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

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MALARIA CONTROL: PESTICIDES VERSUS THE COMPREHENSIVE APPROACH

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Although it is 21 years since I lived in California, I still consider myself a Californian and I am always happy to have an excuse to come back. When I stood before this group to give my presidential address in 1952 I could put a name to 90% of the faces before me. That ratio has reversed itself now, but I do recognize quite a few of my old friends even though, as with me, they may be a little rounder and a little greyer.

In casting about for an appropriate keynote subject for this 1977 AMCA conference, I have chosen "Malaria Control: Pesticides versus the Comprehensive Approach". I feel this is appropriate because those of us working in international malaria control programs now find ourselves in the same dilemma as those of you trying to control mosquitoes in California in that insecticides no longer do an adequate job and we find that we must utilize all available methods in a comprehensive program.

Having worked in mosquito and malaria control for nearly 33 years now, I hope that I will be forgiven for the autobiographical overtones of some of my comments.

I have been an interested spectator and participant in the saga of the rise and the fall of DDT as a major factor in the control of mosquitoes and malaria since 1944 when I took a week-long course on the use of DDT at Orlando, Florida. Since DDT was still a military secret then, that was not the title, but that is what it was nevertheless. Instructors included such names as Knipling, Lindquist and Herald. I recall that the late Dr. Earl Herald showed me a newspaper clipping that went something like this, "The U. S. Army is closely guarding the name of a new miracle insecticide that is so effective that it will revolutionize the whole approach to mosquito control. Simply by spraying this material on the marshes in Canada, the mosquitoes in the United States can be wiped out, because the ducks, geese and other migratory water fowl will pick it up on their feathers and spread it to ponds, marshes, and streams as they migrate south, and in so doing, kill off the mosquito larvae." As we all know, this enterprising and imaginative reporter's prediction came true insofar as revolutionizing the approach to mosquito control, but not in the manner envisaged.

I will come back to the subject of DDT, but first a brief historical perspective on malaria control. Before the DDT Era, control of malaria was largely a matter of filling or drainage, ditch-cleaning; larviciding with oil, or paris green; use of larvivorous or mosquito-eating fish; space-spray adulticiding with pyrethrum sprays in flit guns; and drug treatment, first with quinine and by the 1930's with atabrin. The success of comprehensive programs utilizing a combination of these methods is history and is well-known to all through the work in the early 1900s of Gorgas, Le Prince, Watson and others in Cuba, Panama and Malaya. However, these programs were expensive and beyond the reach of most developing countries in the highly malarious regions of the world.

It was only with the demonstration of the value of DDT as a residual wall spray that it became apparent that here was a safe, cheap and effective method of breaking the cycle of transmission of malaria that was within the financial reach of the malarious countries. The military use of DDT during World War II was largely as a larvicide or a space spray adulticide or both. Immediately following the War in 1945-1946 when DDT became more generally available, demonstrations of its efficacy as a residual wall spray in operational programs were carried out by Rockefeller Foundation in Sardinia, by the U. S. Public Health Service in the 13 malarious states of the Southern United States; and in Cyprus, Greece, Italy, India and Sri Lanka (Ceylon). All were successful. In the early 1950s national malaria control projects utilizing DDT as a residual wall spray were launched in many countries of the world such as Indonesia, India, Ceylon (Sri Lanka), Thailand, Burma, Philippines, Vietnam, Taiwan, Iran, Iraq, Lebanon, Guyana, Venezuela, Brazil, Ecuador, Bolivia, Colombia, Chile, Argentina, and Mexico. Many of these countries obtained technical and commodity assistance from WHO, UNICEF and/or the United States Foreign Assistance Program. By 1955, 29 countries had national malaria control projects and were obtaining technical assistance from WHO and supplies from UNICEF. Nineteen countries were also obtaining strong technical and commodity assistance from the United States International Cooperation Administration (ICA).

In 1955 at the 8th World Health Assembly in Mexico City, the recommendation was made that malaria control programs should be converted to eradication programs. This very important decision was made on the premise that the means of eradication of malaria were at hand and could be achieved by a two-pronged program. This program was envisaged to include residual wall spraying of all the sprayable surfaces of all the houses in the malarious area of a country for three to four years and then a surveillance program for an additional 3 years visiting every house on a regular cycle (bi-weekly or monthly) to detect and treat all remaining cases of malaria. This is a simplified version of a very complex program involving many disciplines, but the subject has been treated exhaustively in the literature, particularly in numerous WHO publications, so I will not go into greater detail at this time.

The Worldwide Malaria Eradication Program promulgated by WHO and assisted in large part by U.N.I.C.E.F., U.N.D.P. and the United States Foreign Assistance Program (E.C.A., M.S.A., T.C.A., F.O.A., ICA and AID), and other donors, reached its peak in the mid 1960s at which time nearly 90 countries had embarked on malaria eradication or control programs. AID provided technical and commodity assistance for 26 of these countries and commodity assistance only for an additional 10 countries.

In the early years and through the mid-1960s most of these programs were highly successful in drastically reducing deaths from malaria and in reducing malaria from the number 1 health problem to number 3, or 6, or 12 or even completely off the list in some cases.

Has the worldwide malaria eradication program been successful? Yes and no. Some 37 countries have been certified by WHO as having achieved eradication of malaria. However, this leaves nearly 50 other countries which attempted eradication and did not succeed. Nevertheless in the course of the attempt, malaria was reduced worldwide from an annual incidence of about 300 million cases to about 120 million cases today of which 80% are to be found in Africa where very little progress has been made.

Perhaps it would be easier to visualize what happened in a single country. Let us take as an example the largest malaria program the world has ever known - - India. In India in the late 1940s and early 1950s malaria cases were recorded as 75 million per year in a total population of 368 million. Five years of a malaria control program starting in 1953 using DDT as a residual spray and covering about half the population reduced malaria from 75 million cases to 25 million cases a year and from 750,000 deaths to 250,000 deaths. Five years of a malaria eradication program covering the total population in malarious areas of 383 million population reduced the number of malaria cases further from 25 million to 150,000 cases with no recorded deaths. This is a 99.8% reduction of malaria in a ten year period.

Unfortunately the number of malaria cases has increased every year since then and reached an estimated 5 million last year out of the total population of 620 million.

During this same period of the late 1960s and the early 1970s resurgence of malaria also took place in Indonesia, Thailand, Nepal, Sri Lanka and Pakistan to name only a few of the major problems. Resurgence of malaria also took place in some of the countries of the American Region, in Ethiopia and to a lesser extent in the Near East. What are the reasons for this resurgence of malaria? There is no single simple answer. In each country a separate combination of circumstances may be responsible. These factors may include insecurity, inadequate finances, delayed delivery of insecticides, operational failures, insecticide resistance, outdoor sleeping habits of the people, drug resistance, and non-cooperation of the villagers. In general, following the initial successes in programs, progress was slowed down, stopped or even reversed for a variety of reasons including complacency, loss of priority due to success, and the administrative management reasons just listed. These problems interfered with the achievement of the eradication objective long enough for the technical problems listed to trigger a resurgence of malaria in many countries. The most significant of these is insecticide resistance. 15 species of anopheline mosquitoes have been reported to be resistant to DDT, 37 to Dieldrin and some anophelines are also resistant to B.H.C., Malathion and Propoxur. More important, however, is the impact of this resistance on malaria programs. In 80% of Pakistan, in several states of India and in most of Sri Lanka, the mosquito vector is resistant to DDT and malathion must be used at 3½ to 5 times the cost of using DDT. In some Central American countries, large scale agricultural usage has resulted in mosquito resistance to both chlorinated hydrocarbons and organic phosphates so that only Propoxur can be used at roughly ten times the cost of DDT. After only a few years of use, resistance has already been reported to Propoxur in at least one Central American country.

What does all of this mean for the malaria programs today? Unfortunately it means that the goal of eradication is fading farther into the future every year. I recall the words of Sam Keeney, recently retired from the Population Council, but the Regional Director for UNICEF in Asia in 1956 when he presented a paper entitled, "Organizing for the Last Battle Against Malaria". His paper was an admirable statement of all of the management problems inherent in conducting a successful malaria eradication program. I particularly remember his closing remarks, "I believe completely that this is the golden moment for an all out attack on malaria and that if it is not seized it may never come again. But just because it is the golden moment, we must not botch the job by over-confidence and careless planning and execution". That was written 21 years ago. In retrospect we will have to admit that in some cases the job was botched. On the other hand, in many countries when the job was carried out on schedule according to plans developed in conformity to the recommendations of the WHO Expert Committees on Malaria, the programs were fantastically successful at a ridiculously low cost per capita. As the director of the India Malaria Eradication Program used to say, "at the cost of a cup of coffee per person per year". The range in cost was from 5 cents to $3.50 per person per year with an average of 15 to 25 cents per person per year. In spite of not reaching the ultimate goal of eradication, the degree of control which saved hundreds of thousands of lives and relieved the suffering of millions of people was worth every cent that was spent in the attempt to eradicate malaria. So much has been said of the failure to eradicate malaria that we tend to forget the tremendous benefits deriving from the program. I am reminded of the time a few years ago when I was on home leave in King City, California and was asked to give a talk to the local Lion's Club. I showed a movie which I had made on the Malaria Eradication Program of Thailand. Some of you may have seen it on the "Science in Action" TV show. After the program, my family doctor came up to me and said, "I am really amazed! Your malaria program in Thailand saves more lives in one year than I as a doctor can expect to save in my entire life. I think I should have gone into preventive medicine". Hearing this kind of appreciation compensates in great measure for the headaches and frustrations involved in overcoming the bureaucratic roadblocks standing in the way of success.

In defense of those programs which fell short of the goal of eradication, I think it should be remembered that successful achievement of eradication depended on near perfection in planning, timing and implementation of a multi-disciplinary program in a sustained effort of not less than seven or eight years. Perhaps the real mistake lay in the overly ambitious planning and the over-optimism as to the prospects for success of the single method of residual house spraying and the neglect of all of the other available weapons such as source reduction, larviciding, etc.

Lest I be accused of exercising hindsight I would like to quote from a memorandum written in December 1956 in Indonesia. I quote two short sections from the summary and conclusion of a lengthy memo: "There would appear to be ample evidence to cast a serious doubt on the ability of the one method of residual spraying to control malaria adequately in Indonesia under existing conditions and in a reasonable period of time. It is imperative to begin investigations and establish pilot projects to determine the effectiveness and proper place of supplementary or alternative methods of control. Every possible supplementary or alternative method of malaria control should be considered and investigated to determine its proper place in the Malaria Control Program of Indonesia when and if residual insecticides fail. Pilot projects should be established to determine the efficiency and cost of each of the various alternative and/or supplementary methods as listed below". The list included larviciding, space spraying, cultural control (referring principally to management of rice fields and fish ponds), source reduction and naturalistic control (referring to use of larvivorous fish). I wrote that memorandum when I had been in Indonesia for about 6 months. The memorandum went on its way through the bureaucratic maze and I never heard another word on it officially. Years later, I heard unofficially that it had been reviewed at various levels with the decision that since the DDT residual spray program was so highly successful none of these things were necessary. This unfortunately was the "party line" of WHO and the U. S. AID from the early 1950s until 1969 when two very significant resolutions were adopted by the 22nd World Health Assembly. The first one was in connection with the Director-General's Report on "Re-examination of the Global Strategy for Malaria Eradication". The resolution urged that eradication be completed where feasible, but for the first time recognized malaria control as a valid interim measure with the ultimate goal still being eradication. The resolution also urged each country to re-examine its own program and develop a strategy suited to its needs and resources. An important, but overlooked part of this resolution recommended "the necessary diversification of means of eradication in accordance with the particular requirements of each country". The second resolution was on Vector Biology and Control and urged an intensification of research and development of alternative methods of vector control.

It is nine years later now and although many of the country malaria programs have changed their objective from eradication to control, only a few are using very many of the supplementary or alternative methods available to them. Why is this? Again, I am sure that there is no simple answer, but I would hazard a guess that one of the principal reasons lies in the lack of personnel adequately trained in the techniques of mosquito control. A whole generation of malariologists has grown up thoroughly indoctrinated in the techniques of malaria eradication, but with no background or experience in larviciding, space spraying or source reduction. At the present time, training facilities to provide this knowledge are virtually non-existent or at best woefully inadequate. The WHO several years ago started conducting 2 to 3 week seminars on anti-larval methods and later expanded them to general vector control. The seminar has been given three times in five years.

A seminar was given on comprehensive mosquito control in California and El Salvador last summer funded by AID, organized and conducted by P.A.H.O. and Tommy Mulhern with able assistance from many California Mosquito Abatement Districts, the University of California and the California State Department of Public Health. This seminar was very valuable for the engineers from the Latin American countries who attended. I have met some of them since and find that they are trying to put into practice what they learned here in California. I want to thank all of you who contributed to the success of the seminar and assure you that it was appreciated by the participants. That seminar was a step in the right direction, but it was only a beginning. Similar courses should be set up for the entomologists and also for the country directors of malaria control programs. I hope that you will be willing to assist again in the future as I feel that California has much to offer in its varied approaches to comprehensive mosquito control.

Let us take stock. Where do we stand in the worldwide malaria program? In Asia and in Central America malaria programs have had serious setbacks. In South America and the Near East there are indications of rising malaria rates. In Sub-Saharan Africa very little progress has been made in controlling malaria and Africa has more cases and more deaths from malaria than all of the rest of the world put together. The technical problems of drug resistance and insecticide resistance cast serious doubts on the possibility of success through the use of the standard techniques of malaria eradication. What then are the alternatives?

I personally feel that the recommendations of the Director-General's Report of the Re-examination of the Global Strategy of Malaria Eradication presented at the World Health Assembly in 1969 are still valid, but have never been given a fair chance. I think that each country should develop its own strategy of malaria control using an appropriate combination of supplementary and alternative methods.

What then is available by way of alternatives?

First it must be recognized that in spite of the resistance problem, residual insecticides are still the most cost-effective single method of malaria control where the mosquitoes are still susceptible. After 20 plus years and the testing of 1400 compounds, WHO has only recommended 6 insecticides for use as residual sprays in malaria programs. They are DDT, Malathion, B.H.C., Dieldrin, Propoxur and Fenitrothion. Resistance to one or more of the mosquito vectors has been reported for the first five and the sixth is not yet in regular large scale use in any malaria program. There is no new panacea in sight in the foreseeable future, so other methods will eventually have to be used.

Larviciding has been utilized as a regular part of some malaria control programs, particularly for urban malaria. In tropical countries during the monsoon season larviciding is too expensive and ineffective. However, there may be a place for the modern larvicides such as abate or dursban in slow release formulations in many malaria control programs. Larviciding cannot be considered an alternative but as a supplementary method when appropriate.

Source reduction had no particular application in malaria eradication since the objective was interruption of transmission of malaria. However, in a malaria control program, source reduction can substantially reduce the number of anopheline mosquitoes and consequently reduce the amount of malaria. Speaking of source reduction reminds me that this is the 25th anniversary of the first use of the term. Some of the old-timers will remember the story, but for the benefit of the rest I will tell you how the term originated. Bob Peters and I were going to participate in a panel discussion of the work we were doing in our respective districts. We met in Merced to discuss the program and were trying to agree on a title for our panel discussion. We rejected Harold Gray's term of permanent control because we felt that in agricultural areas it just wasn't permanent. Source elimination and source minimization were suggested and rejected. I finally said, "We can't expect to get rid of all the mosquito sources, but we can certainly reduce them. That's what we are trying to do anyway. Why don't we call it Source Reduction?" We both agreed and I used the term for the first time on February 14, 1952 at the 20th Annual CMCA Conference in Fresno, California. The term has been adopted and is now used throughout the world.

Source reduction does not have universal application in malaria control programs, but there are cases in which it can well be the method of choice. In urban and peri-urban situations source reduction combined with larviciding can substantially reduce the malaria rate. I recall that in Benkulu, a city in western Sumatra, a combination of ditching, ditch cleaning and installation of a tide gate reduced malaria from 80% to 15%. In most developing countries, the malaria programs and ministries of health do not have the heavy equipment or the budget to do large source reduction projects, but they do have the manpower and they can enlist the support and assistance of their Ministries of Public Works, Agriculture, Transport and others having a mutual interest in water problems. Many malaria programs with problems amenable to source reduction are doing nothing in source reduction because they have no personnel trained in the methods. It must be 7 or 8 years ago that one of your own source reduction specialists George Whitten was asked to go down to El Salvador to advise on establishing a source reduction program. He devoted a great deal of time and effort to establishing relations with every agency and organization in the country that had any interest in water. His report was an excellent basis for developing a cooperative source reduction program. However, there was no one in the government or on the Malaria Research Team who understood what was involved well enough to do anything about it. I hope that last summer's seminar on Comprehensive Mosquito Control will help to remedy that.

Source reduction cannot be considered an alternative to residual spraying and drug treatment, but as a supplementary method it can in the long range reduce reliance on insecticides.

The introduction of mosquito fish to control mosquito larvae has been utilized on a regular operational basis in malaria eradication programs with considerable success in some countries against certain species of mosquitoes, as for example in Iran and India against *Anopheles stephensi*, the principal vector of urban malaria. However, the potential for using larvivorous fish in an integrated malaria control program is much greater. Again this is a supplementary method which can reduce reliance on pesticides.

The use of space spray adulticiding such as ULV by airplane or back-pack has been used experimentally in several malaria programs and has been effective in some cases. Further field trials will be necessary to determine the cost-effectiveness of the method on a regular operational basis, but it appears to have considerable value in epidemic situations.

From this discussion we arrive at the inevitable conclusion that at the present time there are no really viable alternatives in the conduct of a large scale national program of malaria control to the use of the most effective residual wall spray available combined with drug treatment. However, the other methods we have discussed, i.e. larviciding, space spray adultuciding, larvivorous fish, and source reduction are available for use as supplementary methods in national malaria control programs.

What is needed is a comprehensive approach to malaria control utilizing all available methods of malaria control as appropriate for the individual country in an integrated program aimed at reducing the incidence of malaria to a level where malaria is no longer a serious public health problem.

Planning and carrying out such a program requires a well-trained team of a physician, an engineer, and an entomologist working in close cooperation.

What are the future possibilities?

Antimalaria drugs are already a vital part of malaria control programs, but there has been a great amount of research to develop long lasting and more effective drugs. After atabrine, we began using chloroquine but now we have resistance to this drug in *falciparum* malaria in much of Asia. AID has not been putting research money on this problem largely because the U. S. Army has been spending about $10 million per year for a period of 10 years, testing all promising compounds. That program is now down to perhaps $2 or $3 million per year. After having spent about $100 million, the Army has 4 or 5 candidate drugs which show great promise but are not yet ready for operational use.

Malaria vaccines were once considered impossible by immunologists and parasitologists. AID in 1966 proposed a project for development of a vaccine, recognizing that prospects of success were slight. During the first 6 years of that project the biological feasibility of developing a vaccine was demonstrated, however there was no evidence that it could become practical. First it was necessary to develop methods of in vitro cultivation of malaria parasites. Within 6 months after starting a project to do this, one scientist achieved in vitro cultivation of the erythrocytic stages of *Plasmodium falciparum.* Therefore we now believe we may eventually be able to produce a malaria vaccine. Two projects have successfully immunized monkeys against *Plasmodium knowlsi* which is normally 100% fatal to monkeys, and now some monkeys have retained immunity for 3 years after vaccination.

Predators and pathogens – nematodes, bacteria, viruses – are being tried for mosquito control. They are not likely to be alternatives, and they cannot be applied throughout an entire country under all ecological conditions. Our present knowledge indicates they are confined either to a single species or a single habitat.

Insect growth regulators have not been used operationally in malaria control programs, but they may be used in the future.

Genetic control with sterile males or genetically incompatible strains have been tested, but practical use appears to be years away.

We must consider new methods of control, but we also have to go back to methods which were used in the pre-DDT era, basic sanitation, source reduction, larviciding in a comprehensive approach which will make use of all available methods applied to the problems of each specific country.

WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS VIRUSES IN THE FINNEY LAKE HABITAT OF REPETITIVE *CULEX TARSALIS* ACTIVITY[1]

Telford H. Work, Martine Jozan, Gary G. Clark, O. George Berlin and Dario Parra

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Following demonstration of the year-around activity of *Culex tarsalis* mosquitoes in aqueous habitats in Imperial Valley and the elucidation of seasonal transmission of Western equine (WEE) and/or St. Louis encephalitis (SLE) viruses by this vector, quantitative studies of *Cx. tarsalis* virus vector dynamics were initiated at the Finney Lake Wildlife Refuge in January 1973 (Work 1975). Progress in the biological studies of *C. tarsalis* in this arid-zone aqueous habitat, illustrated in Figure 1, has been reported previously (Clark et al. 1974; Clark et al. 1976; Berlin et al. 1976).

Virological studies have recovered SLE virus from *Cx. tarsalis* every year since 1968, with intermittent explosions of WEE virus (Bowen and Work 1973) in some, but not all years. A review of almost ten years data shows that SLE virus has been recovered from *Cx. tarsalis* early in April and as late as the end of November; but, in spite of continuous year-around collection of *Cx. tarsalis*, no SLE or WEE viruses have been recovered in the months of December, January, February or March, a cool-weather period, when California virus has been recovered from *Culiseta inornata* as an indication that winter transmission of an arbovirus has been detected in our field-laboratory system. The patency of this system is also demonstrated by the occasional but consistent isolation of Turlock virus from *Cx. tarsalis* in almost every month of the year.

Collection and examination of four years' data on WEE and SLE virus isolations from *Cx. tarsalis* mosquitoes collected by CDC light-traps (Clark et al 1974) in the Finney Focus defines not only a stable habitat for *Cx. tarsalis* (Berlin et al. 1976) but a reliable focus where WEE and/or SLE virus appears each year. Figure 2 presents the percentage of *Cx. tarsalis* mosquito pools positive for WEE and SLE virus. Pools averaged about 50 mosquitoes, although some were substantially smaller. This is therefore a minimum-infection trend which, by large numbers of mosquito pools inoculated for each year, provides consistent information on type of viruses that appeared, time of appearance, prevalence and duration of activity. Not indicated are the three strains of WEE virus that appeared late in August 1974, an otherwise intensely active year for SLE virus.

In 1975, a transect (Figure 3) parallel to the grid traps closest to the shore and extending out into the tules and over water was added to the nine-trap grid. An inverse relationship of *Culex tarsalis* recovery relative to distance from the grid has been observed, with *Culex erythrothorax* be-

Table 1.–1976 SLE virus isolations from *Cx. tarsalis* mosquitoes collected by CDC light-traps in the Finney Grid, Imperial Valley, California.

	Culex tarsalis	No. Pools	Positive	%Positive
January 29 - Feb. 14	46	7	0	-
February 26 - March 4	239	10	0	-
March 19 - 25	190	7	0	-
April 23 - 29	286	9	0	-
May 21 - 27	868	19	0	-
June 25 - July 1	2,734	55	15	27.2
July 9 - 15	706	24	0	-
July 23 - 29	1,028	24	0	-
August 13 - 19	77	3	0	-
August 20 - 26	1,463	38	5	13.1
August 27 - Sept. 2	431	9	3	33.3
September 3 - 9	69	2	0	-
October 8 - 14	535	12	0	-
October 15 - 21	209	5	0	-
October 22 - 28	145	4	0	-
October 29 - Nov. 4	198	5	0	-
TOTAL	9,224	233	23	9.8

[1]From the University of California Mosquito Research Program of the School of Public Health, Los Angeles, California, 90024. Partially supported by U.S. Public Health Service Training Grant AI-132-11-14.

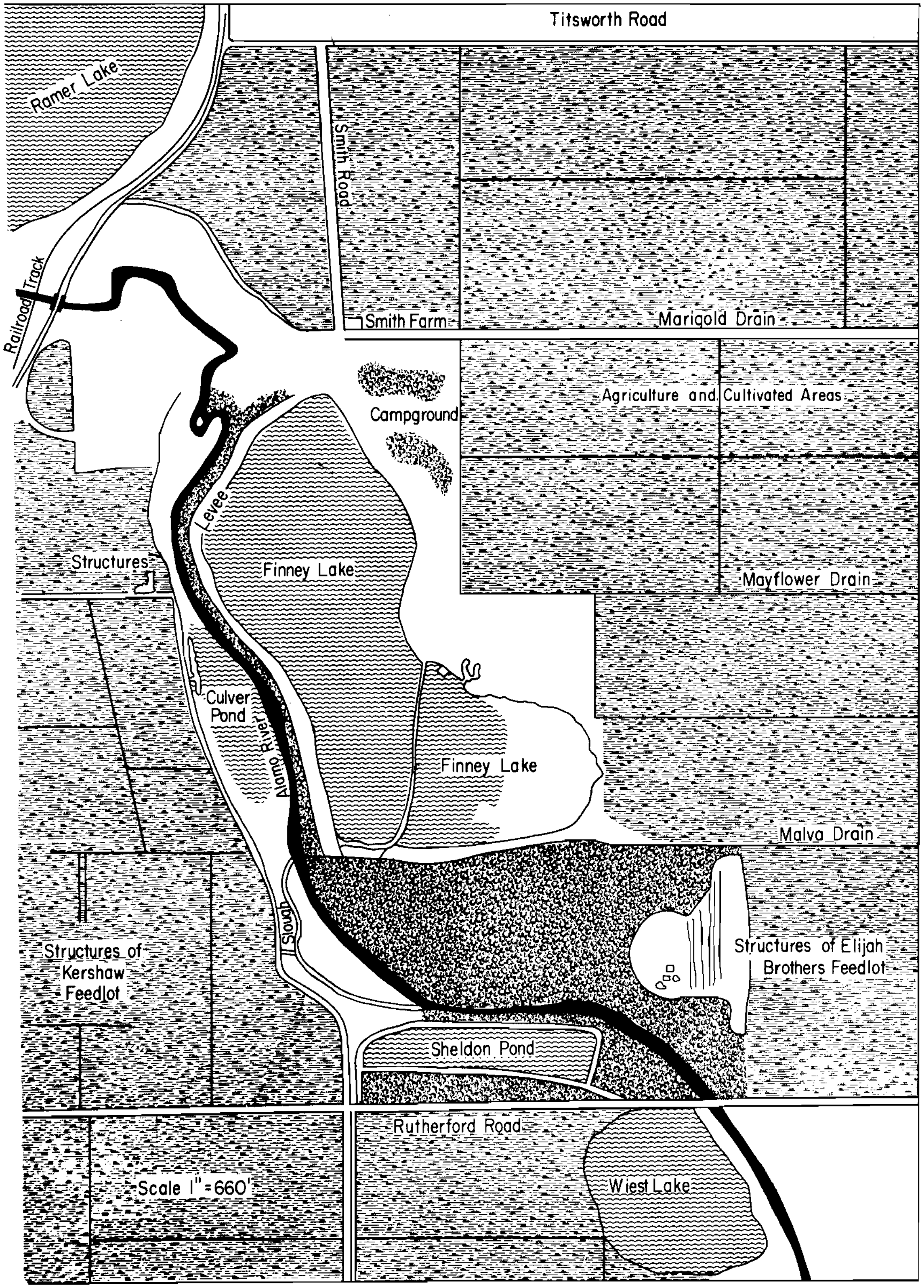

Figure 1.–Finney Lake and adjacent areas of Imperial Valley, California.

coming the more prevalent species in the CDC light-traps over water.

However, the WEE virus infection rates in pools of *Cx. tarsalis* taken far from shore in 1975 were not significantly lower than in the grid. This may reflect dilution of the *Cx. tarsalis* population in the grid by newly emerged mosquitoes and indicates wide dispersal of infected *Cx. tarsalis* in the aqueous habitat where water birds such as coots are abundant.

In 1976, the projected avian host studies in the Finney Lake habitat were abruptly blocked as the result of a fire which swept through much of the grid and lake transect on 20 July as shown in Figures 3 and 4. Although all the collections are not yet identified, it is evident that the *Cx. tarsalis* population drastically declined. Within a month, however, catches once again paralleled previous years (Table 1), particularly in the lake transect. Since high SLE infection rates also resumed (Figure 2), it is difficult to determine

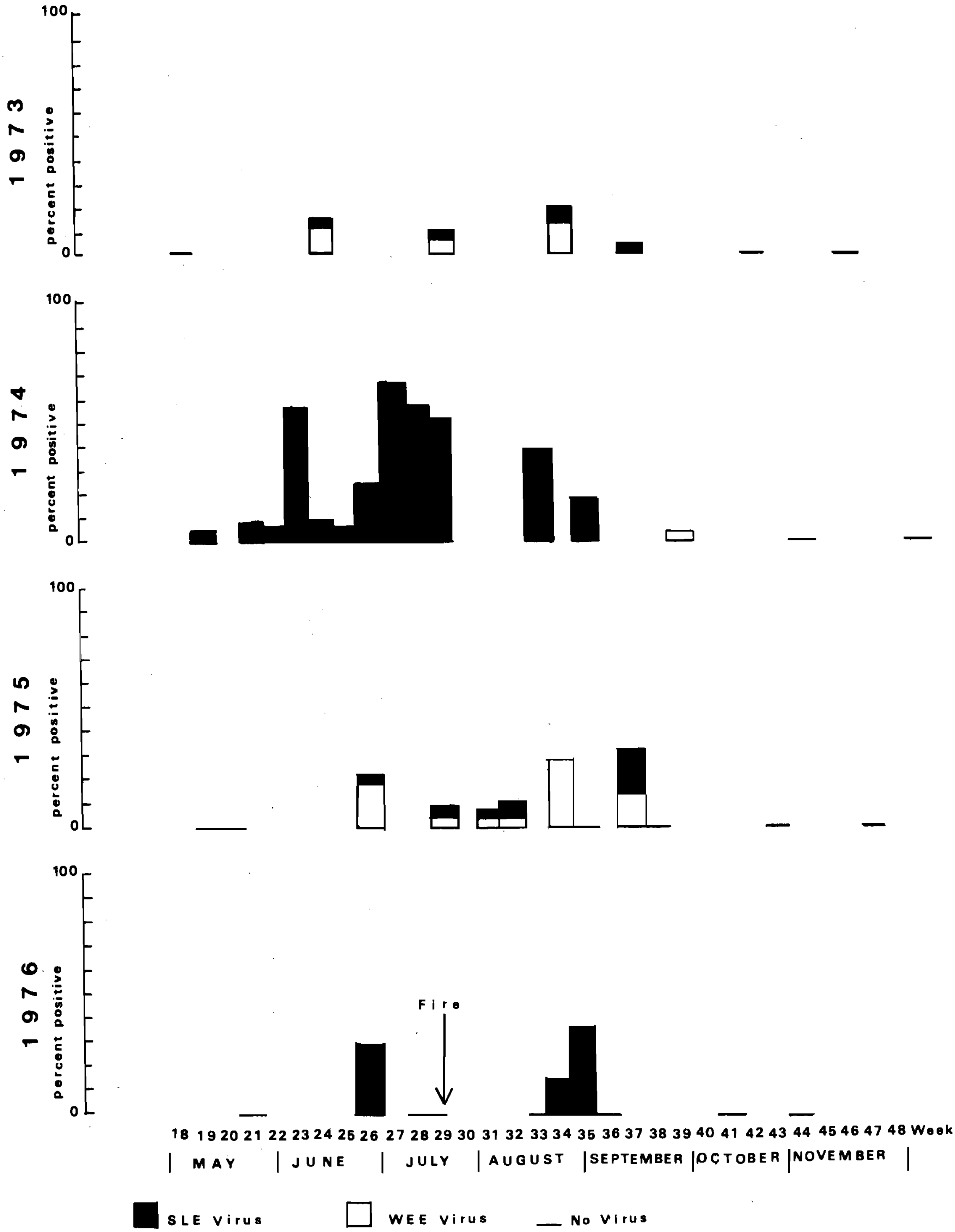

Figure 2.–*Culex tarsalis* mosquito pools positive for SLE and/or WEE arboviruses in Finney Lake grid, 1973 thru 1976.

Figure 3.—Burned tamarisk habitat encompassing (right to left) FG4, FG5, FG7, and FG8 in Finney Focus, one month following fire on 20 July 1976.

whether these *C. tarsalis* came from elsewhere or whether a new breeding cycle produced a new population which became infected during the four weeks following the fire. It does emphasize the reliable focal nature of SLE transmission in the Finney Lake-Grid habitat as a place to search vertebrate sources of virus. WEE virus did not appear in the Finney Focus in 1976.

Accepting mosquito infection as a sensitive indicator for the presence of virus by type, time of appearance, prevalence and distribution, it is concluded that:

1) Repeatable annual patterns for *Culex tarsalis*, only temporarily altered in 1976 by a devastating fire, reflects as stable breeding and vertebrate-feeding habitat.
2) Absence of isolations December through March and varying patterns for SLE and WEE viruses in different years indicates that these viruses are not maintained in the Finney Focus throughout the year.
3) Consistent reappearance reflects a susceptible vertebrate fauna providing amplification within the habitat, which should be an environment where the essential elements of the wild cycle can be elucidated.
4) The annual reappearance or virus may inply that the overwintering habitat is not too far away.

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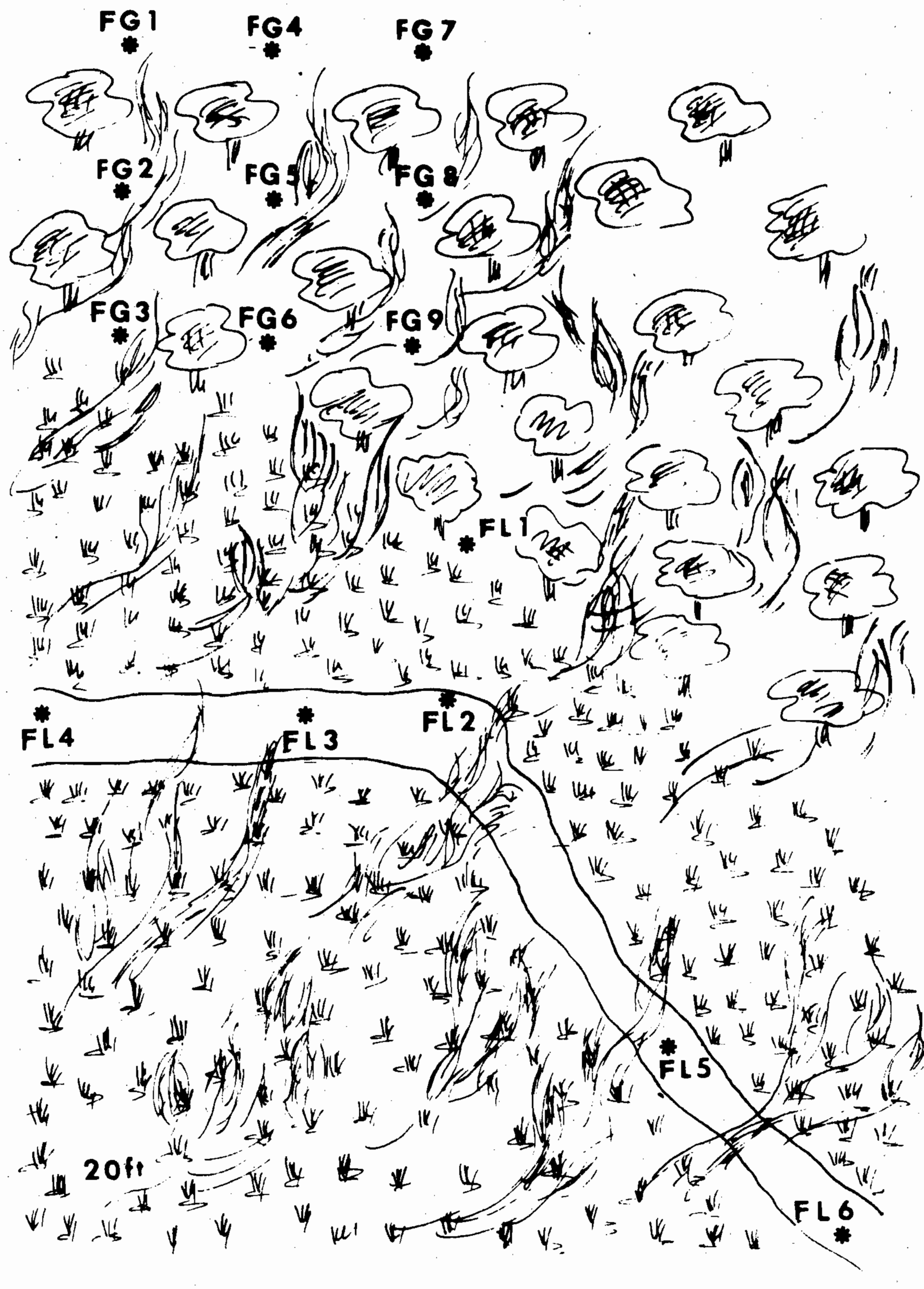

Figure 4.–Schematic presentation of *Culex tarsalis* habitat of Finney Focus burned by fire 20 July 1976; only FG1, FG2, FG3 and FG6 were not destroyed.

PREVALENCE OF ANTIBODIES TO WESTERN EQUINE, ST. LOUIS AND CALIFORNIA ENCEPHALITIS VIRUSES IN RESIDENTS OF IMPERIAL VALLEY, CALIFORNIA[1]

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Zoonotic transmission of Western equine, St. Louis and California encephalitis viruses in Imperial Valley, California, has been demonstrated for the past ten years (Work et al. 1977), yet the potential human impact of such transmission remained to be ascertained. A preliminary antibody study was undertaken for this purpose among a selected group at particular risk of infection, namely the Mexican-Americans who constitute the major labor force in the Valley; in some areas they represent 50 to 60% of the population.

Serum specimens were obtained from the "Clinica de Salubridad de Dampesinos" in Brawley. The clinic, sponsored by the U. S. Department of Health, Education, and Welfare, serves both resident and transient farm laborers. Among 577 sera made available, 452 were from Mexican-Americans; the remaining 125 comprised 103 Whites other than Mexican-American, 11 Blacks, 1 Oriental and 6 of race not stated (Tables 1 and 2). The age category 0-14 years was poorly represented in both sexes and in all ethnic groups. Among males, the highest number of specimens was obtained from men age 40 years and over; among females, most specimens were in the age-bracket 15 years and over (Table 3).

All but nine specimens came from residents of Imperial Valley. The majority were living in the Brawley area (Map 1).

All specimens were sujected to the standard hemagglutination inhibition test against Imperial Valley strains of Western equine (G3132) and St. Louis (G342) encephalitis viruses. Only a few sera were found positive by this method. Final interpretation of the study was based upon the results of the neutralization test, which was performed in weanling mice for SLE, and in BHK21 cells for WEE and CEV virus.

