitis viral activity.. The indices allow an evaluation of success in

Table 5.–WEE serological conversion rates in sentinel chickens, California 1979.

Area	No. flocks	No. birds	% infected[1] Total sample	Range/ flock
Sacramento Valley	15	331	43	17-88
San Joaquin Valley	12	244	41	0-92
Southern California	4	87	9	0-32
Total	31	662	38	0-92

[1]Positive by both immunofluorescent and plaque reduction neutralization tests.

Table 6.–Comparison of sentinel chicken serological conversion rates for WEE virus by mosquito abatement district*.

	1977 - 1979			
		% chickens WEE positive**		
M. A. D.	No. flocks	1977	1978	1979
Butte	4	0	25	52
Glenn	1	0	43	46
Sutter-Yuba	3	0	36	33
Kern	8	0	19	49
Total	16	0	26	47

*Includes only flocks which have not moved

**Plaque reduction neutralization test results only

holding a vector population at or below threshold levels that have been associated with the development of clinical cases or epidemics.

The several direct tests of viral activity each have their uses and limitations. If a sufficient number of mosquito pools can be collected and tested each week, encephalitis viruses can be isolated early in the summer. Such isolates usually will precede or coincide with the first evidence that sentinel flocks have experienced infection. It is not feasible to bleed and test sentinel flocks on a weekly basis and it requires up to 2 weeks after infection for a bird to develop unequivocal levels of antibodies. Weekly sampling of 10 or 20 pools of 50 ***Cx. tarsalis*** each from a site will reliably yield virus when infection rates are at or above 1:500 to 1:100. When infection rates are below these levels, or it is not possible to collect and test mosquito samples of this magnitude, the sentinel flocks offer the most sensitive viral surveillance procedure. For example, there were serological conversions in sentinel flocks at a number of sites in 1979 where light trap indices were below 10 per trap night (Table 4). It would be impossible to collect a sizable number of mosquito pools at such sites. In addition, an isolation from a mosquito pool indicates there is a virus source in the area but does not provide an index of viral transmission rates by the vector population. Development of antibodies in a sentinel bird is a measure of viral transmission. Antibody tests can yield nonspecific reactions but an identified viral isolation from mosquitoes is specific. Thus, the 2 surveillance methods differ in their specificity and sensitivity. They compliment rather than replace each other. The most effective surveillance system should include both measures as well as a program for clinical case finding and diagnosis.

We cannot evaluate the virulence of the viruses that prevail in an area other than to detect and diagnose clinical cases of encephalitis. The Center for Disease Control of the United States Public Health Service in Fort Collins, Colorado, is developing a laboratory virulence marker system for SLE viruses. It would be a major step forward if procedures were available to determine that viruses isolated from mosquito samples are likely to produce clinical disease and deaths in man and equines.

Comments on Malaria.–We would feel guilty of a serious omission if no mention was made of the potential parallel developments in malaria in our state. There has been a significant increase in the number of imported cases. At this writing, the Department of Health Services has reported 318 cases for 1979 and in 1978 they reported 226 cases. Such cases can be a source to infect local *Anopheles*. Simultaneously, the Department of Health Services has summarized the levels of *Anopheles freeborni* per light trap night in the Sacramento Valley area. The counts in 1978 and 1979 were high in many areas and there was some increase in 1979. It will require a major effort and financial investment to maintain surveillance and to prevent the occurrence of malaria transmission in this region. There is always the potential that malaria could again become endemic. Thus, the situation is equally serious as that for encephalitis although more regionally focused.

Conclusions.–In conclusion, we believe that we have an excellent and effective surveillance system for mosquito-borne diseases in California and it should be maintained. The present discussion illustrates how such activity allows the prediction of disease occurrence, assists in evaluating changes in control programs and provides historical documentation of such changes.

The surveillance system is solidly based in a knowledge of the epidemiology of the diseases and demonstrates that the control methods are effective. The maintenance of the surveillance system will not be cheap as it will require investment of increasingly scarce economic resources by both local and state levels of government. It will be difficult at times to decide whether to buy another barrel of insecticide or to maintain a surveillance system.

It is worthwhile to recall that the control of encephalitis in this state was successful (Reeves 1976; Reeves and Milby 1979). The alternative to maintaining this successful program appears to be to accept that there will be a constant decrease in the effort. In such an event, diseases such as encephalitis or malaria probably will return to their former endemic and epidemic levels. We believe the current observations that vector populations and viral activity have increased can only reflect that a resurgence has begun to occur. Further decreases in the resources for control or a year of excess water probably will result in an epidemic unless a costly emergency control program can be financed and carried out at such times. Emergency programs for control of an ongoing epidemic usually are too late and are relatively ineffective. Some of our vector control agencies will have a difficult time controlling the vectors produced in a year of average water availability. The present concerted effort that is being carried out locally and state-wide to assure that financial means will be found to maintain vector control programs must be pursued vigorously.

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SURVEILLANCE FOR ARTHROPOD-BORNE VIRUSES AND DISEASE BY THE CALIFORNIA DEPARTMENT OF HEALTH SERVICES, 1979

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This is the tenth report summarizing the Department of Health Services (DOHS) annual collaborative efforts with other groups on arbovirus surveillance, since an interest in determining mosquito vector infection levels was revived in 1969. As usual, our studies were correlated with the work of local mosquito abatement districts (MADs); County Health Departments; the University of California, Berkeley, School of Public Health, Arthropod-Borne Virus Research Unit (UCBSPH, AVRU); private physicians and veterinarians; California Department of Food and Agriculture; and other concerned individuals and agencies. Despite economic limitations on the extent of field and laboratory work which was possible, a useful surveillance program was accomplished. Special assistance by the UCBSPH,AVRU was provided via supplemental field collections and a microbiologist who did much of the mosquito testing and sentinel chicken serologic tests during the summer period.

During 1979, 342 patients throughout California were tested by the State and County virus laboratories for western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE) and other possible causes of encephalitis/meningitis (herpes, mumps, leptospirosis, enteroviruses, etc.) (Table 1). As usual, a selection of those cases for which the etiology could not be determined by standard tests will be subsequently tested by the UCBSPH,AVRU for antibody to other arthropod-borne viruses besides WEE and SLE. There were 12 human brain samples and 2 human cerebrospinal fluid samples tested in suckling mice for arboviruses, but all were negative.

Only one human case of WEE was found in 1979; a 63 year old woman from Sacramento County, who became ill about September 7 and was hospitalized 8 days later because of vomiting, fever, cranial nerve abnormalities, and progressive aphasia and mental changes. Recovery was slow. Only a single serum sample taken October 1 was available for testing. A complement-fixing (CF) WEE antibody titer of 1:32, an indirect fluorescent antibody (IFA) WEE titer of 1:128, and an IgM-specific IFA WEE antibody titer of 1:32 were shown, indicating recent infection, even though complete serologic studies on paired sera could not be done.

Two persons were found to have rather high but stationary levels of SLE antibody by CF, IFA, and neutralization tests, but no SLE-specific IGM/IFA antibody, thus indicating past infection only (a 41 year old man from Red Bluff and a 51 year old woman from the Sacramento area). A 1-½ year old boy hospitalized with meningitis in a Mexicali hospital in July had paired sera and a rectal swab submitted via the Imperial County Health Department. The rectal swab yielded an isolate of ECHO-type 3 virus, but paired sera also showed high levels of SLE-specific CF, neutralizing, IFA, and IFA-IgM antibody, indicating recent SLE infection. It was not clear whether his disease was due to SLE virus, ECHO-3 virus, or both, but SLE virus infection clearly had occurred during the past year in Mexicali.

There were 74 equines tested serologically for WEE during the season, and 18 equine brain samples were tested in suckling mice (no arboviruses were isolated). There were 18 equines considered positive or presumptive-positive for WEE, based on rising or high-stationary CF and IFA antibody titers. Additional tests (hemagglutination-inhibition method) on these equines and the above-mentioned human sera are in progress by the UCBSPH,AVRU, to help complete the serologic studies. The equine cases occurred in the following counties: Sacramento

[1]Viral and Rickettsial Disease Laboratory Section.

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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 1979.

County	Totals	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Unknown
Totals	342	2	4	5	13	27	41	44	51	64	43	20	2	26
Alameda Co.	8							1	2	2	3			
Berkeley	10								2	2	3			3
Butte	16			2	1		2	1	6	2	1			1
Contra Costa	2			1						1				
El Dorado	7		1					1		1	2	1		1
Fresno*	51				3	4	7	8	10	10	6	2	1	
Humboldt	1										1			
Imperial	11			1		2	1	1		1	1			4
Inyo	1										1			
Kings	1									1				
Lake	2						1			1				
Lassen	1									1				
Los Angeles Co.*	49				1	3	4	4	8	13	11	5		
Mendocino	3						2		1					
Merced	11					1	5	2				1		2
Napa	7							1		1	1			4
Nevada	2					1								1
Orange*	8	1		1	2	2		1		1				
Placer	11		1			1	5	1			2			1
Plumas	1						1							
Sacramento*	17				1	1	4			5	3	2	1	
San Bernardino*	18		2				2	7	4		1	2		
San Diego*	28							5	7	10	3	3		
San Francisco	5							3	1	1				
San Joaquin	9					1	2	1	1	3	1			
San Luis Obispo	7				1	2				2				2
Santa Clara	3													3
Santa Cruz	14					2	2	1	1	3	2	1		2
Shasta	9					2	1		4		1	1		
Siskiyou	1							1						
Sonoma	1													1
Stanislaus	2					1			1					
Sutter	6				1			2		2				1
Tulare	1				1									
Tuolumne	2					2								
Ventura	1							1						
Yolo	15	1			2	2	2	2	3	1		2		

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

Table 2.-- Positive and presumptive positive cases of western equine encephalomyelitis in equines – California 1979.

Case No.	County of Occurrence	Age and Sex	Date of Onset	WEE Vaccination History	Serological Results		
					Date	CF[a]	IFA[b]
1	Imperial (El Centro)	14 mos. M	06-10-79	None	06-14-79	1:32	1:64
					07-16-79	1:128	1:128
2	Sacramento (Elk Grove)	1 yr. M	07-09-79	07-09-79	07-18-79	1:64	1:1024
					07-30-79	1:128	
					09-19-79	1:16	1:256
3	Butte (Chico)	3 yrs.M	07-11-79	None	07-12-79	1:128	1:512
					08-06-79	1:64	1:512
4	Shasta (Cottonwood)	5 yrs.F	08-01-79	None	08-04-79	1:128	1:512
					08-25-79	1:64	1:256
5	Kern (Bakersfield)	2 yrs.M	08-02-79	1978? None in 1979	08-09-79	1:32	1:256
					08-20-79	1:64	1:256
6	Yolo (Davis)	1 yr.F	08-02-79	None	08-03-79	1:64	1:256
					08-14-79	1:64	1:256
7	Sacramento (Galt)	18 mos.F	08-08-79	None	08-09-79	1:64	1:64
					08-24-79	1:256	1:256
8	San Joaquin (Tracy)	1 yr.F	08-09-79	None	08-16-79	1:64	1:128
					08-24-79	1:64	1:128
9	San Joaquin (Acampo)	9 yrs.F	08-12-79	None	08-13-79	1:128	1:512
					08-14-79	1:128	1:1024
10	Stanislaus (Oakdale)	1 yr.F	08-12-79	None	08-14-79	<1:8	1:8
					09-05-79	1:256	1:256
11	San Joaquin	4 yrs.F	08-20-79	None	08-21-79	1:32	1:32
					08-31-79	1:128	1:512
12	Sacramento (Folsom)	4 yrs.M	08-29-79	None	08-29-79	1:32	1:128
					09-13-79	1:64	1:256
13	Stanislaus (Riverbank)	6 yrs.G	08-24-79	Unknown	08-24-79	1:8	1:8
					09-22-79	1:32	1:256
14	Madera (Chowchilla)	6 yrs.F	09-08-79	None	09-08-79	1:16	1:32
					09-21-79	1:64	1:64
15	Solano (Dixon)	6 mos.M	09-03-79	None	09-04-79	1:32	1:32
					09-17-79	1:256	1:512
16	Modoc (Fort Bidwell)	5 yrs.F	08-21-79	None	08-24-79	1:64	1:512
					09-28-79	1:64	1:512
17	Sacramento (Elk Grove)	5 yrs.F	09-22-79	None	09-25-79	1:32	1:64
					10-08-79	1:256	1:1024
18	Fresno (Fresno)	18 mos.M	10-04-79	None	10-04-79	<1:8	1:8
					10-19-79	1:256	1:128

[a]Complement Fixation Test.

[b]Indirect Fluorescent Antibody Test.

(4), San Joaquin (3), Stanislaus (2), and Imperial, Butte, Shasta, Kern, Yolo, Madera, Solano, Fresno, and Modoc (1 each) (Table 2).

In total, 79,785 mosquitoes (1,810 pools) were tested for viruses, almost entirely representing only 4 species (*Culex tarsalis*, *Culex pipiens* complex, *Culex peus*, and *Aedes melanimon*) (Tables 3 and 4). There were 190 virus isolates identified: 114 WEE, 36 Turlock, 26 SLE, 9 Hart Park, 4 California encephalitis group, and 1 Bunyamwera group (Table 5).

The State Virus Laboratory also participated in a chicken flock serologic surveillance program during 1979, in collaboration with the UCSPH,AVRU involving 15 flocks in the Sacramento Valley, 12 in the San Joaquin Valley, and 4 in Southern California. The birds were bled monthly from June through October, and seroconversions to both SLE and WEE viruses were demonstrated in all 3 regions, by both the plaque-reduction neutralization method and the IFA method, which was being evaluated for the first time for this purpose. The

Table 3. Number of mosquitoes (pools) tested by county and species, by the Viral and Rickettsial Disease Laboratory Section, California Department of Health Services, 1979.

County	*Culex tarsalis*		*Culex pipiens* complex		*Culex peus*		*Aedes melanimon*		TOTALS	
Butte	6225	(125)					3980	(80)	10,205	(205)
Colusa	4772	(98)							4,772	(98)
Fresno	130	(3)							130	(3)
Glenn	1000	(20)							1,000	(20)
Imperial	5744	(164)	816	(31)					6,761	(200)*
Kern	7369	(153)	601	(13)			1921	(40)	9,891	(206)
Kings	223	(6)	162	(6)			15	(1)	400	(13)
Los Angeles	15	(1)	10	(1)	25	(1)			50	(3)
Merced	550	(11)					26	(1)	576	(12)
Orange	76	(3)							126	(4)**
Placer	1431	(34)			1099	(28)			2,530	(62)
Riverside	14150	(289)	127	(5)					14,277	(294)
San Bernardino	2540	(57)							2,540	(57)
San Joaquin	1510	(38)	112	(5)	68	(4)	144	(4)	1,834	(51)
San Diego	193	(9)							193	(9)
Sacramento	1196	(33)	22	(1)	23	(2)			1,241	(36)
Shasta	1920	(50)	714	(17)					2,634	(67)
Stanislaus	2927	(78)	526	(14)	100	(6)	183	(6)	3,736	(104)
Sutter	4658	(100)	50	(1)			829	(17)	5,537	(118)
Tehama	1665	(35)							1,665	(35)
Tulare	4476	(96)	201	(4)					4,677	(100)
Yolo	1432	(35)							1,432	(35)
Yuba	2657	(56)	718	(15)					3,375	(71)
Mohave, AZ	93	(4)							93	(4)
Yuma, AZ	102	(2)	8	(1)					110	(3)
TOTALS	67,054	(1500)	4067	(114)	1315	(41)	7098	(149)	79,785	(1810)

*includes 201 (5) *Aedes vexans*
**includes 50 (1) *Culex erythrothorax*

IFA method is rapid and can be performed on a timely basis. Despite some problems with non-specific results at low serum dilutions, which must be worked out, the IFA method appears to be sufficiently sensitive and accurate for use as a serologic surveillance tool in the future. Details of chicken seroconversions by region and month are given in the paper by W. C. Reeves and M. M. Milby in this publication.

A special effort was made this year to provide more timely and current information on the results of the surveillance program to interested groups. Besides prompt telephoned reports to the MADs directly involved of positive mosquito pools, human and horse cases, and chicken seroconversions, a weekly summary report was prepared and mailed to all groups (19 issues from June 1 to October 19).

For the 1980 season, we hope sufficient funding will be available to continue a similar level of mosquito and chicken flock surveillance, and that these indices can be used more selectively and efficiently to get the maximum information at minimum cost. The resurgence of WEE virus activity seen in the past 2-3 years, and the continuing SLE virus activity, along with the difficulties in maintaining adequate mosquito suppression, warn that California's battle with arbovirus encephalitis is not yet done.

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 and Patricia Boehme in conducting chicken flock serologic tests (IFA method) and the mosquito virus-testing portion of the study and summarizing the results is especially noted.

Table 4.–Number of mosquitoes (pools) tested by species and month, by the Viral and Rickettsial Disease Laboratory Section, California Department of Health Services, 1979.

	April	May	June	July*	August	September	October	Totals
Culex tarsalis	1414 (31)	3841 (80)	10,286 (234)	15,647 (342)	18,558 (427)	13,587 (302)	3721 (84)	67,054 (1500)
pipiens complex	161 (6)	10 (1)	660 (27)	67 (3)	1,499 (36)	877 (21)	793 (20)	4,067 (114)
peus			469 (11)	37 (2)	569 (8)			1,315 (41)
Aedes melanimon			1,832 (37)	1,407 (30)	1,865 (39)	648 (15)	1346 (28)	7,098 (149)
TOTALS	1575 (37)	3851 (81)	13,247 (309)	17,409 (383)	22,491 (522)	15,352 (346)	5860 (132)	79,785 (1810)

*July total includes 50 (1) *Culex erythrothorax* and 201 (5) *Aedes vexans*.

Table 5.- Viral isolates from mosquito pools tested during 1979 by the Viral and Rickettsial Disease Laboratory Section, California Department of Health Services.

Identifying Number	County	Place	Date Collected	Species	Number in Pool	Agent Isolated
E-02304	Riverside	Blythe	05-22	*Cx. tarsalis*	50	Turlock
E-02526	Riverside	Mecca	05-22	" "	50	Bunyamwera Gp.
E-02527	Riverside	Mecca	05-22	" "	50	Hart Park
E-02529	Riverside	Mecca	05-22	" "	50	Turlock
E-02532	Riverside	Mecca	05-22	" "	50	Turlock
E-02533	Riverside	Mecca	05-22	*Cx. tarsalis*	30	Turlock
E-02203	San Bernardino	Needles	05-30	" "	50	Turlock
E-02204	San Bernardino	Needles	05-30	" "	50	Turlock
E-02208	San Bernardino	Needles	05-30	" "	50	Turlock
E-02209	San Bernardino	Needles	05-30	" "	50	Turlock
E-02210	San Bernardino	Needles	05-30	*Cx. tarsalis*	50	Turlock
E-02212	San Bernardino	Needles	05-30	" "	50	Turlock
E-02213	San Bernardino	Needles	05-30	" "	50	Turlock
E-02214	San Bernardino	Needles	05-30	" "	50	Turlock
E-02216	San Bernardino	Needles	05-30	" "	50	Turlock
S9-26-013	Butte	Graylodge	06-04	*Ae. melanimon*	50	Calif. Gp.
S9-26-045	Imperial	New River	06-06	*Cx. tarsalis*	50	Turlock
S9-26-048	Imperial	New River	06-06	" "	50	Turlock
S9-26-049	Imperial	New River	06-06	" "	50	Turlock
E-01100	Imperial	Alamo River/Calexico	06-13	" "	50	WEE
E-01001	Imperial	Alamo River/Calexico	06-13	*Cx. tarsalis*	53	WEE
E-01103	Imperial	BrockmanRd/Greeson WA	06-13	" "	23	WEE
E-01108	Imperial	Hoveley Rd/New River Br.	06-13	" "	50	WEE
E-01109	Imperial	Hoveley Rd/New River Br.	06-13	" "	50	WEE
E-01114	Imperial	Brockman Rd/New River Br.	06-13	" "	50	WEE
E-01120	Imperial	Brockman Rd/New River B.	06-13	*Cx. tarsalis*	50	WEE
E-01115	Imperial	IID Village	06-14	" "	50	WEE
E-01132	Imperial	Imperial County Park	06-14	" "	50	WEE
E-01133	Imperial	Imperial County Park	06-14	" "	50	WEE
E-01134	Imperial	Imperial County Park	06-14	" "	50	WEE
E-01138	Imperial	Imperial County Park	06-14	*Cx. tarsalis*	50	WEE
E-01141	Imperial	Imperial County Park	06-14	" "	50	WEE
E-01145	Imperial	Imperial County Park	06-14	" "	29	WEE
E-01147	Imperial	Bard	06-14	" "	12	WEE
E-01154	Imperial	Bard	06-14	" "	33	WEE
E-01150	Yuma, AZ	Yuma Test Station	06-14	*Cx. tarsalis*	50	WEE
E-01151	Yuma, AZ	Yuma Test Station	06-14	" "	52	SLE
E-01178	Imperial	New River Transect Trail	06-19	" "	50	WEE
E-01187	Imperial	Brawley	06-19	" "	50	WEE
E-01091	Imperial	Brawley	06-19	" "	50	WEE

continued -

Identifying Number	County	Place	Date Collected	Species	Number in Pool	Agent Isolated
E-01093	Imperial	Brawley	06-19	*Cx. tarsalis*	45	WEE
E-01094	Imperial	Brawley	06-19	*Cx. pipiens*	32	WEE
E-01140	Imperial	New River/Calexico	06-19	" "	50	WEE
S9-26-038	Sutter	Dean's	06-19	*Cx. tarsalis*	50	Turlock
E-01129	Imperial	Corda Ranch/Heber	06-20	" "	10	WEE
E-11017	Butte	Graylodge Wildlife Area	06-27	*Ae. melanimon*	50	Calif. Gp.
E-11021	Butte	Graylodge Wildlife Area	06-27	" "	50	WEE
E-02535	Riverside	Mecca	06-27	*Cx. tarsalis*	50	WEE
E-02545	Riverside	Mecca	06-27	" "	50	WEE
E-02546	Riverside	Mecca	06-27	" "	50	Turlock
E-02547	Riverside	Mecca	06-27	*Cx. tarsalis*	50	WEE
E-02549	Riverside	Mecca	06-27	" "	50	SLE
E-02564	Riverside	Mecca	06-27	" "	50	WEE
E-02565	Riverside	Mecca	06-27	" "	50	WEE
E-02569	Riverside	Mecca	06-27	" "	50	WEE
E-11025	Glenn	Willows	06-28	*Cx. tarsalis*	50	Turlock
E-13338	Sutter	Rio Oso	07-03	" "	50	WEE
S9-26-074	Sutter	Dean's	07-09	" "	50	WEE
S9-26-076	Sutter	Dean's	07-09	*Ae. melanimon*	50	Calif. Gp.
E-13358	Colusa	Colusa	07-11	*Cx. tarsalis*	50	WEE
E-13367	Colusa	Colusa	07-11	*Cx. tarsalis*	50	WEE
E-06002	Fresno	Mendota	07-11	" "	53	Hart Park
E-11072	Shasta	Panorama Point Rd.	07-13	" "	50	WEE
E-11073	Shasta	Panorama Point Rd.	07-13	" "	26	WEE
E-11075	Shasta	Reading Drive	07-13	" "	15	WEE
S9-26-206	Kern	Meadowbrook	07-15	*Cx. tarsalis*	50	WEE
E-13403	Sacramento	Elk Grove	07-17	" "	45	WEE
E-11045	Tehama	Woodson Bridge State Park	07-18	" "	50	WEE
E-02580	Riverside	Mecca	07-18	" "	50	WEE
E-02581	Riverside	Mecca	07-18	" "	15	Turlock
E-02582	Riverside	Mecca	07-18	*Cx. tarsalis*	50	WEE
E-02586	Riverside	Mecca	07-18	" "	50	SLE
E-02587	Riverside	Mecca	07-18	" "	50	WEE
E-02591	Riverside	Mecca	07-18	" "	50	Turlock
E-13406	Yuba	Wheatland	07-19	" "	50	Turlock
E-13404	Sutter	Rio Oso	07-19	*Cx. tarsalis*	50	WEE
E-13405	Sutter	Rio Oso	07-19	" "	56	WEE
E-13417	Sutter	Trowbridge	07-19	" "	50	Turlock
E-13418	Sutter	Trowbridge	07-19	" "	50	WEE
E-13106	Stanislaus	Denair	07-19	" "	50	Hart Park
S9-26-210	Kern	Meadowbrook	07-22	*Cx. tarsalis*	50	WEE
E-00006	San Diego	Solana Beach	07-24	" "	14	Hart Park
E-13420	Stanislaus	Oakdale	07-24	" "	28	Hart Park
E-13424	Stanislaus	Patterson	07-24	" "	50	WEE
E-16302	Tulare	Earlimart	07-25	" "	50	Hart Park
S9-26-081	Sutter	Rio Oso	07-25	*Cx. tarsalis*	50	Hart Park
S9-26-083	Sutter	Rio Oso	07-25	" "	50	Hart Park
S9-26-100	Sutter	Dean's	07-25	" "	50	WEE
E-11101	Butte	Honcut	07-25	" "	50	WEE
E-11188	Shasta	Reading Drive	07-31	" "	54	WEE
S9-26-117	Yuba	Marysville/Rameys	08-01	*Cx. tarsalis*	50	WEE
S9-26-161	Sutter	Yuba City/Young's	08-02	" "	50	WEE
S9-26-167	Yuba	Shintafers	08-02	" "	50	Turlock
S9-26-180	Kern	Meadowbrook	08-02	" "	50	WEE
E-13456	San Joaquin	Collierville	08-02	" "	34	WEE

continued -

Identifying Number	County	Place	Date Collected	Species	Number in Pool	Agent Isolated
S9-26-191	Kern	Goose Lake	08-05	*Cx. tarsalis*	50	Turlock
S9-26-192	Kern	Goose Lake	08-05	" "	50	WEE
S9-26-213	Kern	Meadowbrook	08-05	" "	50	WEE
E-13468	Sacramento	Sheldon	08-07	" "	30	WEE
E-17002	Sutter	Sutter City/Howard's	08-07	" "	50	WEE
E-13470	Placer	Roseville	08-08	*Cx. tarsalis*	50	Turlock
E-11200	Shasta	Anderson River Park	08-13	" "	23	WEE
E-11205	Shasta	Reading Drive	08-14	" "	32	Turlock
E-01218	Imperial	Imperial Camp Ground	08-14	" "	23	Turlock
E-11299	Tehama	Woodson Bridge State Park	08-15	" "	50	WEE
E-01216	Imperial	Brockman Rd/Greeson Wash	08-15	*Cx. tarsalis*	37	SLE
E-01221	Imperial	Brockman Rd/New River Br.	08-15	" "	50	SLE
E-01226	Imperial	Greeson Wash/New River	08-15	" "	50	SLE
E-01227	Imperial	Greeson Wash/New River	08-15	" "	35	WEE
E-01220	Imperial	Brockman/Greeson Wash	08-16	" "	50	SLE
E-01231	Imperial	Brockman Rd/New River	08-17	*Cx. tarsalis*	50	SLE
E-01236	Imperial	Brawley	08-17	" "	10	SLE
E-01239	Imperial	New River Transect	08-20	" "	50	SLE
E-01240	Imperial	New River Transect	08-20	*Cx. tarsalis*	50	WEE
E-01241	Imperial	New River Transect	08-20	" "	50	WEE
E-01242	Imperial	New River Transect	08-20	" "	50	SLE
E-01244	Imperial	New River Transect	08-20	" "	50	WEE
E-01245	Imperial	New River Transect	08-20	" "	50	SLE
E-01248	Imperial	Mitchell's Camp	08-20	*Cx. tarsalis*	43	SLE
E-01250	Imperial	Gilmore's Camp	08-20	" "	51	SLE
E-13497	Placer	Roseville	08-21	" "	36	Turlock
E-13504	Placer	Lincoln	08-21	" "	50	WEE
E-11224	Shasta	Anderson	08-21	" "	52	WEE
E-11227	Shasta	Anderson	08-21	*Cx. tarsalis*	49	WEE
E-02595	Riverside	Mecca	08-22	" "	50	SLE
E-02597	Riverside	Mecca	08-22	" "	50	Turlock
E-02598	Riverside	Mecca	08-22	" "	50	WEE
E-02602	Riverside	Mecca	08-22	" "	50	SLE
E-02604	Riverside	Mecca	08-22	*Cx. tarsalis*	50	WEE
E-02609	Riverside	Mecca	08-22	" "	50	WEE
E-02610	Riverside	Mecca	08-22	" "	50	SLE
E-02613	Riverside	Mecca	08-22	" "	50	SLE
E-02614	Riverside	Mecca	08-22	" "	50	WEE
E-02625	Riverside	Mecca	08-22	*Cx. tarsalis*	50	SLE
E-02221	San Bernardino	Needles	08-24	" "	50	SLE
E-02223	San Bernardino	Needles	08-24	" "	50	SLE
E-02227	San Bernardino	Needles	08-24	" "	50	WEE
E-02229	San Bernardino	Needles	08-24	" "	50	SLE
E-02230	San Bernardino	Needles	08-24	*Cx. tarsalis*	37	SLE
E-02233	San Bernardino	Needles	08-24	" "	50	SLE
E-02234	San Bernardino	Needles	08-24	" "	50	WEE
E-02235	San Bernardino	Needles	08-24	" "	35	SLE
E-02237	San Bernardino	Needles	08-24	" "	36	WEE
S9-26-223	Kern	Smiths Pasture	08-26	*Cx. tarsalis*	50	WEE
S9-26-225	Kern	Smiths Pasture	08-26	" "	50	WEE
S9-26-228	Kern	Camellia Grove	08-26	" "	50	WEE
S9-26-234	Kern	Goose Lake	08-26	" "	50	Turlock
S9-26-236	Kern	Goose Lake	08-26	" "	50	WEE

continued -

Identifying Number	County	Place	Date Collected	Species	Number in Pool	Agent Isolated
S9-26-238	Kern	Meadowbrook	08-26	*Ae. melanimon*	50	WEE
S9-26-240	Kern	Meadowbrook	08-26	" "	50	Calif. Gp.
S9-26-241	Kern	Meadowbrook	08-26	*Cx. tarsalis*	50	WEE
S9-26-242	Kern	Meadowbrook	08-26	" "	50	WEE
S9-26-243	Kern	Meadowbrook	08-26	" "	50	WEE
S9-26-255	Kern	L. A. Duck Club	08-26	*Cx. tarsalis*	50	WEE
E-13525	Yolo	Zamora	08-27	" "	50	WEE
E-02334	Riverside	Riverside	08-29	" "	50	Turlock
E-02335	Riverside	Riverside	08-29	" "	50	Hart Park
E-02219	San Bernardino	Needles	08-30	" "	50	Turlock
E-16308	Tulare	Earlimart	09-04	*Cx. tarsalis*	50	WEE
E-16311	Tulare	Earlimart	09-04	" "	50	WEE
E-16315	Tulare	Earlimart	09-04	" "	40	WEE
E-11320	Colusa	Colusa	09-05	" "	50	Turlock
E-16213	Kern	F. C. Tracy	09-05	" "	50	WEE
E-16214	Kern	F. C. Tracy	09-05	*Cx. tarsalis*	50	WEE
E-16219	Kern	Tracy's Goose Lake Ranch	09-05	" "	50	WEE
E-16324	Tulare	Teviston	09-10	" "	50	WEE
E-16329	Tulare	Teviston	09-10	" "	50	WEE
E-16330	Tulare	Teviston	09-10	" "	50	WEE
E-16332	Tulare	Teviston	09-10	*Cx. tarsalis*	50	WEE
E-16335	Tulare	Teviston	09-10	" "	50	WEE
E-16336	Tulare	Teviston	09-10	" "	50	WEE
E-16341	Tulare	Teviston	09-10	" "	50	WEE
E-16226	Kern	L. A. Duck Club	09-11	" "	50	WEE
E-01273	Imperial	Gilmores Camp	09-12	*Cx. tarsalis*	50	WEE
E-16348	Kern	Delano	09-17	" "	50	WEE
E-16355	Kern	Delano	09-17	" "	50	WEE
E-16359	Kern	Delano	09-17	" "	50	WEE
E-16360	Kern	Delano	09-17	" "	50	WEE
E-16369	Tulare	Earlimart	09-17	*Cx. tarsalis*	50	WEE
E-16373	Tulare	Earlimart	09-17	" "	50	Turlock
E-16374	Tulare	Earlimart	09-17	" "	50	WEE
E-16376	Tulare	Earlimart	09-17	" "	50	WEE
E-16240	Kern	Northfield Farms	09-18	" "	50	WEE
E-16242	Kern	Northfield Farms	09-18	*Ae. melanimon*	25	WEE
E-16381	Kern	Delano	09-24	*Cx. tarsalis*	50	WEE
E-02239	San Bernardino	Needles	09-26	" "	20	SLE
E-02250	San Bernardino	Needles	09-26	" "	50	WEE
E-11238	Shasta	Anderson	09-27	" "	20	Turlock
E-18008	Kern	Delano	10-01	*Cx. tarsalis*	15	WEE
E-18005	Tulare	Teviston	10-01	" "	50	WEE

DEMONSTRATION OF TRANSOVARIAL TRANSMISSION OF CALIFORNIA ENCEPHALITIS VIRUS IN EXPERIMENTALLY INFECTED *AEDES MELANIMON*[1]

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ABSTRACT

Transovarial transmission of Caliornia encephalitis (CE) virus was shown to occur in *Aedes melanimon* collected in the Sacramento and San Joaquin Valleys and experimentally infected with virus in the laboratory. When parental females were infected with CE virus by intrathoracic inoculation, approximately 16% of their progeny were infected. However, when females were infected orally by feeding on a blood/sucrose/virus pledget only 1.4% of their progeny became infected. *Ae. melanimon* is the natural vector of CE virus in the Central Valley of California.

Over the past 15 years, La Crosse (LAC) virus has been the agent most consistently associated with human cases of arthropod-borne viral encephalitis in the United States. Over 800 cases of encephalitis have been attributed to this virus in reports from the Center for Disease Control (1978) since 1964. Most cases have occurred in the Midwest, especially in the area between Ohio and Minnesota.

La Crosse virus is a member of the California group of arboviruses, and the prototype for this group, California encephalitis (CE) virus, was first isolated in Kern County, California in 1943 (Hammon and Reeves 1952). Serologic studies reported in 1952 showed that approximately 11% of the residents of Kern County had been infected with CE virus (Hammon, Reeves and Sather 1952). In addition, while nearly all cases of infection with CE virus are subclinical, 3 cases of encephalitis have been attributed to this virus (Hammon, Reeves and Sather 1952).

While human infections occur with CE virus, investigations of potential vertebrate hosts have implicated ground squirrels and jack rabbits in Kern County (Hammon, Reeves and Sather 1952) and jack rabbits in the Sacramento Valley (Hardy et al. 1977). From 1970-1974 46 strains of CE virus were isolated from *Ae. melanimon* collected in the Sacramento Valley, and *Ae. melanimon* was found to feed frequently on jack rabbits (Hardy et al. 1977). Thus, the natural cycle of CE virus in the Central Valley of California appears to be between *Ae. melanimon* and jack rabbits or ground squirrels. Human infections probably result from bites of infected *Ae. melanimon*.

One question that remains to be answered concerning the epidemiology of CE virus in the Central Valley of California is "How does the virus survive during the winter when *Ae. melanimon* is inactive?" The most likely mechanism is that the virus is transmitted transovarially by infected *Ae. melanimon* and then overwinters in the diapausing egg. Transovarial transmission has been demonstrated, both under natural and experimental conditions, for several California group arboviruses in their natural vectors (Watts et al. 1973, Cahoon 1978). However, it has not been shown for CE virus in *Ae. melanimon*. In an attempt to demonstrate it, over 25,000 *Ae. melanimon* larvae and pupae were collected in the Sacramento Valley and reared to adults. None of these adults was found to be infected even though females collected in the field during the same time period were infected (Reeves and Hardy, unpublished data). Thus, transovarial transmission did not appear to be involved in the *Ae. melanimon* - CE virus cycle in California.

While CE virus was not isolated from field collected *Ae. melanimon* larvae, it has been recovered from field collected *Aedes dorsalis* larvae in Utah (Crane, Elbel and Calisher 1977) and we have recovered CE virus from about 10-20% of the offspring derived from experimentally infected *Ae. dorsalis* (Cahoon 1978; Turell, unpublished data). Although this supports the existence of transovarial transmission of CE virus in *Aedes*, two problems with these studies must be considered. First, *Ae. melanimon*, not *Ae. dorsalis*, is the vector of CE virus in the Sacramento Valley, and second, the *Ae. dorsalis* used in our studies came from a laboratory colony derived from specimens collected in Sonoma County more than 10 years ago, and may no longer be representative of field mosquitoes.

To evaluate further the possible role of transovarial transmission of CE virus in *Ae. melanimon*, adult female mosquitoes were collected from the Sacramento and San Joaquin Valleys in CDC miniature light traps baited with dry ice. After the mosquitoes had been identified and sorted, female *Ae. melanimon* were inoculated intrathoracically (Rosen and Gubler 1974) with approximately 20 plaque-forming units of CE virus, and placed in an insectary maintained at 27°C and 80% relative humidity with a 16 hour photoperiod including 15 minutes each of simulated dawn and dusk. An uninfected chick was provided 6-7 days after inoculation as a blood source, and eggs were collected on moist paper toweling in a

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100 mm petri dish from 4-7 days after the blood meal. All eggs were allowed to mature for 1-3 weeks at room temperature, and then were hatched under vacuum. The larvae were placed in plastic containers and reared at 27°C. The resulting adults were sorted according to sex, and frozen at -70°C until tested for virus in pools of 2 mosquitoes each by plaque assay in VERO cells (Cahoon, Hardy and Reeves 1979).

CE virus was readily recovered from the offspring of experimentally infected female *Ae. melanimon* (Table 1). Overall, the filial infection rate was 16.2%. The infection rates for progeny of *Ae. melanimon* collected in the Sacramento and San Joaquin Valleys were roughly equal, 16.5 and 15.9% respectively, and there was no difference in the infection rates for male or female offspring (16.0 and 16.5%).

Table 1. Transovarial transmission of California encephalitis to F_1 progeny of experimentally-infected[1] female *Aedes melanimon*.

Sex	Number infected/number tested (% infected)	
	Sacramento Valley	San Joaquin Valley
Males	61/386 (15.8)	36/222 (16.2)
Females	68/398 (17.1)	49/313 (15.7)
Total	129/784 (16.5)	85/535 (15.9)

[1]Parental females infected with CE virus by intrathoracic inoculation.

While this is strong evidence that transovarial transmission of CE virus occurs in *Ae. melanimon*, it does not prove it is a natural phenomenon since these mosquitoes were infected by intrathoracic inoculation. It is possible that the transovarial transmission observed was an artifact of this infection process. In order to examine this possibility, female *Ae. melanimon* were infected by feeding them on a gauze pledget soaked with a blood/sucrose/virus suspension. Eggs were collected 4-7 days after the pledget feeding. At 7 days after infection, an uninfected chick was provided as a blood source for the 2nd ovarian cycle eggs. This process was repeated to obtain 3rd ovarian cycle eggs. Eggs, larvae and adults were handled and tested for virus as described earlier, except that adult progeny were tested in pools of 10-40 mosquitoes each. A sample of 38 of the parental females indicated that only 8 (21%) had become infected.

Examination of the 1st, 2nd and 3rd ovarian cycle progeny of *Ae. melanimon* which fed on virus indicated that the minimum filial infection rates were 3/648 (0.46%), 0/565 and 2/537 (0.37%) respectively. If this is corrected for the fact that approximately 21% of the parental females had been infected, the minimum filial infection rates for the 3 ovarian cycles becomes 2.2, 0 and 1.7%. Since Miller, DeFoliart and Yuill (1979) reported that LAC virus is not transovarially transmitted to the 1st ovarian cycle eggs following oral infection, the 3 recoveries of CE virus from the 1st ovarian cycle probably represent infected progeny of a female *Ae. melanimon* who was infected prior to being captured. At the time these parental females were captured, the field infection rate for CE virus in *Ae. melanimon* was approximately 1/500 (Reeves and co-workers, unpublished data).

The 1.4% filial infection rate observed in progeny of orally infected mosquitoes was a considerably lower rate than the 16.2% that was observed when females were infected by intrathoracic inoculation. This difference may be explained by recent work done in our laboratory by Dr. L. Kramer and coworkers (personal communication). They demonstrated that there are at least 2 barriers which limit the multiplication and dissemination of western equine encephalomyelitis (WEE) virus in *Culex tarsalis* females when they become infected after ingestion of low viral doses. Consequently, the salivary glands of some infected individuals do not become infected, and they fail to transmit WEE virus by bite. Thus, in some CE viral "infected" parental female *Ae. melanimon* the virus may have failed to escape the midgut, or to penetrate the ovaries and infect the eggs.

Even though filial infection rates were low in progeny of orally infected female *Ae. melanimon*, the 1.4% filial infection rate could serve as a potential overwintering mechanism for CE virus. Further studies are needed to determine the importance of this overwintering mechanism, and to clarify further those factors that influence the efficiency of transovarial transmission of CE virus in *Ae. melanimon*.

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EPIZOOTIOLOGY OF CANINE HEARTWORM DISEASE IN NORTHERN CALIFORNIA: A PRELIMINARY REPORT

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ABSTRACT

Rural Pleasants Valley, situated in the foothills of the Vaca Mountains, was utilized as a site for pilot studies of the prevalence and mosquito vectors of canine heartworm in Northern California. A house-to-house survey of 97 dogs for infection with *Dirofilaria immitis* and *Dipetalonema reconditum* was conducted in a 26 km^2 area of the valley during September, 1979. Two autochthonous cases of *D. immitis* were discovered (Knott technique) – 2.1% of dogs examined, older than 5 months of age. *Dip. reconditum* microfilariae were detected in 5.1% of the dogs surveyed.

Microfilarial periodicity in both *D. immitis*-infected dogs was studied over a 24-hr period; dogs were kept in their home environment. Preliminary results indicate high microfilaremia (30,520 - 58,680 mf/ml) and subperiodic tendency in one dog.

Primary mosquito breeding sites discovered in the survey area were 5 reservoirs (0.6-1.4 ha) and Pleasants Creek. Larval mosquitoes collected from these and smaller spillage areas during October, 1979 were: *Anopheles freeborni, An. franciscanus, An. punctipennis, Culex tarsalis, Cx. apicalis, Cx. peus, Cx. pipiens* and *Culiseta incidens.*

Resting mosquitoes were collected from a dog house and other outbuildings associated with 1 positive *D. immitis* case. Four-hundred *An. freeborni, An. franciscanus* and *Cx. tarsalis* were collected from these sites and examined for the presence of filarial worms. Blood-meals of freshly fed specimens were precipitin tested to determine host origin. Of mosquitoes collected from the dog house, 13% of *An. freeborni* contained developmental forms of filariae in the malphigian tubules. Excepting one *Culiseta inornata*, no other mosquitoes were found infected. All *An. freeborni* feedings from the dog house were of canine origin, whereas *An. franciscanus* fed mostly on deer-bovine hosts. Infective (L_3) forms of the deer body worm, *Setaria yehi*, were recovered from the proboscis of 1 *An. franciscanus*.

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DOG HEARTWORM SURVEY IN RIVERSIDE COUNTY

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ABSTRACT

A preliminary survey to establish the prevalence of canine heartworm in Southern California was conducted in the fall months of 1979. Blood samples from pound dogs in western Riverside County and from dogs attending a dog heartworm clinic in eastern Riverside County were examined for microfilaria by a modified Knott technique. *Dipetalonema reconditum* was found in 2% of the 366 pound dogs; *Dirofilaria immitis* was found in 3% of the 162 dogs attending the dog heartworm clinic. A telephone survey of practicing veterinarians in Riverside County was also conducted. Twenty-one cases of *D. immitis* in dogs native to Southern California had been diagnosed by these veterinarians in the past two years.

THE UNIVERSITY OF CALIFORNIA MOSQUITO CONTROL RESEARCH PROGRAM

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This year, 1980, marks the eighth anniversary of the UC Mosquito Control Research Program, first organized and funded by a legislative appropriation in fiscal year 1972. To my knowledge there is no other University mosquito research program comparable in size and scope nationally, or internationally.

A few of the junior members of the audience may need to be reminded that 1971 was not the first year of the University involvement in mosquito research. It actually dates from 1904 when Prof. H. W. Quayle, UCB, researched salt marsh mosquito control at Burlingame, San Mateo County. In July, 1906, he published the results of his work in Bulletin 178, 55 pages long, including 35 figures. Students of mosquito history may find this bulletin interesting reading. Prof. Quayle was followed by a succession of University scientists, dedicated to the study of mosquitoes and mosquito-borne diseases: W. B. Herms, H. F. Gray, S. B. Freeborn, W. C. Reeves and others from UCB, UCD and UCR, who have richly earned wide recognition for their contribution to the control of mosquitoes and mosquito-borne diseases.

However, my interest today is not historical but in focusing on the current University-wide research program. In the brief time available I want to review the basis for its formation and to touch lightly on: what are we doing, what has been accomplished, and what do we foresee for the future?

The basis for the present, comprehensive, expanded research effort was the outgrowth of a twenty-year period of almost exclusive reliance on pesticides as the primary strategy of mosquito control from approximately 1950 to 1970. As I was involved in mosquito control during this period in California and also in Federal and Foreign Service, I can vouch for the fact that pesticides generated spectacular progress in the expansion of mosquito control and the suppression of mosquito-borne diseases. However, as time was to prove, pesticides were not a viable, long-range strategy when used on a massive scale anywhere. It failed in California and also in the United States and overseas. The worldwide malaria eradication program, based on DDT spraying, was a prime example of an international failure of the monocontrol pesticide approach.

The generally recognized causes of failure were mosquito resistance, increases in insecticide costs, the issue of environmental contamination and EPA constraints on use and registration of new pesticides. These factors raised impossible conditions and obstacles to the continuation of a pesticide strategy.

By the late 1960's the high standards of mosquito control in California were being threatened by deterioration. Even a resurgence in mosquito-borne diseases was predicted.

In 1969 the situation was labeled a "crisis in mosquito control", calling for extraordinary measures. One California MAD manager, the late Richard Frolli, in a talk before this Association in January 1971, characterized the problem in this dramatic language:

> "These are crucial times--our pesticides are failing! Our basic solutions for mosquito control are dying! The resistance phenomenon has matured. The pasture mosquito and the encephalitis mosquito have triumphed over sprays in many parts of California.
>
> We must change our basic strategy, we must change our basic solutions, we must change our district images to ones other than spray districts if we are to be effective in mosquito abatement."

Unfortunately, practical alternatives to pesticides that could be quickly introduced into routine operations were virtually non-existent. For twenty years mosquito research had concentrated on pesticide development for which ample funding was almost always available. But only minimal funding could be raised for biological control, genetic control, physical control and cultural control research.

Formation of the Research Program.–In searching for a solution to the California crisis, mosquito abatement districts and this Association in 1969-1970, 1971 urged and supported legislation for an expanded research program in the University system. To meet the crisis head-on, it was proposed that the research be goal-oriented, coordinated, accelerated, and directed toward high-priority control problems of immediate concern. The paramount, overall need was development of methods and strategies that would relieve the MAD's from slavish dependence on broad spectrum compounds and allow adoption of an integrated control strategy.

In 1971-1972 the Legislature appropriated $300,000 as a special fund on top of an earlier appropriation of $100,000 in 1967 from the Water Fund, raising the total to $400,000. Since 1972 additions have been made to the appropriation owing to cost of living increases. The appropriation now stands at $500,000.

To screen and evaluate the research proposals two University mosquito advisory committees were appointed. In addition, the CMVCA research committee was requested to participate in the evaluation to provide input on the relevance of the research to the needs of California mosquito control. The three committees play a necessary role in the selection and funding of research projects and in the technical and policy direction of the program.

Current Research in 1979.–A detailed review of the current research projects will be discussed later in the conference by the investigators directly responsible for the work. I will, therefore, confine my remarks to a few highlights on the research program in 1979.

From the onset of the program, six lines of interlocking research categories were given priority for development as follows: biological genetic, physical and cultural, chemical,

mosquito vector disease control and mosquito biology and ecology.

Mosquito Vector Disease Control. In the area of mosquito vector disease, comprehensive research on arbovirus transmission is continuing under Dr. W. Reeves, UCB, and associates.

In addition two new studies on dog heartworm were approved on the basis of a growing problem in several regions of the state, including evidence of human infections. Dr. E. Platzer, UCR, is studying the disease in Southern California and Dr. E. Lavoipierre, UCD, in Northern California.

Biological and Genetic Control.-Biological and genetic control is our largest research category, receiving the greatest share of funds. There are seven distinct projects, combining mosquito predators (*Gambusia* fish and notonectids), and pathogens (fungus, bacteria, nematodes). Research on vertebrate predators, principally *Gambusia* fish, is receiving considerable support and progress looks good. A new bioagent, *Bacillus thuringiensis israelensis*, is exceptionally promising and will be discussed later by a panel of six researchers who have results to report on laboratory and field trials conducted in 1979.

I'd like also to note that Dr. Ed Platzer, UCR, is prepared to instruct MAD personnel on rearing the nematode, *Romanomermis culicivorax*, for use in mosquito control. Procedures for use in control programs are described by Dr. Platzer in the October 1979 issue of "Mosquito Research Highlights".

Chemical Control.-Chemical control research shows interesting developments which Drs. C. Schaefer, UCB, M. Mulla, UCR, G. Georghiou, UCR, and B. Hammock, UCR, are scheduled to present later in the conference. Although chemical control has fallen into disfavor because of mosquito resistance and problems of environmental contamination, insecticides are generally conceded to be essential in most integrated control programs. The major thrust of current research is evaluation of narrow spectrum insecticides, such as the IGR's and ecological chemicals.

Physical and Cultural Control. Physical and cultural control is undoubtedly the most potentially rewarding approach to mosquito control, but unfortunately our research program has experienced a shortfall of such projects. At this time we have only two current studies: by Vincent Resh, UCB, and William Wildman, UCD.

Biology and Ecology. The importance of research in mosquito biology and ecology is not always fully understood or appreciated. However, effective planning and execution of integrated mosquito control depends on a thorough knowledge of the behavior, habits, flight patterns, and other characteristics of pest and disease vector mosquitoes. Ecological studies are being encouraged and supported wherever possible.

Mini-Grant Program.-To provide opportunities for graduate and undergraduate students to conduct independent research in mosquito biology and control a mini-grant program was approved last year. Grants were limited to $750.00 per applicant. In 1979, five of six applications were approved by the mini-grant research committee at a total cost of $3,700.00. Progress reports on the results of the research will be issued in the "Mosquito Research Newsletter".

Now I would like to make a few personal observations about the University-wide coordinated program from the vantage point of a coordinator.

First, why was the research effort organized as a University-wide coordinated program rather than merely providing grants to individuals to conduct research on an independent basis? This question has been raised and the answer is that the University administration decided that a coordinated, goal-oriented program would provide the most effective means for ensuring compliance with the legislative intent on use of the funds, designed to meet the urgent needs of California mosquito control. To keep the program responsive to the established goal, research coordination, direction, and guidelines were adopted and research monitoring, evaluation and reporting were included as conditions of approval of research awards.

Because the funds are State appropriation, annual progress reports covering the total program are necessary to satisfy legislative inquiries and accountability for the expenditure of public funds. The CMVCA and most MAD's are, of course, vitally concerned with research results and how the information can be applied to improve control operations.

MAD Collaboration.-A great strength of the program is the collaboration and participation of MAD's in much of the University research. This year about 40% of the districts in California participated with the University in various field trials and studies.

I know of no other mosquito research program, institute, agency, or university with a similar expanded liaison, and interaction between researchers and operators. The collaboration-interaction has stimulated the participants and has exerted a positive influence on the direction, trends and progress of the program.

- The question is often raised: what good has the research done for mosquito control? Legislators have made similar inquiries. This is always a troublesome question because the questioner is usually looking for a spectacular breakthrough that will solve all of the problems with a flick of the wrist - like DDT in 1945. Well, we have no spectaculars to report just yet.

However, what is evident are some notable improvements in California mosquito control since the onset of the special program in 1971. Not all, but some of the credit can be claimed by research - past and present. Briefly, here are some highlights on this:

- Public health mosquito control has been uniquely successful. Malaria, excluding imported cases from overseas which exceeded 300 cases in 1979, and Western equine, St. Louis and California encephalitis, have virtually disappeared from the State in recent years. However, routine vigilance is maintained to prevent a disease resurgence, since the factors responsible for disease transmission - - the vectors and reservoirs of infection - - are still present in the State.
- A significant development is the drastic reduction in organophosphorus pesticide use since 1970 - - a decrease of almost 65%. A number of factors besides research have contributed to the decline. These include significant improvements in irrigated agricultural practices, leading to more effective mosquito control on the farm through physical and cultural control factors.
- The Statewide Director of IPM Programs (University-Cooperative Extension) after reviewing mosquito control in California last year, remarked that MAD's have outstripped agriculture in development of IPM. He added that the mosquito control research could serve as a model for IPM research programs in agriculture.

Although I'm normally cautious about predicting the future, it seems to me quite certain that biological control will continue to expand as more information is developed from current research. However, research is valueless unless the information is put to work. In this respect California is fortunate in having progressive minded and resourceful mosquito control managers and staff, willing to recognize and to react positively to the need for change. We see such change in the action taken to reconstruct control programs, to adopt largely unproven methods in control operations and to take the initiative to support and participate in research and development.

So, the attitude of the beneficiary of research is receptive. All that remains is for the University to continue developing the knowledge and methods. We are trying, but research is not easy. If it were, our problems would have been solved long ago. It's a tough game to be played only by patient, persistent and perceptive scientists. I believe we have these attributes in our research team - - so I'm concluding this talk on an optimistic note for the future of California mosquito control research and for the future of mosquito control, for which it was created to serve.

MOSQUITO CONTROL: PAST, PRESENT, AND PROSPECTIVE FUTURE

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One hundred years ago, in the year 1880, the USA was a developing country. Houses were heated by wood or coal stoves, there were few central water supplies, most homes had their own wells or cisterens, their own individual sewage disposal privies or cesspools, and no air conditioning. As residential communities developed, street drains were built, mostly incorporating "catch-basins" to trap gravel that washed off the streets with rains. Untreated industrial liquid wastes often were dumped into the nearest brook or river; ponds for waterpower or stock watering were built wherever suitable topography allowed sufficient water to be caught; borrow pits along railroad and highway rights-of-way usually were not drained; and these together with a great variety of other manmade water-holding sites were important sources of vector and pest mosquito species.

Of course mosquito species initially developed in natural sites, which in season provided ample habitats to produce intolerable "gluts" of mosquitoes, without any help from man. But several species found the assortment of man-made habitats greatly to their liking and quickly adapted their life-styles to take full advantage of man's cooperation - - not overlooking the convenient food supply also made available by man - - his own blood! So, extraordinary pest mosquito infestations and outbreaks of malaria and other mosquito-borne diseases were common and more or less accepted as inevitable.

Now many of the localities that once had severe mosquito problems have ongoing mosquito control by effective local agencies; at this present point in time few people appreciate just how severe seasonal mosquito problems were. However, earlier the mosquito annoyance caused great dissatisfaction among progressive individuals, and although the general public believed that nothing could be done about it, that "there always had been and always would be mosquitoes", sites naturally free of high mosquito populations tended to attract home owners, and these favored places developed more rapidly.

By the turn of the century, the life history of mosquitoes was quite well understood by biologists, and many species had been described. It was known that oil on the water of breeding places would kill larvae and that small fish, birds, spiders, dragon flies, and various aquatic insects were predatory on mosquito life stages using the same habitats. But no good plan for community-wide mosquito control had been advanced until 1889, when in response to an invitation issued to "working entomologists" to compete in a nation-wide essay contest for cash prizes, under the interesting title: "DRAGON FLIES VS. MOSQUITOES. CAN THE MOSQUITO PEST BE MITIGATED? STUDIES IN THE LIFE HISTORY OF IRRITATING INSECTS, THEIR NATURAL ENEMIES, AND ARTIFICIAL CHECKS". The prize money ($200) was provided by Robert H. Lamborn, Ph.D., and there was a great response. The best essays were selected by the staff of the Museum of Natural History (N.Y.) and the selected essays printed as "The Lamborn Prize Essays" in 1890. Authors were Mrs. C. B. Aaron, Archibald C. Weeks, and Wm. Beutenmuller. Also included were a letter from Capt. C. N. B. MaCauley, and a reprint of an article by Henry C. McCook, D.D., "CAN THE MOSQUITO BE EXTERMINATED".

Considerable space here is given to this publication, because the several authors collectively showed that of various natural enemies referred to, all captured some mosquitoes but the fish generally were most effective on a seasonal basis. However, without additional measures, they could not provide sufficient reduction of the mosquito populations. Oils and pyrethrum were given as effective larvicides, and the beneficial effects of water management by drainage, filling and circulation were noted. In short, the principles offered were essentially those we work by today. I was particularly intrigued to see that one author even suggested the use of mosquito light traps, and gave

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sketches of several models, all using kerosene lamps to attract, and a tray of kerosene to kill the mosquitoes. Of special interest is the fact that in this 1890 printing, there is an account of the work of Dr. Carlos Juan Finlay of Havana, who had in 1881 announced to the medical world that *Aedes aegypti* was the guilty culprit in yellow fever transmission and epidemics. Yet it was not until 1900 that the proofs were given by the Walter Reed Commission, and in the 19 intervening years Dr. Finlay was subject to a great deal of peer ridicule - - and doubtlessly many persons died because his medical peers evidently could not accept the "new idea" he advanced in 1881.

Some references had been made by one of the Lamborn authors to the work of Dr. L. O. Howard in the USDA. His fine book on "MOSQUITOES", which contains much more information, appeared about 10 years later (1901). Interestingly enough, he cites the Lamborn Essays in a number of instances.

Of course you are all familiar with the classical projects carried out by General Gorgas and his associates in controlling the vector of yellow fever in Cuba about the turn of the century, and the immediately following control of yellow fever and malaria in the Panama Canal Zone, thereby allowing the USA to successfully dig the "Big Ditch" where the French had failed. Viewed in the light of our present technology, these projects, the first successful large scale community vector mosquito control programs, might well be classified as "comprehensive" mosquito control. And they were immensely valuable as examples to communities and to government in showing that mosquito control could be successful.

Several excellent books, each proposing what we know now as "comprehensive" or "integrated" control contributed immensely to the advancement of mosquito control in the "pre-DDT" period. These include the L. O. Howard 1901 book already mentioned, the John B. Smith 1904 report on the "Mosquitoes of New Jersey and Their Control", a portion of which was updated in 1918 and printed as New Jersey Agricultural Experiment Station Bulletin 348, and in 1945 Dr. Thomas J. Headlee's book "The Mosquitoes of New Jersey and Their Control", wherein the author took practically his entire chapter on biology directly from the Smith work (with only very minimum updating changes), and dedicated his book to Dr. John B. Smith. In 1940 Herms and Gray published their excellent "MOSQUITO CONTROL" which for 20 years or more thereafter was much used as a textbook. Then the development of DDT and other synthetic organic pesticides opened a new chapter in mosquito control by providing the capability to effectively control adult mosquitoes through residual spraying and by space spraying, as well as allowing spectacularly effective and economical control of larvae. It is of particular interest to note that in the pre-DDT era, practical mosquito control programs emphasized prevention, through source reduction and use of larvivorous fish and larvicides, mainly oil, pyrethrum, and Paris green, (the last for anophelines only).

After the introduction of DDT and other synthetic pesticides having the capacity to provide immediate and spectacular elimination of both larval and adult mosquitoes, the emphasis shifted strongly toward chemical control, although source reduction and biological control still continued in limited use where appropriate.

As the shortcomings of DDT and other synthetic insecticides appeared, emphasis once again shifted toward source reduction and biological control, and in 1973 (Revised 1975), the California Mosquito Control Association published "A TRAINING MANUAL FOR CALIFORNIA MOSQUITO CONTROL AGENCIES", written mainly by staff of the California State Health Department's Bureau of Vector Control. This manual was based upon then-current knowledge of the elements of "comprehensive mosquito control" as applied in practical mosquito control in California, taking into account the limitations being imposed by environment protection organizations, etc.

WHAT LIES AHEAD?—My crystal ball is somewhat cloudy but certain trends appear to be developing. We now live in a climate of "limitations" and "regulations" some of which limit freedom to select the most efficacious and economical technology of control, which correspondingly increases costs to the taxpayers as well as wasting energy. Even more alarming, this trend appears to foster lack of mutual respect between highly trained professional workers of differing disciplines.

Mosquito control agencies are in fact "environment improving" agencies, dedicated to eliminating one of the great forces which degrade man's environment. Traditionally, these agencies have chosen to function as "service agencies" rather than as "police agencies". Usually they perform the field operations necessary to prevent or control mosquitoes, reserving their legal "nuisance abatement" powers for application only in flagrant instances where individual landholders create or maintain important mosquito sources with little or no regard for the comfort and health of nearby neighbors. Even where landholders have been shown to be maintaining a mosquito producing "public nuisance", the control agencies have tended to be lenient if the responsible party offers at least a moderate degree of practical cooperation in eliminating the nuisance.

As we move forward in this ever more complex existence, with regulations of other agencies of government imposing ever more restrictive limitations upon what we may do, what chemicals we may apply, and what time and labor consuming procedures we must follow, it appears to me that we may have to more frequently and assiduously enforce the nuisance prevention aspects of the Health and Safety Code, where public nuisances have been detected. Also, we must look to all other public agencies, groups, and individuals that are involved in water and land management to carry on their functions and objectives in a manner that will not create mosquito sources. And where benefits result to other public programs by modifying mosquito control measures, then the additional costs should be shared by the other programs or agencies receiving benefit.

RESEARCH.—Ongoing research may entirely change the complexion of mosquito control in due course of time. It is not inconceivable that the specialists in genetic research may one day be able to manipulate lethal genes in mosquitoes in a manner that will cause populations to self-destruct; or the current efforts to employ the sterile male system may become sufficiently well developed for general application. Research and development with predatory aquatic insects and with fish that prey on mosquitoes has advanced greatly in the past few years, offering new hope that biological control may become satisfactory for much broader application than at present; and

finally, chemical research might well yield more selective, longer lasting, larvicides or adulticides, free of hazard to nontarget animals or plants, and free of other environmental objections.

It appears that encouragement and support should be provided the research workers and organizations, and every promising development should have a fair field trial.

But we are fortunate indeed that among the measures comprising "Comprehensive Mosquito Control" (Table 1) there can be found sufficient technology to provide satisfactory mosquito control in the interim period while we await the results of research.

It appears that the task ahead will surely require even more precision than heretofore, more leadership in obtaining the cooperation of other agencies, and probably more funds and more cooperation.

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Table 1.–Elements of comprehensive mosquito control performed by mosquito control agencies.

COMPREHENSIVE CONTROL

Inclusive of all known control methodology as applicable

INTEGRATED APPLICATION

NATURALISTIC CONTROL

Abets natural limitation through selective use of biological and physical factors similar to those found in the natural environment

TEMPORARY CONTROL

A. Natural Population Limitation

Biological factors:

Predators	Detrimental plants
Parasites	Food productivity
Pathogens	Competitors

Abiotic factors: (physico-chemical factors of the environment affecting mosquitoes, their enemies, or habitat)

Rainfall and runoff	Alkalinity
Percolation	Acidity
Humidity	Sunlight and shade
Evaporation	Turbulence, currents, waves
Temperature	
Salinity	Nature of soils substrate

B. Biologically Oriented Control

Manipulation of living organisms to destroy or limit mosquitoes at all stages

Environmental practices aiding populations of mosquitoe enemies or increasing their effectiveness

Genetic manipulation

C. Physical Control (Source Reduction)–Elimination or Modification of Breeding Places

Water Management	Regulation
Drainage	Circulation
Impoundment	Flow and exchange rates
Contour design	
Reuse	Levels and depth

Organic solids removal

Land preparation and management
- Filling
- Grading
- Drainage

Crop selection and management

Weed control

D. Chemical Control

Ovicides (not usually practicable)

Larvicides (small areas treated to protect large affected areas)

Pupicides (infrequently applied)

Adulticides (particularly useful in emergencies and in areas of chemical resistance by larvae)

Repellents

Growth regulators, physiological inhibitors

Attractants (with other procedures)

Weed Control

E. Mechanical Barriers

Screening of buildings

Temporary barriers as bed nets and mosquito-proof clothing

F. Landholder Motivation to Cooperate

Public information and education

Individual persuasion and cooperative efforts

Legal action and enforcement

Interagency cooperation

From: Training Manual for California Mosquito Control
Developed By: Vector Control Section/California Dept. of Health
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VECTOR CONTROL BY DISTRICTS

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I recently had the opportunity to serve on the AD HOC Committee that has developed the report to the legislature on vector control funding and delivery. The report is being authored by the Vector Biology and Control Section of the Department of Health Services and is in a nearly completed form. I can say it adequately describes the vector problems in the State of California. And, in the face of Proposition 13, it also correctly recommends that a mechanism for funding vector control be established to assist local health agencies, university research and mosquito and pest abatement districts. However, I believe the report may not emphasize some important aspects of the delivery of vector control in California and may be compounding the problems by establishing a tortuous conduit for vector control funding.

As background, I would like to talk a little about some generalities of vector control programs. Vector control programs, for the most part, begin in crises. Either the prevalence of a vector borne disease, or highly pestiferous vectors result in governmental action to control them. Because the program begins in crises, public demand is usually high for immediate results. A vector program in its early developmental stages necessarily depends heavily upon chemical methodologies to obtain the desired results. As the program evolves, it moves along a continuum from the short-term control methodologies to long-term control provided by physical and biological control methodologies. The result, after perhaps many years and significant capital investment, is a cost/effective program. The program is no longer primarily responding to crises caused by the vectors, but through implementation of effective long-term control aimed at the sources, the program has reached a preventive configuration. It is at these latter stages of vector control programs that the public receives the maximum for the least cost. Ironically, it is also precisely at this time in its development that a vector control program is most vulnerable.