As shown in Table 4, seven of 462 specimens tested (1.5%) had antibodies to WEE virus, and five of 456 (1%) to CE virus. In contrast to this low prevalence, 69 among 566 specimens exhhibited SLE antibodies.

As might have been expected, there was an increasing prevalence of SLE antibodies with age; and there was a similar age/sex distribution among Mexican-Americans and non-Mexican-Americans, although antibodies were found at a later age in this last group (after 54 years for males and after 34 years for females).

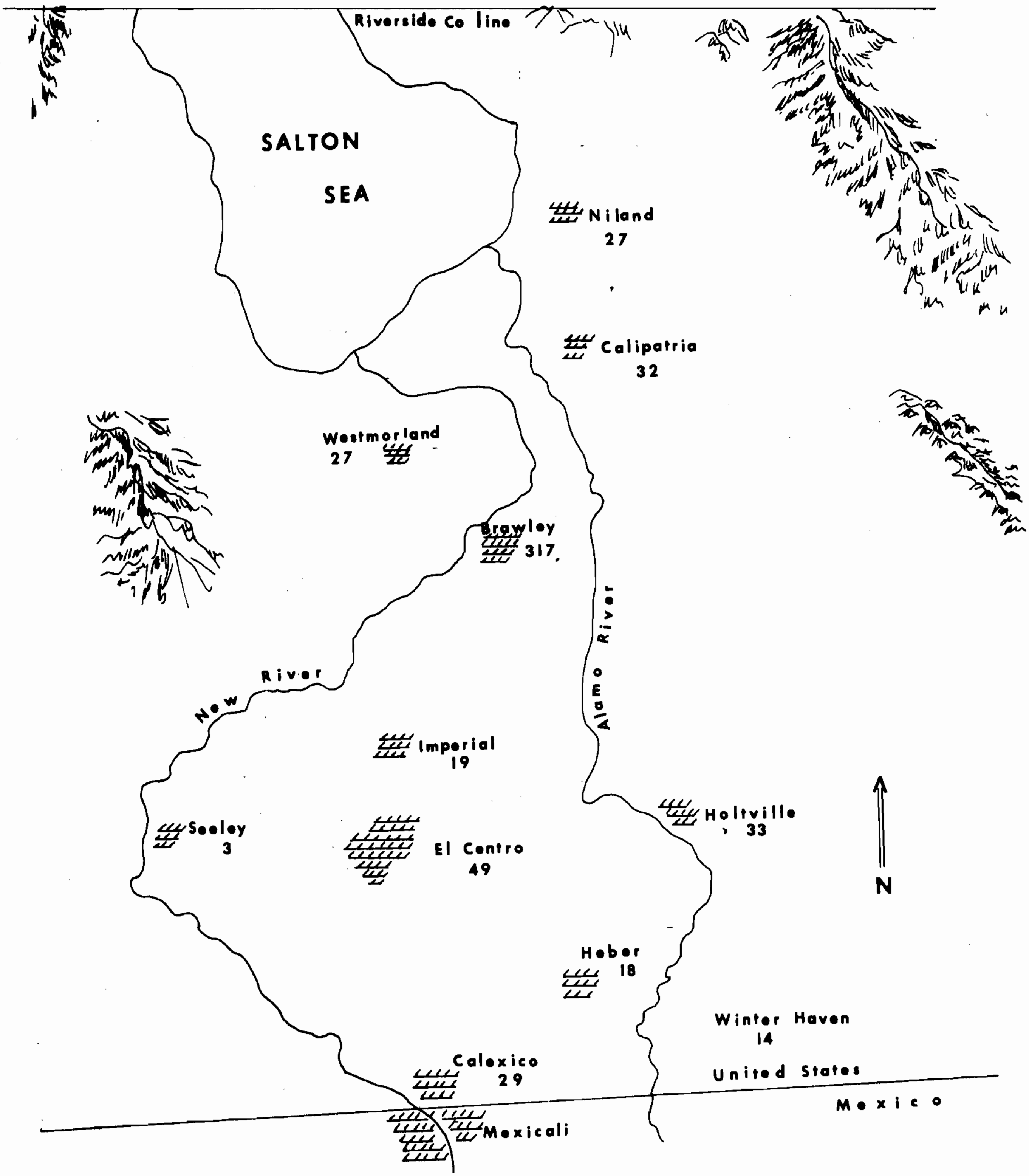
The distribution of positive specimens according to occupation and in relation to farm labor did not show any significant differences among males. Among females, however, 2 of 6 (33%) regular and 7 of 38 (18%) occasional farm laborers were positive as compared to 23 of 251 (9.1%) not involved in agricultural activities (Table 5).

Table 1.—Age distribution of 204 males, Imperial Valley, California according to ethnic group.

Age	Black	White*	Oriental	Amer. Indian	Other	Unknown	Total	Percent
0-4	0	0	0	0	0	0	0	
5-9	0	1	0	0	0	0	1	0.5
10-14	0	5	0	0	0	0	5	2.4
15-19	0	7	0	0	0	0	7	3.4
20-24	0	4	0	0	1	0	5	2.4
25-29	0	6	0	0	1	0	7	3.4
30-34	0	5	0	0	0	0	5	2.4
35-39	0	9	0	0	0	0	9	4.4
40-44	0	13	0	0	0	0	13	6.4
45-49	0	15	0	0	0	0	15	7.3
50-54	0	17	0	0	0	0	17	8.3
55+	1	113	3	1	1	1	120	58.8
All Ages	1	195	3	1	3	1	204	

*Includes Mexican-American

[1]This study was supported by the United States Public Health Service Training grants 5-T0I-AI-00132, and 5-T0I-GM-1141, by the Ch. Carpenter Memorial Fund, U.C.L.A. School of Public Health, and by the University of California Mosquito Control Research Program.



Map 1.–Geographic distribution of human sera obtained from Imperial Valley, California.

The prevalence of antibodies to SLE among both Mexican-Americans and non-Mexican-Americans raises specific questions in regard to the place of infection. It was not surprising to find many positives among those Mexican-Americans who were born in Mexico and had lived there many years prior to their entry into the United States. Yet, of more interest were the positive findings among Mexican-Americans born in the U. S.; the majority in California and others in Texas and Arizona. Among those with SLE virus antibodies, from whom information could be obtained, the duration of residence in Imperial Valley was no less than 12 years and up to 49 years for males, and 1 to 50 years for females, as shown in Table 6. The youngest positive individual was a 17 year-old Mexican-American female from

Table 2.–Age distribution of 373 females, Imperial Valley, California according to ethnic group.

Age	Black	White*	Oriental	Amer. Indian	Other	Unknown	Total	Percent
0-4	0	1	0	0	0	0	1	0.3
5-9	0	4	0	0	0	0	4	1.0
10-14	0	4	0	0	0	0	4	1.0
10-19	0	27	0	0	0	2	29	7.8
20-24	0	68	0	1	0	0	69	18.5
25-29	1	41	0	0	0	0	42	11.2
30-34	0	23	0	0	0	0	23	6.2
35-39	0	28	0	0	0	0	28	7.5
40-44	0	28	0	0	0	0	28	7.5
45-49	2	24	0	0	0	0	26	6.9
50-54	2	35	0	0	0	0	37	9.9
55+	5	77	0	0	0	0	82	21.9
All Ages	10	360	0	1	0	2	373	

*Includes Mexican-American

Table 3.–Distribution by age and sex of 577 residents of Imperial Valley, California.

Age	Mexican-American				Non Mexican-American*			
	Males	Females	Total	Percent	Males	Females	Total	Percent
0-4	0	1	1	0.2	0	0	0	
5-9	1	4	5	1.1	0	0	0	
10-14	5	3	8	1.7	0	1	1	0.8
15-19	7	23	30	6.6	0	6	6	4.8
20-24	4	60	64	14.1	0	9	9	7.2
25-29	6	40	46	10.1	1	2	3	2.4
30-34	5	23	28	6.1	1	0	1	0.8
35-39	8	27	35	7.7	1	1	2	1.6
40-44	10	26	36	7.9	3	2	5	4.0
45-49	12	20	32	7.0	3	6	9	7.2
50-54	12	30	42	9.2	5	7	12	9.6
55+	66	59	125	27.6	54	23	77	61.6
All Ages	136	316	452		68	57	125	

*Includes Blacks, White other than Mexican-American, Oriental, Other, and Unknown.

Brawley, where she had lived since birth. Duration of residence for four non-Mexican-Americans with SLE antibodies was between 25 and 50 years. One oriental, who had lived 35 years in the Valley, had antibodies to both WEE and SLE. Finally, it is noteworthy that 2 of 9 Mexican-American males working as irrigators had SLE antibodies.

The constant interchange with Mexicali, which characterizes the life style of the Imperial Valley residents, makes it difficult to pinpoint the place of some of those SLE infections. Yet, there is evidence of occupational exposure to SLE infections, responsible for the positive findings among the residents and also sometimes natives of Imperial Valley. Because of anticomplementary reactions, detection of a recent infection by complement fixation test could not be accomplished. Only a prospective follow-up would provide this information.

This prevalence study delineates the demographic and serologic attributes of a population group, which, except for the very young, typically represents the farm labor population of Imperial Valley. The presence of SLE antibodies among such a group indicates the need for further evaluation of the disease impact of SLE infections, and for continued surveillance of the zoonotic transmission of the virus.

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Table 4.–Prevalence of antibodies to WEE, SLE, CE encephalitis viruses among residents of Imperial Valley, California.

Age	No. Tested	Negative	Percent	Positive	Percent	Inconc. & Non Spec.	Percent
			Western Equine Encephalitis				
0-19	35	34	97.1	1	2.8	0	
20-34	116	116	100.0	0		0	
35-54	146	145	99.3	1	0.6	0	
55+	165	160	96.9	5	3.0	0	
All Ages	462	455	98.4	7	1.5	0	
			Saint Louis Encephalitis				
0-19	49	43	87.7	1	2.0	5	10.2
20-34	147	122	82.9	13	9.0	13	9.0
35-54	171	120	70.0	22	12.8	28	16.3
55+	199	132	66.3	33	16.5	34	17.0
All Ages	566	417	73.6	69	12.1	80	14.1
			California Encephalitis				
0-19	39	39	100.0	0		0	
20-34	117	116	999.1	1	0.8	0	
35-54	142	141	98.5	1	0.7	0	
55+	158	155	98.1	3	18.9	0	
All Ages	456	451	98.9	5	1.0	0	

Table 5.–Prevalence of SLE neutralizing antibodies among Mexican-American, Imperial Valley, California, according to occupation.

	Agricultural			Occasional*			Non Agricultural**			Unknown		
Age	Total	Pos.	% Pos.	Total	Pos.	% Pos.	Total	Pos.	% Pos.	Total	Pos.	% Pos.
						MALES						
0-19	0	0	0	0	0	0	13	0	0	0	0	0
20-34	6	0	0	2	1	50.0	2	0	0	5	0	0
35-54	17	2	11.7	3	2	66.6	10	2	20.0	12	0	0
55+	30	5	16.6	5	3	60.0	19	6	31.5	12	2	16.6
All Ages	53	7	13.2	10	6	60.0	44	8	17.1	29	2	6.8
						FEMALES						
0-19	0	0	0	0	0	0	28	1	3.5	3	0	0
20-34	2	1	50.0	14	2	14.2	102	8	7.7	5	1	10.0
35-54	3	1	66.6	17	4	23.5	75	8	10.6	8	0	0
55+	1	0	0	7	1	14.2	46	6	13.0	5	0	0
All Ages	6	2	33.3	38	7	18.4	251	23	9.1	21	1	4.7

*Includes housewives, retired, disabled, and unemployed persons who are at certain times involved in farm-related activities.
**Includes all other occupations which do not involve farm labor or farm-related activity.

Table 6.–Place of birth and duration of residence for Mexican-American exhibiting SLE antibodies, Imperial Valley, California.

Age	Born in Mexico: Number Positive	Born in Mexico: Years of Residence in Birthplace – Range	Mean	Born in Mexico: Years of Residence in Imperial Valley – Range	Mean	Born in U. S. A.: Number Positive	Born in U. S. A.: Years of Residence in Birthplace – Range	Mean	Born in U. S. A.: Years of Residence in Imperial Valley – Range	Mean
MALES										
0-19	0					0				
20-34	0					1		11.0		12.0
35-54	3	17-34	21.3	12-16	13.6	3	30-42	37.6	22-42	32.6
55+	7	7-30	17.0	7-49	27.6	1		21.0		38.0
All Ages	10	4-30	16.5	7-49	20.7	5	11-42	29.0	12-42	27.6
FEMALES										
0-19	0					1		17.0		17.0
20-34	4	2m*-20	13.5	1- 7	4.5	3	unk.	unk.	20-23	18.3
35-54	8	5-27	14.8	7-20	13.7	1		34.0		34.0
55+	4	2-25	19.0	4-50	25.2	3	13-35	27.3	35-50	39.6
All Ages	16	2m*-27	14.0	1-50	14.3	8	unk.	unk.	17-50	26.6

*Months

A PRELIMINARY COMPARATIVE STUDY OF *CULEX TARSALIS* AND *CULEX PIPIENS QUINQUEFASCIATUS* FROM THE NEW RIVER, IMPERIAL VALLEY, CALIFORNIA[1]

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Investigations of mosquito transmission of St. Louis encephalitis (SLE), Western encephalitis (WEE) and other arboviruses in the Imperial Valley of Southern California (Bown and Work 1973; Clark et al. 1974; Work et al. 1974; Berlin et al. 1976; Clark et al. 1976; Magy et al. 1976) have elucidated a potential health hazard to the human and equine residents and visitors. Of particular significance were the foci of virus activity in mosquitoes along the Alamo River and at the constant and predictable Finney Lake focus (Work et al. 1977).

Previous studies demonstrated virus recovery from *Cx. pipiens* collected from several sites in the Valley (Bown and Work 1973; Magy et al. 1976). On 4 April 1973, surveillance by the California State Health Department, Bureau of Vector Control, isolated SLE virus from *Culex tarsalis* on the western side of the Imperial Valley along the New River (Work 1975). These cumulative observations prompted a more intensive evaluation of mosquito and virus incidence along the New River north of the Mexican border, the preliminary results and interpretations of which are presented here.

Culex tarsalis and *Culex pipiens quinquefasciatus* occur separately and together in agricultural and urban environments (Magy et al. 1976). In Imperial Valley, peridomestic *Cx. pipiens quinquefasciatus* is often associated with human habitation. Both species produce larvae that are adaptable to aquatic environs containing relatively high concentrations of organic decay products from sources such as irrigation and feed-lot run-off (Magy et al. 1976) and human sewage (Sjogren 1968).

Because of Mexicali's fast growth since 1960, sewage treatment facilities provide inadequate processing of human waste from more than 370,000 Mexican homes. Another 130,000 domiciles discharge untreated sewage into the surroundings. Effluent from both sources are emptied into the New River. The smaller population (75,000) of people adjacent to the river in Imperial Valley produces some inadequately treated sewage that also adds to the problem. In addition, ponding of cattle feed-lot drainage beside the New River adds to the standing untreated water available for mosquito breeding (California Regional Water Quality Control Board 1975).

To ascertain the mosquito- and associated viral activity along the New River, a transect of 16 trap-sites, one mile apart, was laid out along the natural course of the river from Calexico (NR 1) to a point just south of Seeley (NR 16). In March 1976, monthly collections of mosquitoes were commenced at some sites using CDC light-traps each augmented with a two-pound block of dry ice. Lard-can traps (Bellamy and Reeves 1952) baited with pigeons were used in March and April, and then again from August through October. Under optimal conditions collecting was done during the darker phases of the moon and for at least three consecutive nights from dusk until sunrise. Mosquito catching bags were retrieved and placed on dry ice immediately to quick-freeze the live-mosquitoes for virus preservation. Specimens were later transferred to plastic vials and kept on dry ice or at -70°C in a freezer until identified and pooled for viral isolation. Because of varying temperatures, humidities, and wind conditions, analysis of the catches are presented as the average number of mosquitoes obtained per trap night.

The light-trap data demonstrate a bimodal occurrence of *Cx. tarsalis* and *Cx. p. quinquefasciatus* with peaks by both species during late spring and again in early fall (Table 1). These are in agreement with the results of Clark et al. (1974) and Magy et al. (1976), except for a much more pronounced bimodality with *Cx. p. quinquefasciatus*. The September-October peak curve reflects the excess rainfall from Hurricane Kathleen, which struck on 10 September (Magy et al. 1977; Thomas et al. 1977). A similar increase, although of a greater magnitude, also occurred with *Cx. tarsalis*. Numbers of both species diminished in the light-traps one to three days following the storm.

Reluctance of *Cx. p. quinquefasciatus* to enter light-traps has been reported repeatedly. The observations of Magy (1955) and Magy et al. (1976) suggest the same bias operates in Imperial Valley. While the pigeon-baited lard-can traps augmented total numbers of *Cx. p. quinquefasciatus* collected for virus isolations (Work et al. 1977), the smaller numbers per trap night, in comparison to *Cx. tarsalis* tabulated in Tables 2 and 3, indicate that baited traps are also refractory to *Cx. p. quinquefasciatus*. Figure 1 illustrates the similarity of CDC light-traps and pigeon-baited lard-can traps in attracting *Cx. p. quinquefasciatus*, indicating that appreciable increases in numbers will require different methods, including hand-aspiration.

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[1]From the University of California Mosquito Research Program of the School of Public Health, Los Angeles, California 90024. Partially supported by U. S. Public Health Service Training Grant AI-132-11-14.

Table 1.—*Culex tarsalis* and *Cx. pipiens quinquefasciatus* collected from New River CDC Light Trap sites, Imperial Valley, California (1976-1977).

Period	*Cx. tarsalis*	*Cx. p. quinq.*	R*
3/29- 3/31	179 (5.4)**	5 (0.2)	96
4/25- 4/28	1518 (33.0)	150 (3.3)	91
5/28- 5/29	4839 (186.1)	1035 (39.8)	82
6/25- 6/26	2104 (80.6)	551 (21.2)	79
7/ 7- 7/ 8	1712 (61.1)	329 (11.8)	84
7/20- 7/21	1185 (47.4)	92 (3.7)	93
8/ 4- 8/ 5	1059 (96.3)	25 (2.3)	98
8/17- 8/26	2493 (36.7)	187 (2.8)	93
9/12- 9/13	285 (20.4)	0 (0.0)	100
9/21- 9/24	1057 (96.1)	228 (20.7)	83
10/ 1-10/ 3	3917 (97.9)	264 (6.9)	93
10/ 8-10/10	2204 (61.2)	280 (8.2)	88
10/21-10/23	2613 (55.6)	205 (4.4)	79
10/31-11/ 1	130 (6.8)	42 (2.2)	76
11/26-11/28	123 (3.3)	357 (9.6)	26
12/29-12/31	160 (5.9)	15 (0.6)	91
1/29- 1/31	103 (4.1)	1 (0.4)	99

*Percentage of *Cx. tarsalis* per trap relative to *Cx. p. quinquefasciatus*.
**Numbers in parentheses ( ) indicate the mean number of mosquitoes per trap night.

Table 2.—*Culex tarsalis* and *Cx. pipiens quinquefasciatus* collected from New River baited Lard Can trap sites, Imperial Valley, California (1976).

Period	*Cx. tarsalis*	*Cx. p. quinq.*	R*
3/29- 3/31	11 (1.2)**	4 (0.4)	75
4/27- 4/28	212 (26.5)	22 (2.8)	90
8/19- 8/25	505 (15.3)	22 (2.5)	86
9/12- 9/13	201 (18.3)	48 (4.4)	81
9/21- 9/24	273 (15.2)	256 (16.0)	49
10/ 1-10/ 3	3605 (78.4)	592 (12.9)	86
10/ 8-10/ 9	446 (18.6)	147 (6.1)	75
10/22-10/23	225 (15.0)	112 (7.5)	67
10/31-11/ 1	58 (3.2)	97 (5.4)	37

*Percentage of *Cx. tarsalis* per trap relative to *Cx. p. quinquefasciatus.*
**Numbers in parentheses ( ) indicate the mean number of mosquitoes per trap night.

Table 3.—Concurrent collection of *Culex tarsalis* (Ct) and *Cx. pipiens quinquefasciatus* (Cpq) from CDC Light and pigeon-baited Lard Can traps from the New River, Imperial Valley, California (1976).

	Light Traps		Lard Cans	
Period	Ct	Cpq	Ct	Cpq
3/29- 3/31	5.4*	0.2	1.2	0.4
4/25- 4/28	33.0	3.3	26.5	2.8
8/17- 8/26	36.7	2.8	15.3	2.5
9/12- 9/13	20.4	0.0	18.3	4.4
9/21- 9/24	96.1	20.7	15.2	16.0
10/ 1-10/3	97.9	6.9	78.4	12.9
10/ 8-10/10	61.2	8.2	18.6	6.1
10/21-10/23	55.6	4.4	15.0	7.5
10/31-11/ 1	6.8	2.2	3.2	5.4

*Values indicate the mean number of mosquitoes per trap night.

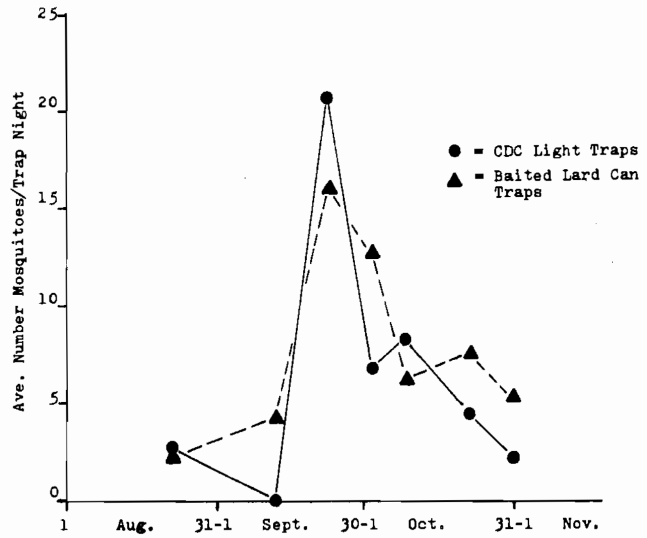

Figure 1.—A comparison of *Culex pipiens quinquefasciatus* collected in CDC light traps and pigeon-baited lard can traps from the New River, Imperial Valley, California (Aug.-Nov., 1976).

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ST. LOUIS ENCEPHALITIS VIRUS TRANSMISSION IN 1976 IN THE BORDER TRANSECT OF THE NEW RIVER OF IMPERIAL COUNTY[1]

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Studies commenced in 1967 have obtained a significant sample of *Culex tarsalis* mosquito populations in virtually every month for the past decade. These have shown that *Cx. tarsalis* is infected with Western equine encephalitis virus (WEE) in some years, and St. Louis encephalitis (SLE) every year. No WEE and SLE viruses have been isolated from December through March. Virus is recovered for varying periods of time between early April and late November, virus activity by weeks differing from year to year. Pool infection rates up to fifty percent have been demonstrated with both WEE and SLE viruses, indicating intensive periods of transmission. Host preference studies (Berlin et al. 1976) indicate that *Cx. tarsalis* feeds primarily on birds in the Finney Lake habitat of WEE and SLE (Work et al. 1977), with only limited attraction to man.

A recently completed serological study of 577 residents at high risk of exposure has shown 8 to 15% prevalence of antibodies to SLE virus, rising with age, indicating local exposure to infection (Jozan 1977). But there is virtually no evidence of WEE virus infection that would be expected if *Cx. tarsalis* were a common vector to man. Therefore, it is concluded that some other vector has transmitted SLE to the human population.

The 1971-72 surveillance for Venezuelan equine encephalitis virus (Work 1973) systematically examined CDC light-trap collections from a one-mile segment of the Alamo River as it enters Imperial Valley from Mexico (Bown and Work 1973). It was remarkable that so many *Culex pipiens quinquefasciatus* mosquitoes were collected in this rural riverine habitat and that pools of these mosquitoes yielded so many isolations of SLE virus. *Cx. pipiens quinquefasciatus* are well recognized as common vectors of SLE virus in states east of California, particularly in a sequence of epidemics in Texas, the Mississippi and Ohio River valleys, and in New Jersey and Pennsylvania in the past twenty years (Work 1971; Monath, personal communication). Previous studies in northern California indicated that *Cx. p. quinquefasciatus* was not an important vector (Reeves and Hammon 1962). However, a survey in 1955 (Magy) and a followup in 1975 (Magy et al. 1976) showed that *Cx. p. quinquefasciatus* was and is a common mosquito in Imperial Valley, prevalent in both rural and urban environments.

Although this species occasionally turned up in light-trap collections at the Wister and Finney Lake study sites, they were so few in number that they did not siginificantly affect the cumulative data on virus-infected pools of *Cx. tarsalis*.

Recognition of the SLE virus isolations from *Cx. p. quinquefasciatus* at the Mexican border, and observations of the proximity to Calexico of the New River entry into California, focussed attention on this habitat as more likely to provide a measure of *Cx. p. quinquefasciatus* activity and transmission of SLE virus in Imperial County.

Webb et al. (1977) described initiation of systematic collections aimed at increasing the yield of *Cx. p. quinquefasciatus* by pigeon- and other animal-baited traps. While *Cx. tarsalis* is still the predominant species in CDC light-trap collections, this new effort has increased total numbers of *Cx. p. quinquefasciatus* mosquitoes for virological examination.

MATERIAL AND METHODS.—Represented in Figure 1 are the sixteen trap sites located approximately one mile apart from the border entry of the New River to just south of Seeley. CDC light-traps were operated a minimum of three nights per period, monthly beginning in April, and at least twice a month from July through October. The frequency in September and October resulted from the importance of measuring the effect of September 12th hurricane Kathleen and subsequent rainstorms on mosquito activity and virus transmission.

In late August, pigeon-baited lard-can traps (Bellamy and Reeves 1952) were placed in proximity of each light trap, specifically to augment collection of *Cx. p. quinquefasciatus*. Pilot trials with newborn mice were also commenced at this time. Wire mesh test tube containers, holding a mother and litter of newborn mice, were suspended from branches near selected light-traps for one night only to test for actual transmission by bite of mosquito vectors. These litters were then observed in the laboratory for 14 days for signs of illness and death.

A borate saline suspension of sick mouse brain was prepared either from those mice inoculated with material from mosquito pools or from sentinel mice which showed signs of illness. Complement fixation tests with specific hyperimmune sera determined the identity of the infecting virus.

RESULTS.—Because no isolations of WEE virus were made from any pool of mosquitoes collected in Imperial Valley in 1976, all isolation data refers to SLE virus, except where Turlock virus is identified.

Table 1 presents data on isolations of SLE virus from *Cx. tarsalis* and *Cx. p. quinquefasciatus* mosquitoes by period of collection in the New River Border Transect.

Virus first appeared from a pool of *Cx. tarsalis* collected at site NR 13 at the end of May. It was identified as SLE. By the end of June and early July, the percentage of pools positive for SLE virus reached 40 percent. Of the 58 SLE virus isolations from 757 pools containing a total of 26,542 *Cx. tarsalis* mosquitoes, all have been identified as SLE. Three strains of Turlock virus were also recovered, but no evidence of WEE virus turned up in the New River or at the

[1]From the University of California Mosquito Research Program of the School of Public Health, Los Angeles, California 90024. Partially supported by U. S. Public Health Service Training Grant AI-132-11-14.

Table 1.–1976 virus isolations from *Culex tarsalis* and *Culex pipiens quinquefasciatus* mosquitoes collected by CDC Light Traps in the New River border-transect of Imperial Valley, California.

Week	*Cx. tarsalis*	Pools	Positives	% Pos.	*Cx. pipiens*	Pools	Positives	% Pos.
April 23 - 29	1,693	54	0	0	154	13	0	0
May 28 - June 3	4,772	104	1	0.8	1,055	34	0	0
June 25 - July 1	2,034	53	14	26.4	335	18	2	11.1
July 2 - 9	1,731	49	20	40.8	312	20	3	15.0
July 16 - 22	1,239	36	3	8.3	90	20	1	5
July 23 - 29	119	14	0	0	6	3	0	0
July 30 - Aug. 5	1,342	25	5	20.0	32	10	0	0
Aug. 13 - 19	1,735	43	1*	2.3	22	6	0	0
Aug. 20 - 26	832	36	12	3.3	129	13	3	23
			1*	2.7				
Sept. 10-16	401	21	0	0	5	1	0	0
Sept. 17 - 23	1,369	44	0	0	422	25	0	0
Sept. 24 - 30	345	13	0	0	149	9	0	0
Oct. 1 - 7	4,089	103	0	0	240	18	1	5.5
Oct. 8 - 14	2,281	62	2	3.2	287	28	0	0
			1*	1.6				
Oct. 15 - 21	634	19	0	0	60	11	0	0
Oct. 22 - 28	1,484	41	0	0	247	21	0	0
Oct. 29 - Nov. 4	155	13	1	7.6	28	28	0	0
Nov. 19 - 25	16	2	0	0	30	1	0	0
Nov. 26 - Dec. 2	260	21	0	0	322	21	0	0
Dec. 24 - 30	18	4	0	0	1	1	0	0
Total	26,542	757	58	7.6	3,926	302	10	0.3
			3*	0.4				

*Turlock Virus.

Table 2.–1976 trap site yield of St. Louis encephalitis virus isolations from CDC light-trapped *Culex tarsalis* and *Culex pipiens quinquefasciatus* mosquitoes collected in the new river border-transect of Imperial Valley, California.

Trap Site	*Cx. tarsalis*	Pools	Positives	%	*Cx. pipiens*	Pools	Positives	%
N° 1	658	34	0	0	398	23	0	0
N° 2	836	33	0	0	315	21	0	0
N° 3	678	33	2	6.6	168	18	0	0
N° 4	1,069	36	4	1.1	423	26	2	2.6
N° 5	526	28	1	3.4	290	22	0	0
			1*	3.4				
N° 6	228	17	0	0	189	20	0	0
N° 7	1,318	46	2	4.3	303	23	0	0
N° 8	1,418	43	2	4.6	268	22	0	0
N° 9	2,623	62	5	8.3	542	26	6	23.8
N° 10	3,191	78	10	12.8	331	18	2	11.1
N° 11	2,294	56	6	10.7	106	18	0	0
N° 12	851	35	3	9.1	38	6	0	0
N° 13	1,303	45	5	11.1	53	14	0	0
N° 14	4,069	83	3	3.6	376	18	0	0
			2*	2.4				
N° 15	1,844	49	6	14.3	34	14	0	0
N° 16	3,643	80	8	10.0	92	13	0	0
TOTAL	26,549	757	57	7.5	3,926	302	10	0
			3*	0.2				

*Turlock Virus

Table 3.—SLE Virus infection of mosquitoes from pigeon-baited lard can traps.

Week	*Cx. tarsalis*	Pools	Positives	%	*Cx. pipiens*	Pools	Positives	%
April 16 - 22	233	6	0	0	19	3	0	0
April 23 - 29	35	1	0	0	22	2	0	0
August 13 - 19	47	2	3	100	1	1	0	0
August 20 - 26	259	24	1	4.2	44	9	1	11
September 10 - 16	209	12	0	0	31	5	0	0
September 17 - 23	34	5	0	0	117	8	0	0
September 24 - 30	282	14	0	0	126	9	0	0
October 1 - 7	3,942	102	3	2.9	544	32	1	3
			1*	0.9				
October 8 - 14	449	20	0	0	143	15	0	0
October 22 -28	342	17	0	0	126	15	0	0
TOTAL	5,832	203	6	2.9	1,173	99	2	2
			1*	0.5				

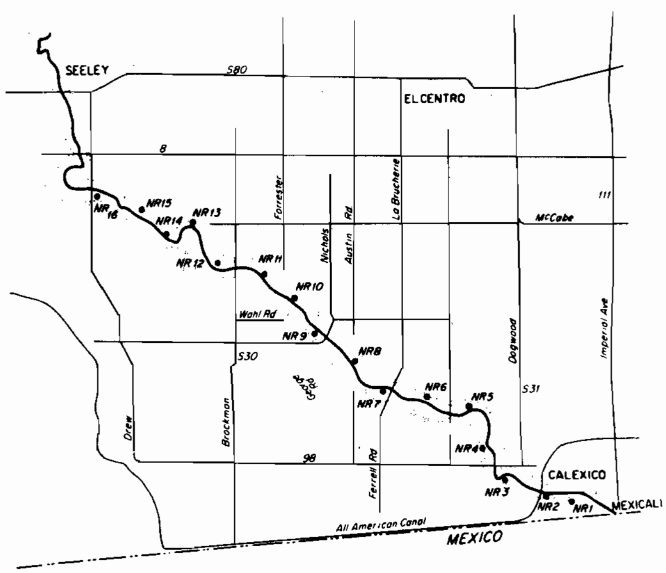

Figure 1.—

Finney focus in 1976, again illustrating the phenomenon of intermittent appearance of WEE in the Imperial Valley.

During the same period, 3,926 *Cx. p. quinquefasciatus* were recovered from the same light traps. Pooled by trap site, as tabulated in Table 2, the actual numbers per pool were significantly smaller (average about 20 per pool) and therefore less likely to yield virus. Recoveries of SLE virus from *Cx. p. quinquefasciatus* correspond to the periods of infection.

The isolation in the first week of October is noteworthy because it occurred three weeks after hurricane Kathleen (Magy et al. 1977), which triggered mosquito breeding all over Imperial Valley (Thomas et al. 1977). The continued presence of SLE in mosquitoes in the New River Transect in the first week of November suggests that the heroic mosquito control measures in the urban areas under aegis of the Imperial County Health Department may have prevented epidemic cases.

Transmissions of SLE virus was demonstrated by isolation of one strain from a mouse litter exposed at NR 4 on the night of 21-22 August and another from a mouse of a litter exposed at NR 5 on 11-12 September.

Success in augmenting the mosquito catch by pigeon-baited lard-can traps is shown in Table 3. Additional strains of SLE were isolated from *Cx. tarsalis* and *Cx. p. quinquefasciatus* recovered by this method. That strains were isolated in the first week of October emphasizes that in spite of the dilution of both species populations by newly emerged imagoes, following the rains of middle and late September, a significant number of infected vectors were seeking blood meals.

Analysis of Table 2 shows that infected mosquitoes occupied the Length of the border transect at some time between May and November. Why no virus was recovered at the two sites closest to Calexico and Mexicali cannot be explained at this stage of the study. It is clear that the habitats between NR 8 to NR 10 and NR 13 to NR 15 were productive of both vector species and SLE virus. More intensive search for vertebrate sources of virus and the link between *Cx. tarsalis* and *Cx. p. quinquefasciatus* will be focussed at these sites.

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SURVEILLANCE FOR ARTHROPOD-BORNE VIRUSES AND DISEASE BY THE CALIFORNIA STATE DEPARTMENT OF HEALTH, 1976

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The large epidemic of St. Louis encephalitis in the eastern 2/3 of the U. S. during 1975 (1,941 confirmed or presumptive cases, 95 deaths) resulted in concern about a possible epidemic during 1976. However, although over 150 cases were detected nationwide, no large outbreaks occurred. Surveillance in California again showed the relatively low level of arbovirus activity characteristic of the past 15 years. Attention was particularly focussed on the Imperial Valley, where late seasonal rainfall and flooding threatened to increase the mosquito population. The results of collaborative efforts by the many state and local agencies and persons interested in arbovirus surveillance in California are summarized here. This is the seventh formal annual report prepared since 1969 on this aspect of the California Department of Health's efforts in preventive medicine.

There were 455 patients (Table 1) who had significant febrile illness, aseptic meningitis, or encephalitis, and were tested serologically for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE), as well as other diseases. This was the lowest number in the last 5 years (peak year was 1973, with 1,037 cases tested). The majority of these cases were found to be caused by enteroviruses, mumps, herpes, other viruses, or leptospirosis; or the cause could not be determined. Physicians caring for patients who live in or have visited endemic areas should be encouraged to improve this aspect of surveillance. Further tests on 102 cases of undetermined etiology will be made in Dr. W. C. Reeves' laboratory (the 12th year for this special study), to see if other mosquito-borne viruses besides WEE and SLE cause human disease in California. There were 13 human brain samples tested in suckling mice, but none yielded arboviruses.

Three cases of SLE were detected during 1976. A 34 year old woman from Cathedral City, Riverside County, was frog-hunting near Mecca, Riverside County, on June 20, and had many mosquito bites. On June 30, she developed severe headache, then disorientation and seizures, and was admitted to a Palm Springs hospital. Paired sera showed rising SLE antibody titers as follows: complement-fixation (CF) <1:8 to 1:16; indirect fluorescent antibody (IFA) 1:256 to 1:2,048; plaque-reduction neutralization (PRNT) 1:1,024 to 1:4,096; and hemagglutination-inhibition (HAI) 1:80 to 1:160.[4] She was discharged July 20 but continued to have memory deficit and hand tremors. This was the first case of SLE acquired in California since 1973, when there were 5 cases. Her probable place of exposure was just 1½ miles from where SLE-positive *Culex tarsalis* were collected on June 9. One flock of 7 chickens was bled on July 16 in Riverside County, and showed SLE antibody in 1, Turlock antibody in all 7, but WEE antibody in none (tested in Dr. Reeves' laboratory).

A 48 year old woman from San Diego became ill August 6 with malaise and fever, then progressive confusion and memory loss, requiring hospitalization. Abnormal cerebrospinal fluid and encephalogram tracings indicated meningo-encephalitis. The SLE antibody titers were: CF <1:16 to 1:16; IFA 1:256 to 1:1,024; PRNT 1:1,024 to ≥1:16,384; and HAI 1:40 to 1:320. She recovered satisfactorily. She and her husband may have been exposed on the weekend of July 2-4 or July 23-24, while fishing near Seeley, Imperial County. SLE-positive mosquitoes were detected from this area during the year. Her husband was not ill, but a single serum sample showed SLE antibody titers of: CF 1:8; IFA 1:1,024; PRNT 1:512; and HAI 1:80 providing evidence of past or current infection.

The third case was a 29 year old woman from Madera, who became ill September 26, with stiff neck, fever, and cells in the cerebrospinal fluid. The SLE antibody titers were: CF <1:4 to 1:8; IFA 1:256 to 1:512; PRNT 1:1,024 to 1:4,096; and HAI pending. Exposure was presumably near her home, but she has been on an extended visit to Mexico since recovering, and detailed information has not been available.

No human cases of WEE were detected in 1976. There were 35 suspect cases of encephalitis in equines reported to the Department, from 25 counties; from 25 of these cases, serum samples were submitted, but none had significant WEE antibody titers confirming it as a case. There were 15 equine brain samples tested in suckling mice, none yielding arboviruses. However, 4 equine brains were positive for rabies by the FRA test.

A total of 1,273 mosquito pools were collected and tested in suckling mice during the year (Tables 2 and 3), with the greatest effort expended in Imperial, Riverside, and San Bernardino Counties. The geographic distribution of the mosquito surveillance effort has varied from year to year, depending on manpower, epidemic potential, and economic factors. Increased bubonic plague activity in the state during 1976 diverted State Vector Control Section personnel from arbovirus surveillance, particularly in central and northern California. Arbovirus isolates from mosquitoes are shown in Table 4: 10 SLE, 11 Turlock, and 1 Hart Park virus strains, all from *C. tarsalis*. Reports by Dr. Reeves and coworkers, and by Dr. T. Work and coworkers, on their more detailed ecologic and surveillance studies will be given separately, and include many more viral isolates. The state mosquito testing program was re-initiated in 1969 (in collaboration with the Center for Disease Control that year). Few tests were done in 1970, but many were done annually since then. From 19,628 vector pools (over 743,132 specimens) tested to date, 650 virus strains have been isolated, including: 159 WEE, 193 SLE, 199 Turlock, and 99 strains of other viruses (Hart Park, California en-

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[4]HAI titers done at School of Public Health, U. C., Berkeley.

Table 1.—Humans tested serologically for arbovirus encephalitis by the Viral and Rickettsial Disease Laboratory, California State Department of Health, and by county health department laboratories, by county of residence and month of illness onset, 1976.

County	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Unk
California Total	455	1	2	4	7	36	78	68	66	58	49	36	2	48
Alameda	6				1	1		1						3
Berkeley - City	17					1	3	3		4	3	2		1
Butte	8					2	1	2	1	1				1
Contra Costa	22					1	2	4	2	5	4	4		
El Dorado	15					5	2	2	1	4				1
Fresno*	20					1		3	6	5	3	1	1	
Glenn	1					1								
Humboldt-Del Norte	4							1				1	1	1
Imperial	0													
Inyo	1										1			
Kern	23			1		1	6	1	4	5	1	2		2
Kings	0													
Lassen	0													
Los Angeles*	22	1	2		1		8	5	5					
Madera	1									1				
Marin	15					3	1	1	2		3	1		4
Mendocino	4							1	2					1
Merced	1						1							
Modoc	0													
Monterey	8							4		1				3
Napa	4				1		1		1					1
Nevada	3							1		1	1			
Orange	5						3	1		1				
Placer	9							3	2	2	1			1
Riverside	14							2	3	3	2	1		3
Sacramento*	15				1	1	5	4	1	1	1	1		
San Bernardino	3						1	1						1
San Diego*	82			3	2	8	13	13	12	9	13	7		2
San Francisco	45				1	2	6	4	8	9	5	4		6
San Joaquin	10					1	4	1	1		1			2
San Luis Obispo	2						1		1					
San Mateo	9					1	6		1					1
Santa Barbara	14					2	2		4		2	3		1
Santa Clara	5					1	1							3
Santa Cruz	6						1		1		1	1		2
Shasta	4					1	1							2
Sierra	0													
Siskiyou	3							1	1					1
Solano	6						1	1	1	1	1	1		
Sonoma	11					1	3		1		2	3		1
Stanislaus	10					2	1	2	1	3	1			
Sutter-Yuba	2							1				1		
Tehama	5								1	1	1	1		1
Tulare	2								1			1		
Tuolumne	3							1	1		1			
Ventura	10						3	3			1			3
Yolo	5						1	1	1	1		1		

*Some or all sera tested by county health department laboratory.

cephalitis group, Bunyamwera group, and a few not yet identified). Some errors in the 1973 summary of viral isolates (Proceedings and Papers of the Forty-Second Annual Conference of the CMCA, Inc.) should be pointed out: 3 viruses listed as California encephalitis virus (CEV) should have been listed as CEV-**group** viruses, since their exact placing in this complex of closely related viruses has not yet been determined; and the Main Drain and Bunyamwera group viruses should have all been designated as Bunyamwera group viruses. Efforts to identify additional unknown viruses from vectors and from several vertebrate hosts are being made in collaboration with the School of Public Health, U. C. Berkeley.

There were 12 cases of Colorado tick fever confirmed during 1976, 3 acquired in Colorado, and the rest in endemic areas of California. Field investigations continue, as time and funds permit, on the above mentioned diseases, and on other arboviruses of known or suspected pathogenicity. The surveillance program for 1977 is anticipated to be about as in 1976.

Table 2.–Number of mosquitoes (pools)* tested, by species and month, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1976.

Species	January	February	March	April	May	June	July	August	September	October	November	December	TOTALS
Culex													
tarsalis		247 (5)	507 (13)	974 (27)	2,566 (65)	4,468 (106)	4,211 (102)	3,451 (92)	3,295 (119)	11,085 (264)	126 (11)		30,930 (804)
erythrothorax	188 (4)	19 (2)	353 (7)	153 (5)	2,347 (50)	1,699 (38)	1,192 (25)	803 (17)	1,694 (38)	1,814 (40)			10,262 (226)
peus							191 (4)	119 (3)	8 (1)	50 (3)			368 (11)
pipiens complex					11 (1)	113 (7)	19 (2)	22 (2)	466 (39)	1,231 (58)	372 (17)		2,234 (126)
Aedes													
dorsalis				34 (2)			5 (1)			4 (1)			43 (4)
vexans				369 (8)				527 (11)	206 (5)	100 (2)			1,202 (26)
melanimon						166 (4)			2 (1)				168 (5)
Anopheles													
freeborni					27 (1)								27 (1)
Culiseta													
incidens									2 (2)	5 (1)			7 (3)
inornata	30 (1)	36 (2)	11 (1)	29 (3)						147 (8)			253 (15)
particeps	50 (1)	12 (1)		13 (1)	37 (1)				1 (1)				113 (5)
Psorophora													
confinnis							118 (3)	687 (15)	1,300 (26)				2,105 (44)
Culicoides													
variipennis		17(1)							56 (1)				73 (2)
Simulium													
vittatum		34 (1)											34 (1)
TOTALS	268 (6)	365 (12)	871 (21)	1,572 (46)	4,988 (118)	6,446 (155)	5,736 (137)	5,609 (140)	7,030 (233)	14,436 (377)	498 (28)		47,819 (1,273)

*Number of specimens tested is followed, in parenthesis, by number of pools tested: species include some other Diptera besides mosquitoes.

Table 3.—Number of mosquitoes (pools)* tested, by county and species, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1976.

County	*Culex tarsalis*	*Culex erythro-thorax*	*Culex peus*	*Culex pipiens*	*Aedes dorsalis*	*Aedes vexans*	*Aedes melanimon*	*Anopheles freeborni*	*Culiseta incidens*	*Culiseta inornata*	*Culiseta particeps*	*Psorophora confinnis*	*Culicoides vari-pennis*	*Simulium vittatum*	TOTALS
Butte	32 (1)						150 (3)								182 (4)
Colusa	646 (13)														646 (13)
Fresno	30 (1)														30 (1)
Imperial	15,883 (415)	1,542 (37)		1,767 (104)		318 (7)				11 (1)		100 (2)			19,621 (566)
Kern	21 (1)			46 (1)											67 (2)
Madera	15 (2)														15 (2)
Merced	174 (4)														174 (4)
Orange		165 (2)													165 (2)
Placer	975 (24)														975 (24)
Riverside	6,263 (142)	1,123 (26)	342 (9)	161 (10)	24 (3)	733 (16)				67 (3)		2,005 (42)			10,718 (251)
Sacramento	413 (12)														413 (12)
San Bernardino	2,538 (78)	2,383 (56)	18 (1)	10 (3)	19 (1)			27 (1)	7 (3)	143 (8)	113 (5)		17 (1)	34 (1)	5,309 (158)
San Diego	134 (12)	2,581 (54)		18 (2)											2,733 (68)
San Joaquin	56 (4)			11 (1)											67 (5)
Shasta	218 (6)														218 (6)
Solano	150 (3)		8 (1)	221 (5)			2 (1)						56 (1)		437 (11)
Stanislaus	266 (9)														266 (9)
Sutter	346 (7)														346 (7)
Yolo	885 (20)														885 (20)
Yuba	343 (8)						16 (1)								359 (9)
Yuma, Arizona	989 (23)	2,027 (41)				151 (3)									3,167 (67)
Mohave, Arizona	553 (19)	441 (10)								32 (3)					1,026 (32)
TOTALS	30,930 (804)	10,262 (226)	368 (11)	2,234 (126)	43 (4)	1,202 (26)	168 (5)	27 (1)	7 (3)	253 (15)	113 (5)	2,105 (44)	73 (2)	34 (1)	47,819 (1,273)

*Number of specimens tested is followed, in parenthesis, by number of pools tested; species include some other Diptera besides mosquitoes.

Table 4.–Viral isolates from mosquito pools during 1976, by the Viral and Rickettsial Disease Laboratory, California State Department of Health.

Mosquito pool no.	County	Place	Date collected	Species	No. of mosquitoes	Virus isolated
RV-0072A	Riverside	Mecca	May 11	*Culex tarsalis*	50	Turlock
RV-1024A	Riverside	Mecca	June 9	*Culex tarsalis*	50	Turlock
RV-1026A	Riverside	Mecca	June 9	*Culex tarsalis*	50	SLE
RV-1029A	Riverside	Mecca	June 9	*Culex tarsalis*	50	Turlock
RV-1031A	Riverside	Mecca	June 9	*Culex tarsalis*	50	Turlock
V5-IV-0096	Imperial	Brawley	June 22	*Culex tarsalis*	56	SLE
V5-IV-0097	Imperial	Westmoreland	June 22	*Culex tarsalis*	50	SLE
V5-IV-0102	Imperial	Calexico	June 22	*Culex tarsalis*	46	SLE
SB-0336	Mohave, Ariz.	Bermuda City	June 25	*Culex tarsalis*	15	Turlock
RV-1043A	Riverside	Mecca	July 12/13	*Culex tarsalis*	11	Turlock
SB-0345	Mohave, Ariz.	Bermuda City	July 27	*Culex tarsalis*	35	SLE
V2-2699	Placer	Roseville	September 3	*Culex tarsalis*	38	Turlock
RV-1049	Riverside	Thermal	August 11	*Culex tarsalis*	41	SLE
RV-1163	Riverside	Corona	August 16	*Culex tarsalis*	50	Hart Park
V5-IV-0173	Imperial	Calexico	August 25	*Culex tarsalis*	50	SLE
SB-0350	San Bernardino	Needles	August 27	*Culex tarsalis*	19	SLE
SB-0351	San Bernardino	Needles	August 27	*Culex tarsalis*	48	SLE
SB-0356	San Bernardino	Neeldes	August 27	*Culex tarsalis*	50	Turlock
IV-0337	Imperial	Seeley	October 2/3	*Culex tarsalis*	50	Turlock
V5-IV-0353	Imperial	Seeley	October 5/6	*Culex tarsalis*	50	SLE
RV-1136	Riverside	Mecca	October 20	*Culex tarsalis*	50	Turlock
SB-0417	Mohave, Ariz.	Bermuda City	October 26	*Culex tarsalis*	16	Turlock

ACKNOWLEDGMENT.–The assistance and cooperation of many staff members of the Viral and Rickettsial Disease Laboratory, the Vector Control Section, and others in the California State Department of Health, as well as of local mosquito abatement districts, the California State Department of Food and Agriculture, and other local, county and state or federal agencies in carrying out the surveillance program are gratefully acknowledged.

UNIVERSITY OF CALIFORNIA'S PROGRAM ON MOSQUITO RESEARCH

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It will come as no surprise to this audience that the University of California has figured prominently in the development of mosquito control through research, investigation, and training starting almost from the time of discovery of mosquito transmission of malaria by Sir Ronald Ross in 1898. At the turn of the century, malaria was a serious problem in the great central valley of California, and the news that malaria could be controlled by an attack against the mosquito was greeted with wild enthusiasm. But oddly enough the first mosquito control campaigns were organized in 1904 and 1905 to control hordes of saltmarsh mosquitoes in Marin County and at Burlingame, San Mateo County under the direction of H. J. Quayle. In this case, the motivation for control was not malaria but protection from saltmarsh mosquitoes which were tormenting the people of the area and depressing property values.

The University of California provided technical assistance to the campaign. A few years later, attention was focused on the malaria problem in the great central valley which was retarding the development of irrigation schemes. Again, the University participated by providing technical assistance, demonstration, and training under the leadership of such UC mosquito control pioneers as W. B. Herms, Harold Gray, and S. B. Freeborn. These early mosquito control campaigns led to the passage of the California Mosquito Abatement Act of 1915. By 1930 seventeen districts had been formed in California primarily for the control of malaria.

Eventually, as the endemic foci of malaria were wiped out and the mosquito-borne encephalitides were suppressed to a low incidence, the public viewed the protection from mosquito annoyance as sufficient justification in itself to support the enormous expansion in mosquito control that took place in California in recent years. Today, we have in California 64 local mosquito control agencies protecting 15,000,000 people at an annual expenditure of about 15 million dollars.

Nevertheless, mosquito-borne diseases are still a viable threat in California and the possibility of severe outbreaks should not be dismissed. Not infrequently, California is the site for locally transmitted cases of malaria and virus encephalitis despite the presence of an extensive network of mosquito control throughout much of the populated areas. Unquestionably, mosquito control is largely responsible for the low level of mosquito transmitted infections reported, but the degree of protection is difficult to assess and could be less than we might like to believe. Recent and current experience in other countries has shown that following the deterioration or abandonment of mosquito control that malaria and other mosquito transmitted infections have quickly resurged from minor to major disease problems.

The question is: could California escape the disease resurgence experienced elsewhere in the event of a deterioration of mosquito control? This was argued in the late sixties when the future of mosquito control appeared very bleak, indeed, because of the long period of overdependence on insecticides which resulted in mosquito resistance to insecticides and the restrictions placed on the registrations and use of new pesticides. The crisis was the inevitable outcome of non-insecticidal mosquito control technology lagging far behind the tremendous expansion of mosquito control following World War II.

Faced with the possibility of deteriorating control and disease resurgence, the state legislature in 1972 appropriated $300,000 exclusively for mosquito control research within the University of California system. This special fund has been approved annually. Additions to the original sum have increased the total to about $350,000 anticipated for fiscal year 1977-78.

Administration of the fund lies with the Office of the Vice President, University of California, Division of Agricultural Sciences. To date, research projects have been supported from the University of California's Schools of Public Health at Berkeley and Los Angeles; the University of California Agricultural Experiment Station; Department of Biology, University of California, San Diego; and the University of California Cooperative Extension Service.

The allocation of funds to the University of California scientists is based on submission of research proposals reviewed annually by two committees: the University of California Entomology Research Coordinating Committee and the University-Wide Advisory Committee on Mosquito Research. The ERCC within the Agricultural Experiment Station reviews Agricultural Experiment Station proposals and recommends priorities to the University-Wide Advisory Committee. Committees for 1977 are: **Entomology Research Coordinating Committee**; Donald L. McLean (UCD); Martin C. Birch (UCD); Evert I. Schlinger (UCB); Robert van den Bosch (UCB); Francis Gunther (UCR); Harold T. Reynolds (UCR). **University-Wide Advisory Committee on Mosquito Research**: James B. Kendrick, Jr. (Chairman); A. Ralph Barr (Public Health UCLA); Richard W. Emmons (Calif. Dept. Health, Berkeley); William C. Hazeleur (CMCA Research Committee); Richard F. Peters (Calif. Dept. Health, Sacramento); William C. Reeves (School of Public Health, UCB); William M. Rogoff (USDA-Fresno); Donald L. McLean (UCD); and Russell E. Fontaine, Secretary.

A note of special interest in the funding of mosquito research is that the special State appropriation represents about 22% of the total of all mosquito research funds expended by the University. In 1976, the research grants and funds from State, Federal, and other sources amounted to approximately $1,406,000. This, incidentally, is 9 percent of approximately $15,000,000 expended for mosquito control in California. A breakdown of the funding by source in FY 1976-77 follows:

Source of Funds	Amount (Thousands)
State Special Fund	336
State Water Fund	100
Other State	510
Federal	442
Mosquito Control Agencies (Calif.)	13
Industry and other Sources	5
TOTAL	1,406

The primary objective of the coordinating committee is to achieve a balanced program of research, responsive to the present and future needs of mosquito control in California. It reviews progress and accomplishments and advises on research priorities and funding. Obtaining a balanced program between basic and applied research is not an easy objective, as priorities may change with time and opinions may differ among committee members as to what constitutes a balance.

The Mosquito Specialist Cooperative Extension serves as a program coordinator advising on program development, strategies and priorities; providing liaison between the University and mosquito control agencies on research; and participating in planning, program evaluation, and reports on program progress.

Since total reliance on pesticides is no longer advisable, the trend is toward integrated control utilizing a variety of methods – biological, chemical, genetic, cultural, engineering, and regulatory. The research reflects the need for integrated control evidenced by the following general categories of research areas receiving support in 1976:

1. **Public Health** received approximately 22% of the special allocation ($60,000). In northern California, the research consisted of arbovirus surveillance, studies on vector competence, and preparatory work on genetic control of mosquito vectors of public health and veterinary importance.
   In southern California, surveillance is continuing on arbovirus activity, and the mechanism of virus transmission in the Imperial Valley is being studied.
2. **Chemical Control** received about 31% of the funds in supporting three projects involving basic and practical studies of mosquito resistance and methods of counteracting the problem; development of novel chemical control strategies; and field trials of new insecticides.
3. **Biological Control** received about 32% of the funds supporting laboratory and field studies of mosquito predators, pathogens and parasites. Five biological agents are being investigated including fish, nematodes, flatworms, fungi, bacteria and viruses. All but the viruses have shown some promise for operational use by mass rearing of the organisms.
4. **Genetic Control** received about 6% of the funds in support of a pilot trial to evaluate the results of field releases of a strain of *Culex tarsalis* refractory to transmission of WEE virus.
5. **Mosquito Biology** was allocated 5% of the funds to ininvestigate the biology of the treehole mosquito, *Aedes sierrensis*, in northern California, and *Culiseta inornata* in southern California.
6. **Pesticide Application** studies was allocated about 4% of the funds for evaluation of aerosol ground equipment. Further work is planned on meteorological and safety considerations.

I believe this summary conveys the comprehensive range of activities covered in this program and I know of no other comparable mosquito research program nationally or internationally with an equivalent scope and diversity of research effort in one institution. You will have the opportunity to hear more about the status and accomplishment of the research projects in the presentations of papers by the individual researchers later in this conference.

In conducting their research, the scientists are not working in isolation out of context with practical operations. There are many examples of collaborative projects in which abatement districts have provided staff, equipment and funds to facilitate the research activity. The trend is growing.

Although progress is being made, difficulties are to be expected in such a large and diverse program. In many instances researchers are dealing with highly complex and dynamic biological systems – particularly in studies in mosquito parasites, pathogens, and predators. No one questions that biological control holds great promise for the future of mosquito control, but the transition from the laboratory to practical application in the field is the most difficult stage in the research process. Moreover, the task of developing new control technology is further complicated by the need to consider environmental impact, resistance to pesticides, safety to man and animals, hazards to nontarget organisms and wildlife, and the economics of the methods. It is understandable that mosquito operational staff grow impatient when answers to pressing operational problems are not available. But solutions to most research problems usually take time and the end result is seldom clearly foreseeable.

In a recent meeting on the research program attended by UC scientists and committee members, representatives of the CMCA Research Committee and others associated directly or indirectly with the research, a number of constructive ideas were proposed:

1. **Communication** between the University research staff and the mosquito control agencies needs to be strengthened. The mosquito workers are genuinely interested in the progress of research but on a more or less informal and continuing basis without waiting for formal publication of results. On the other hand, the researchers are interested in learning more about operational problems as a basis for making their research more responsive to the needs of mosquito control agencies.
2. The research group urged control agencies to propose in specific terms problem areas in need of research and to participate with the researchers in joint planning of collaborative projects. Currently there are such projects underway or being proposed, but more are needed.
3. The notion of categorizing research projects into short and long term was considered unrealistic for most biological control studies dealing with pathogens and predators. While it appears reasonable in principle to set a

time frame on a research project, in the case of biological studies it should be regarded as liberal estimate subject to modification as new information unfolds.

4. To establish a clearer baseline for defining mosquito problems amenable to solution through research or by other means, a district by district inquiry to document the most urgent problems was proposed. Problems could then be categorized according to feasibility for solution either by a research approach, or by alternative means such as a special investigation, study or demonstration involving UC Cooperative Extension, State Vector Control, or other state and national technical resource agency.

In considering the above recommendations in the light of the mosquito problem in California today, I feel we are at the stage in the research program when all possible approaches to control are still in need of investigation. Among the areas of possible investigation in need of priority, source reduction which seems to be the least supported, holds one of the most promising areas for realizing fruitful returns on the investment. Therefore it would appear very prudent to give earnest attention and consideration to the feasibility of strengthening source reduction studies in this program.

MOSQUITO CONTROL IN CALIFORNIA AND THE VECTOR AND WASTE MANAGEMENT SECTION

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I will discuss 208 planning, but I would like first to make a few comments about mosquito control in California and the Vector and Waste Management Section's relationship to it. The chief of the section, Dick Peters, doesn't know what I am going to say, and I may be reprimanded for what I am about to say, but I feel strongly about it.

With the exception of military duty during which he worked on malaria control overseas, Dick Peters has devoted his entire professional experience to the betterment of vector control in California. After World War II when he returned to California, there were 15 MAD's in the state. During the next few years while Dick was working singlehandedly as the Department's technical consultant for mosquito control, 18 additional MAD's were formed as a result of Dick's long hours, many miles of travel, and persuasive testimony.

In 1952 Dick directed a successful campaign against the worst epidemic of encephalitis that we have had in California.

To go into depth in the solution of technical problems facing MAD's, Dick formed a highly productive research unit and made its findings immediately available. He recognized the importance of a professional, precision approach to mosquito control, and promoted a subsidy for local mosquito control programs to provide for entomological services and surveillance. Unfortunately, the California legislature later eliminated our research program and the subvention for local programs.

Dick's philosophy as chief of the Bureau or Section has allowed his staff to be free to carry forward this program of augmentation of the MAD's efforts. I know of no one in the history of California who has contributed so much to the advancement of mosquito control in the state as has Dick Peters.

Thus it is with amazement and disbelief that I hear suggestions, from the very group that has benefited so much, that the section is trying to dominate local programs, with the inference that we have some unwholesome intent.

We are well aware of the profusion of regulatory actions to which you have been subjected recently. We have had the same unfortunate experience.

The source of these regulations is not our Section. Let's examine the record.

Federal laws that have imposed constraints are the Federal Insecticide, Fungicide, and Rodenticide Act, Title 2, Sections 135 et seq.; Federal Water Pollution Control Act Amendments of 1972, PL 92-500, Title 33, Sections 1151 et seq.; Federal Environmental Pesticide Control Act of 1972, PL 92-516; River and Harbor Act, 33USC 403; Federal Environmental Quality Act, Title 42, Sections 4371 et seq.; and Occupational Safety and Health Act of 1970, Chapter XVII of Title 29.

State laws and agencies with regulations are the Porter-Cologne Water Quality Act, Chapter 1241, of the Government Code, Sections 13300-13350; State Lands Commission, Public Resources Code, Sections 6108-8558; State Reclamation Board, California Water Code; California Environmental Quality Act, Public Resources Code, Sections 21000-21174; Fish and Game Commission, Fish and Game Code, Sections 1600-1602.5, 1700-1883; Department of Parks and Recreation, California Public Resources Code, Sections 5012-5093.38; San Francisco Bay Conservation and Development Commission, Government Code, Sections 66600 et seq.; California Coastal Zone Conservation Commission, Public Resources Code, Sections 27400-30900; California Department of Food and Agriculture, California Food and Agricultural Code; California Administrative Code, (Title 3) 2451.5, 2463, 2465, 2476 (d), 2477 (b-c-d), 2479, 2480, 2481, 2487, 2489, 3089.1, 3091, 3092 (d), 3093 (a), 3094 (b-c), 3096; (Title 17) 30054-30061; and state regulations for implementation of various federal laws.

Regional or county agencies with controls are the Regional Water Quality Control Boards, Planning Commissions, and Agricultural Commissioners.

Our activity has been to intercept regulatory actions and reduce the stringency of these laws by obtaining exemptions or lesser requirements through difficult negotiation.

Perhaps our lot is similar to that of the ancient runners who carried messages between governments. If the unfortunate fellow carried bad news, he was killed.

LAND USE PLANNING: 208 PROGRAM

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"208 Planning" is the short title applied to PL 92-500, the Federal Water Pollution Control Act Amendments of 1972, Section 208. According to its sponsors, its main thrust is toward the objective of helping the states set up a management program and institutional arrangements to integrate water quality and other resource management decisions.

The federal government feels that it is necessary to develop a water quality management process at the state and local levels that assures continuous planning for and implementation of pollution control measures.

Management plans are expected to meet two principal mandates of the Federal Water Pollution Control Act: the determination of effluent limitations that would at the least maintain existing water quality (Section 303), and development of State and areawide management programs to implement abatement measures for all pollutant sources (Section 208). Congress established a policy that, by 1983, the nation's waters should be suitable to protect and propagate fish, shellfish, and wildlife, and be suitable for recreation. Congress has required the states to designate beneficial uses for all waters and to establish water quality standards to meet the goals set by the Act.

A fund of ten million dollars has been appropriated for the work in California.

The law has identified two general classes of sources of pollution: point sources and non-point sources. Point sources are specific discharges of pollutants which enter surface waters from such identifiable vehicles as pipes, conduits, fissures, or channels. Non-point sources are those which have no specific point of discharge, such as agricultural return flows or stormwater runoff from a broad area.

Two types of solutions are employed to combat the two types of sources: structural and non-structural. Structural solutions require the building of structures to treat or control the discharges, usually require large capital expenditures, and are normally associated with point source discharges. Non-structural solutions employ management strategies to try to prevent discharges, and are usually associated with non-point discharges.

The Environmental Protection Agency, the administrator for the Federal Water Pollution Control Act, had previously concentrated upon development of solutions to point source discharges, and a sum of 18 billion dollars was appropriated for a grant program for communities to upgrade treatment levels. Since there was a December 1974 deadline for issuance of permits for structures, these permits were issued before comprehensive water quality management planning was begun.

To guarantee that water quality standards were met, the law established two water quality planning mechanisms, basin planning and 208 areawide water quality management planning. The Governor of each state designates a planning agency to implement planning. The 208 planning process moved slowly until the National Resources Defense Council sued EPA, and the court in a decision in 1975 ruled that 208 planning was an essential element of the Pollution Act and that it applied not only to designated areas, but to all areas within a state. EPA made 208 planning its first priority, so now there is feverish activity by all levels of government to accomplish comprehensive management planning that will prescribe abatement actions and control programs.

The 208 planning program must be completed and committed to implementation within two years. EPA expects that it will lead to a continuing planning process for local and areawide environmental decision making. Many agencies will be involved, including, among others, local governments, special districts, State Water Resources Control Board, the Regional Water Quality Control Boards, irrigation districts, soil conservation districts, and public works departments. There are environmental, social, and economic considerations, and technical tradeoffs to be made.

In order for mosquito and vector abatement agencies to have a voice in the directions in which planning and implementation programs are to take, it is most important that you become involved in the political processes by which these vital decisions will be made. We can not expect that urban planners or representatives of special interests other than vector control will promote or even be aware of the requirements of vector prevention or control. Probable effects of the planning program are restoration of "damaged" ecological systems, implementation of controls on solid waste and sludge disposal systems, restoration of rivers, lakes, and bays, protection and improvement of ground water supplies, and methods for disposal of hazardous and toxic wastes.

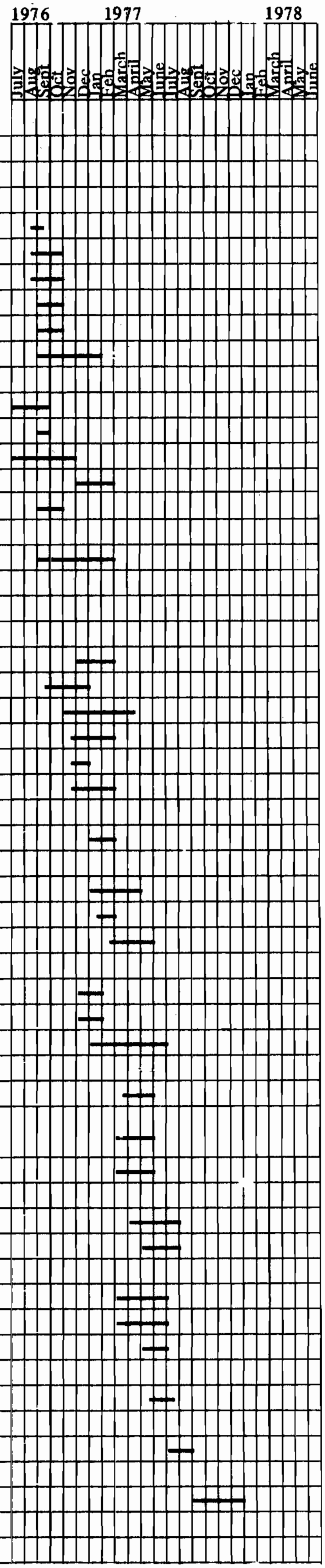
The Act specifically mentions "land use requirement". The use of land is involved in many of the decisions that will be made for the protection of water quality. The 208 planning program is seen by many planners and government officials as a federally-mandated move into comprehensive regional planning.

Seven areas of the state had been identified by the Governor as areas with substantial water problems and have been designated 208 planning areas. They are Lake Tahoe, Sacramento, San Francisco, Monterey, Ventura, South Coast, and San Diego, but as was mentioned earlier, the entire state is now involved in 208 planning.

In the San Francisco Bay Area, Fred Roberts is a member of the 208 planning Advisory Group, and Earl Mortenson and Reuben Junkert are members of the Surface Runoff and Minor Point Sources Management Plan Committee. Fred had obtained the names of contact persons for the various counties within the territory of the Association of Bay Area Governments, the lead agency for the San Francisco Bay Area, and distributed them among the other MAD's in the Bay Area. He has also provided a listing of the functions of his committee and a pert chart for scheduling of the activities (Figure 1). In the Lake Tahoe arêa, Martin Christainsen has been much involved.

Figure 1.—Chart of study and planning functions with time schedule prepared by the Advisory Group for the 208 Planning Study of the Association of Bay Area Governments, 1976-77.

Revised 1/77



1/67 Organize Study
- A. Develop work program - Budget & Control
- B. Advisory Group - Organization.

1/68 Describe Surface Runoff Systems
- A. Select demonstration water sheds.
- B. Describe & delineate urban and rural storm water systems.
- C. Check and/or delineate rural collection systems.
- D. Delineate major water shed boundaries.
- E. Determine land use of major water sheds.
- F. Compile washoff coefficients and hydraulic data for streams and channels.

3/69 Describe Data Needs
- A. Determine type and priority of necessary data.
- B. Select water quality monitoring station.
- C. Develop collection program for model calibration.
- D. Design long-term water quality monitoring program.
- E. Select lab for analysis of water quality samples.

4/217 Implement Water Quality Collection Program
- A. Implement actual collection program.

5/70 Review Land Use Data Base
- A. Verify 1973 land use data base.

6/71 Determine Cause and Extent of Existing Problem
- A. Interview persons with knowledge of water quality degradation and prepare report.
- B. Review and analyze existing quantity/quality data.
- C. Interpret computer model results.
- D. Apply model results to major sheds.
- E. Prioritize problem sheds.
- F. Integrate data and draft report.
- G. Propose tentative measures for pollutant yields.
- H. Review draft report of existing problem.

7/72 Determine Cause and Extent of Future Problem
- A. Forecast future waste loads.
- B. Coordinate estimates of surface runoff's contribution to water quality degradation.
- C. Report on relationship between water quality projections and existing general plans.

8/73 Formulate Control Measures
- A. Revise list of proposed control measures.
- B. Formulate workable combinations (strategies) of control measures.
- C. Analyze pollution abatement effectiveness of alternate control strategies for cities and unincorporated portions of the study area.
- D. Develop a schedule of implementation for near term non-structural controls.
- E. Develop a schedule of implementation for long term structural solutions (reconnaissance level) including anticipated costs.
- F. Estimate probable dollar cost of delaying action on surface runoff problems.

9/74 Develop Institution and Financial Analysis for Near Term Control Measures
- A. Develop alternate institutional and financial mechanisms to implement near term controls.
- B. Allocate alternate planning and management responsibilities among agencies.

10/75 Assess/Evaluate Control Measures
- A. Evaluate environmental and financial impacts.
- B. Evaluate social and land use impacts.
- C. Integrate impact data.

11/ Describe Continuing Planning Process
- A. Prepare report on continuing planning process.

12/76 Prepare Report
- A. Integrate all data and write report.

13/77 Assist In Regional Compilation
- A. Assist in regional integration of county runoff plans.

14/78 Participate In Advisory Committee Meetings
- A. Participate in advisory meetings.

I urge you to make contact with the lead agency in your area and inform that agency of your expertise and willingness to participate. Ask if you can be represented on planning agency policy advisory committees or technical committees. For the rest of the state outside of the designated areas, your Regional Water Quality Control Board will be your agency. The State Water Resources Control Board will be looking at interfaces among agencies.

EPA officials have said that every effort is being made to give local and state governments the opportunity to plan and implement programs, but with the inference that if they fail, the federal government will impose the programs that are necessary, and that these will involve growth and land-use considerations.

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TRUSTEE PARTICIPATION IN MOSQUITO SOURCE REDUCTION

Moderator: W. Donald Murray

Delta Vector Control District

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We in the United States live in a democracy – one of very few remaining in the world today. A basic requirement of a democracy is that the people be treated humanely, with respect for their rights, their interests, and their feelings. The desire for power has been one of the greatest evils in the history of mankind. This has been especially evident in recent years as we have seen one democracy after another succumb to power hungry leaders who then set up dictatorships.

Society at all levels of government has become ever more complex, and laws which have been established to protect the rights of people have sometimes proven to be unsupported and of little value. Attorneys have been notorious for twisting the intent of laws around to favor their clients, regardless of the effect on society as a whole. As administrative leaders have become frustrated in their desire to accomplish goals which at one time may have been meritorious, and as they have acquired power, they have been inclined to avoid or turn against truly democratic procedures and to move towards bureaucracies and dictatorships.

How can we avoid this tendency in our mosquito control program? A person has no right to produce a mosquito nuisance which harms others, and our Health and Safety Code authorizes us to stop him from doing so. Just how we might accomplish this in a democratic system is the subject of the present panel.

"Mosquito Source Reduction" is a motherhood term – everyone agrees it is a good thing. Procedures for achieving it, however, are about as varied as there are districts. Of course there is more than one way to carry out mosuqito source reduction – in fact there certainly are a number of effective ways. On this panel we wish to note several successful ways, but especially we want to emphasize ways in which the Board of Trustees can contribute.

When a district manager tells someone he is using legal procedures to abate mosquitoes, he does not say this to contrast with illegal procedures – rather, he means he is using provisions of the California Health and Safety or other Codes which involve some degree of pressure against mosquito producers, with possible punitive action. One may review volumes of the CMCA Proceedings from almost the first issue to the most recent, and he will note that this subject has been discussed many times, but it is of even greater importance today because of mosquito resistance to insecticides and the increasing governmental controls over insecticide use.

TRUSTEE PARTICIPATION IN NUISANCE ABATEMENT HEARINGS IN THE DELTA VECTOR CONTROL DISTRICT

W. Donald Murray

Delta Vector Control District

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In 1971 all previously useful insecticides for control of the pasture mosquito, *Aedes nigromaculis*, in the Delta Vector Control District (Delta VCD) became ineffective because of resistance. Mosquito populations increased to high levels never before observed in several of our communities. The public was incensed at our ineffectiveness, and our staff was demoralized. We had had an active mosquito source reduction program for 20 years, we had made numerous personal contacts with mosquito producers and had sent them a great many letters and reports, yet somehow we had not been able to achieve an adequate change in the attitudes and practices of most of our high priority producers.

In 1972 we determined to make even more personal contacts with high priority mosquito producers. I invited several to come in and talk with the Board of Trustees, but not one was interested in doing so. The Board thereupon adopted the policy of issuing a formal "Notice to Abate a Nuisance", which provided for Hearings as included in the Health and Safety Code. This procedure proved successful, and 13 individuals were cited during 1972, 18 in 1973, and through 1976 Hearings have been held for 38 persons.

The Delta VCD Staff and Trustees will demonstrate just how these Hearings were carried out. We will emphasize the low key, dedicated service which the Trustees have played in backing up the District policies and which has led to a fantastic degree of corrections of mosquito problems while following what we believe to be the ultimate in democratic procedures. We will portray the Hearings of two actual cases essentially as they occurred in 1972. Four Trustees will play their own parts: Ivan Crookshanks as Chairman of the Board, and Bruce Myers, Ferris LaFond and Ronald Davis as Members. George Whitten will play his own part as Agricultural Engineer, Robert Turner his own part as Superintendent of Operations, and I my own part as Manager. John Combs will play the part of Paul Jones (fictitious name, but all other items true), a farmer one mile northwest of Exeter. Edward Lewis, Foreman of the Kings Mosquito Abatement District, will play the part of Joe Souza (fictitious name), a farmer west of Goshen. I point out that we have never hesitated to have two or more growers appear at the same time – it never has been a problem and it has enabled our program to move much more rapidly than it would have had we taken only one at a time. (Note: an extemporaneous but reasonably factual skit was presented. Kodachrome slides were used to assist in explaining the problem the grower creates for the District. At the conclusion of the discussion, the Board passes two motions – 1) the District Staff will work more closely with the grower, and 2) the District Staff may spray and charge if unacceptable mosquito production continues.)

Summary.–A mosquito control district with a lay board potentially provides an example of democratic procedure at its best. Most mosquito production problems are resolved by the Staff and never come before the Board other than through informational reports. The Board does not get involved in routine operational matters, but sets policies and supports the Staff which carries out these policies, and, finally, serves as a public hearing body, separate from the administration.