If the legislators are going to develop a realistic mechanism for adequately funding vector control in California they should be aware of at least three truisms concerning vector control programs. The first truism is that vector control programs cannot be turned off and on like tap water. Interruption of the programs by inconsistent funding arrests the development of the program condemning it to crises orientation and low cost/effectiveness. The public is not receiving maximum benefit for its money under these circumstances.

The second truism concerning vector control programs is that the more effective it becomes, the less likely it will receive continued and consistent public and political support for funding. For example, in a recenlty published book on malaria control, Gordon Harrison reported that the funding for malaria control in Sri-Lanka was cut in 1954 by a government yielding to the temptation to save money. The result was the resurgence of malaria in 1956. In India, according to Harrison, the need to continue the attack against malaria even after apparent successes could not be translated into practicable political criteria. The resurgence of malaria in India was even more disastrous from a low of less than 100,000 cases in 1960 through 1963 to an estimated 30-50 million cases in 1977. Harrison explains that the key to the malaria resurgence in India was the programs "near success in an environment with an excess of problems clamoring for attention. As malaria receded to a low level other pressing health and social problems exerted irresistible demands for available resources."

The legislators should also be made aware of a third truism about vector control programs. Vector control programs do not compete well for funds in a tight fiscal environment. For example, a report by California's Legislative Analyst in October, of 1979 indicates that local environmental health and sanitation agencies received a 5.3% increase of funds in the fiscal year following passage of Proposition 13. Yet the findings of the AD HOC Committee suggest that vector control programs have lost resources to the other environmental health programs. Quite frankly, my observations are that vector control programs do not do well over the long-term under the aegis of boards of supervisors or within the matrix of general sanitation. The reasons are not pernicious, but it is nearly impossible for vector control programs to obtain adequate funding when competing for limited funds in a highly competitive environment. This, as pointed out earlier, is especially true if the control program is effective.

Another indication that vector control programs do not compete well for funding has been evidenced by state aid allocations to Special Districts following Proposition 13. The county boards of supervisors were charged with the responsibility of distributing the funds to districts within their boundaries. Comparing the 1978/79 distribution to that of 1979/80, mosquito abatement districts received an 18.3% decrease in funding. The mosquito and pest abatement districts, in open competition with library, fire and recreation districts, had lost.

May I conclude by saying that if, as the AD HOC Committee Report has stated, vector borne diseases are a threat to the citizens of California, the State Legislature should be made aware that their actions should establish a funding mechanism that insures sufficient and consistent funding. Such a mechanism is the key to providing effective and efficient vector control programs to the citizens of California. The major failure of the report could be that it does not point out that we already have delivery systems in California that avoid the common pitfalls of other vector control programs. Mosquito and pest abatement districts have been the primary delivery systems for vector control in California since 1915 and have been extremely effective, avoiding these common pitfalls. There are currently 54 of these districts operating in the state. A key element to their success has been the separate funding method inherent in the Special District system. Other important factors are the stewardship of the programs provided by the trustees, the unique operational capabilities of the districts, the close coordination with other agencies, and

the flexibility of the enabling statutes, that, among other things allows boundaries to be established to realistically encompass the problem area.

A realistic and practical solution to the problems of vector control funding in California should begin with the recognition that vector control in California has been delivered, for the most part, by Special Districts and that the districts are uniquely suited to effectively provide vector control for the citizens of California.

UTILIZATION OF A MICROCOMPUTER IN A VECTOR CONTROL PROGRAM – ALAMEDA COUNTY MOSQUITO ABATEMENT DISTRICT

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Information is required in order to accomplish vector control. It is routinely collected, processed and interpreted to provide guidance to vector control programs. It follows logically, that to process the information more efficiently is to increase the efficiency of the programs. It can provide savings in money, and result in increased effectiveness of vector control.

In recent years the costs of automatic data processing have been declining rapidly. A recent article in Science magazine, called "Information - the New Frontier", pointed out that if costs of computers continued to decline at the current rate, a computer would cost 3/10 of a cent 100 years from now. The projection is absured of course, but was intended to indicate the rate of which computer costs are now crashing.

The Alameda County Mosquito Abatement District is currently processing data automatically by means of a TRS-80 microcomputer system. A comparison of the automatic data processing system to the previous punch card system has indicated that we should expect a long-term savings of about $2,400 per year in paper and labor costs. One time costs of hardware and software were not included in the analysis, but the savings should pay for the system in about two years.

The installation of automatic data processing in the District required that we look at the flow of information in the District from the point of view of a computer scientist. The approach is called systems analysis. When the systems analysis is accomplished in the broadest perspective, it provides a logical and practical frameworks for vector management programs. It is relatively easy to accomplish a systems analysis in vector control for a couple of reasons. First, the basic concepts of integrated pest management are compatible with the systems approach. Secondly, vector ecologists have published information in the past that is usable in the systems analysis. Both published and unpublished articles by Richard Husbands, formerly of the California State Vector Biology and Control Section, were quite useful in accomplishing the analysis.

A valuable by-product of the analysis was that before the data processing system could be designed the goals and objectives of the District's programs had to be clearly defined. The analysis also provided rather startling information about the amount of time utilized to gather and process information prior to the installation of the computer system.

The data processing system has been designed to measure the mosquito problem and to determine the impact the control program has on the problem. The basic input data are the same data used in most all vector programs - - light trap data, biting counts, larval sampling data, service request data. The output data are also the same kinds of familiar information - - lineal feet of ditches excavated, number of fish plants, pounds of insecticide per source, pounds of insecticide per species, etc.

Objectives of The System.--The specific objectives to be accomplished by the automatic data processing in the District are the following:

1. To efficiently process data that will measure the effectiveness of the program.
2. To efficiently generate required reports.
3. To develop and utilize models to predict levels of mosquitoes and thereby assist in making treatment decisions.
4. To quantify the work performed by the District.
5. To define "high priority" sources through cost-evaluation and set appropriate work schedules for the physical control program.
6. To measure the insecticide pressure on any given species and avoid resistance problems.
7. To determine the costs of specific program elements and enhance program budgeting.
8. To check current inspection and treatment schedules with those of the past and modify the schedule as required.

The District has already gone a long ways toward meeting the objectives. The "core" program currently in use processes data from the employees' daily reports and generates the monthly reports of the District. The program includes one relatively simple yet informative model that predicts levels of adult *Culex pipiens*. The program can easily be upgraded as the "pipiens" model is elaborated upon and as additional models are developed for other species.

The use of the computer in the District should also provide benefits beyond that of our current stated objectives. The flexibility of the system enables data to be retrieved in a variety of combinations by simple program additions. Other programs could be developed to increase the efficiency of the bookkeeping systems. Existing programs can also be utilized to do statistical analysis if required. In truth, as we gain knowledge of

how increasing amounts of data can be processed rapidly and efficiently, it only increases our expectations of the system.

In summary, automatic data processing has been installed in Alameda County Mosquito Control District at a relatively low cost with long-term savings projected. The system is designed to increase the efficiency of the existing data processing system, to process more data at less cost, and to increase the effectiveness of the vector control program by providing appropriate and timely data to support decision making.

EFFECTIVENESS OF THE BACTERIAL PATHOGEN *BACILLUS THURINGIENSIS* SEROTYPE H-14 AGAINST MOSQUITO LARVAE

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INTRODUCTION.- During the past two decades numerous studies have been conducted on the activity and efficacy of entomogenic bacteria against mosquito larvae. Most of these studies have dealt with laboratory evaluation of strains of *Bacillus thuringiensis* and other pathogenic bacteria recovered from mosquito breeding sources or infected larvae. The activity and potency of a number of bacterial species and strains have been reported by numerous researchers (Davidson et al. 1975, Hall et al. 1977, Kellen et al. 1965, Liles and Dunn 1959, Shaikh and Morrison 1966). Most of these studies dealt with the evaluation of *B. thuringiensis* varieties or strains in laboratory where they were shown to have marginal activity. A few field studies were also implemented on commercial formulations and other strains of this pathogen, and these were all found to show little or no activity at practical rates (Kellen and Lewallen 1960, Liles and Dunn 1959, Shaikh and Morrison 1966). These investigators employed concentrations as high as 200-500 ppm in laboratory evaluations, and rates as high as 0.5-1.0 lb/acre of BT preparations under field conditions without obtaining significant mortality in the treated larvae of several species of mosquitoes.

Evaluation of a BT isolate (designated as BA-068) from dead and moribund *Culex tarsalis* Coquillett larvae from California, showed good activity in laboratory studies (Reeves 1970, Reeves and Garcia 1971). This same isolate was later designated at *B. t.* var. *thuringiensis* (serotype H-1) and was found (preparation HD225/531C) to have an LC_{50} of 0.3 ppm against 2nd instars *Ae. aegypti* (Hall et al. 1977). In the same study, these authors evaluated some 127 strains of *B. thuringiensis* (consisting of 18 varieties/serotypes) against *Aedes* and *Culex* species. Most of the strains showed little or no activity, but some preparations (HD-169/R-567B and HD-96/R574D) in addition to the above possessed good activity, showing an LC_{50} of 0.04-0.06 ppm against 2nd instar *Ae. triseriatus* a most susceptible species as compared to *Culex* and *Anopheles* species.

Recently, Goldberg and Margalit (1977) isolated a strain of *Bacillus thuringiensis* (designated as ONR-60A or WHO CCBC 1897) from mosquito larvae and this isolate showed good larvicidal activity against several species of mosquitoes. This strain was typed by de Barjac (1978) of Institute Pasteur as serotype H-14 and designated as *B. thuringiensis* var. *israelensis* (BT Ser H-14). This strain has been found to be quite effective against *Aedes* and *Culex* species under laboratory conditions, and appears to be the most effective entomopathogenic bacterium studied to date. Its activity against mosquitoes under indoor and outdoor conditions was studied in California by Garcia and Desrochers (1979).

Our current studies were aimed at the laboratory and field evaluation of BT (H-14) against several species of colonized mosquitoes and also a number of species collected from the field. Additionally, field evaluations of semi-commercial preparations of this entomopathogen were conducted in a variety of habitats supporting species of 3 genera of mosquitoes.

METHODS AND MATERIALS.-In the laboratory, 4th instar larvae of *Aedes aegypti* (L.), *Aedes nigromaculis* (Ludlow), *Ae. taeniorhynchus* (Wiedmann), *Anopheles quadrimaculatus* Say, *Culex tarsalis* Coquillett, *Cx. peus* Speiser, *Cx. quinquefasciatus* Say, and *Psorophora columbiae* (Dyar and Knab) were employed. The standard procedures and techniques as reported by Mulla et al. (1966) were utilized. In brief, the procedures used are as follows:

Twenty mosquito larvae were placed in 100 ml tap water (pH 8.0 ± 0.1) in a 160 ml (4 oz squat waxed ice cream) cup. Each treatment was replicated 3 times and run on 2 - 3 different occasions, yielding 6 - 9 replicates per treatment. The treated and control cups were kept at 26°F ± 0.5 and mortality was read after 24 hours of continuous exposure to the bacterial preparations. On account of the short-term exposure period (24 hrs) the larvae were not provided with food.

To determine the range of activity, the bacterial preparations were run at 3 - 4 concentrations, each replicated 3 - 4 times. The average mortality for each concentration was plotted on log probit paper and the LC_{50} and LC_{90} concentrations in ppm were read off the concentration response lines fitted and established through the points. There was little or no mortality in the control larvae, therefore no correction for check mortality was deemed necessary.

Several preparations of H-14 consisting of WP, waxy solid and fluid suspension formulations were evaluated. The solid formulations were suspended in water by the addition of Tween 20 (wetting agent) and blending at moderate speed in

a blender. These stock suspensions were freshly prepared and diluted with water for testing. The particles readily precipitated on standing and required vigorous shaking prior to addition to the cups.

Field evaluations were conducted in experimental ponds and small plots in irrigated pastures. Under field conditions efficacy was studied against *Cx. tarsalis*, *Cs. inornata*, *Ps. columbiae* and *Ae. nigromaculis*. For field application, the solid preparations were suspended in water using a wetting agent and blending. The desired quantity of the suspensions was put in all purpose household spray bottles or ½ - 1 gallon pressurized can sprayers. The volume was brought up to 100 - 200 ml of water depending on the size of the plot and sprayed over the plots.

Mosquito larval counts were taken before, and at intervals after treatment. Five to 10 dips were taken per plot at each sampling time. The level of reduction as a percentage was calculated by comparing all larval instars in the treated plots to those in the untreated. The dosages (lb/acre) for each preparation reported in the table were estimated from field data obtained in these studies.

RESULTS AND DISCUSSION.–The activity and potency of H-14 formulations or preparations as evaluated in laboratory against colonized or field collected larvae are presented in Table 1. The bacterial preparations were evaluated against 3 *Aedes*, 1 *Anopheles*, 3 *Culex* and 1 *Psorophora* species. The source from where the test larvae were obtained are shown in Table 1.

From the data in Table 1, it is evident that the maximum variation in activity of the different preparations against a given species is in the order of 2 - 6 fold. Similarly, a 2 - 6 fold variation in the activity of a given preparation against the various species of mosquitoes is evident. In general, *Anopheles* larvae were much more tolerant, followed by some *Aedes*

Table 1. Laboratory evaluation of formulations of *B. thuringiensis* Serotype H-14 against 4th instar larvae as determined by continuous exposure for 24 hours.

		LC90 (µg/ml) of experimental formulations				
	Larval Source	IPS-78[a]	R-153-78[b]	Sandoz 402	Abbot[c]	Abbot 6108
Aedes aegypti	Lab	1.30	0.38	0.70	0.80	-
Aedes nigromaculis	Irrigated Pasture	0.20	0.14	0.45	0.90	0.70
Aedes taeniorhynchus	Lab	0.60	-	0.85	0.85	-
Anopheles quadrimaculatus	Lab	0.60	-	>2.00	-	-
Culex peus	Daiey Lagoon	0.22	0.13	0.50	0.90	0.70
Culex quinquefasciatus	Lab	0.43	0.35	0.60	1.38	1.00
Culex tarsalis	Lab	-	0.30	0.58	1.00	0.72
Psorophora columbiae	Irrigated Pasture	0.20	0.32	0.42	0.58	0.80

[a]Institute Pasteur, Paris, tentative standard.

[b]Roger Bellon - Biochem, Paris.

[c]3.3×10^{11} spores/gm.

Table 2.–Small-scale field evaluation of *B. thuringiensis* Serotype H-14 preparations against various species of mosquito larvae breeding in a variety of habitats.

			Dosage (lb/acre) yielding 90-100% control		
Mosquito Species	Habitat	Location	R-153-78	Sandoz 402	ABG-6108
Aedes nigromaculis	Irrigated Pastures	S. San Joaquin	0.25 - 0.5	0.5 - 1.0	0.5 - 1.0
Culex tarsalis	Ponds (pH8)	Riverside	0.1 - 0.2	0.1 - 0.25	0.1 - 0.25
Culex tarsalis	Ponds (pH9.5)	Coachella Valley	0.25 - 0.4	0.25 - 0.50	0.25 - 0.50
Culex peus	Dairy Lagoons[a]	Chino Basin	0.5 - 1.0	-	1.0 - 2.0
Psorophora columbiae[b]	Pasture	Palo Verde	0.25 - 0.50	0.5 - 1.0	0.5 - 1.0
Psorophora columbiae[c]	Pasture	Palo Verde	1.0 - 2.0	1.0 - 2.0	1.0 - 2.0

[a]Highly polluted. Incoming barn-wash water facilitate dilution of control agents.

[b]Test conducted when maximum ambient temperature was 105 - 108°F. Larvae at the time of treatment were 3rd and early 4th stages.

[c]These tests were conducted when ambient maximum temperature was 115 - 118°F. Larvae at the time of the treatment were 4th and late 4th instars. Rate of development very fast.

species. In most cases, *Culex* mosquitoes were found to be more susceptible. It should be noted that those preparations which showed lower activity had been diluted and formulated as compared to the more active preparations wich consisted of the original undiluted preparations.

Since BT (H-14) showed good activity in the laboratory, the available preparations were then subjected to preliminary small-scale field experiments against several species of mosquitoes in a variety of habitats. The results of these preliminary small plot evaluations are presented in Table 2.

The three formulations field tested showed different levels of activity against the different species. R-153-78, being the original undiluted product, as expected yielded a higher level of control than the other two diluted formulations. It is important to note that the BT (H-14) formulations manifested lower levels of activity against the same species where water pH was higher than 8. This is apparent from the trials conducted against *Cx. tarsalis* in two different locations where the water pH in the breeding source was quite different. The pH of the mosquito breeding source water, therefore, may have a significant effect on the activity of BT (H-14).

Another consideration in the efficacy of BT (H-14) formulation against mosquitoes is the differential susceptibility of the various larval stages. Younger instars are more susceptible than older larvae, therefore requiring lower rates of application for their control. BT (H-14) toxicity is primarily through ingestion of the spore/crystal mixture containing the toxin. Therefore, as a result, late 4th instars that have ceased feeding will not be killed by applications of this pathogen. In mosquito control, BT (H-14) treatments have to be made when the larvae are 3rd instars or younger. We also noted that efficacy of BT (H-14) is quite low under cold weather conditions when water temperature is below 50°F. At these low temperatures, larval feeding activity is probably minimal.

EFFECTS ON NONTARGET MACROINVERTEBRATES. --During the course of these preliminary studies on the evaluation of BT (H-14) against mosquitoes in the field, data were also gathered on the population density of mayfly, damselfly and dragonfly naiads, diving beetle larvae and ostracods. At the rates of 0.5 - 1.0 lb/acre none of the BT (H-14) preparations studied had any noticeable effects on these abundant groups in the nontarget biota. It is likely that BT (H-14) formulations will have little if any acute effects on these aquatic organisms.

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BACILLUS THURINGIENSIS var. *ISRAELENSIS* (H14), STRAIN ONR60/WHO/CCBC/1897

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ABSTRACT

Bacillus thuringiensis var. *israelensis* (H-14) strain ONR60/WHO/CCBC/1897 has, to date, demonstrated useful larvicidal activity against more than 16 species of mosquito larvae representing 6 genera. Larvicidal activity is generally marked in less than 12 hours. Contrary to the classic pattern noted for *Bacillus thuringiensis*, strain ONR60/WHO/CCBC/1897 does not demonstrate useful activity against the larval stage of Lepidoptera. Hence, this strain of *B. thuringiensis* is unique in its spectrum of larvicidal activity.

Additionally, activity at a level of 10^4 cells/ml has been noted against *Simulium verecundum*, thus suggesting a potential for the control of blackfly larvae.

Bacillus thuringiensis is classically distinguished from *B. cereus* by pathogenicity for larvae of Lepidoptera and by the production of a crystalline protein-body during the phase of spore formation. If one uses *Culex tarsalis* (Coquillett) rather than *Cx. pipiens* complex, one can demonstrate larvicidal activity (Tables 1 and 2). Such activity, however, does not follow any pattern in terms of serotype. Figure 1 summarizes an observed larvicidal activity of *B. thuringiensis* (HD-1). Comparable activity can be observed against *Aedes dorsalis*, *Ae. taeniorhynchus* (Weidman) but no activity was observed against *Cx. pipiens*. It is this spotty characteristic of mosquito larvicidal activity that generated the confusion in published reports (Reeves and Garcia 1970, Norris 1969, Kellen and Lewellen 1960, Liles and Dunn 1959, Rogoff and Ignoffo 1969, Shaikh and Morrison 1966).

In contrast, *B. t.* var. *israelensis* (H14) has, to date, demonstrated useful larvicidal activity against more than 16 species of mosquito larvae, representing 6 genera (Goldberg 1977, Garcia 1978, Weiser 1978). Larvicidal activity is generally marked in less than 12 hours. Contrary to the classical pattern noted for *B. thuringiensis*, this strain (ONR60/WHO/CCBC/1897) does not demonstrate useful activity against the larval stage of Lepidoptera. Professor J. Weiser noted activity against *Aedes cantans* and *Cx. pipiens* autogenesis. He noted some activity on ephemerids, Plecoptera, Trichoptera, Coleoptera and planktonic copepoda. Professor A. H. Undeen has noted activity at a level of 10^4 cells/ml against *Simulium verecundum*. He has recently extended this observation to include four additional strains of blackfly larvae, thus suggesting a potential for field application (Undeen 1978).

The isolation of *B. t.* var. *israelensis* (H14) was obtained following the screening of some 1,000 clones from some 10 soil samples taken at known mosquito larval breeding sites in Israel (Goldberg et al. 1977). Roughly, 1 in 100 such clones demonstrated a larvicidal activity of the same order of magnitude as *B. sphaericus* var. *fusiformis* (SSII-1), but only one, ONR60A, demonstrated a unique activity.

It is important to note that successful screening was dependent upon two factors in screening. The first was the use of a selected nutrient, N2X, which was made available by Nutr-

Table 1.–Isolates found to demonstrate larvicidal activity against *Cx. tarsalis* (K.L.) 1st instar test larvae.

Original Designation	Serotype	Code Number
kurstaki	H3a, 3b*	(2536-9693TW)
kurstaki	H3a, 3b*	(2819-9763F)
aizawai	H7*	(1850-9762C)
tolworthi	H9*	(NPI-460)
sotto	H4a, 4b	(NPI-180-5-2)
thuringiensis	H1	(NPI-186-104)
thuringiensis	H1	(NPI-185-104)
thuringiensis	H1	(NPI-201-113)
thuringiensis	H1	(NPI-197-105)
thuringiensis	H1	(NPI-198-105)
thuringiensis	H1	(NPI-199-105)
sotto	H4a,4b	(NPI-194-101)

*Cultures marked with an asterisk have had recent confirmation. The remaineder are based on information available before 1963, and therefore, there is a possibility those classifications are not accurate.

Table 2. Isolates found to be inactive against *Cx. tarsalis* (K.L.) 1st instar test larvae.**

Original Designation	Serotype	Code Number
kurstaki	H3a, 3b*	(720619A)
B. thuringiensis var.?	H5a, 5b	(730130-1)
thuringiensis	H1*	(1840-182C)
finitimus	H2*	(NPI-451)
subtoxious	H6*	(NPI-456)
entomocidus	H6*	(NPI-457)
aizawai	H7*	(NPI-458)
morrisoni	H8*	(NPI-459)
darmastadiensis	H10*	(NPI-461)

*Cultures marked with an asterisk have had recent confirmation. The remainder are based on information available before 1963, and therefore, there is a possibility those classifications are not accurate.

**Selected from 64 negative results to illustrate range of serotype.

ilite (Nutrilite, Lakeview, California). This media is a standard production media for *B. thuringiensis* (HD-1). This is not to imply that it has unique properties which cannot be duplicated by other nutrient media, but rather, to point out the requirement for the selection of a media which can stimulate entomotoxin production during cell growth. The test organism available for such media selection was *B. sphaericus* (SSII-1) but, as the number of such organisms increase, i.e., strain 1593, one can expand the selection criteria for the screening media.

If, for example, nutrient agar had been the only solid growth media used for clonal selection, only one clone out of the entire set of some 1,000 would have demonstrated marked larvicidal activity during screening. This "media" effect was first noted by Prof. S. Singer in connection with *B. sphaericus* (SSII-1). During screening we actually utilized N2X and nutrient agar (Difco) in parallel but only in the case of the isolate 60A did larvicidal activity occur from surface growth on both medias. Even though the isolates which required special nutrient factors for the production of entomotoxin did not prove to be as dramatic in activity as 60A, positive findings indicated that further screening using other soil samples from the same stream region might prove profitable.

The second factor which I believe is critical is in the selection of the larval screening system. We chose raw sewage water from a sewage pond of Kubbutz Hulda in combination with the use of *Cx. pipiens* (complex) larvae. The sewage liquid

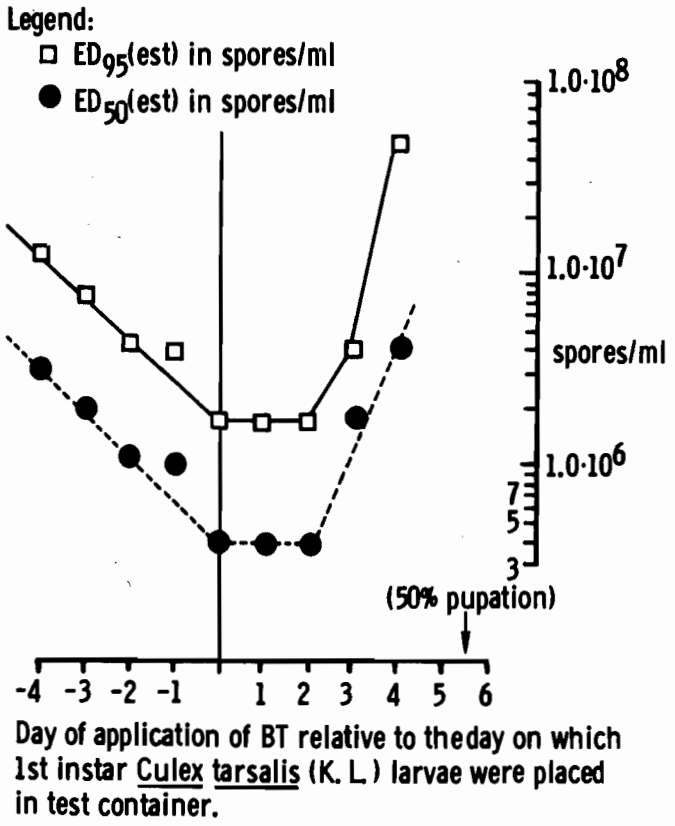

Figure 1.–Estimated ED_{50} and ED_{95} dose, using technical I.M.C. BT (HD-1) for test challenge, as a function of time of application relative to the time at which 1st instar *Culex tarsalis* larvae were placed in the test container.

should not be used as an inoculum for a second screening. In the case of our sewage liquid control, i.e., *B. sphaericus* (SSII-1), the apparent larvicidal activity could increase as much as 10X from such a "second pass". This effect in the case of SSII-1 is due to the fact that this organism can outgrow many other bacterial floras (Figure 2) (Goldberg et al. 1977) and the shift in flora which can occur when a stabilized sewage pond flora is inoculated under different environmental conditions can result in marked changes in measured larvicidal activity. Comparably, all other test larvae were titrated using aliquots of the same stream liquid from which the larvae were obtained. I don't wish to imply that an organism such as SSII-1 is to be rejected on this basis, but rather that this effect be accurately noted, and care be taken to ensure that "worst" field control conditions are anticipated. In the case of SSII-1, such larval test conditions were also used to demonstrate that entomotoxin was formed during vegetative growth and was relatively constant into the spore phase of growth (Goldberg et al. 1977) (Figure 3a, b).

Following the initial mosquito larvicidal screening program using *Cx. pipiens* Linnaeus, further testing was done using *Anopheles sergentii* (Theobald), *Uranotaenia unguiculata* Edwards, *Cx. univitattus* Theobald, and *Ae. aegypti* (Linnaeus) (Figure 4). The isolate ONR-60A demonstrated no significant loss in larvicidal activity after being heat-shocked for 20 minutes at 60°C, lyophilized and reconstituted, or from exposure to 2537 Å (ultraviolet) sufficient to reduce the spore count to less than 0.1 percent of its initial count. The mode of action of ONR-60A can be attributed to an ultraviolet and heat-stable endotoxin (Goldberg et al. 1977).

A single large batch of spores was produced from the original isolate. This was lyophilized and then the dry cell mass was divided into 24 aliquots. One such aliquot was deposited with Dr. A. A. Arata, WHO, Geneva. A portion of this aliquot was sent on to Dr. H. de Barjac at the Institute Pasteur, Paris, France, for definitive identification. I subsequently deposited aliquots of the entire collection of active isolates with Prof. J. D. Briggs, at which time ONR-60A was designated by the acquisition number WHO/CCBC/1897. The entire collection carries the designations 1883 therough 1897. A duplicate set of these isolates was also sent to Prof. S. Singer. Dr. H. de Barjac's excellent work on the identification of ONR-60A WHO/CCBC/1897 as *Bacillus thuringiensis* var. *israelensis* (H14) (H. de Barjac 1978) clearly identifies this organism as a new serotype as well as defining it in biochemical terms.

The problems of safety and field formulation still remain. Since Anopheline larvae, for example, are predominantly surface feeders, the unmodified physical behavior of a spore suspension of ONR-60A/1897, following aquatic field application, would necessitate repeated high dissemination rates in order to provide for a sufficient time duration of a toxic concentration in the upper water layers where larval feeding is predominant. A buoyant colloidal formulation would optimize such larval ingestion and as a direct consequence, larvicidal activity (Goldberg et al. 1977). The formation of a surface scum, however, might be counter-productive since this could minimize larval ingestion by filter feeders. A hydrophilic buoyant colloid should have optimal qualities for surface filter feeders. A formulation with such qualities was tested using

both *An. sergentii* and *Cx. pipiens* (complex) larvae. (Figure 5). Further formulation work will be required, however, to provide a suitable "shelf life".

The observation of rapid selection in mosquitoes to resistance toward the sequential use of broad spectrum chemical insecticides further dictates that future measures might well require the combined use of multiple narrow spectrum control agents in order to obtain a long-term beneficial parasite-predator-pest balance (Goldberg et al. 1974). With the added availability of narrow spectrum growth regulators, ONR-60A/WHO/CCBC/1897 and *B. sphaericus* (1593), and with the possibility of additional new entomotoxins, such multiple (agent) formulations are feasible in the near future.

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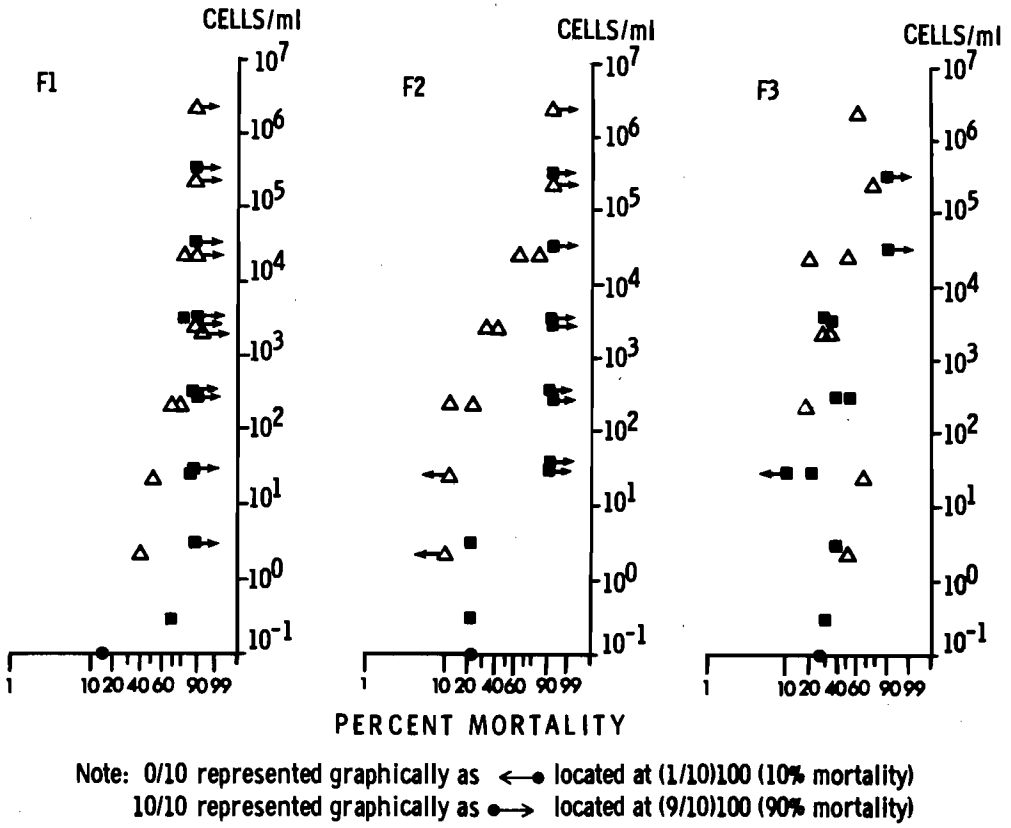

Figure 2.–Lethality of *Bacillus sphaericus* strain SSII-1 for larvae of *Culex pipiens quinquefasciatus* as a function of initial aqueous challenge, presence of different mixed natural microbial flora (F1, F2, F3); and of vegetative (■) or spore (△) phase of the challenge organism. (●) Control.

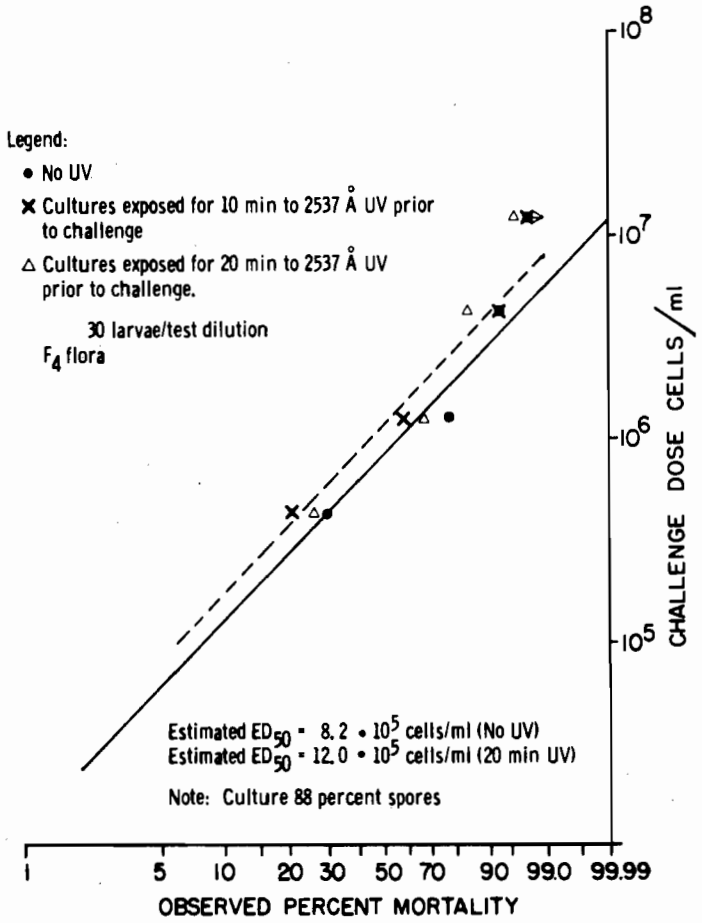

Figure 3.- Comparative dose response of 1st - 2nd instar *Culex pipiens* (complex) larvae to challenge with a 12 hr, 30°C N_2X agar test culture of *Bacillus sphaericus* (SSII-1) culture exposed for 0, 10 and 20 min to 2537 Å ultraviolet prior to use for test challenge.

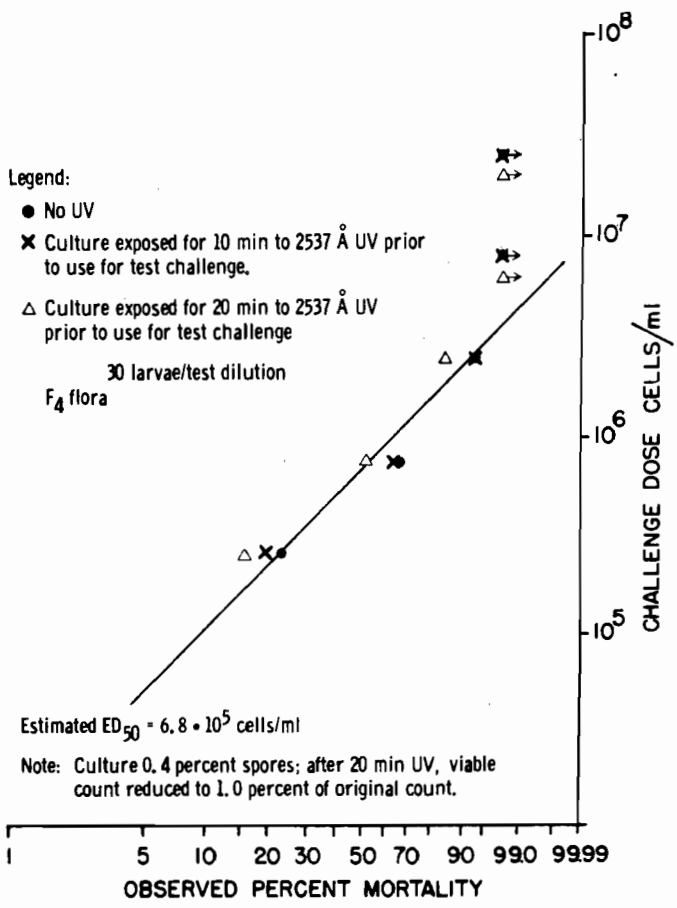

Figure 3(b).- Comparative dose resonse of 1st - 2nd instar *Culex pipiens* (complex) larvae to challenge with a 36-hr, 30°C N_2X agar test culture of *Bacillus sphaericus* (SSII-1); culture exposed for 0, 10 and 20 min to 2537 Å ultraviolet prior to use for test challenge.

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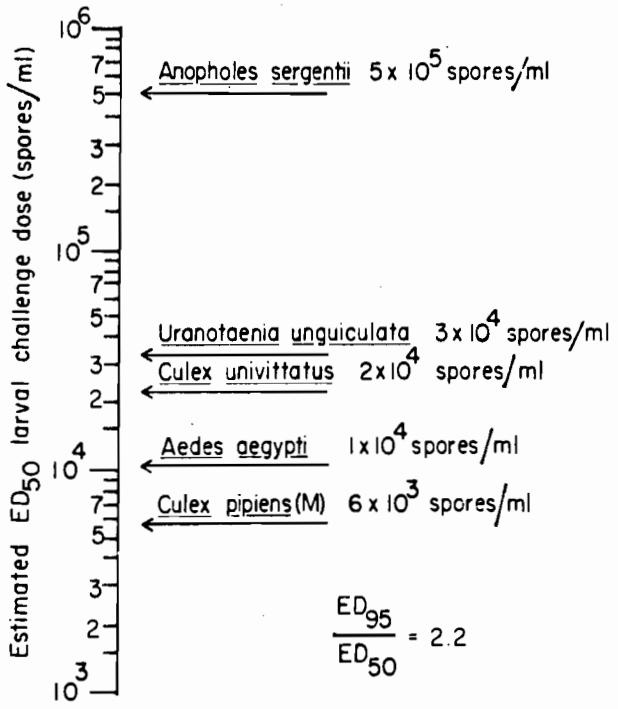

Figure 4. Estimated ED_{50} (spores/ml) for 1st - 2nd instar larvae challenged with a 48 hour, 30°C nutrient agar (Difco) test culture from clone 60A.

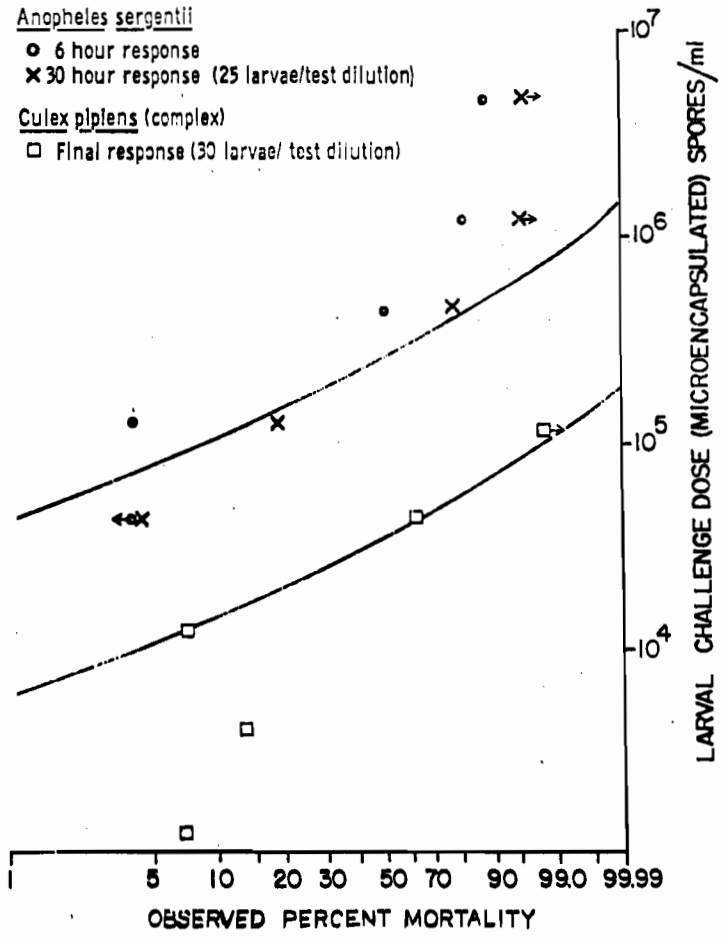

Figure 5.- Dose response of *Anopheles sergentii* and *Culex pipiens* (complex) larvae to challenge with a microencapsulted formulation of isolate 60A.

STUDIES ON THE TOXICITY OF *BACILLUS THURINGIENSIS* var. *ISRAELENSIS* AGAINST ORGANISMS FOUND IN ASSOCIATION WITH MOSQUITO LARVAE

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ABSTRACT

A total of more than 40 non-target species of aquatic animals have been exposed to *Bacillus thuringiensis* var. *israelensis* at concentrations ranging from 50 to several hundred times that required to induce 50% mortality in a laboratory population of third-instar *Culex pipiens*. The non-target species were represented by the following major groups of aquatic animals: three species of amphibians, three species of fish, 10 species of crustaceans and more than 21 species of aquatic insects. Acute toxic effects as determined by mortality differences between test and controls were observed only among the Diptera of the suborder Nematocera. This included, besides the Culicidae and Simuliidae reported elsewhere, species in the family Dixidae, Chironomidae and possibly the Ceratopogonidae. The sources of *Bacillus thuringiensis* var. *israelensis* used during these tests were strains formulated by Sandoz (WDC-I)[1], Goldberg (ONR60)[2], and Abbott (6108)[3].

INTRODUCTION.–The endotoxin of *Bacillus thuringiensis* var. *israelensis* (Bt(i)) has been shown to cause mortality at relatively low concentrations against a wide variety of culicid and simuliid species under laboratory conditions (Goldberg and Margalit 1977, de Barjac 1978, Undeen and Nagel 1978, Garcia and Des Rochers 1979, and Garcia et al. 1980). This paper reports the impact of this pathogen on some wild-caught aquatic organisms found in association with mosquito larvae. These studies were conducted in California and Iran with the majority of species tested indigenous to California. Forty-two wild-caught aquatic organisms were exposed to Bt(i) at concentrations ranging from 50 to several hundred times that required to induce 50% mortality in a laboratory population of third-instar *Culex pipiens*. The sources of Bt(i) used during these tests were strains formulated by Sandoz (WDC-I), Goldberg (ONR60) and Abbott (6108).

METHODS AND MATERIALS.–In California, tests were conducted in a screened outdoor enclosure affording a 50% reduction in sunlight. Plastic tubs with a fluid capacity of approximately 10 l were used in the majority of tests. The tubs were arranged on wooden benches within the screenhouse and each was provided with a separate aeration tube. Hardware mesh (¼ inch) cloth sheets were used as covers for the tubs to prevent certain species from escaping. In Iran, tests were conducted indoors in glass containers. In both places, the water used for maintenance and testing was collected from the same source as the species under examination. All tests were replicated 2 - 6 times, with an average of 3 times for most species. The number of control tubs and the numbers of organisms within them generally equalled the number of organisms and tubs under test. The volume of water used varied from 4 to 8 liters depending on the species under examination and the amount of substrate such as soil, rocks, and vegetation added.

The test dosages of pathogens used were concentrations of 1×10^7, 1×10^6 and 1×10^5 spores/ml. The majority of tests using 1×10^7 spores/ml were with the Goldberg lyophilized strain (ONR60A) which was harvested at a concentration of 1×10^{10} spores/ml. The ONR60A strain was used primarily in Iran. The other aquatic animals were tested with a Sandoz formulation (WDC-I) except for 3 tests with an Abbott formulation (6108). The stock concentration of the Sandoz material at harvest was 2.5×10^{10} spores/ml. The test concentration used was generally 1×10^6 with the exception of a few tests at 1×10^5 spores/ml. The stock concentration of the Abbott material was 1×10^{11} and the 3 tests were conducted at a concentration of 1×10^7 spores/ml.

The LD_{50} for our laboratory strain of *Culex pipiens* (third-instar) averaged $1 \times 10^{3.5}$ spores/ml for the Sandoz preparation and 1×10^4 spores/ml for the Goldberg preparation, which had been stored for over 2 years. Consequently, the dosages received by the non-target test animals in Table 1 ranged from about 50 to several hundred times the LD_{50} for the laboratory strain of *Cx. pipiens*, and likewise for most wild larval *Culex* and *Aedes*, spp. tested elsewhere.

RESULTS AND DISCUSSION.–Table 1 lists the majority of organisms tested during the past 2½ years by species when known, life stage tested, general habitat, number of treatments, number treated per test, formulation used and concentration, observation period and the lethal effect of the pathogen over the observation period. Certain species tolerated the confined conditions better than others which is one of the reasons for the different holding periods.

It is believed that the toxic action of the pathogen for mosquito larvae and other target species appears to be a function of ingestion of the pathogen while feeding. To insure at least some oral intake of the pathogen, all predatory animals were fed mosquitoes which had prior exposure to relatively high concentrations of the pathogen. The vertebrate predators consumed relatively large numbers and showed no mortality that could be construed as due to the result of the pathogen. Parameters such as growth, weight gain and reproduction, which might be affected by the pathogen, were not followed.

[1]Sandoz, Inc., P. O. Box 1489, Homestead, Florida 33030.

[2]Goldberg, L. J., University of California, Berkeley, Naval Biosciences Laboratory, Alameda, California 94501.

[3]Abbott Laboratories, North Chicago, Illinois 60064.

Table 1.- Non-target aquatic organisms exposed to *Bacillus thuringiensis* var. *israelensis*.

Organism	Common Name	Habitat	Number Tested	Formulation & Concentration	Number[1] Treatments	Observation Period	Results
Hyla regilla	tree frog, tadpoles	fresh H_2O pond	30	G* 10^7	one	10 days	no effect
			30	G 10^6	one	10 days	no effect
Bufo sp.	toad,	fresh H_2O pond	15	G 10^7	one	10 days	no effect
Iran	tadpoles		15	G 10^6	one	10 days	no effect
Taricha torosa	California newt	fresh H_2O pond	28	S 10^6	two	22 days	no effect
		fresh H_2O pond	38	S 10^6	one	20 days	no effect
Gambusia affinis Iran	mosquito fish, immatures and adults	fresh H_2O stream	22	G 10^7	one	14 days	no effect
Gambusia affinis	mosquito fish, adults	brackish water	60	S 10^5	three	60 days	no effect
Lucania parva	rainwater killifish, adults	brackish water	45	S 10^5	two	30 days	no effect
			45	A 10^7	two	30 days	no effect.
Gasterosteus wheatlandi	twospine stickleback, adults	brackish water	20	S 10^6	two	30 days	no effect
AMPHIPODA, spp.	scuds, sideswimmers, adults	brackish water	50	S 10^6	two	14 days	no effect
Gammaridae, sp. Iran	immatures and adults	fresh H_2O stream	100	G 10^7	one	4 days	no effect
Hyalella azteca	immatures and adults	sewage ponds	100	S 10^6	two	30 days	no effect
Hyalella azteca	immatures and adults	sewage ponds	40	G 10^7	one	10 days	no effect
			40	G 10^6	one	10 days	no effect
DECAPODA							
Hemigrapsus sp.	purple shore crab	salt water	69	S 10^5	one	10 days	no effect
			10	S 10^6	one	29 days	no effect
ANOSTRACA							
Artemia salina	fairy shrimp, adults	salt ponds	90	S 10^5	one	30 days	no effect
			90	A 10^7	one	30 days	no effect
CLADOCERA							
Simocephalus vetulus	water fleas, immatures and adults	fresh water ponds	100	G 10^7	one	4 days	no effect
			100	G 10^6	one	4 days	no effect
OSTRACODA, sp.	seed shrimps	fresh H_2O ponds	100	G 10^7	one	4 days	no effect
Iran			100	G 10^6	one	4 days	no effect
Cypridae, sp.	seed shrimps	fresh H_2O ponds	15	G 10^5	one	4 days	no effect
			15	G 10^4	one	4 days	no effect
COPEPODA							
Macrocyclops sp.	copepods, immatures and adults	fresh H_2O ponds	50	G 10^7	one	10 days	no effect
			50	G 10^6	one	10 days	no effect
ISOPODA, sp.	marine sow bug	brackish water	28	S 10^6	one	29 days	no effect
EPHEMEROPTERA							
Callibaetis sp.	mayfly nymphs	fresh H_2O reservoir	45	S 10^6	two	22 days	no effect 30% emerged

Organism	Common Name	Habitat	Number Tested	Formulation & Concentration		Number[1] Treatments	Observation Period	Results
ODONATA								
Ischnura sp.	damselfly nymphs	fresh H_2O reservoir	45	S	10^6	two	22 days	no effect molting
Anax sp.	dragonfly nymphs	sewage ponds	10	S	10^6	one	30 days	no effect molting
HEMIPTERA								
Trichocorixa reticulata	water boatmen nymphs	brackish water ditch	60	S	10^5	one	14 days	no effect
Trichocorixa reticulata	water boatmen adults	brackish water ditch	90	S	10^6	one	7 days	50% mortality in controls and tests
Hesperocorixa laevigata	water boatmen adults	fresh H_2O reservoir	40	S	10^6	two	26 days	no effect
Trichocorixa sp.	water boatmen adults	brackish water	10	G	10^7			
			10	G	10^6	one	4 days	no effect
Corixidae, sp.	water boatmen adults	fresh H_2O pond	30	G	10^7	one	3 days	no effect
HEMIPTERA								
Buenoa scimitra	backswimmer nymphs	sewage ponds	30	S	10^6	two	14 days	no effect
Buenoa scimitra	backswimmer adults	fresh H_2O reservoir	50	S	10^6	one	13 days	no effect
Notonecta kirbyi	backswimmer nymphs	fresh H_2O reservoir	25	S	10^6	two	26 days	no effect molted to adults
Notonecta kirbyi	backswimmer adults	fresh H_2O reservoir	24	S	10^6	two	22 days	no effect
Notonecta sp. Iran	nymphs and adults	fresh H_2O pond	9	G	10^6	one	3 days	no effect
Pleidae, sp. Iran	pygmy backswimmer adults	fresh H_2O marsh	26	G	10^6	one	4 days	no effect
COLEOPTERA								
Tropisternus salsamentus	scavenger water beetle larvae	brackish water	8	S	10^5	one	6 days	no effect
Tropisternus salsamentus	scavenger water beetles (adults)	brackish water	45	S	10^5	one	6 days	no effect
			45	A	10^7	one	6 days	no effect
Tropisternus sp.	larvae	fresh water	10	S	10^6	two	22 days	no effect
Tropisternus sp.	adults	fresh water	15	S	10^6	two	30 days	no effect
Dytiscidae, sp. Iran	predaceous water beetle adults	fresh water stream	6	G	10^6	one	4 days	no effect
Gyrinidae, sp. Iran	whirligig beetle adults	fresh water stream	30	G	10^7	one	14 days	no effect
TRICHOPTERA								
Mystacides alafimbriata	caddisfly larvae	fresh water lake	20	S	10^6	three	30 days	no effect emerged to adults and reproduced
DIPTERA								
Dixidae								
Dixa sp.	midge	fresh water pond	9	G	10^6	one	<1 day	100% mortality
Ceratopogonidae	biting midge	treehole	26	G	10^7	one	4 days	100% mortality
Palpomyia sp.			24	G	10^6	one	4 days	42% mortality
Chironomidae, sp.	non-biting midge	fresh water stream	30	G	10^7	one	<6 days	100% mortality
Iran			30	G	10^6	one	<6 days	100% mortality
Chironomidae, sp.	non-biting midge	fresh water reservoir	15	S	10^6	one	24 hours	100% mortality
		late instar	15	S	10^5	one	24 hours	47% mortality
			15	S	10^4	one	24 hours	15% mortality

Organism	Common Name	Habitat	Number Tested	Formulation & Concentration	Number[1] Treatments	Observation Period	Results
Chironomidae, sp.	non-biting midge	sewage ponds	60	S 10^6	one	24 hours	92% mortality
		late instar	55	S 10^5	one	24 hours	58% mortality
Ephydridae *Ephydra riparia* complex	brine fly larvae	brackish water	150	S 10^6	two	30 days	no effect emergence
TUBELLARIA Planaridae *Dugesia dorotocephala*	flatworm	fresh water ponds	20 20	G 10^7 G 10^6	one one	10 days 10 days	no effect no effect
GASTROPODA *Physa* sp.	fresh water snail	fresh water reservoir	30	S 10^6	two	30 days	no effect reproduced
PELECYPODA, sp.	mussel	salt water	20	S 10^6	one	14 days	no effect

*Key to Formulations of *Bacillus thuringiensis* var. *israelensis*:
G = Goldberg (ONR 60)
A = Abbott (6108)
S = Sandoz (WDC-1)

[1]Number of treatments of BTI.

The only non-predatory animal which was fed parcels of food directly was the purple shore crab. These were fed pieces of commercially purchased herring which had been soaked in a water solution of the pathogen. The crabs readily consumed this preparation without apparent harmful effects; however, no molting was observed during confinement.

Of the several different groups of insect predators tested, three groups demonstrated molting during the holding period. These included a notonectid, a damselfly and a dragonfly. The primary food source for these animals was treated mosquito larvae suggesting that growth had taken place with the ingestion of the toxin.

Other non-predatory insect species showing development during the holding period were an ephydrid fly and a species of Trichoptera. This latter species completed its full cycle of development and reproduced while under confinement. The only other animal which was observed to reproduce and develop during the tests period was an aquatic snail of the genus *Physa*.

As noted in Table 1, only members of the families Dixidae, Chironomidae, and Ceratopogonidae of the Diptera seemed to be affected by the pathogen during these tests. Although the non-target tests conducted thus far appear encouraging, they are still preliminary and pertain only to acute toxic effects under the conditions described here. Further testing is required before any reasonable estimate can be made on the total impact of this pathogen on non-target organisms.

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PRELIMINARY FIELD TRIALS WITH *BACILLUS THURINGIENSIS* var. *ISRAELENSIS* AGAINST *AEDES DORSALIS* AND *CULEX TARSALIS* IN SALT MARSHES

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ABSTRACT

Small field treatments with *Bacillus thuringiensis* var. *israelensis* WDC-1 Sandoz formulation were conducted in salt and brackish marsh areas of the San Francisco Bay against *Aedes dorsalis* and *Culex tarsalis*. The pathogen reduced larval mosquito populations by 85% or greater at all dosages applied, with the lowest concentration equivalent to 1 kilogram per hectare.

4,72 – 7,48 lbs/ac

INTRODUCTION.—Laboratory investigations have revealed that the endotoxin of *Bacillus thuringiensis* var. *israelensis* is extremely toxic to mosquito and simuliid larvae (Goldberg and Margalit 1977, de Barjac 1978, Undeen and Nagel 1978, Garcia and Des Rochers 1979, Garcia et al. 1980a). Some preliminary studies on the acute toxicity of this pathogen indicate that it has a relatively narrow host range (Garcia et al. 1980b). Small field trials with this pathogen against several species of California mosquitoes under widely different aquatic and climatic conditions have shown encouraging results (Garcia et al. 1980a). The investigations reported here invovle two small field tests against *Aedes dorsalis* and one against *Culex tarsalis* in brackish and salt marsh habitats of San Francisco Bay.

METHODS AND MATERIALS.—A formulation by Sandoz (WDC-I) of *Bacillus thuringiensis* var. *israelensis* with a stock concentration of 2.5×10^{10} was used in all tests reported here. Bioassay against a laboratory strain of third instar *Culex pipiens* displayed a consistent LD_{50} of $1 \times 10^{3.5}$ during the course of these investigations. Water samples were collected at each test site and analyzed primarily for conductivity (mg/l NaCl) and pH using a Hach Chemical water analyzer kit, DR-EL/2. Other components such as nitrogen, potassium, total hardness (as $CaCO_3$) and others were included in the analysis when suscepted of being present at relatively high levels. Mosquito larvae were collected and identified from each site and bioassayed against the bacterial preparation used in each field test.

The first field trial to be discussed was conducted in late June, 1979, at the San Quentin dump site, Marin County, in a flood water marsh. The water was brackish and receding rapidly into small pools. The salt concentration ranged from 27,000 to 29,000 ppm and the pH was recorded at 9.1. The mosquito population was exclusively *Culex tarsalis* and ranged in numbers from 12 to 66 per dip. The fauna in general was characteristic of a fresh water marsh with the major faunal components consisting of ostracods, copepods, corixids, zygopterans, notonectids, and dytiscids in addition to the mosquito larvae. Plastic buckets with the bottoms removed and a surface area of 1/10 sq. meter were used as test and control enclosures. They were forced into the mud bottom and in this position each contained about 15 liters of marsh water, in addition to the encaptured flora and fauna. Plastic dishes 9 cm in diameter and 3 cm in depth with screened bottoms were used as sentinel stations and floated within the plastic enclosures described above. Each sentinel station contained 40 *Culex tarsalis*, 10 *Trichocorixa* and a mixture of 50 ostracods and copepods. The test enclosures were treated with a water suspension of the Sandoz preparation equivalent to 1.5 and 15 kg/ha. The laboratory LD_{50} for the wild caught *Culex tarsalis* tested in the brackish water from the site was 1×10^3.

Field tests were conducted at two other sites with typical salt marsh characteristics against larval populations of *Ae. dorsalis*. The emergent aquatic and surrounding vegetation at both sites was almost entirely of the salt marsh pickleweed *Salicornia* sp. The first site was located in a small marsh area in Kentfield, Marin County. The test was conducted in mid-June, 1979. Analysis of the water indicated a pH of 8.2 and a sodium chloride content of 32,400 ppm. All other components measured were of negligible significance. The populations of *Aedes dorsalis* were primarily third instar and their numbers ranged from 1 to 11 per dip. Treatment and control sites were measured off in equal segments, each with a surface area of ½ sq. meter and a water depth ranging from 12-19 cm. The water suspension of Sandoz WDC-I was applied with a small hand sprayer at rates ranging from less than 1 to 4 kg/hectare.

The final field test was conducted in mid-August in another salt marsh site near the Larkspur ferry slip in Marin County. Analysis of the water indicated a pH of 8.0 and a sodium chloride concentration of 27,000 ppm. All other measured components were deemed negligible. A high tide had flooded a dike enclosed area of about ½ hectare. Water depth varied from about 26 cm in the areas of emergent *Salicornia* to about one hundred cm in the open water channel. A relatively large population of third instar *Aedes dorsalis* was restricted to the emergent vegetation along the edges of the open channel and in the partially submerged stands of *Salicornia*. Numbers of larvae per dip varied from 5 to 68. The water suspension of Sandoz WDC-I was sprayed with a Root and Lowell Model No. 1977 pressure sprayer at a rate of 2 kg/ha.

RESULTS AND DISCUSSION.—Table 1 shows the results of the treatment of *Culex tarsalis* populations in the San Quentin marsh site. Many dead mosquitoes and an occasional

Table 1.–Field trials using *Bacillus thuringiensis* var. *israelensis* in brackish water, San Quentin dump site, Marin County 1979.

SANDOZ	No. *Cx. tarsalis*/dip in enclosures			No. *Cx. tarsalis* as sentinels			No. corixids/crustaceans as sentinels		
Bt(i) - Kg/HA	1	2	3	1	2	3	1	2	3
Control	21	30	10	40	40	26	10/50	9/50	9/50
1.5	22	1	1	40	6	2	10/50	9/50	8/50
15.0	37	1	1	40	0	0	10/50	9/50	8/50
Exposure period	0 hrs	24 hrs	72 hrs	0 hrs	24 hrs	72 hrs	0 hrs	24 hrs	72 hrs

Table 2. Field trials using *Bacillus thuringiensis* var. *israelensis* in brackish water, Kentfield salt marsh, Marin County, 1979.

SANDOZ	Number of *Aedes dorsalis*/dip						Approximate
Bt(i) - kg/HA	Test	Control	Test	Control	Test	Control	percent reduction
4	10	6	¼	4¼	½	5½	90
2	5	6½	2	4¼	¾	5½	85
1	8	3	½	2	DRIED UP		90
Exposure period	0 hrs		24 hrs		48 hrs		

Table 3. Field trials using 2 kg/HA *Bacillus thuringiensis* var. *israelensis* in brackish water, Larkspur ferry site, Marin County, 1979.

	Number of 3rd instar *Aedes dorsalis*/dip:					
Site No.	Test	Control	Test	Control	Test	Control
1	9.5	11.0	0.4	13.0	0.4	51*
2	11.5	13.0	0.1	51*	*Same Area Used	
3	51*	--	1.0	--		
Exposure period	0 hrs		24 hrs		72 hrs	

live one were observed at the water surface after 24 hrs. Using the standard dipper as a measuring device, it was found that both high and low treatments reduced the larval populations within the plastic enclosures by approximately 95%. Mortality of the sentinel mosquito larvae was about 85% at the low concentration and complete at the exaggerated concentration. The sentinel non-target animals did not seem to be affected at either dosage. Water levels in the pools were receding so rapidly that observations past the 72 hr period were considered not reliable.

Table 2 shows the results of treatment of *Aedes dorsalis* at three dosage rates at the Kentfield marsh site. Reduction of mosquito larvae was approximately the same regardless of concentration. The pathogen remained active at this site despite the highest salt concentration, 3.2%, recorded for any field study. The reduced wind action, due to the heavy stands of *Salicornia*, in combination with the greater buoyancy of the salt water probably accounted for the large number of dead larvae observed floating 24 hrs after treatment. Under these particular conditions the percent reduction in larval populations recorded in Table 2 were approximately the same as the average ratio of dead to live larvae in the post treatment dips, thus giving another verification of the efficacy of the treatments.

The third and final field test indicated an overall reduction of *Aedes dorsalis* larvae by about 95% (Table 3). In this particular test treatment was restricted to the area where mosquito larvae were observed with an additional ½ m added as a buffer zone. Thus, there was opportunity for mixing of the treated area with untreated water which, if it did occur, did not seem to alter the efficacy of the treatment drastically at the treatment rate of 2 kg/ha. The water level in the study area receded rapidly over the 3 day period which accounted for the large number of larvae per dip in the last treatment site.

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LABORATORY AND FIELD STUDIES OF *BACILLUS SPHAERICUS*, STRAIN 1593-4, AND *B. THURINGIENSIS* SEROTYPE H.14

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ABSTRACT

The activities of both *Bacillus sphaericus*, strain 1593-4, and *B. thuringiensis* serotype H.14 (*B. t.* H.14) were lower in raw and autoclaved sewage effluent than those in tap water. Reduction of activity in polluted waters of *B. sphaericus* was caused by the suspended solids fraction of the effluent. Activity of *B. sphaericus* was greatly reduced by exposure to direct sunlight, while the activity of *B. t.* H.14 remained stable. The active ingredient of *B. sphaericus* settled to the bottom of treated, street catch basins where prolonged residual activity was obtained. Reinfesting larvae were not affected; apparently they did not feed from the bottom. The residual action of *B. t.* H.14 was short-lived in the catch basins. Treatment of 0.02-ha pasture plots with *B. t.* H.14 resulted in effective control of *Aedes spp.* and *Culex tarsalis* at 1 kg/ha. An aerial application on a duck club with *B. t.* H.14 at 1 kg/ha produced a 99% reduction of *Cx. tarsalis*, apparently without adverse affect on predator populations.

MERMITHID NEMATODES IN MOSQUITO CONTROL: PERSISTENCE IN SMALL EXPERIMENTAL PONDS

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INTRODUCTION.—The successful release and establishment of a mermithid parasite of mosquitoes, *Romanomermis culicivorax* Ross and Smith, has been demonstrated in Louisiana (Petersen and Willis 1975) and Maryland (Nickle 1979). In 1977, small experimental field releases of *R. culicivorax* were initiated at the University of California, Riverside, to determine the recycling time and the ability of the nematode to persist in the absence of population recruitment. This latter condition was implemented by preventing the entrance of host mosquitoes into the experimental ponds.

MATERIALS AND METHODS.—*R. culicivorax* was propagated in autogenous *Culex pipiens* Linnaeus according to the procedures of Platzer and Stirling (1978). Postparasitic nematodes were collected daily during the emergence phase and the volume of postparasites was measured by centrifuging suspensions of nematodes in 50 ml graduated centrifuge tubes for 2 min at 220 g. After measurement of the volume of postparasites, the nematodes were suspended in 700 ml water and the suspension was divided into 14 parts by dispensing 50 ml aliquots into 100 ml plastic food containers. The suspension was agitated constantly during the dispensing procedure to keep the nematodes evenly distributed in the aliquots.

The small experimental ponds were located in the Midgeville experimental area. The ponds were 1 meter square with redwood borders, and the water level was maintained by automatic valves. The substrate was a medium-coarse sand. The average pH and conductivity of the pond water was 8.0 and 680 micromhos/cm, respectively. All ponds were covered with insect-proof screening to prevent the entrance of mosquitoes during the experiments. All algal mats were removed during the course of the study. Postparasites were introduced into the ponds by distributing one-half of the nematode aliquots at 7 equally dispersed sites on the bottom of the pond. Two ponds were inoculated at each weekly introduction of the postparasites. The soil temperature in one pond was recorded continuously with a Foxboro thermograph. The soil temperature was detected with a thermoprobe inserted into the top 8 cm of the bottom substrate.

The production of infective stages was monitored through the daily use of sentinel mosquito larvae for one month after introduction of the nematodes in the ponds. Thereafter, each pond was tested weekly or biweekly. Twenty larvae of *Cx. pipiens* or *Anopheles freeborni* Aitken were placed in sentinel cages constructed from 1-quart plastic food containers. Three openings, 5.5 x 7.5 cm, were cut into the sides of each container and covered with 80 mesh nylon screen. The mosquito larvae were dissected after 24 or 64 hours exposure in inoculated ponds.