We have been very careful to avoid too legalistic an approach. We have never used an attorney at the Hearing of any of our 38 cases, nor have we needed one. Our appeal is one of social pressure, and the Board and the Staff have handled all issues in a friendly, low key manner. We are not dealing with dangerous criminals – rather, we are dealing with persons who lack understanding and who sometimes fail to show proper respect and concern for the rights of others. Strong legal actions in the preliminary phases can result in unnecessary resentment. We consider our program to be one of pressured education.

What have been the results?

1. We sold our airplane.
2. We eliminated the position of pilot.
3. We reduced sprayed acreage from an average of 70,000 acres per year for the 10-year average (1960-70) to 4,000 acres per year for 1975 and 1976.
4. Insecticide costs have been greatly reduced.
5. Resistance is no longer a major problem.
6. We have reduced the staff and hope to continue.
7. Present personnel are more valuable and receive more pay.
8. *Aedes melanimon* has virtually disappeared from the District.
9. *Culex tarsalis* has been eliminated from most sources in the District.
10. *Aedes nigromaculis*, based on body counts, complaints, larval inspections and service requests in 1976 was for the most part under the best control in our recorded history.

"FUNCTION OF THE BOARD"

Fred DeBenedetti
San Joaquin Mosquito Abatement District
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I recommend that a trustee should not engage in district operational matters during times when he is not in the board room. I have observed very ambitious trustees who think they should function all month. They go into the field, meddle in operational matters, and claim they want to know what is going on as to why the district does this or does not do that. I believe this is not a good practice. The function of the trustee is in the Board room and there only. Trustees should recognize that they hire the Manager. They decide on the person they want, and then they must let him run the district.

There are certain functions the Board should expect of the Manager. In the board room the Manager should provide a full, complete report of what the district has done during the past month, and what is intended for the coming month. The Board needs to know exactly how much money was spent during the past month and how much is contemplated for the coming month. The Board should never be embarrassed by finding that the manager has spent more money than was available in the County Treasury. Auditing of the books is very important. The Board should obtain the best auditors available, and should request a complete audit. The Manager should provide to the Board each month a report of what the district has accomplished during the past month and what it plans to do in the coming month or perhaps more, even 6 months or more.

The San Joaquin Mosquito Abatement District (MAD) seldom has had a Board Meeting when legal counsel was not present. Today more than ever it is essential that the Board has legal counsel to which it can turn at all times.

Many Boards have both new and old members. I have heard comments that a person has been on the Board long enough and should resign and let someone else take his place. I think a Board needs a combination of both new and old members. There is a lot of knowledge and wisdom in members who have served for 15 or 20 or 30 years, yet Boards may need the vitality of new members.

I believe that Board attendance is a must. When a Board Member is absent, he may do several things 1) he does not contribute to the program, 2) he keeps somebody else from being there, 3) he may ask at a meeting "Why didn't you do this at the last Board Meeting?" If a trustee finds that he is too busy or too occupied to attend Board Meetings, he should give the appointing agency which entrusted him with the position the opportunity to put someone else in his place. A Trustee should take his responsibility as a Board Member very seriously. The San Joaquin MAD has a budget which approaches one million dollars, which is a lot of tax dollars, and I feel I owe it to my neighbors, to the rest of the citizens of this district, and to the Board of Supervisors which appointed me, to do the very best job I possibly can with those tax dollars.

I have had the occasion of serving on Boards on which the Chairman has served in that chair for so long he thinks he owns it. I have been a strong advocate that after one has served as chairman for 2 years, he should move over and let another take that position. This gives everyone an opportunity to participate in this position, and at the same time no one becomes a fixture. If a man has served as Chairman for 10 or 20 years, the chair becomes his little kingdom, and he may reach a state in which he demands that everyone does just what he wants them to do. The Board Chairman should change every 2 years, in my opinion.

In the past several years there have been many changes in this world. Problems are getting bigger and more difficult. We must face politics, labor, environmental impact. I sometimes wonder how we are going to function at all. Our Manager used to be out in the field routinely, but today he spends more time on political and administrative matters: book work, paper work, matters of communication, labor, OSHA, EPA, etc. It is very important that the Board supports the Manager, and the Manager has a right to anticipate this support. If the Board cannot support the Manager, then it should replace him. The Board sets the policies and the rules, and the Manager must be certain that the Board will support him as he carries them out.

The Board should provide the Manager with the tools he needs. It should not expect him to tear down a mountain with a spoon. There is no use providing a Jeep if gasoline money is not also provided. However, money alone will not solve problems – policies to support funding must also be provided. One of the most important words in the dictionary is "reason". I believe that if something is reasonable, we will do a good job with it. We must be reasonable with money, with labor, with management – and sometimes it is important that we be reasonable with ourselves.

Question: Whom do you have for your legal counsel?

DeBenedetti: We have an attorney on a retainer. We insist that he attend our Board Meetings. Time after time he will sit through the meeting and never say a word, but many times we turn to him and ask "What are the legal ramifications of this decision?" Occasionally he will stop us and state: "There is a risk involved, and perhaps we should consider another approach." Also, we are including a consultant to whom we can turn in developing our labor relations. We cannot know all the answers, so perhaps we should bring in a professional person for these needs. Our employees belong to the County Employees Association. We found that our Manager was taking a lot of his time on labor relations matters, especially at budget time, so we appointed and retained a labor coordinator. He serves the District at a cost of $1,500 per year, which is money well spent. He will give us advice, let us know how things stand, and submit recommendations. Of course we also listen to the Manager. Then we decide on raises, fringe benefits, etc. Then he does the negotiating. We did not really want to go this way, and we do not really like it, but it is part of our changing times and we have had to change with them.

FUNCTIONS OF MANAGEMENT

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Management is responsible for the entire program in a mosquito abatement or vector control district. He or she is where the buck stops. If the program is good, he gets the credit; and if a program sours, he takes the blame.

Management has to provide the leadership, guidance and direction to the staff and the Board of Trustees to accomplish the goals and objectives of the District. The management has to discuss with the Board and both have to agree on the goals and how to accomplish those goals.

The following five functions are usually considered of primary importance in management: Planning, Organizing, Staffing, Directing, and Controlling. I define these functions as follows:

Planning: The setting of long term goals and short term objectives for the District. This means, "What does the District want to accomplish and how to do it." It must be written down whether it is the main goal of the District, or 25 year plan, or the objectives for a season, or the objectives for a special survey.

Inherent in Planning is the attitude of the Board of Trustees! Will they agree on the goals and will they adopt policies to attain these goals? Obviously these should be discussed at Board meetings. Ideally, the Manager should be spending a good deal of his time planning for the District operations and programs. Selected staff members should be involved in this planning because it will affect them directly. They can help work out the obstacles in the plan and ultimately they will make the plan work.

Organizing: The systematic arrangement of our resources--staff, finances, equipment and pesticides to accomplish our goals and objectives. You have to know the capability, adaptability and proper use of all your resources. I would say this requires a written plan for the year on personnel, which would include an organization chart showing positions, relationship between positions, number of positions, salaries, etc. A financial resource plan would be a budget and accounting method to show current account balances. The plan for vehicles would include use, assignment, estimated life for District. These plans are made available to the Trustees and discussed at Board meetings.

Staffing: The Board hires the Manager and gives him the responsibility to hire the rest of the staff. The staff works for the manager--not the Board. This gives the manager the right to hire and fire. The manager hires staff with the education, experience and aptitude for the intended job. You have to hire enough staff but not too many. You have to train them in all aspects of the job.

1. Technical
2. Public Contact
3. Safety
4. Certification

You can help develop staff through tuition reimbursement programs so that they can take courses pertinent to their growth at the District. I think one of the downfall's of Districts is not developing management in our own staffs, especially when our Managers are usually promoted from within the organization. We should provide time and money to develop managers and, after they are appointed, for their continuous education.

Directing: Providing day to day supervision to accomplish goals. Checking to make sure use of resources is accomplishing goals. Establishing procedures to get job done. Collecting data for review of programs and evaluation of program. I believe most managers spend too much of their time in this capacity. That is they use a great deal of their time directing day to day operations which should be left to supervisory personnel.

Controlling: Regulating the use of resources (personnel, finances, equipment, pesticides). Seeing that District doesn't exceed resources, such as, budget, overtime, proper use of pesticides, etc.

Examples of These Functions

1. Hires the most knowledgeable and experienced people to be the vector ecologists, mechanics, foremen, secretaries, certified technicians, engineers, and pilots. However, make sure that you have at least one minority group member of each minority to fulfill your affirmative action plan.
2. Prepares job descriptions, salary & benefit plan and organization plan that tell people what to do, where to do it, why they are to do it, when they are to do it. If you don't, the Equal Employment Opportunity Commission & Occupational Health and Safety Administration, Fair Labor Standards Board and others will be glad to help you.
3. Sets up a budget plan to pay the bills, pay the employees, and a system to account for every penny so that the

County Treasurer, Auditor, Tax Collector, your own auditor are satisfied, and the Grand Jury, a city council, the newspapers, the taxpayers association and lastly the senior citizens on fixed incomes will be satisfied and won't want a full scale investigation of your District.

4. He sets up a pay plan for the employees. Enough so that they are happy, don't want a union, but not so much that the Board of Trustees thinks it's a giveaway of public funds. Or else the AFSCME, Teamsters, United Farm Workers, SEIU, or Employees Association will be happy to provide a salary and benefit plan.

5. Provides leadership to the Board of Trustees by recommending policies, such as, a vector abatement policy that will eliminate the production of vectors of which the biggest producer is one of his trustees or Chairman or the Board of Supervisors.

6. Procures all the supplies, such as, vehicles, gas and oil, paper, typewriters, etc. from people who are not either a trustee, employee, friend of an employee, or have a financial interest with a trustee or employee so that a conflict of interest doesn't occur.

7. Then you have to purchase a pesticide that is:
   a. Still effective
   b. Approved by the State Health Department
   c. Approved by EPA
   d. Has on the label the bug you want to kill
   e. Is acceptable to the Sierra Club and/or the Department of Fish and Game
   f. And can be used on property controlled by either the Coastal Commission, State Lands Commission, or the Corps of Engineers.

8. Act as Secretary to the Board of Trustees: He has to figure out if he thought you said what he understood you to say and if not then figure out what you thought you meant to say.

These facetious remarks point out the wide variety of functions of management and how decisions are affected by outside influences. Management, at sometime, has to act in the following areas: technical, supervision, accountant, mechanic, personnel, labor negotiator, secretary, purchasing agent, public relations expert, and psychiatrist.

Relationship to Board of Trustees: The Manager has to provide a comprehensive, efficient and effective mosquito abatement program that is technically sound and that the Board of Trustees will support. Two elements that are most important in the Board-Manager relationship are: Communications with the Board and the results of that communication, the development of written policies and guidelines.

1. Communications: There has to be an open line of communication between the Board of Trustees and Manager. A Board member should call the Manager whenever a question arises. Manager should be funneling information on District activities to Board members. This can be through written and/or verbal reports at Board meetings, or special reports, copies of important letters, pamphlets. This information enables the trustee to become knowledgeable on all aspects of the District. I believe a trustee is one of the greatest assets of the District, especially in public education. You are community leaders and are respected, you should tell people about the District, what is going on, and how it benefits the community and individual.

2. Policies: The Manager has to provide, as a result of this communication, written policies and guidelines for:

   a. Abatement
   b. Personnel & Pay
   c. Purchasing
   d. Travel
   e. Budgeting
   f. Board By-laws
   g. Control of other vectors

   These written policies provide both the Board and Management with procedures on these items and make sure each has a procedure to follow.

Management and the Board have responsibilities that working together accomplish the goal of the District. With mutual respect and pride in accomplishment, this relationship will benefit the community, the Board, and the staff.

"POLICIES"

Marguerite Leipzig
San Mateo County Mosquito Abatement District
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Policies should be made to use, not to be broken. During 1976 the San Mateo County Mosquito Abatement District has been putting together a manual of bylaws and policies. One of the trustees researched all the board minutes, going back 50 years to 1916, pulling out items which could be considered policies. We then accepted, modified, added to or deleted the various items and put them together into a book or manual. The ad hoc committee which worked on this matter during the past year was appointed by the president to do just this task. Finally, the board analyzed all policies and organized them for acceptance as current policies. They have been grouped into specified areas:

Employer-employee relations policy.—Within our district, we have a certain amount of discretion. A committee is established to coordinate these relations. This committee meets with the employees, then reports to the full board with its recommendations. This may not be as efficient a procedure as with an outside labor negotiator, but it is less expensive.

Budget policy.—A trustee committee is appointed by the president to assist in budget matters.

Fiscal policies.—This relates to signing of warrants, funding, etc.

Travel and Conference policy.—This subject has caused the greatest concern in our district. Some of the trustees have believed that no one should go any place at any time. Others have believed that everyone should be entitled to attend any meetings or conferences he wished. Some have believed these conferences are very enlightening and beneficial and lead to a better board. Some have believed that only the manager should go, or only one trustee, etc.

Legal approach to abatement.—This covers the procedures the district may take in relation to the district powers.

Insurance policy.—

Approval policy.—This approves the program which the manager has developed for the coming year. This provides checks and balances.

Use of vehicles and water craft.—This matter has stimulated much discussion. At one time in the past, vehicles were driven home from work. Some trustees believed the vehicles should be left on the district grounds and be used only for actual district business. Thus they would not be readily available for emergency calls.

Purchasing and disposal of property.—This guides the board and the manager on what sometimes prove to be rather technical matters.

The district has developed a rather complete set of procedures relative to how the board should act in any given situation. We believe we have covered almost every contingency, but we know there will be changes in the future. I believe it would be worth the effort for each mosquito abatement district to develop policies in this manner.

Question: Does your district have an attorney?

Leipzig: The District Attorney is our counsel. He is not at each meeting. Our policies were presented to the District Attorney's Office, and he found them in good order. We did check each item to assure we were operating in accordance with the Health and Safety Code. This Code, however, is not necessarily specific – such as relative to election of officers, conducting meetings, etc., so we added many items to our bylaws not covered by the Code.

TRUSTEE LIABILITY IN DECISION MAKING PROCESSES OF THE MOSQUITO ABATEMENT DISTRICT

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The layman, particularly a trustee, may have some difficulty in appreciating the complex legal problems confronting counsel for a governmental district, such as mosquito abatement districts. Counsel for such districts must be prepared to change their positions from day to day, because rules are continually changing. This is especially true with the matter of district responsibility and liability in connection therewith.

A trustee of a mosquito abatement district may well ask "What can happen to me personally in connection with liability for any acts as trustee?" Twenty years ago a person seeking to recover money would, in a litigation, name and seek out the failures of an individual because the district immunity made that public agency practically untouchable. This is no longer true. The courts have whittled away at that immunity shield until the public agency is left with but little of that immunity protection. The pedulum, then, now is at the other end of the swing and the claimant is looking to the liability of the district rather than the liability of the trustee. A person seeking to litigate has a greater opportunity to recover, not only some funds, but more funds, by suing the district, since the funds and resources of the district are often far greater. Thus, today, said complainant is virtually aiming at the district and not the trustee.

The conduct of a trustee outside the scope of his authority makes that trustee personally liable. What then is outside the scope of that trustee's authority? It would be those acts that do not implement the district's function and authority. Thus, if a trustee votes to expend money for a particular project or item which counsel for the district has advised is an illegal expenditure, and, despite the legal advice, the trustee votes foı the expenditure, then that vote or act is outside the scope of the trustee's authority in the event a court subsequently determines the expenditure to be wrongful. Personal liability would likely attach. Also, suppose a trustee knowingly and wilfully sprays a dog with insecticide. Any of such acts are unlikely and remote. These are extreme examples, but the illustrations for personal liability present such a limited field of examination that I realistically believe that it is a minimal risk possibility. Each trustee wants to do the very best job possible and the districts are anxious to have nothing but the best and this, in itself, is one of the greatest safeguards because the district is liable if the trustee acts within the scope of his authority. Insurance companies still cling to the 20 year old concept of personal liability of trustees and sell some policies based thereon, but at the present cost of insurance this seems an unwise expenditure of funds.

Can we now turn to the liability of a district? Too much of our concern is governed by stimulated fear and unrestricted imagination of what liabilities can result from publicized unrelated happenings. The realistic evaluation of the district's losses over the past 20 years is a better measurement of liability losses than the emotional appeal to what may happen in the future. The subtle propaganda of the insurance companies is to impress trustees and managers that those great future liabilities are a real possibility. We cannot deny the possibility, but its true remoteness and frequency must be directly related to the known historical data for that district. If reserves are built on known historical information and not on projected future occurrences correlated with unrelated insurance company formulated average coefficients, then those reserves would be substantially less than the insurance carriers progressive business-oriented reserves.

In years gone by, the cost of insurance was relatively low and districts and individuals could afford to buy insurance to cover liabilities. Today the costs have become excessive. What can be done about this? There should be an objective evaluation of losses. Perhaps an attorney may be required to examine the district's program. Many people may believe that districts are no longer immune from anything, but this is not true. For instance, districts are immune from the act of an independent contractor. The Government Code states that a public entity is not liable for punitive damages resulting from the act of an employee, that is, a district is not liable for damages as punishment. A public entity is not liable for failing to adopt a particular enactment or procedure, or for failing to enforce any such enactment or procedure. If the statute requires a district to do a specific act relating to which the employee or officer has discretion, then failing to enforce or to adopt that requirement, does not make the district liable. A public entity is not liable for an injury caused by the issuance, denial, suspension or revocation, or by the failure or refusal to issue,deny, suspend or revoke any permit, license, certificate, approval, order, or similar authority, where the public entity or an employee of the public entity is authorized by enactment to determine whether or not such authority should be issued, denied, suspended or revoked. The district is not liable for making an inspection required by law, but what is more important, the district is not liable for making an inadequate inspection. This is of particular interest to the mosquito abatement districts, because an employee or an officer may make an examination and determine that no mosquitoes or larvae are present in a particular place, but, in actual fact, there are mosquitoes and larvae present. This rule would make the district immune from liability for that failure to determine that mosquitoes or larvae were present. A misrepresentation by an employee creates no liability for that district, not even if the employee is negligent or willful. If the employees of a district perform their duties pursuant to an unconstitutional statue, their wrongful acts do not establish liability against the district.

Will you examine the liability data for your district over the past 20 years? If you do, you may find an extremely interesting paradox because the comparison of that information with the total insurance premiums for the period may create a "conversation piece" for any board meeting. The immediate foreseeable objection is the task of compiling the information. Insurance companies have that information but are unwilling to disclose the necessary analytical breakdown required for a true comparison. I recommend that each district itself go to its own records and examine each claim filed with the board of trustees and rejected by that board, then follow that claim to its ultimate resolution by the insurance company. Analysis of some claims may be easy, some will be difficult and time consuming, but the final accumulated information may be even startling. One word of caution, however, to have the investigation instituted and prosecuted by clerical or inexperienced individuals can only produce distorted and unsatisfactory results.

In the good old days, the reliable insurance carrier engaged the services of the most qualified attorneys and investigators. However, within the past 10 years the cost of doing business by the insurance companies has spiralled to such a degree that those carriers have not only had to increase premiums, but cut corners in whatever manner possible. One of the most obvious directions of such saving techniques is to bargain for the services of attorneys and investigators. Inevitably quality suffers. I have personal experience with the fact that inexperienced attorneys dealing with complex governmental administrative processes have erred seriously. For the carrier to avoid those pitfalls it would be most economical for the insurance carrier to settle the claim and pass the cost of that settlement on to the customer. The unfortunate position of the district is that it has nothing to say about whether a claim or lawsuit is settled or is not settled. In fact, I am familiar with one case where the district did not want to settle under any circumstances, but the carrier ignored the wishes of the district and settled the case simply because it was expedient for the district since the acts complained of in connection with the employees of the district were litigated in a criminal action and the employees were therein acquitted. It is a sad commentary but the district must, in some way, participate in settlements if the cost spiral is to be slowed. Obviously, the processing of claims and the ordinary administration connected therewith is included within a premium. We have guessed that 40-50% of the premium is devoted to that administrative cost. We cannot confirm that estimate data. There is no question that inflation has touched the insurance carrier, as it has all other businesses, but, in addition thereto, there has been a greater tightening of governmental control which also increases cost. We must not overlook the fact that the governmental control requires the piling of reserves for unlitigated claims. This, in itself, is a drain upon the carrier's ability to function effectively and cheaply. A glimpse of the future for district liability insurance is afforded us by the malpractice insurance difficulties now being experienced by hospital districts. We are not there yet, but how soon the precipice?

We recognize that insurance companies are conducting a legitimate business. Be that as it may, we still must reexamine the matter of paying liability insurance at today's rapidly escalating prices. It is obvious that excessive claims, mounting administrative costs and perhaps stock market losses have lead to premiums the district can no longer consider economical.

In light of the foregoing, let me present an alternative proposal, and, in connection with that, preface my remarks by pointing out an illustration. The Chowchilla District Hospital was about ready to close its doors because of the tremendous increase in the malpractice insurance premiums. We went to the State Legislature to try to get some help. Peculiarly, the insurance industry has stated that it really did not want to provide this insurance, yet it did not appear they wanted the hospital to become self-insured. The industry resisted by refusing to be of any real assistance. The Legislature asked the insurance industry for information such as the amount of the premium to cover administration, or about actual losses, etc., but we are advised that meaningful answers were not provided. Peculiarly, the insurance companies are still selling insurance, even though they say it is so hard to do and they still resist self-insurance. We did get our proposed legislation finally approved. Obviously the hospital districts are being forcibly propelled toward self-insurance and, by reason of such compulsion, self-insurance as an alternative has acquired a stigma which it does not deserve. Therefore, will you look at self-insurance with a free, open mind in connection with this presentation.

First, we should exclude fire insurance and industrial accident insurance since those subjects should be considered separately and under somewhat different proposals.

The trustee of a small district such as the Madera Mosquito Abatement District, would have strong divergent views when he considers that one cent ($.01) on the tax rate for one (1) year will raise $19,000, and, in the event normal activity continues, no problem would result since that district has not suffered any substantial loss in the last 20 years. However, a $50,000 loss may occur tomorrow. Under normal circumstances a district could self-insure easily, but the problem is to meet an unfortunate situation which could happen to anyone. One might say, "a large district can take care of itself," but this is not necessarily true, because mathematically the frequency of occurrences in the large district should be the same as in the small district.

The mosquito abatement districts in California have never been surveyed to determine what their industry-wide costs and losses have been. I will make the same suggestion I made to the Chowchilla Hospital District. It will turn some people on and other people off, but it has possibilities and at least should be examined. Two or more districts could agree on a joint powers agreement. Thus District A and District B would, by agreement, form a third agency which may be called X. X is a public agency just as much as A and B are public agencies, it is a joint powers agency. What authority does it have? It has the authority which A and B grant it, limited to a single matter if desired, or extended to broad powers which would permit it to perform many things for A and B. X would operate only as A and B agree.

How would this apply to the self-insurance field? Your first impression may well be that there is a pot into which everyone puts his money and then into which some reach and grab out what they need. That is not my proposal. Perhaps we should examine what has been accomplished to date in this area. The State of California is self-insured, the P.G.&E. is self-insured, the hospitals have commenced a plan of self-insurance led by the Chowchilla Hospital District, the cities in the Richmond area are putting together a plan for self-insurance. School districts have adopted joint powers for certain self-insurance projects.

All of these joint powers programs are now provided for by statute and the implementation by legislation is simply an attempt to carry governmental agencies over the critical period from insurance to self-insurance. There is no reason to believe that the mosquito abatement districts would not easily fit into the joint powers self-insurance pattern.

This is a change of concept and will require time to eliminate problems and confusion. Insurance companies themselves have introduced confusion into the present understanding of the problem. This happened when some small counties decided to look into the matter and, against our advice, went to insurance brokers to get a paper prepared on the subject. Unfortunately, the insurance people did not understand the problems or the limitations of joint powers or the procedures to be used. The insurance broker prepared a proposition designated "reverse funding", which proposal only created confusion. "Reverse funding" is the name they applied to this joint powers concept which means nothing. It only indicates that counties involved would not pay the losses until the joint powers agency borrowed the money for such payment. The joint powers was not authorized to borrow the money for them. The problem was that they had not properly provided the method by which the joint powers agency would get its money to pay back the borrowed money. In my estimation it will take 2 to 3 years before these counties are straightened out and get on the right track. Unquestionably, to develop joint powers, an attorney who is an expert and skillful in the field should be engaged.

May we here note some of the advantages of joint powers self-insurance. 1) The actual administrative costs would be known by each district; 2) Each district would have a voice in determining the person or persons making investigations and their qualifications; 3) The most important factor is that each district would participate in the selection of the attorney or attorneys; 4) The district would control the litigation in every stage, including that stage relating to settlement of the claim; 5) Each district would most certainly energetically participate in risk management since it would be directly beneficial to the district.

The primary problem to be resolved is the establishing of the fund by the joint powers agency. Each of us recognize that each district will contribute its proportionate share. The contribution creates no difficulty. The question is, how are the claims paid from that fund? If you will think of a total fund departmentalized into 10 segments, each segment representing a district. Thus District No. 1 would contribute $1,000 to a compartment; District No. 2, $2,000 to a compartment; District No. 3, $3,000 to a compartment, etc., remembering that the total 10 departments constitute the total fund, but each district is contained within a department. Thus, the smallest district might contribute $1,000 while the largest district would contribute $10,000, and the others in between. Assume that No. 1, the smallest district, has a loss of $2,000. The total sum of money in the departmental reserve is $54,000. The small district would be able to draw from the reserve the additional $1,000 to pay the claim. But the next year or two, or more, depending on the amount of money, District No. 1 would have to pay back into the reserve the amount that was paid out on the judgment. If No. 10 is sued for $20,000, it could borrow from the reserve the additional $10,000 beyond what it had originally contributed. That amount would be paid back in the same manner as was noted for No. 1. A great many different plans could be developed – but the concept is not changed. Basically each district would pay for its own costs. The exception is that there would be one common cost – the cost to all districts alike for administration of the joint powers, which would include the personnel to administer the program. But again – the actual cost of handling a claim for any district would be charged to that district. With this plan the districts could work up to a total sum to cover all contingencies. The plan also has the advantage that one district is not paying for the losses of another district.

When one thinks about reserves for a district, he thinks about cash in the county treasury. But there is more - the district has a reserve it can draw on, the taxing power. Should a claim be filed today, it might be two or more years before it gets into court. In addition, if a judgment were issued which required the district to make payment, there would be many months before the payment would have to be made. Thus, the tax rate might be raised in the next fiscal year to cover part or all of the costs. Also, courts have authority to permit payments over a period of years. The question arises - what if the claim is so large that the district cannot do anything about it at all? Is there any experience with districts that were caught in a bind and could not pay the judgment? It has happened. In 1932 the irrigation districts had bonds which they could not pay because of the depression. In effect they were bankrupt. There is a provision in the law, in effect since 1932, whereby the court can make provision for paying off the claims. Thus, even if a district got into the worst possible position, I believe there is relief which could be found.

I am informed that there are 49 mosquito abatement districts who are members of this Association. If we were to take the total assessed valuation for the 49 districts and levy a one cent ($.01) tax for one tax year, it would raise the sum of $4,400,000. That is based upon a conservative estimate of 44 billion dollars assessed valuation. I would guess that a historical check of all liability losses would show that the interest alone on that amount would more than pay for all liabilities. The precise amount to be raised could be calculated rather precisely once the historical information was compiled.

A disadvantage of moving to self-insurance would be the difficulty in getting into operation. If such is well planned, this objection could be overcome.

What can be expected in the future? I believe a plan should be developed. Perhaps there are other procedures than the joint powers, but this is the only one I see now that has real possibilities. In the event some of the districts decide to investigate this proposal, I would strongly recomment that you not engage the services of insurance people who are oriented to the insurance business and not to self-insurance and risk management, which is a different field of endeavor. Use good sound business management, together with well founded specialized legal advice. Plans must be worked out at the district level, not on the business level of an insurance company. Above all, the districts should obtain expert assistance from people who have a working knowledge of the district's powers and the use of the joint powers program. I really believe time is of the essence. We may not be in deep trouble today, but there is no telling about tomorrow.

AN UNUSUAL LABOR PROBLEM

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Probably no one enjoys the prospect of having to dismiss or fire an employee, but there may come a time when there is no choice. District managers and trustees should be prepared for such an occasion. The occasion came for my District and I thought we were prepared - but we were not!

In the spring of 1975 our District began to have a problem with one of the Inspector-Operators. This man had been with the District for about 2½ years. At first his work in the field and in the shop area was above average, thus he was never a borderline case. Then we began to notice a change in his personality, his work habits and his performance. Even his fellow employees began to notice this change and began shying away from him. We found after we had suspended this man that he had verbally threatened several of his coworkers out in the field.

We asked him to come to the office to find if he had any grievences, and we hoped to get him back on the right track. However, his reaction to the session was negative – he claimed we were picking on him, that he had not done anything wrong and that we were at fault. From this point on we made very certain that his personnel file was kept up to date and that we would document all of his actions as much as possible.

On October 21, 1975, following another incident, a case in which he failed to obey orders, Mr. Robert Peters, manager at that time, called this employee into the office and informed him of his immediate dismissal. He explained to this man why he was being dismissed, and gave him 2 weeks severence pay. Approximately one week later we received a phone call from Robert Cole, manager of the San Joaquin County Employees' Association. He requested a meeting to discuss the action we had just taken. At this meeting he was quite upset with us, and informed us that an employee cannot be fired legally without first receiving the facts. He stated he could order us to put the employee back to work, citing a case from the State Personnel Board which had ruled that before an employee could be fired, suspended, demoted or receive a reduction in pay he must first receive a notice of intention. After that, the employee had 5 days in which to appear before the management or be represented by the Employees' Association. Then, if the employee and the Employees' Association believed that the hearing was unfair, they could request a formal hearing before the trustees of the District. Mr. Peters had been manager of the District for some 27 years, and he had never had a history of firing an employee. Operators would generally leave to better themselves in higher-paying jobs, get into a field they liked better, or to retire.

We were not familiar with the changes in the law, and did not realize that we could not fire an employee without the possibility of getting legally involved. Immediately upon being contacted by the manager of the Employees' Association, we asked our attorney for legal advice. On his advice, the employee received full pay and full fringe benefits from the first day of suspension, October 21, until the final decision was given by our trustees on December 2, 1975. Thus for the two month period this man received full pay and full fringe benefits. This did not make our staff and board happy, but this was the reason we hired legal advice.

We had not been familiar with the Skelly case and had not followed the proper procedure in giving the employee a notice of intention. A date of hearing was set for December 2, and for the next 3 weeks we spent approximately 8 hours per day, conferring with our attorney and going back through records and reports, preparing ourselves for the case. There were several items which had been entered into the personnel files, but our attorney advised us very strongly against using them at the hearing, stating that if the case went to court they would eventually hurt our case. The employee believed he no longer needed a haircut, and he forgot what a razor was for. There were racial incidents between him an another employee. At sessions of the employees, this man felt he was a self-appointed speaker because he wanted to be the leader and he had no consideration for the other employees. At employee sessions he gave the impression he wanted everything he could get, and if such were not available he wanted to strike, even though the majority of employees did not feel this way. Other employees would avoid him. We carefully avoided personality clashes which eventually could bind us if we had to go to court – things that we did not have in writing.

Our hearing was held on December 2, 1975, at the District office. The employee was represented by legal counsel, a member of the San Joaquin County Employees' Association. The District was represented by legal counsel, and a second attorney, from the same law firm, acted as referee or judge. We also had a court recorder. The charges which the District brought against the employee were insubordination and incompetence. Under insubordination, one of the items that was very strong for our case was when he was given a specific order not to take a vehicle into a certain area. This area was wet and boggy, and he proceeded to take the vehicle into it and to bury it there. He then tried to get it out and tore out the transmission, resulting in a cost to the District of over $200. There were other items.

Under incompetence, we cited failure to control mosquitoes at several locations. His records indicated he had gone onto private property on inspections and reported no mosquito breeding, yet our foreman went to these places on the same day and found all stages of mosquitoes. His records showed he had sprayed certain areas, yet mosquitoes were still present, not because of resistance but because it was a careless job of spraying. Also, we had several hundred separate charges of illegible and improper record keeping and faulty entries. It was not because this man could not write – he just did not care. We went out with him on numerous occasions, and our secretary counseled him and tried to get him to change, but he would not do it.

The board hearing took the entire day, from 10:00 a.m. to about 5:00 p.m. Our trustees met with the referee and went over each issue that had been presented. The final decision was that this man should no longer be an employee of our District. The attorney of the Employees' Association thoroughly reviewed the transcript of the hearing, which became available within several weeks, and the Association agreed that its client had received a fair trial and it would not pursue the case further. On March 15, 1976, the Association withdrew its petition for a writ of mandate and notified the employee it would not continue his case. He was advised that if he wished to continue to fight to regain his employment, he would have to seek legal counsel on his own. This he immediately did, and his new attorney applied for an alternate writ of mandate.

Our first court hearing was held in April, 1976. The judge wanted to know if the employee was fired or suspended, because, under the Skelly Act, the employee has the right to be given a notice of intention, which we had not done because we were not familiar with the law. The judge also wanted to know if the employee had received full pay and fringe benefits up to the board of trustee hearing. This employee's attorney challenged the fact that at the trustee hearing the referee was an attorney from the same firm as our legal counsel. The judge ruled that this is done frequently and is legal.

Mosquito abatement districts, as with many other special districts, are not part of the civil service system, but they may follow the system rather closely so that it might be called quasi-civil service system. Under the Myers-Milias-Brown Act, an employee has the right to speak at a public hearing. In reading the transcript of our hearing, the judge detected what he believed was a personality conflict between the employee and manager Peters. At no time at this or subsequent hearings did the judge ever challenge our reasons for firing the employee, that he was incompetent and insubordinate. Rather, he seemed to pick on incidental matters. We found that this judge leaned towards the employee as opposed to management. He indicated he would notify us of his decision, but then we received word that he had gone to Europe to have eye surgery, so we had another delay, from the April 19 court hearing to a second hearing on September 27.

At the second hearing, the first thing the judge attacked was the right of Mr. Peters and the board of trustees to fire the employee, so we had to go through this entire matter again. Again he asked if the employee had received full pay and fringe benefits. He stated that if the employee had not received full pay and benefits, that he would order us to put him back to work. That is how close we came! The judge again asked if Mr. Peters was prejudiced against the employee speaking out at our District meetings. He asked if Mr. Peters or I talked to the board of trustees before the December 2, 1975 hearing, saying we wanted this man fired. One thing that Mr. Peters did when we started this action, was to advise the board that we had a problem with one of the employees, and that a hearing may be necessary. Details were not discussed. This probably saved us later on, because at the close of our second court hearing the judge wanted to know if we had at any time before the District hearing told the trustees the details of what this man had done and that we wanted him fired. There was still a third hearing, at which the judge requested that each of our trustees appear to take the stand individually. Each was examined and cross-examined by the judge himself, by our attorney, and by the employee's attorney. There were picky questions, but our trustees answered them truthfully and correctly. The judge asked questions such as "did any of the trustees know this man personally, had they ever talked with him, did they know members of his family." There was a final question: "Anytime Mr. Peters appears before you, do you always do as he requests?" One trustee responded that when the manager requested something, if the trustees liked it they approved, if not they rejected it or delayed action. He stated that the board would not be dictated to. He declared "our District hired this employee, and we have the power to fire this employee." This had an impact, and on January 14, 1977, our attorney received a letter from the judge stating that the alternate writ of mandate granted to the employee had been discharged, that judgment had been entered for the district and against the employee.

This decision was 1 year and several months after the original action. However, the employee's attorney stated to us that the employee had 60 days in which to appeal to the 3rd Appeals Court if he so desired. Our attorney advised us that the chances are very great that the trial judge's decision would be upheld, and that this becomes a very costly procedure from this point on. This ordeal cost the district possibly $5,000 for attorney fees and costs. This does not include staff time working on the matter. Our District is now working up a written policy on how to proceed in case we should ever need to discharge another employee.

COMMUNICATION: HOW TO AVOID CONFUSION

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Communication can be defined as the act of conveying information among two or more people. This is not always a simple process because quite often little regard is given to how well we actually transmit these messages. Poor communications frequently cause information to become misunderstood, misconstrued, modified and even confused beyond comprehension. Recognition of these causes and corrective steps to avoid poor communication are the subject of this paper.

In recent years the San Mateo County Mosquito Abatement District has been directly involved in several projects requiring in-depth communications with governmental agencies and private institutions. On several occasions the District's method of communication was inadequate and, ultimately, confusion lead to misunderstanding. In some instances, the responses to imparted information were not clearly understood. Many times the results of efforts to communicate are gratifying and rewarding but often they may end up frustrating and discouraging. After closely reviewing various communication techniques, several areas were selected which will enhance the ability to communicate effectively and efficiently with people. These important categories are outlined and the reasons for their selection are highlighted. While all points may not be pertinent for every situation, all should be reviewed before ruling them out.

Remember that you are dealing with people, not organizations or agencies. – Work at understanding people. Remember that they are, first of all, people; and that they assume different roles. There are similarities but many differences between people, so treat each person as a distinct individual. Human beings are by nature exceedingly complex creatures and we shouldn't, therefore, expect to completely understand them. However, that is not a justification to avoid gaining a better understanding of them. Ultimately, in a close association, people will develop both a greater understanding and appreciation for each other. Don't be surprised when requests are rejected or ignored if you failed to keep an open mind during discussions. Respect and accept people as unique individuals, each having the right to differ. A lack of ability to understand people usually indicates losing the fight before it has begun.

Select a leader (communication specialist). – Designate an individual to take charge of the project. Generally, in mosquito abatement districts, this would be the manager but it does not necessarily have to be. Success is often dictated by the time available for the project and frequently the manager may be involved in pressing administrative duties. The chosen individual must take charge of organizing, coordinating and controlling the communication process to see it through to completion. He must know explicitly the roles, duties and responsibilities of all persons involved; and must know what has to be done, why, when, how and by whom. The leader should seek assistance from colleagues and others who may contribute new and diversified ideas, techniques and other valuable information which may achieve better project coordination. The ability of the leader to accomplish work objectives for each project is closely centered around District unity. If the core of the District is solid, harmonious and working toward a common goal, the success rate should be high.

Identify the problem. – Keep the problem in its proper perspective from the beginning. Before communicating, clarify and define each problem as it arises. Criteria employed to assess and identify various problems may be distinctly different; therefore, be selective and accurate in choosing and applying criteria to a specific problem. Once a problem has been identified, don't delay or unnecessarily postpone its correction. Procrastination will only compound the situation.

Develop and prepare project strategy with formulated alternative plans for exceptions and emergencies. "FAIL TO PLAN = PLAN TO FAIL". – When discussing someone else's problem, provide an opportunity to present solutions without divulging your previously formulated plan. This technique removes the risk of not allowing him any chance to correct the problem on his own. Prematurely revealing the District's plan may indicate that no alternate proposal to the District plan would be acceptable. The objective is to solve or reduce a problem. Allowing others to exhibit pride in their input will strengthen working relationships immensely.

Develop a plan of action. Plan the work - work the plan. – Don't get involved in guessing or word games, or threatening people for their work plans. Have a carefully thought out plan and be prepared and in a position to exercise it. Have the plan outlined and written down so there is a smooth step-by-step sequence. If conditions warrant a possibility of legal abatement, it is imperative that the Board of Trustees have adopted a policy governing the necessary administrative and legal procedures. This portion of the work plan is the responsibility of the Board and **not** that of the project coordinator. Keep legal counsel appraised of progress throughout the duration of the project.

Define objectives and authority provided by State statutes. – As previously mentioned, provide the individual with an opportunity to present solutions to problems but, at the same time, make available your expertise during the planning process. Defining the authority of mosquito abatement districts will, hopefully, eliminate uncertainties about the powers granted by State statute. Listen to all ideas before making decisions. Mutual concerns and conflicts should be dealt with as they appear. Where possible, offer professional knowledge and experience. It is better to receive requests for recommendations rather than to force them on someone without recourse. During this stage of communications, try to settle differences without resorting to forceful action.

Compile pertinent data. – Include records both past and current, such as: light trap and larval collections; area maps; outline problem boundaries; service requests; and citizen complaints. Develop a chronological sequence of events leading up to and including the present; describe the geographical area; outline the species of pests or vectors of public health significance; provide research data; cite technical papers and references; and give cost estimates.

Documentation. – Keep copies of all written correspondence related to the problem. Take notes in every meeting and associate comments with specific people. Record who was present and the agency they represented. Have work summary reports and inspection records available for review. List phone calls, dates, times and any responses. Public opinions should be recorded and dated. Develop the habit of signing and dating all documents for quick reference and identification.

How to handle written communications. – If possible, answer written communications within two or three days. Be prompt, courteous and accurate when responding. Answer or clarify questions upon request. Be available and willing to discuss these matters in greater detail. Don't put in writing anything which you may regret later. Written communications may become legal evidence and could be determining factors in the eventual success or failure of a project. A good practice is to have another person edit letters for clarity, simplicity and accuracy. Constructive criticism from collegues may preclude embarrassment.

Seek professional advice from other agencies. – Use knowledgeable authorities in fields outside your area of expertise. It is often beneficial to have specialists debate controversial or complex issues. Written correspondence from highly skilled individuals is encouraged. Meetings are more productive when experts provide testimony. This may prove to be the most valuable information, so document it. The need to thoroughly examine and research any problem cannot be over-emphasized – this can only further the credibility of the District's position.

Degree of involvement. – Anticipate and be fully prepared to spend considerably more time resolving problems than estimated during the initial planning stages. Unforeseen situations frequently unfold during the communication process and usually require additional input. The leader should keep himself available and his schedule flexible.

Establish a time frame schedule. – Adopt reasonable guide lines and criteria for time schedules. When dealing with medically important mosquito species, timing may be extremely important – this is another valid reason for avoiding procrastination. Remember, requests must be first heard and then assimilated before they can be clearly understood. To accomplish this, allow a reasonable period of time. Adhere to time schedules, yet provide for some flexibility. The lack of versatility in a time schedule is threatening and definitely undesirable.

Periodically re-evaluate District's position. – Invariably the chain of events is disrupted; therefore, do not be disappointed when things fail to go smoothly. Routinely evaluate progress to determine whether you are meeting objectives, time schedules and project direction. The leader must keep the project progressing because valuable time lost is difficult to make up.

Contact the appropriate people. – The variability of approaches is prescribed by each particular situation. Decisions not involving large expenditures may frequently be made by supervisorial rather than administrative personnel. Corrective work requiring capital expenditures generally requires an administrative decision. Save time by communicating with the appropriate personnel. When a person needlessly hesitates in decision making, go to someone with greater authority. If other agencies, organizations, special committees, etc., have an interest in the project, advise them of meeting dates and provide progress reports.

Holding meetings. – The leader must take charge and conduct meetings according to his ground rules. Schedule meetings in your own facilities as foreign surroundings may give the edge to opponents. Adversaries naturally feel more confident in their home territory and may attempt to down-play the problems as outlined by District leader in this type of environment.

The significance and urgency of the problem carries more meaning when presented in the District's office. Defending program recommendations before people in their territory, who are neither knowledgeable about nor sympathetic with your goals, is extremely difficult and quite often exasperating and frustrating.

As the lead agency, strive to resolve conflicts in a comfortable setting.

Personal attitude. – Be understanding, cooperative and responsive to individual needs but, at the same time, not neglectful of your responsibilities. A person with a cheerful, friendly and cooperative attitude will succeed in communications. Cooperation means doing with a smile that which has to be done anyway.

Resolving misunderstandings. – Messages are more readily received and understood if presented on a level which the listener can grasp. Simplify the communication channels to avoid repetition of statements. Reduce scientific jargon to a minimum. Use the art of expressing words in the form of questions so as to ascertain whether messages are comprehended. It is best to clarify misunderstandings as they occur – supplementary information will only compound any confusion.

Remain cool, calm and practice patience.– Remember he who loses his temper usually loses. Arguments often lead to a break-down in communications while discussions lead to mutually acceptable solutions. Communication failures can render null and void any progress previously achieved. People who talk too fast often say things not thoroughly thought out. While labor and material costs are high, the most expensive product we deal with is people; therefore, be sensitive, respectful and thoughtful.

Informing the Board of Trustees. – The responsibility of management to make necessary technical and operational decisions does not end here - - they must also keep the Board of Trustees fully appraised of conditions which may eventually require Board action. During the initial discussion phase of communication, request time on the Board meeting agenda for an informal presentation and discussion. The leader is responsible for a thoughtful and accurate presentation. Insure that all homework is done and the necessary data gathered. Be prepared to present an opinion and, if requested, recommendations. If the Board chooses to implement policy action, i.e., to issue a legal abatement notice, it is imperative that the governing body be provided information in chronological sequence. Do not give the Board the entire package during a single meeting. If legal interpretation or clarification is necessary, request prior to the meeting that legal counsel be in attendance at the Board meeting. Trustee Boards and legal counsel can act more readily on district affairs when kept abreast of developing problems.

There are many things involved in the art of communication. Recognize this challenge of effective communication and strive to develop good community communication channels. Present program with authority and in a positive manner. Anticipate the unexpected and be prepared to respond with sound judgment, timely reaction and experience. There is a Chinese proberb which goes as follows:

What I hear, I forget.
What I see, I remember.
What I do, I understand.

If the communication specialist has the gift of persuasion and understanding, you will witness your agency making things happen, not merely watching and wondering what happened. It is said that if you tell a man there are 300 billion stars in the universe, he will believe you, but if you tell him a bench has just been painted, he has to touch it to be sure. There are a multitide of ways to communicate; but to achieve the highest degree of credibility and respect, be sincere, factual and truthful. Confusion in communications can be avoided.

COOPERATIVE DEVELOPMENT OF INSTRUCTIONAL TELEVISION AS A MEDIUM FOR PUBLIC EDUCATION

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INTRODUCTION.–One of the most popular entertainment devices found in the American home is the family television receiver. The medium has also proven itself valuable in a broad range of educational applications. Television as a public education medium became apparent to the senior author when he and the Manager of the Orange County Vector Control District were invited to participate in a videotaped panel discussion concerning the biology and control of mosquitoes. Shortly thereafter the senior author was appointed to the Board of Trustees of the Orange County Vector Control District. As a Trustee of the District and Professor at Fullerton College, he recognized the possibilities of combining the resources of the College's Instructional Television Department and the Vector Control District for the purpose of producing a series of educational videotapes on the subject of vector control. The videotape could be used as an instructional aid in the classroom, as well as a public education tool for the District. After consultation with the Vector Control Staff, it was decided to submit a proposal to the Fullerton College Committee on Instructional Development, requesting authorization and funding for production of two educational videotapes. The first tape to be produced was "Rat Problems in Orange County" followed by a second tape entitled "Fly Control". Both the College Committee and the Vector Control District Board of Trustees approved the project and work began on the first videotape effort.

PRODUCTION METHODS AND COSTS.–The College's Instructional Television Department assigned Mr. Michael Moore as the Director of the two tapes. After an orientation meeting between Mr. Moore and the authors, a script outline of the "Rat Problems In Orange County" videotape was constructed. From this starting point, the task of writing the actual script was begun. It was agreed that the script would be divided into five separate segments consisting of the titles sequence, the Vector Control District Laboratory segment, the residential backyard sequence, the review and conclusions segment, and the ending credits portion. The "Fly Control" production script was written in four sections, including the titles segment, introduction sequence, the Fullerton College Laboratory portion, and a review and conclusions segment.

The scripts were written by the authors, with assistance from District Vector Ecologists Roy E. Eastwood and Stuart J. Long. The Director then arranged the script to coordinate with camera movements and other production requirements.

The stage properties required for production of the rat problems tape ran the gamut from live rats to fresh examples of rodent gnawed oranges and avocados. Other props depicting rat runways on fences (fence runs), woodpiles, and examples of smudge marks were also constructed.

The properties needed for the "Fly Control" production were not as elaborate as those used in the first effort. Four species of live flies, in all stages, were used extensively as well as enlarged line drawings of adult flies. Photographs and actual examples of fly breeding habitats were used along with several charts depicting the fly's life cycle and anatomy.

The titles and credits portion of both tapes were recorded at the Fullerton College Instructional Television Department Studio. The Fullerton College Laboratory sequence was also recorded on campus. The remaining segments were produced at off campus sites utilizing the Department's newly acquired remote television production van.

In the initial planning stages it was decided that the Vector Control District would contribute as much staff time as was necessary for production of two videotapes. It was further agreed that the District would provide all secretarial services, paper, and duplication costs stemming from script preparation. All required still photography and photograph processing was provided by the District. Figure I illustrates the costs in actual cash and expended man hours contributed by the Vector Control District to the project.

Fullerton College bore the largest proportion of the cost of the two tapes. The cost to the College came in the form of salaries for several required production crew persons, special equipment costs (telephoto lens and adaptors for the fly production), blank videotapes and background music. This cost amounted to approximately $5,000 for the production of the two tapes. It is anticipated that some of these monies will be recovered by Fullerton College with the sale of the tapes to agencies or other educational institutions.

Actual recording of "Rat Problems in Orange County" began in March of 1976 and the videotape was completed near the end of May of the same year. Taping of the "Fly Control" production was started in October of 1976 and finished in January of 1977.

DISCUSSION.–The Orange County Vector Control District participated in the production of these videotapes for a number of reasons. In reviewing various sources of audio-visual material, no current vector control related films or videotapes were found. It was also found that the expense involved in having such material commercially produced is extremely high.

The cost of the television production equipment necessary for good quality videotapes is high. As a rule, only commercial television production companies or educational institutions invest in this type of equipment. The equipment owned by the Fullerton College Instructional Television Department is valued in excess of $150,000.

The single most important effort contributing to the success of such a project is planning. Such details as script preparation and coordination, site location, lighting problems, traffic, video camera placement and accessability, and insuring the availability of live vector specimens were all vital to the project's success.

When recording the "Rat Problems in Orange County" videotape, equipment limitations allowed only a few attempts at recording a perfect take. If too many retakes were required, the risk of erasing part of a preceding segment was great. This required that all details of each segment be well thought out and rehearsed before actual recording could take place. The "Fly Control" videotape was somewhat less difficult because the addition of a specialized editing device allowed precision insertion of prerecorded taped sequences onto the master tape. Even with this added flexibility, planning was still important if valuable crew time was to be utilized efficiently.

In an attempt to solve production problems as they occurred, periodic meetings were scheduled between staff members from both organizations. In addition to the meetings, a large amount of individual time was spent planning the two productions.

Orange County Vector Control District Contribution To Production of Two Videotapes

	"Rat Problems In Orange County"	"Fly Control"
I. Actual Cash Expenditure		
A. Art Supplies	$ 4.29	$ 0.00
B. Photographs and Supplies	113.20	46.20
C. Properties Material	59.87	10.00
D. Xerox Copies @ .0315 cents each/560	17.64	18.96
TOTAL	$195.00	75.16
II. Man Hours Expended		
A. Beams (script preparation, remote site location, production coordination)	130.0 hours	150.0 hours
B. Blaylock (secretarial)	12.0 hours	4.0 hours
C. Eastwood (Script preparation, still photography, on-camera work)	70.0 hours	190.0 hours
D. Geck (live fly specimen collection and handling)	30.0 hours	24.0 hours
E. Keller (secretarial)	0.0 hours	6.0 hours
F. Long (script preparation, still photography, on-camera work)	66.0 hours	150.0 hours
G. Sherick (property preparation)	8.0 hours	0.0 hours
TOTAL	316.0 hours	524.0 hours

Cost to Fullerton College for Production of Two Videotapes

	"Rat Problems In Orange County"	"Fly Control"
I. Actual Cash Expenditure		
A. Crew	$ 929.00	$1,000.00
B. Tape	100.00	42.00
C. Production & Direction	1,271.00	263.00*
D. Music	200.00	0.00
TOTAL	$2,500.00	$1,305.00
II. Man Hours Expended		
A. Leo F. Kohl**	1,200.0 hours**	1,000.0 hours**
B. Michael Moore	300.0 hours	350.0 hours
C. Production	100.0 hours	120.0 hours
TOTAL	1,600.0 hours	1,470.0 hours

*Reduction of this cost was due to the fact that the Director was appointed a full-time Classified Staff member.

**The total hours for both productions shown above were actual hours spent on the project over a fifteen month period.

Upon completion of the first two videotapes, it was found that the original cost estimates were below the actual expense incurred. Additional production time accounted for most of the added cost, while art supplies, stage property fabrication, and a charge for background music accounted for the remainder of the increased cost. The total actual cost of producing the two tapes was double the original estimate of $2,500. The added cost was absorbed by the College's Committee on Instructional Development, as well as the schools Educational Resources Division.

Staff members of the District and the College are presently planning the production of two additional videotapes. A videotape on the subject of mosquitoes and midges is planned, as well as a final production on general vector control. With the completion of two videotapes, a much more accurate cost estimate is possible for the final two productions.

The videotape on "Rat Problems in Orange County" addresses itself to three of the rodent species found in Orange County. Biology, habitat, and physical characteristics of the native wood rats, *Neotoma* sp., and the two nonnative species, the roof rat, *Rattus rattus* L., and the Norway rat, *Rattus norvegicus* L., are discussed. The tape then focuses on the roof rat as the most prominent problem species in the County. The roof rat's unique habits, food sources, infestation evidence, and control measures are further noted. The tape is concluded with a review of measures which the homeowner can implement in order to reduce roof rat populations on his property. The "Rat Problems" videotape is just over thirty minutes in length.

In the "Fly Control" production, four species of common flies are discussed. The life cycles, habits, and physical description of the housefly, *Musca domestica* L., the lesser housefly, *Fannia canicularis* (l.), the blow flies, *Phaenicia sp.*, and the biting stable fly, *Stomoxys calcitrans* L., as well as others, are mentioned. The "Fly Control" production runs approximately twenty minutes. All videotapes produced are in full color.

In return for its participation in the production of the videotapes, the Orange County Vector Control District received one copy of each videotape for use in the District's public education program. The tapes are available to other agencies on either a lease or a purchase basis. A copy may be leased for $175 per year or the cassettes are available for sale at a cost of $235 each. The tapes may be ordered directly from Jane Armstrong, Associate Dean of Instruction, Fullerton College, 321 East Chapman Avenue, Fullerton, California 92634.

The authors believe that the project is an excellent example of public agencies working together toward a mutually beneficial goal. We also believe that these tapes will ultimately benefit that very important group, the public we serve.

ACKNOWLEDGMENTS.–The authors wish to express their gratitude to Shirley Bosen, Associate Dean of Educational Resources and Communications, Fullerton College; Jane Armstrong, Associate Dean of Instruction and Coordinator of the Curriculum Instructional Development Committee, Fullerton College; Allen G. Brown, Ph.D., Division Chairman, Life Science Division, Fullerton College; Gilbert L. Challet, District Manager, Roy E. Eastwood and Stuart J. Long, Ph.D., District Vector Ecologists, and members of the Board of Trustees, Orange County Vector Control District for the help, encouragement, and faith rendered in completing these projects.

THE HIGH SCHOOL FARM SANITATION AWARD PROGRAM: AN APPROACH TO PUBLIC EDUCATION

B. Fred Beams

Orange County Vector Control District
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One of the major elements within this District's vector control program is public education. When the responsibility for vector control was transferred to the District in 1975, emphasis was taken in the area of public education. The Farm Sanitation Award Program is just one of the District's new approaches.

The program was originated in 1968 by Ken Birkbeck, R.S., formerly of the Orange County Health Department, and consists of an ongoing competition among the eleven Orange County high schools offering instruction in agricultural sciences. Each school's farm is inspected monthly and graded on the basis of general cleanliness, and the students efforts directed toward prevention and elimination of vectors.

The program begins in October of each school year with classroom presentations to each of the four to six class periods per high school. This amounted to fifty-four one hour presentations with 1,350 high school students in attendance for the 1976-77 school year.

The incoming freshman agricultural students are given introductory presentations which focus on vectors, disease associated with common vector species, and the relationship between agricultural operations and vector production. The presentation consists of approximately twenty minutes of lecture, followed by a twenty to twenty-five minute 35 mm. color slide presentation. The remainder of the period is used to answer questions from the class.

The sophomore, junior and senior levels receive a somewhat shorter presentation in which vector control is reviewed and updated, and is concluded with a question and answer period. Grading criteria and procedures are discussed and reviewed with all class levels so that the students know how each monthly score is determined.

The monthly school farm inspection and grading phase begins in November with an inspection by the Vector Control District Educational Coordinator and the District Vector Control Technician in whose zone the school is located. A standardized grading sheet (Figure 1) is used for each inspection, a copy of which is left with each school. The grading sheet contains catagories such as manure cleaning and removal, manure storage, water usage (irrigation, animal watering, pen grading), refuse storage, rodent control methods, tool care and storage, tack care and storage, and the general physical condition of facilities. During the inspection a check mark is made for each situation or condition in these categories which is actively producing, or will in the near future produce flies, mosquitoes, or other vectors. The care of tools and other equipment is also evaluated. Each check mark is valued at 0.1 points. When the inspection is complete, each horizontal column of check marks is added and the total recorded in the right hand column of the grading sheet. These are then totaled at the bottom of the sheet. If the District receives a vector complaint from the surrounding residential area, an additional 2.0 points are assessed for each complaint that can be verified as having been generated by a source on the school farm. The total points are then deducted from a beginning score of 30.0, this number being the schools score for the month. As an illustration, if a high school is found to have nine infractions, 0.9 points are deducted. If the school farm has generated one bonafide vector complaint within the month, an additional 2.0 points will be deducted. Thus, the school's ending score will be 30.0, minus 0.9 for infractions, minus 2.0 for complaints, equalling 27.1 points for that month. In July, at the end of the grading period, each school's monthly scores are added to determine the first and second place winners. A third school is awarded the most improved honors after demonstrating the greatest degree of improvement during the grading period. The first place winner is awarded a fifty dollar cash award, a trophy plaque, and award certificate. The second place and most improved awards consist of twenty-five dollars cash, a trophy plaque, and award certificate for each school. Certificates of participation are sent to all eleven of the high schools.

Presentations of the awards are made to the Future Farmers of America Chapter President and an agricultural instructor from each of the three schools honored. These awards are made in July at the Annual Orange County Fair Future Farmers of America Awards Ceremonies. A second award ceremony is held at the Vector Control District's July Board of Trustee Meeting.

The Orange County Farm Bureau cosponsors the program by contributing a $100 cash grant to be used as the cash awards. The Vector Control District makes its contributions in the form of trophy plaques, award certificates, and staff time.

The total amount of staff time expended in the 1975-76 school year amounted to approximately fifty-five man hours for classroom presentations, and 160 man hours for the inspection phase of the program. Another five hours should be added for administrative and clerical work associated with the program. This amounts to about twenty-six man days per year that the District devotes to the program.

If just one neighbor complaint per school a month is avoided as a result of the Farm Sanitation Award Program, then the project's worth is evident. The author believes many more complaints are prevented every month because of the effort. In addition, the information gained by the high school students is useful to them not only as private citizens concerned with their own health and the health of the community, but also as future agriculturalists responsible for dealing with vector production on their farms.

Various publications and pamphlets are made available through the District. **Identification of Common Flies Associated with Livestock and Poultry**, a University of California agricultural extension service publication, is one of several pamphlets given to each high school's agricultural science department.

With urban growth and residential development encroaching on not only high school agriculture farms, but large scale agricultural operations as well, it is vital that the future agriculture producers have at least basic knowledge of vector prevention and control techniques.

Form No. 187
Revised 11/75

FFA Chapter Farm Sanitation and Vector Prevention Program Grading Sheet

Figure 1

Each ✓ indicates an area where 0.1 of a point has been deducted for a practice which is or may produce flies, mosquitoes or other vectors, and can be corrected by the students. Each complaint received by the District will result in a 2 point deduction for the month if it can be determined that an FFA Project was the source of the vector.

High School SUNNY HILLS For Month of JANUARY

														Total Points Deducted
Manure Removal	8 Pts. Total													
Corrals	(1)	✓	✓											.2
Fence Lines	(1)													
Alleyways	(1)													
Show Ring	(1)													
Chicken Pens	(1)	✓												.1
Pig Pens	(1)													
Sheep Pens	(1)													
Horse Stables	(1)													
Manure Storage	4 Pts. Total													
Storage Facility	(2)													
Manure Spreader	(2)	✓												.1
Water Usage	8 Pts. Total													
Grading of Pens	(2)													
Animal Watering Fac.	(2)	✓												.1
Casual Standing Water	(4)													
Refuse Storage	2 Pts. Total													
Rodent Control	2 Pts. Total													
Tool Care & Storage	2 Pts. Total	✓	✓	✓	✓									.4
Tack Care & Storage	2 Pts. Total													
Physical Structure	2 Pts. Total													
Total Beginning Score 30 Points														30.0
Minus Total Deducted:	No. of pts. deducted .9, plus 2.0 pts. deducted for 1 complaints received. Reference S.R. Nos. 12862													2.9
Total Score For Inspection														27.1

Comments: EXCESSIVE MANURE BUILDUP IN 2 CORRALS AND 1 CHICKEN PEN. FLY LARVAE IN MANURE SPREADER. MOSQUITO LARVAE IN CATTLE TROUGH. RAKES AND FORKS ON GROUND.

Technician: Date: 1-12-77

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THE EFFECTS OF SUBACUTE CONCENTRATIONS OF THE INSECT GROWTH REGULATORS, DIMILIN™ AND METHOPRENE, ON THERMAL TOLERANCE AND BEHAVIOR IN THE MOSQUITOFISH *GAMBUSIA AFFINIS*

Clifford Ray Johnson

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ABSTRACT

Gambusia affinis were exposed for 24 hours to the insect growth regulators Dimilin™ and Altosid™ (methoprene) at the concentrations of 5, 10, 25, 50, 100, and 200 ppb. The thermal tolerance was significantly lowered for males at concentrations of 10 ppb and above for Dimilin and 50 ppb and above for methoprene, whereas in female fish concentrations of 100 ppb and above and 200 ppb were required for a depression in heat resistance by Dimilin and methoprene, respectively. Thus Dimilin was slightly more active to adult *G. affinis* than the compound methoprene with males being slightly more susceptible to both compounds than female fish. No mortality occurred at the concentrations tested and loss of orientation was not observed.

INTRODUCTION.–Mulla and Isaak (1961) and Mulla et al. (1963, 1966) reviewed the literature on the effects of organophosphorus and organochlorine insecticides on various fishes, including species of the genus *Gambusia*. Boyd and Ferguson (1964) found that mosquitofish were highly tolerant to a number of insecticides.

Such studies have dealt mostly with acute toxicity; little emphasis has been placed on the effects that pesticides have on physiological or behavioral changes at subacute levels. Most studies dealing with subacute effects on fishes have concerned the effects of DDT on the salmonids (see review by Johnson and Prine 1976). Exposure of adult *G. affinis* to low concentrations of five organophosphorus insecticides resulted in lowering thermal tolerances and impairment to orientation and reflexes (Johnson 1977a, in press). Information is scarce on the effects of insect growth regulators on non-target organisms. Johnson and Prine (1976) found that thermal tolerance was significantly lowered and activity depressed in juvenile western toads, *Bufo boreas*, exposed to methoprene at concentrations of 100 and 200 ppb. Here information is presented on the effects of sublethal exposure of *G. affinis* to Dimilin™ and methoprene.

MATERIALS AND METHODS.–Specimens of *G. affinis* were collected from large culture ponds near Concord, California. These ponds had originally been stocked with fish from ponds near Pleasanton, California. The males ranged in standard length from 18 to 26 mm; females from 27 to 50 mm. The fish were acclimated at 18 to 19°C and light: dark 12:12 for 24 hours before toxicant expposure. After 24 hours acclimation, the fish (about 35-50 per test) were exposed in aquaria (10 l of solution, without aeration) to the insect growth regulators Dimilin and methoprene at concentrations of 5, 10, 25, 50, 100, and 200 ppb. The trade names, formulae, and common field application rates of the compounds tested are shown in Table 1. After 24 hours exposure, the fish by treatment were removed and transferred to a 1000 ml beaker filled with 900 ml of water. The water was heated with a 250w infrared heat lamp at a rate of 0.3°C/min, continually aerated and agitated; at no time did a temperature gradient exist within the beaker. Heating was done between 1000 and 1400 hours (Pacific Standard Time) to avoid a diel fluctuation in heat resistance (Johnson 1976). Thermocouples (38 gauge constantan-copper) were attached to the inside of the test beaker at various depths within the water column, and water temperature was continually monitored with a Comark electronic thermometer (model 1625; accuracy ± 0.05°C) through a Comark selector unit (model 1698). Death was assumed to be the point of cessation of gill activity, and with the onset of death, temperature was recorded for each individual tested.

Table 1.–Trade name, formula, and field applied rates as recommended in California for mosquito control, for the chemicals tested in this study.

Trade Name	Chemical Name	Field Applied Rate
DIMILIN™	N(4-chlorophenyl) amino] carbonyl]-2,6-Difluorobenzamide	Not yet registered in California for mosquito control; registered only for experimental use.
Altosid™	[Isopropyl (E,E)-methoxy-3,7,11-trimethyl-2,4-dodecadienoate]	4-8 fluid oz/acre

Observations were made prior to heating to determine if any fish were dead and to ascertain if there were any behavioral differences between the control and exposed fish. The controls had been subjected to the same experimental procedures with the exception of not being exposed to the insect growth regulators.

All research was conducted at the University of California *Gambusia* research facility near Concord, California during the month of September 1976.

RESULTS AND DISCUSSION.–The thermal tolerance was significantly lowered (5%, non-overlap of $S_{\bar{x}}$ for male *G. affinis* at concentrations of 10 ppb and above and at 50 ppb and above for Dimilin and methoprene respectively, whereas in female fish thermal tolerances were significantly lowered at concentrations of 100 ppb and above for Dimilin and at 200 ppb for methoprene (Table 2). These data suggest that male *G. affinis* are more susceptible to

Table 2.–Thermal lethals for male (M) and female (F) *Gambusia affinis* exposed for 24 hours to the insect growth regulators Dimilin TM and methoprene.

Toxicant, Concentration and Sex of Fish	D(°C)			
	$\bar{X}$	Range	$2 S \bar{X}$	N
control				
M	38.2	36.9-39.8	0.4	52
F	38.5	37.4-39.7	0.4	58
Dimilin TM				
5 ppb				
M	38.1	37.3-39.0	0.3	37
F	38.4	37.7-39.6	0.2	35
10 ppb				
M	37.4	36.8-38.5	0.2	40
F	38.2	37.7-39.0	0.4	35
25 ppb				
M	37.5	36.9-38.5	0.2	35
F	38.1	37.5-38.8	0.2	34
50 ppb				
M	37.3	36.8-38.3	0.2	44
F	38.1	37.6-39.0	0.2	38
100 ppb				
M	37.0	36.1-38.3	0.3	45
F	37.6	36.5-38.9	0.4	51
200 ppb				
M	36.8	35.9-37.9	0.2	36
F	37.6	36.9-38.7	0.2	49
methoprene				
5 ppb				
M	37.9	37.4-38.8	0.4	35
F	38.4	38.1-38.9	0.2	43
10 ppb				
M	37.9	37.1-38.5	0.2	38
F	38.4	37.6-38.7	0.3	37
25 ppb				
M	37.7	36.9-38.3	0.2	39
F	38.3	37.0-38.6	0.3	38
50 ppb				
M	37.3	36.1-38.0	0.2	40
F	38.2	36.9-38.7	0.2	43
100 ppb				
M	37.4	36.3-37.9	0.2	41
F	38.0	36.5-38.5	0.2	44
200 ppb				
M	37.1	36.0-38.0	0.2	42
F	37.9	36.6-38.7	0.1	39

these compounds than females and that Dimilin was slightly more active to *G. affinis* than was methoprene. No mortality was observed for either sex at the concentrations tested. Loss of orientation (the loss of coordinated swimming movements as exemplified by rolling behavior) was not observed as it has been with the exposure of *G. affinis* to organophosphorus insecticides and copper sulphate (Johnson 1977b, c.). Although no behavioral differences could be detected in these tests between the control and exposed fish, it can not be safely assumed that these chemicals did not affect other physiological responses, along with the lowering of heat resistance. Other test data appear essential to properly assess the sublethal effects of these insect growth regulators on non-target organisms.

ACKNOWLEDGMENTS.–This research was supported by funds from the California State Legislature Special Allocation for Mosquito Research. William G. Voigt, University of California, and Ernst P. Zboray, State of California Department of Health, offered suggestions concerning the manuscript.

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THE EFFECTS OF FIELD APPLIED RATES OF FIVE ORGANOPHOSPHORUS INSECTICIDES ON THERMAL TOLERANCE, ORIENTATION, AND SURVIVAL IN *GAMBUSIA AFFINIS AFFINIS* (PISCES:POECILIIDAE)

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ABSTRACT

Gambusia affinis were exposed for 24 hours to the insecticides temephos, fenthion, chlorpyrifos, methyl parathion, and malathion at various concentrations consistent with the recommended field application rates for mosquito control in California. The thermal tolerance was significantly lowered for male and female *G. affinis* by the toxicants methyl parathion (25, 50, 100 ppb), chlorpyrifos (25, 50 ppb), and malathion (50, 100 ppb). Malathion at 500 ppb resulted in 100% mortality for both male and female fish. However, no mortality occurred for male and female fish with the toxicants at the other concentrations tested. Loss of orientation was observed with male *G. affinis* with methyl parathion at all concentrations tested (25, 50, 100 ppb) and with 100 ppb malathion. Loss of orientation was not obaserved with female fish, suggesting that male fish were more susceptible to the toxicants. Fenthion and temephos did not affect *G. affinis* at field application rates. A notable depression in activity was observed with exposure to methyl parathion (25, 50, 100 ppb), chlorpyrifos (25, 50 ppb), and malathion (50, 100 ppb) suggesting that other physiological responses besides the lowering of thermal tolerance were also affected.

INTRODUCTION.–Mulla and Isaak (1961) and Mulla et al. (1963, 1966) reviewed the literature on the effects of organophosphorus and organochlorine insecticides on various fishes, including species of the genus *Gambusia*. Boyd and Ferguson (1964) found that mosquitofish were highly tolerant to endrin, aldrin, dieldrin, toxophene, hepatochlor, BHC, chlordane, and Strobane®.

Past studies on pesticides have dealt mostly with acute toxicities with little emphasis on subacute physiological or behavioral alterations. The majority of subacute studies on fish have considered the effects of DDT on salmonids (see review by Johnson and Prine, 1976). Johnson (1977) found that 24 hour exposure of *G. affinis* to the toxicants methyl parathion and chlorpyrifos resulted in a significant lowering of the thermal tolerance at concentrations as low as 5 ppb and that activity was restricted with fish exposed to methyl parathion and chlorpyrifos at concentrations of 10 ppb for 24 hours and at 5 ppb for 48 hour exposure.

This paper presents information on the effects of the insecticides temephos, fenthion, chlorpyrifos, methyl parathion, and malathion on *G. affinis* when applied at the field rates as recommended for mosquito control in California.

METHODS AND MATERIALS.–Specimens of *Gambusia affinis* were collected from large culture ponds near Concord, California (original stock fish collected from near Pleasanton, California). The *G. affinis* ranged in standard length from 18 to 26 mm for males and from 27 to 53 mm for females. The fish were acclimated at 22 to 23°C and LD 12:12 for 24 hours prior to insecticide exposure. After the 24 hour acclimation period, the fish (approximately 10 per test) were exposed in aquaria (10 l of solution) with no aeration to the insecticides methyl parathion (25, 50, 100 ppb), chlorpyrifos (25, 50 ppb), malathion (25, 50, 100, 500 ppb), fenthion (25, 50, 100 ppb), and temephos (25, 50 ppb). The common name, formula, and common field application rates of the compounds tested are shown in Table 1. After exposure, the fish were removed and transferred to a 1000 ml beaker filled with 900 ml of water. The water was heated with a 250w infrared heat lamp at a rate of 0.3°C/min. The water was continually aerated and agitated; at no time did a temperature gradient exist within the beaker. Heating was done only between 1000 and 1400 hours (Pacific Standard Time) to avoid a diel fluctuation in heat resistance. Such a diel fluctuation in thermal tolerance has been demonstrated to occur in *Gambusia affinis* (Johnson 1976). Thermocouples (38 gauge constantan-copper) were attached to the inside of the test beaker at various depths within the water column and water temper-

Table 1.–Common names, formulae, and field applied dosage rates recommended for mosquito control in California for the chemicals tested in this study.

Common Name	Chemical Name	Field Applied Rate
temephos	0,0,0',0'-tetramethyl 0,0'-thiodi-p-phenylene phosphorothioate	0.05 lb/acre
fenthion	0,0-Dimethyl 0-[3-methyl-4-(methylthio) phenyl] phosphorothioate	0.1 lb/acre
chlorpyrifos	0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate	0.05 lb/acre
methyl parathion	0,0-dimethyl 0, p-nitrophenyl phosphorothioate	0.1 lb/acre
malathion	0,0-dimethyl S-(1,2-dicarbethoxy) ethyl phosphorodithioate	0.5 lb/acre

ature was continually monitored with a Comark electronic thermometer (model 1625; accuracy ± 0.05°C) through a Comark selector unit (model 1698). Death was assumed to be the point of cessation of gill activity and with the onset of death the temperature was recorded for each individual fish being tested.

Observations were made prior to heating on the number of dead fish and on behavior and activity of the live individuals and these data compared with the controls which had been treated in like manner with the exception of not being exposed to toxicants. Loss of orientation was exemplified by rolling behavior and the loss of coordinated swimming movements.

All of the experimentation was conducted during the months of June and July, 1976 at the Concord *Gambusia* research facility set up by the University of California.

RESULTS AND DISCUSSION.—Thermal tolerance was lowered significantly (5%, non-overlap of 2 $S\bar{x}$) for male and female *G. affinis* by the toxicants methyl parathion at concentrations of 25, 50, and 100 ppb, by chlorpyrifos at 25 and 50 ppb, and by malathion at 50 and 100 ppb (Table 2). Exposure to 500 ppb malathion resulted in 100% mortality for both male and female fish within 24 hours. However no mortality occurred for male and female *G. affinis* with the rest of the concentrations and toxicants tested. Next to 500 ppb malathion, methyl parathion and malathion both at 100 ppb placed the greatest stress on the fish. Some field studies have shown that malathion was more toxic to *G. affinis* than parathion causing 48 to 70% loss of caged fish at 0.5 lb/acre. Furthermore, fenthion at 0.1 lb/acre caused little or no mortality, but at a rate of 0.4 lb/acre resulted in 18% mortality in 48 hours. Parathion at 0.1 lb/acre caused 18 to 30% mortality to caged fish while methyl parathion showed no mortality (Mulla and Isaak, 1961; Mulla et al., 1963, 1966). Johnson (1977) showed that when the concentrations are equal, methyl parathion placed the greatest amount of stress on *G. affinis* followed closely by chlorpyrifos. The results were consistent with this study in that in order of decreasing effects of stress placed on *G. affinis* were the chemicals methyl parathion, chlorpyrifos, malathion, fenthion, and temephos (Table 2)

Loss of orientation was observed with male *G. affinis* with methyl parathion at all concentrations tested and with 100 ppb malathion. Such a loss of orientation was not observed in female fish suggesting that male fish are more susceptible to these toxicants. Fenthion and temephos did not apparently affect *G. affinis* even at field concentration rates. A notable depression in activity occurred with 24 hour exposure to methyl parathion (25, 50, 100 ppb), chlorpyrifos (25, 50 ppb), and malathion (50, 100 ppb) suggesting that other physiological responses besides the lowering of thermal tolerance were also affected. In a recent study, activity and behavioral changes were apparent in 48 hour exposed *G. affinis* to methyl parathion and chlorpyrifos at concentrations as low as 5 ppb (Johnson 1977). Previously, a depression in activity was found when juvenile western toads, *Bufo boreas*, were exposed to the insecticides methyl parathion (25, 50 ppb), chlorpyrifos (30, 60 ppb), chlorpyrifos-methyl (30, 60 ppb), fenthion (30, 60 ppb), temephos (30, 60 ppb), and the insect growth regulator methoprene (100, 200 ppb)(Johnson and Prine 1976).

It is apparent that even though mortality may not occur with some field application rates, impairment to physiologi-

Table 2.—Thermal lethals for male (M) and female (F) *Gambusia affinis* exposed for 24 hours to various insecticides. % LO, percent of fish observed in loss of orientation (computation based upon live fish after 24 hours); % MO, percent of mortality after 24 hours (computation based upon total number of fish exposed).