RESULTS AND DISCUSSION. During 1977, 3 separate introductions of postparasites into 6 ponds were made. Ponds 2 and 3, 6 and 7, and 4 and 5 received 4.5, 9.5, and 7.5 ml, respectively, of *R. culicivorax*. There were 1,700 nematodes per milliliter of postparasites. In 3 ponds (Table 1) infectious larvae were detected in sentinel mosquitoes after an average inoculation period of 26 days (range 19 to 37 days) at a mean temperature of 27°C. Infection levels rose to 100% (Figure 1). In pond 4, inoculated in late September, the infectious larvae appeared after 69 days at a mean soil temperature of 19°C. Infectious larvae did not appear in two ponds until the following summer (Table 1). Production of infectious larvae continued in two ponds until mid-December when the average soil temperature fell below 13°C.

The temperature of the pond substrate was monitored continuously during the winter months and sentinel mosquitoes were used on a weekly basis to check for the resumption of parasite activity. Low parasite activity was detected in a single pond late in February after an abrupt change in daily maximum and minimum temperatures. However, no further parasite activity was detected until late May despite steadily increasing soil temperature.

Sentinel mosquitoes were infected in May through June, 1978, in all six ponds inoculated in the previous year (Figure 1). The average pond soil temperature was 21°C (17 to 26°C daily range) at the time of resumption of parasite activity. Parasite activity in 3 of these ponds continued until late October to mid-November for a total period of 15 months from the time of the introduction of the postparasites. During this time, no mosquito breeding was allowed in the inoculated ponds and therefore the infectious progeny were produced by the initial population of postparasitic nematodes.

During June to August, 1978, 8 more ponds were inoculated with postparasites (4-10 ml per pond). In these introductions, *R. culicivorax* was successfully established in 5 or 60% of the ponds. In three ponds, infectious nematode larvae were detected with sentinel mosquitoes 26 days (range 23 to 29 days) post-introduction after an average temperature exposure of 27°C (Table 1). In the remaining 2 ponds, appearance of infectious larvae was delayed 37 and 87 days although the average temperature was 25 to 27°C (Figure 1). These delays were not related to temperature and must have reflected the effects of some unknown properties of these particular ponds upon the mermithids. Ostensibly, all ponds were alike with respect to soil type, water depth, exposure to sunlight and types of aquatic organisms present.

The production of infectious progeny in 1978 was not as much as that of the 1977 introductions (Figure 1). Production of infections continued until the end of November when the average soil temperate fell to 12°C.

Table 1.–Chronological record of *Romanomermis culicivorax* introduction in small ponds and persistence of infections.

Pond #	Inoculation Date	Avg. Temp.* °C.	Infectious Larvae: Interval to 1st Appearance	Infectious Larvae: Date of Last Appearance
2	08-05-77	27	19 days	06-12-78
3	08-05-77	27	23 days	10-30-78
6	08-10-77	–**	338 days	11-16-78
7	08-10-77	27	37 days	08-30-78
4	09-27-77	19	69 days	06-12-78
5	09-27-77	–**	221 days	11-01-78
9	06-23-78	27	29 days	08-01-78
10	06-23-78	–	ND***	ND
13	07-06-78	28	25 days	11-03-78
14	07-06-78	–	ND	ND
11	07-20-78	25	87 days	06-29-79
12	07-20-78	27	37 days	06-01-79
15	08-03-78	–	ND	ND
16	08-03-78	27	23 days	06-06-79

*Average daily temperature after introduction of postparasites to first appearance of infectious larvae.

**Average temperature not applicable because of winter interval before appearance of infectious larvae.

***ND - No detection of infections with *R. culicivorax*.

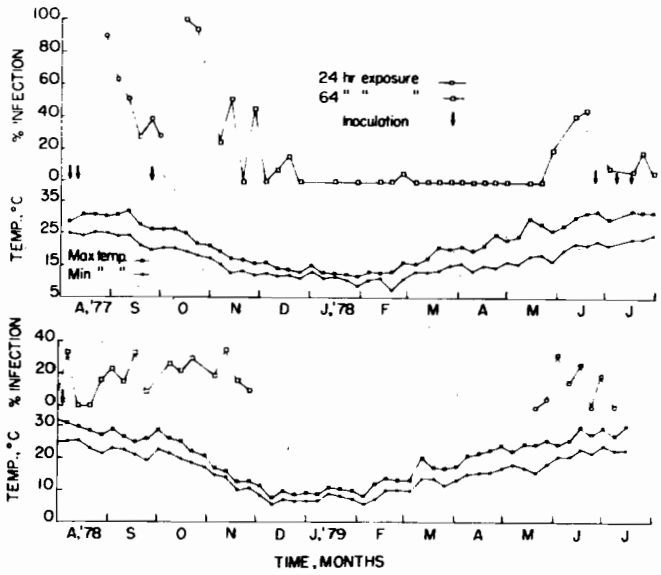

Figure 1.–Recycling of *Romanomermis culicivorax* in small ponds at Midgeville, UCR, during 1977 to 1979. The infectivity curve represents a weekly average of infections in all ponds. Infections were measured after 24 or 64 hr exposure of sentinel mosquitoes. The temperatures were recorded from the bottom sediment of one pond and represent the average weekly maximum and minimum temperatures.

During the early summer of 1979 production of infectious nematodes resumed in three of the 5 successful ponds inoculated in 1978 after the temperature had risen to an average 22°C (Figure 1, Table 1). No infections were detected in ponds inoculated in 1977. The experiment was terminated in early July 1979.

This experiment demonstrated the ability of *R. culicivorax* to over-winter in Southern California. The optimal average temperature for development of the postparasites was 27°C. Maximum daily temperatures in pond soils did not exceed 34°C during hot summer periods. Production and hatching of preparasites is prevented when sustrate temperatures fall to 12 to 13°C. After overwintering, soil temperature must approach 21 to 22°C before preparasite activity resumes.

The extended persistence of the parasite through 2 mosquito seasons for 15 months was shown. This persistence was due undoubtedly to the variable developmental rates of the postparasites and eggs that can be observed readily in laboratory mass cultures. This variability suggests substanial genetic heterogeneity in *R. culicivorax*. Such persistence in mosquito habitats suggested that in the case of mosquito eradication, the treated habitat would be further protected from new mosquito immigration.

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MASS MARKING OF MOSQUITOFISH: PRELIMINARY RESULTS

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ABSTRACT

Three marking techniques were tested to determine their applicability for mosquitofish. Tetracycline drugs and DCAF administered in the diet successfully marked laboratory-cultured mosquitofish, but exposure to direct sunlight in outdoor tanks resulted in the rapid disappearance of the marks. Preliminary data on fluorescent marks from a polystyrene pigment in a melamine-sulfonamide-formaldehyde resin forced into the dermal tissue with compressed air are more promising. At an optimal deliver pressure of 7.3 m Hg (140 p.s.i.) and spraying time of 15 sec., marking percentage is maximized and fish mortality is minimized. Mark retention time was up to 80 days in outdoor tanks at this writing. Long retention times and the availability of four colors increases the potential of this marking technique as a useful tool in field population studies of mosquitofish.

INTRODUCTION. Although the mosquitofish (*Gambusia affinis*) has been introduced throughout California as a biological control agent for mosquitoes, many aspects of the microdistribution and population dynamics of this species are poorly understood. Accurate assessments of mosquitofish population densities, survival rates, dispersal rates, and dispersal distances are important for effective mosquito control while minimizing disturbance to local fish communities.

Marking fish in some manner (e.g. tags, fin clips) is often the most straightforward technique used in calculating population parameters (see Laird and Stott 1978). Tags are inappropriate for marking mosquitofish due to the small size of these animals and the large numbers which must be marked to obtain accurate population data. A desirable mark for mosquitofish would (1) be rapidly and easily applied to large numbers of fish, (2) not affect behavior or increase mortality rates through predation, and (3) be distinctive and retained for several months. The objective of the present study was to develop a marking technique having the above characteristics.

Three marking techniques which have been used successfully for other fishes were tested on mosquitofish. The tetracycline series of drugs was used by Weber and Ridgeway (1962, 1967) to mark skeletal structures and scales of Pacific salmonids. Tetracycline added to the diet, is incorporated into collagen fibrils of bones and scales. The deposited antibiotic is detectable by a yellow fluorescence when exposed to ultraviolet (UV) light (Weber and Ridgeway 1962). The second compound tested was 2,4 bis (N, N' di-carboxymethyl aminomethyl) fluorescein (DCAF) which is also administered in the diet. Hankin (1978) reported DCAF was incorporated into growing scales of guppies, *Poecilia reticulata* and is detected as a yellow-green fluorescence when subjected to UV light. Finally, we tested a fluorescent polystyrene pigment in a melamine-sulfonamide-formaldehyde resin which is forced into the dermal tissue with compressed air from a small sandblasting gun (Jackson 1959, Scidmore 1961, Phinney et al. 1967, Phinney and Matthews 1969). Detection of the pigment requires exposure to UV light.

METHODS. Tetracycline - - Oxytetracline and chlorotetracycline (Sigma Chemical Co.) were used to test the applicability of the tetracycline antibiotic series as a marker for mosquitofish. These chemicals, and in many cases a potentiator, were incorporated into the diet by mixing them with an aqueous slurry of a commercially available food (Basic Flake®) in a blender, then drying the slurry on foil sheets to reform a flake diet. Tetracycline was administered at three levels: 100, 250 and 500 mg tetracycline/kg of mosquitofish/day. Weber and Ridgeway (1967) state that 300 mg/kg/day fed to young salmonids resulted in good marks. Glucosamine was used as a potentiator (Weber and Ridgeway 1967) in conjunction with tetracycline at four levels 0, 25, 50 and 200 mg glucosamine/kg of mosquitofish/day.

Mosquitofish (*Gambusia affinis*) for all experiments were collected from a local stream and held in flow-through tanks, receiving a continuous flow of tap water. Temperatures of the non-chlorinated tap water supply varied seasonally between 18 and 23°C. Groups of twenty males or females of two qualitative sizes (small-ca. 15-20 mm and large-ca. 25-30 mm standard length) were used in the feeding trials. As small females exhibit the fastest growth rates and, therefore, presumably incorporate tetracycline at the highest rate, this group was also tested at various tetracycline/potentiator concentrations (Table 1). Other sex-size combinations were chosen for comparison with small females at the highest concentrations only. Each group of 20 fish was weighed in a tared beaker of water and placed in 15 1 flow-through aquaria. Each experimental group was fed at 20% initial average body weight/day in two daily feedings for 21 days.

The treated fish and a control group receiving only Basic Flake® were examined at 4, 10, 15 and 21 days after the initial feeding to assess onset and intensity of marking. Each group of fish was placed in a shallow pan (1 cm water depth) and viewed under UV light (G. E. 40W fluorescent tube, spectral emission peak 360 nm).

After 21 days each experimental group was divided in half. Ten fish were moved to outdoor 15 1 aquaria receiving direct sunlight throughout the day. After 24 hours these fish were examined and compared to the remaining experimental fish held inside the laboratory under normal fluorescent lighting.

DCAF - - 2, 4 bis (N, N' di-carboxymethyl aminomethyl) fluorescein (DCAF) was administered orally in combination with glucosamine and Basic Flake®. Two ration levels 100 and

1000 mg DCAF/kg of mosquitofish/day were fed in conjunction with 10 mg glucosamine/kg/day (Table 1). DCAF was dissolved in 0.5N NaOH and then incorporated into the flake diet in the same manner used for tetracycline. Experimental fish were fed at 20% initial average body weight/day in two daily feedings for ten days.

Fish were viewed under UV light source at 4 and 10 days after initial feeding to assess the rate of DCAF incorporation in the scales. After 10 days, half of the fish were placed outside in 15 l aquaria. Fish were examined after one day exposure to sunlight and compared to experimental fish held inside under laboratory lighting.

Fluorescent Polystyrene Pigment - - The equipment used for the forced, external application of this powdered substance includes: (1) a low pressure spray gun and hose (Port-a-Blast, Lindberg Products Co.), (2) a compressed air tank with single stage regulator, (3) a container to hold fish during marking, and (4) the fluorescent polystyrene pigment in a melamine-sulfonamide-formaldehyde resin available from Scientific Marking Materials, Seattle, Washington.

One-hundred fish (>15 mm standard length) were used in each marking trial. The fish were placed in a 30 X 30 X 5 cm box with wood sides and plastic screen bottom (1 mm x 1 mm mesh) and top (3 x 4 mm mesh) to retain fish inside. Four delivery air pressures were tested (100, 120, 140 and 160 p.s.i.) at two time (10 and 15 sec) durations (Table 2). Immediately following pigment application fish were rinsed with water and placed into a water bath. Each group was divided in half and placed into 15 l flow-through aquaria. A single potassium permanganate ($KMnO_4$) treatment (2 ppm) was flushed through the aquaria to retard bacterial infection.

After pigments in the external mucus coat had sloughed (3-4 days) the fish were examined under UV light. Marks clearly visible from above when the UV source was placed 45 cm above the fish were considered acceptable.

After initial inspection, treatment groups and a control group of fish were placed in outdoor glass aquaria to determine marking-induced mortality rates and the effects of sunlight on mark duration and intensity. Deaths occurring within 24 hr of pigment application were classified as "initial" mortalities. A continuing record of mortalities was mantained to determine "long term" mortality rate.

RESULTS.–Tetracycline and DCAF - - All fish fed tetracycline drugs and DCAF exhibited a visible fluorescence when exposed to UV light. Within 10 days the scales of all fish receiving DCAF in their diet were fluorescent, while 21 days was required for the tetracycline-fed mosquitofish. This fluorescence persisted in fish held inside the laboratory for at least an additional 24 hr. However, exposure to direct sunlight for one day resulted in the disappearance of fluorescence from mosquitofish fed either tetracycline or DCAF.

Fluorescent Polystyrene Pigment - - Marking success was positively correlated with delivery air pressure and time duration. The percent mosquitofish marked increased from 20% at 100 p.s.i. to approximately 60% at 160 p.s.i., based on percentage of the initial sample of 100 fish (Table 2). Initial mortality percentages were also positively correlated with delivery pressure and time duration. Percent initial mortality was very low at 100, 120, and 140 p.s.i., but increased dramatically at 160 p.s.i. (Table 2). On the other hand, extended mortality percentage appeared to be uniformly low for all delivery pressures and time durations, except for the treatment

Table 1.–Summary of experimental design of the concentration, sex and size combinations of tetracycline drugs and DCAF administered to mosquitofish in the diet.

Marking Chemical	Concentration mg/kg/d	Potentiator mg/kg/d	Size-sex	Number of fish	Mean fish Weight (g)
Tetracycline	100	25	small female	20	.342
Tetracycline	250	25	small female	20	.276
Tetracycline	500	25	small female	20	.299
Tetracycline	250	0	small female	20	.365
Tetracycline	250	50	small female	20	.355
Tetracycline	250	200	small female	20	.293
Tetracycline	250	25	large female	20	.943
Tetracycline	250	25	large male	20	.202
Tetracycline	250	25	small male	20	.124
Tetracycline-HCL	100	25	small female	20	.329
Tetracycline-HCL	250	25	small female	20	.318
Tetracycline-HCL	500	25	small female	20	.345
DCAF	100	10	small female	10	.524
DCAF	100	10	small female	10	.433
DCAF	1000	10	small female	10	.544
DCAF	100	10	mixed male	10	.209
DCAF	100	10	mixed male	10	.217
DCAF	100	10	large female	10	.904
DCAF	100	10	large female	10	1.211

Table 2. Marking success and mortality rates of mosquitofish at different delivery air pressures and time durations with fluorescent polystyrene pigment forced into dermal tissues. Initial mortalities were recorded after 24 hr, extended mortalities after 80 days.

Delivery pressure (p.s.i.)	Time duration (sec)	Marking Success (%)	Initial Mortality (%)	Extended Mortality (%)
100	10	20	1	–*
120	10	38	0	16
140	10	28	3	3
140	15	45	4	–*
160	10	59	15	3
160	15	54	34	2
Control				20

*Extended mortality not measured.

at 120 p.s.i. The mortality percentage at 120 p.s.i. was five times higher than other treatments, but still below the number of mortalities observed in the control group (Table 2). Delivery pressures of 140 p.s.i. for 15 sec. seems to be the optimal marking protocol at this time. Marking success is near 50%, while mortality rates are low.

Mark retention studies are still underway on two groups initially marked at 100 p.s.i. for 10 sec. and 140 p.s.i. for 15 sec. These fish have retained clear and distinctive marks for 80 days at this writing, even with exposure to sunlight.

DISCUSSION. Weber and Ridgeway (1962) and Hankin (1978) using tetracycline and DCAF respectively, obtained fluorescent rings on scales of fishes. In both cases individual scales were viewed using a microscope. Marks were retained at least three months using either chemical. To allow mosquitofish life history parameters to be evaluated with minimal laboratory time, marked mosquitofish must be immediately recognized. Thus, the tetracycline and DCAF dosages in the present study were increased an average of three-fold over those used in past studies in order to mark the whole fish. Nevertheless, the fluorescent mark disappeared from *Gambusia* after one day in direct sunlight. Mosquitofish typically inhabit surface layers of shallow, warm waters. Thus, they are exposed to comparatively intense solar radiation, including some UV (Wetzel 1975) which apparently obliterates the marks produced by the tetracycline and DCAF techniques. In contrast, the preliminary results using the sprayed polystyrene pigments meet all the criteria desired for mosquitofish marks. That is, the mark is easily and rapidly applied, is not visible under visible light and is retained for a sufficient period to obtain pertinent life history data. Pribble (1976) reports that 35,000 fish can be marked per hour and that the sprayed pigment marks were retained at least two years.

The availability of 4 colors of fluorescent pigments increases the sophistication of potential mark-recapture data. For example, movements of fish simultaneously planted at different locations could be distinguished. Also the growth and mosquito predation performance of different genetic stocks could be quickly assessed.

As these preliminary data do not include replicates testing spray delivery pressures, time durations, or effects of fish size or sex, further studies should be conducted prior to wide scale field applications. Field experience should streamline the marking, handling and identification of large numbers of mosquitofish in stocked habitats. Reliable estimates of marking success and resulting mortalities should enhance the precision of mosquitofish population size calculations.

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THE FOOD DEMANDS OF MOSQUITOFISH, *GAMBUSIA AFFINIS*

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ABSTRACT

Using respiratory metabolic measurements, the oxidative food demand (calories/day) for mosquitofish (*Gambusia affinis*) was determined under "resting" conditions at six temperatures and three concentrations of dissolved oxygen. Gravimetric and bomb calorimetric measurements of *Culex tarsalis* instars allowed calculation of mosquitofish food demands in terms of feeding rates on mosquito larvae. Food demands ranged from 4.4 "large" mosquito larvae (or 35.2 "small" larvae) day^{-1} in water at 10°C and 25 torr dissolved oxygen tension to 58.1 "large" mosquito larvae (or 463.3 "small" larvae) day^{-1} in 35°C water at air-saturation for a mosquitofish weighing 0.5 g.

INTRODUCTION.--As classic ectotherms ("cold-blooded" animals), most fishes show increased rates of respiratory metabolism when in warmer environments (Schmidt-Nielsen 1979). At warmer temperatures, the rates of intracellular biochemical reactions speed up and the demand for food (as a substrate for these reactions) increases. The demand for oxygen increases in proportion to the food demand as long as oxidative biochemical pathways are used for energy conversion. As part of our proposed five-year program to develop a bioenergetic model for mosquitofish, *Gambusia affinis*, we undertook a series of measurements of *Gambusia* respiratory metabolism to quantify the food and oxygen requirements at various temperatures and dissolved oxygen levels.

METHODS.--A multiple-chambered, flow-through respirometer was constructed from polystyrene, vinyl, and PVC plastic tubing. The apparatus was situated in an insulated water bath which provided the source of flowing water as well as temperature control (Figure 1). Experimental mosquitofish were obtained locally or from the Sutter-Yuba Mosquito Abatement District and held in fiberglass laboratory tanks (125 l). Fish were held at the experimental temperature, which approximated ambient field conditions. Fish were fed 1-3 times daily on an ad lib. ration of Tetramin until 24-48 h prior to experimentation. Individual mosquitofish were placed in each chamber, and the top of the apparatus was mounted to shield the fish from movements of the investigators and other laboratory-based stimulation. The fish were given an overnight period to accustom themselves to their surroundings. Over the following two-day period, oxygen consumption rates (as measurements of respiratory metabolism) were determined as mosquitofish were exposed to normoxic and two levels of hypoxic water, 40 and 25 torr $PO_2 \approx$ 25% and 15% of air saturation, respectively. Hypoxic water was generated by regulation of countercurrent flows of tank water and nitrogren gas in a vertical "oxygen-stripping" column (Cech et al. 1979).

Oxygen consumption rates (mg O_2/kg body mass/h) were calculated by multiplication of the oxygen concentration differences across each respirometer (inflowing-outflowing) by the water flow rate with adjustment for body mass. Water flow was measured by timed, volumetric collection of water flowing out of each respirometer chamber. Dissolved oxygen concentrations were calculated from oxygen tension (PO_2) measurements (Radiometer PHM71, D616, E5046 electrode/meter system) applied to an oxygen solubility nomogram (Green and Carritt 1967). "Wet" body mass determinations were made after the oxygen consumption measurements by 10 sec dips in acetone followed by 30 sec of paper towel blotting of the immobilized fish and immediate weighting to the nearest 0.1 mg (Mettler H-10 balance). Confidence in this method is enhanced by the .97 correlation between wet and dry weights. Dry weights for each fish were determined after 48 h in a drying oven at 70°C.

Oxygen demands were converted to food energy (caloric) demands by multiplying mg of O_2 used by the oxycalorific coefficient, 3.22 cal/mg O_2. Forty-two individual mosquitofish were used to calculate mean food demand rates at each temperature.

Dry weights of fifteen "large" *Culex tarsalis* larvae (3rd and 4th instars) and fifteen "small" larvae (1st and 2nd instars) were determined to the nearest 0.1 mg (Mettler H-10 balance) after drying them 96 h in a 60°C drying oven. Also, several hundred *Cx. tarsalis* mosquito larvae (mixed larval stages) were ground in a motorized blender and a mortar and pestle, and pelletized for triplicate bomb calorimetric determinations of mean energy content of the larvae (Phillipson 1966). No estimate of indigestible organic percentage was made.

RESULTS.--The average dry weight of a "small" mosquito larva was 0.02 mg, where as the "large" larvae averaged 0.16 mg apiece. Calorimetric analyses yielded 5.0810 ± 0.0284 ($\bar{x}$ ± SD) calories mg-1 larvae. Thus, each small larva contained an average of 0.1016 calories while each large larva contained an average of 0.8130 calories. By dividing the daily caloric (food energy) demands of a 0.5 g *Gambusia* (determined by respiratory metabolic data) by the caloric value of the mosquito larvae, the daily food demands at the experimental temperatures and dissolved oxygen concentrations were calculated.

Under normoxic conditions, the demand for oxygen and for food increases with increasing temperature by approximately 2.28-fold per 10°C increase in temperature (Figure 2). The relationship at 40 torr PO_2 is similar except at 35°C

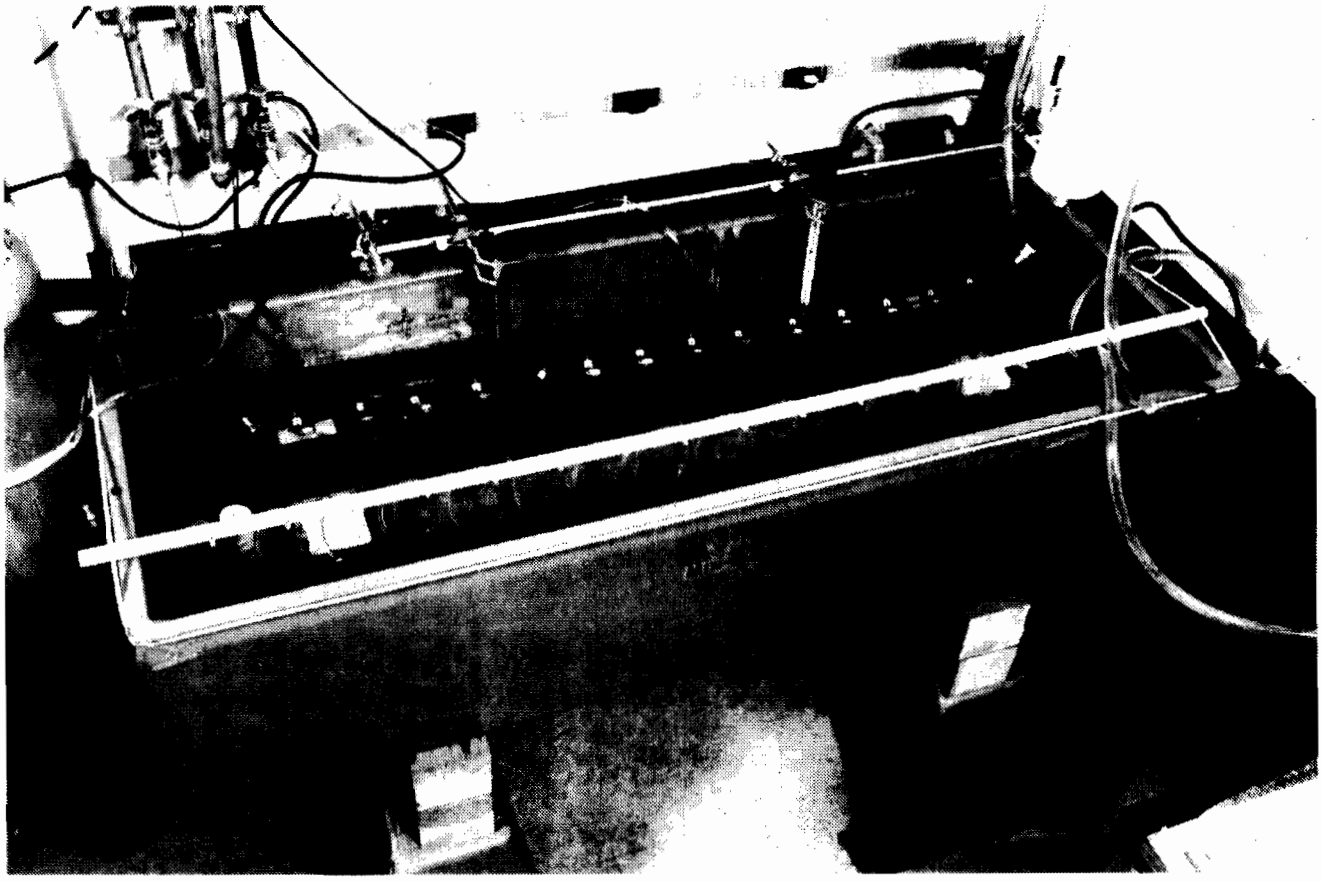

Figure 1.- Mosquitofish Flow-through Respirometer Apparatus.

where food demand fails to increase (Figure 3). When dissolved oxygen is reduced to 25 torr, significant metabolic depressions (from the 40 torr level of hypoxia) are demonstrated ($P < .01$ by t-test) at 25, 30 and 35°C (Figure 4).

Overall, we measured an approximate 12-fold difference in food demand extremes between the fish in the 10°C and 25 torr P_{O_2} habitat at the lower end and those at 35°C and normoxic conditions at the upper end.

DISCUSSION. The results presented in terms of number of large or small larvae consumed per day are an approximation of a feeding rate under fairly quiescent conditions. The respirometer chamber size and design did not allow extensive movement by the experimental animals. The actual food consumption rate over a 24 h period in a habitat such as a rice field will depend on the availability of prey of various sizes and species (Wurtsbaugh et al. 1980, Hess and Tarzwell 1942, Harrington and Harrington 1961). Also the metabolic demand for food is expected to increase with larger body size and higher activity levels. Rajagopal and Kramer (1974) measured elevated respiratory metabolic rates for bigger Utah chubs (*Gila atraria*) and speckled dace (*Rhinichthys osculus*) and at elevated activity levels for both species.

Gambusia energy costs for swimming activity, growth, and development of reproductive products will be elucidated in current and future laboratory experiments to more fully develop the bioenergetic model of this species. Early summer water samples from rice fields in the Sacramento and San Joaquin Valleys showed dissolved oxygen saturation levels in excess of 200% (Cech et al. unpublished data). Thus, the effect of hyperoxic as well as hypoxic environmental conditions on mosquitofish energy conversion rates needs to be examined. The ultimate predictive value of the bioenergetic model to mosquito abatement and public health personnel should be enhanced by refinements emanting from future field tests.

ACKNOWLEDGMENTS. We gratefully acknowledge the support from the State of California and the CMVCA and the assistance of Bob Washino and his group in the Department of Entomology at UCD for supplying mosquito larvae, Bob Coykendall of the Sutter-Yuba Mosquito Abatement District for help in supply *Gambusia*, and to several other individuals (especially those at the Sutter-Yuba and Fresno Westside Mosquito Abatement Districts) for their information and field support. The expert technical asisstance of Mr. R. Pimentel and Dr. D. Baltz and typing of Ms. D. Lombardo are also appreciated.

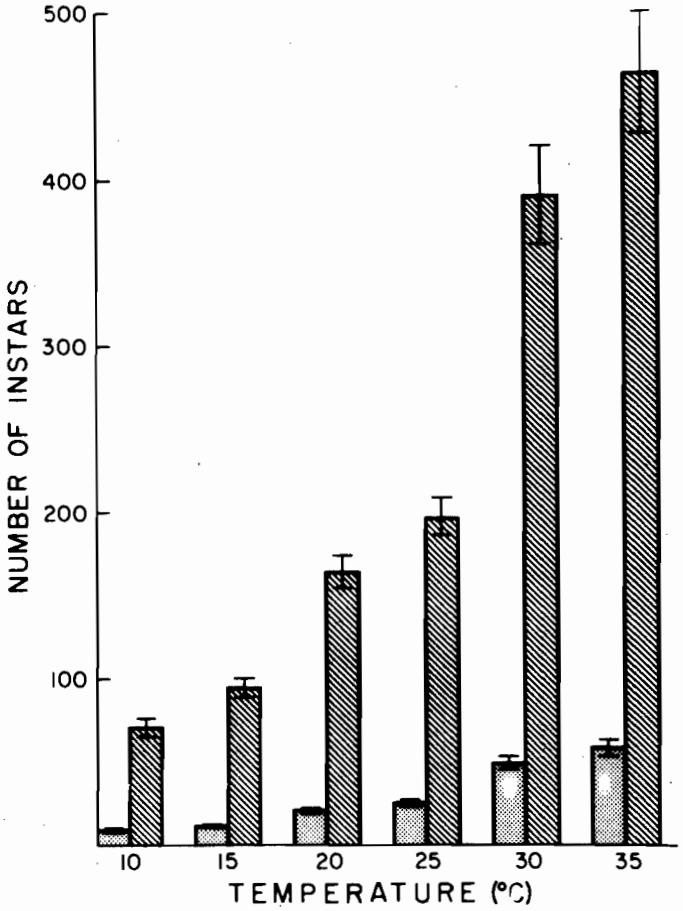

Figure 2.–Number of small (hatched bars) and large (dotted bars) mosquito larvae required per day by a 0.5 g mosquitofish (*Gambusia affinis*) in a "resting" state at various temperatures, while exposed to air-saturated water ($\bar{x}$ S. E.).

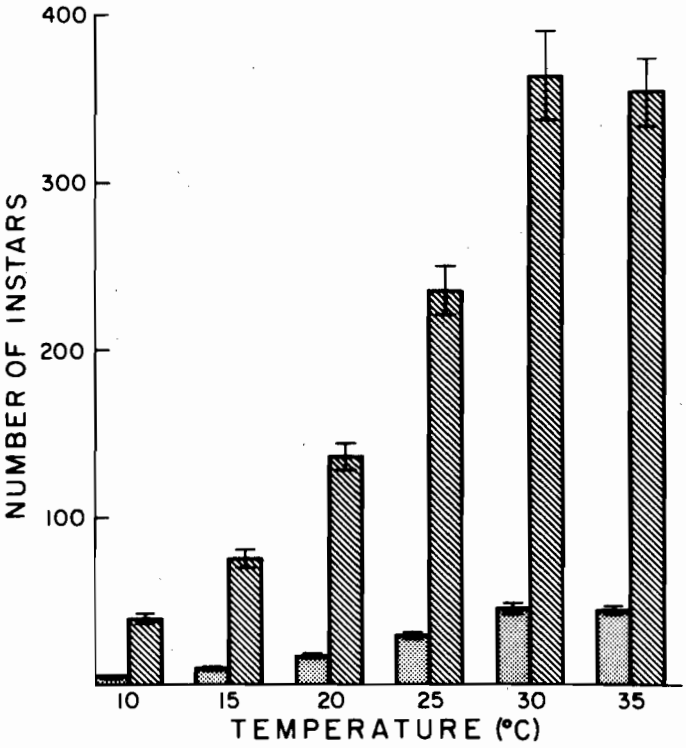

Figure 3.–Number of small (hatched bars) and large (dotted bars) mosquito larvae required per day by a 0.5 g mosquitofish (*Gambusia affinis*) in a "resting" state at various temperatures while exposed to dissolved oxygen concentrations approximating 25% of air saturation ($\bar{x}$ ± S.E.).

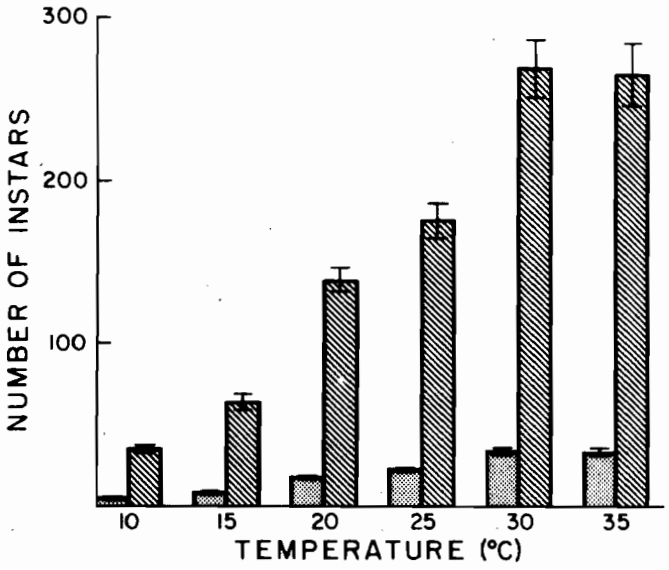

Figure 4.–Number of small (hatched bars) and large (dotted bars) mosquito larvae required per day by a 0.5 g mosquitofish (*Gambusia affinis*) in a "resting" state at various temperatures, while exposed to dissolved oxygen concentrations approximating 15% of air saturation ($\bar{x}$ ± S.E.).

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EFFECT OF FISH SIZE ON PREY SIZE SELECTION IN *GAMBUSIA AFFINIS*

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ABSTRACT

Food size selection of the mosquitofish, *Gambusia affinis affinis*, was measured in aquaria using juvenile stages of the mosquito, *Culex tarsalis*, as prey. Fish size varied from recently born fry to large adult females.

Food size selection was positively correlated with fish size. Mosquitofish fry (6-8 mm standard length) attacked and ate primarily first and second instar larvae. Fry attacked larger instars, but attack success on these was low (0 - 50%). Fish larger than 20 mm attached primarily pupae and third and fourth instar larva. No first instar mosquitoes were eaten. Attack success for these fish was above 65% for all instars.

INTRODUCTION.–*Gambusia affinis* is widely used to control mosquito populations, but the efficacy of these fish often varies widely between habitats. Although some information is available on the diets of *Gambusia* (Barney and Anson 1920; Hess and Tarzwell 1942; Harrington and Harrington 1961; Washino and Hokama 1967; Maglio and Rosen 1969; Walters and Legner - in press) relatively little is known about factors influencing predation rates or prey choice, and consequently, little is known about factors affecting the efficacy of the control process. It is extremely difficult to study the predation process in the field since so many factors affecting predation are uncontrolled (i.e., fish size, hunger state, and experience; prey availability, distribution and escape mechanisms; environmental temperature and light levels). By studying predation in the laboratory, we can isolate specific factors and determine their importance to the feeding process. This approach has been used profitably by others studying fish predation (O'Brien 1979, Werner 1977). When enough factors have been investigated we may begin to understand *Gambusia* predation in a complicated natural environment.

An important factor affecting fish feeding is size-selective predation (Brooks and Dodson 1965). There has been a considerable amount of work in aquatic ecology which demonstrates that most fish feed on specific sizes of organisms and that this size selectivity is so important that it can modify community structure (for review, see O'Brien 1979). Most work in this area has emphasized the feeding behavior of large fish, and the conclusion often reached is that "planktivorous fish consume many more large-sized prey than would be the case if the feeding were random" (O'Brien 1979). Unfortunately, the diets of fish larvae and fry are often ignored, so that little is known about their size selectivity. *A priori*, we would not expect a larval fish to consume the largest prey in the environment when that prey might be the same size or larger than the predator. Werner (1974) has shown that fish size is important in determining handling time in sunfishes, and Elston (1975) has shown how fish size influences prey size selection in *Menidia audens*.

This study reports preliminary results on the selective feeding behavior of *Gambusia affinis* ranging in size from recently born fry to large adult females. The prey organisms tested were the five instars of the mosquito, *Culex tarsalis*.

METHODS.–The prey selection experiments were conducted in glass aquaria (5 gallon) filled with 17 liters of water. Surface areas of the rectangular aquaria were 820 cm^2. Temperatures were maintained at 25° ± 2°C during acclimation and during the feeding trials. Flourescent room lights illuminated the aquaria from above.

The experimental fish were from a wild stock obtained at the Wheatland, California, Sewage Treatment Plant. The fish were maintained in the laboratory on prepared flake diets. Fry (6-8 mm) used in the experiments were born in the lab and were between 6 and 60 days old. The larger fish, taken from the field, may have had some feeding experience on mosquito larvae, but they had been kept in the laboartory for over 120 days without access to natural prey organisms.

The *Culex tarsalis* (Davis strain) were reared in synchronous cultures. In most trials 20 of each instar (1-4 and pupae-P) were placed in a 25 mm diameter petri dish before an experiment. In the first set of trials only instars 1-4 were used. Ten feeding trials were conducted on three separate dates.

Before a trial, the fish were allowed to feed for one hour on a flake diet. Five similarly sized fish were then placed in a test aquarium and kept for three hours without food to provide a moderate, standardized, hunger level. The prey organisms were introduced by sinking the petri dish in the center of the aquaria. Behavioral observations were made throughout the feeding bout. The number of attacks and attack success on particular insects were recorded during the trial. A larvae was considered "attacked" if the fish contacted it. A successful attack resulted in an ingestion. Unsuccessful attacks occurred if the instar evaded the predator or if the predator discharged the prey from its mouth. The accuracy of this behavioral observation is limited due to the difficulty in correctly differentiating the five instars during an attack. The fish usually began feeding within thirty sec. after the prey were introduced, and feeding

activity often decreased considerably after the first 3 to 5 min. This was probably due to the fish becoming satiated and to the fact that after several minutes, many of the mosquitoes had moved to the periphery of the tank and were relatively inconspicuous there in the meniscus. After the fish had fed for ten minutes, they were netted from the tank and preserved.

The actual ingestion of the various instars was determined by dissecting the fish and measuring the head capsule widths of the prey at 30X magnification. The standard length of each fish was also recorded. Many of the larvae in the guts were partially digested. However, head capsules of the larvae were usually intact and were used to identify and count instars. The head capsule widths of the different instars were determined on freshly preserved larvae (Figure 1B), which allowed us to assign a prey item to an instar category. The relationship between head capsule width and total body length (tip of head of the end of the abdomen with siphon excluded) was also determined (Figure 1A). This regression was used to assign lengths to the prey eaten.

RESULTS.–The feeding trials demonstrated that *Gambusia* size effects prey-size selection. Fish less than 8 mm ate primarily first and second instar larvae (Figure 2). The few third instar larvae eaten had an estimated size of 3 mm. No fourth instar or pupae were eaten by fry. *Gambusia* between 12 and 18 mm ate approximately equal proportions of all instars. The body lengths of first instars through fourth instars ranged from 1 to 5 mm. Fish larger than 20 mm ate primarily late instars with very few second and no first instars being chosen. The corresponding size range of prey eaten was 2 to 5 mm. Fish larger than 30 mm chose primarily fourth instar larvae. However, we have only experimented with five fish of this size, so our results are only tentative.

The behavioral observations indicated that *Gambusia* will attack a wider range of prey sizes than they are capable of eating. *Gambusia* fry attacked second instar larvae more than any other prey (Figure 3) and attack success was maximal for this instar (Figure 4). Fry also attacked fourth instars and pupae, but all of these prey escaped and consequently did not appear in the diet. Fry also attacked a high proportion of third instar larvae, but attack success was only 50% (Figure 4) and consequently relatively few third instars appeared in the diet.

In contrast to fry, intermediate sized *Gambusia* (18-26 mm) attacked primarily the third, fourth, and pupal instars (Figure 3). Only 7% of the attacks were on second instar larvae. We also recorded several successful attacks on first instar larvae. However, since no first instars appeared in the guts of these fish (Figure 2), this may have been due to incorrect instar identification during the observations. Attack success varied from 100% on second instar larvae to 68% on fourth instars (Figure 4). Many of the unsuccessful attacks we observed were

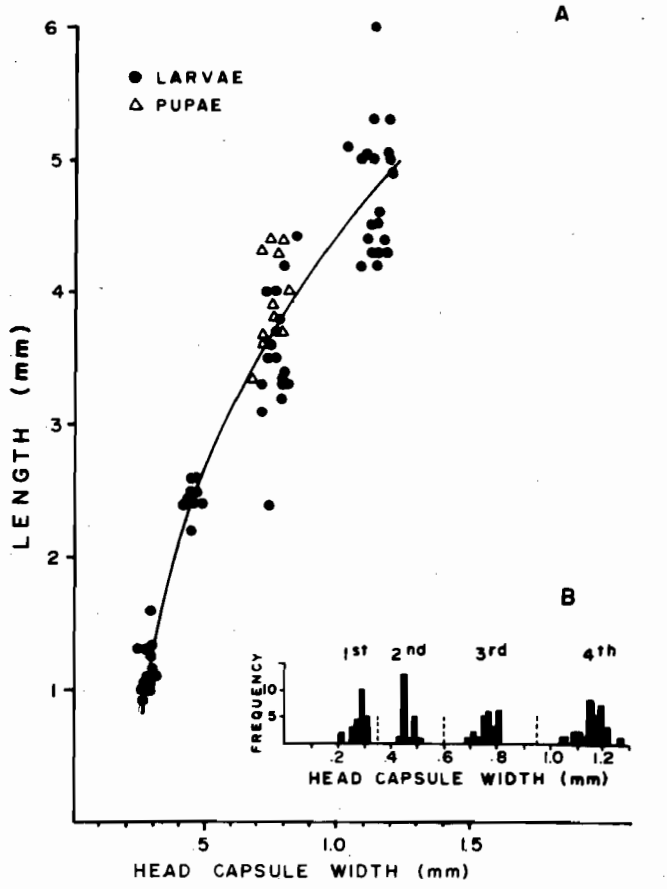

Figure 1.–A. Relationship between head capsule width and total length (less siphon) in *Cx. tarsalis* (curve fitted by eye). B. Frequency distribution of head capsule widths of larval *Cx. tarsalis*.

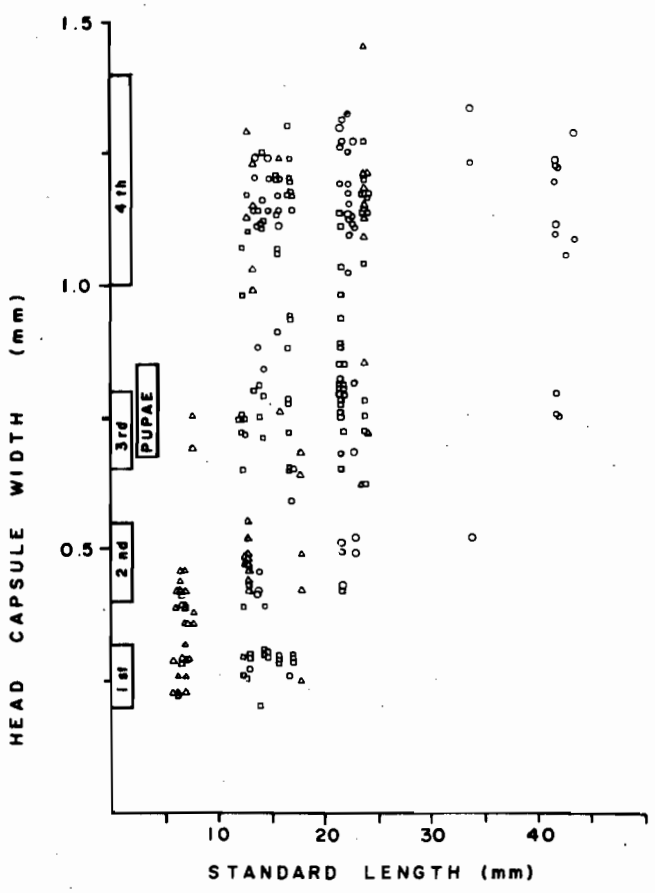

Figure 2.–Relationship between *Gambusia* length and head capsule widths of ingested *Culex tarsalis*. The bars near the ordinate indicate the approximate size range of each instar.

due to fish discharging the prey from their mouths. This was particularly evident for attacks on pupae in late stages of development.

DISCUSSION.–Our data and observations indicate that several factors influence prey choice in *Gambusia*, even in the simple experimental systems we used. The data demonstrate that both fish size and prey size interact to determine prey choice. While small fry attacked some large prey, they were unsuccessful in capturing them and the diet consisted primarily of early instars. Larger fish had little difficulty eating either early or late instar larvae. However, most of their attacks were directed at the largest prey available. Consequently, it appears that *Gambusia* will choose the largest prey they can successfully capture. O'Brien et al. (1976) have developed a model for prey choice in planktivorous fish which predicts that because large prey are more visible than small prey, they will be attacked proportionately more often than the smaller organisms. Our results indicate that this model must be modified for fish larvae and fry, which reduce attacks on large prey which they could not consume.

Our behavioral observations indicate that relative size alone does not determine prey choice. As suggested by Zaret and Kerfoot (1975), factors affecting visibility will influence predator choice. One factor which may have influenced prey visibility in our tests was prey movement. Larvae or pupae were attacked more often if they moved in the visual field of a fish. O'Brien et al. (1976) report similar findings for bluegill sunfish. In some trials, pupae were quickly discharged by the fish after they were captured. Pupae may become physically or chemically unpalatable during the period when they are immobile and easily captured. Kerfoot (1979) has recently identified cases of unpalatability and aposematism in some aquatic invertebrates.

Since different sized fish show differences in the size of mosquito larvae they consume (Harrington and Harrington 1961; and our data), all size classes of fish must be considered to determine the dynamics of this predator-prey interaction. The importance of juvenile fish in mosquito control should not be underestimated since both juvenile fish and early instar larvae will be more numerous in most habitats than the larger fish and late instar mosquitoes. Additionally, since fry can consume nearly 100% of their body weight per day (W. Wurtsbaugh, unpublished data), they may have a high impact on prey populations despite their small size.

ACKNOWLEDGMENTS.–The mosquito larvae used in the trials were provided by R. K. Washino. R. L. Coykendall provided many of the mosquitofish used. T. E. Wragg assisted in several technical aspects of the study, and D. Lombardo prepared the final typescript. Funding for the study was provided by the State of California.

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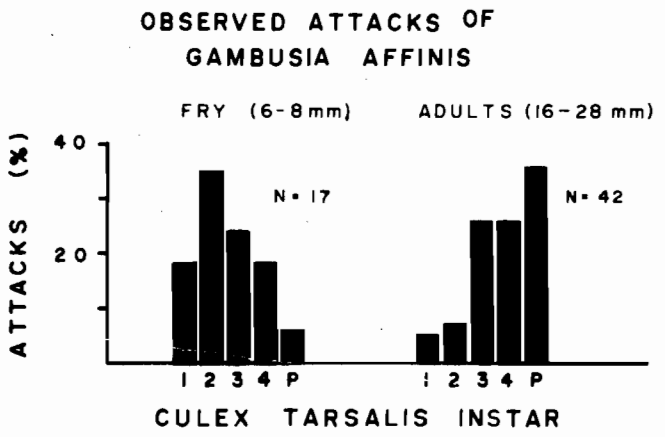

Figure 3.–Frequency distribution of attacks on different instars of *Culex tarsalis* by fry and intermediate sized *Gambusia*.

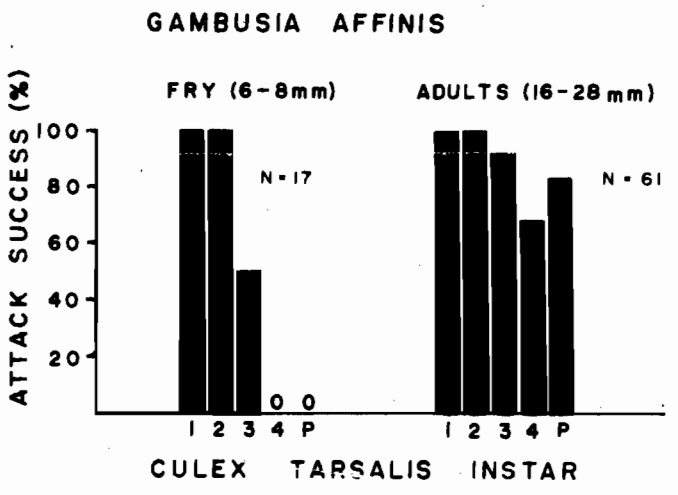

Figure 4.–Attack success of *Gambusia* on the instars of *Culex tarsalis*.

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PREY SELECTION BY THE MOSQUITOFISH *GAMBUSIA AFFINIS* IN FRESNO COUNTY RICE FIELDS

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INTRODUCTION. The mosquitofish *Gambusia affinis* is currently the only agent widely utilized for biological control of mosquitoes (Washino and Hokama 1967, Hoy and Reed 1970). California mosquito control agencies reported using in excess of 23,000 pounds of mosquitofish to control mosquitoes in 130,842 acres of potential breeding sources during 1978 (CMVCA Year Book 1979). Rice fields comprised the bulk of acreage stocked.

It was once thought that the main diet of *G. affinis* was mosquito larvae, hence the name "mosquitofish". However, many authors (Hess and Tarzwell 1942, Bay and Anderson 1966, Washino and Hokama 1967, Ahmed et al. 1970, Miura et al. 1979) have shown that mosquitofish are diverse feeders and that mosquito larvae comprise only a small part of their diet. Reddy and Pandian (1972) showed that mosquitofish cannot even survive on an exclusive diet of mosquito larvae.

The fact that mosquitofish are diverse feeders can make them more effective in controlling mosquitoes than if they ate only mosquito larvae. They can consume other prey items when mosquito larvae are absent from a source and continue to increase their numbers. Populations of predators which depend on a single prey species fluctuate with the size of the prey population. Such predator populations are slow to react to dramatic increases in their prey. In the case of mosquitoes which transform from an aquatic to a terrestrial existence, the slow population reaction of an aquatic predator would mean ineffective control since the mosquitoes would be out of the aquatic environment when the predator population peaked.

Conversely, the diverse feeding habits of mosquitofish could be a detriment to their effectiveness in controlling mosquitoes. If mosquitofish prefer other prey species they might exclude mosquito larvae from their diet until the preferred species are eliminated. It is therefore important to understand the feeding habits of mosquitofish in a particular type of source before predictable results can be obtained.

Bay and Anderson (1966), Reed and Hoy (1970), Hurlbert et al. (1972), and Farley and Younce (1977) all looked at the effects of *G. affinis* on the aquatic biota in various sources. However, no gutting work was done to correlate the fish's diet with the observed changes in prey populations. Washino and Hokama (1967), Ahmed et al. (1970), and Washino (1969) published the results of gutting studies of mosquitofish in California rice fields but little was published concerning the aquatic biota present in the fields. Hess and Tarzwell (1942) and Muira et al. (1979) published good studies of the feeding behavior of *G. affinis* in reservoirs and small ponds. Both correlated the results of gutting and aquatic sampling to give a good idea of feeding preferences. The present paper is a study of the feeding habits of *G. affinis* in Central California rice fields.

MATERIALS AND METHODS.–Ten rice fields ranging from 30 to 113 acres were selected for study. Eight fields were stocked with mosquitofish at 0.2 pounds per acre. The fields were stocked by the three-drop method (Farley and Younce 1978). Two fields were left untreated by either mosquitofish or pesticides and served as experimental controls.

Three sites in each field were selected for sampling: the fourth paddy from the inlet, the center paddy, and the fourth paddy from the outlet. Fish were collected from each sampling site for gut analysis between June 19 and June 26, 1979, and between July 25 and July 31, 1979. All fish were collected in 1/8 inch mesh Gee minnow traps fished for a maximum of four hours at each site. The alimentary tract from each fish was removed in the field and preserved in 70% isopropyl alcohol for examination in the lab.

Aquatic insects and crustaceans were collected in the sampling sites between June 19 and June 29, 1979, and on August 1, 1979. Six to eight samples were taken from each site by transecting the site from shallow to deep end. A water column sampler consisting of a six inch i.d. square box 12 inches high made of 0.25 inch thick plexiglass was used (Figure 1). The box is open at both ends and encloses an area of 0.25 square foot. One end is fitted with a steel band extending 0.5 inches beyond the rim of the box. A slot is cut in one side of the sampler 1.5 inches above the bottom. A six inch square shelf can be inserted into the slot.

The sampler is coupled to a ten-foot long aluminum pole so that it can be plunged into the rice paddy ten feet ahead of the operator. The sharpened steel band cuts through the vegetation and digs into the substrate enclosing a .25 foot square column of water. The sampler is pushed into the substrate until the mud is even with the slot. The shelf is then inserted into the sampler completely enclosing the column of water.

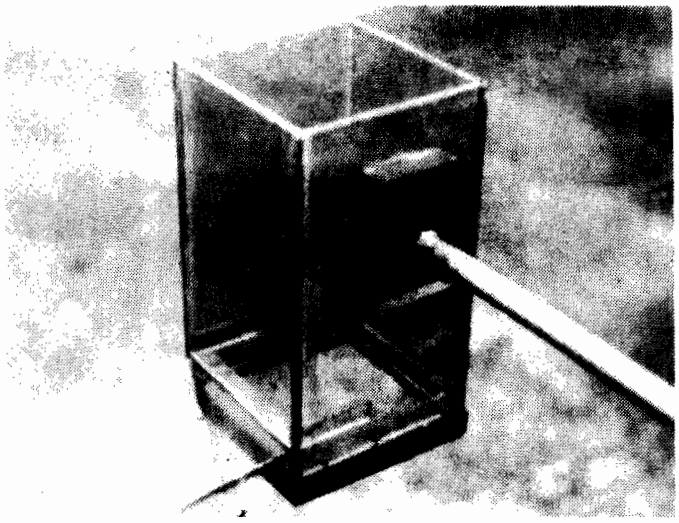

Figure 1.- Water column sampler.

The unit can then be lifted from the water and the contents poured through a funnel and concentrator (Husbands 1969). All samples were placed in Whirl-Pak plastic bags containing 70% isopropyl alcohol for later examination in the lab.

Ivlev's Selection Index (1961) was utilized to determine the degree of preference mosquitofish had for each prey species. The ratio is calculated as follows:

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

Where U = number of any prey in the guts (in %)
V = number of the prey in the pond (in%)

An S. I. of 0.00 indicates no preference or rejection. Preference increases as the S.I. approaches +1.00, and rejection is indicated as the S.I. approaches -1.00. Statistical significance of differences in abundance of aquatic organisms in stocked and control fields was determined using Student's t-test.

RESULTS AND DISCUSSION.–Results of the water column sampling are listed in Table 1. Crustaceans (Cladocera, Podocopa, Eucopepoda) were the most abundant organisms in the rice fields during both sampling periods representing 95.56% of the organisms collected in stocked fields and 94.83% in control fields during the June sampling period. During the August sampling period they represented 98.38% and 98.84% respectively.

The species composition of the crustacea changed drastically from one sampling period to the other (Table 2). Cladocera represented 52.95% of the organisms collected from stocked fields in June (35.21% in control fields) but only 15.31% in August (6.85% in control fields). Ostracods (Podocopa) represented 40.96% of the organisms collected from stocked fields in June (56.60% in control fields) and 82.41% in August (91.81% in control fields).

Populations of *Notonecta unifasciata*, *Corisella sp.*, *Laccophilus spp.*, and *Callibaetis sp.* were significantly lower in the stocked fields during June when compared to the experimental controls. This generally agrees with results published by Farley and Younce (1977), but differs with the results of Reed and Hoy (1970), concerning *Callibaetis sp.* Statistically significant differences in populations were observed only for the tipulid *Limonia sp.* during the August 1 sampling, their numbers being higher in stocked fields than in controls. No explanation is proposed for their higher numbers in stocked fields especially since gutting records show that they were selected by the fish (S.I. of .88 and .91 for fish greater than and less than 3 cm. respectively) and composed slightly over one percent of the diet (Table 2).

The Selection Index (Table 2) indicates that even though crustaceans composed a large part of the diet, they were not eaten in the same proportions as they existed in the environment. The smaller fish ate cladocerans at a higher rate than the larger fish, but both groups ate ostracods and copepods at the same rate. Both the smaller and larger fish depended on crustaceans more during the August sampling period. This was probably due to the 64% increase in Crustacea from June to August in the rice fields. The other organisms decreased by 42% during the same period.

During the June sampling period the smaller fish showed a preference for most of the dipterans as well as the beetle *Laccophilus sp.* Both small and large fish preferred *Notonecta sp.* and *Corisella sp.* while the larger fish had a fairly high selection index for *Callibaetis sp.* It is interesting to note that *Laccophilus sp.*, *Notonecta sp.* and *Callibaetis sp.* population means were all significantly lower in stocked fields than in controls during the June sampling period. Preference for dipterans was still evident in August for both fish groups. The larger fish had shifted from *Notonecta sp.* and *Corisella sp.*, which were nearly non-existent in August, to the odonates *Anax sp.* and *Enallagma sp.* Both fish size groups dramatically shifted to a diet composed of smaller organisms, especially Crustacia.

Terrestrial organisms were a large percentage of the diet of the larger fish during both sampling periods. Plant seeds were eaten in abundance but most were found to be undigested. The seeds may have actually been sought after and ingested, but more likely they were accidentally ingested while gulping prey organisms at the water surface. Animals generally don't select items which they are incapable of disgesting.

Chironomids were highly selected by the small fish size group and represented a significant percentage of the diet (26.45% and 6.83% in June and August respectively). Numbers of chironomids collected in water column samples indicated no significant reduction in field populations due to fish predation. Bay and Anderson (1966) also found *G. affinis* to be ineffective in reducing chironomid populations. Their observation was that although most chironomids stay within their protective tubes in the mud, a few leave their tubes and become free-swimming. These evacuees are subject to predation by the fish. However, the free-swimming individuals would generally die anyway, so their destruction by the fish is of no control importance. Though the fish don't eat a significant number of chironomids when compared to the massive numbers in the mud, they do prey heavily on those which are available to them and show definite selective tendency.

Not enough mosquito larvae were found in the aquatic or gut samples to make a good conclusion about their desirability as a prey item. Two fourth-instar *Culex tarsalis* were found in fish fields during the June sampling period, but none were

Table 1.–Abundance of organisms collected duirng water column sampling.

Organism	ABUNDANCE (No. per Ft2)			
	6/19-29/79		8/1/79	
	Fish	Control	Fish	Control
Cladocera	533.33	504.76	245.03	152.52
Podocopa	412.53	811.43	1318.73	2043.00
Eucopepoda	16.64	43.32	10.51	4.00
Diptera				
Unidentified	0.19	0.00	0.24	0.00
Tipulidae				
Limonia sp.	0.25	0.00	0.92**	0.12
Chironomidae	13.29	18.86	3.67	0.60
Ceratopogonidae				
Dasyhelea sp.	0.05	0.00	0.81	0.00
Culicidae				
Anopheles freeborni	0.00	0.00	0.05	0.00
Culex tarsalis	0.05	0.00	0.00	0.02
Hemiptera				
Notonectidae				
Notonecta unifasciata	0.14***	0.76	0.15	0.12
Corixidae				
Corisella sp.	0.41*	1.81	0.00	0.00
Belostomatidae				
Belostoma fluvineum	0.07	0.00	0.09	0.00
Gerridae	0.02	0.19	0.00	0.00
Odonata				
Anisoptera				
Anax sp.	0.32	0.48	0.32	0.40
Erythemis sp.	0.02	0.19	0.17	0.92
Zygoptera				
Enallagma sp.	23.70	34.57	17.92	16.12
Coleoptera				
Dytiscidae				
Sygrotus sp.	1.36	2.76	0.47	0.12
Laccophilus sp.	1.49*	5.16	0.00	0.00
Dytiscus sp.	0.02	0.00	0.00	0.00
Hydrophilidae				
Unidentified	0.00	0.00	0.05	0.00
Tropisternus sp.	0.41	1.04	0.15	0.20
Hydrochus sp.	0.18	0.00	0.69	0.00
Laccobius sp.	0.21	0.00	0.05	0.12
Berosus sp.	0.05	0.20	0.00	0.00
Ephemeroptera				
Baetidae				
Callibaetis sp.	2.85*	7.04	0.57	0.32
Oligochaeta	2.09	1.04	10.25	6.52

* Significantly different P = 0.05
** Significantly different P = 0.025
*** Significantly different P = 0.01

Table 2.–Summary of the food habit study showing species abundance in the rice fields and alimentary tracts and the resulting Selection Index (S.I.).

	6/19-29/79					8/1/79				
		Fish >3 cm		Fish <3 cm			Fish >3 cm		Fish <3 cm	
Organism	%Field	% Diet	S.I.	% Diet	S.I.	% Field	% Diet	S.I.	% Diet	S.I.
Cladocera	52.95	26.40	(-.33)	53.10	(.00)	15.31	15.05	(-.01)	32.01	(.35)
Podocopa	40.96	9.14	(-.64)	8.07	(-.67)	82.41	50.90	(-.24)	45.66	(-.29)
Eucopepoda	1.65	0.00	(-)	0.00	-	0.66	0.12	(-.69)	0.09	(-.76)
Diptera										
Limonia sp.	0.02	0.00	(-)	0.19	(.81)	0.06	0.96	(.88)	1.29	(.91)
Chironomidae	1.32	1.52	(.07)	26.45	(.90)	0.23	1.43	(.72)	6.83	(.93)
Dasyhelea sp.	0.01	0.00	(-)	0.19	(.95)	0.05	0.00	(-)	0.18	(.75)
Anopheles sp.	0.00	0.00	(-)	0.00	(-)	0.003	0.00	(-)	0.19	(.94)
Hemiptera										
Notonecta sp.	0.01	11.68	(.99)	2.81	(.99)	0.01	0.00	(-)	0.00	( )
Corisella sp.	0.04	4.57	(.98)	.38	(.81)	0.00	0.00	(-)	0.09	(-)
Odonata										
Anax sp.	0.03	0.00	(-)	0.00	(-)	0.02	0.24	(.85)	0.00	(-)
Enallagma sp.	2.35	3.05	(.13)	0.94	(-.43)	1.12	2.75	(.42)	0.18	(-.72)
Coleoptera										
Hygrotus sp.	0.14	0.00	(-)	0.19	(.15)	0.03	0.12	(.60)	0.00	(-)
Laccophilus sp.	0.15	0.00	(-)	0.38	(.43)	0.00	0.12	(-)	0.00	(-)
Ephemeroptera										
Callibaetis sp.	0.28	0.51	(.29)	0.00	(-)	0.04	0.00	(-)	0.09	(.38)
Terrestrial Organisms		36.55		4.69			11.59		3.78	
Molluska		2.03		0.00			1.19		0.00	
Plant Seeds		4.57		0.38			15.29		9.32	

found in the gut contents. One third-instar *Anopheles freeborni* larva was found in a fish field during the August sampling and one fourth-instar *An. freeborni* larva was found in a fish during that same period. In addition, two *Cx. tarsalis* larvae were found in control fields during the August sampling. Mosquito larvae do not appear to compose a significant part of the total rice field biota in Central California.

Our records indicate that although *G. affinis* is a diverse feeder, it does demonstrate certain preferences with prey size being particularly important. The larger fish generally prefer larger prey while small fish prefer smaller prey. This can best be demonstrated in the case of the odonate *Enallagma sp.* which was the largest of the prey items found in the alimentary tracts. The larger size group of fish showed a definite selective tendency towards *Enallagma sp.* (.13 and .42 in June and August respectively), while the smaller size group rejected the odonoate as a prey item (-.43 and -.72 in June and August respectively). In all prey groups except Crustacea, the larger fish ate later instars than did the smaller fish. Both size groups of fish showed a lack of preference for Crustacea, even though they composed the bulk of the aquatic biota in number of individuals. Their small size evidently makes them less attractive as food items.

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AN EVALUATION OF THE GUPPY (*POECILIA RETICULATA* PETERS) FOR MOSQUITO CONTROL

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The guppy, *Poecilia reticulata* Peters 1859, formerly *Lebistes reticulatus* (Peters 1859), is similar in size and shape to its close relative the mosquitofish, *Gambusia affinis* spp. (Rosen and Bailey 1963). Like the mosquitofish, guppies have a superior mouth ideal for feeding on mosquitoes at the water's surface.

Male guppies are very colorful even in the "semiwild" populations used for mosquito control in California. The slender males cease growing when they reach sexual maturity. This limits their maximum length to about 28 mm or 1.1 inches.

Females, however, grow throughout their lifetime and can attain 48 mm or 1.9 inches. The olive grey females are heavier bodied than the males and often show a dark "gravid spot" on the abdomen just ahead of the anal fin. The gravid spot indicates that a brood is nearly ready to be dropped. Fertilization is internal and the young are born alive. While female guppies closely resemble female mosquitofish, the guppy can be readily identified by the absence of spotting on the almost transparent fins. Mosquitofish (*Gambusia affinis*) have transverse rows of dark spots on the fins.

Water temperature tends to be the most important limiting factor for guppies. They do best at temperatures in the 68° - 80°F range. Guppies can survive up to 100°F but die if the temperature drops to 55°F.

A greenhouse or some means of overwintering guppies is, therefore, necessary in most of California. Early efforts at the Butte County M.A.D. along this line using plastic sheeting proved too costly in terms of labor. Yearly recovering was necessary as ultraviolet light rotted the plastic. Therefore, the Butte County MAD went to a fiberglass greenhouse with a thermostatically controlled fan and self closing vent. Heating cables in the tanks are a necessary supplement to the solar heating. These cables are also thermostatically controlled to avoid overheating and wasting energy.

Why would a MAD go to all this effort to overwinter a tropical relative of the cold hardy mosquitofish? Guppies are valuable because they can survive and feed in highly polluted waters that exclude other fishes, even the mosquitofish. They have been found living and controlling mosquitoes in sewage-fed ponds in Thailand and Burma (Bay and Self 1970). In American sewage treatment plants, guppies now thrive in many secondary treatment ponds containing high levels of organic waste and metal ion pollution. At one facility using copper sulfate for algae control, the guppies thrived despite a copper concentration of 12 ppm in the water and 4 ppm in their tissues (Hiscox 1972).

In larger treatment plants, such as the ones in Chico and Lodi, there is enough heat generated by decomposition for guppies to survive year round. They can be netted in large numbers in such a facility even in January. Despite the ability of such facilities to overwinter guppies it is advisable for the local MAD to maintain a stock of guppies. Occasionally chemicals toxic to fish are introduced into sewage treatment plants resulting in the elimination of fish populations.

There are many small sewage ponds that can be readily controlled by guppies but where guppies can not successfully overwinter. These, naturally, need to be restocked yearly. In such primary sewage ponds, besides feeding voraciously on mosquito larvae, eggs, and pupae, guppies will also feed on human feces which speeds up the natural purification process (Sasa et al. 1964).

It is not necessary to hold large numbers of guppies for restocking as they have a tremendous reproductive potential. Guppies are also known as "Barbados Millions" due to the phenomenal population levels attained. In similar ponds, in the same length of time, guppies reach and maintain higher population densities than do mosquitofish. Guppies tend to be less cannibalistic towards their fry than mosquitofish, which is one reason they can attain such large numbers. Also, guppies will produce young throughout the year if the water in the plant or greenhouse is warm enough. This suggests a reproductive cycle independent of photoperiod.

On the international scale, it has been found that guppies will feed on the cercariae of the blood flukes *Schistosoma mansoni* and *S. japonicum* (De Maria and Pellegrino 1966). These are the causative agents of schistosomiasis or bilharzia-

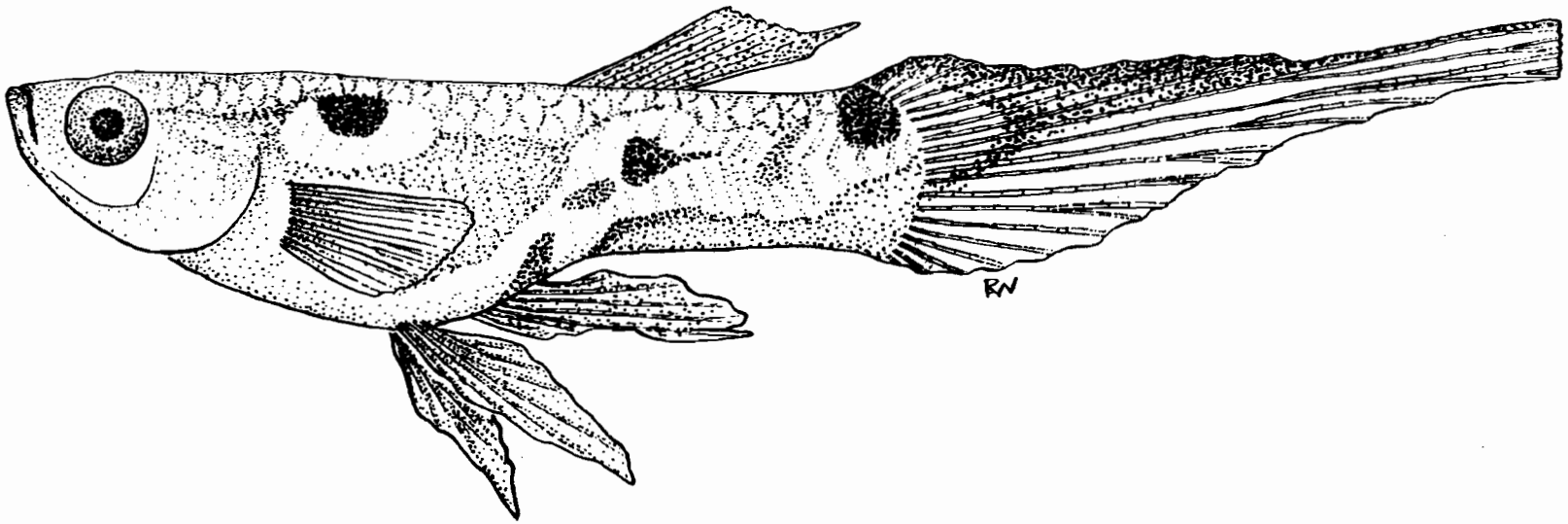

Figure 1.- Male Guppy, *Poecilia reticulata* Peters - - 2.5cm TL.

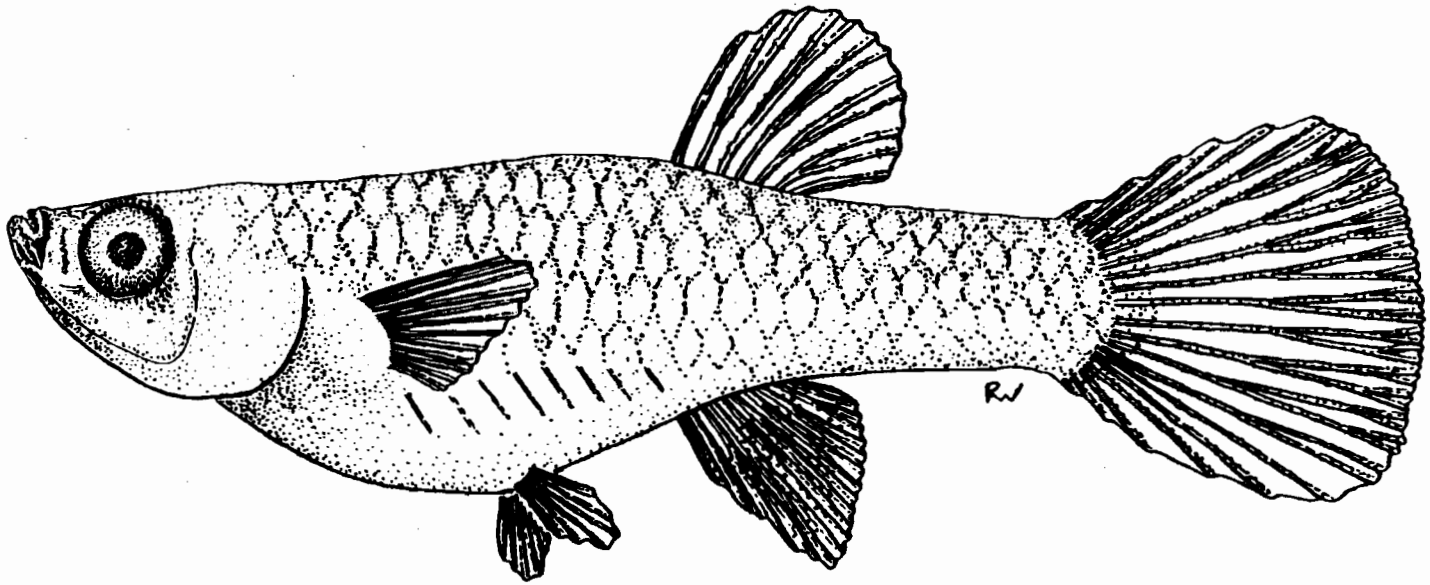

Figure 2. - Female Guppy, *Poecilia reticulata* Peters - - 3.4 cm TL.

sis, a severe disease which occurs in the tropics. Schistosomiasis affects 180-200 million people worldwide and is on the increase. Guppies appear to have great potential for interrupting the life cycle of this parasite in that they feed on the cercariae of the flukes after they emerge from the snail intermediate and before they can infect vertebrate hosts.

In California, guppies are relatively easy to control as they can not overwinter without help. Although they have been introduced into many waters for mosquito control or by the release of aquarium stocks, they have only been able to establish in warm artifical environments i.e., the sewage treament facilities at Lodi, Chico, and U. C. Davis. Also, guppies appear to lack the competitive edge. Bay and Self (1970) reported that guppies in Southeast Asia were confined to polluted habitats as they could not survive in natural watersheds containing piscivorous fishes. In California, where environmental conditions have permitted mosquitofish survival, guppies have been eliminated. In light of these factors, the California Department of Fish and Game has been readily issuing planting permits for guppies.

Guppies are therefore an alternative that should be considered when dealing with polluted waters incapable of supporting mosquitofish. Guppies only need to be planted once for season long control. With the cost of insecticides and labor constantly rising, overwintering guppies is becoming more and more cost efficient.

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FACTORS INFLUENCING THE OPERATIONAL USE OF EFFECTIVE BIOLOGICAL MOSQUITO CONTROLS

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ABSTRACT

The absence of commercial and or mosquito abatement district production of mosquito biological control agents capable of field control levels comparable to the annual cost/acre/year of alternative control means, limits greater use of these agents. Varying physical and chemical conditions of mosquito breeding areas are expected to limit practical large scale use of invertebrate biological agents to mosquito breeding sites <50 acres.

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AN IMPROVED METHOD FOR HARVESTING MOSQUITOFISH FROM RICE FIELDS

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In order to achieve adequate biological control of mosquitoes in California rice fields, dependable sources of mosquitofish *Gambusia affinis* must be developed. One method of assuring large numbers of fish is to culture them in ponds utilizing the technology being developed by the aquaculture industry. This method is effective but generally requires a sizeable cash outlay for construction and maintenance of the facility as well as for water, food and manpower. Another effective method has been the use of sewage treatment facilities and commercial or agricultural ponds. These are not as dependable, but if several within a District are stocked, a few can be counted on to produce fish each year.

Since the Fresno Westside Mosquito Abatement District (FWMAD) has neither the facilities for a large fish-culture operation, nor adequate usable sewer ponds, we have embarked on a program of retrieving mosquitofish from draining rice fields for overwintering and subsequent re-stocking in the spring.

Most of the rice fields in the FWMAD are surrounded by small drains that collect any seepage water emanating from the rice fields. When the rice field is drained in the fall, the farmer makes cuts in the outside border of the field allowing water to pour into the seepage collection drains which empty into a series of deep drains. The deep drains take the water away from the rice areas to the grasslands area where the water is used to flood duck clubs. When the field begins to drain, the mosquitofish avoid the fast-moving water and stay within the rice field. However, when the water level in the field reaches a critical point, the fish enter the collection drains en masse and are carried into the deep drains. Fish movements begin during the late morning and peak during mid-afternoon and can occur for one or two days depending on the speed at which the field is draining.

The old method of harvesting fish involved seining isolated pockets of fish in the deep drains. The method was effective but it required a three man crew and was very time consuming and tiring. The fish were naturally stressed during seining due to the stirring up of the anoxic bottom ooze associated with rice field deep drains.

A new, more efficient method of harvest was developed at the FWMAD in 1979. This new method involves trapping the fish in the collection drains before they enter the deep drains. This new method of operation is economical and efficient because:

1) It takes only a two-man crew to harvest large numbers of fish.
2) Fish are captured in clean water and are not stressed.
3) Much less land is needed for overwintering ponds than for production ponds.
4) Commercial food is needed only in the winter when the fish's metabolic demands are depressed.

The basic tool used is a simple hoop trap or "fike" trap made of two 24-inch reinforcing steel hoops and 1/16 inch nylon netting (Figure 1). One hoop is placed 24-inches in front of the other and serves as the "mouth" of the trap. A 24-inch diameter funnel or "fike" made of 1/16 inch nylon netting is attached to the front hoop and terminates in a three-inch opening within the circumference of the rear hoop. A 24-inch diameter tube made of the 1/16 inch nylon netting is also attached to the front hoop and extends back to a point 48-inches behind the rear hoop. The rear hoop serves to keep the tube from collapsing on the funnel. The back of the tube is fitted with a drawstring so that it may be closed to form a bag. The trap is placed in the collection ditch with the mouth facing upstream (Figure 2). As the fish are carried downstream by the current, they are directed into the funnel "mouth" of the

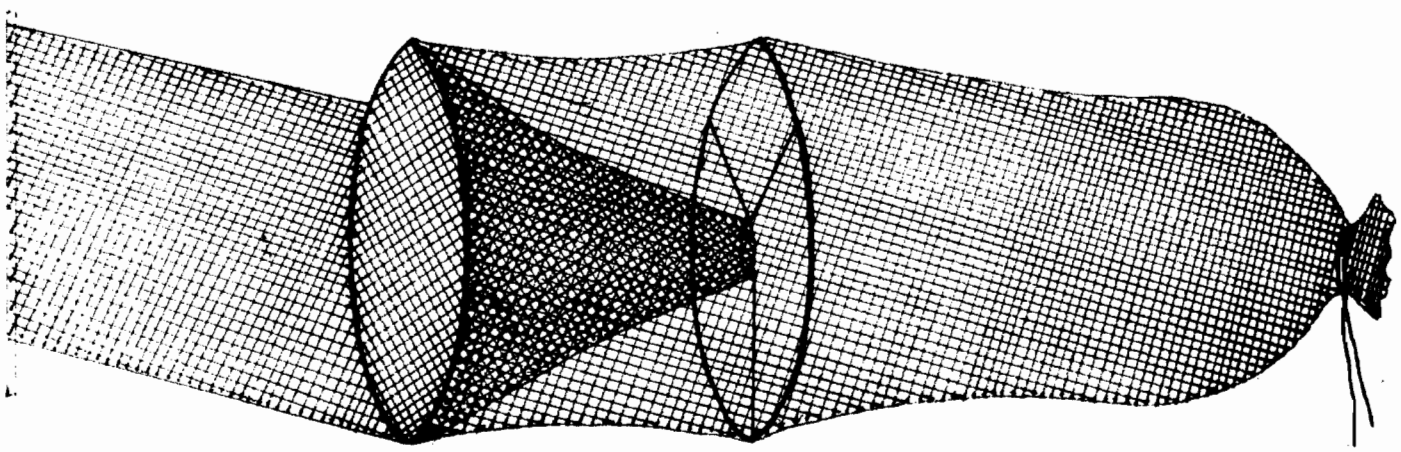

Figure 1.–Hoop trap with one wing extended.

trap by two three-foot long nylon mesh wings or weirs which are attached to the front hoop and are extended to the sides of the collection ditch. The wings insure that no fish escape around the trap. The fish are carried through the funnel and into the bag by the current. Once in the bag of the trap, the fish have difficulty finding the small opening of the funnel and are effectively trapped. The small net size slows the current somewhat so that the fish do not become excessively fatigued while in the trap.

To empty, the trap is removed from the water and held such that all the fish are concentrated at the drawstring end of the bag. The drawstring can then be opened and the fish placed in the transport system (Figure 3).

Once the fish are brought to the overwintering ponds, they are placed in a floating cage made of ½-inch square hardware cloth. The cage acts as a separator allowing the mosquitofish to pass through into the pond but retaining all larger or deeper-bodied fish such as sunfish, carp, etc.

During 1979, the collection ditches of 15 of the 142 stocked rice fields were trapped. A total of 1,088 pounds of mosquitofish were collected in 55 trap hours for an average of 20 pounds of fish per trap hour. Trapping was terminated each day at 2:30 p.m. which is about the peak time of fish movement. By extending the trapping into the late afternoon, significant increases in the amount of fish harvested should result.

Figure 2.--Hoop trap in collection ditch.

Figure 3.--Emptying trap.

THE BIOLOGY OF A PARASITE FOUND IN THE MOSQUITOFISH *GAMBUSIA AFFINIS*

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During the past year, we have conducted research on a parasite found in the mosquito fish *Gambusia affinis* from a stock in Orange County, in Southern California. This parasite is a protozoan of the order Microsporida that commonly infects invertebrates and lower vertebrates. In this report, we present some general results of our pathological investigations and a brief outline of the parasites biology.

The infective stage of the parasite is the spore, a small, cylindrical body that is shaped somewhat like a grain of rice. Measuring 8 by 3.4 microns, the spore is opaque with an apparent vacuole at one end (Figure 1). Its protective coat of chitin makes it resistant to environmental stress, thereby allowing the spore to remain viable for months in water or mud.

Microsporidian infections normally occur after an animal ingests some spores. Once in the gastrointenstinal tract, the spores are activited by certain conditions involving osmolarity and pH. This activation involves rapid, explosive extrusion of a hollow filament stored inside the spore. If the spore is positioned correctly, its filament will pierce the membrane of a cell in the intestinal mucosa. This punctuation allows the germ of the spore, the sporoplasm, to move through the filament into the mucosal cell. Once inside the cell, the parasite controls the nucleus and diverts the cell's normal functions to produce the vegetative form of the parasite, a form that is found only in host cells and that eventually will produce more spores.

The most obvious characteristic of microsporidian infections is host cell hypertrophy. The magnitude of the increase in size is staggering, and we commonly encountered xenomas (enlarged host cells filled with the parasite) measuring several millimeters in diameter. Such large internal masses give the host a peculiar external appearance, usually a distended, lumpy abdomen (Figure 2). The small xenomas absorb nutrients from the surrounding abdominal fluids, whereas larger xenomas develop connections with the host vascular system and draw nutrients directly from the blood (Figure 3).

In the laboratory, we successfully infected adults and fry of northern California stocks of mosquito fish by mixing spores with the fish's food. Fry were the most susceptible to infection.Within a month after exposing 2-week-old fry, we observed a 100% infection rate, with the infections being heavy and usually terminal (Figure 4). Some resistance may be present in adults which exhibited lower levels of infection and less virulence of the parasite than did the fry.

To examine the parasite in the wild, we surveyed several infected ponds in Orange County. We found that 15% of the mosquitofish populations in these ponds were infected. The infections ran from light to heavy, at which point 25% of the host's body weight could be attributed to the parasite. We also observed that a high proportion of infected females exhibited ovarial atrophy. Although we did find some infected females with broods, we believe their fecundity may be lowered by the diversion of nutrients to the parasite and by space limitations in the xenoma packed abdominal cavity.

Thus, this microsporidian parasite demonstrated a destructive potential to mosquitofish, and it is capable of infecting northern California stocks. To prevent spread of the infection to other portions of the state, the control of infected populations should be considered.

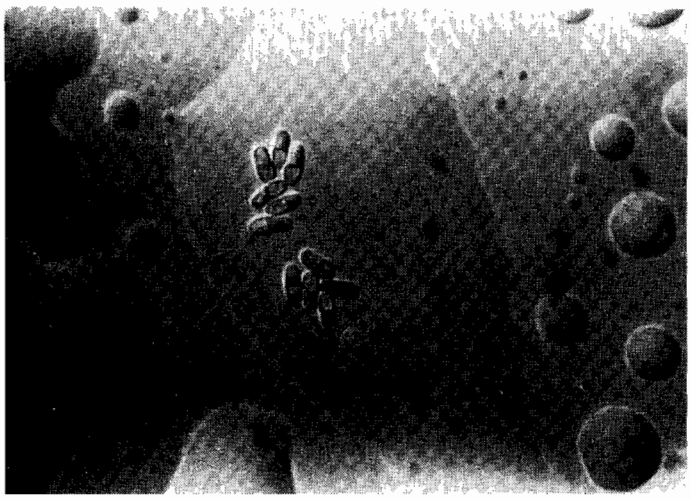

Figure 1. Spores of the microsporidian parasite.

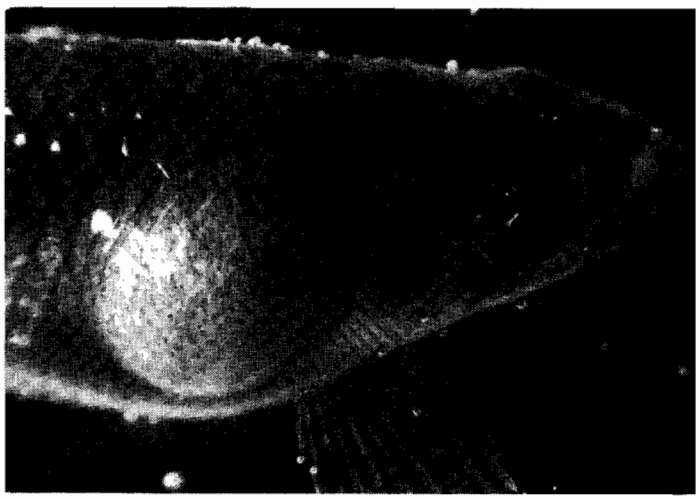

Figure 2. Deformity of body caused by underlying xenoma.

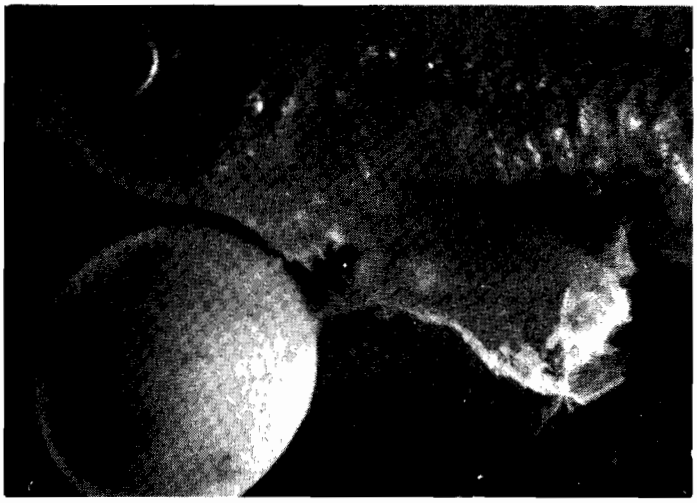

Figure 3.–Large xenoma with well-developed vascularization.

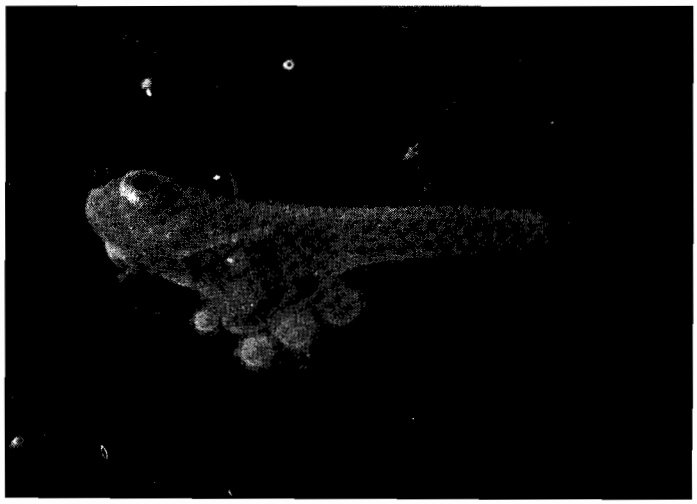

Figure 4.– Gut of fry with multiple xenomas.

MATING BEHAVIOR OF *AEDES SIERRENSIS*

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ABSTRACT

By pairing 10 hourly age combinations ranging from 12:12 to 48:48 at a 1:1 sex ratio for two hours it was found that males first mated when 24 hours old. Females were not receptive until 32 hours old, at which time 56% paired for two hours had spermathecae full of sperm. In studies of possible monogamous mating behavior, receptive, virgin females were first paired for 24 to 48 hours with irradiated, sterilized males. They were subsequently paired with normal males for 48 hours after 0, 1, or 2 gonotrophic cycles. Control groups were paired with sterilized and normal males. Individual females were held for oviposition and the eggs bleached to determine the sterile:fertilized ratio. Females lastly were dissected and the spermathecae examined for sperm. In all experimental combinations females exhibited monogamous mating behavior, with only 4 of 21 (3%) possibly having mated a second time. No females remated after completing either 1 or 2 gonotrophic cycles. In other experiments there was no significant difference in the number of eggs laid by females mated with normal males ($x = 90.9$) versus those mated with sterile males ($\bar{x} = 104.2$). The monogamous mating behavior of female *Aedes sierrensis* establishes that sterilized males could be used as one control component in the IPM program for this species.

LABORATORY CAGE TRIALS WITH POTENTIAL GENETIC CONTROL STRAINS OF *CULEX TARSALIS*[1]

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ABSTRACT

Laboratory cage trials with males of two mutant-marked translocation homozygote stocks of *Culex tarsalis* indicated that both types were at a competitive disadvantage compared to unmarked laboratory stock males. The hybrid males created from crossing the two stocks appeared to be as competitive as the unmarked non-translocated type.

Research with translocations as possible genetic control mechanisms has progressed rapidly over the last several years. We have tested the competitiveness of a male-linked translocation system designed to be used for population suppression (McDonald, Asman, Milby, Bruen and Ainsley 1978, Terwedow et al. 1977). This paper reports the development of genetic translocation systems to carry desirable genes into a *Culex tarsalis* population.

In our present efforts to develop genetic transport systems two recessive mutants of *Cx. tarsalis*, carmine and black eye (Asman 1976), were associated with translocation stocks to facilitate their isolation and laboratory maintenance (McDonald, Asman and Terwedow 1978). Males of 2 such mutant-marked stocks had been tested for competitiveness against unmarked non-translocated males in laboratory cages.

MATERIALS AND METHODS.—The following designations were applied to the stocks employed in these studies: 1) Wild (= non-mutant): the Poso West Colony or Chico stocks, homozygous wild type for the carmine and black eye genes. 2) Mutant: carmine and black eye stock, homozygous for these two recessive markers. 3) Mutant translocation: stocks homozygous for carmine and black eye and also homozygous for either T(2;3) 15A or T(2;3) 16A autosomal translocations. 4) Wild heterozygote: the F-1 of mutant females and wild males, heterozygous for carmine and black eye.

Competition tests were carried out in pairs of 17 cm x 17 cm cylindrical cages. The cages were kept in an insectary maintained at 24°C, 70% RH, and with a 15L, 9D photoperiod. The first cage contained mutant females with wild males in competition with mutant males. The companion cage contained mutant females and wild males in competition with mutant translocated males. The mosquitoes were 2-day-old virgins when the competition cages were initiated, and 4 day-olds when the females were bloodfed. On the day following bloodfeeding females were separated from the males and placed individually into smaller cages for subsequent oviposition in shell vials with water. The translocation systems used in these tests were of normal fertility so the hatchability of rafts would not indicate the type of mating. The rafts were hatched and the progenies raised. The type of mating that had occurred was determined by progeny examination. Mating with wild males would result in wild progeny and mating with mutant or mutant translocated males would result in mutant progeny. Matings with wild heterozygote males would result in both wild and mutant individuals in the same progeny family.

RESULTS AND DISCUSSION.—In the first pair of crosses the T(2;3) 15A translocation homozygote stock was tested (Table 1, crosses A and B). Progeny examination revealed that 14 of 24 rafts resulted from matings with mutant males. In the companion cage with mutant translocated males, only 7 of 21 rafts were attributed to matings involving mutant translocated males. These results show that mosquitoes with this translocation were at a competitive disadvantage.

In the second pair of crosses the T(2;3) 16A translocation homozygote stock was tested (Table 1, crosses C and D). The first cage again had mutant females with wild and mutant males. The mutant males showed a competitive advantage over wild with 15 to 20 matings attributed to them. In the companion cage only 3 of 23 matings involved the mutant translocated males. And, quite surprisingly, all 3 of the latter progeny groups gave evidence of multiple insemination. Apparently the T(2;3) 16A translocation system was associated with a deficiency in monogamy as well as being at a competitive disadvantage.

In the third pair of crosses a hybrid translocation system, derived from crossing T(2;3) 15A homozygotes and T(2;3) 16A homozygotes, was tested (Table 1, crosses E and F). The first cage contained mutant females and wild and mutant males. In this case the wild males were heterozygotes, carrying both recessive mutant genes but of wild appearance. Only 3 of 14 matings were attributed to mutant males, indicating a distinct advantage of the wild heterozygote males. In the companion cage the wild heterozygote males outcompeted the mutant translocated males comprising 14 of 20 matings. Nevertheless the mutant translocated males represented themselves better than the mutant males had in the companion cage. By

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Table 1.–Progeny produced from competition cage crosses between mutant females and either wild vs. mutant or wild vs. mutant translocated males of *Culex tarsalis*.

Cross	Females (no.)	Males (no.)	Progeny (no. families)
A	mutant (44)	wild (44)	wild (10)
		mutant (44)	mutant (14)
B	mutant (44)	wild (44)	wild (14)
		mutant-translocated (44) T(2;3) 15A	mutant (7)
C	mutant (45)	wild (45)	wild (5)
		mutant (45)	mutant (15)
D	mutant (45)	wild (45)	wild (23*)
		mutant-translocated (45) T(2;3) 16A	mutant (3*)
E	mutant (64)	wild heterozygote (64)	wild and mutant (11)
		mutant (64)	mutant (3)
F	mutant (64)	wild heterozygote (64)	wild and mutant (14)
		mutant-translocated (64) T(2;3) 15A/T(2;3) 16A	mutant (6)

*Includes 3 progeny groups resulting from insemination by both types of males.

comparison with the other competition tests these hybrid translocated males were superior to the regular wild males. The hybrid translocated males will be considered for possible use as transport mechanisms in genetic replacement schemes. They will now be tested in larger laboratory and outdoor cages.

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MATING COMPETITIVENESS AND LONGEVITY OF STERILIZED VERSUS NORMAL SIBLING AND WILD MALE *AEDES SIERRENSIS*

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ABSTRACT

In two experiments at the University of California Russell Tree Farm Field Station, laboratory-colonized and laboratory-reared wild mosquitoes were released into screen tents having natural turf floors. The tents contained oviposition boxes, sucrose cubes and rotting fruit, and the female mosquitoes periodically obtained blood meals from chickens or humans. In the first experiment (May 10 through July 12) each of six tents (3 pairs) received 50 normal males and 50 sterile males on day one; one pair of tents also received 50 females on day one. A second pair of tents received 50 females on day 10 and a third pair of tents received 50 females on day 15. The experimental design for the second experiment (July 21 through September 25) was similar, except that we used 75 females per tent along with 75 normal males and 75 sterile males. Eggs laid by females were collected from tents each week and bleached to determine the sterile:fertilized ratio.

Observations of released mosquitoes revealed that mating began to occur within the first few minutes after release of the females. Bleached eggs from the first experiment showed that: 1) we obtained the expected sterility rate in all six tents (47 to 50% sterile eggs), and 2) the longevity of sterilized males was equal to that of normal males. Results of the second experiment were better than expected, with the 50 to 60% sterility rate of eggs indicating that sterilized males survived slightly better than normal males. At low densities in tents, therefore, both the mating competitiveness and the longevity of sterilized males was equal to that of normal males.

A FIELD RELEASE OF IRRADIATED MALE *CULEX TARSALIS* IN CALIFORNIA[3]

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ABSTRACT

Over 13000 *Culex tarsalis* males irradiated at 6.0 kR were released on 2 August and 3 August, 1979, into a native population in the Sierra foothills about 13 km E. of Bakersfield, CA. The study site consisted of 3 adjoining canyons, each containing a series of evaporation ponds.

A mark-release-recapture experiment was conducted 1 month prior to the release of irradiated males. Recovery collections indicated extensive intercanyon migration. A relative measure of *Culex tarsalis* abundance was provided by operating CO_2/light traps biweekly in May and June, and then weekly through October at trap sites in all 3 canyons.

Vapona was applied aerially on the day prior to the initial release of irradiated males to eliminate inseminated females from the release area, and to lower the existing male population. No more than 42% mortality was observed among caged mosquitoes in the treated canyon, although mortality was significantly higher for all age and habitat categories from the treated canyon except those mosquitoes in cartons placed in tamarisk trees.

Irradiated males were released in the central canyon. Prior to the release, only 2% of embryonated egg rafts had low or medium hatch. Sterility increased to 42% 3-weeks post release, then declined to 3% 6-weeks post release. Initially high numbers of high hatch egg rafts following the release can be attributed to intercanyon movement of females inseminated by unirradiated males, and by the failure of the adulticide to eliminate previously inseminated females in the central canyon. The sterile to normal male release ratio could not be determined due to insufficient numbers of marked irradiated males recaptured.

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[3]These studies were supported in part by special State funds for mosquito control research appropriated annually by the California Legislature.

PREDICTING THE OUTCOME OF GENETIC CONTROL STRATEGIES FOR *CULEX TARSALIS*[1]

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ABSTRACT

A computer model has been developed which simulates a fluctuating mosquito population with overlapping generations. The model can be used to predict the effect of releasing sterile or genetically altered adults into the wild mosquito population. Sterile males are most effective for immediate reduction of a population, but males which are homozygous for an autosomal translocation will have a greater residual impact on population size. Adults of both sexes must be released in order to achieve replacement of the native population with a more desirable strain. The number of adults which must be released depends largely on the mating competitiveness of the released strain.

[1]These studies were supported in part by Research Grant AI-03028 from the National Institute of Allergy and Infectious Diseases and General Research Support Grant I-SO1-FR-05441 from the National Institutes of Health, and by special State funds for mosquito control research appropriated annually by the California Legislature.

EFFECTS OF FLUORESCENT MARKER DUSTS ON *CULEX TARSALIS*, A FACTOR IN MARK–RELEASE–RECAPTURE STUDIES[1]

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ABSTRACT

Fluorescent marker dusts had little effect on the survival and mating success of *Culex tarsalis* Coquillett in laboratory and large outdoor cages. When dust-marked and unmarked mosquitoes of a mutant marker strain were released and recaptured in the field, dispersal and recapture rates of the 2 groups were very similar.

Mark-release-recapture techniques are basic to many studies of insect field biology. The many ways that these techniques have been used in mosquito studies were reviewed at last year's Association meetings (Milby 1979). Mark-release-recapture technology has been applied extensively to populations of *Culex tarsalis* Coquillett in connection with genetic control trials in Kern County, California (Nelson et al. 1978, Asman et al. 1979). A continuing problem has been an inability to recapture enough marked males to develop reliable estimates of the size and survival of male populations. Recaptures of marked females have been more than adequate. It was speculated that the behavior or survival of the males, and perhaps of the females, may have been affected by the fluorescent dust markers. If so, this would violate a basic requirement for successful mark-release-recapture studies. Concerns over the possible effects of marker dusts on the survival, mating success, and dispersal of *Cx. tarsalis* led to the present observations.

Fluorescent dusts of 4 basic colors, - - - yellow, red, blue, and chartreuse - - -, have been used singly or in combination to produce 8 distinct markers. Colors obtained from the combinations are aqua, gold, silver, and purple. The yellow dust is a "Helecon" product, from U. S. Radium Corp., and has a zinc sulfide base. Bailey et al. (1965) and Dow et al. (1965) successfully used Helecon dusts in *Cx. tarsalis* flight dispersal studies in the 1960's. In an earlier evaluation of methods for marking *Cx. tarsalis*, Bailey et al. (1962) noted that "heavily dusted individuals appeared to be unaffected and reacted normally". The red, blue, and chartreuse dusts are relatively new "Radiant" products from Hercules Inc. and have a triazine aldehyde amide base.

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One of the Radiant dusts (blue) was applied to about 200 male and 200 female *Cx. tarsalis*, from a laboratory colony, to test for possible effects on survival in the laboratory. The mosquitoes were introduced to a 3.4-ft^3 cage covered with moist toweling, supplied with sucrose-soaked pads, and lined on the bottom with white paper. An unmarked control group was set up identically in an adjacent cage, and all dead mosquitoes were recorded and removed from the 2 cages daily. Logarithmic survival curves for males of the 2 groups are shown in Figure 1. There was little difference between the 2 curves, although survival of unmarked specimens was somewhat greater in the 2nd and 3rd weeks, but later was less. Over 90% of the

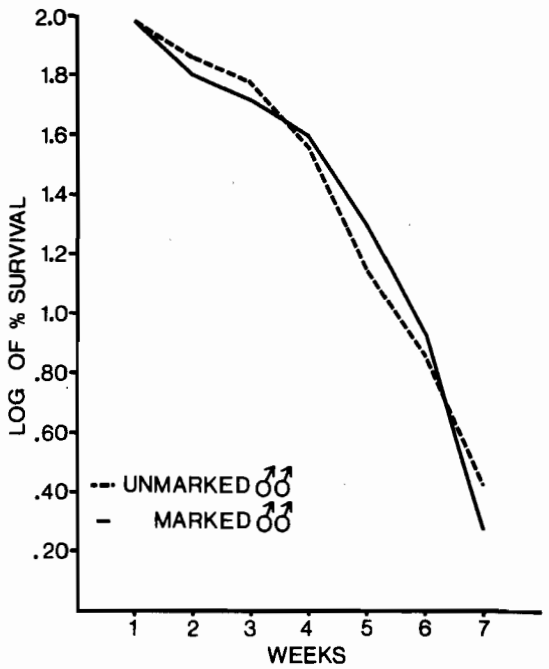

Figure 1.–Logarithmic survival curves of fluorescent-dust marked and unmarked male *Cx. tarsalis* in the laboratory.

marked males survived 9 days and 50% survived 23 days. Similar patterns were observed for females (Figure 2), which survived better than males. These findings suggested that the dusts had little effect on survival.

Some additional data on survival were obtained from a trial in an outdoor, quonset-type cage that was 5.5 m wide, 6 m long, and 3 m high. In this trial, 500 males reared from field-collected pupae were marked with Radiant red dust and released in the cage with 500 unmarked but otherwise identical males. A light trap was run for the next 5 nights and the percentage of marked males was determined for each collection. The results, which presumably reflected short-term survival and mobility, failed to reveal any adverse effect of the marker. Marked males predominated in 4 of the 5 collections and, overall, comprised 55% of 465 males collected.

Observations next were made on the mating success of marked and unmarked males in laboratory cages. In the 1st test, 2 groups of *Cx. tarsalis* from a laboratory colony were set up identically in two 3.4-ft^3 cages, except that the males in one cage were marked with Radiant blue dust. The ratio of males to females was about 1 to 2. After 3 days, 50 females from each cage were checked for sperm, with the results shown in Table 1. Insemination rates for the 2 groups were nearly identical. The rather high insemination rates represent an average of 1.9 inseminated females per male. Another test that involved 4 groups in 1-ft^3 cages, and a male:female ratio of 1 to 3, produced similar results (Table 1).

In another trial, reported previously (Milby 1979), 1,000 males and 1,000 females reared from field pupae were released into each of 2 quonset cages, and all of the males in 1 cage were 1st marked with Radiant chartreuse dust. A daily sampling of 25 females by aspirator revealed that insemination was initially delayed in the cage with the marked males. However, overall insemination rates of females of the 2 groups did not differ significantly. In a similar test, in which 750 males and 750 females were released in each cage, females were sampled only once 4 days after release. Insemination rates were 57 and 52% for females with marked and unmarked males, respectively.

Laboratory observations were not made on the effects that marking of females might have on their mating success. However, limited observations were made on the field insemination of females that were marked, released, and recaptured. In one case, virgin females were marked and released in the morning and recaptured the following night. Of 13 females examined, 11 had mated. On another occasion, 8 out of 8 females were found to have mated on the 1st night after their release. These findings indicated that marker dust did not inhibit mating under field conditions.

Finally, several studies were made of the field dispersal of marked and unmarked *Cx. tarsalis*. These studies utilized a unique mutant strain of *Cx. tarsalis* that can be distinguished by its outward appearance from the normal or wild type. This strain, known as charcoal, was developed and made available by S. M. Asman, Division of Entomology and Parasitology, University of California, Berkeley. By release of equal numbers of dust-marked and unmarked charcoal *Cx. tarsalis*, it was possible to directly compare dispersal and recapture rates of the 2 groups in the field and thereby test for marker effects.

The 1st of the comparisons, all of which were at the Poso West study site 16 km N. of Bakersfield, was in May, 1979.

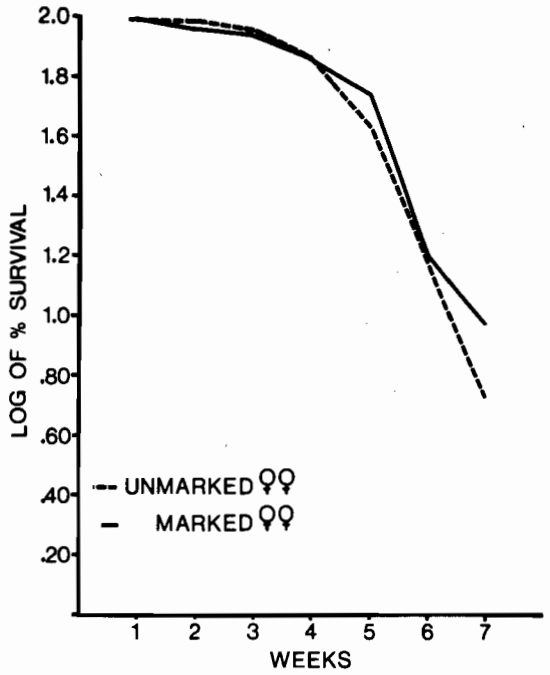

Figure 2.--Logarithmic survival curves of fluorescent-dust marked and unmarked female *Cx. tarsalis* in the laboratory.

Table 1.--Insemination rates of female *Cx. tarsalis* in laboratory cages with marked or unmarked males.

Cage size	No. mosquitoes		Mark status	♀♀ inseminated	
(ft^3)	♂	♀	of ♂♂	%	no./♂
3.4	86	190	Blue	86	1.9
	86	190	Unmarked	88	1.9
1.0	30	90	Yellow	70	2.1
	30	90	Unmarked	74	2.2
	30	90	Red	64	1.9
	30	90	Aqua	86	2.6

Table 2.--Recapture rates and dispersal of marked and unmarked "charcoal" *Cx. tarsalis* at Poso West, 1979.

	Releases			Recaptures		
Date	Sex	No.	Mark status	No.	(%)	Mean distance (m)[a]
May 21	♂	504	Yellow	32	(6.3)	108
		504	Unmarked	21	(4.2)	89
	♀	450	Yellow	121	(26.9)	125
		450	Unmarked	138	(30.7)	127
Sept. 17	♀	237	Aqua	29	(12.2)	134
		237	Unmarked	34	(14.3)	133
Oct. 22	♀	135	Red	20	(14.8)	110
		135	Yellow	23	(17.0)	105
		135	Unmarked	23	(17.0)	91

Helecon yellow-marked and unmarked adults were released shortly after sunrise at the middle of a row of 21 trap sites that extended from N. to S. along a ravine bottom. CDC-light traps with dry ice were run for 5 consecutive nights after the release, and collections were checked for marked and unmarked charcoal specimens. The 2 groups did not differ significantly with respect to recapture rates or dispersal (Table 2). The values for males were less than those for females, but no differences could be ascribed to the marker dust. Two similar releases, that involved 3 different dusts, were made in September and October with similar results (Table 2). Nearly identical numbers of the different categories of charcoal females were taken and dispersal of the different groups was very similar.

From the preceding observations, it appeared that survival, mating success, and dispersal of ***Cx. tarsalis*** were not seriously affected by the marker dusts. These findings are encouraging and increase confidence in the dusts and marking methods. However, the low recapture rates of males, referred to at the outset, remain unexplained. Since estimates of male population size and survival are critical to control programs that involve the release of genetically altered males, further efforts should be made to increase recoveries of males.

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VARIATIONS IN THE DEGREE OF HOMOZYGOUS RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES IN *CULEX QUINQUEFASCIATUS* SAY[1]

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ABSTRACT

A comparison of the data obtained on organophosphate (OP) resistance in two field populations of *Culex quinquefasciatus* Say after different periods of temephos selection revealed three significantly distinct levels of resistance. The LC_{50} values were 0.21, 0.58 and 1.85 ppm, and the dosage-mortality lines, including that of the susceptible reference strain (LC_{50} = 0.002 ppm) were all parallel to one another.

The inheritance of resistance in the strains that exhibited the two highest levels was found to be monofactorial; OP resistance at all three levels was due to increased detoxication, caused by a highly active esterase that was detectable by starch gel electrophoresis. These observations suggest the existence of an OP resistance gene whose level of effectiveness is controlled by a regulatory mechanism, such as a modifier, or by gene amplification.

INTRODUCTION.–Resistance to xenobiotics in general, and to insecticides in particular, can arise through a large variety of biochemical, physiological and ethological mechanisms (review by Oppenoorth and Welling 1976). These mechanisms represent genetic changes that are advantageous to the organism and, therefore, are selected for in the presence of insecticide.

In the present report, we show that three distinct levels of OP resistance were reached in different strains of *Culex quinquefasciatus* Say after various periods of temephos selection, and give evidence, based on a critical review of all published and unpublished data, that this was not achieved by the selection of different mechanisms, but by the action of regulatory mechanisms or possibly by gene amplification.

MATERIAL AND METHODS.–Three strains of *Cx. quinquefasciatus* were considered: a susceptible reference strain, S-Lab, which had been reared under insecticide-free conditions since 1954, and two OP-resistant strains, HAN'74 and HAN'75, which were selected in the presence of temephos from two field populations collected in 1974 and 1975, respectively, near Hanford, California.

The HAN'74 strain is still maintained in this laboratory under selection pressure in each generation with 0.01 ppm temephos. Data on synergism, inheritance and linkage of OP resistance and on correlation to esterase-B^A were obtained in 1976 after nine generations of selection (Ranasinghe 1976, Ranasinghe and Georghiou 1979, Georghiou and Pasteur 1978), and in 1978 after some 40 generations of selection (Georghiou et al. 1980). Details on the experimental procedures are given in these references.

The HAN'75 strain was selected during nine generations with temephos concentrations that induced approximately 50% mortality. The evolution of OP resistance during selection was determined as reported by Georghiou et al. (1966), and esterase-B^A was studied in a small number of individuals from each generation by starch gel electrophoresis as described by Georghiou and Pasteur (1978).

RESULTS AND DISCUSSION.–Status of OP-resistance in HAN'74 and HAN'75 strains after various periods of selection by temephos. - - Selection by temephos resulted in a rapid increase of OP resistance in the HAN'74 and HAN'75 strains. In each case, a dosage-mortality line parallel to the line of the susceptible reference strain, S-Lab, was obtained within a few generations. This line remained unchanged for five generations in HAN'74 (see Table 1 of Ranasinghe and Georghiou 1979), suggesting that this strain was homogeneous for the resistant factor(s) involved. Despite this apparent homogeneity, OP resistance increased by a factor of 3 after two years of further selection, i.e., about 40 generations.

The data obtained in HAN'75 after selection by temephos during nine generations (HAN'75-9) and in HAN'74 during nine and forty generations (HAN'74-9 and HAN'74-40) disclosed three distinct levels of OP resistance for temephos, as well as for chlorpyrifos, methyl parathion and parathion (Figures 1 and 2, and Table 1).

Inheritance of OP-resistance. - - Two independent investigations on the inheritance of OP resistance were conducted on the HAN'74 strain, the first on HAN'74-9 when the temephos LC_{50} was 0.58 ppm (Ranasinghe 1976), and the second on HAN'74-40 when the LC_{50} had increased to 1.85 ppm

[1]This research was supported in part by State of California Special Funds for Mosquito Control Research.

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Table 1. LD_{50} and resistance ratios (RR) of different strains and of their offspring after crosses with the S-Lab strain, and their genotypes under gene duplication hypothesis (see text).

Strains	Temephos[1]		Chlorpyrifos		Methyl-Parathion		Parathion		Genotypes
	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR	
S-Lab	.0022[2]	-	.0032	-	.0061	-	.0038	-	0/0
HAN'75-9	.210	95.4	-	-	-	-	-	-	A/A
HAN'74-9 x S-Lab	.200	90.9	.059	18.4	.084	13.7	.056	14.7	AA/O
HAN'74-9	.580	263.6	.120	37.5	.180	29.5	.135	35.5	AA/AA
HAN'74-40 x S-Lab	.520	236.4	.150	46.9	.235	38.5	.100	26.3	AAAA/O
HAN'74-40	1.850	840.9	.280	87.5	.360	59.0	.140	36.8	AAAA/AAAA

[1]Insecticide used for the selection of the resistance factor.

[2]In ppm.

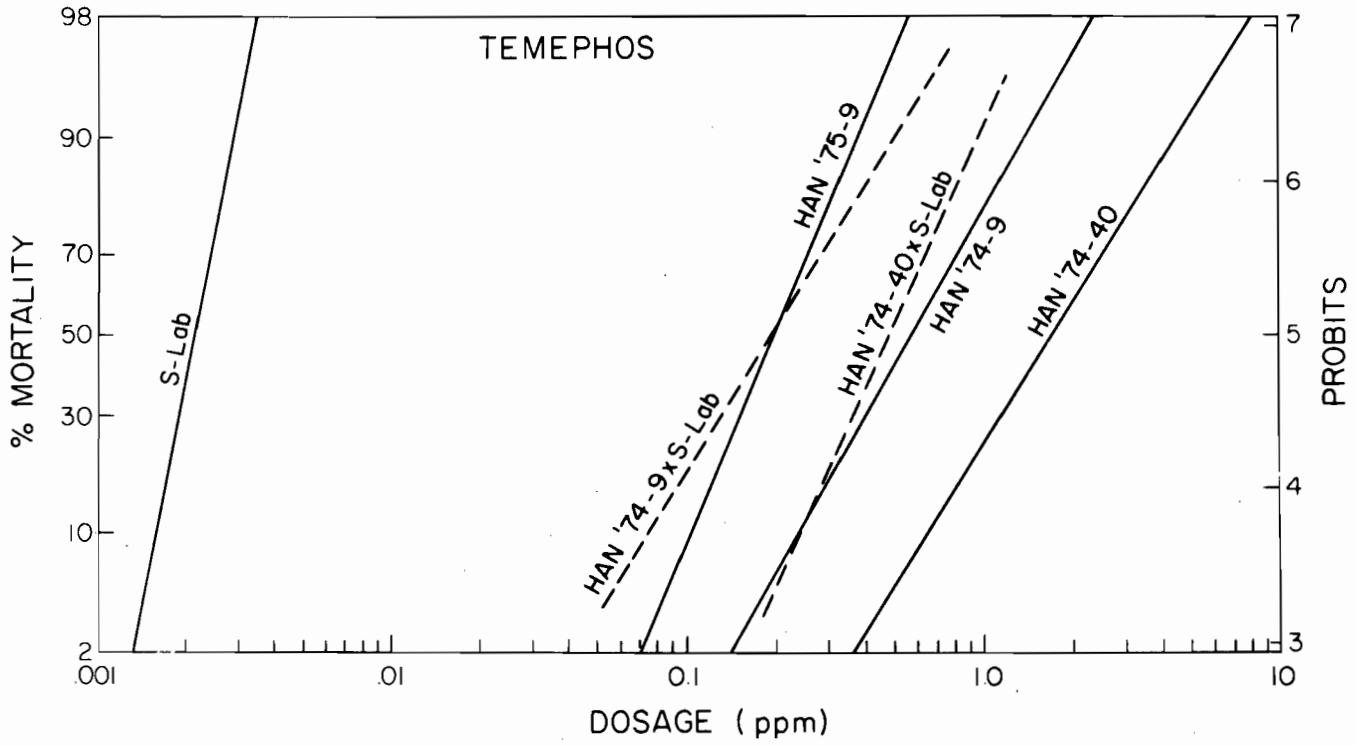

Figure 1. Temephos dose-mortality relationships of susceptible (S-Lab), temephos-selected, and F_1 populations of *Culex quinquefasciatus*: HAN'74-9, HAN'74-40 and HAN'75-9 were collected at Hanford, CA in 1974 and 1975 and selected by temephos during 9, 40 and 9 generations, respectively.

(Georghiou et al. 1980). In both cases, resistance to temephos, chlorpyrifos, parathion and methyl parathion was inherited as a monofactorial character that was dominant (R) over susceptibility (S) (Figure 3).

Monofactoriality of resistance to these insecticides remained evident in HAN'74-9 after four repeated backcrosses to the S-Lab strain (SS homozygotes) of RS heterozygotes that had survived discriminating dosages, i.e., dosages that are lethal for SS susceptible but do not affect RS resistant genotypes (Table 2). Thus, the possible existence of closely linked genes can be excluded in this strain.

The dosage-mortality lines of HAN'74-40 x S-Lab and HAN'74-9 x S-Lab offspring were almost identical to the lines of HAN'74-9 and HAN'75-9 populations, respectively, for temephos, chlorpyrifos and methyl parathion (Figures 1 and 2). Concomitantly, the LC_{50} values of these two offspring were comparable to those of HAN'74-9 and HAN'75-9 (Table 1). This situation was not as evident for parathion (Figure 2 and Table 1), but it must be pointed out that the dosage-mortality line of HAN'74-40 x S-Lab offspring with this insecticide was neither linear nor parallel to those of the parental strains, suggesting some experimental aberration.

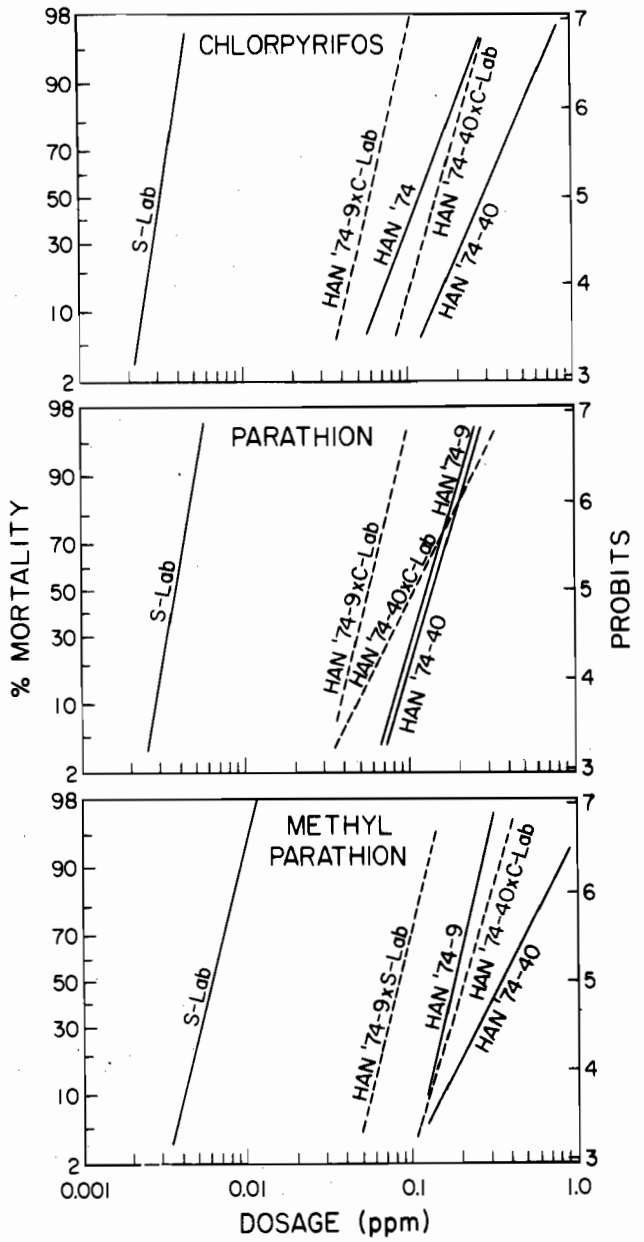

Figure 2.–Chlorpyrifos, parathion, and methyl parathion dose-mortality relationships of susceptible (S-Lab), temephos-selected, and F_1 populations of *Culex quinquefasciatus*. (C-Lab = S-Lab; other details as in Figure 1).

Biochemical mechanisms of OP-resistance. - - Synergism studies. . .Most synergism studies were done on HAN'74-9 (Ranasinghe and Georghiou 1979). Temephos resistance was shown to be completely suppressed by DEF, an inhibitor of esterase and glutathion-S-transferase (GSH) activity, but was not affected by PB, an inhibitor of mixed-function oxidases.

That there are DEF-inhibited mechanisms in OP resistance was further supported by the fact that resistance did not develop when the parental HAN'74 strain was selected in the presence of both temephos and DEF, but that it reached a high level that was completely suppressible by DEF when temephos selection was accomplished in the presence of PB

Table 2.–Mortality data at discriminating dosages that are lethal for susceptible homozygotes. Tests performed on offspring of four consecutive backcrosses using HAN'74-9 as source of resistant homozygotes (see text).

Insecticide	Dosage (ppm)	% Mortality BC1	BC2	BC3	BC4
Temephos	0.004	48	44	48	48
	.02	56	37*	52	44
Chlorpyrifos	.01	55	44	43	51
	.02	55	44	52	43
Parathion	.01	50	50	57	50
	.02	41	43	38*	48
Methyl parathion	.007	44	43	55	46
	.01	48	36	47	48
	.04	50	41	48	59

*Differences significant from the expected 50% at P = 0.05 ($F > 3.841$ for 1 df).

Table 3.–Changes in LC_{50} values and Est-B^A frequency during selection of HAN'75 population with temephos.

Generations	Temephos LC_{50} (ppm)	Est-B^A frequency	
Parental	.0058	0.15	(33)[a]
Gen. 1	.012	-	
Gen. 2	.011	0.83	(36)
Gen. 4	.019	0.88	(16)
Gen. 5	.045	-	
Gen. 6	.033	0.88	(32)
Gen. 7	.060	1.00	(16)
Gen. 8	-	1.00	(16)
Gen. 9	.210	1.00	(16)

[a]Sample.

(Ranasinghe and Georghiou 1979). It can be concluded, therefore, that the HAN'74 parental strain, and consequently HAN'74-9, contained only OP resistance mechanisms that were inhibited by DEF, i.e., they contained esterases or GSH.

Electrophoretic studies. - - Georghiou and Pasteur (1978) analyzed the esterase pattern of individual *Cx. quinquefasciatus* adults by starch gel electrophoresis, and found that all members of the HAN'74-9 strain, as well as those of the strain selected with temephos and PB by Ranasinghe and Georghiou (1979), possessed a highly active esterase, later named esterase-B^A. In contrast, this esterase was absent in susceptible mosquitoes, or in mosquitoes selected with either temephos + DEF or with other non-OP insecticides. Esterase-B^A, which is inhibited by DEF *in vitro*, was present in some 80% of the mosquitoes of the parental HAN'74 strain.

The evolution of the esterase-B^A frequency was studied during the selection of HAN'75, and all mosquitoes had this

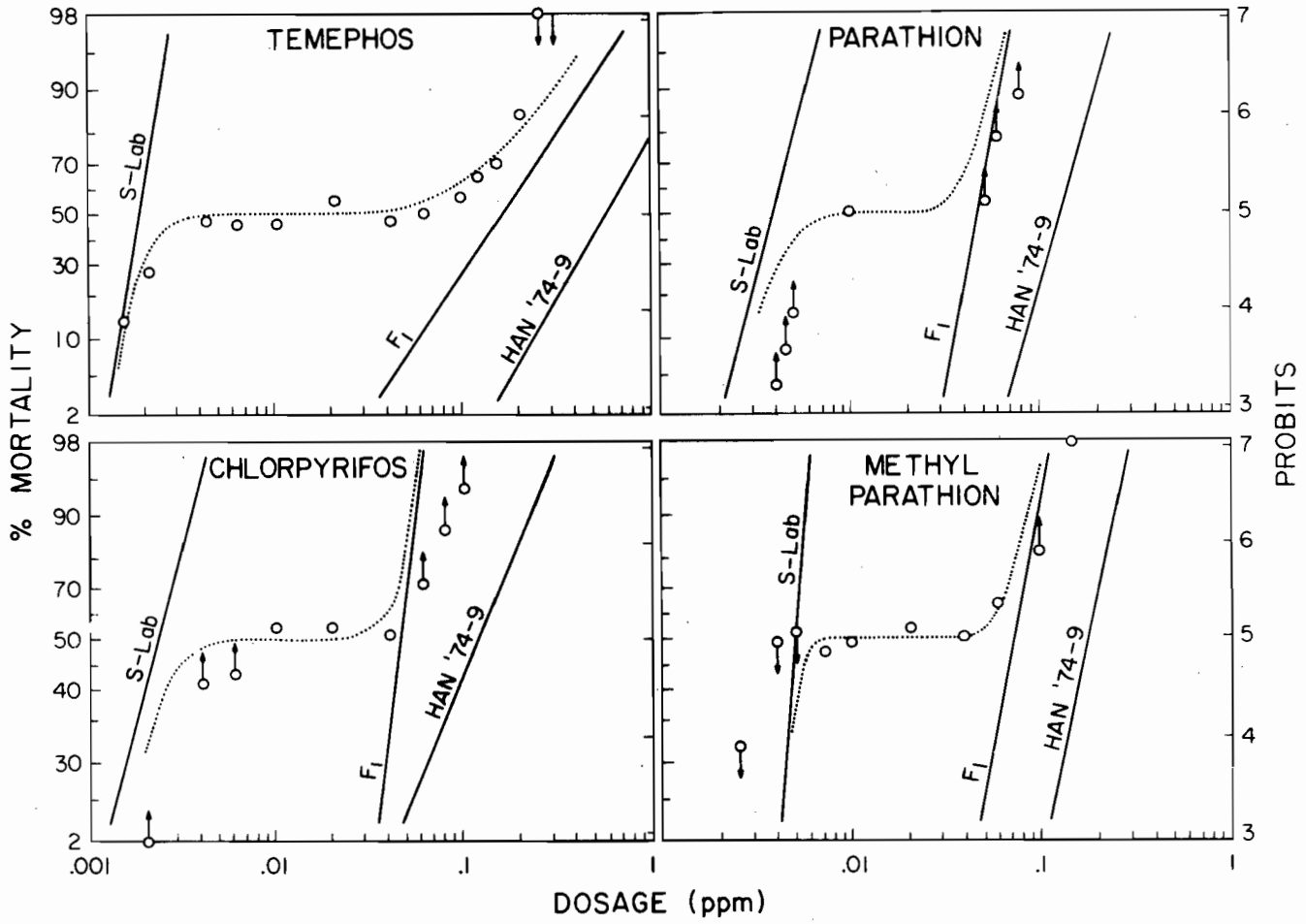

Figure 3. Dose-mortality relationships toward organophosphates of *Culex quinquefasciatus* susceptible (S-Lab), temephos-selected (HAN'74-9), F_1 (S♂ x R♀), and backcross population (F_1 ♀ x S♂). Dotted line indicates expected mortality of backcross on basis of single gene inheritance. Circles indicate observed mortality. Arrows signify significant deviations of observed from expected mortality.

enzyme from the seventh generation onward (Table 3), indicating that its presence was highly favorable for survival.

Finally the inheritance of esterase-B^A was studied by Georghiou et al. (1980) using HAN'74-40. This enzyme is coded by a dominant gene, as is resistance, and both factors could not be dissociated from one another. Thus, the Est-B gene and the OP resistance gene are either identical or tightly linked.

CONCLUSIONS. Comparison of data obtained on OP resistance in two different populations after temephos selection have disclosed that at least three levels of OP resistance can be reached. All our observations indicate that the same physiological mechanism (i.e., increased detoxification) is responsible for the different levels of OP resistance that have been observed. Investigations on the inheritance of resistance on the intermediately resistant HAN'74-9 strain, and more particularly the results obtained in successive backcrosses, proved that a single gene was involved in the mechanism of resistance. Synergism and electrophoretic studies indicated that most probably this gene codes for increased detoxification due to esterase-B^A.

HAN'75-9 mosquitoes, which displayed the lowest resistance, also possessed this enzyme. Therefore, the difference in OP resistance observed between HAN'75-9 and HAN'74-9 cannot be attributed either to the selection of different mechanisms or to the combined action of several mechanisms in HAN'74-9, so the gene that causes OP resistance must have two different states of efficiency. Most probably, OP resistance in HAN'74-40 represents a third state of efficiency: resistance and esterase-B^A could not be separated from one another and no other mechanism of resistance than the one suppressed by DEF was present in the HAN'74 parental collection. This conclusion is in agreement with observations that the activity of esterase-B^A varies in field populations, mosquitoes with the less active enzyme consistantly giving a less resistant offspring (Georghiou and Pasteur 1980).

The differential expression of genes has been reported in many organisms and is generally believed to be caused by the action of modifier and/or regulatory genes (McCarron et al. 1979, McDonald and Ayala 1978). Continuous temephos exposure may have induced changes in the polymorphism of different modifier genes at different times and thus may have

modified the expression of the OP resistance gene. If this is the case, a large number of modifier genes (or a modifier gene with a large number of alleles) regulate OP resistance. We have recently isolated two stains with two resistance levels lower than that recorded in HAN'75-9.

Recently, gene amplification has been suggested as another mechanism to explain variations in gene expression (Schimke et al. 1978, Devonshire and Sawicki 1979). Repetitive gene duplication is also compatible with our own observations on OP resistance in *Cx. quinquefasciatus*. Thus, if we assume that the genotype of HAN'75-9 mosquitoes is A/A, and that of HAN'74-9 is AA/AA (i.e., a 2-fold duplication of what is found in HAN'75-9), heterozygotes between HAN'74-9 and S-Lab (O/O genotypes) will be AA/O and should, therefore, present the same resistance characteristics as the A/A insects of HAN'75-9. This was indeed the case (Table 1). Similarly, if a duplication has occurred between HAN'74-9 and HAN'74-40, individuals of the latter strain will be AAAA/AAAA and their offspring with S-Lab will be AAAA/O. AAAA/O genotypes should then display a resistant pattern similar to that observed in AA/AA mosquitoes from HAN'74-9, and again this was the case for temephos, chlorpyrifos and methyl parathion, as reported in Table 1.