Toxicant, Concentration, and Sex of Fish	D (°C) $\bar{X}$	Range	$2 S_{\bar{X}}$	N	% LO	% MO
Control						
M	38.9	37.8-39.7	0.3	80	0.0	0.0
F	39.1	38.4-40.1	0.2	94	0.0	0.0
methyl parathion						
25 ppb						
M	35.9	28.7-38.5	1.0	45	4.4	0.0
F	37.2	36.4-38.6	0.4	49	0.0	0.0
50 ppb						
M	35.1	32.2-36.9	0.4	36	11.1	0.0
F	36.9	32.2-38.6	0.5	48	0.0	0.0
100 ppb						
M	31.6	26.5-35.2	1.0	28	17.8	0.0
F	35.3	28.1-37.4	0.9	39	0.0	0.0
chlorpyrifos						
25 ppb						
M	36.2	35.7-36.9	0.2	41	0.0	0.0
F	36.7	35.2-38.1	0.2	45	0.0	0.0
50 ppb						
M	35.1	34.6-36.6	0.2	35	0.0	0.0
F	35.5	34.9-38.0	0.2	42	0.0	0.0
malathion						
25 ppb						
M	38.4	37.9-38.6	0.1	53	0.0	0.0
F	38.9	38.6-39.5	0.1	40	0.0	0.0
50 ppb						
M	38.0	37.5-38.5	0.2	36	0.0	0.0
F	38.6	37.3-38.9	0.2	40	0.0	0.0
100 ppb						
M	31.4	28.2-35.7	0.6	33	24.2	0.0
F	35.2	33.9-37.7	0.2	44	0.0	0.0
500ppb						
M				42 dead		100.0
F				39 dead		100.0
fenthion						
25 ppb						
M	38.9	38.3-39.2	0.1	52	0.0	0.0
F	39.5	39.0-39.9	0.1	55	0.0	0.0
50 ppb						
M	38.5	37.8-39.2	0.4	49	0.0	0.0
F	39.0	38.7-39.2	0.2	42	0.0	0.0
100 ppb						
M	38.2	37.4-38.7	0.4	35	0.0	0.0
F	38.7	37.7-39.3	0.3	38	0.0	0.0
temphos						
25 ppb						
M	39.1	38.9-39.5	0.1	45	0.0	0.0
F	39.1	38.7-39.5	0.2	50	0.0	0.0
50 ppb						
M	38.5	37.8-39.1	0.4	49	0.0	0.0
F	38.6	38.0-39.3	0.3	39	0.0	0.0

cal functions may result from exposure to field concentrations or those much less than field application dosages as suggested by this and some previous studies (Johnson and Prine, 1976; Johnson, 1977).

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THE EFFECTS OF THE HERBICIDE DIQUAT ON *GAMBUSIA AFFINIS* AND SOME ASSOCIATED FAUNA IN FRESHWATER PONDS

Clifford Ray Johnson[1] and Paul A. Gieke[2]

ABSTRACT

Two ponds were treated with diquat at a concentration of 0.5 ppm AI/acre applied to the surface. Floating live cages containing *Gambusia affinis, Rana catesbeiana* tadpoles, and the gastropods *Physa virgata* and *Planorbella tenui* were immersed in the treated ponds and inspected at 12, 24, and 48 hour intervals for survival and abberant behavior such as loss of orientation or uncoordinated movements. No deaths were recorded for *Rana catesbeinana* tadpoles and *Planorbella tenui*; only one dead *Physa virgata* was found. Male *G. affinis* were more susceptible to diquat than female fish, but mortalities in both sexes were low (4.7% for males and 8.6% for females) and diquat can be used within *G. affinis* habitats with relative safety at an application rate of 0.5 ppm.

INTRODUCTION.–Diquat fish toxicity LC_{50} values for 24 hours exposure range from 15.5 ppm for lake emerald shiner, *Notropis atherinoides*, to 315 ppm for striped bass, *Morone saxatilis*, (Swabey and Schenk 1963; Wellborn 1969). Longnose killifish, *Fundulus similis*, exposed to 1.0 ppm of diquat over a 48 hour period showed no noticible effects (Butler 1963) and biueguills, *Lepomis macrochirus*, lake chubsuckers, *Erimyzon sucetta*, and smallmouth bass fry, *Micropterus dolomicui*, survived a concentration of 2.5 ppm of diquat for 3, 2, and 1 days, respectively (Hiltibran 1967). Little is known about the toxicity of diquate to amphibians and molluscs. Butler (1963) reported that exposure of eastern oysters to 1.0 ppm of diquat for 96 hours had no noticible effect on shell growth.

This study provides information on the observed effects of diquat application to freshwater ponds containing mosquitofish *Gambusia affinis*, bullfrogs *Rana catesbeiana*, and the molluscs *Physa virgata* and *Planorbella tenui*. Other fauna were present but not monitored in these experiments. Such information is important in understanding which chemicals can be used with safety in vegetation management with relation to *Gambusia affinis* production.

METHODS AND MATERIALS.–The experiments were conducted at the Eastside Mosquito Abatement District, Modesto, California in November 1976. Floating live cages (61 x 61 x 15 cm) were placed in two elongated ponds (69 m long, 3.3-5.7 m wide, 1.2 m deep). Three live cages were placed in each pond, one at each end and one in the middle. A total of 483 male and 252 female *Gambusia affinis* were distributed between the floating cages. *Physa virgata* (96), *Planorbella tenui* (48) and *Rana catesbeiana* tadpoles (24) were placed in the live cages.

In another pond (69 m long, 4.5 m wide, 1.2 m deep) three floating live cages were set up for a control containing a total of 122 male and 109 female *G. affinis*, 12 *Rana catesbeiana* tadpoles, 43 *Physa virgata*, and 49 *Planorbella tenui*. The control pond was approximately 30 m away from the treated ponds to prevent drift of the chemicals during application. Diquat (diquat dibromide [6,7-dihydrodipyridol (1,2-a:2′, 1′-C) pyrazidiinium dibromide]) was applied to the water surface in the test ponds by hand carried spray cans at the dosage rate of 0.5 ppm AI.

The live cages were inspected at 12, 24, and 48 hour intervals and counts made of dead individuals and surviving fauna by species and by sex for *G. affinis*. Observations were also made on individuals for aberrant behavior such as the loss of orientation or uncoordinated movements.

RESULTS AND DISCUSSION.–The application of diquat at 0.5 ppm did not affect the *Rana catesbeiana* tadpoles, *Planorbella tenui*, and only resulted in one death to *Physa virgata* (Table 1). All the tadpoles and live molluscs appeared normal in behavior. Male *G. affinis* were more susceptible to diquat than females with 8.6% mortality occurring with 48 hour exposure compared to 4.7% mortality for females over the same time span. Greater mortality has been found in male *G. affinis* than in female fish when exposed to other pesticides (Johnson 1977a, b). No loss of orientation (rolling behavior) was observed with any of the *G. affinis*. It would appear that diquat can be used with relative safety at a concentration of 0.5 ppm in *G. affinis* habitats as the mortality observed within this study was within reasonable limits.

Table 1.–Accumulative mortality by species over a 48 hour period in control and treated ponds. Numbers in parentheses refer to % mortality.

Species and Treatment	Accumulative Deaths 12 hr	24 hr	48 hr
Control			
male *G. affinis*	0	0	1(0.8%)
female *G. affinis*	0	0	0
Rana catesbeiana tadpoles	0	0	0
Physa virgata	0	0	0
Planorbella tenui	0	0	0
Diquat Treated			
male *G. affinis*	3(0.6%)	24(4.9%)	42(8.6%)
female *G. affinis*	0	6(2.3%)	12(4.7%)
Rana catesbeiana tadpoles	0	0	0
Physa virgata	0	1(1.0%)	1(1.0%)
Planorbella tenui	0	0	0

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FOOD AND FEEDING OF BULLFROGS, *RANA CATESBEIANA*, WITHIN *GAMBUSIA AFFINIS* CULTURE PONDS

Clifford Ray Johnson[1], William G. Voigt[1] and Paul A. Gieke[2]

ABSTRACT

Concern over the possible predation of bullfrogs on mosquitofish prompted us to conduct a stomach analysis of these bullfrogs and determine what if any effect bullfrog predation had on the population of mosquitofish within rearing ponds. Medium-sized frogs (66-90 mm) were the largest consumers of *Gambusia affinis* (frequency of occurrence, FO = 18.5%). *G. affinis*, although an abundant prey, did not constitute a great number of prey items. Crayfish were the most abundant prey and their size increased with the size of the frogs. In this study bullfrogs did not feed upon significant numbers of *Gambusia affinis* so as to justify frog eradication or restriction programs on this factor alone. However, if the crayfish had not been present in the ponds, a switch in feeding preference to *G. affinis* might occur. Whether this predation would significantly affect fish production, however, is uncertain. Certainly the reproductive potential of bullfrogs would make any eradication program very costly and would probably exceed the cost of fish lost to bullfrogs by predation alone.

INTRODUCTION.–Since it was first detected in the early 1900's, the bullfrog, *Rana catesbeiana*, has dispersed throughout the central valley of California. Its spread and great success in the valley has been attributed to the lack of native frogs filling its niche (Stebbins 1962). Studies of the feeding habits of this frog have shown a great variety of food items including insects, mulluscs, crustaceans, annelids, fish, snakes, young turtles and alligators, small birds and mammals. Frost (1935) noted that although a great variety of food items have been recorded for *R. catesbeiana* it has a "singular method of accepting its food, preferring to take it under water. This has been observed a number of times in nature and in captivity".

A large population of bullfrogs has established itself in the experimental ponds at Eastside Mosquito Abatement District where a study of mosquitofish productivity was being conducted by the authors. Concern over the possible predation of bullfrogs on mosquitofish prompted us to conduct stomach analyses of these bullfrogs and determine what if any effect bullfrog predation had on the population of mosquitofish.

MATERIALS AND METHODS.–Frogs of all size groups were captured with dip nets, trout flies, and bow and arrow during one day of each month from May through September 1976. Sampling periods were usually in the morning (approximately 1000-1200 hrs), afternoon (1400-1600 hrs) and after dark (2200-2400 hrs). More frogs were sampled at night during the latter part of the study due to the increased wariness of the remaining frogs. After the sampling period, the stomachs were removed and contents examined immediately. Intestinal contents were not examined due to their advanced state of digestion. Data were analysed only for frequency of occurrence (i.e. percentage of stomachs containing each prey item) and total number of each item encountered.

RESULTS AND DISCUSSION.–Tables 1 through 4 summarize the food items identified from the three size classes of bullfrogs and present the total food contents encountered within all size classes combined. Table 5 shows that an increasing number of aquatic prey as compared to terrestrial or aerial prey were taken with increasing size of frogs.

Medium-sized frogs (66-90 mm snout-vent length) were the largest consumers of *Gambusia affinis* (FO = 18.5%). *G. affinis*, although an abundant prey, did not account for a great number of prey items. Prey items are probably eaten while on the bottom or when the items emerged from the water surface. Surface activity of *G. affinis* therefore keeps the animals (*G. affinis* and bullfrogs) separated. Some surface feeding by *R. catesbeiana* may account for fish appearing in the frog stomachs, or they may be ingested when fish are feeding along the bottom. Evidence for bottom feeding exists based upon a study of the food and feeding of *Gambusia affinis* within these ponds being carried out by the authors. The fish stomachs have been found to contain high numbers of ostracods and chironomid larvae (unpublished studies).

The size of crayfish increased with the size of the frogs. Often the stomachs would be greatly distended in frogs with one or two large crayfish present. The number of empty stomachs in all size categories indicates a possible fasting in the frogs. It may also represent frogs which had not yet fed prior to sampling.

In this study bullfrogs did not prey on such significant numbers of *Gambusia affinis* as to justify frog eradication or restriction programs. However, if the crayfish had not been present in the ponds, a switch in feeding preference to *G. affinis* might occur. Whether this predation would significantly affect fish production, however, is uncertain. Certainly the reproductive potential of bullfrogs would make any eradication measures very costly and would probably exceed the cost of fish lost to bullfrogs, if such predation was the only effect to be considered.

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Table 1.–Digestive tract contents of *Rana catesbeiana* within the size group 25 to 65 mm snout-vent length. FO = frequency of occurrence; N = total number of food items encountered.

Item	May		June		July		August		September		Total	
	% FO	N	% FO	N	% FO	N	% FO	N	% FO	N	% FO	N
INSECTA												
Odonata												
Anax junius (larvae)	–	–	–	–	–	–	6.6	1	15.3	2	7.5	3
Coenagrionidae (adult)	33.3	1	–	–	20.0	1	6.6	1	–	–	7.5	3
Dermaptera												
Labia minor	–	–	–	–	60.0	3	–	–	–	–	7.5	3
Hemiptera												
Belostoma bakeri	–	–	–	–	–	–	6.6	1	7.6	1	5.0	2
Pentatomidae (unident.)	–	–	–	–	40.0	2	–	–	–	–	5.0	2
Diptera												
Tipulidae (adult)	–	–	–	–	–	–	6.6	2	–	–	2.5	2
Coleoptera	–	–	–	–	–	–	–	–	15.3	2	5.0	2
Tropisternus lateralis	–	–	–	–	–	–	6.6	1	–	–	2.5	1
Chrysomelidae	33.3	1	–	–	–	–	–	–	–	–	2.5	1
Hymenoptera												
Apis melifera	–	–	–	–	–	–	6.6	1	7.6	1	5.0	2
Unidentified insect remains	–	–	–	–	–	–	–	–	15.3	–	5.0	–
ACARINA	–	–	–	–	40.0	2	–	–	–	–	5.0	2
CRUSTACEA												
Decapoda												
Procamberus clarkii	–	–	50.0	2	–	–	26.6	4	15.3	2	17.5	8
CHORDATA												
Rana catesbeiana (tadpoles)	–	–	25.0	1	–	–	–	–	–	–	2.5	1
Gambusia affinis	–	–	–	–	–	–	20.0	3	–	–	7.5	3
DETRITUS	–	–	–	–	–	–	6.6	–	–	–	2.5	–
No. empty	33.3	1	25.0	1	20.0	1	26.6	4	38.4	5	30.0	12
Total number of frogs examined	3		4		5		15		13		40	

Table 2.–Digestive tract contents of *Rana catesbeiana* within the size group 66 to 90 mm snout-vent length. FO = frequency of occurrence; N = total number of food items encountered.

Item	May		June		July		August		September		Total	
	% FO	N	% FO	N	% FO	N	% FO	N	% FO	N	% FO	N
MOLLUSCA												
Gastropoda												
Physa virgata	–	–	–	–	–	–	20.0	5	–	–	5.5	5
Planorbella tenui	–	–	–	–	–	–	26.6	5	–	–	7.4	5
INSECTA												
Odonata												
Anax junius (larvae)	7.1	1	–	–	–	–	–	–	–	–	1.8	1
(adult)	–	–	–	–	–	–	6.6	2	13.3	3	5.5	5
Coenagrionidae (adult)	–	–	–	–	–	–	6.6	1	–	–	1.8	1
Orthoptera												
Acrididae	–	–	–	–	–	–	–	–	6.6	1	1.8	1
Hemiptera												
Belostoma bakeri	–	–	–	–	–	–	–	–	6.6	1	1.8	1
Lepidoptera												
Danaus plexippus (larvae)	–	–	25.0	1	–	–	–	–	–	–	1.8	1
Coleoptera	–	–	–	–	–	–	–	–	6.6	1	1.8	1
Hydrophilus triangularis	7.1	1	–	–	16.6	2	–	–	6.6	1	5.5	4
Hymenoptera												
Apis melifera	–	–	–	–	–	–	–	–	6.6	2	1.8	2
ACARINA	–	–	–	–	–	–	–	–	6.6	1	1.8	1
CRUSTACEA												
Decapoda												
Procamberus clarkii	64.2	10	50.0	4	16.6	1	13.3	2	26.6	4	33.3	21
CHORDATA												
Rana catesbeiana (tadpoles)	–	–	25.0	1	16.6	1	13.3	2	6.6	1	9.2	5
Gambusia affinis	14.2	3	25.0	1	33.3	2	26.6	12	6.6	1	18.5	19
DETRITUS	–	–	–	–	–	–	–	–	6.6	–	1.8	–
No. empty	21.4	3	–	–	16.6	1	13.3	2	26.6	4	18.5	10
Total number of frogs examined	14		4		6		15		15		54	

Table 3.—Digestive tract contents of *Rana catesbeiana* within the size group 91 to 150 mm snout-vent length. FO = frequency of occurrence; N = total number of food items encountered.

Item	May		June		July		August		September		Total	
	% FO	N	% FO	N	% FO	N	% FO	N	% FO	N	% FO	N
MOLLUSCA												
Gastropoda												
Physa virgata	7.6	2	–	–	25.0	5	–	–	–	–	4.0	7
Planorbella tenui	–	–	–	–	–	–	13.3	3	–	–	4.0	3
INSECTA												
Orthoptera												
Acrididae	–	–	–	–	–	–	6.6	1	–	–	2.0	1
Hemiptera												
Belostoma bakeri	–	–	–	–	–	–	6.6	1	8.3	1	4.0	2
Coleoptera												
Hydrophilus triangularis	–	–	–	–	–	–	6.6	1	–	–	2.0	1
CRUSTACEA												
Decapoda												
Procamberus clarkii	84.6	14	60.0	3	–	–	40.0	6	58.3	8	55.1	31
CHORDATA												
Rana catesbeiana (tadpoles)	–	–	20.0	1	50.0	3	20.0	6	–	–	12.2	10
Gambusia affinis	7.6	1	–	–	25.0	1	6.6	1	–	–	6.1	3
DETRITUS	–	–	–	–	–	–	20.0	–	–	–	6.1	–
No. empty	15.3	2	20.0	1	25.0	1	13.3	2	33.3	4	20.4	10
Total number of frogs examined	13		5		4		15		12		49	

Table 4.–Total digestive tract contents of *Rana catesbeiana* within all size groups. FO = frequency of occurrence; N = total number of food items encountered.

Item	% FO	N
Mollusca		
Gastropoda		
Physa virgata	3.4	12
Planorbella tenui	4.1	8
Insecta		
Odonata		
Anax junius (larvae)	2.7	4
(adult)	2.0	5
Coenagrionidae (adult)	2.7	4
Orthoptera		
Acrididae	1.3	2
Dermaptera		
Labia minor	2.0	3
Hemiptera		
Belostoma bakeri	6.9	5
Pentatomidae	1.3	2
Lepidoptera		
Danaus plexippus (larvae)	0.7	1
Diptera		
Tipulidae (adult)	0.7	2
Coleoptera		
Hydrophilus triangularis	2.7	5
Tropisternus lateralis	0.7	1
Chrysomelidae	0.7	1
Hymenoptera		
Apis melifera	2.0	4
Unidentified insect remains	1.3	–
Acarina	2.0	3
Crustacea		
Decapoda		
Procamberus clarkii	36.3	60
Chordata		
Rana catesbeiana (tadpoles)	8.3	16
Gambusia affinis	11.1	25
Detritus	3.4	–
No. empty	23.1	32
Total number of frogs examined	143	

Table 5.–Numbers of prey items broken down to terrestrial and aerial versus aquatic by size groups of bullfrogs.

Food Items	25-65 mm s-v	66-90 mm s-v	91-150 mm s-v
Terrestrial + Aerial	17	11	1
Aquatic	18	41	51

THE EFFECTS OF COPPER SULPHATE EXPOSURE ON THERMAL TOLERANCE, ORIENTATION AND SURVIVAL IN ADULT MOSQUITOFISH, *GAMBUSIA AFFINIS*, AND JUVENILE THREESPINED STICKLEBACKS, *GASTEROSTEUS ACULEATUS*

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ABSTRACT

Adult male and female *Gambusia affinis* and juvenile *Gasterosteus aculeatus* were exposed for 24 hours to copper sulphate at concentrations of 0.05, 0.10, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, and 10.00 ppm. Thermal tolerance was significantly lowered in *Gambusia affinis* at concentrations of 5.00 ppm and greater and male fish were more susceptible than female fish. Mortality was considerable for males at concentrations of 8.00 and 10.00 ppm. The thermal tolerance was significantly lowered for juvenile *G. aculeatus* at concentrations of 4.00 ppm and above and mortality was observed at this concentration and greater with 63.8% mortality occurring at 10.00 ppm. Loss of orientation was observed with male *G. affinis* at concentrations of 5.00 ppm and above and with juvenile *G. aculeatus* at concentrations of 6.00 ppm and above at exposure temperatures. Except for the loss of orientation which occurred at higher concentrations at exposure temperatures, no other behavioral or activity differences were noted between the exposed and control individuals.

INTRODUCTION.—The toxicity of copper sulphate to fish varies by species and with the physical and chemical composition of the water. McKee and Wolfe (1963) presented a wide variety of toxicities for various fish species with trout being the most sensitive. Bond (1960) had previously found that copper sulphate was particularly toxic to trout in soft water. Dosages of copper sulphate for weed control range from 0.05 to 10.00 ppm for various plant species (USDA 1954).

In the past few studies on pesticides have considered little but acute toxicity levels although some have dealt with the sublethal effects of DDT on fishes, mostly salmonids (see review by Johnson and Prine 1976). Other studies have found hyperactivity and skeletal abnormalities in anuran tadpoles exposed to chronic low doses of DDT (Cooke 1970, 1972, 1973) and Weis (1975) found that in *Rana pipiens* exposed to 25 ppb DDT retardation of regeneration occurred. Recently, a series of studies on anurans, on *Gambusia affinis*, and on the copepod, *Macrocyclops albidus*, have shown that temperature tolerance was significantly lowered, and in some cases orientation and reflexes were impaired with exposure to subacute concentrations of some organophosphorus insecticides, some herbicides and the insect growth regulator methoprene (Johnson and Prine 1976; Johnson 1976a; Johnson 1977a, b).

This paper presents information on the physiological and behavioral responses of the mosquitofish, *Gambusia affinis*, and the three-spined stickleback, *Gasterosteus aculeatus*, after exposure for 24 hours to copper sulphate in a wide range of concentrations consistent with field application rates.

MATERIALS AND METHODS.—Specimens of *Gambusia affinis* were collected from large culture ponds near Concord, California. The original stock of *G. affinis* was collected from ponds near Pleasanton, California. The juvenile *Gasterosteus aculeatus* were collected from permanent ponds near Concord. The *G. affinis* ranged in standard length (SL) from 18 to 26 mm for males and from 27 to 52 mm for females. The juvenile *G. aculeatus* ranged in size from 10 to 35 mm SL. The fish were acclimated at 22 to 23°C and LD 12:12 for 24 hours prior to copper sulphate exposure. after the 24 hour acclimation period, the fish (approximately 10 per test) were exposed in aquaria (10 l of solution) with no aeration to copper sulphate at concentrations of 0.05, 0.10, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, and 10.00 ppm for 24 hours. Alkalinity and hardness of the test water ranged from 50 to 85 ppm and 60 to 120 ppm, respectively. After exposure, the fish were removed and placed in a 1000 ml beaker filled with 900 ml of water. The water was heated at a rate of 0.3°C/min with a 250 w infrared heat lamp. The water was continually aerated and agitated and no temperature gradient existed within the beaker. Heating was done only between the hours of 1000 and 1400 (Pacific Standard Time) to avoid the diel fluctuation in heat resistance which occurs in *Gambusia affinis* (Johnson 1976b) and might occur in *Gasterosteus aculeatus*. Thermocouples (38 gauge constantan-copper) were attached to the inside of the test beaker at various depths and water temperature was continually monitored with a Comark electronic thermometer (model 1625; accuracy ± 0.05°C) through a Comark selector unit (model 1698). Death was assumed to be the point of cessation of gill activity and with the onset of death the temperature was recorded for each individual fish being tested.

Observations were made before heating on the number of dead fish and behavior and activity of the live individuals and these data were compared with the controls treated in like manner with the exception of not being exposed to the toxicant. Loss of orientation was exemplified by rolling and loss of coordinated swimming movements.

All research was conducted at the University of California research facility for the study of *Gambusia* near Concord and was conducted during the months of July and August 1976.

RESULTS AND DISCUSSION.—With *G. affinis*, thermal tolerance was lowered significantly (5%, non-overlap of 2 S $\bar{x}$) at concentrations of 5.00 ppm and greater (Table 1). Male fish were more susceptible than female fish as demonstrated by greater mortality rates at concentrations of 5.00 through 10.00 ppm. Johnson (1976b) has shown female *G. affinis* to be more heat tolerant than males. Also

Table 1.–Thermal lethals for male (M) and female (F) *Gambusia affinis* exposed for 24 hours to $CuSO_4$ at various concentrations. % LO, percent of fish observed in loss of orientation (computation based upon live fish after 24 hours); % MO, percent of mortality after 24 hours (computation based upon total number of fish exposed).

Concentration and Sex of Fish	D(°C) $\bar{x}$	Range	2 S $\bar{x}$	N	% LO	% MO
Control						
M	38.9	37.9-39.7	0.3	70	0.0	0.0
F	39.2	38.5-40.0	0.2	84	0.0	0.0
0.05 ppm						
M	38.8	37.6-39.8	0.4	42	0.0	0.0
F	39.1	38.5-39.6	0.4	45	0.0	0.0
0.10 ppm						
M	38.6	37.8-39.3	0.4	54	0.0	0.0
F	39.2	38.7-39.6	0.4	54	0.0	0.0
0.25 ppm						
M	38.7	37.6-39.4	0.4	58	0.0	0.0
F	39.3	38.6-39.7	0.4	59	0.0	0.0
0.50 ppm						
M	38.5	38.3-39.2	0.2	58	0.0	0.0
F	39.4	38.7-39.6	0.2	66	0.0	0.0
1.00 ppm						
M	38.1	37.6-38.8	0.5	36	0.0	0.0
F	39.0	38.4-39.5	0.4	42	0.0	0.0
1.50 ppm						
M	38.2	37.9-38.8	0.4	51	0.0	0.0
F	38.7	37.8-39.1	0.5	54	0.0	0.0
2.00 ppm						
M	38.2	37.7-39.2	0.5	48	0.0	0.0
F	38.9	38.1-39.4	0.4	48	0.0	0.0
2.50 ppm						
M	38.3	37.8-38.7	0.4	46	0.0	0.0
F	39.0	38.4-39.3	0.4	37	0.0	0.0
3.00 ppm						
M	38.2	37.7-39.0	0.4	38	0.0	0.0
F	38.9	38.1-39.3	0.4	35	0.0	0.0
4.00 ppm						
M	38.1	37.6-38.4	0.5	32	0.0	0.0
F	38.8	38.3-39.0	0.4	38	0.0	0.0
5.00 ppm						
M	37.0	34.4-38.3	0.9	27	0.0	12.9
F	38.0	37.5-38.6	0.5	32	0.0	0.0
6.00 ppm						
M	32.8	30.9-34.0	0.7	33	0.0	13.1
F	35.4	34.4-38.0	0.7	35	0.0	0.0
8.00 ppm						
M	33.0	30.9-35.2	0.7	28	7.1	45.1
F	36.2	35.2-36.9	0.6	30	0.0	14.2
10.00 ppm						
M	29.3	27.3-31.4	0.8	14	14.2	70.8
F	33.0	30.6-36.9	0.6	28	0.0	40.4

only males were observed in a state of loss of orientation at concentrations of 5.00 ppm and above at exposure temperatures (22-23°C), whereas females were not so affected. Mortality was considerable for males at 8.00 and 10.00 ppm and at 10.00 ppm for females.

The thermal tolerance was significantly lowered (5%, non-overlap of 2 S $\bar{x}$) in *Gasterosteus aculeatus* at concentrations of 4.00 ppm and above (Table 2). Mortality was also observed at 4.00 ppm and greater with 63.8% mortality occurring at 10.00 ppm. Loss of orientation occurred at exposure temperatures at 6.00 ppm and above. As previously mentioned, thermal tolerance has been significantly lowered by subacute exposure to some organophosphorus insecticides, some herbicides, and the insect growth regulator methoprene in a variety of animals.

A depression in activity and/or behavioral changes such as the observed loss of orientation and/or loss of reflexes would suggest that other physiological responses were also affected. This has been suggested in some previous studies (Johnson and Prine 1976; Johnson 1976a, 1977a).

Table 2.–Thermal lethals for juvenile *Gasterosteus aculeatus* exposed for 24 hours to $CuSO_4$ at various concentrations. % LO and % MO as in Table 1.

Concentration	D (°C) $\bar{x}$	Range	2 S$\bar{x}$	N	% LO	% MO
Control	34.1	33.0-35.5	0.1	90	0.0	0.0
0.05 ppm	34.3	33.2-34.9	0.3	48	0.0	0.0
0.10 ppm	34.2	33.5-34.8	0.2	45	0.0	0.0
0.25 ppm	34.3	33.4-34.6	0.2	46	0.0	0.0
0.50 ppm	34.1	32.5-34.8	0.3	51	0.0	0.0
1.00 ppm	34.2	33.2-34.7	0.2	60	0.0	0.0
1.50 ppm	33.9	33.1-34.3	0.2	42	0.0	0.0
2.00 ppm	34.1	32.1-34.5	0.3	49	0.0	0.0
2.50 ppm	34.0	33.1-34.5	0.3	51	0.0	0.0
3.00 ppm	33.9	32.9-34.4	0.3	49	0.0	0.0
4.00 ppm	33.4	32.6-34.0	0.4	38	0.0	15.5
5.00 ppm	33.5	32.9-34.4	0.3	35	0.0	18.6
6.00 ppm	32.9	30.9-34.0	0.4	34	14.7	32.0
8.00 ppm	33.0	32.1-33.3	0.6	22	18.1	56.0
10.00 ppm	29.7	28.2-30.8	0.7	17	29.4	63.8

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THE EFFECT OF EXPOSURE TO THE ORGANOPHOSPHORUS INSECTICIDE CHLORPYRIFOS ON THE FEEDING RATE IN THE MOSQUITOFISH, *GAMBUISA AFFINIS*

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ABSTRACT

Adult female *Gambusia affinis* were exposed to 50 ppb chlorpyrifos for 24 or 48 hours. The exposed fish's feeding rate on *Daphnia pulex* and *Culiseta incidens* was examined and compared to the feeding rates on these food items by unexposed control individuals. The predation rate on *D. pulex* was significantly reduced by exposure to chlorpyrifos for both 24 and 48 hours, and similar results were found for the predation rate on *Cs. incidens* for 48 hour exposure. Such was not the case for the feeding rate on *Cs. incidens* with the 24 hour exposed fish. The results are discussed in relation to food preferences of *G. affinis* and with density, behavior, and size of the food items.

INTRODUCTION.–Little is known concerning the subacute effects of pesticide exposure on behavior and physiology of fishes. The majority of such studies have dealt with the sublethal effects of DDT on salmonid fishes (as reviewed by Johnson and Prine 1976). A depression in activity has been found to occur in *Gambusia affinis* when exposed to methyl parathion and chlorpyrifos at concentrations as low as 10 ppb with 24 hour exposure and at 5 ppb at 48 hour exposure (Johnson 1977). The exposed fish remained stationary and movements were restricted as compared to the control individuals. Since activity was depressed by exposure to the above organophosphorus insecticides, the question was raised if such exposure would also affect the feeding rate; and therefore the following experiment was designed to test for this effect using a concentration of 50 ppb of chlorpyrifos which is the recommended field application rate for mosquito control in California.

MATERIALS AND METHODS.–Only female *G. affinis* ranging in size from 35 to 55 mm in standard length were used in the experiments, so as to maintain a more uniform size range of fish. The fish were collected from stock ponds near Concord, California. Females in the latter stages of gestation were not selected for experimentation. Some of the female *G. affinis* were exposed to the organophosphorus insecticide chlorpyrifos 0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl phosphorothioate) at a concentration of 50 ppb for 24 hours and other fish were exposed to 50 ppb for 48 hours. Ten fish were exposed at a time in 10 l of solution. After exposure, five fish were placed in each aquaria with 20 l of clear water. Food was introduced into each of the tanks. Prior to experimentation, none of the fish had been fed for 48 hours. The food items tested were adult cladocerans *Daphnia pulex* and 1st and 2nd instar larvae of the mosquito *Culiseta incidens*. Each type of food item was tested individually with samples of 50 being introduced into each tank. All experiments were commenced at 0800 hrs (Pacific Standard Time). Counts of the uneaten food items were made at intervals of 2, 6, and 12 hours. The tanks were also examined at these intervals for any dead food items lying on the bottom or present in the water column; such items were rejected from the sample. Control fish were tested in like manner with the exception of not being exposed to chlorpyrifos. Calculations of the number of each food species preyed upon per female *G. affinis* per 12 hour period were based upon the above counts. All experimentation was conducted during the months of June and July, 1976.

RESULTS AND DISCUSSION.–The predation rate on *D. pulex* was significantly reduced in female *G. affinis* by exposure to 50 ppb chlorpyrifos for both 24 and 48 hours (5%, non-overlap of 2 S $\bar{x}$) (Table 1). Although similar results were found with the predation rate on *Cs. incidens* by 48 hour insecticide exposed *G. affinis*, such was not the case with those individuals exposed for 24 hours.

Table 1.–Number of food items preyed upon per control and chlorpyrifos exposed female *Gambusia affinis* over a 12 hr period. N = number of fish tested.

Treatment	Food Item	$\bar{X}$	Range	2 S $\bar{x}$	N
Control	*D. pulex*	10.20	2.01-30.00	1.91	100
	C. incidens	4.74	0.98-10.80	0.89	100
24 hr 50 ppb	*D. pulex*	5.88	3.60- 9.24	1.64	60
chlorpyrifos	*C. incidens*	5.60	4.80- 7.20	0.53	60
48 hr 50 ppb	*D. pulex*	4.38	0.38- 9.00	1.40	60
chlorpyrifos	*C. incidens*	2.38	1.14- 3.60	0.37	60

A depression in activity has been found in *G. affinis* following chlorpyrifos exposure (Johnson 1977) and may therefore be responsible for the reduction in predation rate as observed here. Food preferences of *G. affinis* and the behavior of the prey species may also influence the results seen here. Since this fish is predominately a sight feeder, any movement or continous swimming movement of a prey item, such as with *D. pulex*, would undoubtedly increase their vulnerability. Density may also play an important role, and even though the predation studies involved the same number of prey items in each tank, the *D. pulex* were more evenly distributed within the water column, whereas the *Cs. incidens* remained mostly in the surface cm. These factors (behavior, density, and size of the food items along with food preferences of *G. affinis*) probably relate to the fact that the female *G. affinis* preyed more readily upon *D. pulex* than on the *Cs. incidens* larvae, and the general observed reduction in feeding rate after exposure to the insecticide probably relates to the depression in activity of the exposed *G. affinis*.

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SUGGESTED POND DESIGN FOR OVERWINTERING POPULATIONS OF *GAMBUSIA AFFINIS*

Clifford Ray Johnson[1] and Paul A. Gieke[2]

Maintaining stable populations of *Gambusia affinis* in shallow rearing ponds in California has usually met with little success. Within Stanislaus and Alameda Counties it has been observed that some natural ponds have demonstrated an abundance of *G. affinis* throughout the year. The majority of these ponds affording such stable populations were alike in two important respects: all were deep, ranging in depth from 8 to 20 feet, and all were spring fed.

It has been documented that *Gambusia affinis* has adapted to environments with fluctuating temperatures. Growth and fecundity are increased in environments with fluctuating temperatures (Hubbs 1971; Johnson 1976a). A diel rhythm in heat resistance also occurs in *G. affinis* (Johnson 1976b). Midday heat resistance has demonstrated survival value (Johnson 1976b). Hubbs (1971) found that populations of *Gambusia affinis* were most abundant in fluctuating temperature environments in summer and in constant temperature habitats during winter. Observations by the authors have further supported this fact in California. Rearing ponds with shallow design demonstrate fluctuating temperature environments in the summer, but lack stenothermal conditions in the winter. By constructing a pond with deep sections and by supplying pump or spring water, a pond suited to overwintering *G. affinis* could be established.

In the fall when ambient temperatures drop, *G. affinis* may move offshore to stenothermal habitats. To avoid predation during the offshore movement the ponds should be devoid of deeper water piscivores.

After reviewing the preceding it was determined that the following designs for pond construction would help in maintaining overwintering populations of *G. affinis* (Figures 1 and 2). Ponds of similar design and dimensions are presently under construction at the Eastside Mosquito Abatement District site. They will be stocked with *G. affinis* and the populations will be monitored throughout the year as to their productivity and overwintering capabilities, in conjunction with the ponds' physical and chemical conditions.

ACKNOWLEDGMENTS.–This research was supported by funds from Eastside Mosquito Abatement District and

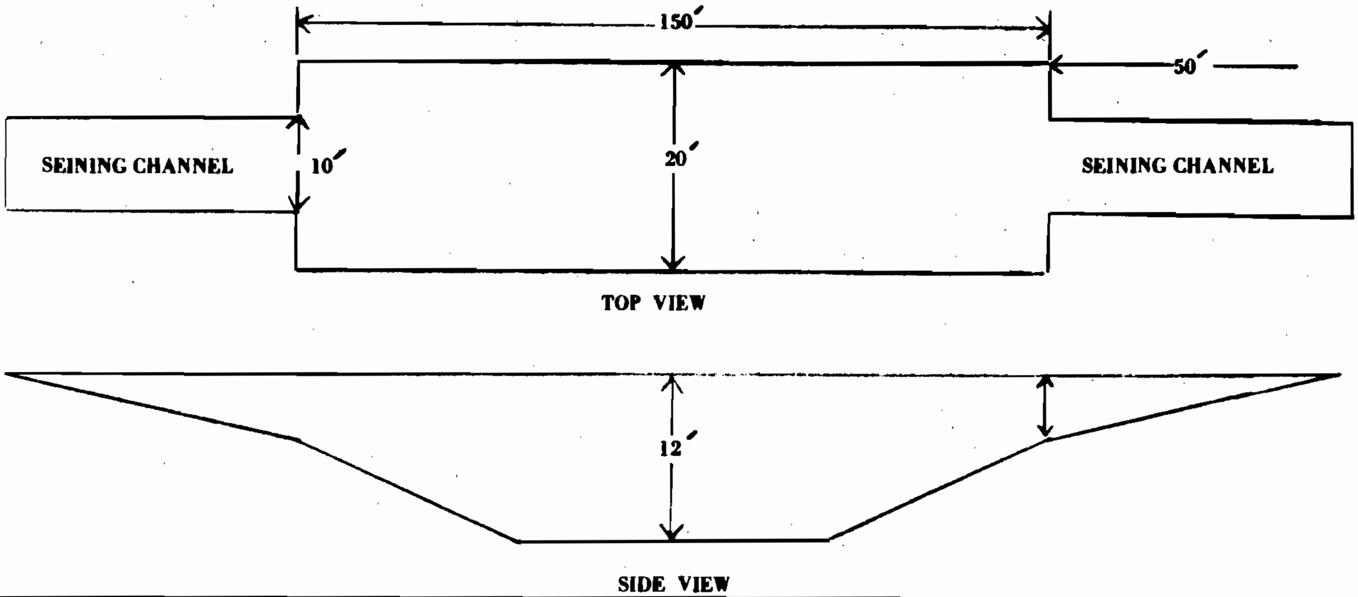

Figure 1.–Suggested pond design for overwintering *Gambusia affinis*.