Although we have no proof that gene amplification rather than gene regulation is the mechanism involved in the increase of OP resistance of *Cx. quinquefasciatus*, we note that the former hypothesis was also suggested by Denvonshire and Sawicki (1979) to explain the variations of OP resistance and of the highly active esterase associated with it in a series of strains of *Myzus persicae*. Likewise, Schimke et al. (1978) have emphasized the possibility of the large scale occurrence of such phenomenon in those cases of resistance in which increased detoxification mechanisms have been observed.

If these hypotheses are confirmed, they could modify our views on the evolution of resistance and cross resistance; the fact that a large number of copies of the same gene may be accumulated in one individual enhances the probability of point mutations and, consequently, the rapidity of appearance of more efficient enzymes against a large variety of insecticides.

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ANALYSIS OF ESTERASES AS A MEANS OF DETERMINING ORGANOPHOSPHATE RESISTANCE IN FIELD POPULATIONS OF *CULEX PIPIENS* MOSQUITOES

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Several studies have shown a close association between organophosphate (OP) resistance and esterases that are highly active in hydrolyzing α- and β-naphthyl acetates (Table 1). In *Myzus persicae* (Devonshire 1977), *Nephotettix cincticeps* (Miyata and Saito 1976) and *Laodelphax striatellus* (Miyata et al. 1976), it was demonstrated that these enzymes cleave ester bonds of OP compounds, thus conferring OP resistance. In *Culex tarsalis*, *Cx. pipiens* and *Cx. quinquefasciatus*, the evidence of strict association between OP resistance and the highly active esterases was revealed by studies on field populations (Pasteur et al. 1980, Georghiou and Pasteur 1980), as well as by genetic studies in the laboratory (Georghiou et al. 1980, Pasteur 1977). It may be reasoned, therefore, that techniques indicating the proportion of individuals with the highly active esterases would also reveal the frequency of OP resistance in a given population.

Here we review the experimental evidence for this statement and discuss the feasibility of substituting esterase analysis for classical bioassay as a means of determining the frequency of OP resistance in field populations of certain *Culex* species.

Determination of frequency of resistance by bioassay tests. -- OP resistance in France and California is due to a single gene (Ranasinghe 1976, Pasteur and Sinègre 1978) and is coded by an R allele, which is dominant over the susceptible allele S. In strains from both countries, dosage-mortality lines of susceptible (SS) mosquitoes and of resistant (SR or RR) mosquitoes do not overlap (Figure 1), and therefore, a range of "discriminating dosages" is available at which all SS individuals are killed while SR and RR insects remain unaffected. If a complete dosage-mortality curve is established on a population composed of SS, SR and RR, a plateau becomes evident within the range of the discriminating dosages and the mortality registered at this plateau equals the proportion of susceptible SS individuals in the population. The following discriminating dosages (in ppm) have been determined as suitable for use on *Cx. quinquefasciatus* in California (Georghiou et al. 1980): temephos, 0.01; chlorpyrifos, 0.01; fenthion, 0.02; malathion, 0.4; parathion, 0.01; methyl parathion, 0.02.

Table 1.- Insect species in which OP resistance is associated with esterases that are highly active in hydrolyzing α- and/or β-naphthyl acetates.

Species	Geographic origin	References[a]
Aedes aegypti	Japan	Yasutomi, 1980
Culex pipiens fatigans	East Africa	Curtis & Pasteur, 1980
Cx. p. pallens	Japan	Yasutomi, 1970
Cx. p. pipiens	Southern France	Pasteur, 1977
Cx. p. quinquefasciatus	California	Georghiou & Pasteur, 1978
Cx. tarsalis	California	Georghiou & Pasteur, 1978
Cx. tritaeniorhynchus	Japan	Yasutomi, 1971
Laodelphax striatellus	Japan	Osaki and Kassai, 1970
Myzus persicae	England	Beranek, 1974
Nephotettix cincticeps	Japan	Ozaki & Koike, 1965

[a]First description.

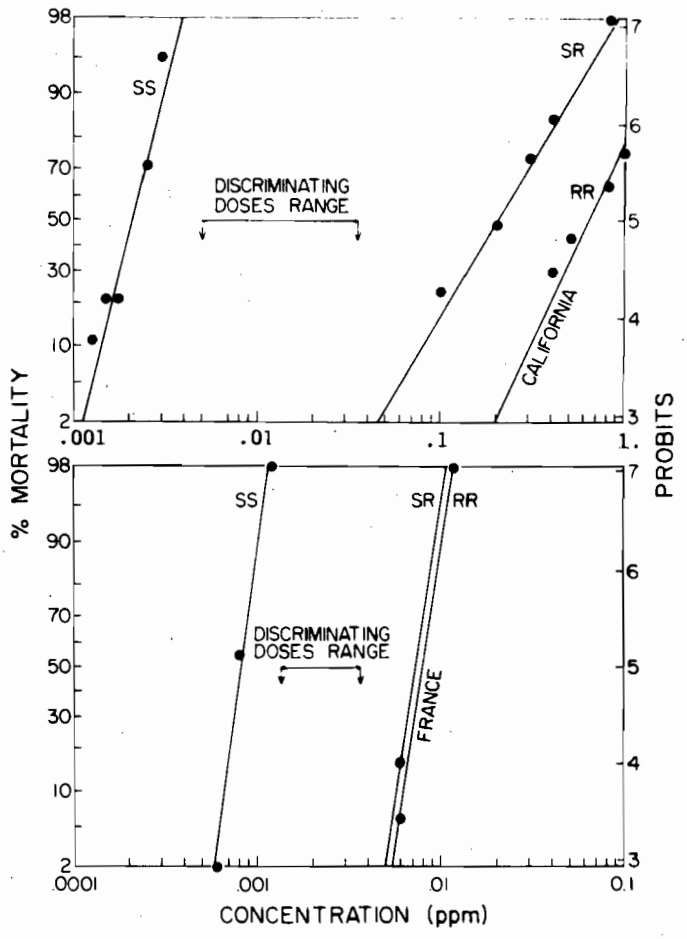

Figure 1.--Dosage-mortality lines of SS, SR and RR genotypes for temephos in *Cx. quinquefasciatus* from California and for chlorpyrifos in *Cx. pipiens* from France.

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Esterases in OP-resistant mosquitoes. - - In both France and California, OP-resistant *Culex* mosquitoes (either RS or RR) possess an esterase that appears as a very strongly stained spot after electrophoretic separation and subsequent staining. Susceptible SS mosquitoes have only faintly staining spots (Figures in Georghiou et al. 1980, Curtis and Pasteur 1980). As noted by Georghiou and Pasteur (1978) each of these esterases has different biochemical properties and thus they have been given different names: Esterase-B in strains from California, and Esterase-3 in strains from France. Investigations on the inheritance of these enzymes have shown that each is coded by a single gene with two alleles (Georghiou et al. 1980, Pasteur 1977): allele A (Est-B^A or Est-3^A) is dominant and codes the highly active esterase observed in OP-resistant mosquitoes; allele O (Est-B^O or Est-3^O) is recessive and codes either an esterase of low activity (Est-B^O) or no detectable enzyme (Est-3^O).

Linkage studies, performed independently on both Californian and French strains, have shown that all offspring of backcrosses between F_1 and susceptible mosquitoes that had survived exposures to discriminating dosages possessed the highly active esterase. These results indicate that the genes coding these esterases are either identical to the OP resistance genes, or that the two characters are so closely linked that they seldom dissociate (Georghiou et al. 1980, Pasteur 1977).

Determination of frequency of resistance by esterase tests. - - It is evident from the above that determination of the number of individuals that do not possess the highly active esterases is equivalent to the determination of the proportion of susceptible insects that are killed by discriminating dosages.

In France, some 100 populations of *Cx. pipiens* have been analyzed over a four year period using both techniques, and the results were never significantly different. As shown by Figure 2, the correlation between the two characters is excellent ($r = 0.98$ for about 100 df). In California, toxicological and genetic studies on *Cx. quinquefasciatus* led to the same conclusion. A field population from Pixley in the San Joaquin Valley displayed a 56 ± 8% mortality with discriminating dosages of temephos, and 61% of the population lacked the highly active esterase (Table 2, Figure 3).

Table 2.–Occurrence of the highly active esterase, Est-B^A, in Californian populations of *Culex quinquefasciatus*[a].

Locality of Abatement District	Date of Collection	% Mosquitoes with Est-B^A	
Camara	June, 1975	87%	(38)
Foster	November, 1976	71%	(7)[b]
San Mateo	November, 1976	43%	(7)[b]
Sacramento	November, 1976	0%	(8)[b]
Pixley	October, 1977	39%	(56)
Delta	October, 1979	98%	(64)
Fresno	October, 1979	100%	(52)
L.A. West	October, 1979	58%	(37)
Southeast	October, 1979	85%	(53)
Riverside	August, 1979	40%	(36)

[a]In parentheses, size of sample tested.

[b]Samples unreliably small.

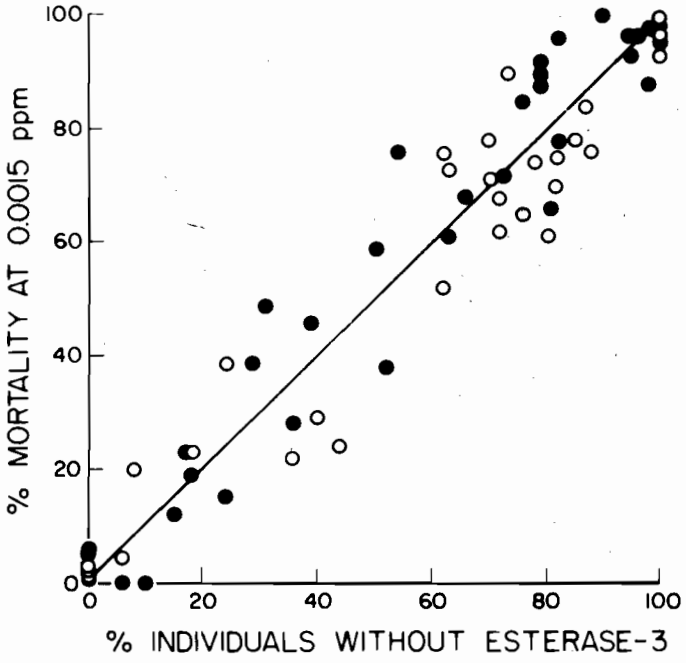

Figure 2.–Relation between mortalities observed at a discriminating dosage of 0.0015 ppm chlorpyrifos and the absence of the highly active Esterase-3 in natural populations of *Cx. pipiens* from France. O represents populations collected in 1974-75 (Pasteur and Sinegre 1978), ● populations collected in 1978 (Pasteur et al. 1980).

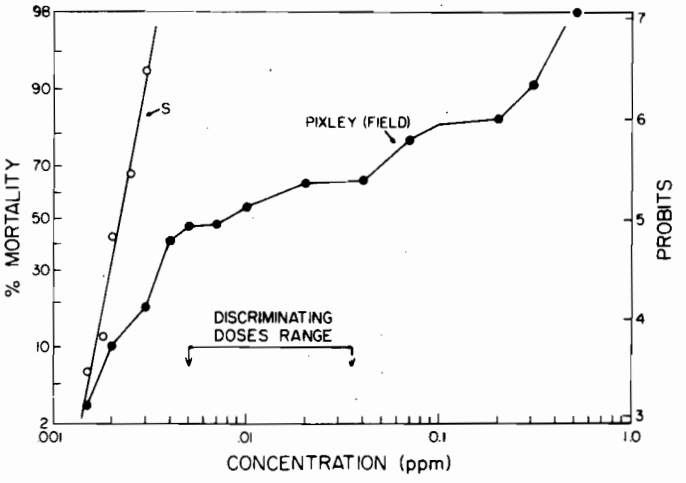

Figure 3.–Dosage-mortality curve for temephos of a field population of *Cx. quinquefasciatus* from Pixley, San Joaquin Valley, compared to that of a susceptible strain (S).

In the studies described thus far, esterases were analyzed by means of starch gel electrophoresis. Although the technique is simple, it is rather time-consuming and requires specialized equipment, so that a skilled investigator cannot analyze more than 150-200 mosquitoes per day. We recently have elaborated a new technique ("filter-paper test") that does not require specialized equipment and can be performed rapidly. Briefly, the test consists of depositing crude homogenates of single mosquitoes on filter paper and then immersing the paper in a staining solution. The appearance of a dark stain reveals the presence of the esterase and hence, of resistance. The test may be employed with either adults or larvae, but adults are preferred since excessive larval fat may interfere with homogenization (Figure 4).

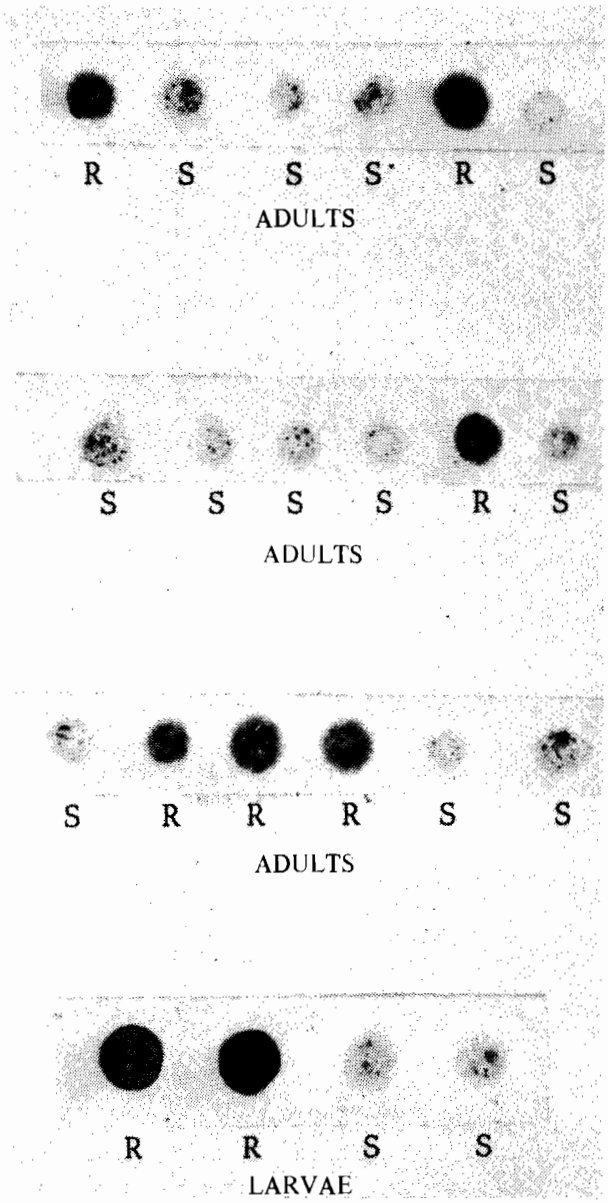

Figure 4.--"Filter-paper test" on *Cx. quinquefasciatus* adults and larvae, susceptible (spots faintly stained) and resistant (spots strongly stained) to temephos.

DISCUSSION.--The analysis of esterase frequency is analogous to the determination of mortalities at discriminating dosages by bioassay. However, there are distinct differences concerning the practicality of each method and the reliability of results, especially where resistance occurs at very low frequencies. The determination of mortalities at discriminating dosages requires the testing of several replicated groups of 20-25 larvae each. If only a few survivors are found, questions arise as to alternative reasons for such results, including the nutritional status of the insects, error in insecticide application, environmental conditions, etc. The presence of moribund individuals further complicates the results. In contrast, the unequivocal nature of esterase analysis can provide a good estimate of the frequency of resistant insects, even with a total sample of only 100 individuals. With the development of the "filter-paper test", results can be obtained in less than an hour, and the rapidity and simplicity of this technique enables the screening of large numbers of mosquitoes per day even when the detection of resistance at very low frequencies is desired. Thus, the method enables more efficient surveillance and monitoring of resistance than is possible by bioassay.

Another application of the esterase technique could be for investigation of the geographic distribution of the resistance gene. In southern France, it was found that this gene could be detected as far as 300 km from the zone under chemical control, and that its frequency varied according to a regular north-south cline. In California, the highly active esterase was found in 9 out of 10 samples that were examined from different areas of the State. In 6 of these samples, resistant mosquitoes occurred at frequencies greater than 58% (Table 2).

Despite its distinct advantages, the esterase method of detecting resistance is presently deficient in two important respects. First, it does not provide information on the degree of resistance that is present in the resistant individuals, i.e., the insecticide dosages required for mortality. We now have evidence that in California, the resistant allele R occurs in different forms, R_1, R_2, R_3, etc., each form conferring a different degree of resistance. The first indication that the R allele might not be unique was the observation that resistance in our

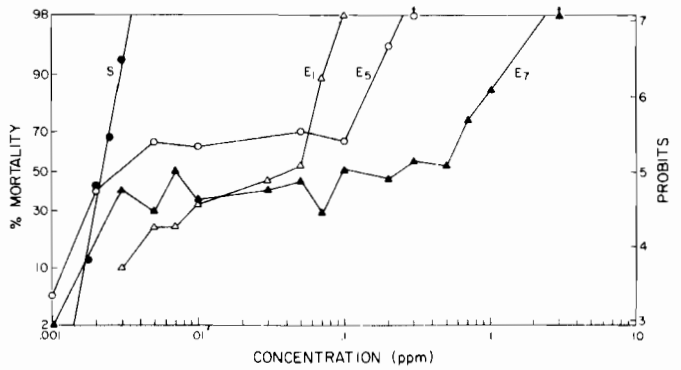

Figure 5.--Dosage-mortality curves for temephos of four strains of *Cx. quinquefasciatus* (E_1, E_5, E_7) containing different R alleles (see text). Note also the plateaux indicating different ratios of susceptible (SS) and resistant (SR, RR) genotypes.

temephos-resistant strain continued to increase under selection pressure, although genetic investigations indicated that the strain was homozygous for a single factor of resistance. We have recently isolated strains from a number of single crosses (♀RS x ♂SS) and have selected these for the presence of the highly active esterase. Although none of the strains is yet homozygous (mortality lines show the presence of plateaux at discriminating dosages), it is evident that the degree of resistance varies widely among the different strains (Figure 5).

A second limitation of the esterase method is that it detects only resistance associated with the esterase gene. Although no other important mechanism of OP resistance has been found to date in *Cx. pipiens* populations from either France or California, this condition may not persist indefinitely.

CONCLUSIONS.–Until now, the analysis of esterases for determination of the frequency of OP-resistance genes has been confined to research laboratories because the electrophoresis technique of esterase determination is difficult to use in routine tests. However, if the "filter-paper technique" proves as simple as it appears, it could partially replace bioassays in resistance surveillance and monitoring programs for cases in which the association of the abnormally active esterase and OP resistance has already been verified. Esterase analysis cannot replace bioassays entirely, however, since it only enables the determination of the frequency of resistant individuals, but not the degree of their resistance.

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LARVICIDAL AND PUPICIDAL ACTIVITY OF ALKANAMIDES AGAINST *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Studies on the structure-activity relationship of various aliphatic amides showed that only primary 3-methylalkanamides possessed excellent larvicidal activity against the first instars of *Culex quinquefasciatus* Say whereas their N,N-dimethyl derivatives were inactive. While primary and secondary straight-chain alkanamides did not show any measurable activity, tertiary N,N-dimethylalkanamides displaced excellent activity against the first instars of the mosquito.

Some N,N-dimethylalkanamides demonstrated a wide spectrum of activity affecting all stages of immature mosquitoes. On the other hand, 3-methylalkanamides had a narrow spectrum of activity affecting only the very young larvae.

INTRODUCTION. We previously reported that straight-chain and branched-chain aliphatic amides demonstrated larvicidal activity against mosquito larvae (Hwang et al. 1979). Our studies showed that, among the various types of amides, 3-methylalkanamides and N,N-dimethylalkanamides were the only types that were active against first instar *Culex quinquefasciatus* Say (Hwang and Mulla 1980). Here, we present detailed study on the biological activity of alkanamides against mosquito larvae and pupae.

An amide can be considered as a derivative of a carboxylic acid with an amino or a substituted amino group replacing the hydroxyl in the carboxyl functional group, thus forming an amide linkage between the carbonyl and amino groups. If the amino group of an amide has two hydrogens, it is a primary amide. In case one of the hydrogens is substituted by an alkyl group, such as methyl or isobutyl, the amide is secondary. If both amino hydrogens are substituted by two alkyl groups, such as N,N-dimethyl, the amide is tertiary. As will be discussed later, the substitution at the amide nitrogen is crucial in determining whether or not an amide possesses biological activity. The amides under current investigations also have a long-chain alkyl group attached to the carbonyl functioning. The alkyl group can be a straight-chain n-alkyl, a 3-methylalkyl, a 2-alkylalkyl, or a 2-haloalkyl. Therefore, the amides in our studies can comprehensively be termed alkanamides which include straight-chain alkanamides (or plainly alkanamide in a strict sense), 3-methylalkanamides, 2-alkylalkanamides, and 2-haloalkanamides.

SYNTHESIS.–The synthesis of an alkanamide was conveniently achieved by the solvolysis of the corresponding acid chloride (Buehler and Pearson 1970). Thus, a carboxylic acid was treated with boiling thionyl chloride. After removal of excess thionyl chloride, the residual acid chloride was added dropwise into a cooled solution of an amine or ammonia. The mixture was vigorously stirred for a few hours, and the resulting amide was separated by filtration or solvent extraction. The crude amide was purified by distillation or recrystallization.

BIOASSAY METHOD.–The alkanamides thus synthesized were dissolved in acetone, and the solutions were serially diluted with the same solvent to the desired concentrations. Aliquots of the solutions, usually no more than 1 ml, were added to each polyurethane dish containing 200 ml tap water and 20 larvae or pupae. The tests were replicated and continued until adult emergence. Mortalities of the mosquitoes were recorded every other day, and the dosage-response data were processed by a Compucorp model 145E computer for the log-probit analysis. The biological activity is expressed in terms of LC_{50} and LC_{90} in parts per million. For clarity and convenience, the activity is ranked as excellent (LC_{50} and LC_{90} <1ppm), good (1 ppm <LC_{50} and LC_{90} <5 ppm), moderate (5 ppm <LC_{50} and LC_{90} <10ppm), and poor or none (LC_{50} and LC_{90} >10ppm) in this paper. The highest concentration used in our evaluation was 10 ppm.

RESULTS AND DISCUSSIONS.–The first group of amides that showed promising activity was primary 3-methylalkanamides. They were primary amides with a methyl group attached to the 3-position in the acid moiety of the amides. Representative compounds were 3-methyltetradecanamide, 3-methylheptadecanamide, 3-methyloctadecanamide, and 3-methylnonadecanamide, all of which displayed excellent activity against first instars of *Cx. quinquefasciatus*. Among the 3-methylalkanamides, 3-methylnonadecanamide was the most active with an LC_{50} of 0.16 ppm and an LC_{90} of 0.33 ppm.

Secondary N-isopropyl-3-methylalkanamides did not show any activity with the exception of the N-isobutyl-3-methyltetradecanamide which was moderately active. Of the tertiary N,N-dimethyl-3-methylalkanamides, N,N-dimethyl-3-methylnonadecanamide was moderately active, but N,N-dimethyl-3-methyleicosanamide did not display any activity. The corresponding 3-methylalkanoic acids and esters were previously reported to show excellent activity (Hwang et al. 1977, 1978b).

An analysis of the larvicidal activity of the 3-methylalkanamides and their N-alkyl and N,N-dialkyl derivatives with regard to their chemical structures distinctly indicated that the primary 3-methylalkanamides were the only group of amides that manifested excellent activity. Mono- or disubstitution at the amide nitrogen of the 3-methylalkanamide would severely impair the activity.

Various primary 2-methyl-, 2-ethyl-, 2-butyl-, and 2-hexylalkanamides from C_{10} and C_{20} and their secondary N-methyl derivatives did not show any activity. However, some of their tertiary N,N-dimethyl derivatives did exhibit activity. Thus, N,N-dimethyl-2-ethylhexadecanamide demonstrated excellent activity, N,N-dimethyl-2-butyltetradecanamide showed good activity, and N,N-dimethyl-2-butyltetradecanamide showed moderate activity. Conclusively, this type of 2-alkylalkanamides required dimethyl substitution at the amide nitrogen to manifest larvicidal activity. Nevertheless, the activity of the resulting N,N-dimethyl-2-alkylalkanamides was variable and did not bear a clear-cut structure-activity relationship due to the lack of adequate experimental data.

2-Halogen-substituted amides, such as 2-chloro-, 2-bromo-, and 2-iodooctadecanamides and their N-methyl and N,N-dimethyl derivatives, did not possess any measurable activity despite that their parent 2-haloalkanoic acids were reported to be active against first-instar larvae (Hwang and Mulla 1976, Hwang et al. 1978a).

The primary straight-chain alkanamides from C_4 to C_{18} did not show appreciable larvicidal activity. Neither did any secondary straight-chain alkanamides from C_{10} to C_{18} demonstrate toxicity against the first instars.

The tertiary straight-chain alkanamides or N,N-dimethylalkanamides surprisingly showed larvicidal activity against the first instars. The lowest homologue synthesized, N,N-dimethyldecanamide (C_{10}) was moderately active, and the next higher homologue, N,N-dimethylundecanamide (C_{11}) showed good activity. As the chain length of the amides lengthened further, the higher amides became more active. Thus, the activity of the next higher amide, N,N-dimethyldodecanamide (C_{12}) increased mide (C_{13}), N,N-dimethyltetradecanamide (C_{14}), N,N-dimethylpentadecanamide (C_{15}), N,N-dimethylhexadecanamide (C_{16}), N,N-dimethylheptadecanamide (C_{17}), and N,N-dimethyloctadecanamide (C_{18}), all exhibited excellent activity against first-instar larvae with the C_{16} amide showing the highest degree of activity. According to the log-probit analysis, all the highly active N,N-dimethylalkanamides showed very steep slopes of probit regression lines, ranging from 3.73 to 11.73. This, of course, indicated that the difference between the LC_{50} and LC_{90} values were considerably narrow. In the case of the C_{16} amide, the difference between the LC_{50} and LC_{90} was as narrow as 0.08 ppm (0.24 ppm-0.16 ppm = 0.08 ppm).

This tendency of increasing activity as a function of the carbon-chain elongation abruptly ceased in N,N-dimethylnonadecanamide (C_{19}) which displayed no activity at all. Its higher homologues, C_{20}, C_{21}, and C_{22} amides, were all inactive.

As a result of our studies on the structure activity relationship of the straight-chain alkanamides, we concluded that the highly active alkanamides should have two methyl groups attached to the amide nitrogen. In other words, the tertiary amides with straight-chain alkyl moieties from C_{12} to C_{18} always demonstrated excellent activity against first instars.

The larvicidal activity of the parent straight-chain fatty acids has been emphatically scrutinized. Decanoic acid was reported to show larvicidal activity against *Cx. restuans* Theobald (Maw 1970), but the concentrations used were exceptionally high ranging from 150 to 300 ppm. Fatty acids from C_6 to C_{12} induce 100% mortality in *Aedes aegypti* (L.) again at the extremely high concentration of 500 ppm (Quraishi 1972). The LC_{50} values of decanoic acid, dodecanoic acid, and tetradecanoic acid against young larvae of *Aedes triseriatus* (Say) were 14, 7, and 4 ppm, respectively (LaLonde et al. 1979). With the ranking system used in this report, the three acids were only moderately active at the most. When tested against *Cx. quinquefasciatus*, nonanoic acid, decanoic acid, dodecanoic acid, hexadecanoic acid, and octadecanoic acid caused less than 7% mortality at the 9 ppm concentration (Ikeshoji and Mulla 1974). Compared with those straight-chain alkanoic acids, the N,N-dimethylalkanamides demonstrated a much higher level of larvicidal activity despite their structural similarity to the acids.

The alkanamides discussed above were bioassayed against the first instars of *Cx. quinquefasciatus*. Similar to previously reported substituted alkanoic acids and esters (Hwang and Mulla 1976, Hwang et al. 1977, 1978a, b), most alkanamides were particularly effective against the first instars but showed no activity against the fourth instars and the pupae. When the alkanamides were tested against the older larvae and the pupae, the alkanamides showed some specificity. 3-Methylheptadecanamide, 3-methyloctadecanamide, and 3-methylnonadecanamide were typical primary 3-methylalkanamides that exhibited excellent larvicidal activity against the first instars but did not show any measurable activity against the fourth instars and the pupae. Among tertiary N,N-dimethylalkanamides, N,N-dimethyldodecanamide displayed excellent activity against the first instars, good activity against the fourth instars, but no activity against the pupae. Its higher two homologues, N,N-dimethyltridecanamide and N,N-dimethyltetradecanamide, not only showed excellent activity against the first instars but surprisingly also displayed good activity against both fourth instars and pupae. These studies have therefore confirmed the stage-specificity relationship of the amides. Thus, some N,N-dimethylalkanamides (tertiary straight-chain alkanamides) demonstrated a wide spectrum of activity affecting all stages of immature mosquitoes whereas the 3-methylalkanamides (primary branched-chain alkanamides) had a narrow spectrum of activity affecting only the very young larvae.

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FUMIGANT TOXICITY TO EGGS OF *AEDES SIERRENSIS*

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ABSTRACT

When eggs from laboratory colonies and fumigants were exposed in laboratory contrainers of 1735 or 26,000 cm^3 fitted with loose lids, the percentage mortality of eggs was related to time of exposure and size of container. Mortalities of 53 to 100% resulted from one to four hour exposures to 1/6 of a Shell No-Pest Strip (20% Vapona), whereas 8 cm strips of Hartz Dog Flea Collars (13.7% active ingredient) resulted in mortalities of only 4 to 6%. Paradichloro-benzene (PDB) crystals produced 100% mortality after 24 hours in the small container, but only 16% mortality in the large container.

In field studies tree holes received 1/3 of a NPS or a 57 gm packet of PDB along with a perforated pill box of laboratory-derived eggs of *Aedes sierrensis*. The tree holes were closed with a sheet of polyethylene for 24, 48 or 67 hours, after which the eggs were removed and their hatch compared with field-exposed controls. Exposure to PDB was ineffective, but most holes treated with the NPS showed a 95 to 99% mortality of exposed eggs after 48 to 67 hours. When water in the naturally-filled tree holes was siphoned in January 1980, results showed no larvae in one hole, one dry hole, most holes with 10 to 25 larvae/250 ml, and one hole each with 100 and 150 larvae/250ml. Most of the 10 untreated holes also had 100 to 150 larvae/250 ml of water. These results suggest that a longer exposure or more powerful fumigant would kill all residual eggs in temporarily dry tree holes.

EVALUATION OF ALKANAMIDE FORMULATIONS AGAINST IMMATURE MOSQUITOES

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ABSTRACT

For field assessment, larvicidal and pupicidal N,N-dimethyltetradecanamide and N,N-dimethylhexadecanamide were formulated into emusifiable concentrates which were then evaluated in the laboratory against the fourth instars of *Culex quinquefasciates* Say. Formulations consisting of 20% N,N-dimethyltetradecanamide and 1% Triton X-100 in either Velsicol AR-60 or xylene demonstrated good larvicidal activity. Formulations containing 10% of N,N-dimethylhexadecanamide and 1 or 2% Tween 20 in xylene were also effective.

INTRODUCTION. Chemical and biological investigations of mosquito autoinhibitors led us to develop a new type of larvicides and pupicides collectively termed alkanamides (Hwang et al. 1979, 1980, Hwang and Mulla 1980). Of the various alkanamides, N,N-dimethylalkanamides were generally active against all stages of immature mosquitoes. Effective formulations of the N,N-dimethylalkanamides needed to be developed and tested in the laboratory before proceeding to undertake field trials.

Pesticide formulations include wettable powders, emulsifiable concentrates, dusts, granules, water-soluble concentrates, flowable concentrates, solutions, and microencapsulated preparations. For applying the active ingredients to aquatic mosquito breeding sources and because of the immiscibility of the amides in water, preparations of emulsifiable concentrates seemed to be the most appropriate for this purpose. An emulsifiable concentrate usually consists of a toxicant, an organic solvent or solvents, and a blend of emulsifiers. An emulsifier system is used for causing the concentrate to disperse spontaneously into small, stable droplets when mixed with water.

Here we report the larvicidal activity of formulations of N,N-dimethyltetradecanamide and N,N-dimethylhexadecanamide against the fourth instars of *Culex quinquefasciatus* Say.

MATERIALS AND METHODS. N,N-dimethyltetradecanamide, mp 27-28°C, and N,N-dimethylhexadecanamide, mp 38-39°C, were synthesized according to Hwang and Mulla (1980). All solvents and surfactants were obtained from commercial sources. Xylene is of reagent grade. Velsicol AR-60, an aromatic solvent, has a flash point of 104.4°C and a boiling range from 237 to 293°C. Minas diesel fuel (Chevron Oil Company) is mainly a mixture of aliphatic and aromatic hydrocarbons.

Pluronic L-121 is a nonionic difunctional block polymer composed of polypropylene glycol fatty acid derivatives. Triton X-100 is a condensation product of ethylene oxide with an alkylphenol. Tween 20 was composed of polyoxyethylene sorbitan monolaurate.

In preparing emulsifiable concentrates, the prescribed amount of an alkanamide was dissolved in a solvent. A surfactant was then added to the amide solution. The resulting mixture was shaken until a homogeneous formulation was obtained.

The emulsifiable concentrates thus prepared were evaluated against the fourth instars of *Cx. quinquefasciatus* according to the procedure reported elsewhere (Hwang et al. 1974). In short, the desired amount of an emulsifiable concentrate was added to test dishes, each containing 200-ml tap water and 20 larvae. Aqueous mixtures of the emulsifiable concentrate with various concentrations of the active compound were thereby prepared in the dishes. The bioassay tests were replicated and continued until adult emergence. Mortalities were read every other day. The dosage-response data were processed by a Compucorp model 145E computor for the log-probit analysis. The larvicidal activity was expressed in terms of LC_{50} and LC_{90} in ppm; however, in some cases, it was expressed as percent mortality at given concentrations.

RESULTS AND DISSCUSSIONS.—Table 1 shows the compositions of the formulations of N,N-dimethyltetradecanamide and their larvicidal activity against fourth-instar *Cx. quinquefasciatus*. In experiment 1, a 1% solution of N,N-dimethyltetradecanamide in acetone (formulation 1) showed considerable larvicidal activity with an LC_{50} of 2.3 ppm and an LC_{90} of 3.1 ppm. A 20% solution of the amide in Velsicol AR-60 without any surfactant (formulation 2) displayed about the same level of activity as formulation 1. When the amide was formulated as a 20% solution in Velsicol AR-60 with 1% Pluronic L-121 as a surfactant (formulation 3), the resulting formulation did not show increased activity. The substitution of Triton X-100 for Pluronic L-121 in formulation 3 yielded formulation 4 which did show enhanced activity. However, in the absence of the amide, a mixture of Velsicol AR-60 and Pluronic L-121 (formulation 5) caused 46% mortality, and a mixture of Velsicol AR-60 and Triton X-100 (formulation 6) caused 53% mortality. These mortalities caused by formulations 5 and 6 inevitably made the activity of fermulations 2, 3 and 4 unreliable. In both formulations 5 and 6, the concentration of Velsico AR-60 applied to the test dishes was about 20 ppm, which was equivalent to the highest concentration of

Table 1. Compositions of N,N-dimethyltetradecanamide formulations and their larvicidal activity against fourth-instar *Cx. quinquefasciatus*.

Formulation no.	Amide %	Solvent	Surfactant (%)	ppm LC_{50}	ppm LC_{90}	Concn. of amide or solvent (ppm)[1]	% Mortality
			Experiment 1				
1	1	Acetone	--	2.3	3.1	--	-
2	20	Velsicol AR-60	--	1.8	5.2	--	-
3	20	Velsicol AR-60	Pluronic L-121 (1)	1.8	4.6	--	-
4	20	Velsicol AR-60	Triton X-100 (1)	1.2	2.1	--	-
5	0	Velsicol AR-60	Pluronic L-121 (1)	-	-	20 (solvent)	46
6	0	Velsicol AR-60	Triton X-100 (1)	-	-	20 (solvent)	53
			Experiment 2				
7	20	Minas Diesel	--	2.8	3.9	--	-
8	20	Minas Diesel	Triton X-100 (1)	2.0	3.7	--	-
9	20	Velsicol AR-60	--	1.5	2.7	--	-
10	20	Velsicol AR-60	Triton X-100 (1)	1.3	1.7	--	-
11	20	Xylene	--	2.0	2.5	--	-
12	20	Xylene	Triton X-100 (1)	-	-	2 (amide)	100
13	0	Xylene	Triton X-100 (1)	-	-	12 (solvent)	0
14	0	Velsicol AR-60	Triton X-100 (1)	-	-	12 (solvent)	0

[1]The highest concentrations of the solvents at the final dilution in test dishes.

the solvent used in the formulations containing the amide. The mortality might be caused by the high concentration of Velsicol AR-60. Some petroleum products are well known to show some insecticidal activity (Pearce et al. 1948, Trammel 1965, Micks et al. 1967, 1968).

In experiment 2, the maximum concentration of the solvents was reduced to 12 ppm to moderate their toxic effects. A 20% solution of N,N-dimethyltetradecanamide in Minas diesel fuel (formulation 7) displayed the same degree of activity as the acetone solution (formulation 1) or the Velsicol AR-60 solution (formulation 2). The addition of 1% Triton X-100 into the Minas diesel fuel solution of the amide did not increase the activity of the resulting formulation 8. In this experiment, the Velsicol AR-60 solution of the amide (formulation 9) showed higher activity than that of formulation 2 in experiment 1. This type of discrepancy sometimes took place in bioassay tests. The addition of 1% Triton X-100 into formulation 9 resulted in obtaining formulation 10 which demonstrated improved larvicidal activity. Formulation 11 consisted of a 20% solution of the amide in xylene which was as active as other solutions. A formulation of 20% amide in xylene with 1% Triton X-100 (formulation 12) also showed good activity causing 100% mortality at the 2 ppm concentration of the amide. Formulations 13 and 14 did not induce any mortality probably due to the low concentration of the solvent used in this experiment. The lack of activity by formulations 13 and 14 confirmed that the toxicity shown by formulations 9, 10, 11 and 12 was caused by the amide.

As previously reported (Hwang et al. 1980, Hwang and Mulla 1980), N,N-dimethylhexadecanamide did not display any measurable activity at or below the 10 ppm concentration (Table 2, experiment 3, formulation 15); however, a solution of the amide in Velsicol AR-60 (formulation 16) showed some activity. Although the incorporation of 1% Pluronic L-121 in the Velsicol AR-60 solution (formulation 17) did not enhance the activity, the addition of Triton X-100 indeed increased the activity of the resulting formulation 18. These results should be interpreted with care due to the toxic effects shown by the solvent alone in formulations 19 and 20.

In experiment 4 (Table 2), a 10% solution of the amide in xylene (formulation 21) displayed some activity which was, nonethless, higher than that of the acetone solution (formulation 15). Formulations containing 10% solution of the amide in xylene with 1 and 2% Tween 20 (formulatins 22 and 23, respectively) considerably increased the activity. When the concentration of Tween 20 was increased to 4%, the resultant formulation 24 was not as effective as formulations 22 and 23. In the absence of the amide, a mixture of xylene (90 ppm) and Tween 20 (1%) did not induce any mortality. It clearly indicated that it was the amide, not the solvent, that caused mortality in the mosquito larvae.

We have thereby prepared several effective emulsifiable concentrates which are being assessed under field conditions. Typical effective formulations are as follows:

1) Formulation 10: 20% N,N-dimethyltetradecanamide and 1% Triton X-100 in Velsicol AR-60.
2) Formulation 12: 20% N,N-dimethyltetradecanamide and 1% Triton X-100 in xylene.
3) Formulations 22 and 23: 10% N,N-dimethylhexadecanamide and 1 and 2% Tween 20 in xylene.

Table 2. Composition of N,N-dimethylhexadecanamide formulations and their larvicidal activity against fourth-instar *Cx. quinquefasciatus*.

Formulation no.	Amide %	Solvent	Surfactant (%)	ppm LC_{50}	ppm LC_{90}	Concn. of solvent (ppm)[1]	% Mortality
			Experiment 3				
15	1	Acetone	--	>10	>10	--	-
16	20	Velsicol AR-60	--	2.5	6.8	--	-
17	20	Velisicol AR-60	Pluronic L-121 (1)	3.6	4.0	--	-
18	20	Velsicol AR-60	Triton X-100 (1)	1.6	2.9	--	-
19	0	Velsicol AR-60	Pluronic L-121 (1)	-	-	20	46
20	0	Velsicol AR-60	Triton X-100 (1)	-	-	20	53
			Experiment 4				
21	10	Xylene	--	7.3	13.6	--	-
22	10	Xylene	Tween 20 (1)	2.4	3.6	--	-
23	10	Xylene	Tween 20 (2)	2.0	3.5	--	-
24	10	Xylene	Tween 20 (4)	3.5	5.1	--	-
25	0	Xylene	Tween 20 (1)	-	-	90	0

[1]The highest concentrations of the solvents at the final dilution in test dishes.

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THE OCCURRENCE OF p-CHLOROANILINE AND p-CHLOROPHENYLUREA FROM THE DEGRADATION OF DIFLUBENZURON IN WATER AND FISH

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ABSTRACT

When diflubenzuron was applied to a pasture, a lake, and a pond, the parent compound degraded in water to p-chlorophenylurea. Small amounts of p-chloroaniline were apparent but this material was only a minor degradation product. Fish accumulated diflubenzuron from water up to 160X, but the tissue concentration declined steadily with time. The fish tissues contained moderate amounts of p-chlorophenylurea but only trace levels of p-chloroaniline.

INTRODUCTION.–Research to define the potential of diflubenzuron (Dimilin™ or TH6040) as a mosquito control agent in California was initiated in 1973. Since then extensive studies have been conducted to determine its efficacy against target dipterans (Schaefer et al. 1974, 1975, 1976, 1977), its effects on nontarget organisms (Apperson et al. 1978; Miura and Takahashi 1974, 1976, Takahashi and Miura 1975) and its persistence in the environment (Schaefer and Dupras 1976, 1977; Schaefer et al. 1979). A petition for the registration of this insecticide is pending.

Generally, diflubenzuron is highly effective against mosquitoes, midges and gnats, has only short-term effects against sensitive nontargets and is not persistent in habitats where it would be applied. However, one established metabolite formed from diflubenzuron is p-chloroaniline (Figure 1). This compound is classified as a mutagen by the National Cancer Institute and the Cancer Assessment Group of EPA. Data is needed to establish the extent of conversion of diflubenzuron to p-chloroaniline in the habitats where it would be used by mosquito abatement districts. This study was undertaken to quanitatively measure the conversion of diflubenzuron to p-chloroaniline in the waters of typical breeding habitats (a pasture, a pond and a lake) and in the edible tissues of fish, since accumulation of diflubenzuron from water into fish tissues has been established (Schaefer et al. 1979).

MATERIALS AND METHODS.–On August 20, 1979 a 0.2 ha experimental pasture in Kern County was treated with 0.08 kg AI/ha diflubenzuron, using a 25% WP formulation applied in water. Before treatment, at 1-hr, and at 1, 2, 3 and 4 days after treatment, two 600 ml water samples were collected for analysis.

In order to study the degradation of diflubenzuron in the water of Clear Lake, a series of glass tubes (Kimax® beaded process pipe-15.2 cm i.d. x 3.0 m) were inserted through the water column (1.7 m depth) and into the mud bottom (ca. 30 cm depth). The tubes were anchored to a pier (Figure 2) and covered with ventilated acrylic resin (Lucite®) covers to avoid deposition of excrement by birds. On June 18, 1979 the tubes were treated with 0.5% (AI) sand granules with amounts calculated to give 0, 5, 25 and 50 ppb diflubenzuron. Water samples (600 ml) were collected from each tube before treatment, at 3 hr and at 7, 14 and 21 days following treatment. On August 22, 1979 another set of tubes, as above, was treated with diflubenzuron 25% WP to give calculated concentrations of 0, 20 and 200 ppb. In the latter test, the 25% WP was slurried in water, poured into the tube and then mixed into the upper two-thirds of the water column. Water samples (600 ml) were taken from each tube before treatment, at 1 hr and at 7 and 14 days following treatment.

On July 20, 1979 an experimental pond (12 x 17.5 x 1.7 m) in Lake County was treated with 0.5% (AI) sand granules to give a calculated concentration of 200 ppb diflubenzuron. The pond contained unknown numbers of bluegill (*Lepomis macrochirus*). In order to ensure sufficient numbers of fish for sampling, bluegill were collected from another pond and these were placed in a live car (61 x 61 x 61 cm) in the pond to be treated. Fish were collected for residue analysis from the pond by hoop net (73.7 cm diameter, 2.54 cm apertures), by seine 9.1 x 1.0 m, 2.0 mm apertures) and also from the live care, at intervals up to 19 days posttreatment. Fish were placed in plastic bags which were sealed in cans and then frozen and held at -20°C, or below, until analysis. Water samples (600 ml) from the pond were taken from the top and mid-depth levels before treatment and at 3 hr, and at 5, 12 and 19 days posttreatment.

The separation of diflubenzuron, p-chloroaniline and p-chlorophenylurea was accomplished by pH adjustments and partitioning into organic solvents (Diprima 1976). Diflubenzuron and p-chlorophenylurea were then hydrolyzed under acid conditions to p-chloroaniline; the latter was derivatized with heptafluorobutyric anhydride to the anilide and then analyzed by gas chromatography using an electron capture detector (Rabenort et al. 1978).

All water samples were stabilized immediately after collection with HCL (<pH 2.0) and the addition of hexane. They were transported to the laboratory and extracted on the same day.

In order to determine the possible loss of diflubenzuron (AI) from water through crystallization, a glass column (4.6 x 60.0 cm) containing 900 ml of Clear Lake water was treated

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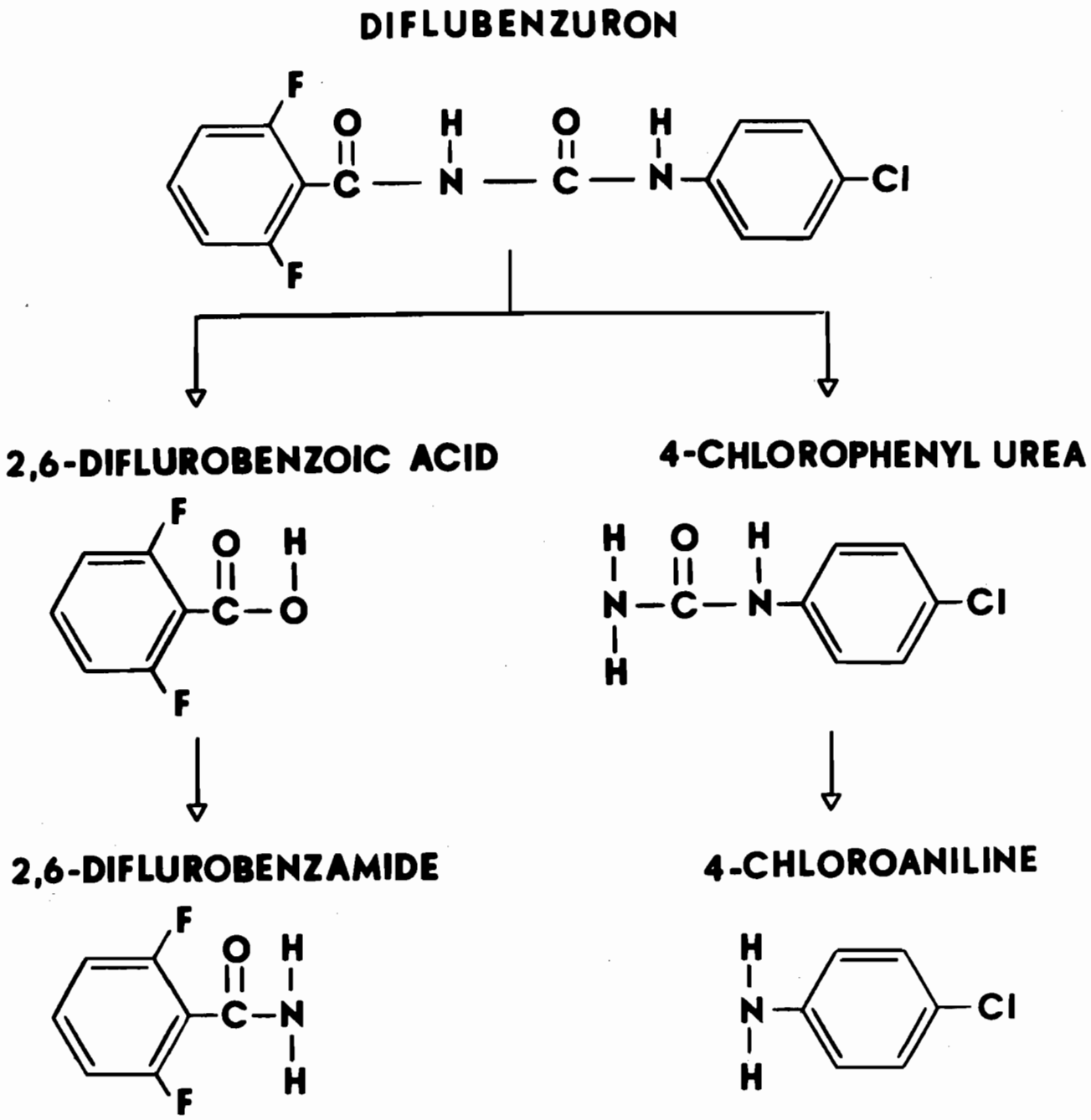

Figure 1. Degradation pattern for diflubenzuron.

with 25% WP to give a calculated concentration of 200 ppb. After 1 hr the upper, mid and lower 300 ml portions of the water column were collected and analyzed for diflubenzuron. The fritted glass disc at the bottom of the column was rinsed with acetone, this was collected and analyzed. The walls of the column were washed with acetone and this was also analyzed. The experiment was conducted in the laboratory at room temperature (22°C).

RESULTS AND DISCUSSION.--Recovery from water - - Pretreatment water samples from each study site were fortified with 10 ppb diflubenzuron, p-chlorophenylurea and p-chloroaniline (separately). In all cases recoveries were very high (>95%) and therefore the observed data were not corrected for loss during analysis.

Degradation in pasture water - - The pasture plot (treated with 0.09 kg AI diflubenzuron) was ca. 30 cm deep at the time of treatment; the pH of the water was 8.2 and the temperature was 23°C. Afternoon water temperatures of the pasture plots reached highs of 38-40°C during this period. The degradation of diflubenzuron in this habitat is shown (Table 1) for a 4 day period, after which the water level was too low to allow sampling. It is apparent that hydrolysis to p-chlorophenylurea is the primary means of degradation; this is consistent with other studies (Schaefer and Dupras 1976; Ivie et al. 1980). It is also

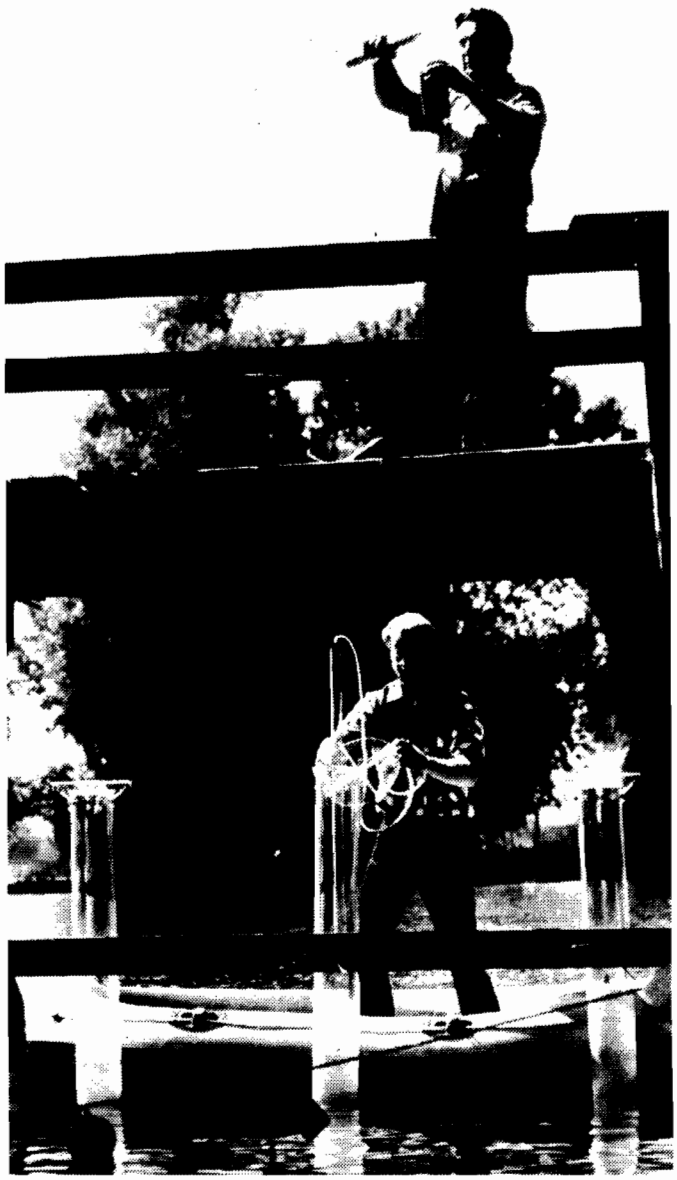

Figure 2. Sampling glass tubes anchored in Clear Lake (photograph by J. K. Clark).

Table 1.--Residues of diflubenzuron, p-chlorophenylurea and p-chloroaniline in pasture water following treatment of 0.02 ha pasture plot with 0.09 kg/ha diflubenzuron on 8/20/79.

Sample time	Concentration in water (ppb)		
	diflubenzuron	p-chlorophenylurea	p-chloroaniline
pre	N.D.[1]	N.D.[2]	N.D.[3]
1 h	15.8	5.9	0.2
	24.8	5.4	1.1
	20.3[4]	5.6	0.6
1 d	20.1	5.1	0.5
	23.1	4.3	0.3
	21.6	4.7	0.4
2 d	10.3	7.5	2.6
	16.8	6.3	1.6
	13.6	6.9	2.1
3 d	3.1	7.2	2.0
	5.7	6.8	0.6
	4.4	7.0	1.3
4 d	1.3	7.4	3.7
	3.4	7.1	1.4
	2.4	7.2	2.6

[1]N.D. = <0.2 ppb.
[2]N.D. = <0.1 ppb.
[3]N.D. = <0.1 ppb.
[4]Average.

apparent that small amounts of p-chloroaniline form, but that it is not a major breakdown product.

Degradation in Clear Lake water - - The studies of diflubenzuron degradation in glass tubes in Clear Lake are summarized in Tables 2 and 3. The pH of Clear Lake water (adjacent to and within the tubes) varied from 7.6 to 8.6 during these tests and the water temperatures were ca. 27°C on July 25, 1979, and 22°C on 8/22/79. The concentrations of diflubenzuron in the water following both treatments (the first with 0.5% sand granulares and the second with 25% WP) were much lower than the calculated values. However the same degradation pattern was observed as in the pasture plot, i.e. significant formation of p-chlorophenylurea with only small amounts of p-chloroaniline being apparent.

Degradation in water of a Lake County pond - - Table 4 summarizes data on the degradation of diflubenzuron applied to a Lake County pond. The highest concentration of diflubenzuron found was only 5 ppb (5 days), while the total amount applied on the sand granules was calculated to give 200 ppb. Concentrations of diflubenzuron in the upper and middle levels of the pond were similar at each sampling time, indicating an even dispersal of the active ingredient that was present. The degradation pattern in this pond to p-chlorophenylurea with only small amounts of p-chloroaniline is similar to the results from the other habitats studied.

Uptake and Degradation in fish tissues in the Lake County pond. - - The maximum accumulation from water into bluegill tissues was ca. 160X (5 to 800 ppb) and there was a steady decline as the study period progressed (Table 5). Tissue levels of diflubenzuron were approximately equal between fish collected from the pond and those held in the live car for days 1 and 3; thereafter, the residues were higher in fish from the live car (days 5-19). It is possible that free-living fish were able to metabolize diflubenzuron faster than those confined in the live car. Fish tissues also contained p-chlorophenylurea and its concentration remained moderately steady throughout the 19 day sampling interval. Only small amounts (most values just above detection limit) of p-chloroaniline were apparent and there was no evidence that it accumulated in fish tissues.

Table 2.- Residues of diflubenzuron, p-chlorophenylurea and p-chloroaniline in water following treatment of glass tubes in Clear Lake on 7/18/79.

Sample time	Tube	Treatment (ppb)	Concentration (ppb)		
			diflubenzuron	p-chlorophenylurea	p-chloroaniline
3 h	1	0	N.D.[1]	N.D.[2]	N.D.[3]
3 h	4	5	2.7	N.D.	N.D.
3 h	5	25	9.9	0.1	N.D.
3 h	6	50	10.4	0.2	N.D.
7 d	1	0	N.D.	N.D.	N.D.
7 d	4	5	1.3	0.2	N.D.
7 d	5	25	1.8	0.8	0.1
7 d	6	50	6.0	0.7	0.1
14 d	1	0	N.D.	N.D.	N.D.
14 d	4	5	1.2	0.4	0.1
14 d	5	25	2.7	1.9	0.1
14 d	6	50	2.0	1.9	0.1
21 d	1	0	N.D.	N.D.	N.D.
21 d	4	5	1.1	0.3	0.1
21 d	5	25	3.2	0.5	0.1
21 d	6	50	4.2	2.3	0.1

[1]N.D. = < 0.2 ppb.
[2]N.D. = < 0.1 ppb.
[3]N.D. = < 0.1 ppb.

Table 3.- Residues of diflubenzuron, p-chlorophenylurea and p-chloroaniline in water following treatment of glass tubes in Clear Lake on 8/22/79.

Sample time	Tube	Treatment (ppb)	Concentration (ppb)		
			diflubenzuron	p-chlorophenylurea	p-chloroaniline
Pre	1	0	N.D.[1]	N.D.[2]	N.D.[3]
Pre	2	20	N.D.	N.D.	N.D.
Pre	3	200	N.D.	N.D.	N.D.
1 h	1	0	N.D.	N.D.	N.D.
1 h	2	20	15.3	7.2	N.D.
1 h	3	200	44.5	17.0	0.9
7 d	1	0	N.D.	N.D.	N.D.
7 d	2	20	7.7	8.2	0.3
7 d	3	200	19.1	10.0	0.5
14 d	1	0	N.D.	N.D.	N.D.
14 d	2	20	0.5	4.9	0.2
14 d	3	200	29.8	7.4	0.9

[1]N.D. = < 0.2 ppb.
[2]N.D. = < 0.1 ppb.
[3]N.D. = < 0.1 ppb.

Table 4.- Residues of diflubenzuron, p-chlorophenylurea and p-chloroaniline in pond water following treatment with diflubenzuron on 7/20/79.

Sampling time	Sampling depth	Concentration (ppb)		
		diflubenzuron	p-chlorophenylurea	p-chloroaniline
Pre	top	N.D.[1]	N.D.[2]	N.D.[3]
Pre	middle	N.D.	N.D.	N.D.
3 h	top	1.3	0.6	N.D.
3 h	middle	1.0	0.1	N.D.
5 d	top	5.1	3.1	0.2
5 d	middle	4.6	2.4	0.4
12 d	top	2.0	1.3	0.1
12 d	middle	1.5	1.2	0.1
19 d	top	1.1	2.4	0.1
19 d	middle	1.5	3.1	0.2

[1]N.D. = < 0.2 ppb. [2]N.D. = < 0.1 ppb. [3]N.D. = < 0.1 ppb.

Table 5.- Residues of diflubenzuron, p-chlorophenylurea and p-chloroaniline in tissues of bluegill sunfish from a Lake County pond treated with diflubenzuron on 7/20/79.

Sample time	Collection method	Concentration (ppb)		
		diflubenzuron	p-chlorophenylurea	p-chloroaniline
Pre	Seine	N.D.[1]	N.D.[2]	N.D.[3]
3 h	Hoop Net	118.9	1.6	0.8
1 d	Seine	812.5	8.0	2.1
	Live Car	748.5	7.4	0.9
3 d	Live Car	591.9	17.2	1.1
	Seine	602.5	58.4	2.5
4 d	Live Car	589.2	68.0	2.0
	Seine	276.0	17.6	2.1
5 d	Hoop Net	252.1	9.6	1.3
	Live Car	546.8	39.6	1.3
6 d	Live Car	599.8	41.6	1.3
	Hoop Net	289.3	25.6	N.D.
7 d	Live Car	477.8	31.6	2.3
	Seine	281.3	30.8	2.1
8 d	Live Car	525.5	29.4	4.8
	Hoop Net	254.8	23.2	1.8
10 d	Live Car	302.6	36.8	1.7
	Hoop Net	169.9	25.6	0.9
12 d	Live Car	196.4	30.8	1.1
	Seine	153.9	32.4	0.9
14 d	Seine	109.4	28.0	0.9
17 d	Seine	104.0	30.8	0.9
19 d	Live Car	86.0	32.6	1.3
	Seine	57.9	33.2	0.9

[1]N.D. = < 2.2 ppb. [2]N.D. = < 1.2 ppb. [3]N.D. = < 0.8 ppb.

Loss of diflubenzuron from water by precipitation - - When diflubenzuron was added to a cylindrical glass column containing Clear Lake Water at 200 ppb and then allowed to stand for 1 hr, only 46% of the active ingredient applied was found in the water, and this was evenly dispersed with depth. The fritted-glass disc on the bottom of the column had 45% and the remaining 9% was recovered from the tube wall. Thus, in this simple laboratory experiment less than one half of the diflubenzuron applied was in the aqueous phase even though the water solubility is ca. 200 ppb. Diflubenzuron, and other substituted benzamides, have peculiar solubility-crystallization properties and as one attempts to approach the solubility limit the active ingredient is likely to form small crystals and precipitate. This may explain the problems encountered in trying to obtain high (relative to the solubility limit) concentrations of diflubenzuron in field waters. Earlier field applications to large water bodies (Apperson et al. 1978) at relatively low doses (2.5 - 10.0 ppb) resulted in measured concentrations very close to the calculated values.

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A PROGRESS REPORT ON THE IMPACT OF JOINT, ROTATIONAL, AND SEQUENTIAL USE OF INSECTICIDES ON THE DEVELOPMENT OF RESISTANCE BY MOSQUITOES

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Resistance is traditionally countered by the following three principal measures, which are usually introduced in the sequence shown:

- More frequent applications of the insecticide
- Application at higher dosages
- Substitution of the insecticide with an alternative chemical whose toxicity is not affected by cross resistance.

As the number of available chemicals was gradually decreased due to the development of resistance, it became imperative that alternative procedures of insecticide usage be formulated that could extend their useful life. To this end, three main measures have been considered:

- Use of an insecticide with a synergist
- Use of mixtures of insecticides
- Use of insecticides in a rotational sequence.

Commercial synergists are still limited to piperonyl butoxide, which is suitable only for indoor use. In this brief paper, we report on the progress of research that is being carried out in our laboratory with mixtures and rotations of chemicals as possible countermeasures for resistance.

The principle use of mixtures assumes that genes for resistance to each chemical in the mixture are so rare that the possibility of their being present together in the same insect is very remote. Thus, a mosquito that is capable of surviving one insecticide will be killed by the other insecticide in the mixture. This requirement makes it almost mandatory that the constituents of the mixture have dissimilar modes of action and that they be subject to dissimilar detoxication pathways.

In rotational applications, two or more different insecticides are selected and are applied individually according to a predetermined schedule. If one chemical is repeatedly applied and is not replaced until resistance begins to develop, the system is called "sequential." The challenge in this case is to determine the optimal sequence of use of the available chemicals.

The principle of rotation and sequential usage is based on the premise that resistance to a given chemical declines in the absence of selection pressure from that chemical, due to the lower reproductive fitness of the resistant individuals. Assuming that this decline is relatively fast and that the same chemical is used at sufficiently wide intervals, resistance should theoretically not evolve, or should remain stationary.

The hoped for results of a system of rotational use of four compounds, I-IV, in three cycles over a period of 12 generations, are shown diagrammatically in Figure 1. Resistance to each compound rises slightly in the generation following the one in which it is used (generations 1, 5, and 9 in the case of compound I), but declines in the intervening three generations during which the unrelated compounds, II, III and IV are used.

In examining the literature, we find that both principles, mixtures and rotations, have been tried previously, mainly on agricultural pests, and that the results have been negative, positive, or inconclusive. In retrospect, the problem appears to have been the lack of an adequate choice of chemicals that would meet the requirements for independent modes of action and dissimilar pathways of detoxication.

Recent discoveries of new toxic agents of disparate structures and modes of action, such as juvenile hormone mimics, chitin inhibitors, *Bacillus thuringiensis* toxin, pyrethroids, and certain "derivatized" carbamates and organophosphates, have prompted us to re-examine the validity of these approaches. We should stress that this is long-term research, and that certain aspects, especially rotations, require selection for several generations before definitive conclusions can be reached.

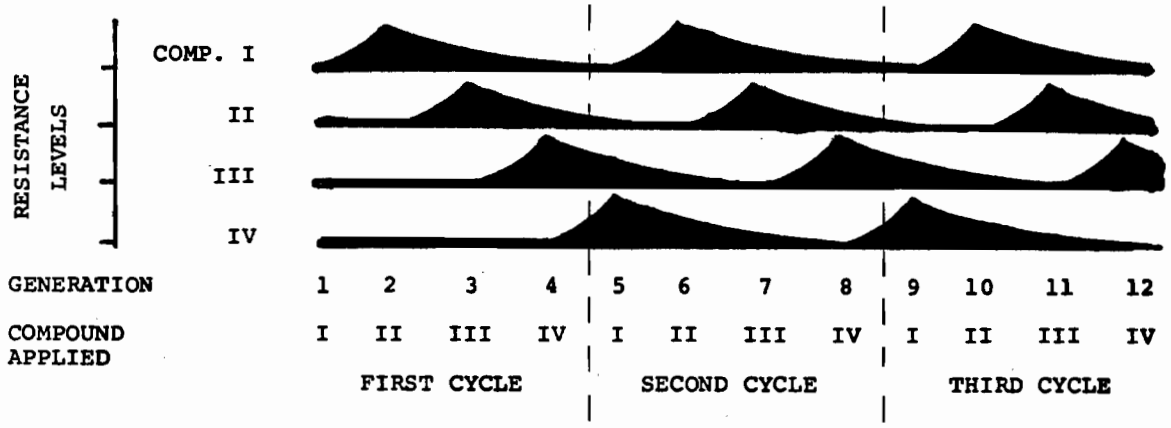

Figure 1. Diagrammatic representation of expected fluctuation of resistance to compounds I, II, III and IV employed consecutively in three cycles during 12 generations of an insect population (Adapted from Georghiou 1980).

We have employed three insecticides: temephos, propoxur and permethrin. Each of these is known to induce high resistance to the others. To begin with, we synthesized a strain of *Culex quinquefasciatus* by incorporating 2% of the genome of three resistant strains, Temephos-R, Propoxur-R and Permethrin-R, into a susceptible strain of this species. This was accomplished by repeatedly backcrossing each resistant strain to the susceptible strain and subsequently combining the resulting three lines. Subcolonies from this synthetic strain were selected with each insecticide applied either singly or jointly, as well as in various rotational sequences.

Although this study is still incomplete, a number of trends are emerging:

Each strain that was selected with a single chemical promptly developed resistance toward that chemical thus confirming that the factors for resistance from strains Temephos-R, Propoxur-R and Permethrin-R had indeed been incorporated in the synthetic strain. There was no cross resistance evident toward the other two chemicals.

Upon suspension of selection pressure, the level of resistance began to decline. This regression was faster in the temephos-selected strain, less so in the permethrin strain, and least in the propoxur strain.

Selection by combinations involved temephos + propoxur, temephos + permethrin, propoxur + permethrin, and temephos + propoxur + permethrin. After selection for nine consecutive generations, a significant degree of resistance to propoxur evolved in all strains in which propoxur was included as a selecting insecticide; however, resistance to temephos and permethrin was inhibited by the combinations.

Sequential usage consisted of selection of a strain by one chemical until significant resistance had appeared, at which time it was replaced by a second chemical. After resistance to this had also appeared, it was substituted by the third chemical. The following sequences were studied:

Temephos - Propoxur - Permethrin
Temephos - Permethrin - Propoxur
Propoxur - Temephos - Permethrin
Propoxur - Permethrin - Temephos
Permethrin - Temephos - Propoxur
Permethrin - Propoxur - Temephos

The most interesting observation emerging from the results of sequential usage was the rapid regression of resistance to temephos or permethrin when selection by one of these two chemicals was followed by selection by the other. Thus, temephos resistance regressed rapidly when temephos was replaced by permethrin as the selecting agent, and vice versa, and this regression appeared to be somewhat faster than was observed in strains that were removed completely from selection pressure. This reciprocal relationship did not appear to exist between propoxur and permethrin or between propoxur and temephos. Whether a negative relationship exists between permethrin and temephos resistance is now being examined by genetic tests.

The concept of use of insecticides in mixtures, rotations or in optimal sequences may be limited in many instances by economic or practical considerations. However, where control practices are applied on a large scale and are centrally coordinated, this concept might present a distinct advantage as a means of delaying or averting the evolution of resistance.

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Table 1. Resistance experiments with pesticide combinations.

Author	Site	Pest	Chemicals	Benefit
1961 Asquith	(Field)	*Panonychus ulmi* *Tetranychus urticae*	Dicofol + Tetradifon	(+)
1960 Burden et al.	(Lab)	*Blattella germanica*	Chlordane + Malathion	(+)
1964 Asquith	(Field)	*Panonychus ulmi*	Dimethoate + Dicofol	(~)
			Dimethoate + Tetradifon	(~)
1973 Ozaki et al.	(Lab)	*Laodelphax striatellus*	Malathion + Carbaryl	(+)
			Malathion + Tsumacide	(+)
			Fenitrothion + Carbaryl + Methomyl	(+)
1976 Pimentel and Bellotti	(Lab)	*Musca domestica*	NaCl; $Mg(NO_3)_2$; KOH; $CuSO_4$; $NH_4H_2PO_4$; $H_3C_6H_5O_7H_2O$	(+)

Table 2. Resistance experiments with pesticide rotations.

Author	Site	Pest	Chemicals	Benefit
1958 Jeppson et al.	(Field)	*Panonychus citri*	Demeton/Ovex	(-)
			Demeton/Aramite	(+)
1961 Asquith	(Field)	*Panonychus ulmi* *Tetranychus urticae*	Dicofol/Tetradifon	(+)
1960 Burden et al.	(Lab)	*Blattella germanica*	Chlordane/Malathion	(+)
1962 Graves & Roussel	(Field)	*Anthonomus grandis*	Toxaphene/DDT	(+)
1967 Graves et al.	(Lab)	*Anthonomus grandis*	Endrin/Azinphosmethyl	(+)
1973 Ozaki et al.	(Lab)	*Laodelphax striatellus*	Malathion/Carbaryl	(≃)

NEW SYNTHETIC PYRETHROIDS – EFFECTIVE MOSQUITO LARVICIDES

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Mosquito fauna in California is quite diverse and the many species found in this State utilize a variety of habitats as breeding sources and shelter sites. To manage the populations of such diverse fauna in a variety of habitats, it is necessary that a multitude of management techniques be developed for effective mosquito control.

The use of chemical larvicides play an important role in the overall mosquito control programs in California. However, the development of widespread resistance to many of the conventional synthetic larvicides has resulted in many failures using the common and currently labelled larvicides. To overcome this difficulty, continuous and systematic effort has to be directed toward the development of more specific, effective and relatively safe larvicides. As a result of concerted effort in this direction, research on some of the newer synthetic pyrethroids has been expanded in the last two or three years.

During the past 20 years a fairly large number of synthetic pyrethroids (related to the natural pyrethrins) have been studied for insect control. However, practically all of these manifested high level of activity against adult mosqitoes while showing little or no activity against mosquito larvae in aquatic habitats. Since most of the mosquito control operations in California are aimed at the immature mosquitoes, the availability of synthetic pyrethroids provided no relief for the OP resistant mosquitoes.

However, very recently a number of newer synthetic pyrethroids have become available for experimentation against insect pests. These pyrethroids, unlike their older relatives, are photostable and are highly effective against mosquito larvae. An additional bonus effect of these compounds is their high level of activity against mosquito pupae, with a little increase in the effective larvicidal rates; pupal populations are also controlled effectively. This unique feature of the synthetic pyrethroids renders this new class of compounds highly desirable in mosquito control programs. Using the synthetic pyrethroids, one can delay the treatment for 2-3 days, a period after which the extent of the breeding sources may be greatly diminished, thus requiring less effort and material for application.

In the earlier stages of our research we found that several synthetic pyrethroids were highly effective in the laboratory, but due to their photo and environmental sensitivity, one of these materials yielded satisfactory larval control at practical rates in the field. As a follow-up of this systematic research; recently several synthetic pyrethroids have become available for evaluation against mosquito larvae. Among the new generation of synthetic pyrethorids there are several compounds that manifested very high level of activity (Table 1). The most

Table 1.–Activity of pyrethrins and synthetic pyrethroids against mosquito larvae in the laboratory.

Synthetic pyrethroid	24-hr LC_{90} (ppb) to mosquito species	
	Cx. quinquefasciatus	*Ae. nigromaculis*[1]
allethrin	30 - 40	-
bioresmethrin	3.0	-
decamethrin	0.045	0.5 - 0.9
dimethrin	50 - 65	-
fenvalerate	7 - 8	10 - 12
neopyanmin	10 - 15	10 - 14
permethrin	2 - 3	0.8 - 1.0
pyrethrins	15 - 20	-
resmethrin	5 - 6	8 - 9
sumithrin	10 - 20	5 - 10
ethyl parathion	2 - 5	70 - 700
methyl parathion	3 - 8	600 - 700

[1]OP resistant strain from San Joaquin Valley.

effective of these are decamethrin, permethrin and sumithrin. The LC_{90} (lethal concentration killing 90% of the exposed population) of the first mentioned is in the range of 0.045-0.9 parts per billion. This is the most effective compound ever tested against mosquitoes. Permethrin and sumithrin are also highly effective, especially against OP-resistant mosquitoes (see Table 1).

Although resmethrin showed a very high level of activity against mosquito larvae in the laboratory (see Table 1), it failed to control larval mosquitoes at practical dosages in the field (Table 2). As in the laboratory, decamethrin yielded complete control of larvae at the very low rates of 0.00025-0.005 lb/acre (less than a teaspoonful/acre), under a variety of field conditions. Permethrin and sumithrin, although producing good results at higher rates, are still considered to have excellent activity against field populations of mosquitoes (see Table 2).

Cx. tarsalis and *Cs. inornata* both were highly susceptible to decamethrin. It took from 0.00025-0.0005 lb/A of active ingredient to produce 90-100% control of these mosquitoes. This rate of application is equivalent to an amount of less than half a gram of ai per acre. Bioresmethrin and resmethrin were 1000-2000 times less active than decamethrin under field conditons.

Against *Ps. columbiae*, decamethrin was not as effective as against the above species. It took almost 4-10 times as much material to control OP-resistant populations of *Ae. nigromaculis* and *Ps. columbiae*, but still, decamethrin gave excellent control of floodwater mosquitoes at the rates of 0.001-0.005 lb/acre (less than 2.5g/acre).

Fenvalerate, permethrin and sumithrin were effective in the range of 0.025-0.20 lb/acre. The latter two materials were essentially the same in their efficacy against mosquito populations.

As mentioned earlier, the synthetic pyrethroids, unlike many other mosquito larvicides, have excellent activity against mosquito pupae. From the standpoint of mosquito control programs, this attribute of the synthetic pyrethroids has numerous practical implications. The synthetic pyrethroids if developed and cleared for use in mosquito control programs will definitely mitigate some of the current difficulties encountered in mosquito control programs in California and elsewhere.

It should be pointed out, that mosquitoes will very likely develop resistance to the synthetic pyrethroids, as they have to other synthetic larvicides. It is therefore very advisable to limit the use of these new compounds to situations where other larvicidal compounds are ineffective. Some of the synthetic pyrethroids possess a high level of toxicity to game and warmwater fishes. Therefore, great caution has to be exercised in the use of synthetic pyrethroids in large-scale mosquito control programs.

Table 2. Efficacy of synthetic pyrethroids against various species of mosquitoes under field conditions.

Mosquito Species	Habitat and Location	Synthetic pyrethroid and formulation	Lbs/A yielding 90 - 100% control
Cx. tarsalis	Ponds -	ABG-6070 EC 4	0.05 - 0.10
Cs. inornata	Riverside &	decamethrin EC 0.21	0.00025 - 0.0005
	Coachella Valley	bioresmethrin EC 1	0.25 - 0.50
		fenvalerate EC 2.4	0.025 - 0.05
		permethrin EC 0.8	0.01 - 0.025
		resmethrin EC 2	0.50 - 1.0
		sumithrin EC 2	0.05 - 0.10
Ps. columbiae	Irrigated pastures	decamethrin	0.001 - 0.005
	Palo Verde Valley	fenvalerate EC 2.4	0.1 - 0.20
		permethrin EC 0.8	0.025 - 0.05
Ae. nigromaculis[1]	Irrigated pastures	decamethrin EC 0.21	0.0025 - 0.005
	San Joaquin Valley	fenvalerate EC 2.4	0.025 - 0.05
		permethrin EC 2.0	0.01 - 0.025
		EC 3.2	0.025
		WP	0.01 - 0.025
		sumithrin EC 2	0.025 - 0.05

[1]OP-resistant. Those populations with high OP-resistance wree more susceptible to these pyrethroids than less resistant populations.

THE STORAGE STABILITY OF ALTOSAND® DURING THE SUMMER MONTHS IN CENTRAL CALIFORNIA

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ABSTRACT

When Altosand® is prepared according to the manufacturer's instructuions and stored in covered but uncooled facilities during the hot summer months, a loss of active ingredient will occur. This loss is generally 10% or less after 2 weeks, 10-15% by 4 weeks and 20% by 6 weeks. During periods of very high temperatures (hottest periods of year), a loss rate of 5% per week should be expected. Storage of Altosand under these conditions has no apparent effect on the rate of release of the active ingredient into water.

INTRODUCTION. During the past several years many Mosquito Abatement Districts have been applying Altosid SR 10® using a sand formulation-Altosand®. The method for on-site preparation of the sand is given by the manufacturer. To prepare a 100-pound (45 Kg) batch:

1) Measure the time required for a level funnel full of sand to empty.
2) Into a rotating-type mixer, place 96 pounds (43.2 Kg) of dry (20-45 mesh) sand. While the mixer is rotating, slowly pour 2 pounds (0.9 Kg) or 30 fluid ounces (840 ml) of Altosid SR 10 into the sand. (If better wetting is required, the Altosid SR 10 may be diluted in up to an equal volume of water.)
3) Mix until the sand is uniformly coated with the Altosid SR 10 (usually 5 to 10 minutes).
4) Stop the mixer and add 2 pounds (0.9 Kg) of Hi-Sil 233 (silicone dioxide). Cover the mixer to reduce dust problems. Start the mixer and run for approximately five minutes. (The quantity of Hi-Sil 233 necessary to achieve a dry, free-flowing mixture will vary depending on the particle size distribution and moisture on the sand.)
5) Compare the flow rate of the Altosand mixture with the untreated sand. Add more Hi-Sil if it flows significantly slower and reduce the amount of Hi-Sil in subsequent batches if the mixture flows at the same or a faster rate and is excessively dusty.

MATERIALS AND METHODS.–The batches of Altosand studied were prepared by personnel of the Fresno MAD during the regular course of their operational activities.

Two storage tests were conducted at the Fresno Laboratory. The sand formulations were freshly prepared at the Fresno MAD and batches were then divided into 2 lots; one was stored in an air-conditioned laboratory and the other in an outside metal shed resting on asphalt (without any cooling unit). All samples were placed in plastic bags held tightly closed by rubber bands throughout the storage intervals.

This method yields a formulation having a theoretical concentration of 0.20% active ingredient. The storage characteristics of this formulation during the hot summer months in the Central Valley are not known and Mosquito Abatement Districts have tried to use each batch within a few days of the date of preparation. Therefore a study was made to define (1) the rate of loss of the active ingredient during outside storage during the summer and (2) the effect of storage on the release-rate.

Two chemical analyses were performed. First, to determine the concentration of active ingredient of each lot, a 10 gm Altosand sample was placed in a 100 ml flask and hexane was added; the solvent was mixed with the sand and then an aliquot of the hexane was analyzed by gas-liquid chromatography. Second, to determine the release-rate of the active ingredient of each lot, 200 mg of sand was placed into 4L of water and allowed to stand for 4 hr. Two 600 ml samples of the water were then measured and 200 ml acetonitrile was added to each. After mixing, 100 ml of petroleum ether was added and the active ingredient partitioned into the upper phase. The petroleum ether phase was then dried over Na_2SO_4 and concentrated to ca. 0.5 ml in a rotoevaporator; hexane was added to a final volume of 5 ml for each sample. These were then subjected to analyses by gas-liquid chromatography. The latter was accomplished using a ¼-inch diam. and 6 ft. long Pyrex column packed with 3% OV-101 on 100/120 mesh Chromosorb W-AW-DMCS. A Hewlett Packard Model 5750 gas chromatograph equipped with a hydrogen flame ionization detector was used. The column temperature was 180°, the injection port 190°, and the detector 330°C. The carrier gas was helium and the flow rate was 60 ml/min.

RESULTS AND DISCUSSION.–Table 1 shows results of the first storage test. During the 42-day period there was no apparent loss of active ingredient from the sand stored in an air-conditioned 26.6 ± 1°C (80 ± 2°F) laboratory. The lot stored outside showed no loss for 14 days and then during the

next 4 weeks a total loss of 20%. It should be noted that during the latter half of July 1979, the ambient air temperatures in Fresno were at their highest for the year (highs 105-111°F/40-45°C). The temperatures in the storage shed were frequently above the maximum level recorded by the hygrothermograph (110°F/43°C). Thus, the maximum loss of 20% occurred after 42 total days of outside storage and included 2 weeks of very hot weather. In all cases (inside and outside), there was no apparent reduction of the release-rate, i.e. the amount of active ingredient on the sand at any date that was released into water after a 4-hr period.

A second 42-day storage trial, beginning on the day that the first test ended, is summarized in Table 2. The data on release-rate obtained during the first test was clear-cut and therefore it was not measured during the second test. For reasons we cannot explain, there was a 10% loss of active ingredient of the sand stored inside after 3 weeks of storage; however this loss did not continue during the next 3 weeks. In the outside shed, loss of active ingredient was also 10% at three weeks and this increased to 20% by 6 weeks.

When Altosand is stored in covered but uncooled facilities under the hot summer temperatures of Central California, the loss of active ingredient is relatively small (10% or less during the first 2 weeks, is 10-15% by 4 weeks and 20% by 6 weeks). Storage under such conditions does not alter the release-rate.

Table 1. Storage stability of Altosand held indoors and outside from 6/18/79 until 7/30/79 at Fresno, CA.

Date (1979)	Days in storage	Indoor storage[1]		Outside storage[2]	
		Concentration (% a. i.)	Release rate (%)[3]	Concentration (% a. i.)	Release rate (%)[3]
6/18	0	0.20	97.3	0.20	97.3
6/25	7	0.20	96.8	0.20	98.4
7/2	14	0.20	93.9	0.20	90.0
7/9	21	0.20	96.7	0.19	94.6
7/16	28	0.19	97.7	0.18	98.8
7/23	35	0.20	98.2	0.17	99.1
7/30	42	0.20	99.4	0.16	98.9

[1]Under air-conditioning.

[2]Uncooled metal shed.

[3]Based on content of active ingredient present on each date.

Table 2. Storage stability of Altosand held indoors and outside from 7/30/79 until 9/10/79 at Fresno, CA.

Date (1979)	Days in storage	Indoor storage[1]		Outdoor storage[2]	
		% a. i.	% loss[3]	% a. i.	% loss[3]
7/30	0	0.20	--	0.20	--
8/6	7	0.20	0	0.18	10
8/13	14	0.19	5	0.18	10
8/20	21	0.18	10	0.18	10
8/27	28	0.18	10	0.17	15
9/3	35	0.18	10	0.16	20
9/10	42	0.18	10	0.16	20

[1]Under air-conditioning.

[2]Uncooled metal shed.

[3]% reduction of original concentration.

A TECHNIQUE FOR CONTROLLING MOSQUITO BREEDING IN UNDERGROUND STORM DRAINS USING METHOPRENE: ALTOSID® (CALIFORNIA SLN-780183)

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INTRODUCTION. The estimated thousand miles of underground storm drains ("undergrounds") throughout the Southeast Mosquito Abatement District collectively represent a complex of systems that are important seasonal breeding and overwintering habits for *Culex quinquefasciatus*. Virtually every street in the District seemingly is undermined by a mosquito infested underground. As a difficult to treat mosquito source, undergrounds are apparently common to most urban and some rural mosquito abatement agencies. However, control strategies are not abundantly documented for a generalized or typical system.

A typical system of the underground complex at our District is composed of 1) usually several open catchbasins connected to 2) covered lateral drains leading to one or more 3) covered mains or run-off conduits that ultimately carry water to flood control channels or rivers. The structural elements of the system may vary dimensionally as much within a system as between systems. The system is infrequently full or backed up with water, but depressions due to poor construction, partial blockage due to debris, improper drainage, and physical changes as a consequence of earth movement can create temporary or permanent mosquito breeding sites. Controlling mosquitoes in the relatively open catchbasins by conventional chemical techniques is usually easily accomplished. However, obvious control difficulties are encountered when attempting treatment of the inaccessible subterranean portion or covered laterals and mains.

Historically, the Southeast Mosquito Abatement District began including undergournds in its larviciding operations when it became apparent that they were producing mosquitoes. Available insecticides were introduced at accessible, i.e., catchbasins and manholes. Disappointing results in many systems led years later to a "flushing" program, a regime that included flusing priority undergrounds with hydrant water every 10 to 14 days followed by larviciding. Control was good to excellent, but only temporary, yet with adequate manpower available "flushing" was continued until recently. Due to the 1977-78 severe drought and the negative financial impact of Proposition 13, the District discontinued "flushing", relying only on larviciding. Currently, larviciding includes the use of Golden Bear 1111 and both emulsifiable concentrate and granular formulations of Abate and Dursban. Today, after two years, larviciding as before is producing less than satisfactory results. This coupled with recently determined incipient Abate and Dursban resistance in *Cx. quinquefasciatus* associated with the undergrounds generated the need to test untried chemicals or treatment methodologies or both. Therefore, the purpose of this field study was to try to find a new strategy or alternative effective chemical methods for combating *Cx. quinquefasciatus* inhabiting the undergrounds.

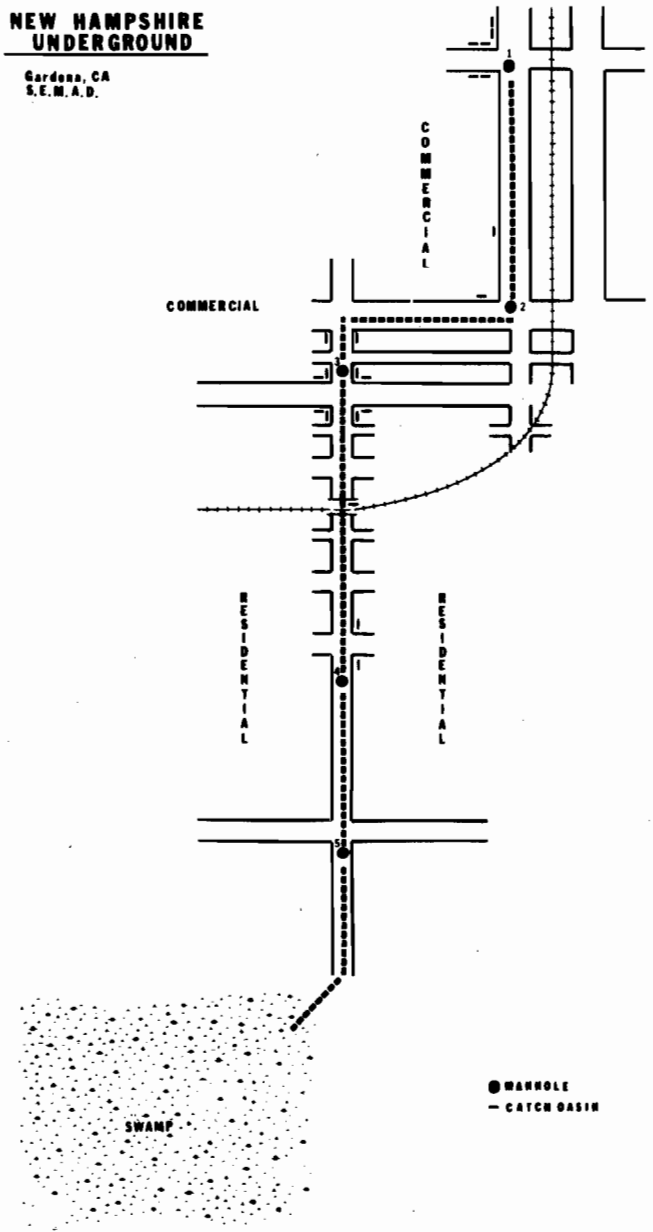

Figure 1.—A schematic representation of the underground storm drain system used by the authors. The system is approximately one-mile long and contains 21 catchbasins, access through 5 manholes, an undetermined number of laterals and one main conduit leading to an 8 to 10-acre vernal swamp.

In contemplating this study, we dismissed those alternative strategies requiring frequent periodic treatment, which would necessitate a complement of inspector-operators now unaffordable by virtue of Proposition 13. Adulticiding was considered impractical. Following an unsatisfactory attempt at using Altosid briquets (see Results and Discussion), we decided to investigate the efficacy and feasibility of the Altosid disc formulation (SLN-780183). In 1978, we obtained excellent results using this formulation experimentally in a flood control channel (Hazelrigg and Pelsue 1979). We presumed it would work as well and longer within the sunless confines of an underground.

MATERIALS AND METHODS. The study site, the New Hampshire underground system, is shown in Figure 1. It is a noncontinguous underground storm drain system, approximately one-mile long situated in a high-density urban area and typical of most of the District's undergrounds. Its components include 21 catchbasins, an undetermined number of laterals, and a single main run-off conduit serviceable through 5 manholes. Water flows from the system to a 8 to 10-acre vernal swamp that empties only during extreme flooding into a nearby flood control channel. Data on the dimensions of the subterranean portion were not available nor determined.

This site was selected because it was believed that the underground portion contained an abundant population of *Cx. quinquefasciatus*. This system and two associated but separate undergrounds were purged as a result of a 2-inch, mid-August rain in 1977. Within 5 days, the previously dry swamp contained larval and pupal counts of *Cx. quinquefasciatus* exceeding 100 per standard dip sample, evidencing our suspicion that the *Cx. quinquefasciatus* had been flushed from the underground and not recruited from ovipositiong adults.

In the summer of 1978, we implemented a passive, sticky-trap adult sampling technique to determine the kinds and relative number of mosquitoes within the system. Two Fly Catchers® ribbons (Aeroxon Products, Inc.) at equal length were suspended side by side approximately one meter above the main at manholes 3, 4, and 5 (Figure 1). The ribbons were supported by a metal dowl cut to fit the lower inside diameter of a manhole opening. The dowl ends rested on the inner lip of the manhole-cover retainer, permitting removal and replacement of the heavy steel cover. Traffic hazards precluded using manhole sites 1 and 2. Ribbons were monitored irregularly, but usually once a week. At each monitoring, the ribbons were changed. Data were recorded as total number of adults (males and females) trapped on the ribbons at each manhole site. In addition to adult sampling, a high-low temperature was recorded occasionally at manhole sites 3 and 5 using a standard daily high-low recording thermometer.

Preliminary sampling begun July 25, 1978, preceded a treatment of the catchbasins of this sytem with Altosid briquets at the recommended dosage rate on August 17, 1978. In 1979, this same sampling technique was used to quantify treatment effects on *Cx. quinquefasciatus* following application of one Altosid disc (SLN-780183) on July 2.

Prior to applying the 544g disc, it was modified for placement. A hole was made in the center of the disc allowing the insertion of a two-inch long, ¼-inch bolt with a small hole at the free end. Fish line, 25 pound test, was threaded and tied-off through the hole in the bolt. The disc was then suspended in the main at manhole 2 (manhole 1 was not safely accessible) and then supported by a metal dowl like those used for supporting the sticky traps. This arrangement of the disc was done to prevent its loss while permitting a sustained release of methoprene to be transported through the system.

RESULTS AND DISCUSSION. The results of this study are shown in Figure 2. Each point on the graph represents the sum of the traps' mean number of adults, both male and female, captured per trap night. The three traps varied in the number of adults they captured, but all were consistent throughout the study in their variability. Trap 3 consistently captured more adults than either traps 4 or 5, the latter capturing the least number of adults. These observations were presumed due to the concentration of mosquito breeding sites, i.e., catchbasins, laterals, and expanded vault-like main associated with manhole 3, and the increased distances from trap 3 of traps 4 and 5 (manholes) encountered by dispersing adults.

It is doubtful, that the Altosid briquets used in 1978 produced significant control. Compared to the 1979 Altosid disc study, the 113 briquets used may have suppressed *Cx. quinquefasciatus*, but only temporarily. Because of the lack of any lag time between treatment and adult suppression unlike that observed in the disc study, we considered the immediate and subsequent diminution of the population following briquet treatment anomalous and probably not the result of released methoprene. Yet, assuming the briquets had some effect, it was marginal and concluded by late September. The mid-September peak rising from the downward population trend was presumed the result of a 0.5-inch rain occurring several days earlier (September 5) that increased adult flight activity in the subterranean portion of the underground, increasing the chance of adults contacting the traps. The authors did notice a number of briquets in the drain culvert near the swamp following the September 5 rain, indicating movement of this formulation through the system. It is possible, that after this rain there were insufficient briquets remaining in the system to sustain control. One of the drawbacks of the briquet formulation, is its sometimes loss or displacement in moving water, a condition common to underground storm drains. In general, the 1978 data were too inconclusive to substantiate or deny some control by the briquets and the explanation for the temporarily reduced *Cx. quinquefasciatus* population remained undetermined.

The contrasting 1979 study demonstrated that the Altosid disc, after a lag time of approximately 5 weeks following its application, was capable of significantly reducing *Cx. quinquefasciatus* and then sustaining control in the New Hampshire underground. The lag time led us to presume that environmental factors associated with this system were favoring adult mosquito longevity. The system is without significant predators and innately an excellent shelter. Humidity is presumably high as a consequence of ever present water. Summer daily high-low temperatures measured between an ideal maximum of 27°C and minimum 21°C. Winter temperatures, although lower, remained mild, measuring between 19°C and 12°C. Therefore, given those optimum conditions, established adults of *Cx. quinquefasciatus* monitored from the underground prior to larviciding would not appear to diminish until their senescence and death, or some extended time after incipient larval and pupal mortality.

The disc was effective for nearly 3 months, the formulation having completely dissolved by 20 October, or 139 days following application. Adults were maintained at fewer than 6 per trap night during mid-September through early November compared to 60 or more adults per trap night during the same period of 1978.

Figure 2 shows that adult *Cx. quinquefasciatus* diminish significantly in November due presumably to weather. The capture rate dropped to less than one adult per trap night and did not rise again until May or early June. Therefore a treatment program involving disc placement should ideally begin in late May or early June and not before the end of heavy winter-spring rains. And while it was not possible in this study, discs should be placed to expose all underground water to dissolved methoprene. Perhaps the failure of this study to achieve absolute control was the unavoidable placement of the disc in manhole 2 and not manhole 1, leaving a portion of the system containing mosquitoes untreated.

Throughout this study, many of the adults captured were teneral, indicating definite underground breeding. However, the degree of extent of adult mosquito dispersal, migration, and emigration in relation to the system, the general ecology of the system and the behavior and bionomics of *Cx. quinquefasciatus* inhabiting undergrounds needs investigation. The authors suggest that further study is needed in these areas in order that reliable alternative control strategies and specialized application techniques and equipment for this important urban source can be developed.

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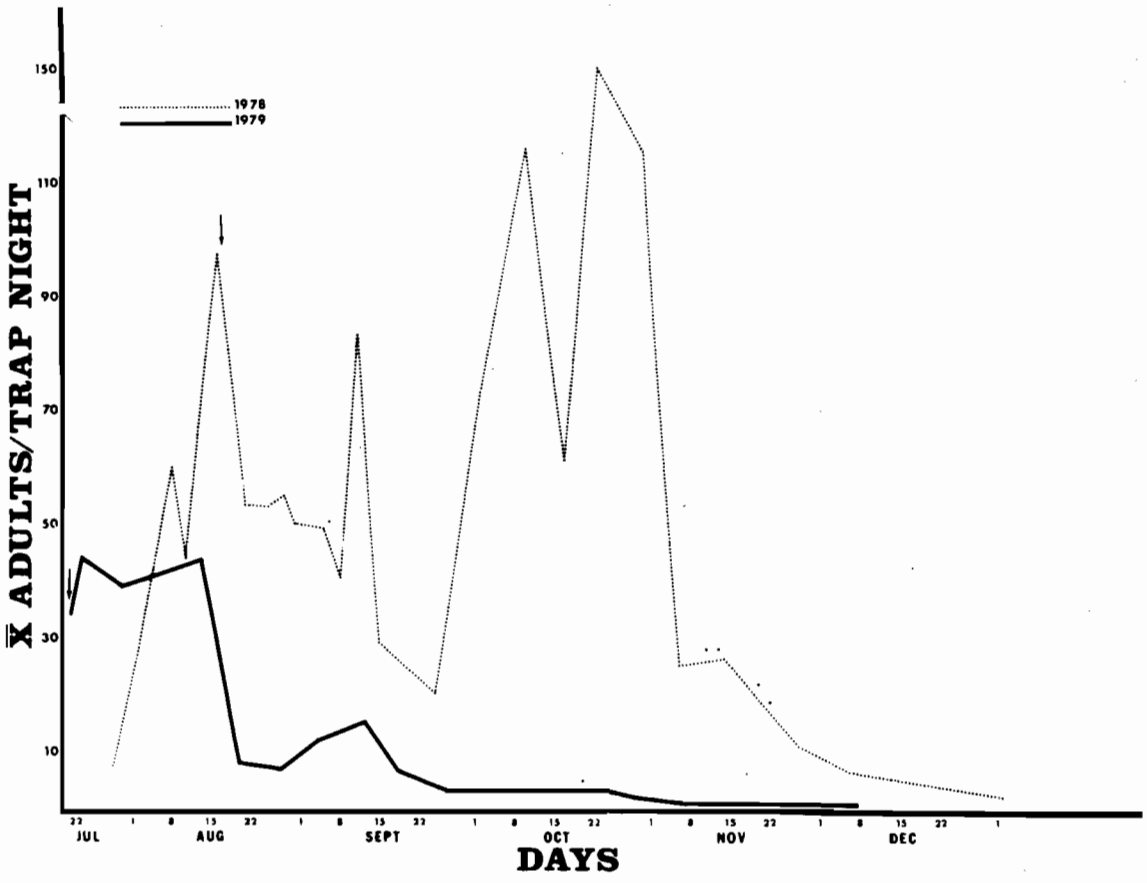

Figure 2.--A graph illustrating the population changes in *Culex quinquefasciatus* adults during successive years of treatment using Altosid briquet (1978) and Altosid disc (1979) formulations in the underground storm drain system shown in Figure 1. Each point on the graph represents the sum of the traps' mean number of adults, both male and female, captured per trap night.

↓ indicates time of Altosid application.
* indicates measureable rainfall (1978 only).
● indicates completely dissolved disc.

EVALUATION OF INSECT GROWTH REGULATORS AGAINST FLORIDA CHIRONOMIDS: EFFECTS ON AQUATIC NONTARGET INVERTEBRATES

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ABSTRACT

Three IGRs, diflubenzuron, Bay SIR-8514 [1-(4-trifluoromethoxyphenyl)-3-(2-chlorobenzoyl)-urea], and Stauffer MV-678 [2-methoxy-9(4-isopropylphenyl)-2,6-dimethylnonane], and JHA Stauffer R-20458 [1-(4-ethylphenoxy)-6, 7, epoxy-3, 7-dimethyl-2-octene] were bioassayed against field-collected 4th instars of *Chironomous decorus* Johannsen, and *Glyptotendipes paripes* Edwards.

A 25% WP and a 0.5% G of SIR-8514, a 25% WP of diflubenzuron, and an EC 4 of MV-678 were evaluated against natural populations of *C. decorus*, *Goeldichironomus holoprasinus* (Goeldi), and *Tanytarsus* spp. in experimental ponds. The WP of SIR-8514 was also applied for midge control in a sewage polishing pond, while MV-678 (EC 4) was applied in a natural pond. Pre- and posttreatment midge larval populations, adult midge emergence, and populations of nontarget invertebrates in each treated habitat were assessed.

Diflubenzuron and SIR-8514 caused 90% mortality of *C. decorus* and *G. paripes* at 4-22 ppb in the laboratory, while LC_{90} of MV-678 for the 2 species ranged from 50-69 ppb. R-20458 was the least active with LC_{90} ranging between 0.24 to 0.7 ppm.

In experimental ponds, WP and G of SIR-8514 at 56 and 112 g AI/ha affected *Tanytarsus* spp. and *G. holoprasinus* more than *C. decorus*, giving an excellent overall control of these midges for ca. 3 wk. Diflubenzuron at 28 and 56 g AI/ha was slightly more effective than SIR-8514 and far more than MV-678 applied at comparable rates. MV-678 gave a maximum of 30% control of total midges at 56 g AI/ha and 70% at 112 g AI/ha. MV-678 applied at rates higher than 0.12 kg AI/ha may produce better results. In the sewage pond, SIR-8514 at 70 g AI/ha completely suppressed midge emergence for at least 10 days after treatment.

The WP and the G of SIR-8514 at 56 and 112 g AI/ha adversely affected *Cyclops* spp., Collembola, *Chaoborus* larvae, nymphs of *Baetis* sp., notonectids and corixids, and larvae of Coleoptera in the experimental ponds but they recovered within a few days to a few weeks after treatment.

The WP of diflubenzuron and SIR-8514 at 28 and 56 g AI/ha reduced *Cyclops* spp. in the experimental ponds. The latter IGR affected the copepod more severely. Collembola, *Chaoborus* sp. and *Baetis* sp. were suppressed by both IGRs. Coleoptera were more sensitive to SIR-8514 than to diflubenzuron. The EC of MV-678 at 56 and 112 g AI/ha proved the least harmful of the 3 IGRs to the various invertebrates in these ponds.

In the sewage pond, SIR-8514 (WP) at 70 g AI/ha adversely affected *Cyclops* spp. and *Hyalella azteca* (Saussure) but they recovered after 3-5 wk of treatment. *Cypridopsis* sp. and Oligochaeta were not affected.

The EC of MV-678 at 0.22 kg AI/ha had no significant ($P>0.05$) adverse effects on *Diaphanosoma brachyurum* (Lieven), *Bosmina coregoni* Baird, *Ceriodaphnia* sp., *Diaptomus* spp., Hydrachnellae, Hirudinea, and Oligochaeta in the natural pond. The 3 IGRs adversely affected some nontarget organisms in the habitats studied, but these organisms recovered within a few days or weeks after treatment.

DISTRIBUTION OF ARTHROPOD POPULATIONS IN RELATION TO MOSQUITO CONTROL RECIRCULATION DITCHES AND NATURAL CHANNELS IN THE PETALUMA SALT MARSH OF SAN FRANCISCO BAY

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As part of a study to evaluate the impact of mosquito control recirculation ditches on the ecology of two San Francisco Bay salt marshes (Resh and Balling 1979), the terrestrial arthropod fauna has been examined in terms of community structure, biomass, and the distribution patterns of specific populations. This paper deals with the analysis of population distribution patterns.

The addition of recirculation ditches physically changes the open marsh habitat. However, these ditches appear similar to the smaller natural channels that occur throughout the marsh. It could be hypothesized that these two features, the recirculation ditches and the natural channels, exert similar influences upon the terrestrial arthropod fauna of the marsh, and that the addition of recirculation ditches merely results in an increase in the number of natural channels. Therefore, a study was designed to determine: 1) the major terrestrial arthropod populations of a San Francisco Bay salt marsh; 2) which of these populations exhibit distribution patterns that indicate a distance response (positive or negative) to the natural channels; and 3) if these latter species exhibit the same resonses to the presence of ditches as they do to natural channels.

MATERIALS AND METHODS.--This study was conducted at the Petaluma salt marsh (Figure 1) located along the Petaluma River, 7 mi north of where it enters northern San Francisco Bay. The vegetation in this marsh is nearly a monoculture of pickleweed, *Salicornia virginica*, a succulent vascular plant.

Arthropod samples were taken with a D-vac suction device at distances of 1 m, 3 m, and 10 m from a 2-yr old ditch, a 5-yr old ditch, and a natural channel (Figure 1). Thus, 9 strata (3 sites X 3 distances) were sampled on each date. Each stratum was sampled by placing the D-vac on the substrate for approximately 5 sec; this was repeated at 1-m intervals, 30 times. Arthropods were extracted (24-h) using Berlese-Tullgren funnels and preserved in 70% ethanol. Samples were collected at approximately two-week intervals from November, 1977 through November, 1978.

The Fauna - - Occurrence and abundance of selected arthropod species over an annual cycle from the Petaluma marsh are presented in Figure 2. Although not intended as an exhaustive faunal list, the majority of the dominant Petaluma marsh populations, particularly in terms of biomass dominance, are included.

Herbivores . . Brineflies (Diptera:Ephydridae) comprise one of the most ecologically diverse families of the cyclorrhaphous Diptera with large numbers of species occurring worldwide in a variety of aquatic and semi-aquatic habitats. Of the 22 species of ephydrids collected as adults in this study (Figure 2, *Scatella* to *Ilythea*), five were abundant.

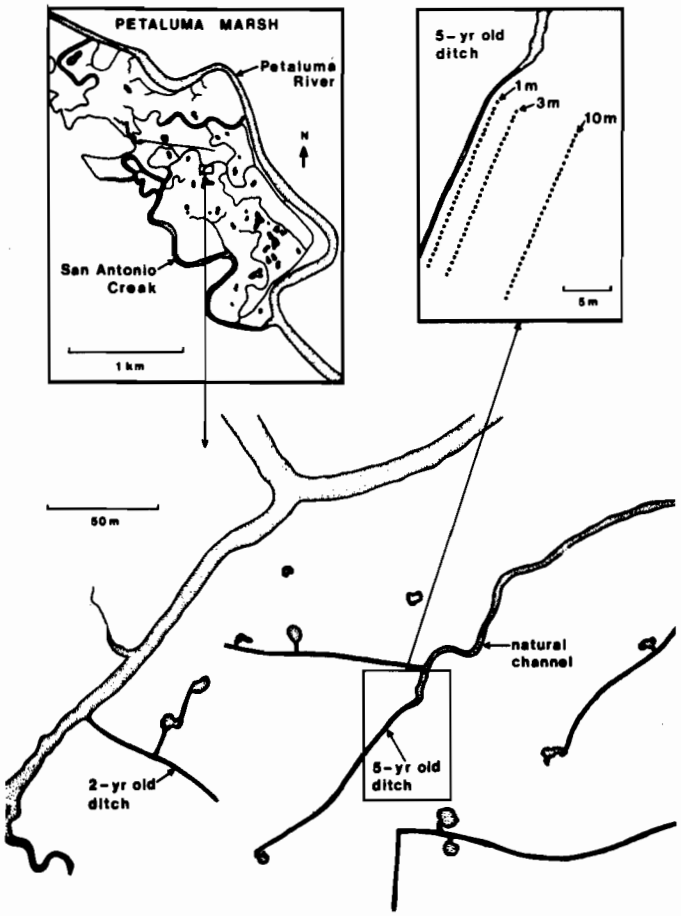

Figure 1.--Map of study site.

Of the 8 species of leafhoppers (Homoptera:Cicadellidae) collected in the marsh (Figure 2, *Idiodonus* to *Aceratagallia*), only one species, *Streptanus confinis*, was abundant.

Carnivores . . . . Spiders are important arthropod predators in salt marshes (Davis and Gray 1966, Foster and Treherne 1976). The wolfspider *Pardosa ramulosa* (Aranеida:Lycosidae) was the numerically dominant spider in the Petaluma marsh (Figure 2).

Saprovores . . . .Two terrestrial crustaceans were very abundant (Figure 2), the amphipod *Orchestia traskiana* and the isopod *Littorophiloscia richardsonae*.

DISTRIBUTION ANALYSIS.--Arthropod species representing different trophic groups were chosen based on both

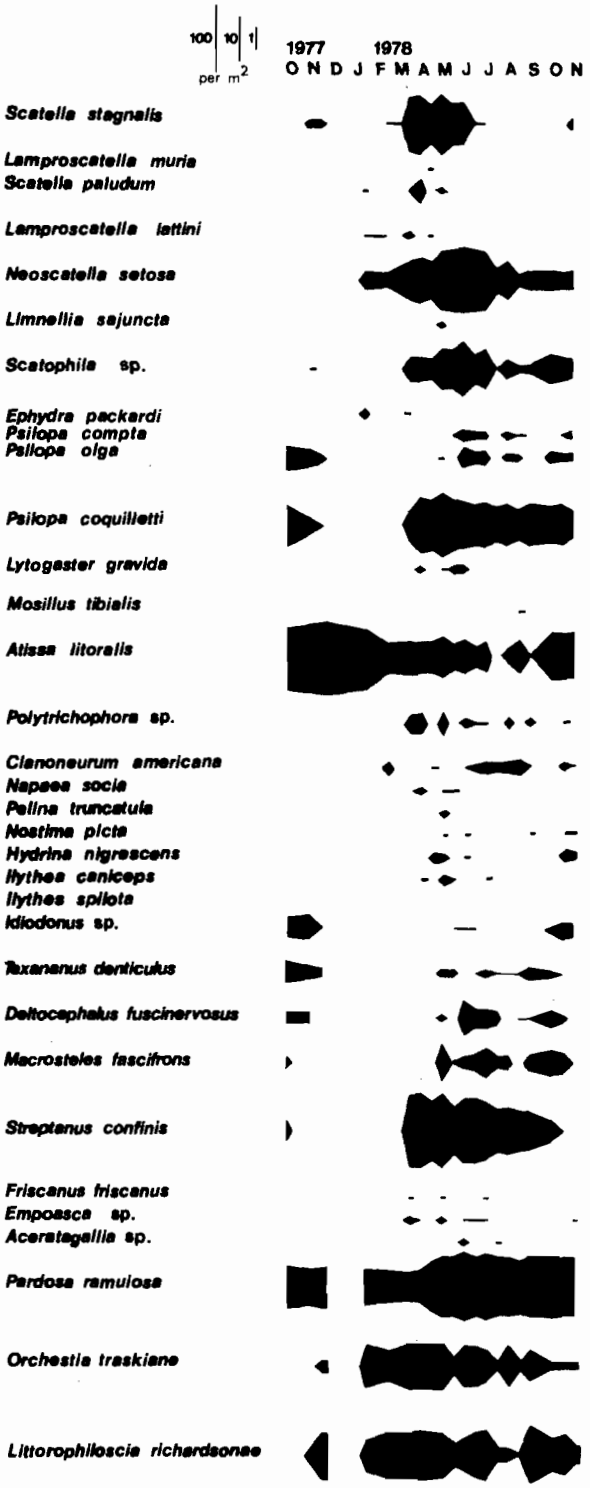

Figure 2.—Adult (cf. *S. confinis* and *P. ramulosa* include immature specimens) occurrence and abundance, November 1977-78, in D-vac samples from all habitats and distances, Petaluma marsh.

their numerical abundance and biomass; the former for statistical considerations and the latter as reflective of their role as components in the food chain via invertebrate and vertebrate predators.

STATISTICAL TREATMENT. For each species, population counts from a sample were expressed as a proportion of the total count for that species collected on that date. Therefore, relative abundance for each sampling date was calculated for specific sites and distances (e.g., 2- and 5-yr old ditches and a natural channel at 1-, 3-, and 10-m). By giving each sampling date equal weight, the seasonal variation in the counts was reduced.

Distances were compared for each site and sites were compared at each distance by analysis of variance. The Student-Newman - Keuls procedure ($p = 0.05$) was used for *a posteriori* multiple comparisons tests (Table 1A) following the ANOVA tests.

RESULTS.—In Table 1, arthropod species selected for distribution analysis and their responses to the presence of recirculation ditches and natural channels are presented. Two points were considered for each of these populations:

1) Is there a distribution response at the natural channel?
2) If there is a response at the natural channel, is it repeated at the recirculation ditches?

CONCLUSIONS.—Distribution patters of salt marsh arthropods have been observed to correspond closely with distance from natural channels, marsh ponds, or other water bodies (e.g., Hull et al. 1934, Williams 1938, Evans et al. 1971, Garcia and Schlinger 1972, Foster and Treherne 1975). Therefore,

Table 1.—Responses of selected arthropod populations from Petaluma marsh to the presence of a natural channel and 2-yr and 5-yr old recirculation ditches. Column A: Is a distribution pattern evident at the natural channel? Column B: Is there a similar pattern at the 2-yr ditch and (Column C) the 5-yr ditch? A Yes represents an ANOVA $p \leq 0.05$; NA = Not available.

Population	A (natural channel)	B (2-yr ditch)	C (5-yr ditch)
S. stagnalis	Yes ($p = 0.05$)	yes	yes
N. setosa	Yes ($p = 0.01$)	no	no
Scatophila sp.	No	no	no
P. coquilletti	Yes ($p = 0.03$)	no	no
A. litoralis	Yes ($p = 0.04$)	yes	yes
S. confinis	Yes ($p = 0.01$)	yes	yes
O. traskiana	No	no	NA
L. richardsonae	Yes ($p = 0.02$)	no	NA
P. ramulosa			
(immatures)	Yes ($p = 0.01$)	yes	NA
(adults)	Yes ($p = 0.01$)	yes	NA
(total)	Yes ($p = 0.01$)	yes	yes

a sampling program designed to evaluate whether a population response to recirculation ditches is the same as it is for natural channels has an appropriate biological foundation.

It is clear that some of the terrestrial arthropod populations examined in the Petaluma salt marsh demonstrate significant distribution patterns when examined with respect to three distances from a natural channel (five of six herbivores, one of two saprovores, and a single carnivore). Of these, certain ones exhibited similar distribution patterns at ditches (*S. stagnalis*, *A. litoralis*, *P. ramulosa*, and *S. confinis*), whereas others did not (*N. setosa*, *P. coquilletti*, and *L. richardsonae*). Thus, for some populations, the addition of ditches is not different from an increase in the number of natural channels. However, for others, either the ditches or some factor(s) associated with them do not simulate natural channels in their effect on population distribution patterns. This information, coupled with results from community and biomass analyses, should yield a clearer understanding of the effects of mosquito control recirculation ditches on the terrestrial arthropod fauna of San Francisco Bay salt marshes.

ACKNOWLEDGMENTS. We thank Steven S. Balling and Joshua N. Collins for field and laboratory assistance. Identifications of Ephydridae were confirmed by Dr. Wayne Mathis, and Cicadellidae by Dr. Ray Gill. 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 and Dr. R. Garcia of the University of California, Berkeley, has been invaluable to this project.

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THE EFFECTS OF IRRIGATION WATER SOURCE AND CROP ROTATION ON THE ABUNDANCE OF *CULEX TARSALIS* IN CALIFORNIA RICE FIELDS

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INTRODUCTION. The mosquito *Culex tarsalis* is widely distributed throughout most of the United States, although it is uncommon or rare east of the Mississippi (Bohart and Washino 1978). Over much of the western U. S. it is an important vector of WEE and SLE viruses, particularly in the agricultural valleys of California where it has also been implicated in the transmission of CE, Turlock, and Hart Park viruses (Emmons et al. 1979).

One of the major breeding habitats of *Cx. tarsalis* in California is the more than half a million acres of flooded rice fields and associated irrigation and seepage ditches (Markos 1951, Markos and Sherman 1957). The density of *Cx. tarsalis* larvae varies considerably among rice fields, with a relatively small proportion of fields being responsible for most of the mosquito production (Case and Washino 1979). Because of the vast amount of acreage under rice cultivation, control of larval mosquitoes in this habitat is feasible only if the highly productive fields can be easily identified.

Previous work suggests that fields newly returned to rice culture after a period in an alternate crop tend to have higher densities of *Cx. tarsalis* larvae than fields cultibated for two or more consecutive years in rice (Collins and Washino 1979). In this same study, the mean seasonal *Cx. tarsalis* sampling densities were significantly correlated with the mean specific conductance of the rice field water ($r_s = 0.415$, $p<0.001$). To assess the value of the above two variables as predictors of *Cx. tarsalis* production by rice fields, the following study was undertaken.

MATERIALS AND METHODS. From June through September of 1979, a central check in each of thirty north Sacramento/south Sutter County rice fields was sampled on twelve occasions for preimaginal stages of *Cx. tarsalis*. On each sampling date, two transect samples of 25 dips each (in groups of 5 at intervals of three meters and thirty seconds) were taken parallel to the levy at distances of one meter and ten meters into the check. The two individuals who performed the dipping followed this procedure which was standardized for method and timing. The two transect samples were taken simultaneously, and the individuals alternated their locations every sampling period to minimize the effect of bias. Egg rafts were sampled once every four weeks (four times during the season) by a visual search of the water surface demarked by a one square meter frame. Two such areas, one adjacent to the levy and one ten meters into the check, were searched on each sampling date. Specific conductance of the rice field water at each sampling station was determined concurrently with the egg raft sampling.

To assess the survival and development rates of preimaginal *Cx. tarsalis* in each field, cohorts of 25 first instar laboratory strain *Cx. tarsalis* were placed in two liter field sentinel cages and monitored during the succeeding eleven days for survival rate and developmental stage. Seven such cohorts (one every two weeks) were followed throughout the season at each station. The sentinel cages were constructed of plastic buckets, the top, bottom and side panels of which were replaced with a Tekto® monofilament nylon screen with a mesh opening (53 microns) small enough to exclude all mosquito predators. The cages were placed two meters into the check.

The rice fields in the study had the following distribution of ages (number of consecutive years under rice cultivation):

Age of Field	Number of Fields
first year	6
2 years	6
3 years	6
4 years	3
5 years	3
6+ years	6

Four of the fields were irrigated with water from deep wells. The remaining twenty six fields were irrigated with surface water, either from the Camp Far West irrigation system or the Sacramento River.

RESULTS AND DISCUSSION. An examination of the frequency distribution of the total number of preimaginal *Cx. tarsalis* sampled per field clearly reveals a bimodal pattern of mosquito density among the thirty fields examined (Figure 1). The nine most productive fields accounted for more than 90% of the mosquitoes sampled during the season. In eight of the stations, preimaginal *Cx. tarsalis* were never present in the samples. The seasonal trend of mosquito density as reflected by the total number of mosquitoes captured in each of the two transect samples on each sampling date (Figure 2) suggests that the interior of the field supports heavy *Cx. tarsalis* populations only during the early part of the season. The marked decline in the mean size of the interior samples after the 25 June sampling period may be a consequence of either a decrease in the abundance of plankton, which results as the developing rice stand blocks the pentration of sunlight, or the inability of oviposition females to penetrate the dense vegetation. The edge of the field, where rice growth is inhibited by the deep borrow pit adjacent to the levy, supports *Cx. tarsalis* populations throughout the season with no pronounced seasonal trend (Figure 2). Although not enough egg rafts were found to reveal any seasonal oviposition pattern, only one of the total of thirty egg rafts collected was from an interior sample, an observation which suggests that females tend to oviposit in the first suitable site they find on their encounter with a rice field. Since the rice stand near the periphery of a check is quite sparce during the early part of the growing season (thus permitting unimpeded flow of the irrigation water), interior sites may be more suitable at this time. Such an interpretation is consistent with the tendency of edge sample larval densities

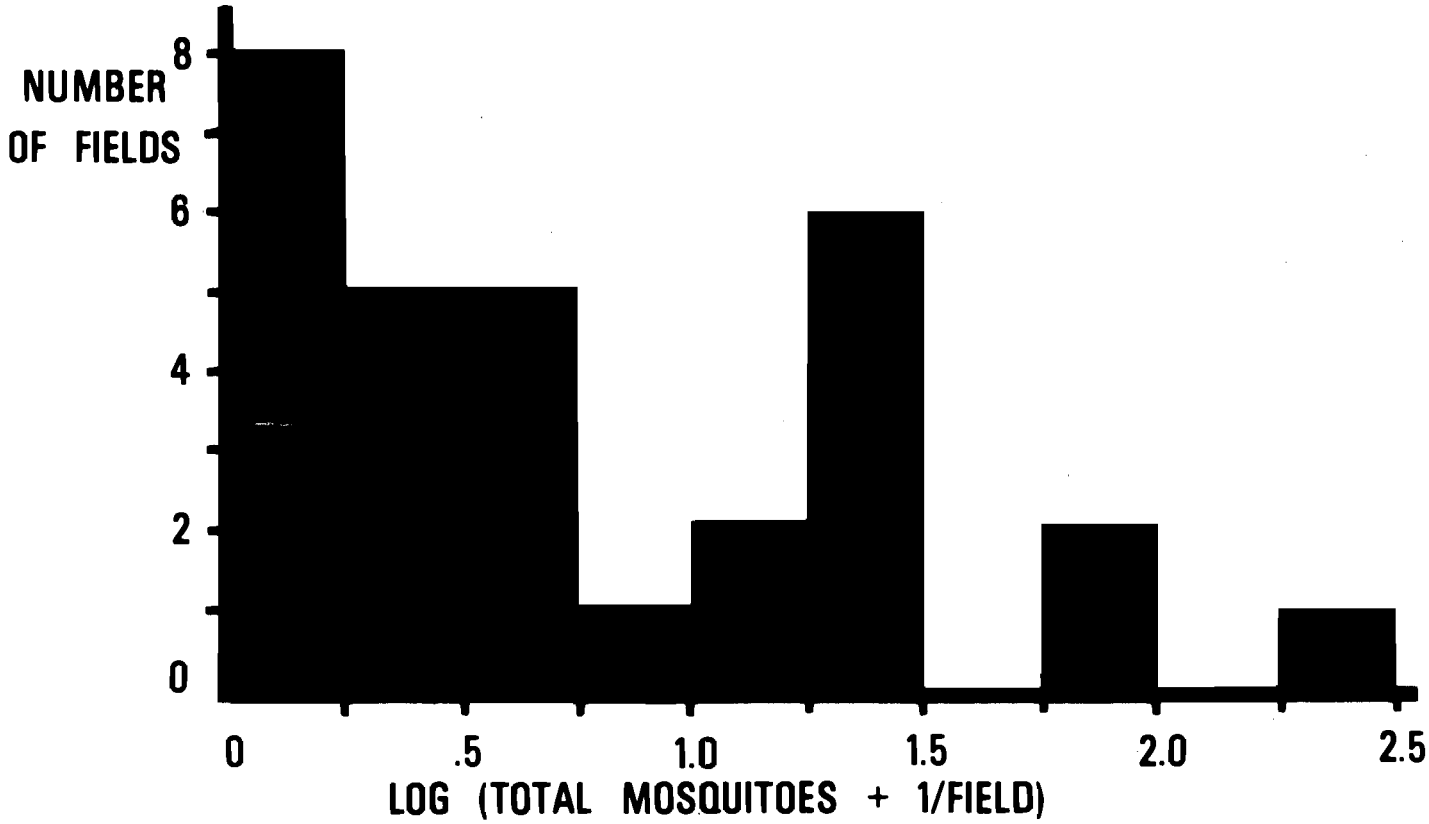

Figure 1.--Frequency distribution of total number of preimaginal *Culex tarsalis* sampled in 30 rice fields. (12 samples/field, 50 dips/sample). Rice field study, Sacramento/Sutter Counties, California, 1979.

to be at their lowest during the period when interior samples are highest (Figure 2).

The spatial distribution of preimaginal *Cx. tarsalis* is not random over a rice field. Previous work has shown that not only does the density vary considerably among the different checks of a given field (Collins and Washino 1979), but it also varies markedly within a given check. Larval populations seem to occur in discontinuous pockets, probably as a result of several factors, including the contagion of larvae emerging from one egg raft and the observed tendency for rafts to be deposited in clusters (possibly a consequence of ovipositional preference for certain microhabitats). There are also very pronounced temporal discontinuities. In our samples, larvae were rarely abundant in a given location for more than three weeks; they typically would appear suddenly as a bloom of early instars which were replaced over the succeeding weeks with decreasing densities of later instars.

As a result of these discontinuities, most dip samples were negative, but more than 10% of the positive samples yielded 20 or more mosquitoes. Since the total number of preimaginal *Cx. tarsalis* sampled in a given field is extremely sensitive to the effect of these occasional large samples, we have included (in addition to the total number of preimaginal *Cx. tarsalis* sampled from each field) a measure of *Cx. tarsalis* abundance based on the ranks of the samples. Specifically, we ranked all the samples taken in the thirty fields over the season and then calculated the mean sample rank for each field. The fields were then ranked from least to most productive on the basis of this measure (Table 1). Since this method sacrifices degree of difference information for an improved estimate of the order of mosquito productivity by the thirty fields, both estimates are retained for analysis and used where appropriate.

Also included in Table 1 are the following measures for each field: (i) the mean of the four measures of specific conductance (micromhos/cm corrected to 23°C) and (ii) two indicator variables which distinguish (respectively) well water irrigated fields from surface water irrigated fields and fields in their first year under rice cultivation from fields which have been in rice for two or more consecutive years. The specific conductance of irrigation water is determined largely by the source (water from deep wells in the study area typically ranges between 450-750 micromhos/cm while the conductance of surface water rarely exceeds 350 micromhos/cm), although evaporation and, to a lesser extent, soil type are probably important as well.

The pairwise associations among the variables listed in Table 1 were determined by calculation of Spearman's rank correlation coefficients (Table 2). The significant correlation between the rank based measure of *Cx. tarsalis* abundance and mean specific conductance supports the validity of this observation in our previous study (Collins and Washino 1979). Also notable is the significant correlation between the *Cx. tarsalis* abundance variable and egg raft totals, a result which suggests that ovipositional preference, as opposed to larval survival or development rates, may be the principal determinant of mosquito abundance in a given rice field.

It is also important to observe that while the correlation between egg raft totals and mean specific conductance is not significant, high egg raft counts are significantly associated with well water fields. In fact, when the same correlation coefficients are calculated for surface water irrigated fields only

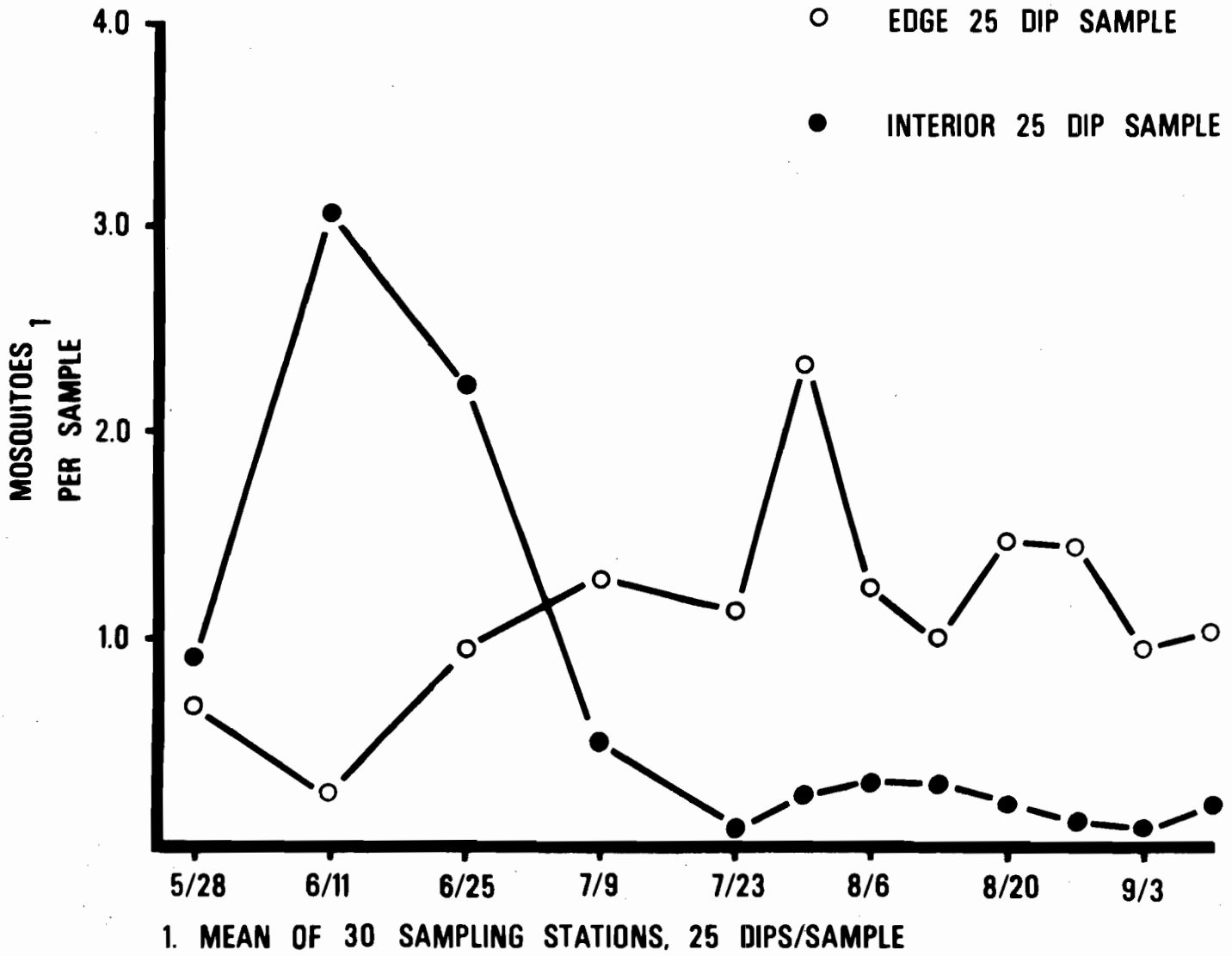

Figure 2.–Seasonal abundance of preimaginal *Culex tarsalis* sampled in 30 rice fields. Rice field study, Sacramento/Sutter Counties, California, 1979.

(Table 3), the *Cx. tarsalis* abundance is no longer significantly correlated with either mean specific conductance or egg raft totals. This strongly implies that the high conductivity well water irrigated fields account for the significant overall correlations of *Cx. tarsalis* egg raft and preimaginal stage abundances with specific conductance measures.

The actual cause of this phenomenon is uncertain, but any of several factors may contribute. Ovipositing females may prefer high conductivity well water fields over surface water irrigated fields (a possibility supported by a preliminary ovipositional preference study with a laboratory strain of *Cx. tarsalis*); mosquito predators or competitors may be less abundant in well water fields (although this would not explain the higher egg raft counts); or well water fields may have characteristics (i.e., nutritional physiochemical) that contribute to higher rates of autogeny among emerging adults, which, unconstrained by the requirement of seeking a blood meal, may oviposit in the same site where they developed.

Analysis of the survival and development rates of the sentinel cohorts reveals a significantly more rapid rate of sentinel development in fields with higher levels of specific conductance (Table 4). There is, however, no significant relationship between specific conductance and overall sentinel survival rates.