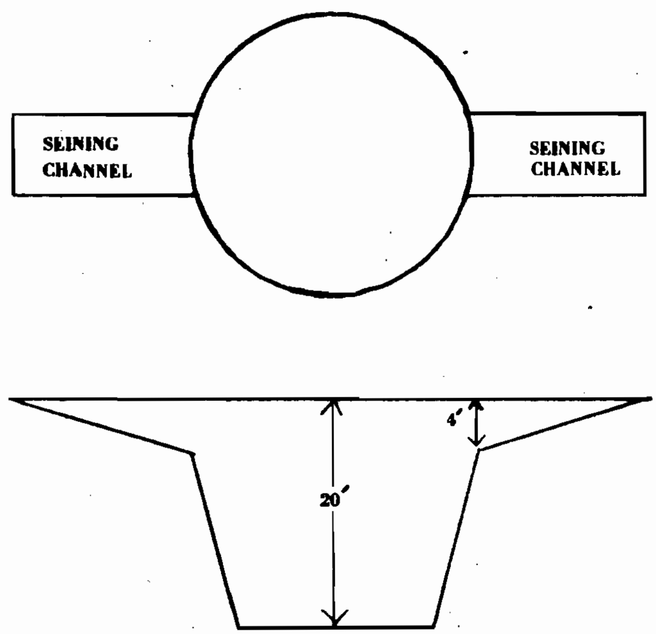

Figure 2.–Suggested pond design for overwintering *G. affinis*.

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California State Legislature Special Allocation for Mosquito Research.

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A HEAT EXCHANGE SYSTEM FOR USE IN WARM WATER AQUACULTURE

Clifford Ray Johnson[1] and Paul A. Gieke[2]

Large quantities of heated water are required for year around production of *Gambusia affinis*, *Tilapia zillii*, and *T. mossambica* in California. Such year long production of these fishes is important to provide stock fish for large spring and summer production programs. The following is a description of a system involving heat exchangers that will provide warm water at temperatures necessary for continuous production of these fishes and can be used for the culture of other warm water aquatic animals.

The system was first set up and tested on a small scale at the Eastside Mosquito Abatement District (Figure 1). Figure 2 presents a diagram of the system. In this system water was heated by a commercial pool heater supplied with propane, but natural gas could also have been used. The heated water (80°F) was circulated (pool pump) through a rectangular network of galvanized pipe 2 inches in diameter submerged 3 feet beneath the water surface of an 18,000 gallon pond. The pipe network was supported within the pond on concrete blocks. Thus an enclosed water system which could act as a heat exchanger was constructed. Since the pond was only 4 feet deep and uncovered, heat loss to the atmosphere was considerable. The test was to be made with the simplest heat exchanger under the most adverse conditions. However, even with the substantial heat loss, the average temperatures within the pond were maintained some 12°F ($\bar{x}$ = 57.9°F, range 48-68°F) above the surrounding unheated ponds $\bar{x}$ = 45.5°F, range 34-55°F) during the winter months (January-February

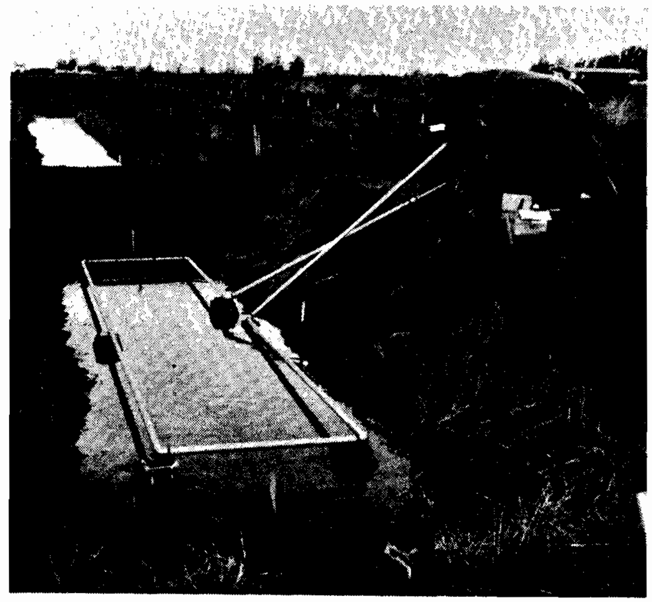

Figure 1.–Photograph of the test heat exchanger system at the Eastside Mosquito Abatement District.

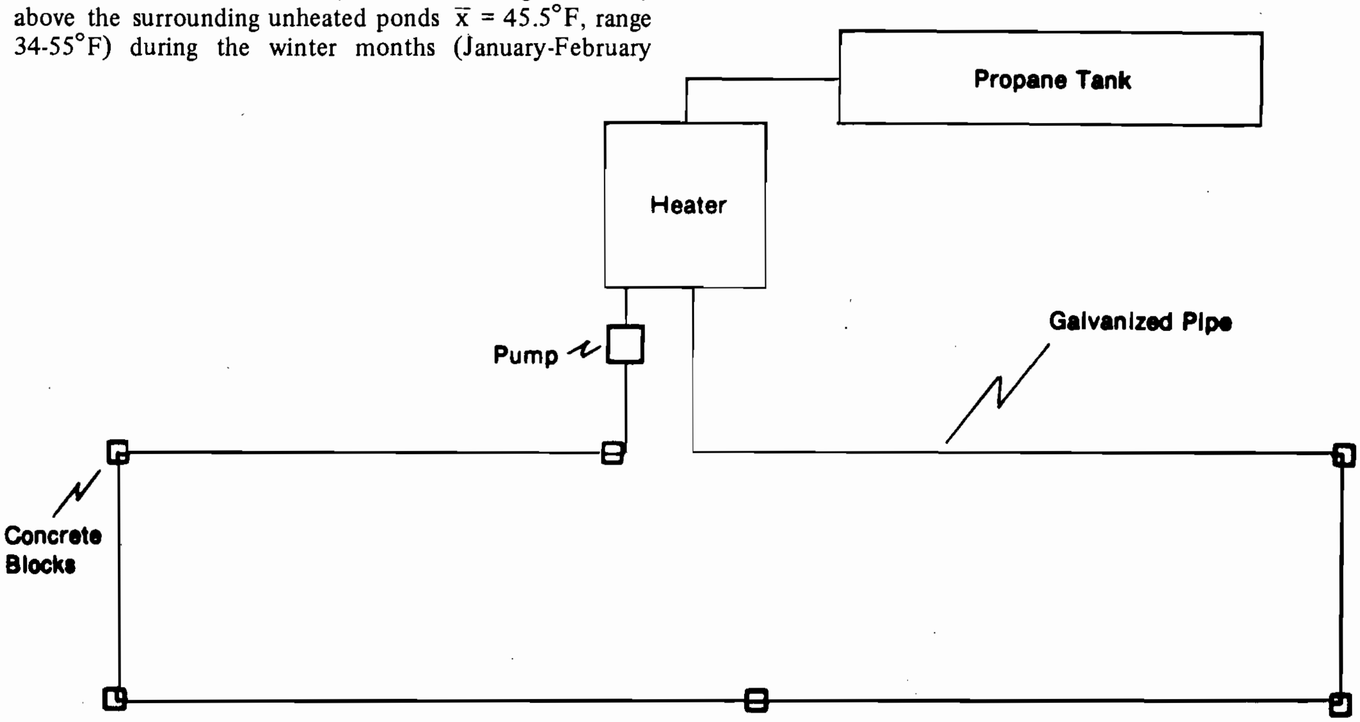

Figure 2.–A diagram of the heat exchanger system tested at the Eastside Mosquito Abatement District.

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1976). As mentioned before, the test was made under minimum operational design to test the feasibility of the system, and since heat loss was considerable the cost of operating the system was excessive. However, it did prove that the system would work and therefore modifications could be made which would minimize heat loss and cut costs. To this end, the pond should be covered at night and during cloudy weather.

A more efficient heat exchanger could be used within the pond. In the above case, the galvanized pipe itself was the heat exchanger. Short pieces of wire (6 inches to 1 foot in length) could be soldered at intervals along the entire network of pipe such as shown on a heat pump collector (Figure 3). Another efficient heat exchanger is the car radiator, although problems of metal toxicity and leakage should be evaluated. Radiators could be installed in series or at intervals along the pipe. Heated water passing through these radiators would impart maximum heat flow to the surrounding pond water. Such a system has the advantage of being able to heat a small volume of water with either propane or natural gas and in turn heat a larger volume of water, in this case the fish pond itself. A system such as described above could be used for heating small rearing ponds which could supply the needs of a district with small fish requirements. If fossil fuels are used, the size of the pond that can be heated is limited due to the increasing operating costs with increasing pond size. The use of fossil fuels is also limited by their ever increasing cost and limited supply.

This system can be used on a larger scale, if a heat source other than fossil fuels is used. It requires being located adjacent to an industrial site which uses cooling ponds or a warm water discharge in its operation. Within most California districts such plants or industrial facilities exist such as canneries, other food production plants, and power companies which have not switched to a cooling tower system. Arrangements are being made to install this system within the Eastside Mosquito Abatement District in conjunction with a cooling pond at an Oakdale food manufacturing plant. A diagram of the system is presented in Figure 4. Various modifications can be made to attain the desired temperature range required for various fish or aquatic species and for varying pond dimensions. The cost of installing a large system is of course more costly than a smaller one, but operational costs are minimal because it is using hot water as a heat source which was originally heated by some industrial process. Therefore animals are being reared at a private company's energy expense.

The system operates by passing heated water from the cooling pond through an elaborate network of efficient heat exchangers, such as described above, which in turn transfers the heat to the pond water. One of the main advantages of this system, besides the low operational costs, is that the water quality of the cooling pond is unimportant because this water is being circulated within an enclosed system and never contacts the culture environment. However, if the water is of a corrosive nature it can cause problems with the heat exchanger system. Another advantage is that only electrical power to drive the pumps is being used and a dependency upon fossil fuels is eliminated. The system is almost limitless, provided large cooling ponds can be found and arrangements made to use them. Such large cooling ponds usually have water in sufficient quantities and high temperatures to supply heated culture ponds of between 30,000 to 100,000 gallons in capacity.

This system can also be backed up with a solar or heat pump system which could be useful to maintain desired temperatures during extremely cold weather or other adverse conditions, such as times of high winds. Winds are a prime factor as they increase heat loss at the water surface and efforts should be made to place the ponds in a sheltered aspect, if at all possible.

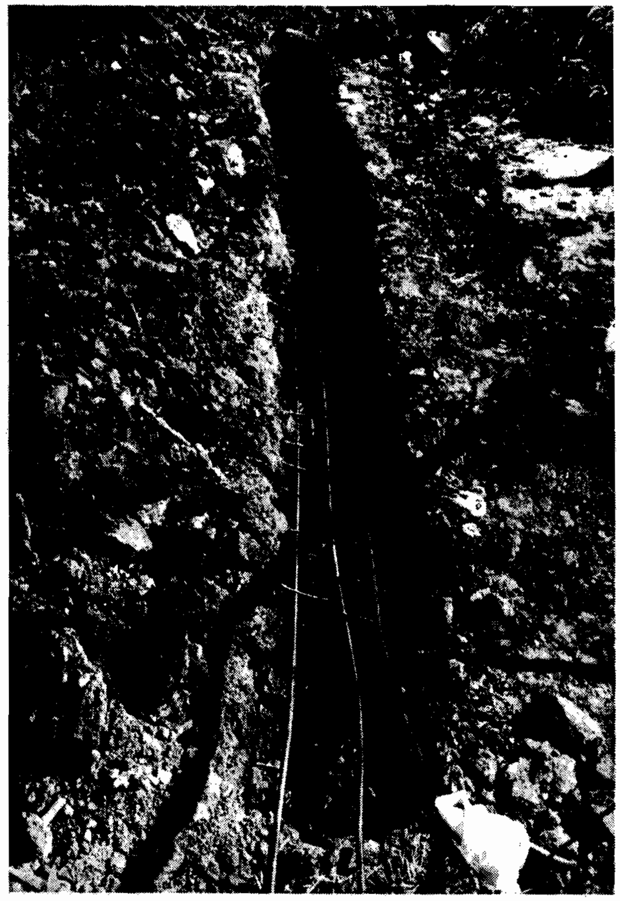

Figure 3.—Photograph of a heat pump collector showing the short pieces of wire soldered at intervals along the copper tubing to increase heat collection.

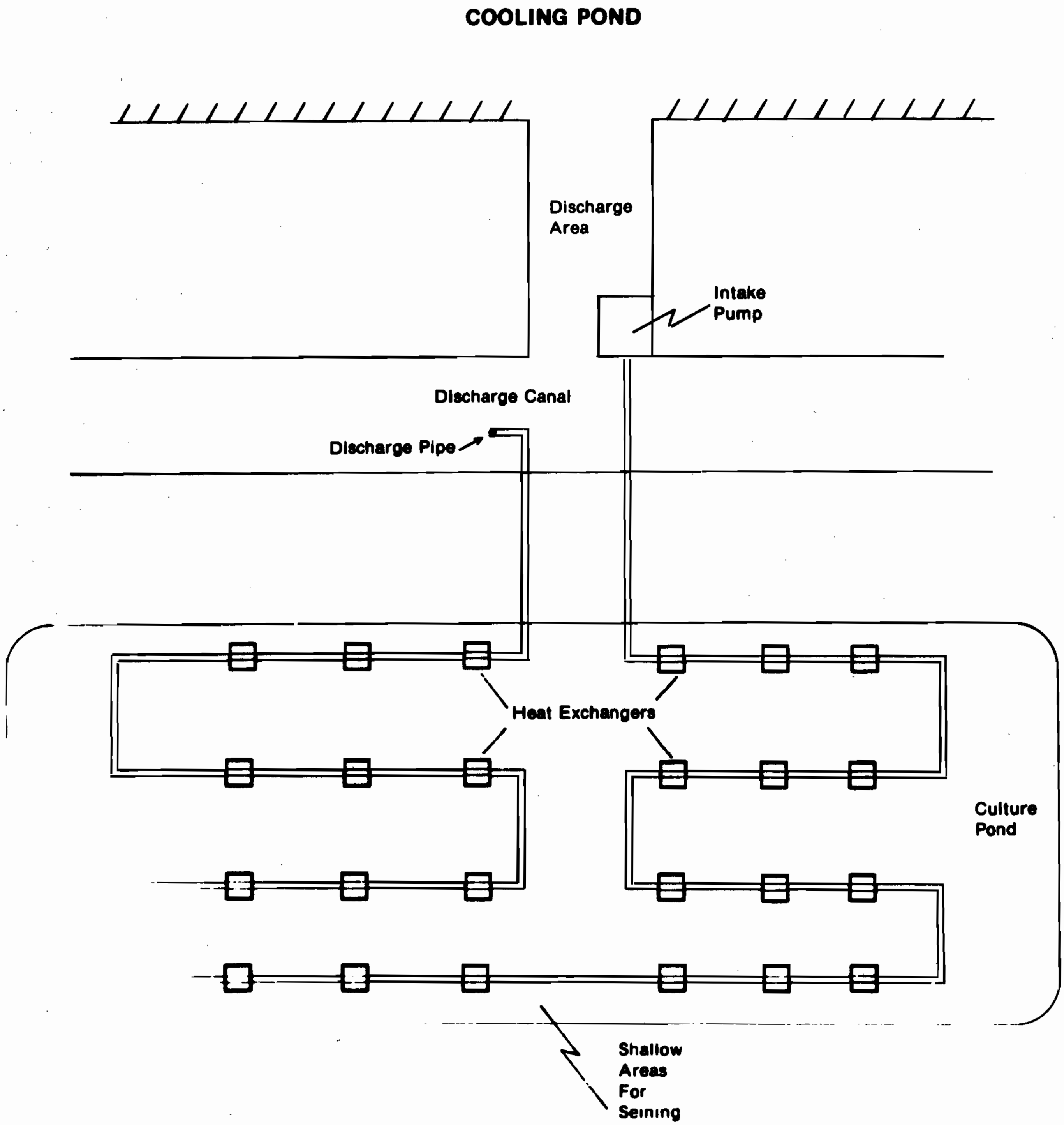

Figure 4.–A diagram of a heat exchange system used in conjunction with an industrial site cooling pond and discharge.

PILOT PROJECT FOR THE INTENSIVE CULTURE OF *GAMBUSIA AFFINIS* (BAIRD AND GIRARD)

PART IV – THREE YEAR EVALUATION OF OPERATION AND PRODUCTION EFFICIENCIES

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INTRODUCTION.–This paper presents the summation of a three year pilot project for the intensive culture of the mosquitofish, *Gambusia affinis* (Baird and Girard). A description of the specialized facility and operational methods were reported in Challet and Rohe 1974, Challet et al. 1974, and Reynolds 1975.

Updated fish production and cost estimate data are included. Improvements to the facility are suggested to increase production and efficiency. In addition, modification of this system to meet the needs of agencies requiring smaller production levels or lower costs is discussed.

The pilot project began in 1973 at the Orange County Vector Control District, then known as the Orange County Mosquito Abatement District. Our primary objective was to produce and hold large numbers of *Gambusia* for early spring stocking of temporary and permanent sources. Maximum production of fry in our heated ponds is required during winter when little or no *Gambusia* breeding occurs in local natural ponds.

METHOD OF OPERATION.–The techniques for collecting, sorting, rearing, and weighing mosquitofish were comparable during the three year program. Adult *Gambusia* were collected from golf course ponds, lakes, and our own District holding ponds. Gravid females were sorted by hand and placed in culture cages with 3.2 mm. square (8 x 8 mesh) fiberglass screen. As *Gambusia* fry were born they passed through the screen into a pond area void of large mosquitofish.

Fry were moved from the culture cage section to a rearing section biweekly, except in 1974 when fry were moved weekly. Then females that had dropped their young were counted and replaced with gravid females. When fry were four to six weeks old, they were harvested, weighed and counted by the scale method (Leitritz 1959).

Gambusia fry and adult fish were fed Purina® Trout Chow No. 1 at 3 percent of their body weight as a dietary supplement.

Pond water was circulated, filtered, and heated to 24-27°C. In addition, a photoperiod of fifteen hours was maintained by using overhead incandescent light.

RESULTS AND DISCUSSION.–*Gambusia* Fry Production – Yearly fry production showed a steady increase from 25,826 in 1973, to 38,720 in 1974, and 43,064 in 1975 (Figure 1).

Fry production in 1973 was low. Use of culture cages and records of *Gambusia* fry production did not begin until May 14, 1973. In 1974 and 1975 fry production records began on January 1. *Gambusia* production record for 1975 is found in Table 1.

On a monthly basis, the greatest number of fry were harvested during August of 1973 and July of 1974 and 1975. However, since the time for harvesting and weighing fry lags two to six weeks behind the actual production period, greater fry production actually occurred in July of 1973 and June of 1974 and 1975. Another study also indicates a larger brood size for *Gambusia* in July than August (Wu et al, 1974).

As expected, fewer fry were observed during the winter months from October to April. Fry production at our facility is reduced in the winter because gravid *Gambusia* for stocking our culture cages are not available in quantity from the field.

The average number of fry surviving from birth to the time they were harvested, weighed and counted (two to six weeks) was 10.7 fry per female in 1973 and 9.3 fry per female in 1975. In 1974, the average was 5.3 fry per female. This may relate to the fact that in 1974 fry were moved from the culture cage section to a rearing section on a weekly basis rather than biweekly, as in 1973 and 1975.

Summary of *Gambusia* Fry Production Data

Year	Gravid Females that dropped Fry	Total Fry Harvested	Fry/ Female	Total Weight of Fry	Average Weight Per Fry
1973	2,419	25,826	10.7	2,890.6 g.	0.11
1974	7,293	38,720	5.3	2,700.7 g.	0.07
1975	4,648	43,064	9.3	2,645.0 g.	0.06

Cost Summary for *Gambusia* Fry Production

	50% of Total Operational Costs [(a)]	Fry Production	Cost per Fish
1973	\$3,179.50[(b)]	25,826	\$0.12
1974	\$3,103.25	38,720	\$0.08
1975	\$3,720.23	43,064	\$0.09

[(a)]Fifty percent of fish rearing facility is used to directly breed *Gambusia* fry.

[(b)]Costs do not include capital cost of \$4,725.18 for pond construction, which raises cost per fish to \$0.22 for 1973. Cost and fish production data for 1973 and 1974 were published in Challet et al. 1974 and Reynolds 1975. Cost data for 1975 are found in Table 2 of this paper.

Based on a three year average (1973 - 1975), \$3,334.33 was spent to produce 35,870 fish costing \$0.09 per fish.

CONCLUSION.–Evidence of *Gambusia* fry production throughout the year by artifical means has been established. The outdoor fish rearing facility operated well during the project and has met the author's expectations in terms of function, reliability and adaptability for various projects.

Table 1.–*Gambusia affinis* production record - rearing tank no. 1.

Rearing Record 1975							Batch Production			Accumulative Production		
Female Drop Period				Rearing Period								
Batch No.	Start Date	End Date	Total Days for Female Drop	Harvest Date	Total Days Drop to Harvest	No. Gravid Females that Dropped	No. of Fry	Total Weight of Fry	Weight per Fish (grams)	Total no. of Fry	Total Weight	Average Weight per Fish
1	12-11-74	12-18-74	7	1-2-75	15-22	30	313	25.0	0.08	313	25.0	0.08
2	12-18-74	1-2-75	15	1-15	13-28	40	438	35.0	0.08	751	60.0	0.08
3	1-2	1-15	13	1-29	14-27	34	363	29.0	0.08	1,114	89.0	0.08
4	1-15	1-29	14	2-13	14-28	47	500	40.0	0.08	1,614	129.0	0.08
5	1-29	2-13	15	2-26	13-28	55	875	87.5	0.10	2,489	216.5	0.09
6	2-13	2-26	13	3-12	14-27	50	833	75.0	0.09	3,322	291.5	0.09
7	2-26	3-12	14	3-26	14-28	53	856	77.0	0.09	4,178	368.5	0.09
8	3-12	3-26	14	4-9	14-28	218	1,742	104.5	0.06	5,920	473.0	0.08
9	3-26	4-9	14	4-23	14-28	241	1,643	115.0	0.07	7,563	588.0	0.08
10	4-9	4-23	14	5-7	14-28	230	1,683	101.0	0.06	9,246	689.0	0.07
11	4-23	5-7	14	5-21	14-28	245	1,757	123.0	0.07	11,003	812.0	0.07
12	5-7	5-21	14	6-4	14-28	388	3,767	226.0	0.06	14,770	1,038.0	0.07
13	5-21	6-4	14	6-18	14-28	489	3,825	229.5	0.06	18,595	1,267.5	0.07
14	6-4	6-18	14	7-2	14-28	352	2,990	149.5	0.05	21,585	1,417.0	0.07
15	6-18	7-2	14	7-16	14-28	331	3,240	162.0	0.05	24,825	1,579.0	0.06
16	7-2	7-16	14	7-30	14-28	290	2,808	168.5	0.06	27,633	1,747.5	0.06
17	7-16	7-30	14	8-13	14-28	283	2,840	142.0	0.05	30,473	1,889.5	0.06
18	7-30	8-13	14	8-27	14-28	210	2,400	144.0	0.06	32,873	2,033.5	0.06
19	8-13	8-27	14	9-10	14-28	194	2,250	135.0	0.06	35,123	2,168.5	0.06
20	8-27	9-10	14	9-24	14-28	201	2,325	139.5	0.06	37,448	2,307.5	0.06
21	9-10	9-24	14	10-8	14-28	150	1,333	80.0	0.06	38,781	2,387.5	0.06
22	9-24	10-8	14	10-22	14-28	126	933	56.0	0.06	39,714	2,443.5	0.06
23	10-8	10-22	14	11-5	14-28	87	617	37.0	0.06	40,331	2,480.5	0.06
24	10-22	11-5	14	11-19	14-28	70	567	34.0	0.06	40,898	2,514.5	0.06
25	11-5	11-19	14	12-3	14-28	81	750	45.0	0.06	41,648	2,559.5	0.06
26	11-19	12-3	14	12-17	14-28	74	683	41.0	0.06	42,331	2,601.0	0.06
27	12-3	12-17	14	12-31	14-28	79	733	44.0	0.06	43,064	2,645.0	0.06

Table 2.–Operational cost estimate for 1975.

A. Utility costs from January 1 - December 31, 1975:

Utility	Units Used	Cost
Gas	3,798 Thermal Units	$ 459.65
Electricity	7,888 Kilowatt-Hours	299.76
Water(a)	0.413 Acre Feet	7.05
	Total	$ 766.46

B. Wage and benefit expenses for fisheries biologist(b)	$6,510.00
C. Water softening tank refills (24 ea)	108.00
D. Purina® Trout Chow (150 lbs)	32.00
E. Sand for filter (3 sacks)	11.00
F. Muriatic acid (3 gals)	3.00
G. Logwood oil (2 gals)	10.00
Total operational costs.	$7,440.46

(a)Our water is from a well on District property. A more realistic cost of water from Garden Grove Water Department would be $36.70.

(b)Salary for fisheries biologist was determined by percent of actual time spent on this project (50%).

In order to achieve our objective, a fry production rate of 50 percent of the District's total stocking program was desired. The yearly total of *Gambusia* stocked from 1973 to 1975 averaged 200,234. The average number of *Gambusia* fry produced annually at our fish rearing facility during this same period was 35,870. Therefore, the facility provided only 18 percent of the fish used in our *Gambusia* stocking program. In terms of fry production, the facility did not produce the desired 50 percent level. However, our heated ponds held large numbers of *Gambusia* during the winter for early spring stocking.

The facility made a significant contribution to our overall mosquito biological control program. It provided a large number of readily accessible, healthy fish to stock in small sources such as fish ponds, ornamental ponds, portable circular pools and swimming pools. Actually, if we consider only small sources, the District stocked a yearly average of 37,446 *Gambusia* from 1973 to 1975. Thus, the facility produced sufficient fish to stock 96 percent of those sources. If we compare this with a yearly average of 162,788 *Gambusia* stocked in large sources, the facility provided only 22 percent of the fish required in those sources. This may be important since urbanization in Orange County has reduced the number of large sources while increasing the quantity of small sources.

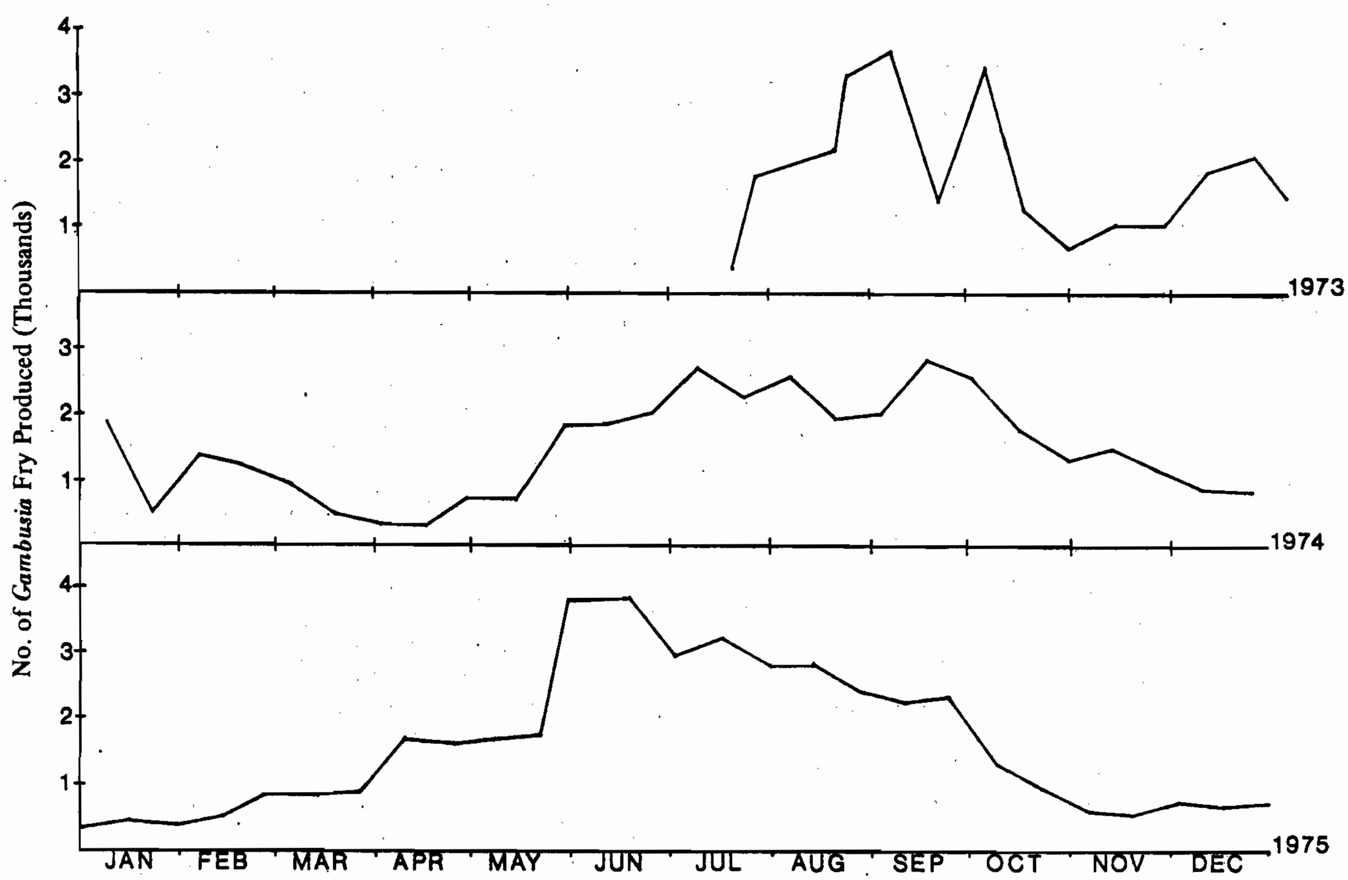

Figure 1.–*Gambusia* fry production record 1973-75.

A major problem in *Gambusia* fry production is encountered during the winter months. There is a lack of gravid females from the field to stock our culture cages. This is reflected by our 1973 to 1975 data, showing only 37 percent of total fry production occuring from October thru April (Figure 1). Our heated ponds provide some gravid females during winter but the expense of heating larger ponds to supply adequate gravid females would be prohibitive. Solar heating may solve this problem in the near future. Investigation in this area is being conducted by Johnson (1976). Low cost solar heating would allow us to heat large ponds not only to provide more gravid females but for holding thousands of *Gambusia* for early spring stocking.

Additional projects under investigation at the Orange County Vector Control District, which are intended to reduce cost and increase *Gambusia* production, include: the use of shallow soil bottom ponds using warm water from power or sewage treatment plants, natural sunlight compared to various types and intensities of artificial light, and low cost vinyl-lined holding ponds. In addition, since labor is a major cost, a sorting device for gravid females is being studied. This pilot project will be used to determine the feasibility of a permanent facility. Comments will be directed toward this topic as well as modification of this facility for districts requiring fewer mosquitofish or lower operational costs.

A permanent facility can be justified by using solar heat, a sorting device for gravid females, and low cost holding ponds. A single concrete raceway 50′ x 4′ x 2′ is recommended for low operating cost and harvesting efficiency. This would be used as a breeding pond with at least ten culture or breeding cages in a thirty foot section with a separate twenty foot section for rearing fry until time of harvesting and weighing. Circular portable pools can be substituted for the concrete raceway and wooden breeding trays used instead of culture cages (Johnson 1976). However, a series of long shallow ponds are recommended for rearing fry and holding large numbers of *Gambusia* to early spring stocking. This will facilitate easy removal of *Gambusia* by seining as well as lowering heating costs. An algacide may be required to keep these ponds free of algae or to maintain the desired level of plankton.

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AQUACULTURAL STUDIES OF MOSQUITOFISH, *GAMBUSIA AFFINIS*, IN EARTHEN IMPOUNDMENTS: STOCKING RATE OPTIMIZATION FOR YIELD, PROTECTION OF OVERWINTERING FISH STOCKS

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ABSTRACT

A mosquitofish stocking rate of 50 lbs per acre in fractional acreage ponds resulted in the highest mean yield and the most efficient use of feed and fertilizer under the given conditions of this summer field study. Additionally, the mean daily growth rate approached 1%, while a food conversion ratio of 1.88 was achieved with this stocking rate. A stocking rate of 25 lbs of fish per acre provided the highest growth rate (1.0% per day), but mean fish densities were lower than those attained with the 50 lbs per acre stocking rate. Stocking rates of 75 lbs per acre resulted in less growth and signs of excessive habitat exploitation were apparent.

In the following winter, fish populations in ponds having bird exclusory barriers constructed of plastic netting in the form of fencing and overhead covers had a spring survival rate of 25.2% (76% of all recovered fish). Ponds having only netting fences provided a 7.8% survival rate, while unprotected control ponds had only a 0.1% survival rate. Bird counts were conducted throughout the winter and incursion by birds was concluded to be deleterious to overwintering mosquitofish stocks. Practical recommendations for summer and winter fish culture and protection are provided.

INTRODUCTION.–Continuing field experimentation is being conducted regarding the mass culture of mosquitofish, *Gambusia affinis*, in outdoor earthern impoundments at the Sutter-Yuba Mosquito Abatement District. The District hopes to eventually rear the majority of fish needed for our biological control program.

In 1975 and early 1976, two studies were completed which have enabled the District to better manage its mosquitofish stocks. The first experiment was designed to provide information concerning optimal stocking rates for summer fish production. These data would allow the District to maximize summer fish yields with the least amount of initially-stocked fish. The second experiment was designed to reveal information pertaining to the deleterious effects migratory and resident bird populations had upon our overwintering mosquitofish. In addition, data from this study could be used to compare the effectiveness of certain types of physical barriers to bird incursion.

MATERIALS AND METHODS.–Stocking rate/production study - Three stocking rates were selected for this experiment; these were 25, 50 and 75 pounds of adult mosquitofish per pond surface acre. Nine ponds were utilized; thus each of the three treatments was twice replicated. All ponds were of a similar construction, although their measurements differed somewhat. Pond configuration was rectangular with westwardly-increased water depths. A central drainage channel was excavated as was a pit at the west terminus of each pond to facilitate fish harvest activities. On the average each pond was 301 feet in length, 63 feet in width, 1.7 feet in depth and encompassed 0.43 surface acres.

All ponds received water from a common well and their respective levels were maintained as similar as possible. Mean pond volume was 0.74 A-ft. Granular fertilizer (16-20-0, NPK) was applied to the ponds at an initial 100 pounds per acre rate, followed by periodic 50-pound per acre applications whenever Secchi disk turbidity measurements exceeded 1.5 feet. The use of this fertilizer encouraged the growth of plankton which was to serve as food and protective cover for the fish. The fish were fed daily; however, the quantity of food applied at each feeding varied with respect to water temperature and the fish biomass in an individual pond. This ration ranged between five and ten percent of the current biomass. Sample seinings of three ponds, each representing a different stocking rate, were conducted monthly to determine the biomass present through the growing season.

Undesirable aquatic vegetation was controlled through the use of simazine (80%, wettable) at a dosage rate of three parts per million. This material promoted subsequent phytoplankton blooms after each die-off; thereby contributing to the proliferation of plankton. The period of this study was 2 May - 31 October, 1975.

Bird exclusion study - Three levels of exclusory protection, as provided by the varied use of Conwed Plastic Bird Netting (supplied in 14′ x 5000′ rolls, ¾″ x 11/16″ mesh), were tested in this winter experiment. The same nine ponds of the preceding study were used in this study; therefore the basic physical parameters of the ponds were essentially unchanged.

In terms of experimental design, three ponds (controls) didn't receive any exclusory netting structures, while three others (low protection treatments) had net fencing installed parallel to the shoreline at a 4.7-foot height. The remaining three ponds (high protection treatments) had, in addition to the aforementioned fences employed in the low protection treatment, horizontal overhead net barriers stretched perpendicular to the length of each pond in eight evenly-spaced, 14-foot wide rows. Thus, the experiment consisted of a control and two experimental variables twice replicated. Actual layout of the experiment provided that no two similarly treated ponds were situated adjacent to each other.

The quantity of fish initially stocked in this second study differed from pond to pond, as the fish harvested in the preceding experiment were simply weighed and restocked into the individual ponds from which they had been harvested. Supplemental feeding was conducted on a fairly regular schedule, which was controlled primarily by prevailing weather conditions on a given day. The actual quantity of food applied at any feeding corresponded to the fish biomass and water temperature; this usually amounted to between one and four percent of the estimated fish biomass. Bird counts were conducted regularly and all waterfowl and shorebirds sited in or near a given pond were recorded. This experiment was conducted from 10 December to 6 May, 1976.

RESULTS AND DISCUSSION.–Stocking rate/production study - The results of the periodic sample seinings are charted in Figure 1. The major results of this experiment are presented in Table 1.

As a treatment variable, the intermediate stocking rate (50 lbs/A) provided the highest mean yield with respect to surface area and volume and the best utilization of food and fertilizer. For these reasons it was selected as the optimum stocking rate of those tested. The low stocking rate (25 lbs/A) provided the best growth, but maximum densities were apparently not attained because food, nutrients and habitable space were incompletely exploited by the fish populations comprising this treatment. Conversely, the high stocking rate (75 lbs/A) resulted in signs of excessive exploitation, as nutrient conversions and growth were apparently impeded. Under the cultural conditions imposed in this experiment, the optimum stocking rate would most likely be placed somewhere between the low and intermediate rates. Other indirect data not presented suggest that in terms of practical management the following recommendations could be forwarded:

1. Provide at least a six-month summer growth period.
2. Predators and competitors, such as bullfrogs, their tadpoles, and birds should be excluded from the summer culture sites. The August decline in the seined biomass representing the high stocking rate (Fig. 1) was most likely the result of the tremendous numbers of tadpoles in that pond which competed with the fish for available food and habitat.