High levels of *Cx. tarsalis* abundance are also significantly associated with first year fields, but high egg raft counts are not (Table 2). This correlation remains significant event when well water irrigated fields are removed from consideration (Table 3), in spite of the fact that the field with the highest *Cx. tarsalis* density (field 17, a well water/first year field - see Table 1) is not included in the reduced data set. The relatively high *Cx. tarsalis* densities found in first year fields are probably a consequence of very low levels of mosquito predation by a *Mesostoma* species of microturbellarian which is generally quite abundant in fields that have been under rice cultivation for two or more consecutive years (Collins and Washino 1978,

Table 1. Measures of in-field abundance of preimaginal *Culex tarsalis* and associated variables at selected stations in 30 rice fields. Rice field study, Sacramento/Sutter Counties, California, 1979.

Field Number	Estimates of *Cx. tarsalis* productivity (lowest to highest)		Mean specific conductance micromhos/cm @ 23°C	Total *Cx. tarsalis* egg rafts sampled	Indicator variables	
	Sample rank estimate	Total mosquitoes sampled			Well water irrigated fields	first year fields
15	4.5	0	165.0	0	0	0
32	4.5	0	190.0	0	0	0
31	4.5	0	210.0	0	0	0
28	4.5	0	225.0	0	0	0
19	4.5	0	242.0	0	0	0
25	4.5	0	332.5	0	0	0
3	4.5	0	200.0	1	0	0
14	4.5	0	227.5	1	0	0
33	10	1	230.0	0	0	0
34	10	1	247.5	0	0	0
7	10	1	95.0	0	0	0
23	12	3	192.5	0	0	0
16	13	20	197.5	0	0	0
4	14.5	2	265.0	0	0	0
21	14.5	2	217.5	0	0	0
18	17	3	230.0	0	0	0
26	17	3	335.0	0	0	0
24	17	3	207.5	1	0	0
8	19	4	212.5	1	0	0
11	20	7	280.0	0	0	1
27	21	21	335.0	0	0	0
1	22	15	235.0	1	0	0
6	23	59	497.5	1	1	0
29	24	16	187.5	0	0	1
9	25	25	485.0	3	1	0
20	26	29	270.0	2	0	0
13	27	22	317.5	4	0	1
22	28	28	255.0	0	0	1
5	29	106	475.0	11	1	0
17	30	283	705.0	4	1	1

Table 2.–Measures of correlation among levels of in-field *Culex tarsalis* abundance and associated variables listed in Table 1. (Spearman's correlation coefficients). (N = 30). Rice field study, Sacramento/Sutter Counties, California, 1979.

	Mean specific conductance	Well water irrigated fields	1st year fields	Total *Cx. tarsalis* egg rafts sampled
Mean rank of 50 dip *Cx. tarsalis* samples	.569[1]	.515[1]	.491[1]	.546[1]
Total *Cx. tarsalis* egg rafts sampled	421[2]	.607[1]	.045	

[1] $p < .005$ (significant at an overall $\alpha = 0.05$).

[2] $p < .025$ (not significant at an overall $\alpha = 0.05$).

Table 3. Measures of correlation among levels of in-field *Culex tarsalis* abundance and associated variables listed in Table 1; well water irrigated fields removed. (Spearman's correlation coefficients). (N=26). Rice field study, Sacramento/Sutter Counties, California, 1979.

	Mean specific conductance	1st year fields	Total *Cx. tarsalis* egg rafts sampled
Mean rank of 50 dip *Cx. tarsalis* samples	.386[2]	.542[1]	.300
Total *Cx. tarsalis* egg rafts sampled	.090	-.025	

[1] $p < .005$ (significant at an overall $\alpha = 0.05$).

[2] $p < .05$ (not significant at an overall $\alpha = 0.05$).

categories. The four well water irrigated fields account for 72.3% of all preimaginal *Cx. tarsalis* sampled during the season, the six first year fields account for 54.8%, and the one first year/well water field is responsible for 43.3% of the total. Thus the nine fields identified by these two characteristics are responsible for 83.8% of the total *Cx. tarsalis* production, as compared with only 16.2% from the remaining 21 surface water/older fields (Table 5).

A comparison of the relative *Cx. tarsalis* productivity of each of the four categories of fields (Table 6) clearly indicates that (i) fields newly returned to rice after a growing season in an alternate crop yield *Cx. tarsalis* densities three to four times greater than fields planted in rice for two or more consecutive years, (ii) *Cx. tarsalis* densities are ten to fifteen times as great in fields irrigated with well water as in those irrigated with surface water. The effects of the above two factors when combined seem to be multiplicative, a first year field irrigated with well water producing roughly fifty times as many mosquitoes as an older, surface water irrigated rice field.

Table 4. Measures of in-field survival and development rates of cohorts of 25 first instar *Culex tarsalis* sentinals monitored for 11 days. (N = 196). Rice field study, Sacramento/Sutter Counties, California, 1979.

	Total survivors at day 11	Sentinals reaching Adult	Sentinels reaching pupal stage	Sentinels reaching fourth instar	Sentinels reaching third instar
Mean	17.79	2.09	5.14	18.19	19.92
S.D.	5.91	3.74	6.09	5.97	5.05
Pearson's correlation coefficient with specific conductance	0.025	0.276[1]	0.236[1]	0.157[2]	0.028

[1] $p < 0.001$ (significant at overall $\alpha = 0.05$).

[2] $p = 0.026$ (not significant at overall $\alpha = 0.05$).

Table 5.–Proportion of the total number of preimaginal *Culex tarsalis* in 30 rice fields classified by irrigation water source (well vs. surface) and rice field age (first year fields vs. older fields). Rice field study, Sacramento/Sutter Counties, California, 1979.

	1st year field	Older field	Total
Well water field	0.433 (1)[1]	0.290 (3)	0.723
Surface water fields	0.115 (5)	0.162 (21)	0.277
Total	0.548	0.452	1.000

[1] (N) = number of fields in each category.

While we do not propose that our measurements of the magnitude of these effects are applicable in general, we are fairly certain of their overall implication. In particular, we believe that allocation of control resources such as *Gambusia affinis* or larvicides on the basis of these two readily identifiable characteristics can produce effective and relatively efficient control of *Cx. tarsalis* in northern California rice fields.

1979). In first year fields, *Mesostoma* populations are usually very low, probably due to the slow rate at which this flatworm recolonizes fields that have been returned to rice after one or more seasons in an alternate crop. Flatworms overwinter by means of heavily encapsulated eggs which apparently are largely destroyed by dessication in fields which are not flooded during the summer.

On the basis of irrigation water source (deep well versus surface) and the status of a rice field in the cycle of crop rotation (first year in rice versus two or more consecutive years in rice), the thirty fields in this study can be grouped into four

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Table 6. Density of preimaginal *Culex tarsalis* in 30 rice fields classified by irrigation water source (well vs. surface) and rice field age (first year field vs. older fields). Rice field study, Sacramento/Sutter Counties, California, 1979.

Field type	Average mean density[1]	Standard error	Number of fields	Relative density[2]
Well water/first year	23.58	--	1	56
Well water/older	5.28	1.96	3	13
Surface water/first year	1.25	0.40	5	3
Surface water/older	0.42	0.15	21	1

[1]Average over number of fields in category of seasonal mean sample size for each field (seasonal mean sample size based on 12 samples of 50 standard dips/sample).

[2]All relative densities based upon average mean density of surface water/older fields.

ESTIMATION OF MOSQUITO POPULATION SIZE IN CONFINED NATURAL BREEDING SITES

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ABSTRACT

Population sizes of immature stages of *Culex quinquefasciatus* Say populations breeding in the 1-m^2 pond, catch basin, treehole and cemetery flower vase were estimated by using the removal method.

Estimation of the population size of an animal in its habitat is the most fundamental step in population ecology. Especially for mosquito control agencies, it is one of the important factors for making decisions such as chemical applications and application time.

It is time-consuming work, but it is possible to derive estimates of absolute population sizes of mosquitoes by sampling. In the present study I have examined the efficiency of the removal sampling method (Zippin 1956, Wada 1962a, 1962b, Southwood 1966, Service 1976) to estimate absolute populations of mosquitoes breeding in small ponds, catch basins, treeholes and cemetery flower vases.

Description of the Removal Sampling Method.–The removal of a known number of larvae on each dip from a breeding site affects the number of larvae which will be captured in each subsequent dip. The rate of decline is related to the original population size (unknown) and to the number of larvae removed from the site (known).

The assumptions underlying the method are that (A) the population must be stable, i.e., no birth or death during the sampling period, (B) there is an equal chance for each animal to be caught, and (C) this probability of capture remains constant during the sampling period (Zippin 1956, Wada 1962a, b).

MATERIALS AND METHODS.–Naturally occurring *Culex quinquefasciatus* Say populations in a 1-m^2 pond located in the back yard of the laboratory (Schaefer et al. 1974) and in a catch basin (54 cm diam) in the City of Kerman were studied by dipping, using a dipper (450ml capacity) with a long handle. Mosquito larvae from each dip were carefully poured into a larval concentrator (Husbands 1969) and each collection was kept separately in a vial containing 85% alcohol. Collections were then brought back to the laboratory for identification and counting.

In order to analyze the data by the regression method, several dipper collections were combined and designated as "unit of catch" (super-unit of Wada 1962a); for the pond study, 5 consecutive dips were combined and for the catch basin study, 15 dips were combined to make a unit. Each unit of catch was plotted against the previous total catch. The regression lines were drawn by eye.

Table 1. Dipper collection data from the 1-m^2 pond showing the relationship between dip and unit collections.

Dip no.	No. larvae/dip	No. larvae/unit	Dip no.	No. larvae/dip	No. larvae/unit
1	123		19	8	
2	52		20	18	82
3	54		21	8	
4	40		22	16	
5	32	301	23	19	
6	35		24	9	
7	38		25	13	65
8	28		26	6	
9	22		27	11	
10	57	180	28	13	
11	34		29	8	
12	34		30	5	43
13	18		31	3	
14	27		32	6	
15	18	131	33	10	
16	21		34	12	
17	18		35	0	40
18	17				

Table 2.–Stage composition of *Cx. quinquefasciatus* breeding in catch basins, Kerman, CA.

	Larvae					
Sample No.	I	II	III	IV	Pupae	Total
1	121	63	40	21	4	249
2	196	76	58	34	7	371
% (mean)	51.13	22.42	15.81	8.87	1.77	100

Population sizes calculated by the regression method were also cross-examined by the estimations obtained by a graphical method (Zippin 1956, Southwood 1966).

Cx. quinquefasciatus populations breeding in fabricated treeholes (4 liter capacity, Lewis and Tucker 1978) and cemetery flower vases (1 liter capacity) were also calculated by the removal method. This time, larvae were collected by a medicine dropper and number of larvae collected were recorded each minute, a 3 minute catch was designated as a unit of catch.

Population sizes calculated by the regression method and graphical method were reexamined by comparison to the absolute number of the true population counts.

RESULTS AND DISCUSSION.–Thirty-five dip collections were made from the 1-m^2 pond, removing 842 mosquitoes (Table 1). They were then grouped into 7 units to show a gradual decline in numbers caught in each unit.

In order to estimate the population size, the data were plotted and the population size was calculated as 1030 larvae. (Figure 1A). On the same day, the remaining population in the pond was reestimated to double check the initial population estimation, the remaining population size was estimated as 385 (Figure 1B). Therefore, if these 2 numbers (842 initially removed and 385 remaining population size) are added together, this also estimates the population size in the pond. By this way the population size was 1227. Finally this population was estimated by using the graphical method (Zippin 1956) as 925. These 3 estimations, 1030, 1227, and 925 are close enough to satisfy the reliability of the removal method to estimate population size.

Figure 2 shows the estimation of the population size breeding in the catch basin in the city of Kerman. By the regression method it was calculated as 19,800 mosquitoes and by the graphical method 19,525.

It was surprising to find so many mosquito larvae in such a small area. This does not mean that all mosquitoes present in the catch basin would emerge as adults. Mortality rate must be high, especially among young instars; 2 samples were taken randomly from the alcohol preserved collection and stage composition of the population was examined (Table 2). More than ½ of the mosquitoes collected were 1st instar larvae and less than 2% of the mosquitoes were pupae.

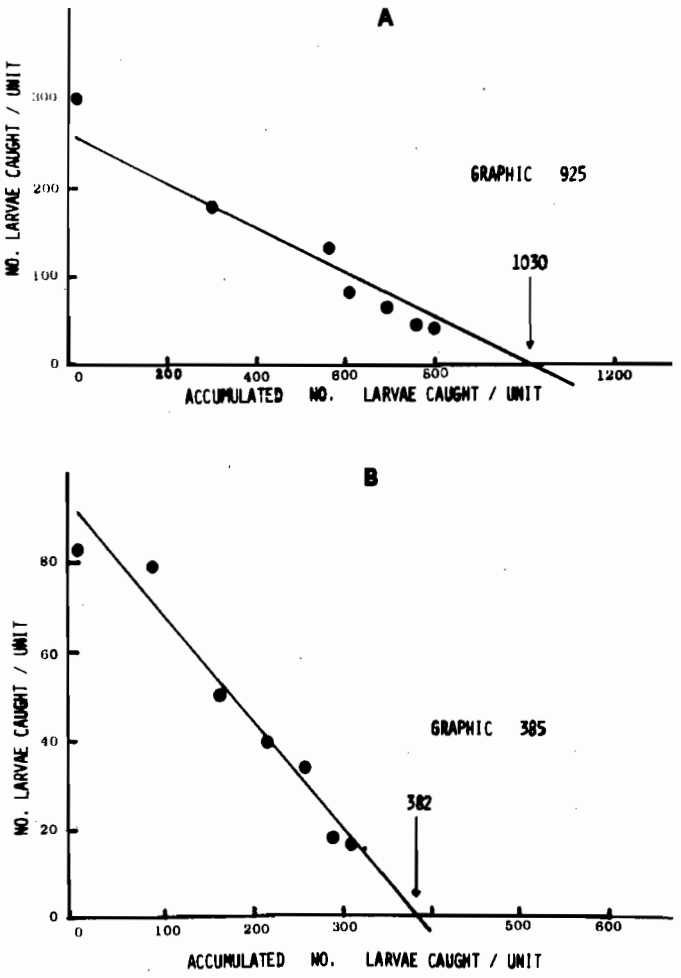

Figure 1.- Estimation of larval population in a 1-m^2 pond by the removal method. Showing estimated sizes by the regression and graphical methods.
A. First estimation of the initial population size.
B. Second estimation of the remaining population size.

Figure 3 shows the estimation of *Cx. quinquefasciatus* breeding in a fabricated treehole on the ground. The population size was estimated as 775 by the regression method and 720 by the graphical method. At the end of the study the entire population was counted as 756.

Figure 4 shows the estimation of *Cx. quinquefasciatus* breeding in the cemetery flower vase. By the regression method it was 405, by the graphical method 436, and absolute population was 355.

In summary, these results show that the removal method for estimating mosquito population size in habitat is satisfactory. The data collected for the removal method can be analyzed either by the regression method or by a graphical method.

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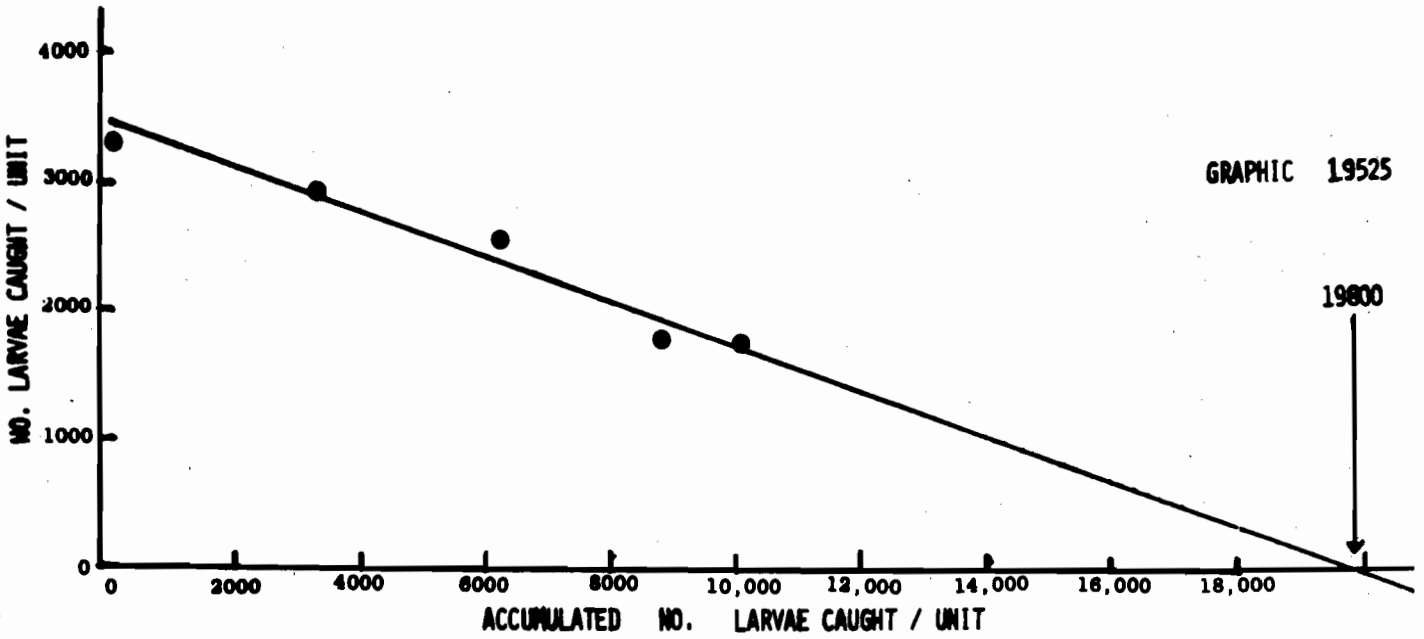

Figure 2.- Estimation of larval population in a catch basin by the removal method, showing estimated sizes by the regression and graphical methods.

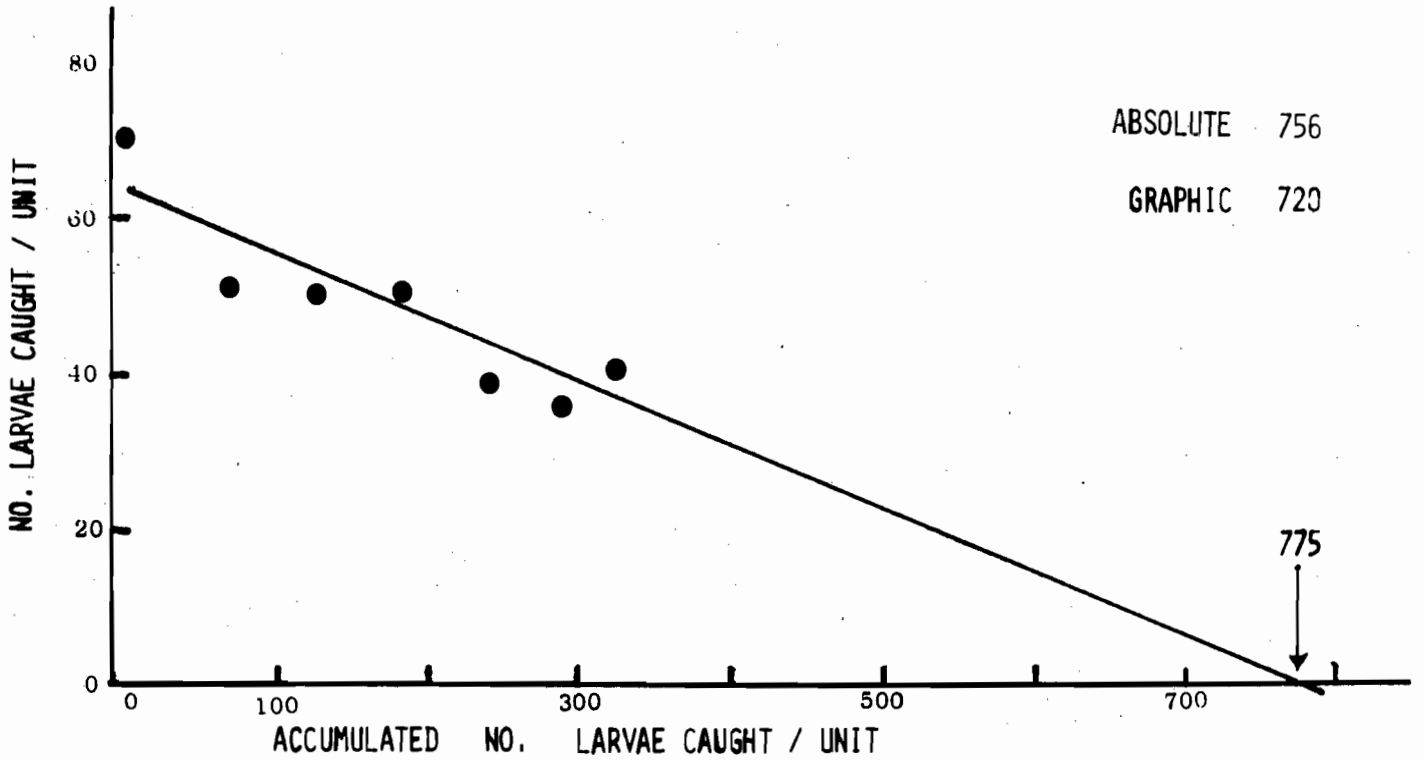

Figure 3.--Estimation of larval population in a fabricated treehole by the removal method showing the absolute population size and estimated sizes by the regression and graphical methods.

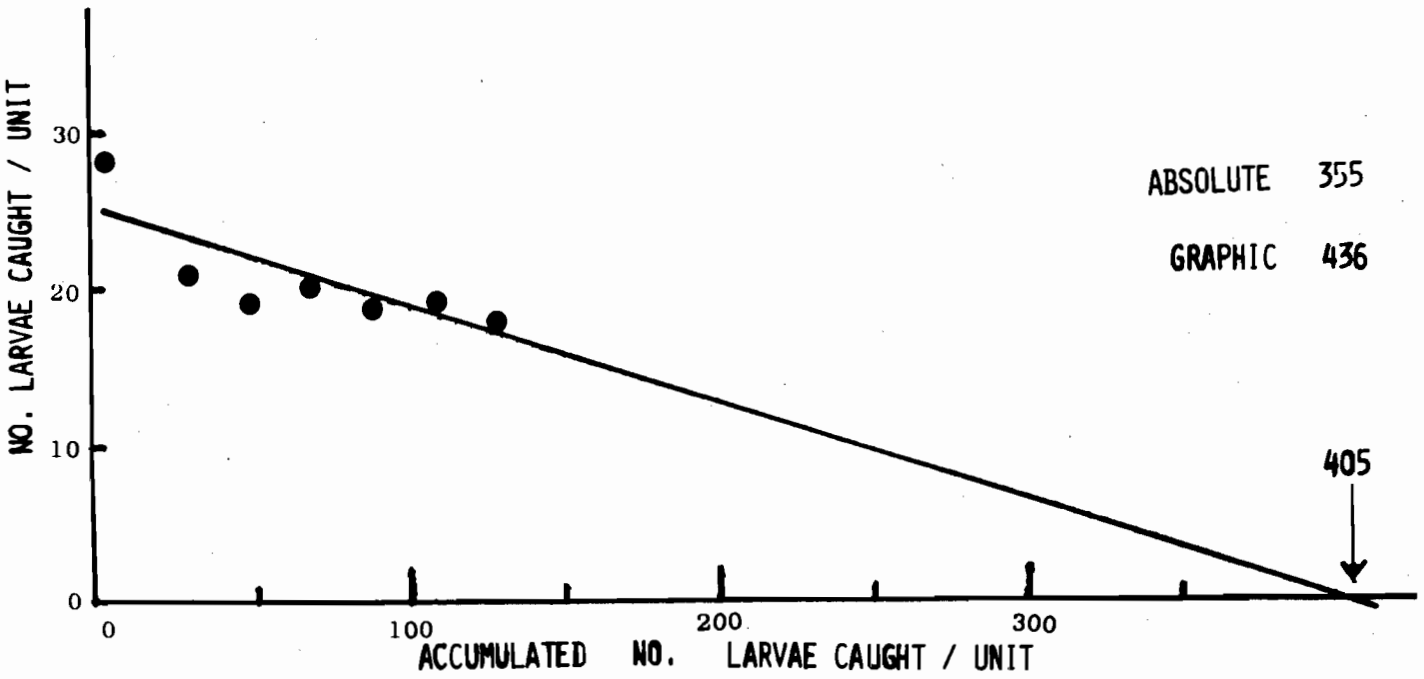

Figure 4.–Estimation of larval population in a cemetery flower vase showing the absolute population size and estimated sizes by the regression and graphical methods.

COMPARATIVE MOSQUITO COLLECTION DATA FROM THE SOUTHERN MARIANA ISLANDS (DIPTERA: CULICIDAE)

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ABSTRACT

The distribution of mosquito species on the four southernmost islands in the Mariana Archipelago is reviewed. The initial collection data for each species on each island are included and attention is directed to the introduction of new species and interisland distributions. Emphasis is placed on the necessity for effective air and surface craft quarantine programs to stop both the increase in introductions and dissemination of the species throughout the Mariana Islands.

Considerable attention has been directed to the Mariana Islands in modern times because of their relative geographic isolation and localized fauna; their strategic importance to both the Japanese and American military planners during World War II; and their use as a stop-over point by both aerial and surface craft in trans-Pacific travel. This is a study of the culicid fauna of Guam, Rota, Saipan and Tinian, the 4 islands comprising the southernmost end of the Marianas chain.

The earliest American references to mosquitoes of the Marianas were written at the beginning of the century by the senior medical officers assigned to the U.S. Naval Station, Guam, and included in their annual reports to the Surgeon General of the U.S. Navy. The initial entomological surveys of Guam and Saipan were achieved in 1911, with subsequent studies being accomplished on Guam in 1936, and Saipan in 1932 and 1939. The invasions by American military forces on

Saipan, Tinian and Guam during World War II reemphasized the requirement for information on the mosquitoes and mosquito-borne disease potential on those islands. As a result reports of surveys, collection records, bionomic notes and vector data were accumulated for each of the four southernmost islands starting in 1944.

The island of Guam received the most attention because it had been a U.S. territory since 1898 and historically provided the primary ports of entry to the Mariana Islands. The presence of military personnel on Andersen Air Force Base and the U.S. Navy facilities at Apra Harbor, along with the increasing tourist business on the island, continued to focus attention on the mosquitoes and their disease potential.

The record shows the mosquito fauna of Guam has increased through introductions from other Indo-Pacific areas. Each survey showed an increase in the number of species and noted the presence of vector species (Nowell 1977). It is suspected that distribution among Guam, Rota, Saipan and Tinian is associated with interisland transport of cargo and passengers.

This paper brings together the key mosquito collection data for each of the 4 islands so that they may be compared by species and sequence of recovery. This bibliographic review indicates that only the fauna of Guam has been collected and documented in detail; that the mosquito fauna of the Mariana Islands is increasing in number of species through inter-Pacific introductions; that there is interisland distribution of the species throughout the southern 4 islands; and that the mosquito fauna of the archipelago, with the exception of Guam, is not completely known.

There are early references to *Stegomyia fasciatus* Fabricius (= *Aedes aegypti* (Linnaeus)) by Leys (1905), McCullough (1908), and Bagg (1927) on Guam, but the first entomologic survey on that island was made by D. T. Fullaway in 1911. Fullaway (1912) recorded 2 species of mosquitoes, *Stegomyia scutellaris* and a "*Culex*, species near *vishnui*." His *Stegomyia scutellaris* was later determined to have been *Ae. guamensis* (in part) by Farner and Bohart (1944), and *Ae. pandani* (in part) by Stone (1939), while Swezey (1942) was of the opinion that his "*Culex* sp. near *vishnui*" was *Culex pipiens quinquefasciatus*. A later study of Fullaway's specimens revealed one female *Ae. vexans vexans* (Bohart 1957). In 1937 O. H. Swezey reported that *Cx. quinquefasciatus* and an *Aedes* sp. occurred on Guam, and in 1939 A. Stone described *Ae. oakleyi* and *Ae. pandani* from that island. Another early reference was that of P. Schnee (1912) who pointed out that only mosquitoes of the genera *Culex* and *Stegomyia* were to be found on Saipan. S. Sogen (1941) places *Ae. albopictus* on Saipan in 1934 and this species was collected by U.S. military entomologists on Guam, Saipan and Tinian during the surveys of 1944 and 1945. On the basis of these records, the mosquito fauna of the southern Marianas up to the start of World War II comprised 7 species: *Ae. aegypti*, *Ae. albopictus*, *Ae. guamensis*, *Ae. oakleyi*, *Ae. pandani*, *Ae. vexans vexans*, and *Cx. pipiens quinquefasciatus*. The number of known species doubled to 13 during the World War II years and then increased steadily with each succeeding survey.

The documented collection data for all 41 species known to date are summarized in Table 1, and the first recorded year of collection for both the adult and larval forms is given for each island.

Species distribution on Saipan, Tinian and Rota shows a similarity in number but the same species do not occur on each of the islands. The distribution patterns indicate similarities between the faunas of Guam and Rota and of Saipan and Tinian. However, 7 of the 9 species which occur on Rota are also shared by Saipan and Tinian.

Table 1 shows 41 species collected on Guam, 9 on Rota, 12 on Saipan, and 13 from the island of Tinian. As for distribution, none of the species recovered from the other islands is different from those reported from Guam. Furthermore, the collection dates reflect interisland dispersal of the species from Guam.

That Guam should be the source for the other islands is understandable. Guam has provided the major air and surface vessel ports in the Marianas for inter-Pacific transport departing Manila, Tokyo, Honolulu and San Francisco. Guam is also the terminal for intra-Marianas travel. The dispersal of the species from Guam to the other islands should have been expected since there have been repetitive interisland sailings and flights from Guam over a sustained period of time and without the protection of a quarantine or inspection program.

The collection data in Table 1 show that the mosquito fauna in the southern Marianas appeared during 3 increments: prior to World War II, during World War II, and subsequent to World War II. Dispersal of the species among the islands of Guam, Rota, Saipan and Tinian occurred primarily during the WWII and post-WWII periods. The pairing between the species lists for Guam and Rota show 5 WWII and 4 post-WWII dates, while the Saipan relationships are 8 during WWII and 3 post-WWII pairs with Tinian, and 1 pre-WWII, 7 WWII, and 4 post-WWII pairings with Guam.

Seven of the 41 species are indigenous to the Marianas. These are: *Ae. guamensis*, *Ae. neopandani*, *Ae. oakleyi*, *Ae. pandani*, *Ae. rotanus*, *Ae. saipanensis*, and *Cx. annulirostris marianae*. Two species, *Ae. aegypti* and *Cx. pipiens quinquefasciatus*, are cosmopolitan in their distribution, while the 2 *Toxorhynchites* species were released on Guam in 1954 to control other mosquitoes (Hu 1955).

It appears that while all of the 41 species known from the southern Marianas have been recovered from Guam, at least 3 (*Ae. neopandani*, *Ae. rotanus* and *Ae. saipanensis*) were introduced to Guam from Rota and Saipan. Seven species have been found on all 4 islands, but only 4 of the initial 7 species known from Guam are in this group; of the remainder, *Ae. pandani* has been restricted to Guam and Rota, *Ae. oakleyi* has been recovered from Guam and Saipan, while *Ae. vexans vexans* has yet to be found on Rota Island.

World War II collections confirmed the presence of each of the initial 7 species and added 6 more to the fauna. In addition to *Ae. neopandani*, *Ae. rotanus*, *Ae. saipanensis* and *Cx. annulirostris marianae*, all indigenous to the Marianas, *Cx. litoralis* was found on each of the 4 islands in 1945 and *Cx. sitiens* was collected on Guam during the same year. *Cx. litoralis* was too well established to have been introduced during the war, but *Cx. sitiens* was prevalent throughout the western Pacific zone and introduction was probable.

The genus *Anopheles* was not known in the islands until *An. indefinitus* (Ludlow) was found on Guam in 1948. Introduction of the species from the Philippines, its type locality, was suggested by Hull (1952). The wide distribution and high

Table 1.–Mosquito collection records; south Mariana Islands.

Species	Initial Collection Dates and Distribution Records							
	Guam		Rota		Saipan		Tinian	
	L	A	L	A	L	A	L	A
Subfamily Anophelinae								
Genus *Anopheles* Meigen								
Subgenus *Anopheles* Meigen								
baezai Gater	1971							
barbirostris Van der Wulp	1976	1975						
lesteri Baisas and Hu	1971							
sinensis Wiedemann	1971	1970						
Subgenus *Cellia* Theobald								
indefinitus (Ludlow)	1948	1948			1970	1970		1973
litoralis King	1975	1975						
subpictus Grassi	1970	1970						1973
tessellatus Theobald	1971							
vagus Dönitz	1970	1970						
Subfamily Culicinae								
Genus *Aedeomyia* Theobald								
catasticta Knab	1967	1958						
Genus *Aedes* Meigen								
Subgenus *Aedimorphus* Theobald								
oakleyi Stone	1938	1938			1945	1945		
vexans vexans (Meigen)	1945	1911			1944	1944	1973	1945
vexans nipponii (Theobald)	1971							
Subgenus *Stegomyia* Theobald								
aegypti (Linnaeus)	1937	1905		1945	1944	1934	1944	1944
albopictus (Skuse)	1944	1948	1976	1976	1934	1934	1944	1945
burnsi Basio and Reisen	1971	1971						
dybasi Bohart		1970						
guamensis Farner and Bohart	1936	1911	1945	1945	1944	1944		1944*
hensilli Farner		1970						
marshallensis Stone and Bohart		1970						
neopandani Bohart		1976		1976	1951	1944		1945
pandani Stone	1938	1911		1976				
rotanus Bohart and Ingram	1971	1970	1945	1945				
saipanensis Stone		1970			1944	1944	1944	1945
scutellaris (Walker)		1970						
Genus *Culex* Linnaeus								
Subgenus *Culex* Linnaeus								
annulirostris marianae Bohart and Ingram	1945	1945	1945	1945	1944	1944	1945	
fuscocephala Theobald	1969	1969						
hutchinsoni Barraud		1970						
litoralis R. Bohart	1945	1945	1945	1945	1945	1945	1944	1945
pipiens quinquefasciatus Say	1936	1911	1976	1945*		1925	1973	1973
pseudovishnui Colless		1970						
sinensis Theobald		1970						
sitiens Wiedemann	1945	1945						
tritaeniorhynchus Giles	1962	1962						1973
vagans Wiedemann		1970						
Subgenus *Culiciomyia* Theobald								
papuensis (Taylor)		1970						
Subgenus *Lutzia* Theobald								
fuscanus Wiedemann	1971	1968			1970	1970	1973	1973
Genus *Mansonia* Blanchard								
Subgenus *Mansonioides* Theobald								
uniformis (Theobald)		1962						
Genus *Armigeres* Theobald								
Subgenus *Armigeres* Theobald								
subalbatus (Coquillett)	1969	1969						
Subfamily Toxorhynchitinae								
Genus *Toxorhynchites* Theobald								
Subgenus *Toxorhynchites* Theobald								
amboinensis (Doleschall)	1954	1954						
brevipalpis Theobald	1954	1954						
TOTAL SPECIES RECORDED	41		9		12		13	

*Collection was indicated or presence of species inferred through disease vector association.

degree of adaptation to local conditions at the time this species was collected caused the collectors to comment that the mosquito had probably existed on Guam for an appreciable period of time, and that it did not seem likely that it was introduced during or following WWII (Yamaguti and LaCasse 1950).

The list of post-WWII introductions and interisland dispersals of mosquitoes began with the discovery of *An. indefinitus* in 1948. *Aedeomyia catasticta* was found on Guam in 1958 and it has remained restricted to that island. *Cx. tritaeniorhynchus* and *Mansonia uniformis* were both discovered during routine surveillance on Guam in 1962, with *Cx. tritaeniorhynchus* being taken also on Tinian in 1973. *Cx. fuscanus*, first reported from Guam in 1968, was subsequently collected on Saipan along with *An. indefinitus* in 1970, and on Tinian in 1973. *Cx. fuscocephala* and *Armigeres subalbatus* were added to the Guam list in 1969.

A total of 17 species new to the Marianas were recorded during 1970 and 1971 by Reisen et al. (1972), and the input culminated with the collection of *An. barbirostris* and *An. litoralis* in 1975. All of these 19 species were discovered on Guam and all were introductions. This population included 8 *Anopheles*, 1 *Aediomorphus*, 5 *Aedes* including a new species, *Ae. burnsi* Basio and Reisen, and 5 *Culex* species.

The surveys on Tinian in 1973 and Rota in 1976 disclosed evidence of interisland distributions since the initial collections made in 1944-45. Five additional species, including 2 anophelines, were taken on Tinian, and 3 species plus confirmation of the existence of *Cx. pipiens quinquefasciatus* were added to the Rota list. An adult *Ae. neopandani* taken during routine mosquito surveillance at the air force base on Guam in 1976 was the first collection record for that island.

The interisland collections suggest introduction of *An. indefinitus* onto Saipan and Tinian, and movement of *An. subpictus* from Guam to Tinian. Since *Ae. albopictus* has been known to occur on the other 3 islands since 1944, it is possible that it existed on Rota at the same time but was missed during the wartime survey of that island. The recovery of *Ae. neopandani* from both Guam and Rota in 1976 suggests interisland introduction, as does the finding of *Ae. pandani* on Rota in 1976, and the record of *Ae. saipanensis* on Guam in 1970. The recovery of *Cx. pipiens quinquefasciatus* and *Cx. tritaeniorhynchus* on Tinian were new collection records for that island, and the taking of *Cx. fuscanus* on Saipan and Tinian so soon after its initial collection on Guam in 1968 strongly suggests interisland introduction.

The capability for introduction of mosquitoes into the Marianas exists and the probability for entry is constant. A U.S. naval facility was established on Guam following acceptance of the island from Spain in 1898, and the island became a major stopover for ships sailing from Manila to the United States and from Hawaii to the Philippines. In 1935 Pan-American Airways selected Guam to be one of the landing points for its China Clippers and as the number of flights increased, so did the opportunity for insect introductions. World War II saw the taking of Guam by Japanese forces and later the invasions of Saipan, Tinian and Guam and capture of Rota by the Americans. Guam became a center for the return of U.S. armed forces personnel from the western Pacific following the conclusion of WWII. Both the aerial and surface ports were reestablished and Philippine Airlines inaugurated its trans-Pacific flight in 1947.

The flow of American military manpower and resources to the Far East resumed during the Korean Conflict and later the Vietnam Campaign, and for several years following both crises ships and aircraft laden with retro grade cargo from the combat zones continued to be routed eastward via Guam. This was also the period when Japan Airlines developed its flight schedules through Guam. The post-war demand for interisland commuter service resulted in the chartering of several Guam-based airlines. The current schedule of 9 flights daily from Guam to Saipan and return, 6 with a stopover on Rota and 2 with landings on Tinian, enhances the probability of interisland dispersal of mosquitoes.

The most effective way to preclude introduction of unwanted flora and fauna into the Marianas is by establishment of a quarantine program. Such a program administered by the Government of Guam and applying to all travel and commerce at each aerial and surface port on Guam, coupled with a comparable program in effect at the airports on Saipan, Rota and Tinian, would provide the protection which is required.

Quarantine activities at aerial ports in the Pacific have been discussed by Basio et al. (1970) for Manila and Joyce (1961) for the Hawaiian Islands. The requirement for a program to preclude the introduction of mosquitoes and other pest animals onto Guam was recognized by early authors (Farner 1944, Reeves 1953, and Joyce 1961). Holway (1964) and Reisen et al. (1972) recommended increased emphasis be placed on mosquito surveillance around both the sea and aerial ports. The most comprehensive quarantine proposal for Guam was presented by Hayes and Whitworth (1969). The history of introductions and interisland dispersals of mosquito species substantiates the need.

The periodic surveys made on Guam beginning in 1948 have shown a constant augmentation in the number of mosquito species occurring on that island. This number increased from the 13 known at the end of WWII to a total of 41 by 1975. In addition, collections on Saipan and the mosquito surveys made on Tinian in 1973 and on Rota in 1976 showed gains in the number of species for each island. This indicates a steady influx of species onto Guam with subsequent interisland dispersal. Both the influx and dispersal can be attributed to aerial and vessel traffic, and until a screening program to stop this flow is established it is anticipated that the traffic will continue.

Both the health and economy of the southern Mariana Islands can be affected adversely by mosquito introductions. An island community is particularly vulnerable to disaster through introduction of pest and vector species of mosquitoes. Without a history of repeated exposures human populations tend to be more susceptible to new infective agents. Since the probability of introduction of vector species exists for each of the islands, then the possibility of infection and epidemic must be expected. This form of disaster has already been documented for the Marianas. Dengue was encountered by the American invasion forces on Saipan, Tinian and Guam during 1944, and an epidemic of Japanese B encephalitis occurred on Guam in 1947-48. Guam suffered its first cases of autochthonous malaria in 1966, and additional cases were diagnosed on the island in 1969. Vectors of these and other mosquito-borne diseases occur throughout the Indo-Pacific area and the potential exists for their spread into the Marianas.

The number of mosquito species on each of the 4 southern Mariana Islands has increased during the period 1945-1980, and mosquito-borne disease outbreaks have already been recorded on Guam, Saipan and Tinian. It is anticipated that the influx of mosquitoes will continue and eventually threaten human health if it is not controlled.

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MYRIOPHYLLUM GROWTH IN IRRIGATION CANALS FOLLOWING REDUCTIONS OF COMPETITIVE *POTAMOGETON* BY *TILAPIA ZILLII*

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ABSTRACT

Significant reductions of competitive *Potamogeton pectinatus* L. by *Tilapia zillii* (Gervais) were not followed by increases in the *Myriophyllum spicatum* var. *exalbescens* Jepson density, in irrigation canals of southeastern California. Both species of aquatic weeds may be controlled at low density levels when adequate numbers of the herbivorous fish are present.

The use of herbivorous African fish of the genus *Tilapia* [Cichlidae] in southeastern California irrigation canals and drains has produced substantial to complete biological aquatic weed control during the past several years, although certain obstacles related to fish availability and management continue to exist (Legner 1979, Legner and Pelsue 1977). One species, *Tilapia zillii* (Gervais) is favored in biological control because of its adaptation to rapidly flowing canal waters (0.9 - 1.5 m/sec) and low water temperatures (10°C).

Aquaria studies have revealed a strong preference of *T. zillii* for *Potamogeton pectinatus* L. over *Myriophyllum spicatum* var. *exalbescens* Jepson (Hauser et al. 1977, Legner unpublished data), so that the latter weed might be selectively excluded by *T. zillii* in nature, leaving it as a persistent monoculture. These results led to the establishment of a temporary *T. zillii* quarantine by the California Department of Fish and Game, from the agricultural areas adjacent to the Colorado River. The presumption was that since the fish would probably not control *Myriophyllum*, it would be useless to weed control agencies. Thus, the annual release of large numbers of these "useless" herbivores would not justify the threat of their possible establishment in the lower Colorado River, where important waterfowl habitats might be reduced.

The results of 7 years of research with *T. zillii* in the Imperial and Coachella Valleys now show that *M. spicatum* is also effectively controlled along with *P. pectinatus* and other weed species (Legner and Fisher 1980, Legner unpublished data). Discrimination by *T. zillii* in the usually mixed stands of both weed species is not complete, considerable nourishment being attained from the often significant numbers of crustaceans, larval mosquitoes and chironomid midges, and aquatic snails that seek refuge in the *Myriophyllum* mass. Thus, *T. zillii* may be considered on an experimental basis for the biological control of aquatic weeds in irrigation canals of the Palo Verde Valley and other areas adjacent to the Colorado River. Large scale reproduction in the relatively cool waters of the Colorado River system is not expected, given the minimum 22.5°C spawning requirements of this species (Hauser 1977). Also, extensive winter mortality would probably be common in water temperatures below 10°C. The high energy consumption of alternative mechanical canal cleaning operations is not justified when a possible effective fuel efficient biological control exists.

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INVESTIGATIONS ON NUISANCE MIDGES IN WATER PERCOLATION BASINS, MONTCLAIR, CALIFORNIA

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During 1979 an investigation was initiated regarding nuisance levels of chironomid midges produced from the San Antonio Flood Channel and Montclair percolation basins. This particular flood control system operated by the Chino Basin Municipal Water District, Cucamonga, CA, consists of 4 percolation or spreading basins which receive water from a concrete-lined flood channel. These basins are used for percolating water into the ground for replenishing subsurface aquifers. However, during flooding of the basins, nuisance midges are produced in large numbers, and complaints regarding swarms of adult midges have been registered at the Chino Basin Municipal Water District by residents neighboring the flood control system.

The thrust of this investigation was to make a quantitative and qualitative determination of midge fauna in the fast-moving, shallow waters of the flood channel and the deep waters of the percolation basins. Additionally, it was desirable to ascertain how the midge species composition changes seasonally and how this information might be incorporated into a successful midge abatement program. During the course of this study applications of insecticides were made along with installation of electrocuting zap traps and adult light traps. Here we present a summary of the 1979 studies and offer suggestions for future studies to alleviate the acute pest problem produced by chironomid midges.

The concrete-lined San Antonio Flood Channel transports water from Silverwood Lake through Devils Canyon into the Montclair percolation basin system (Figure 1). When not diverted into the basins this water continues south to Orange County, CA. At maximum water depth these basins vary in size from 4.4 to 12.3 ac with basin 3 being the smallest and basin 2 the largest. Basin 1 and 4 each cover about 8 ac. Two of the 4 percolation basins, basins 1 and 2, are bordered on the east and west by residential dwellings. Basin 3 is bordered on the west by residential homes and on the east by an elementary school, while the fourth basin, located south of Interstate 10, is surrounded by trees and other terrestrial vegetation.

Beginning in April and extending through November a routine larval sampling program was established where 3 of the 4 percolation basins were sampled by lifting 5 "6x6x2" mud samples from the bottom of each basin with an Ekman dredge. Due to the isolation of basin 4 it was excluded from the sampling program, since it was not considered to significantly contribute to the midge problem. The collected mud samples were processed according to Mulla et al. (1971). The contents of each mud sample was taken to the laboratory where the number of larvae were counted and taxonomic determinations made according to Mason (1968) and Oliver et al. (1978).

The results of this monitoring program are presented in Figure 2. For the majority of the sampling season basin 1 contained the greatest numbers of midge larvae followed by basin 3 and basin 2. In the first basin the midge numbers started out at about 100 larvae/ft^2 of bottom sediments in April and increased to over 600 larvae/ft^2 by mid-June. During this same period the larval densities in basins 2 and 3 showed a similar increase reaching approximately 125 and 170 larvae/ft^2 of mud, respectively. The midge species of quantitative importance in basin 1 from April-mid-June were species of *Chironomus*, *Tanytarsus* and *Procladius*, while *Tanytarsus* spp. and *Procladius* spp. were most prevalent in basins 2 and 3.

Based on the increasing densities of midge larvae in the 3 basins through June, and the number of complaints registered to the Chino Basin Water District by residents east of basin 1, it was deemed necessary to treat the basins with the organophosphorus insecticide Abate, the only registered material currently available for aquatic midge control. Thus on June 21, 1979 basins 1 and 2 were treated with Abate EC4. The insecticide was dripped into the water coming into basin 1 from the concrete-lined channel. This basin having an average depth of 23′ at the time of treatment was treated at a rate of 1/3 lb A.I./surface acre. Basin 2 was treated at a rate of 0.25 lb A.I./surface acre by dripping the liquid formulation into the water coming over the spillway. This basin had an average depth of 21′ at the time of treatment. Basin 3 was not treated since it was not considered a significant contributor to the annoyance problem primarily due to the distance separating this basin and the area inundated by swarming midges.

The immediate effectiveness of this larvicide on the midge larval populations can be seen in Figure 2. By 1 wk posttreatment the larval populations in basin 1 were reduced from about 650 larvae/ft^2 to approximately 140 larvae/ft^2, while in basins 2 and 3 the avg. no. larvae/ft^2 dropped from about 125 to 25 and 170 to 27, respectively. The reduction in larval numbers in basin 3 was probably due to the diffusion of treated water from basin 2. The efficacy of the compound was short lived with the larval densities increasing 2 wks posttreatment. Based on the posttreatment samples it was found that Abate affected species of *Chironomus* and *Tanytarsus*, however, *Procladius* was not reduced by this treatment. This phenomenon of natural resistance by species of *Procladius* to this and other O-P insecticides has been reported by Ali and Mulla (1978).

Following the June 21 Abate treatment the larval populations in basin 1 remained below 200 through September and were well below 100 larvae/ft^2 in basin 2. As shown in Figure 2 the larval densities increased in basin 3 to about 300 larvae/ft^2 through July and August, after which they declined to about 75 larvae/ft^2 in September. During July resident complaints east of basin 1 were steadily increasing and, although it was believed unwarranted, an insecticide application was made

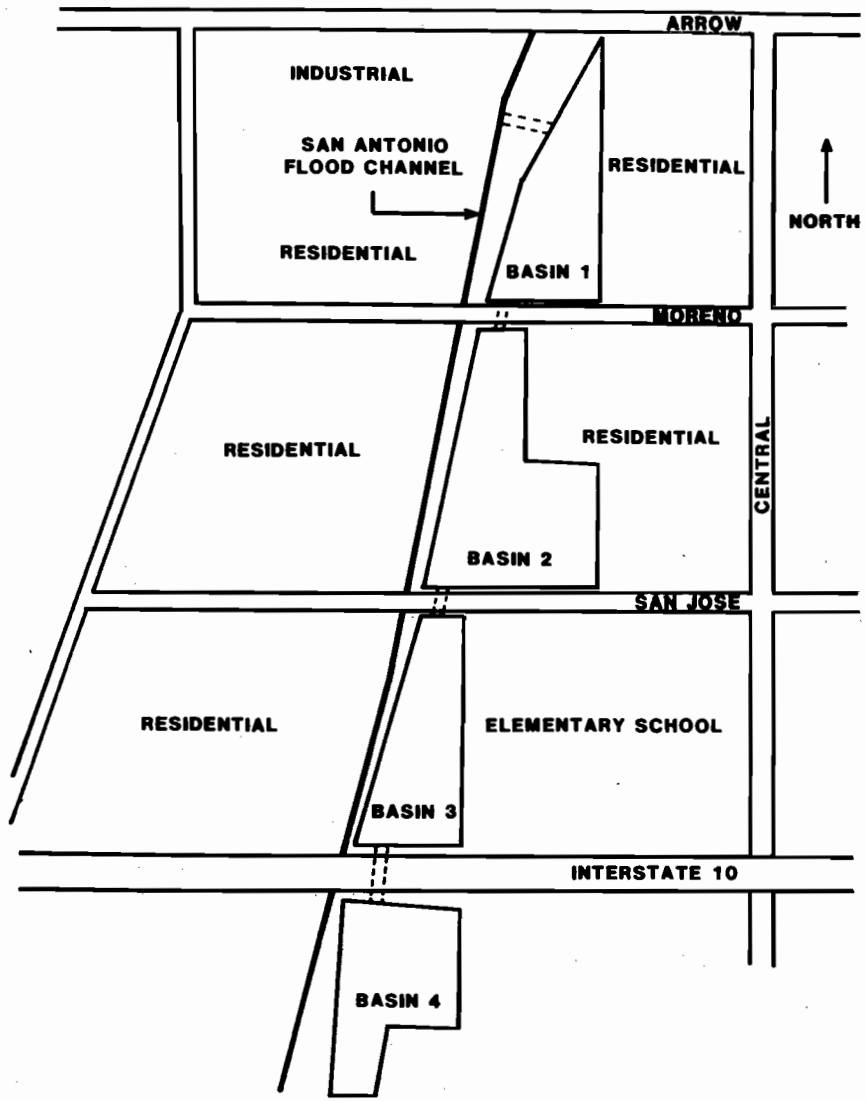

Figure 1.–San Antonio flood channel and percolation basins in the City of Montclair, California.

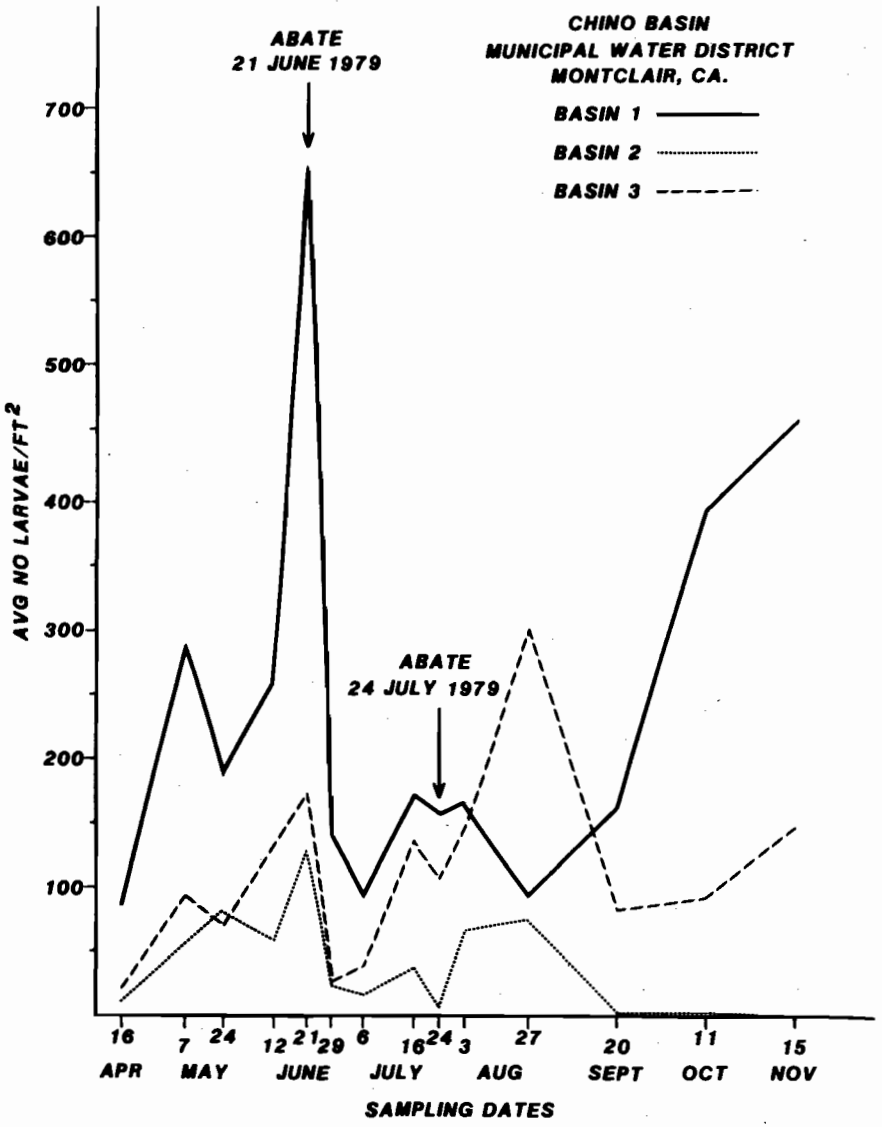

Figure 2.–Averge no larvae/ft2 from percolation basins in the City of Montclair, California.

to basin 1. On July 24 this basin was treated at 0.5 lb A.I./surface ac using the treatment methods previously described. This treatment had no affect on the midge larval densities (Figure 2) nor reduced the frequency of complaints by the residents bordering this basin. This was primarily due to the fact that the midge population at this time were predominantly species of *Procladius* and as previously indicated this midge is resistant to the O-P Abate.

Through July when midge larval densities were under 200 larvae/ft^2 complaints were continually being registered by residents located east of basin 1. In order to gather quantitative and qualitative data regarding adult midges in this area 2 New Jersey light traps were set up. One adult trap was located behind home A which is immediately adjacent to basin 1 and the second trap was situated at home B in the center of the residential area where adult swarms had been reported. The traps were placed in these areas overnight for 3 sampling dates. The results are presented in Table 1.

By looking at the total number of midges collected from the 2 areas on the 3 sampling dates it can be seen that the overnight chatches in the center of the residential neighborhood was roughly ½ that collected behind home A just next to basin 1. This was expected since this dwelling is adjacent to basin 1 and in the direction of the prevailing wind. As with the larval samples the midge species of quantitative importance were *Tanytarsus*, *Chironomus* and *Procladius* with *Tanytarsus* spp. usually predominating (Table 1). However, a large number of *Cricotopus* adults were collected in the light traps although larvae of this genus were quantitatively unimportant in the collected mud samples. The source of these *Cricotopus* adults was most likely from the concrete-lined flood channel which was found to exclusively harbor *Cricotopus* spp.

To provide local relief for the residents in close proximity to basin 1, 4 electrocuting zap traps were set up at 4 residences in the midge infested area. The intention of installing these traps was to provide the residents temporary relief from the nuisance swarms of midges away from windows and doors, allowing unobstructed passageway to and from their homes. It has been shown previously that these traps will provide local relief, however, they have been found to have negligible effects on the overall midge population (Mulla and Axelrod, unpublished data).

One of the main problems encountered in controlling midge populations has been natural and acquired resistance of midge species to organophosphorus insecticides (Ail and Mulla 1978). Consequently, midge larvicides should be applied only as a last resort and at a time when the population densities have increased sufficiently to yield maximum control. Naturally, the midge species will also determine the efficacy of insecticide treatments.

Due to the problems associated with insecticide - e.g., escalating prices, unavailability of effective compounds, only yielding short-term control - it would be more beneficial in the long run to select other suppression tactics to deal with the chironomid midge problem in this flood control channel. The first method would be the stocking of larvivorus fish such as carp which can thrive in deep water reservoirs, living on or close to the bottom and their diets include bottom dwelling insect larvae. Naturally, water must be present in the basins throughout the year. Intermittent flooding and drying of basins lead to heavy production of midges (Ali and Mulla 1978b). As the basins remain full for long periods and remain in stable condition, midge productivity declines markedly. Consequently the use of natural predators and limited use of insecticides might be the best solution for controlling midges in this flood water system.

ACKNOWLEDGMENT.—These studies were conducted in cooperation with the Chino Basin Municipal Water District. The assistance and encouragement of Fran Brommenshankel and Don Peters of that District is duly acknowledged. John Chaney of the Department of Entomology, University of California, Riverside, also assisted in this study.

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Table 1.—Number of adult midges collected in New Jersey light traps at Chino Basin Municipal Water District Spreading Grounds, Montclair, CA.

Date (1979)	Trap Location[1]	Midge Species					
		Chironomus	Tanytarsus	Cricotopus	Procladius	Other	Total
August 2	Home A	16	137	95	156	0	404
	Home B	6	128	71	56	2	263
Sept. 6	Home A	104	202	135	80	6	527
	Home B	22	153	42	8	9	234
Sept. 20	Home A	321	267	91	77	35	891
	Home B	130	231	44	14	50	469

[1]Home A, adjacent and right behind Basin 1, home B further away in the center of the residential development.

PREVALENCE AND POTENTIAL PROBLEMS OF NUISANCE AND VECTOR INSECTS IN MALIBU CREEK, LOS ANGELES COUNTY, CALIFORNIA

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INTRODUCTION.–The Malibu Creek drainage is located 30 miles west of downtown Los Angeles in the Santa Monica mountains of Southern California. It receives runoff from Las Virgenes and Cold Creeks and also from Liberty Canyon. Lakes in the drainage area including Westlake, Malibu Lake and 20th Century Lake. The creek discharges into the Pacific Ocean by the way of a lagoon located near the community of Malibu, California.

Prior to July 1978 the Las Virgenes Municipal Water Districts's Tapia Facility discharged reclaimed water (treated to the secondary level) into Malibu Creek at a point 5 miles upstream from the lagoon (discharge point 003 - Figure 1). The reclaimed water was discharged into the creek only during the cooler months of the year from mid-November to mid-March. Under the influence of this seasonal discharge and the prevailing winter rains Malibu Creek flowed in the winter, spring and summer months and during the late summe and fall water flow became discontinuous and isolated pools of water were created.

As part of a pilot study, the Tapia facility has been discharging reclaimed water into Malibu Creek on a year-round basis at discharge point 003 since July 1978. Concern has been expressed that such a discharge during the summer months may possibly result in the production of pest and vector insects in the creek and in the terminal lagoon area.

The purpose of this study was to complete an assessment of the production of nuisance and vector insects in the Malibu Creek area. The study included mosquitoes (Culicidae), blackflies (Simuliidae) and aquatic midges (Chironomidae). Quantitative data was gathered on the abundance of these insects over a 4 month period (Aug. 1-Dec. 1, 1979 at the various sampling stations utilized in the study. Within the relatively short duration of the study period we also endeavored to evaluate potential problems created by the specific vector and pest insects found in the study area and to suggest specific remedies for the elimination of high populations of mosquitoes, blackflies and midges should the potential for such populations be found to exist in the Malibu Creek area.

Additionally we wished to determine if the discharge of reclaimed water increases the production of mosquitoes, blackflies and midges and to evaluate the relationship between algae and macrophyte growth and the production of nuisance insects.

METHODS AND MATERIALS.–All sampling was completed between August 1, 1979 and December 1, 1979 during the 4 month study period. Sampling sites utilized in this study are listed below and shown on the map of the Malibu Creek area in Figure 1.

Upstream Site 1 . R9. .Malibu Creek, Malibu Creek State Park
Upstream Site 2 R12 Malibu Creek, confluence with Cold Creek
Downstream Site 3 R10. Malibu Creek, upstream Rindge Dam
Downstream Site 4 R3 Malibu Creek, downstream Rindge Dam
Downstream Site 5 . R4 . Malibu Creek, at Cross Creek Road

Because of dredging activities carried out between Cross Creek Road and Malibu Lagoon during the study period, site R4 could only be utilized during November 1979.

Sampling Plan - - Larval mosquito populations were sampled on a weekly basis at each sampling site. Samples were taken in suspected larval habitats along the stream margin as well as potential habitats (isolated pools, discontinuous depressions) in the area of each site to determine the presence of mosquito larvae. Larval samples were taken by the standardized dipper technique (Hagstrum 1971) in which 10 dips/site were taken and returned to the laboratory where all larvae were identified and counted.

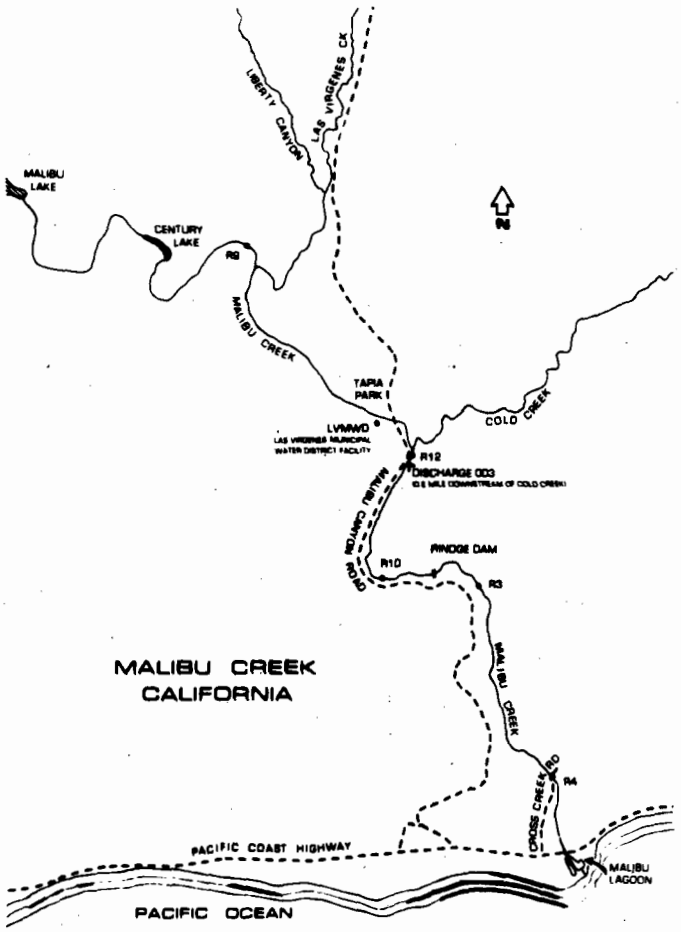

Figure 1.–Schematic diagram of Malibu Creek and associated tributaries, with sampling sites shown as R003-R12.

Adult mosquito populations at each site were sampled on a biweekly basis beginning September 13, 1979 using CDC miniature light traps baited with dry ice (Newhouse et al. 1966).

Immature blackflies (Simuliidae) were sampled using a method similar to that described by Williams and Obeng (1962). Larval and pupal numbers were estimated utilizing polyethylene strips as artifical substrates. The strips measured 38.1 x 1.3 cm (15 x ½ in.) and were attached to wire spikes with staples. Three strips were placed each week at each creek sampling site. The following week, allowing 7 days for colonization, 2 strips from each sampling site were removed and placed in 70% alcohol for future counting and identification of larvae and pupae.

Larval chironomid midges were sampled by benthic scoop samples taken at weekly intervals. This scoop removed a 6"x6"x2" area of the substrate and this material was placed in a bucket and mixed thoroughly with water. The sample was then sieved through a 52-mesh screen, transferred to a container and transported to the laboratory. The larvae were recovered, floated, and counted in the manner described by Mulla et al. (1971).

RESULTS.–The results of all samplings completed in the Malibu Creek area during the study period are presented in Table 1.

A. Mosquitoes - - The larval and adult mosquitoes collected from the Malibu Creek area during the course of the study period include the following species: ***Anopheles franciscanus, An. freeborni, Culex apicalis, Cx. tarsalis, Culiseta incidens*** and ***Cs. inornata.***

The mosquitoes found in the greatest number during the study period (August 1, 1979 - December 1, 1979) were in the genus *Anopheles*. Both larval and adult samples taken during the study indicate the presence of *An. franciscanus* and *An. freeborni* along various portions of Malibu Creek (both above and below discharge point 003). The higherst populations of *An. franciscanus* larvae and adults located in the Malibu Creek drainage area during the study period were found at R9 (upstream of discharge) while the highest populations of *An. freeborni* larvae and adults were found at R3 (downstream of discharge).

Larvae of *An. franciscanus* were found to be associated with mats of filamentous algae (*Cladophora*, *Ulothrix*, and *Vaucheria*) usually found along the margins of slow moving sunny sections of the creek. Occasionally *An. franciscanus* larvae were also found in mats of algae floating in the center of slow moving portions of the creek. *An. freeborni* larvae, however, were found in sheltered sites along the margins of slow moving sections of the creek. At site R3 *An. freeborni* larvae were found to be associated with grasses (*Cynodon* and *Echinochloa*) growing along the creek margin. At the present time the extent of growth of this grass is limited, occurring only in a very few locations. Expanding growth of these grasses could result in growing populations of this mosquito.

The other mosquito species sampled during the study were usually found in isolated pools or depressions separated from the main body of the creek. These isolated pools of water were created after periods of peak discharge and subsequent receding, and were formed in flat or sunken areas located adjacent to the stream margin. The main mosquito found in these habitats during the early part of the study was *Cx. tarsalis* while in the last month, November, as expected *Cs. incidens* and *Cs. inornata* had begun to appear in some of these sites. *Cx. tarsalis* is the most common and widespread mosquito in California and the immature stages of *Cx. tarsalis* are found in practically all types of freshwater habitats. *Cs. incidens* and *Cs. inornata* are essentially cool weather mosquitoes and the larvae are also found in a variety of freshwater habitats similar to those of *Cx. tarsalis*.

Populations of larval mosquitoes declined during the last month of the study period probably as a result of cooler temperatures although *Culiseta* sp. became more numerous. Similarly adult trap catches were high at site R9 in September but much lower in October and November.

B. Blackflies - - Two blackfly species, *Simulium virgatum* and *S. argus*, were found at each of the Malibu Creek sampling sites. *S. virgatum* was found in greater numbers at sites characterized by a faster and more turbulent current. Larvae and pupae were found in the swiftest part of the stream, with large numbers congregating on rocks directly under the rushing water. On the other hand, *S. argus* larvae and pupae were found in greater numbers in currents of lower velocity and were found to be attached to almost any type of substrate. At the various sampling sites both species could be found but the relative numbers of the two species colonizing the polyethylene strips seemed to be influenced by the placement of the strips at each site and the immature blackfly micro habitat preferences previously described. Therefore, care was taken to place polyethylene tapes in the same portion of the stream at each site on successive sampling dates to measure relative population trends during the course of the study period.

As can be seen from Table 1, the highest observed weekly colonization rates for immature blackflies were at sites R10 and R3 where the main species present was *S. virgatum* while the sites characterized by lower colonization rates, R9, R12 and R4, were dominated by *S. argus*.

In contrast to the general decline in mosquito populations noted during the later part of the study period, populations of immature blackflies at the study conclusion remained at relatively high levels at sites R10, R3 and R4 when compared to previous samples at the same sites.

C. Midges - - Aquatic midges in the genera *Cryptochironomus*, *Polypedilum* and *Procladius* were found to dominate in the larval scoop samples taken to monitor populations of these insects. Site R10 showed the highest numbers of larval chironomids sampled in Malibu Creek but populations of these insects found were low in relationship to numbers found in other aquatic situations.

DISCUSSION.–Mosquitoes propagate in stagnant water or portions of streams that are slow moving and clogged with vegetation. The potential for mosquito production in the Malibu Creek area is greater where the water flow in the creek is impaired or minimal, resulting in the creation of pools, puddles and discontinuous depressions. Such conditions are similar to those which are seen in Malibu Creek above the discharge point during the summer months or below discharge point when the creek is receiving no discharge.

In general, creeks and streams with continuous flow are not good mosquito habitats. In such situations suitable habitats for mosquito larvae are scarce and additionally in continuous and deeper water, predators have a greater capability to eliminate mosquito larval populations. Therefore, continuous substantial

Table 1.—Population densities of nuisance insects collected in the Malibu Creek area, Los Angeles County, California (1979).

Sampling date	Larval Mosquitoes No./10 dips			Adult Mosquitoes No./trap				Larval blackflies No./tape		Larval
	Culex	*Anopheles freeborni*	*Anopheles franciscanus*	*Culiseta*	*Culex*	*Anopheles*	*Culiseta*	*Simulium virgatum*	*Simulium argus*	Chironomidae No./sq. ft.
SITE R-9 (upstream from discharge)										
Aug. 10	0	0	15	-	-	-	-	-	-	68
Aug. 16	0	0	11	-	-	-	-	-	-	-
Aug. 27	0	0	14	-	-	-	-	0	5	-
Sept. 6	0	4	33	-	-	-	-	1	0	-
Sept. 13	0	5	23	-	4	179	0	-	-	16
Sept. 21	0	4	3	-	-	-	-	-	-	12
Sept. 27	0	17	9	-	3	131	0	2	34	0
Oct. 4	1	8	2	-	-	-	-	2	4	28
Oct. 11	0	1	2	-	no sample		4	8		0
Oct. 18	0	2	0	-	-	-	-	4	6	0
Oct. 24	0	0	0	-	1	3	1	6	8	0
Nov. 1	0	2	5	-	-	-	-	33	6	-
Nov. 15	0	0	1	-	0	0	0	4	13	-
SITE R-12 (upstream from discharge)										
Aug. 10	0	0	1	0	-	-	-	-	-	20
Aug. 16	0	0	0	0	-	-	-	-	-	-
Aug. 17	16	0	2	0	-	-	-	-	-	-
Sept. 6	6	0	13	0	-	-	-	-	-	-
Sept. 13	9	0	2	0	1	0	0	-	-	32
Sept. 21	7	0	1	0	-	-	-	-	-	44
Sept. 27	9	0	3	0	1	0	0	2	10	4
Oct. 4	8	1	2	0	-	-	-	1	4	8
Oct. 11	8	0	0	0	1	0	0	6	12	8
Oct. 18	8	0	1	0	-	-	-	4	17	24
Oct. 24	15	0	1	12	0	0	0	12	48	0
Nov. 1	9	0	0	7	-	-	-	15	61	-
Nov. 15	0	0	0	0	no sample		3	18		-
SITE R-10 (downstream from discharge)										
Aug. 10	9	0	3	-	-	-	-	-	-	28
Aug. 16	6	0	6	-	-	-	-	-	-	-
Aug. 27	0	0	0	-	-	-	-	377	38	-
Sept. 6	3	0	8	-	-	-	-	58	1	-
Sept. 13	0	0	9	-	no sample		98	2		52
Sept. 21	0	0	10	-	-	-	-	190	0	72
Sept. 27	0	0	8	-	2	0	0	100	3	76
Oct. 4	1	0	0	-	-	-	-	156	2	52
Oct. 11	0	0	8	-	1	0	0	151	6	36
Oct. 18	0	0	5	-	-	-	-	83	10	63
Oct. 24	0	0	2	-	0	0	0	no sample		80
Nov. 1	0	0	1	-	-	-	-	57	4	-
Nov. 15	0	0	0		no sample			50	2	-
SITE R-3 (downstream from discharge)										
Aug. 10	9	18	0	-	-	-	-	-	-	16
Aug. 16	2	6	0	-	-	-	-	0	1	-
Aug. 27	0	10	0	-	-	-	-	no sample		-
Sept. 6	0	25	0	-	-	-	-	155	24	-
Sept. 13	0	19	0	-	0	16	0	139	66	48
Sept. 21	0	32	0	-	-	-	-	2	13	20
Sept. 27	0	36	0	-	3	13	0	24	32	8
Oct. 4	0	41	0	-	-	-	-	10	12	20
Oct. 11	0	62	0	-	0	1	0	84	39	4
Oct. 18	0	29	0	-	-	-	-	26	3	12
Oct. 24	0	38	0	-	0	0	0	24	17	20
Nov. 1	0	10	0	-	-	-	-	8	54	-
Nov. 15	0	7	0	-	0	3	5	29	33	-
SITE R-4 (downstream from discharge)										
Oct. 4	0	0	3	-	-	-	-	-	-	
Oct. 11	0	0	0	-	-	-	-	-	-	
Oct. 18	0	3	0	-	-	-	-	4	44	
Oct. 24	0	10	9	-	0	0	1	6	30	
Nov. 1	0	0	0	-	-	-	-	8	8	
Nov. 15	0	0	0	-	0	1	3	9	38	

water flow will result in no noticeable increase in mosquito production along most of Malibu Creek.

Immature blackflies are found only in well oxygenated lotic water habitats. Larvae and pupae attach themselves to various substrates in portions of the stream where the current is favorable for their development. The discharge of secondary effluent from the Tapia Facility will certainly increase the populations of larval blackflies in the running water situations downstream from the discharge point. Blackflies occur naturally in those sections of Malibu Creek which flow during the winter, spring and early summer of the year but they would probably not be present in the late summer and fall months throughout most of Malibu Creek since without effluent discharge the water in many of these areas would cease to flow and isolated pools would be created. Forutnately, the blackfly species currently found in Malibu Creek are not anthropophilic and pose little nuisance potential to humans.

Mosquito Breeding Potential - - Although we have mentioned previously that the year round discharge of effluent into Malibu Creek would proably result in no noticeable increase in mosquito populations, there remain certain areas along the creek that would be subject to low-level flooding created by the increased flow brought about by effluent discharge. These areas could then produce mosquitoes, so care must be taken with regard to monitoring both flow discharge rates and areas subject to inundation for potential problems. On those occasions when large quantities of water are discharged in short periods of time, some low areas located along sections of Malibu Creek near sites R10 and R3 may be inundated. Most of Malibu Creek possesses good incline, facilitating flow within the main stream channel, and those areas subject to inundation are not extensive in any sense.

Another potential problem exists in the low-lying areas near Malibu Lagoon. Channelization of a section upstream from the lagoon has created good flow into the lagoon but it might be necessary from time to time to breech the berm between the lagoon and the ocean at regular intervals so that the water level in the lagoon does not rise to the extent that it floods the extensive potential mosquito breeding areas bordering the lagoon. These salt marsh areas have been known to produce large numbers of *Aedes squamiger* in previous seasons and, with increased creek flow as a result of effluent discharge, the water level of Malibu Lagoon should be closely monitored to eliminate the potential flooding of these marsh areas.

Aquatic Vegetation and Mosquito Breeding - - There was a definite relationship found to exist between the presence of *Anopheles* larvae and aquatic vegetation. *An. franciscanus* larvae were found associated with filamentous algae and *An. freeborni* larvae were found to be associated with grasses (*Cynodon* and *Echinochloa*). Since it is known that the effluent discharge is rich in nutrients, it could be postulated that this discharge could lead to a downstream increase in algae and macrophytes which may serve as potential mosquito habitats. Such conditions during the current discharge period, however, were not noted on a large scale.

We examined this question by comparing creek sites upstream (R9 and R12) from the effluent discharge point (003-Figure 1) with those downstream (R10 and R3).

The amounts of algae and macrophytes observed at the upstream sites were equivalent to those observed downstream and hence the creation of increased vegetative growth could not be attributed to the nutrient-laden secondary effluent.

Pests Identified in the Study Area - - The main mosquito species collected in the study area, *An. freeborni* and *An. franciscanus*, are known to feed preferentially on mammals but are not especially anthropophilic in nature. *Cx. tarsalis* feeds on man to some extent, and it plays an important role as a potential vector of WEE (Western Equine Encephalitis) and SLE (St. Louis Encephalitis) in the Western United States (Bohart and Washino 1978). The populations of this mosquito sampled during our study at Malibu Creek are not large as compared to some other areas in the State.

Of the other mosquitoes samples during the four month study period, *Cx. incidens* is known to be anthropophilic and at times a significant pest species. *Cs. inornata* feeds primarily on large domestic mammals and seldom bites man.

Blackflies are similar to mosquitoes in that females are bloodsucking and certain species can be very annoying to man. The two blackfly species collected at Malibu Creek, *S. virgatum* and *S. argus* are not known to be significant pests to man. These species seem to feed preferentially on horses and other large mammals (Hall 1972).

Chironomid midges can constitute a human ruisance problem when the adults emerge in large numbers from aquatic sites and congregate near human dwellings. The populations of these insects observed in the Malibu Creek area are not deemed to pose such a problem since they occur in fairly low densities along only limited stretches of Malibu Creek.

Nuisance Insect Control Procedures -- In the event that insect production during water discharge periods is materially increased, several control methodologies might be employed to manage their populations in the area.

Flushing - - The flusing action of moving water can be utilized for mosquito control. If a large head of water is discharged, for example at weekly intervals, the current can wash the immature stages of mosquitoes downstream. Mosquitoes are much more sensitive to this management practice than other organisms. This management practice could be easliy coordinated with effluent discharge patterns.

Biological Control Agents - - Mosquito fish and other biological control agents are more efficient in deeper water than in small discontinuous bodies of water. With substantial water flow, not only predaceous fish but also game fish, will probably thrive. If necessary, the stream or portions of it can be stocked with mosquito fish from time to time.

Chemical Control Measures - - At times it might be necessary to treat isolated pools and puddles with relatively safe and innocuous granular chemical larvicides that will leave no lasting residues in the ecosystem.

Two registered materials temephos (Abate) and fenthion are currently available for use in aquatic situations and are routinely employed to control high populations of mosquitoes. Temephos is registered for the control of blackflies and aquatic midges. The extent and magnitude of these treatments would be quite limited as only those small portions of the total creek habitat which show problems need be treated. We believe that these treatments would have to be resorted to very infrequently.

Channelization - - Most of Malibu Creek possesses good incline facilitating channelized flow. However, there are a few

locations where the stream bed becomes wide and clogged with vegetation. Such a situation existed near R4 between Cross Creek Road and Malibu Lagoon. Extensive channelization in this area, completed in September and October 1979, has eliminated potential mosquito breeding sites by confining the stream flow to several small waterways. Such channelization practicies are employed routinely for flood control purposes.

CONCLUSIONS.--In discussing the results of our study, it must be remembered that this study was done only over a 4 month period from August 1, 1979 to December 1, 1979. We did not have the opportunity to observe the populations of the insects surveyed (mosquitoes, blackflies and midges) over a complete seasonal cycle and hence it is difficult to provide positive conclusions regarding their possible abundance at other times. It is known that mosquitoes and blackflies not found in this survey do occur in the Malibu Creek during other seasons of the year. Mosquitoes such as *Culex thriambus*, *Cx. boharti* and *Cx. peus* have been found in the study area by personnel from the Los Angeles County West Mosquito Abatement District and hence this study must not be taken as a complete survey of the nuisance potential known to occur in this area.

Considering the data gathered from this study, several concluding statements can be made with regard to the nuisance insects observed in the Malibu Creek area and the influence of effluent on populations of these insects.

1. It is our feeling that year-round discharge of effluent from the Tapia Facility into Malibu Creek will not materially increase the populations of nuisance insects in the areas downstream from the discharge.
2. Most likely overall populations of mosquitoes will not increas while populations of blackflies will increase when compared to seasonal populations of these insects before the creek received year-round discharge.
3. Areas of potential mosquito breeding do exist in the Malibu Creek area and should be monitored for production of mosquitoes. These breeding potentials exist whether there is reclaimed water discharge or not. The salt marsh area bordering Malibu Lagoon can create a significant problem under flooded conditions, but this can be avoided by breeching the berm between the lagoon and the ocean at regular intervals. Additionally, the effluent discharge flow pattern should be of an irregular nature to provide flushing of mosquito habitats and yet not be extremely large in volume so that depressions and pools adjacent to the creek bed are flooded. Flooding these sites will contribute to unnecessary mosquito breeding.
4. Several specific control remedies including biological, physical and chemical control treatments could be used to handle potential outbreaks of nuisance insects in sections of Malibu Creek.

ACKNOWLEDGMENTS.--These studies were conducted in cooperation with EDAW, Las Virgenes Water District and the Los Angeles County West Mosquito Abatement District.

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FLIGHT AND DISPERSAL OF NATIVE MOSQUITO SPECIES IN THE NEW RIVER BASIN, IMPERIAL COUNTY, CALIFORNIA[1]

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ABSTRACT

Two mark-release-recapture studies were performed to extend the knowledge of *Culex tarsalis* bionomics in the New River area of Imperial County, California. Under the direction of the Imperial County Health Department, the studies were designed to determine movements of mosqutioes within and out of the river bottom, and utilized a trap arrangement consisting of three concentric circles centered on the New River. Adults were collected for marking and release by CDC traps with CO_2, and releases were made at the center and four points on the inner circle.

Although movement was predominantly up and down the river bed, approximately 20% of *Culex tarsalis* recaptures in each of the two studies were made at trap locations on the bluffs surround the release sites, indicating that this species would disperse out of the river bottom. Life table and population estimates were not possible because either climatic or logistic problems prevented extension of trapping and observations for a ten day period. The findings achieved the primary pupose of demonstrating significant movement from the river bottom to adjacent areas of human habitation.

[1]These studies were supported in part by funds from the California State Appropriations for Mosquito Control Research in the University of California and by Research Grant AI03028 from the National Institute of Allergy and Infectious Diseases, General Research Support Grant I-SO1-I'R-05441 from the National Institutes of Health, Education, and Welfare.

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SPATIAL AND TEMPORAL DISTRIBUTION AND DIET OF LARVAE OF THE CLEAR LAKE GNAT

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ABSTRACT

Clear Lake gnat (*Chaoborus astictopus* Dyar and Shannon) larvae were more than twelve times as abundant in the center of a pond (depth 3.2 m) as they were at shallower locations near the shore. Late instar larvae exhibited diel vertical migrations, moving from the bottom sediment during the day into the water column during the night. The migrating larvae did not follow a specific dissolved oxygen or temperature zone as these changed over a 24-hr period. However, the larvae did remain in regions of low light intensity. The daily movement up into the water was also associated with feeding. At 2100 hr, >80% of the larvae in the water had ingested matter (including dinoflagellates, rotifers, and copepods) in their crops while >95% of the larvae in the mud had empty crops. Electivity indices indicated the larvae were more successful in ingesting small (<0.5 mm) smooth-bodied prey than other organisms in the pond.

EQUIPMENT FOR STUDYING THE AQUATIC STAGES OF THE CLEAR LAKE GNAT (*CHAOBORUS ASTICTOPUS*)

N. L. Anderson[1], A. E. Colwell[1] and R. K. Washino[2]

INTRODUCTION.–Immature stages of *Chaoborus astictopus* (Clear Lake gnat) inhabit both the water column and the bottom sediment of ponds and lakes in Lake County and other regions of California. These habitats necessitate the employment of a variety of sampling apparatus in order to study the abundance and distribution of *C. astictopus*. The equipment used for routine monitoring of *C. astictopus* is similar to apparatus used to assess some lentic chironomid midge populations. Ekman Dredges, Schindler Bottles, plankton nets, and floating and submerged emergence traps are all used regularly. However, this equipment is not suitable for sampling the region of flocculant ooze which is present at the lower end of the water column in many chaoborid habitats. Also, this apparatus is not suitable for determining the vertical distribution of *C. astictopus* in the sediment at the lake bottom. An ooze sampler and a sectional coring device were developed for these purposes. In addition, an underwater observatory was constructed to permit direct observations of larvae and pupae in the water and the mud.

OOZE SAMPLER.–The first sampling device is similar to a Schindler (1969) plankton trap. To function properly, the Schindler trap must be lowered very rapidly, and jerked upwards for a distance before it will close. These actions disturb the ooze and make it difficult to sample the ooze region just above the mud-water interface. To overcome this difficulty, a messenger-activated trigger mechanism was developed so that the unit could be gently lowered to the desired level and the sample collected just above the lake bottom. Since the side ring, plankton net, and plankton bucket present on a Schindler trap can create turbulence or vibrations which could cause avoidance by *C. astictopus*, these items were not included on the ooze sampler. A separate funnel bucket for concentration and collection of the plankton sample was constructed for use with the ooze sampler.

The ooze sampler (Figure 1) was constructed of 7.9 mm Plexiglas® acrylic sheets glued together with ethylene dichloride (E.D. cement). The top, bottom, and corners were reinforced with additional Plexiglas®. The metal handle, trigger mechanism, compression spring hinges, and tension springs were all attached with flat-headed stove bolts.

SECTIONAL CORER.–The second device is a K.B.™ design core sampler which was modified by the addition of a plunger and a core sample collection vessel. The core plunger handle was made of steel, the head of aluminum. The aluminum head was turned on a lathe so it would slip fit into the core tube with a maximum clearance of 0.076 mm, thus preventing the more liquid sediments from spilling out of the core tube. The collection vessel was constructed from polyvinylchloride pipe fittings and flangs. These pieces were glued together with polyvinylchloride pipe cement. The collection vessel was threaded to mate with the core tube. During field operation, the corer is lowered into the bottom sediment of a pond or lake and upon retrieval, the plunger is inserted into the bottom

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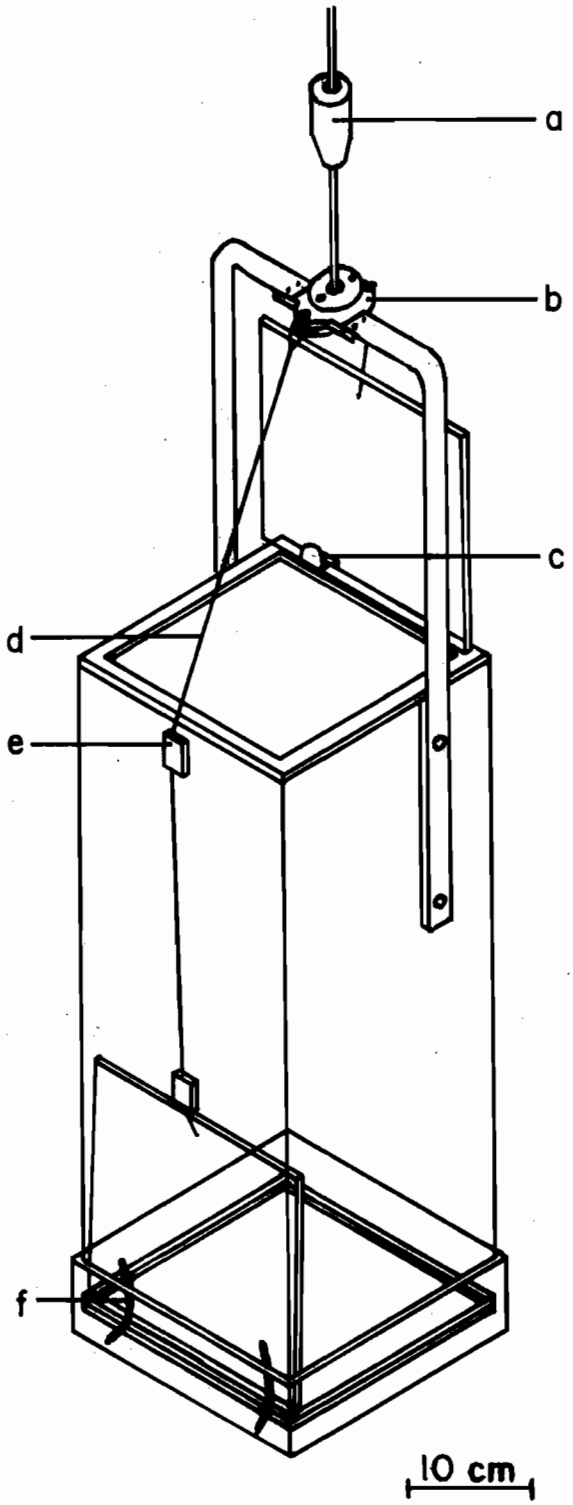

Figure 1.–Diagram of ooze sampler. a. messenger, b. trigger mechanism; c. compression spring hinge; d. 22.7 kg test line; e. guide for line; f. tension spring.

of the core tube. After the trip head assembly is removed, the core sample collection vessel is screwed onto the core tube. The core sample can then be easily separated into discrete vertical fractions by pushing the sample up into the collection vessel. The plunger handle is marked in centimeter increments so that samples can be collected from known depth intervals. The mud is scraped with a straight edge into the collection vessel and rinsed with a squeeze bottle into a sample cup for subsequent laboratory analysis.

BENTHOBSERVATORY. The benthic observatory or benthobservatory (Figure 2), was constructed on dry land and then set into a specially dug pond. The design of the benthobservatory was modified from the wooden, shallow-water model of Bay (Strong 1972). Construction of the benthobservatory was initiated by building forms for the slab floor and bending and tying the steel reinforcing rods. The steel work included 1.27 cm rebar for most of the structure except for 1.59 cm rebar in each corner to provide loops for hoisting. Also, welded wire mesh (15.2 cm squares) was tied to the rebar for added rigidity. The concrete walls were constructed with two pours. The first included the slab base and 61 cm of the wall. The forms were made from plywood and consisted of an interior form and an exterior form. The interior form for the first pour was filled with sand to counter the buoyant force of the liquid concrete. Future window openings were framed with a reverse lip of clear redwood so that in the finished concrete wall the windows could be set on a smooth surface. The two lower window frames were left intact after the first pour and provided an anchor for the upper forms. The next step was completing the upper forms. Once the interior and exterior forms were in place and braced securely, the upper portion was poured by bucketing the concrete into the forms while standing on a scaffold. The concrete contained a cement to aggregate ratio (wt./wt.) of 25/133, and the benthobservatory required ca. 3.8 cubic meters of concrete.

Observation windows were placed in three walls. Two windows (61 cm x 122 cm) were placed in a lower position and one window (61 cm x 152 cm) was placed higher in the wall of the observatory. The caulking compound used in mounting the glass was "Elastoseal 227", a two part epoxy mixture that was applied with a special caulking gun. The 1.9 cm thick tempered plate glass was wiped down with acetone just prior to setting it in place. Neoprene spacers (6.4 mm thick) were used to hold the glass up off the bottom lip while the sealant set up.

Provisions were made to waterproof the benthobservatory as much as possible before submerging the structure. Thoroseal®, a cementitious waterproof coating, was applied to the cold joint on both inside and outside concrete surfaces. The entire benthobservatory was then plastered on the outside using a mixture of one part cement to three parts sand. The plaster was then covered with two coats of coal tar epoxy. Two cranes were necessary to lift the benthoservatory and set it into the pond. A small second story (utilized primarily for equipment storage) was subsequently constructed out of recycled form lumber. Metal shields were coated to prevent oxidation and hung over the windows for protection from vandalism and to inhibit algal growth on the glass.

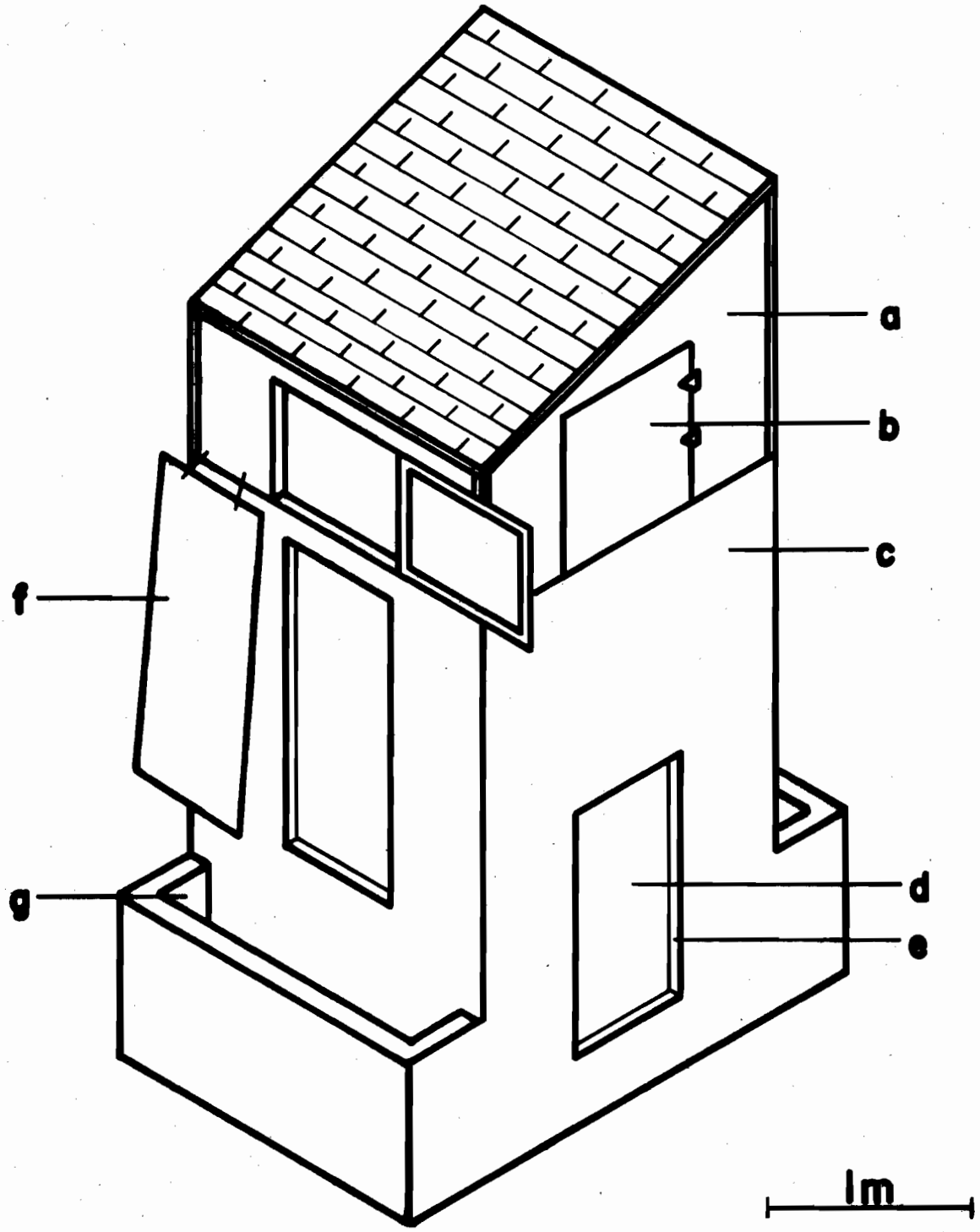

Figure 2.--Diagram of benthobservatory. a. second story of wood; b. lockable doors; c. multiple exterior coatings over 15.2 cm thick conrete walls; d. 1.9 cm thick plate glass; e. recessed lip for window; f. window shield; g. ballast tank.

The benthobservatory presently in use does not leak or float and is not easily vandalized. The construction materials for the benthobservatory cost less than $2,000. It provides 2.4 square meters of viewing area for observing the bottom mud, the mud-water interface, a water column of up to 2.1 m, the water-air interface, and the aerial space above the water.

The benthobservatory was designed to facilitate direct observations of possible competitors or predators of *C. asticto-pus* and to determine what characteristics might be necessary for a biological agent to be effective in controlling this species. Bay (Strong 1972) has discussed the utilization of a benthobservatory in mosquito and chironomid research, and in other limnological studies.

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STUDIES OF *CULICOIDES OCCIDENTALIS* AT BORAX LAKE, CALIFORNIA

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ABSTRACT

Culicoides occidentalis Wirth and Jones is a public health pest in Lake County, California. Field tests during 1979 of Lethane® 384, temephos, methoprene, and *Bacillus thuringiensis* var. *israelensis* de Barjac at Borax Lake proved ineffective in controlling *C. occidentalis*. Seasonal emergence occurred from March to November and appeared cyclical; diel emergence apparently was influenced by moonlight and by low temperatures. Adult aggregations of *C. occidentalis* were observed and collected. Females aggregated around a gaseous vent; males aggregated in shoreline "mating swarms" at sunset.

INTRODUCTION. The blood feeding of *Culicoides* spp. (Diptera:Ceratopogonidae) can cause human discomfort and affect economic development and tourism (Linley and Davies 1971). The widely distributed *C. variipennis* Coquillett was recently split into two species (Downes 1978), one of which, *C. occidentalis* Wirth and Jones is of public health importance in Lake County, California . *C. occidentalis* also is a vector of bluetongue arbovirus of livestock (Price and Hardy 1954; Luedke et al. 1967).

The *C. occidentalis* larval population center in Lake County is Borax Lake, an alkaline body of water approximately 50 ha in area. Natural biological control does not maintain the population below nuisance levels. The lake is fishless, and during studies in cooperation with Oregon State University (unpublished), no fish, including *Gila* spp., *Hesperoleucas symmetricus* (Baird and Girard), *Catostomus* sp., *Ictalurus nebulosus* (Lesueur), *I. punctatus* (Rafinesque), *Cyprinodon macularius* Baird and Girard, *Gambusia affinis* (Baird and Girard), *Poecilia reticulata* Peters, and *Gasterosteus aculeatus* Linnaeus, proved capable of long term survival in water from Borax Lake. Other biological agents and chemical pesticides tested also have been ineffective in controlling the larvae at Borax Lake (Apperson 1975; Schaefer et al. 1976).

This paper will discuss field tests of potential control agents conducted in 1979 and the monitoring methods developed for these tests. Also presented are studies during 1979 of emergence (seasonal and daily) and adult aggregations of *C. occidentalis*.

MATERIALS AND METHODS. A 0.06 ha pond (mean depth: 0.6 m) adjacent to Borax Lake was treated with the potential control agents; the lake itself (for chemical treatments) served as the control.

On June 8, 180 m^2 of the pond shore was sprayed with Lethane® 384 (2.72 kg AI/ha) in heavy aromatic napthalene. On June 26, Abate® (temephos) 1% granules were spread over the pond surface in an amount calculated to yield a concentration of 200 parts per billion. On August 14, 409 Altosid® (methoprene) 4% briquets were suspended midway in the water column.

A biocontrol agent, *Bacillus thuringiensis* var. *israelensis* de Barjac, was tested in a metal cylinder (57 cm diameter) which was embedded in the substrate at the pond shoreline. On

September 5, 1.0 ml of formulated (SAN 402I WDC) *B. t. israelensis* was applied to the water (10.01 liters) in the cylinder. A second cylinder was the untreated control.

The temephos treatment was monitored (in part) by sampling larval and pupal populations of *C. occidentalis* from shoreline mud. Six mud samples were taken twice weekly from the pond and lake from June 15 to August 3. The sampling location was just below the water line. Lateral position of the samples was selected with a random number table. A polyethylene vial (5 cm long, 3 cm diameter) was used to obtain each 7.1 cm^2 mud sample. The samples were rinsed into a cup and immediately transported to the laboratory. There, larvae and pupae were separated from the mud by $CaCl_2$ flotation. Each mud sample was transferred into a 250 ml polypropylene cup. The cup was then filled with saturated $CaCl_2$ solution and stirred. As the mixture settled, pupae and large larvae were picked off the surface with forceps; when the mixture had settled the liquid was poured through side holes of the cup and examined under a dissecting microscope for small larvae. All larvae and pupae were counted. This sampling procedure was adapted to an area of high *Culicoides* density, as one 7.1 cm^2 sample from Borax Lake mud could contain up to 5000 larvae.

Mud samples were also taken during the *B. t. israelensis* test from each cylinder 1 hr pretreatment and 1, 2, and 6 days posttreatment (2, 3, 3, and 4 samples respectiviely). Only larval numbers were recorded.

The temephos treatment (in part), and the Lethane® 384 and methoprene treatments were monitored by sampling adult

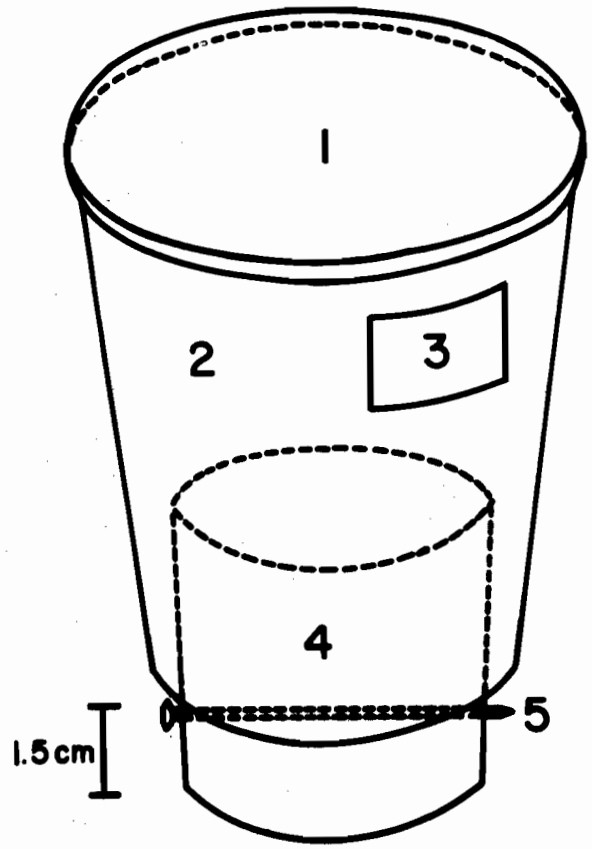

Figure 1.–Diagram of emergence trap for *Culicoides*. 1. lid; 2. outer 300 ml cup; 3. window; 4. inner 250 ml cup part; 5. nail.

C. occidentalis emergence. Each emergence trap (Figure 1) consisted of a 300 ml Corning $_{TM}$ polypropylene cup (with bottom and two 2 x 1 cm windows cut out) and lid, and a 250 ml Falcon$_{TM}$ polypropylene cup (with bottom cut out and top half cut off) held together by a nail. The ventilation windows were covered with screening (30 meshes/cm). The inner surfaces of the 300 ml cup and lid were coated with Tanglefoot®. The traps were designed to allow the pupae to wriggle up the inner 250 ml cup part (free of Tanglefoot®) and emerge, the adults then flew upward and became stuck on the sticky surfaces. This trap sampled 17 cm^2; larger conical emergence traps (e.g., Davies 1966) were not necessary because of the high density of *C. occidentalis* at Borax Lake. The traps used in the present study were inconspicuous (less subject to vandalism), easily transportable, inexpensive, and quick to construct.

Emergence traps were placed along the shore (just above the water line) of the pond and Borax Lake. Lateral position of the traps was selected with a random number table. Traps were embedded 1.5 cm into the mud to prevent migration into the area under the trap. Four to ten traps were set at both locations: twice weekly from May 3 to July 5, July 31 to August 28, September 14 and 18, October 5 to November 6; approximately weekly from March 9 to April 24; and on December 4. Traps were removed after 3 to 5 days (sufficient time for the adults to emerge from the pupae). After retrieval, traps were sealed in plastic bags, immediately transported to the lab, and frozen until counted by removing adults with forceps.

Seasonal data on emergence at Borax Lake were obtained as described above. Weekly mean air temperatures were computed from National Weather Service data. Major emergence peaks and the time intervals between them were determined from Figure 2, and the mean temperature during each interval was calculated. Thermal input (in degree-days) for a complete life cycle was calculated by multiplying each time interval (in days) by the mean interval temperature less 5°C (the assumed developmental threshold). This 5°C threshold was used because it allowed the least variation in calculations of thermal input (Johnson et al. 1979).

Diel emergence at the pond was monitored by setting and removing 10 emergence traps every 2 hrs from 0900 September 27 to 0900 September 28. Adults in the traps were removed and preserved in 90% ethanol. Times of sunrise and sunset, moonrise and moonset at the pond site were recorded. Physical measurements were taken at standard locations every hour: temperatures (air, pond water, and shore mud) were measured with a mercury thermometer, relative humidities with a certified hygrometer, and wind speeds with an anemometer (Kurz Instruments, Model 441M).

Chance observations and collections (made with rapid swings of an insect net and preserved in 90% ethanol) of adult aggregations were made sporadically during the year. On October 4, "mating swarm" aggregations were systematically observed for: location, character of substrate beneath swarm, time present, size, shape, height, and diameter. Nine swarms were collected on this date.

RESULTS AND DISCUSSION.–None of the experimental treatments tried in 1979 proved effective enough to be considered for practical use in the control of *C. occidentalis*. Despite the treatments, emergence patterns of *C. occidentalis*

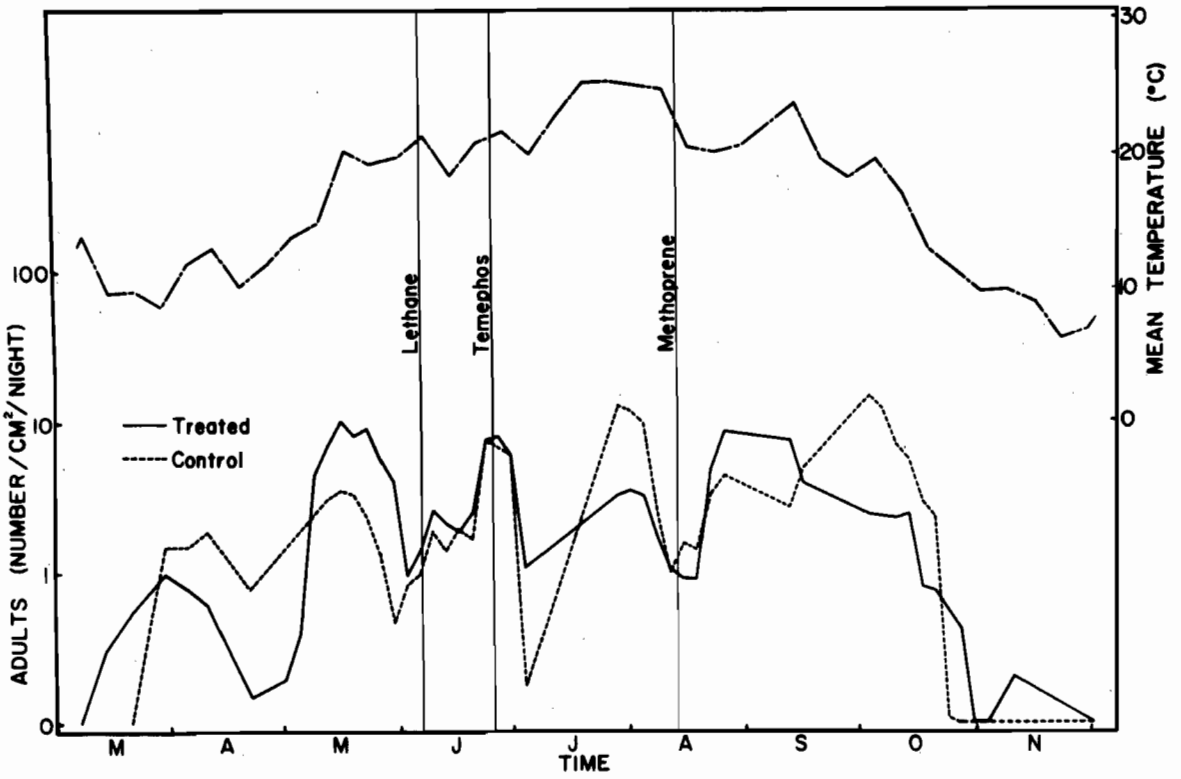

Figure 2.–Upper graph, 1979 weekly mean temperature at a location 3 miles ESE of Borax Lake. Lower graph, seasonal emergence (3 point running means) of *C. occidentalis* at the pond (treated) and Borax Lake (control). Vertical lines indicate treatment dates.

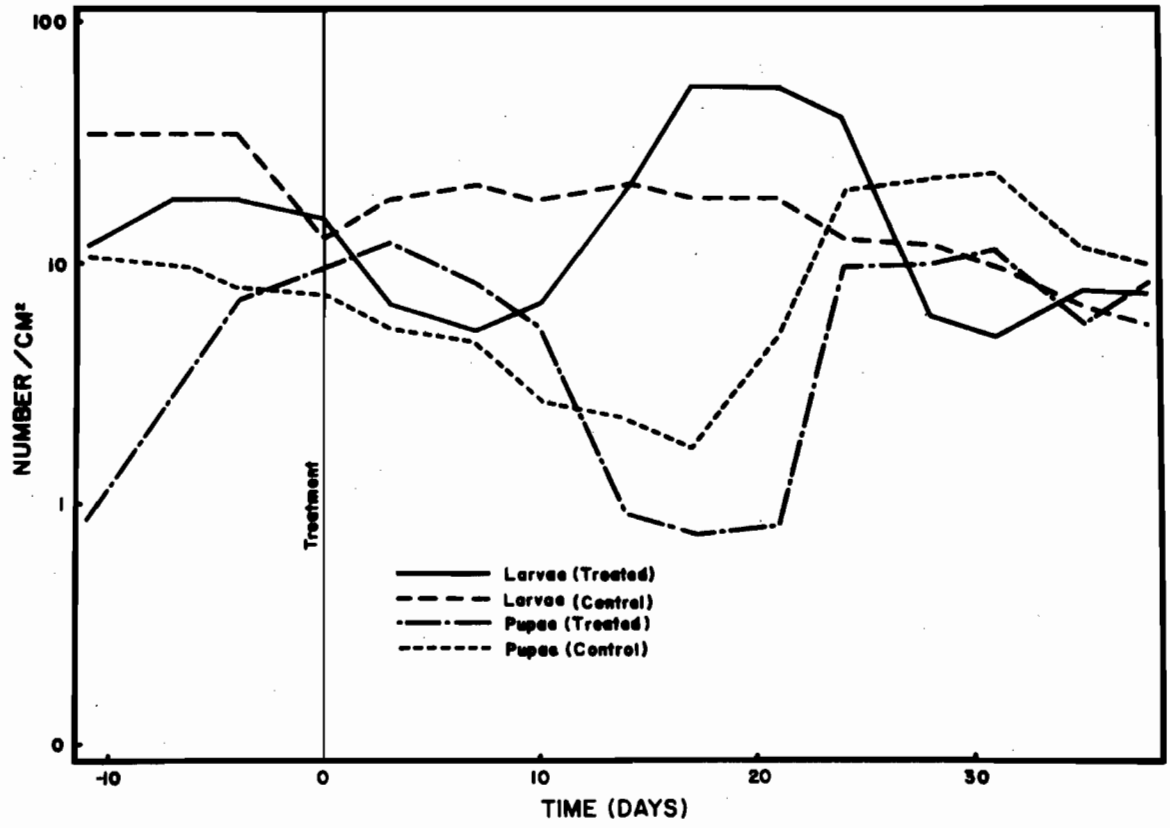

Figure 3.–Larval and pupal numbers (3 point running means) of *C. occidentalis* at the pond (treated) and Borax Lake (control) before and after the June 26, 1979 temephos treatment.

remained similar at the treated and control locations (Figure 2). Larval numbers declined temporarily after the temephos treatment (Figure 3), but this change was not of the magnitude nor duration necessary for practical control. Temephos had no apparent effect on pupal numbers (Figure 3). The *B. t. israelensis* treatment also was ineffective as larval numbers actually increased following treatment.

The discouraging results of field tests at Borax Lake may be attributable to the water's high pH (ca. 10.0) which degrades some chemical compounds, to the abundance of macrophytes and algae which tend to adsorb compounds, and to the habits of the *C. occidentalis*. A large proportion of the larvae and pupae stay in the mud and are thus protected from the highest concentrations of the control agents.

Figure 2 indicates *C. occidentalis* emerged into the traps from March 16 to November 13 (slowly developing larvae are the overwintering stage of this species at Borax Lake). The emergence counts increased (or decreased) noticeably at a mean temperature threshold of ca. 15°C. No emergence occurred below 8°C. Nelson and Bellamy (1971) noted that temperatures below 10°C appeared to suppress flight activity of *C. occidentalis* in Kern County, California. Temperatures below 10°C could inhibit the upward flight necessary for capture in the emergence traps or could directly prevent emergence.

Table 1 indicates the generation time was shortest (30 days) during the summer months. Average thermal input for a complete life cycle was 536 degree-days. These thermal input findings might be useful in predicting when during a year *C. occidentalis* could become a serious problem.

The majority of *C. occidentalis* emergence on September 27-28 (Figure 4) occurred after sunset (1708 hrs, 0.0 crep units) and peaked during a period of full moonlight at 1930 to 2120 hrs (5.3 to 9.5 crep units). No emergence peak occurred about sunrise (0736 hrs, 0.0 crep units). The peak period suggests that on this date, *C. occidentalis* emergence was positively affected more by full moonlight than by sunrise or sunset. Nelson and Bellamy (1971) found that for flight activity of *C. occidentalis* moonlight could obscure the usual sunrise and sunset activity peaks.

If emergence were only dependent on light intensity (or changes in light intensity), a peak should have occurred about sunrise. In the summer months, many newly emerged individuals (teneral adults) were observed in early morning at Borax Lake. The absence of a sunrise period emergence peak in this diel study was probably attributable to temperature as low as 10°C (Figure 5).

The majority of the diel emergence (1800 hrs to 2200 hrs) was at a time of low wind velocity (≤1.0 m/sec) and rising relative humidity (40% to 80%). Males and females emerged at

Table 1.–Dates of major emergence peaks of *C. occidentalis*, time intervals between these peaks, and thermal inputs.

Emergence Peaks (Dates)	Peak Intervals (Days)	Thermal Inputs (Degree-Days)
March 31	48	369
May 17	42	640
June 28	35	641
August 2	32	529
September 3	30	500
October 3		
	$\bar{x}$ = 38	$\bar{x}$ = 536

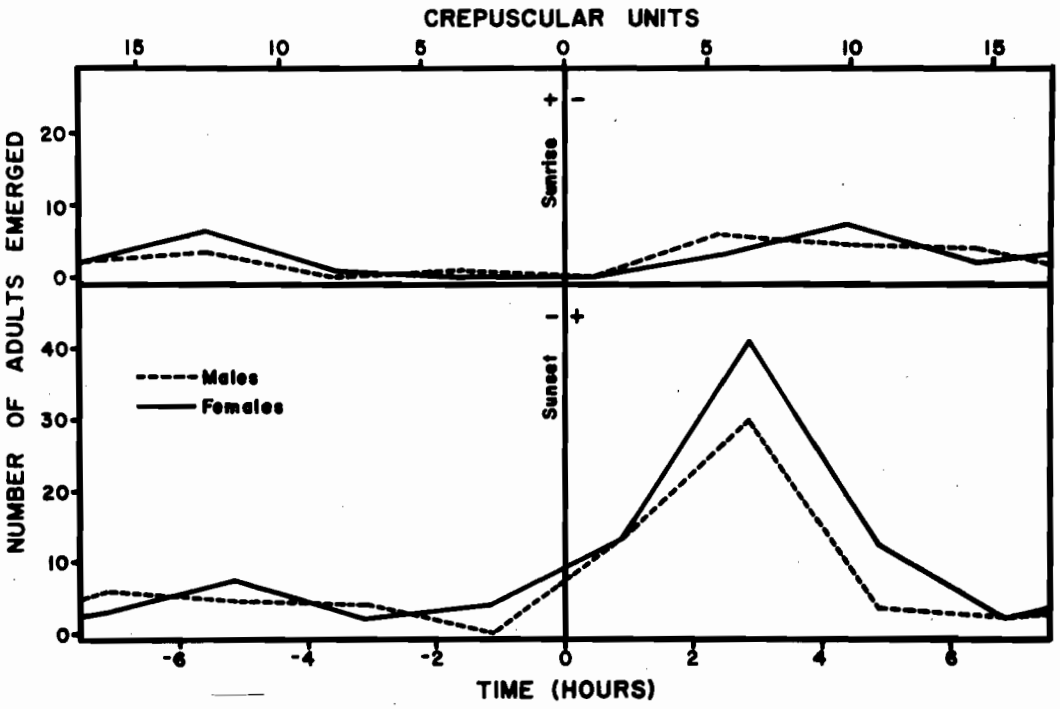

Figure 4.–Diel emergence (10 trap totals) of *C. occidentalis* on September 27-28, 1979 at the pond. Upper graph centered on sunrise, lower graph centered on sunset. Crepuscular units as defined in Neilson (1961).

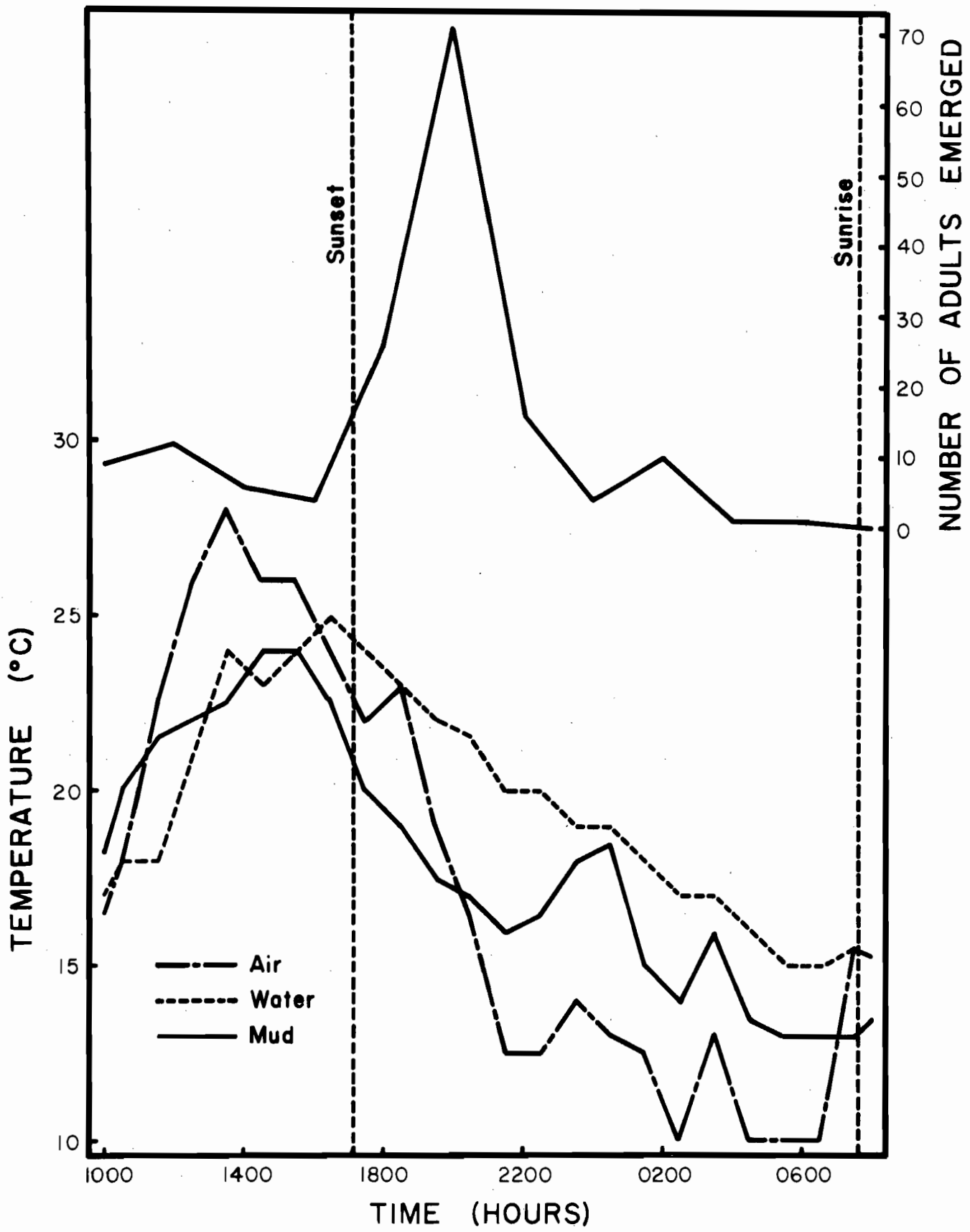

Figure 5.- Upper graph, 24 hr emergence (10 trap totals) of *C. occidentalis* on September 27-28, 1979 at the pond. Lower graph, temperatures of air, pond water, and shore mud at the pond.

generally the same times, although females outnumbered males at 2000 hrs (41 to 30) and at 2200 hrs (12 to 3). The 24 hr emergence total of females outnumbered males 92 to 67.

Two types of adult *C. occidentalis* aggregations were observed; one type composed predominantly of males, termed a "mating swarm", and another type composed only of females. One female aggregation was present during the entire emergence season, at least during daylight hours, at a gaseous vent on the southeastern side of Borax Lake. Three collections from above the vent on different dates contained only females. The females coming to this spot were probably responding to CO_2 emitted from the vent. Nelson (1965) showed that CO_2 was an attractant for female *C. occidentalis* in Kern County, although some males were also attracted.

Mating swarms were composed predominantly of males: of 2678 individuals collected in the nine swarms, 2668 were males. Three of the swarms collected contained no females. Presumably, when a female entered a mating swarm she almost immediately contacted a male (Downes 1955). At Borax Lake, joined couples were observed to fall down, out of the swarms, and rest upright on the substrate facing in opposite directions.

Other insects were collected in *C. occidentalis* swarms. In one swarm (1735 hrs October 4, 0.6 to 1.2 m over a light gray gravel road) were two species of chironomids (including *Paralauter borniella* sp.). Other swarms contained cecidomyiids, empidids, aphidids, cicadellids, and ephemeropterans.

Most swarming occurred near sunset (1803 hrs at Borax Lake) on October 4; swarming commenced at 1728 hrs (-1.3 crep units) and had ceased at all but one location by 1930 hrs (2.1 crep units). Most recognizable swarms consisted of 20 to 20,000 individuals, were columnar in shape, were 0.6 to 3.0 m in diameter and occurred 0.6 to 6.0 m above the ground. The majority of swarms observed were within 30 m of the lake and all were within 150 m. Swarms frequently formed along the shore at the interface of bare, bright white salt deposits and a cover of dull green salt grass, *Distichlis spicata* (L) Greene. This interface may have functioned as an "optische marke" (as defined in Campbell and Kettle 1979) to which the *C. occidentalis* oriented. Mating swarms also formed over pickup trucks, a light-colored insect net, and the observer's head. On October 26, a mating swarm of about 100 individuals formed over a blue truck at 1000 hrs. This was the only mating swarm of *C. occidentalis* seen during the morning.

ACKNOWLEDGMENTS.–We thank N. L. Anderson, Dr. C. S. Apperson, Dr. C. E. Bond, L. W. Davidson, J. A. LaDieu, F. N. Lucas, J. E. Williams, and D. L. Woodward for their participation in various aspects of this study.

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INTRODUCTION OF SCORPIONS TO ORANGE COUNTY

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INTRODUCTION.- Over the past few years, the Orange County Vector Control District has become aware of a problem of apparently increasing occurrence, that being the importation of poisonous scorpions (*Centruroides sculpturatus*) into the Orange County area. This importation is notable for a number of reasons. Foremost is the reputation this scorpion shares as being "deadly" poisonous. Although this implies death may occur to any stricken person, this usually is not the case. As a rule, infants and aged persons are the persons most susceptible to this scorpions's venom. Most persons in good health will survive envenomation from a sting without serious consequences. Secondly, the introduction of this scorpion has occurred frequently enough that we have come to expect it each summer. From our observations and investigations, we feel an obligation to alert appropriate agencies (vector control districts and health departments) and inform the public about this problem as well as provide some preventative measures. The following discussion is a summary of our findings.

INVESTIGATION. Since the fall of 1975, the District has identified five *Centruroides sculpturatus* scorpions brought in from the public. At first, the occurrence seemed to fit a pattern: the scorpions were usually encountered in the fall, and without exception, all have been found inside houses. This led us to believe that the scorpions had been brought in during the latter part of the summer and found their way into the homes from the garage area where camping equipment is usually stored. It appears, however, after a number of interviews and communication with the residents, that the scorpions may have been brought in earlier in the summer (with the exception of one) and moved into the home as a result of the first cold weather in October and November. A chronology of the occurrences is the following:

September 4, 1975 . . . . . Garden Grove
October 1976 . . . . . Huntington Beach
November 15, 1977 . . . . . Fountain Valley (2 specimens)
January 6, 1978 . . . . . .Fountain Valley
October 17, 1978 . . . . . .Anaheim

The tendency of these scorpions to seek shelter during cold periods is unquestioned and its ability to climb structures, trees, or enter homes is well known. To compound things further, our fears have been that this species could establish itself in the county. This possibility has been confirmed to us by Dr. Stan Williams of San Francisco State University.

MODE OF INTRODUCTION.- In general, it is believed that scorpions find their way into other areas by way of concealment in rolled up tents, camping equipment, etc. Indeed, we are certain the first scorpion we identified arrived in this manner, our investigations revealed that in all cases the scorpions were transported in by either the person finding the scorpion or by their neighbors (inadvertantently). The differences in present day camping styles and equipment suggests that there are other modes of introduction in addition to those previously mentioned. This is borne out by the fact that a substantial percentage of the residents in the areas where these scorpions have been found either go camping in motor homes or pickup type campers. This applies to most of the county and Southern California as well. In essence, the boom in R.V. travel has resulted in unprecedented numbers of people going out to the desert areas of Southern California and Arizona including their mutual boundary, the Colorado River. Although motor homes and campers do not lend themselves readily to transporting scorpions, items such as trailers do (the type used for hauling motorcycles and dune buggies). Many of these trailers have ramps which are left down during the campers stay. This permits easy access into the trailers with much paraphernalia to take refuge in. This is the suspected mode of introduction in some of the cases, however, we are not excluding other possibilities. Because of the tremendous use of the deserts and Colorado River area by campers and their diverse activities, the District has taken the posture that informing the public of the possibility of bringing in this scorpion is the best means of coping with the problem.

PREVENTION AND CONTROL.- Public awareness has been accomplished by submitting press releases to the local newspapers. The article advises the public of precautionary steps to take prior to departing for home. Although the article is aimed primarily at *Centruroides* scorpions, we mention other arthropods and snakes, etc. because they may also be unwelcome guests. This year we will add to this by printing up bulletins for distribution to motorcycle and off-road vehicle clubs and trailer storage yards. In the event that a scorpion is found it is essential to inquire whether that homeowner has been vacationing and where. If the probable source is a neighbor, they should be tactfully informed of the potential hazard they are creating. Detection within a neighborhood is virtually impossible due to the creatures nocturnal and reclusive habits. The best remedy is to recommend pesticide application in the yard and around the foundation of the home. Several general purpose insecticides are registered for this type of application.

CONCLUSION.- The introduction and establishment of a potentially dangerous scorpion is a very real possibility within the Southern California coastal region; increased recreational use of the areas where these scorpions originate can only serve to increase this possibility. In view of this, the Orange County Vector Control District is certain that other Southern California counties will experience this problem. It is hoped that this paper will serve as an alert to a potentially serious problem.

DEVELOPMENTS IN TRIALS IN CALIFORNIA ON ANIMAL WASTE MANAGEMENT PRACTICES IN RELATION TO MOSQUITO CONTROL

J. L. Meyer[1] and E. H. Olson[2]

Using water to move dairy manure to a storage pond is economical, efficient, clean, mosquitoes can be controlled, and the process can be automated. In the Central Valleys of California recycling of nutrients from waste ponds by irrigation of crops has gained wide acceptance and is the most economical and pollution-free method yet developed.

In 1970 a few dairies were using waste ponds to capture waste from milking parlors. About 10 percent of the animal waste occurs during cattle holding and washing in preparation for milking. The advent of pollution control regulations in 1970 created a need for research in pond sealing, crop nutrient requirements with manure, mosquito control, and prevention of manured water from entering waterways or ground water supplies.

Manure ponds were found to be self-sealing, after research in a series of ponds under sand to clay soil conditions. Manure and the anaerobic byproducts formed an impermeable seal. In the early stages of development of ponds, very few mosquitoes were observed.

Large dairy animals produce about 120 wet pounds of waste per day. This waste contains valuable nutrients. These nutrients, when applied to cropped soils at the rate of 3 to 5 cows/acre/year were found not to cause groundwater eutrophication and supply a large portion of crop nutrient requirements.

Dairymen found ponds so efficient many began to flush waste from feeding and loafing areas. Much larger amounts of manure solids, up to 100 pounds/cow/day were flushed into the holding ponds. The solids in the form of fiber can create odor and floating materials on ponds. At this point in time mosquitoes began to breed in the floating materials, in liquids near weedy banks, and in all probability a type of mosquito developed which was able to reproduce in the saline, anaerobic ponds. Weed control on the banks became necessary.

Solids separators began to be used to remove the large material. The solids separators have materially reduced floating materials and mosquito breeding. The fiberous materials are sun dried and may be used for bedding, the liquids with nutrients are returned to the soil as fertilizer on neraly all Central Valley dairies, today.

Other than oil surface control in holding ponds, more frequent irrigation from the ponds is a management tool available to dairymen and poultrymen for mosquito control.

We have concluded that weed bank control, fiber removal, and irrigation intervals of two to three weeks from the ponds, are the best management practices. Only occasional pond treatment is then necessary.

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GRAY LODGE WILDLIFE AREA (CALIFORNIA) - - CONTINUING STUDIES OF MOSQUITO PRODUCTION AND CONTROL

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ABSTRACT

Studies toward developing an integrated control program against aedine mosquitoes (principally *Aedes melanimon* and *Aedes nigromaculis*), begun in August 1978, were continued in 1979. Previous work was reported by Hanna (1979) and an historical perspective was provided by Lusk (1979).

Tentative conclusions or findings in this ongoing study include the following:

a. A basic distinction between fields with sparse emergent vegetation and those with dense emergent vegetation is centrally important for both experimental and control purposes.
b. For fields with sparse emergent vegetation treatment with *Gambusia affinis* at a stocking rate of 1 lb. fish per surface acre of field pond resulted in at least a 75% reduction in aedine larval populations (as compared to control fields). This effect was equivalent to treatment with 2% granular chlorpyrifos at 2 lb. per acre. No significant differences were found between 1 lb. per acre and 3 lb. per acre *G. affinis* stocking rates for these fields.
c. For fields with dense emergent vegetation, results of treatment with both *G. affinis* and chlorpyrifos continued to be ambiguous due to the high variation in larval densities between fields.
d. Relatively low larval densities were associated with specific fields containing dense growth of watergrass (*Echinochloa crusgalli*). However, this association may have been due to field location rather than vegetation type and further testing is necessary. (Watergrass is an important species encouraged in wildlife management.)
e. Intensive and extensive sampling throughout two seasons indicated that for most fields at least 75% of the larvae were located within 50 ft. of the edge of the field pond. (Exceptions occurred where fields were slowly flooded and/or where a large amount of high ground was left unflooded within the field.)
f. Treatment with chlorpyrifos at 2 lb. per acre around a 30 ft. perimeter swath rather than the entire field appears to be sufficient for adequate control in many situations and further testing of this strategy is planned.
g. Results of removal of perimeter vegetation (by discing a 30 ft. swath), to enhance fish dispersal into critical larval habitats and to enhance penetration of chlorpyrifos granules, were inconclusive. Further studies of this strategy are planned.

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