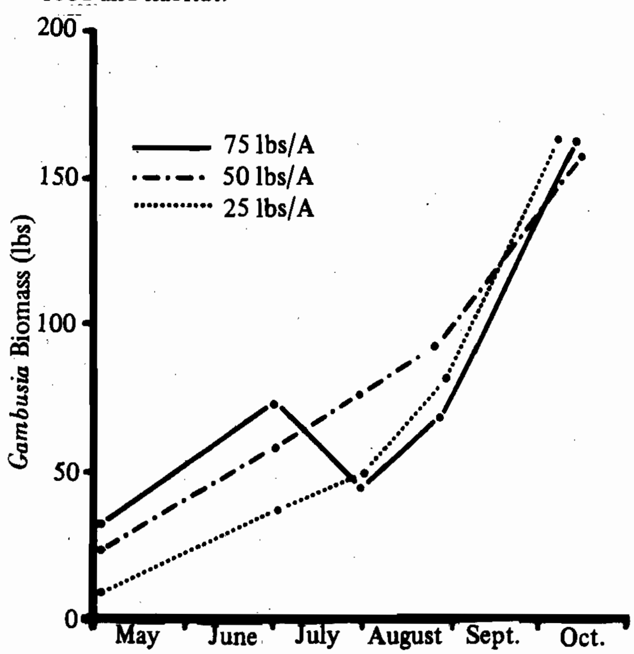

Figure 1.–Periodic sampling of *Gambusia affinis* in three ponds where each impoundment represented a different experimental stocking rate.

Table 1.–Results of stocking rate/production study grouped by experimental treatment mean values, May through October, 1975.

Evaluation Criteria	Stocking Rate (lbs/A) 25	50	75
Net Fish Yield (lbs harvested)	200.4	193.2	187.4
Net Fish Yield/Area (lbs/A)[a]	431.4	467.3	438.6
Final Fish Density (lbs/A-ft)[a]	219.1	301.5	275.3
Food Conversion (lbs food/lbs fish)	2.13	1.88	2.36
Fertilizer Conversion (lbs fert/lbs fish)	0.83	0.75	0.80
Growth Factor, K (% increase/day)	1.07	0.98	0.88

[a]Weighted with respect to differences in individual pond configuration.

Table 2.–Results of bird exclusion study grouped by experimental treatment means, December, 1975 to May, 1976.

Evaluation Criteria	Treatment: Control	Low Protection	High Protection
Fish Yield (lbs)	0.2	16.7	53.4
Percentage Yield, by treatment (%)	0.2	23.8	76.0
Overall Survival (%)	0.1	7.8	25.2

Table 3.–Bird observation data compiled and grouped by protection method.

Bird Species Enumerated	Actual Number of Sitings: Control Ponds	Low Protection Ponds	High Protection Ponds
American bittern, *(Botaurus lentiginosus)*	4	4	5
American coot, *(Fulica americana)*	618	138	339
Ducks, (Anseriformes)	145	133	50
Pied-billed grebe, *(Podilymbus podiceps)*	5	4	0
Herons, (2 species), *(Ardea herodias & Butorides virescens)*	3	0	0
Total Sitings =	775	279	394

3. Filamentous algae, emergent grasses, and floating aquatic plants should be adequately controlled; however, most planktonic algal forms should be encouraged.
4. Muskrats, squirrels and other burrowing rodents should be excluded because they cause much damage to pond

levees and roadways. Crayfish have been useful in the control of noxious weeds and their burrowing hasn't harmed levees or roadways.

Bird exclusion study - The results of this experiment are presented in Table 2. Table 3 provides a compilation of bird observation data.

Date collected in this latter experiment strongly suggest that birds were the cause of poor overwinter survival, at least during this particular winter season. There was practically no fish survival in the unprotected controls (0.1% survival); whereas perimeter fencing of the low protection treatment provided a small measure of exclusory protection (7.8% survival). However, the best survival (25.5%) occurred in the high protection treatment ponds which had both perimeter fences and overhead rows of netting. Over 76% of all fish recovered in the spring were seined from those ponds receiving this highest level of protection. Unfortunately, the yield data and bird siting counts clearly show that all birds weren't excluded in the high protection treatment. Since open gaps existed in the overhead netting structures, it was only a short time before birds learned that they could penetrate those gaps. In fact, one pond representing the high protection treatment whose great width didn't lend itself to an effective netting installation had very high bird counts and poor fish survival. Had this pond's barriers been as effective as those of the other two ponds comprising this treatment, there would probably have been a rough inverse correspondence in bird counts versus fish survival.

It wasn't possible within the scope of this experiment to conclude what bird species or how birds actually caused the reduction in fish biomass. Predators, such as American bitterns, *Botaurus lentiginosus*; pied-billed grebes, *Podilymbus podiceps*; great blue herons, *Ardea herodias* and green herons, *Butorides virescens* were always difficult to observe and as a possibility, consumed large numbers of fish despite our harrassment activities. Another possible factor was that birds and fish competed for submerged vegetation and continual grazing by American coots, *Fulica americana* and ducks, (Anseriformes) may have reduced vegetative shelter to the degree that the habitat was less able to support the fish and the reduced vegetation aided predators. A third potential factor was that there may have been a reduction in metabolic reserves in the fish brought about by the continual disturbances caused by the large number of grazing birds swimming about each pond. This potential loss of reserves may have weakened the fish so they may have been easier prey and more susceptible to disease and parasitic infestations. Each of the aforementioned factors may have been operative and the combination of them may have been very influential with respect to fish survival.

After considering observations and results of this experiment, the following recommendations can be advanced:

1. All future pond construction and/or modification in this locale should facilitate the installation of netting barriers to deter bird incursion.
   a. Narrow ponds (less than 30′ in width) are much easier to screen than ponds having a greater width.
   b. Pond depth should ideally be five feet or greater because certain emergent weed species will be inhibited in deeper waters. Deeper ponds also offer greater overwinter temperature stability which may provide a better habitat for the fish.
2. Even if netting barriers are employed, some supplemental bird harassment will probably be necessary.
3. The choice of a specific netting material should be made only after consideration of the following criteria.
   a. Prevailing wind and direction at the pond site necessitates proper materials and placement.
   b. Pond configurations should be small to facilitate installation of barrier netting.
   c. Levee construction should be such that the netting cover will not be excessively exposed to wind.
   d. Netting material selected for pond use should have a high tolerance to ultraviolet radiation. It should also be selected with respect to mesh size, wind velocities and tensile strength. Useful lifespan and cost of the netting will probably be the most important factors to be considered.
4. Netting installation criteria.
   a. Netting runs should be as short as possible to minimize loading on fasteners, guys and support anchors.
   b. The netting should cover as much of the water surface as is practicable.
   c. Netting should be placed as close to the water surface as possible to reduce wind loadings.
   d. Support systems should be secure and free from undue flexing.
   e. Netting should be installed with removal and storage ease in mind.
   f. Terrestrial and emergent weed control should be conducted along all netted shorelines, as once the vegetation grows through the netting its removal is very difficult.

STOCKING DATE VERSUS EFFICACY OF *GAMBUSIA AFFINIS* IN FRESNO COUNTY RICE FIELDS

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ABSTRACT

The effect of early (April 26-30), middle (May 15-21), and late (June 14-18) stocking of *Gambusia affinis* (Baird & Girard) in rice fields was evaluated. Thirty fields were stocked with *G. affinis* at 0.25 pound per acre. Six additional fields were selected as experimental controls. Biweekly fish trapping and mosquito sampling established curves for fish populations and mosquito prevalence in the fields. Data indicate that the field age (days after seeding of rice) is a more important consideration than calendar date in fish stocking. Stocking fields 15 to 25 days after seeding gave the best fish population growth and mosquito control.

Many studies have been published concerning effective stocking rates of mosquitofish, *Gambusia affinis* (Baird & Girard), to achieve mosquito control in rice fields (Davey et al. 1974, Hoy & Reed 1970, Hoy et al. 1971, Hoy et al. 1972). However, to date few studies have been undertaken to determine the optimum stocking time for mosquitofish. Stocking date generally varies from early May (Hoy & Reed 1971) to early July in the San Joaquin Valley (Norland & Bowman 1976). Population studies have been carried out with fish stocked at various periods within this range, but no attempts have been made to correlate the results. The present study is designed to evaluate the effects of different stocking dates on the rate of fish population increase and subsequent mosquito control, and to determine whether calendar date or field age (days since seeding of rice) is the most important criterion.

MATERIALS AND METHODS.–Thirty fields were selected to receive mosquito fish at the rate of ¼ lb. of fish per acre. This rate has been shown to give adequate control of mosquitoes in Fresno County rice fields, especially when combined with occasional chemical control (Hoy and Reed 1970). The fields were divided into three groups of 10, each group being stocked at a different time; early (April 26-30), middle (May 15-21), and late (June 14-18). Table 1 lists pertinent data for each group of fields. The fields were selected such that the early stocking was 0-10 days after rice seeding, the middle stocking was 15-25 days after seeding, and the late stocking was more than 30 days after seeding. Six additional fields were selected as experimental controls.

Fish were obtained from natural populations in Fresno Westside, Kern and Coalinga-Huron Mosquito Abatement District sources. Batches from all three sources were mixed before stocking. Size selection was accomplished by seining the fish from holding ponds using 3/16 inch mesh seines. Fish were not sexed or counted, but based upon our previous experience it is presumed that they were predominantly sexually mature females at 400-600 fish per pound.

Fish populations were monitored biweekly using 8 mesh minnow traps (Reed and Bryant 1974, Norland and Bowman 1976) set for approximately 24 hours. An open topped, screened box was placed in the outflow of each field in conjunction with the trapping to determine the number and size composition of fish leaving the field. Experimental control fields were trapped in the same way to check for contamination by natural mosquito fish populations. All trapping was initiated two weeks after stocking with the exception of five of the fields in the third stocking period which were first trapped three weeks after stocking. Only mature females were considered in the population curves.

Larval mosquito sampling was on a biweekly basis and was concurrent with the fish trapping. Sampling was done using a standard 475 ml. dipper and a hand concentrator (Husbands 1969). Three 10-dip samples were taken in each field; one sample from each of three predesignated areas. Larvae were preserved in 50% alcohol and counted and identified in the lab.

In our district, 0.1 *Culex tarsalis* Coquillett per dip and 0.05 *Anopheles freeborni* Aitken per dip have been considered high enough larval counts to warrant pesticide application. Generally, study fields exhibiting these counts were sprayed with parathion. Exceptions included experimental control fields and fish fields containing only first and second instar larvae or only fourth instar larvae and pupae. The former were not sprayed since fish are better predators of later instar larvae, and the latter were not sprayed since most of the mosquitoes would have emerged by the time spraying occurred. Unless otherwise stated, all results of mosquito larval sampling are for *Cx. tarsalis*.

RESULTS AND DISCUSSION.–Fish Populations - Mean population increase for Groups I and II were nearly equal for all sampling periods (see Table 1 and Figure 1). Mean population growth for Group III was more rapid than Group I or II considering days from date of stocking, however the total population at the end of the study (August 27) never reached the same magnitude since Group III was stocked 7 and 4 weeks after Group I and II, respectively.

Increasingly good fish production in later stockings is probably due to several factors. Cover is much improved later in the season as the rice seedlings emerge and begin to tiller. Mosquito fish are much easier prey for piscivorous birds before seedling emergence. Food organisms are also more abundant as the rice season progresses.

Water temperature would seem to be a factor for increased reproductive rates in later stocked fish. However, studies indicate that mean water temperatures remain nearly constant throughout the season. Much greater temperature fluctuation occurs early in the season due to direct

Table 1.—1976 summary of *Gambusia affinis* population increase and *Culex tarsalis* prevalence.

Stocking Period	Seeding Dates	Stocked Days After Seeding		1 May 10-21	2 May 24 Je. 4	3 Je. 7-18	4 Je. 21 Jy 2	5 Jy. 5-16	6 Jy 19 20	7 Aug. 2-13	8 Aug. 16-21
1	April 20-28	0-7	Mean fish per trap	.71	.31	.29	.83	4.23	9.36	16.23	34.40
			% Sprayable inspections	0	20	70	30	10	10	0	0
2	April 20- May 6	15-25	Mean fish per trap	–	.37	.29	1.54	3.45	11.25	20.34	30.52
			% Sprayable inspections	0	0	20	30	20	10	0	0
3	April 19 May 17	30-57	Mean fish per trap	--	--	--	.98	1.79	4.28	6.11	10.62
			% Sprayable inspections	20	0	30	40	10	10	10	30
Experimental control	April 19 May 26		Mean fish per trap	0	0	0	.01	0	.05	.31	.63
			% Sprayable inspections	0	50	17	20	33	50	67	83

1. Mature females only
2. Sprayable inspection equals 0.1 per dip for *Culex tarsalis*

radiation but according to Johnson (1976), temperature fluction is not detrimental to mosquito fish. Therefore, water temperature is not likely to have caused the slower growth rate of the Group I fish.

Data from outflow trapping indicates that three times as many mature females were leaving the Group I fields 2 weeks after stocking as compared with Group II fields in the same relative time period. The increased loss of early stocked fish is likely due to the lack of cover and food. Loss of mature females from the original stock can do significant damage to the initial reproductive rate of the population. Indeed, fish stocked at 3/16 lb/acre in a group of fields with no drainage outlet showed a more rapid mean population buildup than the Group III fields stocked the same week.

Mosquito Populations - The proportion of fields in each stocking group containing sprayable concentrations of larvae (0.1 larvae per dip or more) are shown in Figure 1. The rice fields with fish generally had a peak in *Cx. tarsalis* populations in mid to late June followed by a gradual decline to low counts through July and August. Group III fields had a rise in *Cx. tarsalis* prevalence in mid to late August which is probably due to low fish populations.

Experimental control fields had a peak for *Cx. tarsalis* early in June and then dropped during periods 3 and 4. Natural invertebrate predators were abundant in the fields at this time. As the natural invertebrate predators declined, mosquito prevalence increased steadily until the end of the study. Five of the six experimental control fields developed low mosquitofish populations late in the season, but the results of the one fishless field indicates that fish in these low concentrations had little effect on the mosquito population.

Very few *Anopheles freeborni* larvae were found utilizing the methods described for the present study. However more intensive examination revealed sprayable (more than 0.05 per dip) populations of *An. freeborni* in eight of the study fields during late July and early September. These findings were made in fields with fish populations high enough to produce good control of *Cx. tarsalis*. This is an indication that *G. affinis* produces better control of *Cx. tarsalis* and *An. freeborni* in Fresno County rice fields.

The large peak in *Cx. tarsalis* prevalence in Group I fields early in June may be due to several factors. The concentration of fish in the Group I fields was the lowest during periods 2 and 3. A rapid buildup of fish in the Group I fields was the lowest during periods 2 and 3. A rapid buildup of mosquito larvae at this time could overwhelm the depressed fish population. Also, Ahmed et al. (1970) and Hess and Tarzwell (1942) have shown that the bulk of the diet of *G. affinis* consists of microcrustacea and chironomid larvae and pupae when these species are found in abundance. Our study indicates that chironomid populations peaked in early June and cladoceran populations peaked in mid June. Thus, the fish may have been feeding on more preferred organisms at the time of the mosquito buildup. In addition, we have found that early stocked fields have significantly reduced notonectid populations. This reduction of an important predator may account in part for the mosquito prevalence in Group II fields in mid June. Similar mosquito prevalence curves were found in 1974 (Reed, unpublished data).

Group II fields demonstrated the best mosquito control of the 3 groups tested. Fish population growth in Group II was nearly identical to that of Group I even though Group I fields were stocked 5 to 15 days earlier. Since the most probable reasons for the depressed population growth in

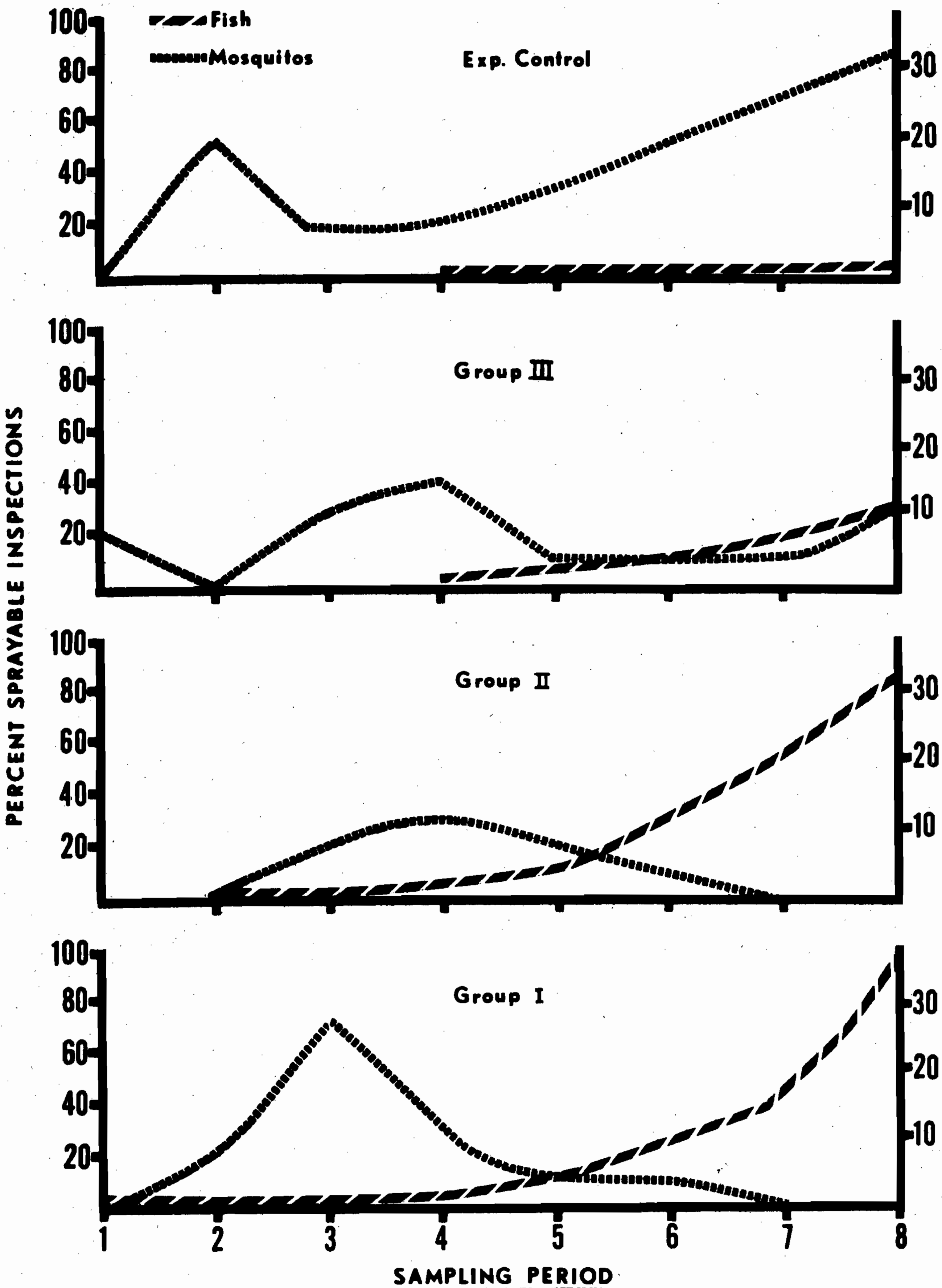

Figure 1.–1976 summary of *Gambusia affinis* population increase and *Culex tarsalis* prevalence.

Group I was lack of food and cover, it appears that the age of the field is the most important aspect to consider when stocking fields which have drainage outlets. The optimum stocking time appears to be 15 to 30 days after seeding, and preferably before June 15.

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EFFECTS OF *GAMBUSIA AFFINIS* (BAIRD & GIRARD) ON SELECTED NON-TARGET ORGANISMS IN FRESNO COUNTY RICE FIELDS

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ABSTRACT

The effects of *Gambusia affinis* (Baird and Girard) introduction on selected non-target organisms was studied. Thirty rice fields were stocked with *G. affinis* at 0.25 lb/acre. Six additional fields were selected as experimental controls. Non-targets were sampled biweekly. Backswimmer, damselfly, and dragonfly populations were significantly reduced in the presence of *G. affinis*. Hydrophilid adults, chironomids, corixids, and mayflys had lower population means in the presence of *G. affinis*, but not significantly different at the .05 level. Hydrophilid larvae, belostomids, and dytiscid larvae and adult populations were not affected by the presence of fish.

INTRODUCTION.–There has been an interest in the use of mosquitofish, *Gambusia affinis* (Baird and Girard), for mosquito control since the turn of the century in the United States. For many years, mosquitofish have been introduced and utilized throughout the world. Many of the early workers published accounts of the success of mosquitofish in controlling mosquito larvae; however, not until Hess and Tarzwell (1942) published their treatise on the feeding habits of *G. affinis* were the potential effects of the fish on non-target invertebrates known.

Since that time, many studies on the real or potential effects of mosquitofish on invertebrate groups in rice fields and other habitats have been published (Ahmed et al. 1970; Bay and Anderson 1966; Hurlbert et al. 1972; Reed and Hoy 1970; Washino and Hokama 1967). All of the studies deal either with gut samples from the fish or invertebrate samples from fields stocked with fish. The present paper is a report of aquatic invertebrate sampling in a large number of fields stocked with *Gambusia*. By studying mean population curves for invertebrates in many fields, more reliable results on the effects of mosquitofish might be obtained.

MATERIALS AND METHODS.–Thirty fields were selected to receive unsorted mosquitofish at 0.25 pound of fish per acre. The fields were divided into three groups of ten, each group being stocked at a different date during the season: Group I (April 26-30), Group II (May 15-21), and Group III (June 14-18). Six additional fields were selected as experimental controls. These were not stocked with fish or treated with public health pesticides during the course of the study.

Fish populations were monitored biweekly using 8-mesh minnow traps set for approximately 24 hours (Reed and Bryant 1974, Norland and Bowman 1976). Counts of selected aquatic insect groups in the traps were also recorded. In addition, concurrent biweekly aquatic samples were taken using a standard 475 ml. dipper and a hand concentrator (Husbands 1969). Ten dip samples were taken in three different parts of the field giving a total of 30 dips per field. The samples were preserved in alcohol and identified in the lab.

General invertebrate groups were selected for study rather than individual species. This made the identifications of large numbers of samples less time consuming. The groups selected were: dragon flys, damselflys, mayflys, chironomids, cladocerans, copepods, notonectids, belostomids, dytiscid larvae and adults, hydrophilid beetle larvae and adults and corixids. Correlating the results of concurrent dipping and trapping of invertebrates gave an indication of the abundance of each invertebrate group in the field.

RESULTS AND DISCUSSION.–Population trends of each invertebrate group based on stocking and sampling periods are shown in Figures 1 - 11. The vertical lines indicate range and the bisecting lines indicate mean population level. Numbers appearing above the vertical population range lines indicate the number of fields sampled. Expanded areas indicate twice the standard error of the mean on each side of the mean. Non-overlap of the expanded areas indicates significant difference at the 95% confidence level.

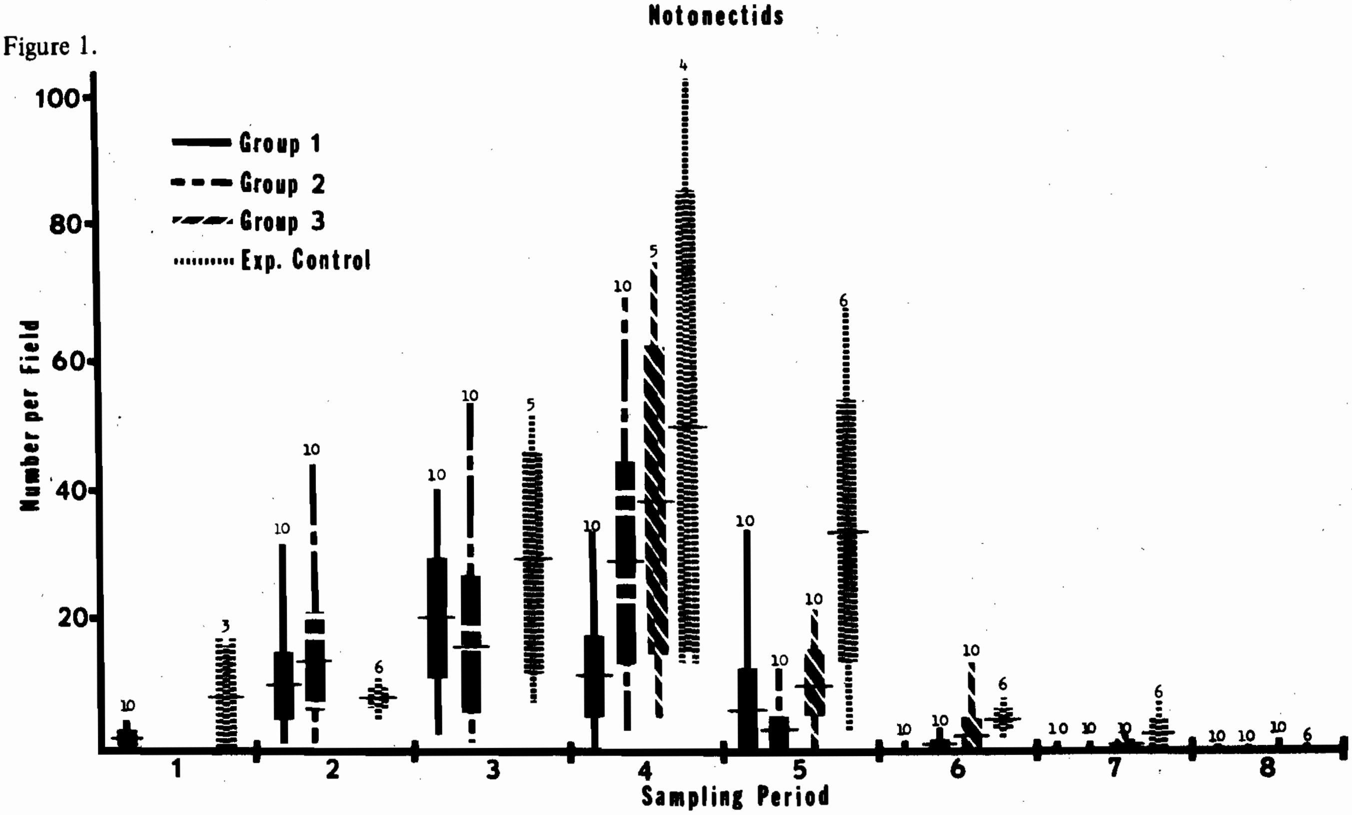
Generally, the greatest mean population differences for any sampling period were between the Group I fields and the experimental controls. Invertebrate population means for Group II and III fields usually ranged between the Group I and experimental control fields.

The population means for the notonectids (Figure 1) in the experimental control fields were generally higher than those in the stocked fields and were significantly higher (.05) than the Group I and II fields during periods 5 and 6. *Gambusia* appears to be directly responsible for the drop in notonectid populations as seen by the higher population means in the experimental controls and later stocked fields. Hess and Tarzwell (1942) indicate that hemipterans are actively selected as food based on forage ratios. However, they do not indicate which hemipterans were studied.

The population means for damselfly naiads (Figure 2) demonstrated a pattern similar to that of the notonectids. Population means in experimental control fields were higher than any of the fish fields during periods, 1, 2, and 5, and significantly higher (.05) during periods 6, 7, and 8. Reed and Hoy (1970) found significant reductions in paddies stocked at 1000 fish per acre (approx. 1.25 lb) but found no such reductions in paddies stocked at 200 fish per acre (approx. 0.25 lb).

Dragonfly naiad populations (Figure 3) were nearly equal in Group I and experimental control fields during periods 1 and 2, but period 3 brought an explosion of naiads in the stocked fields. Mean naiad populations were nearly equal in all fields during periods 4, 5 and 6. Population means in experimental control fields were significantly higher than Group I fields during periods 7 and 8.

Figure 1.



Figures 1-11.–Non-target invertebrate populations in study fields. Vertical lines indicate range; bisecting lines indicate population mean. Expanded rectangles indicate twice the standard error of the mean on either side of the mean. Numbers indicate the numbers of fields sampled. Sampling periods are as follows:

Period 1 – May 10-21	Period 5 – July 5-16
Period 2 – May 24 - June 4	Period 6 – July 19-30
Period 3 – June 7-18	Period 7 – August 2-13
Period 4 – June 21 - July 2	Period 8 – August 16-27

Figure 2.

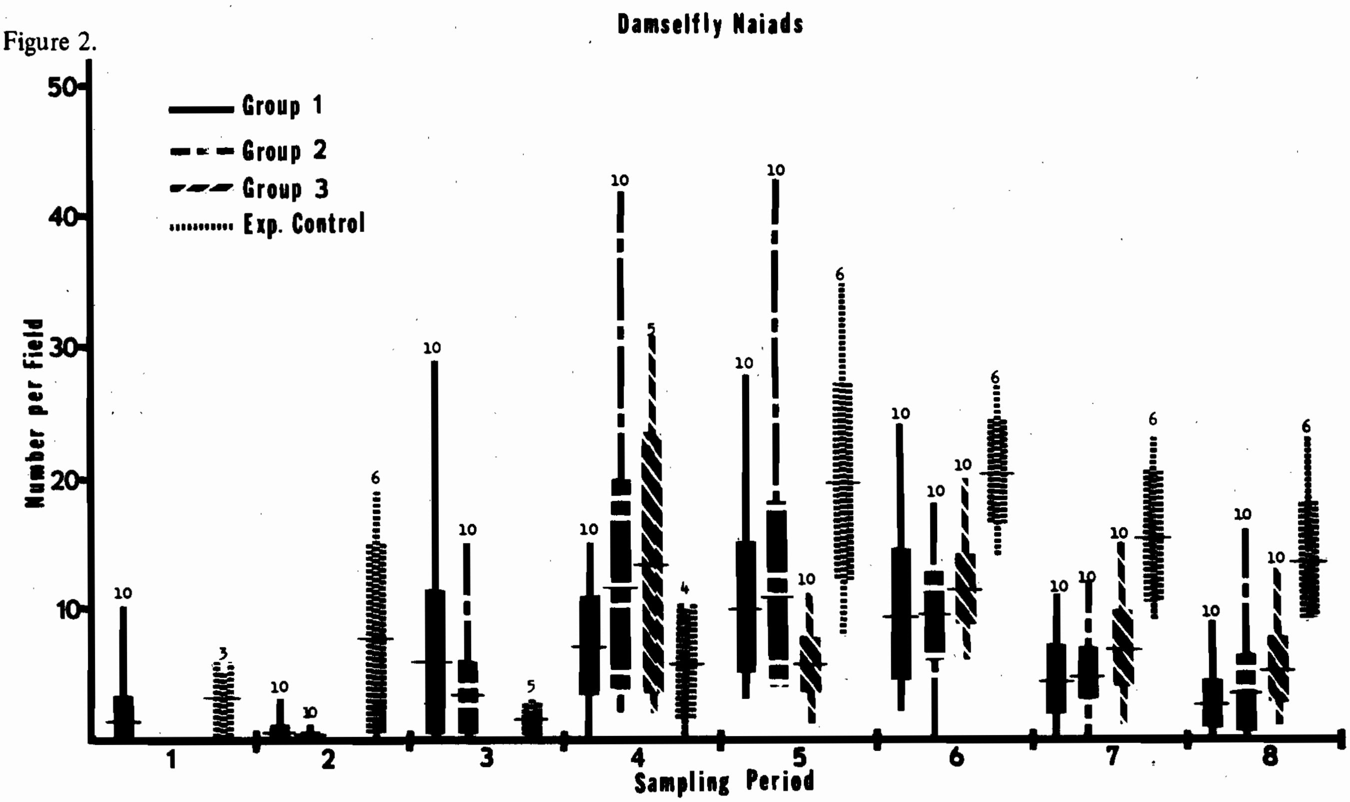
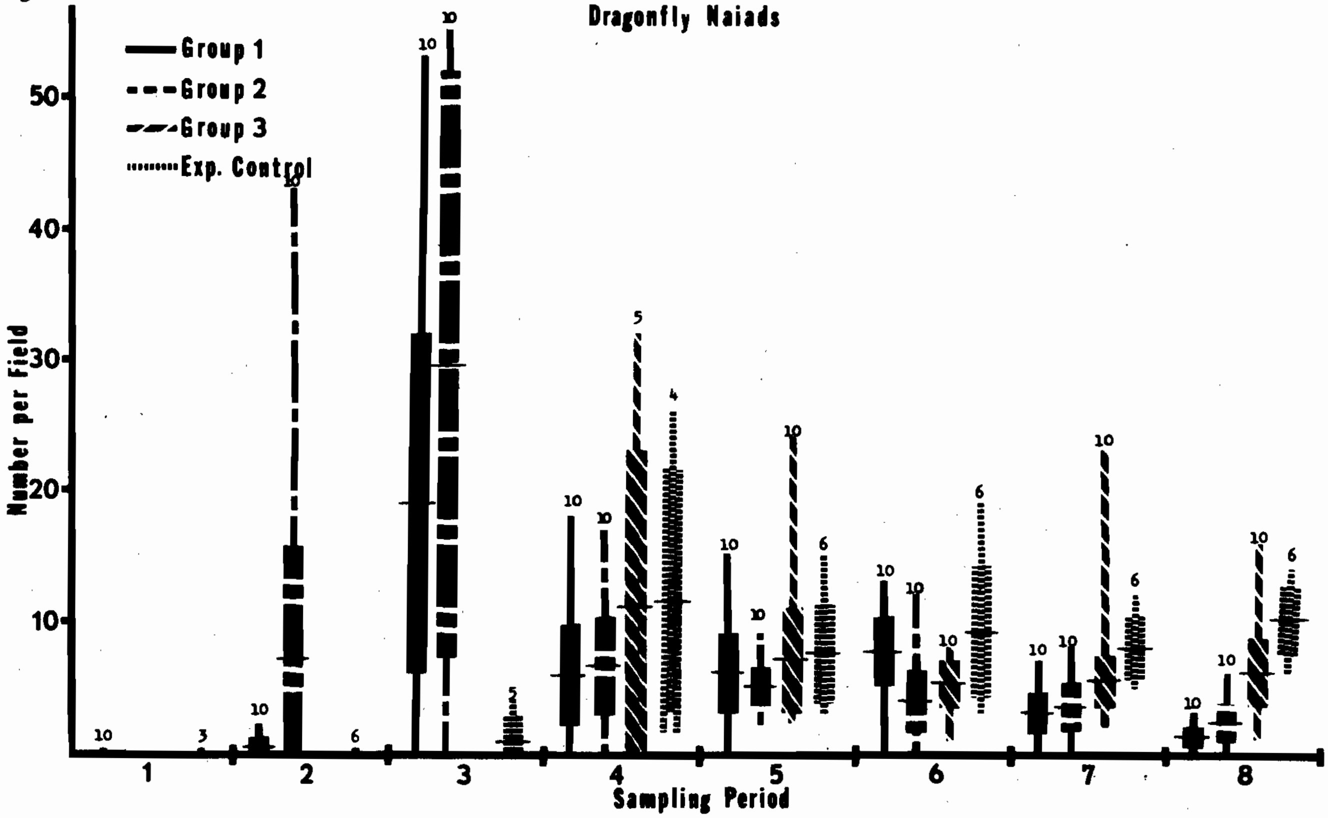


Figure 3.



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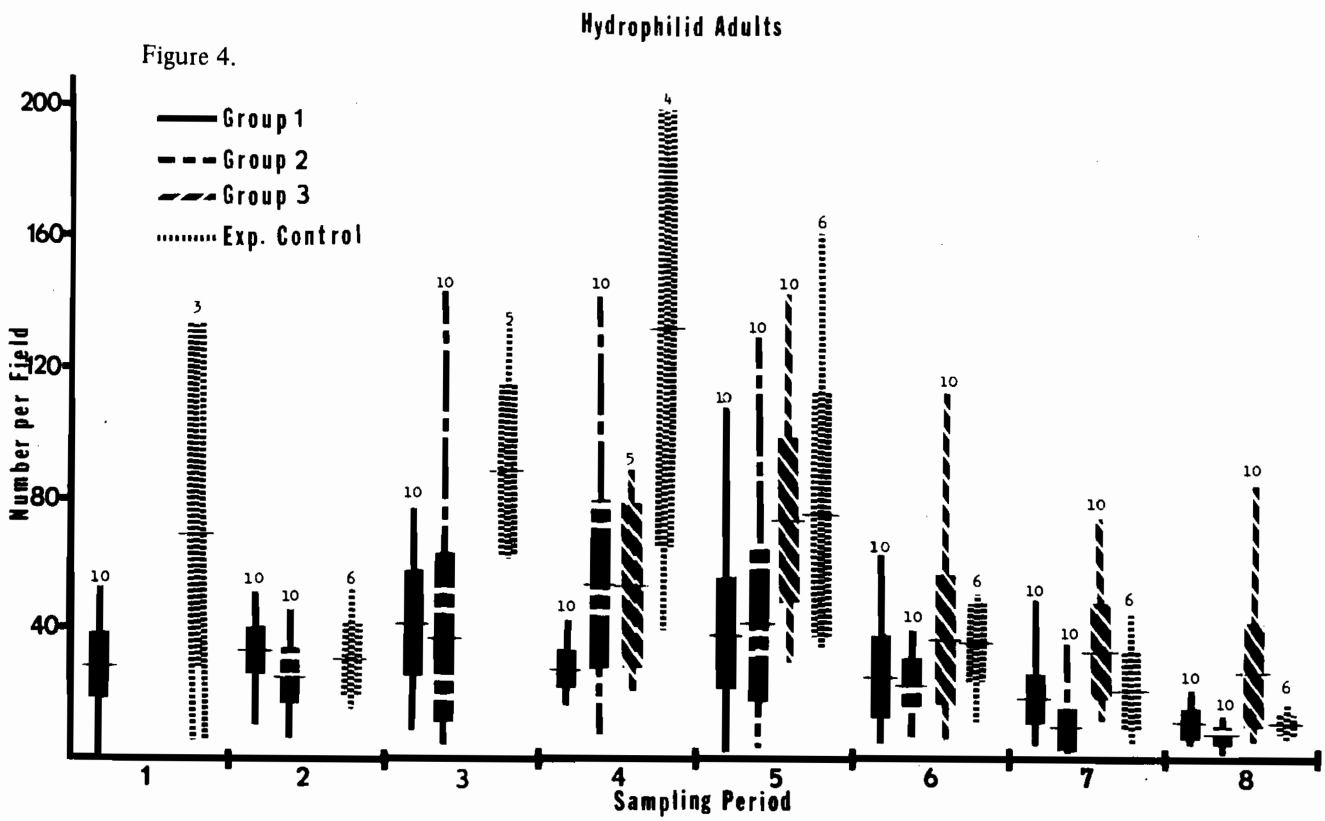

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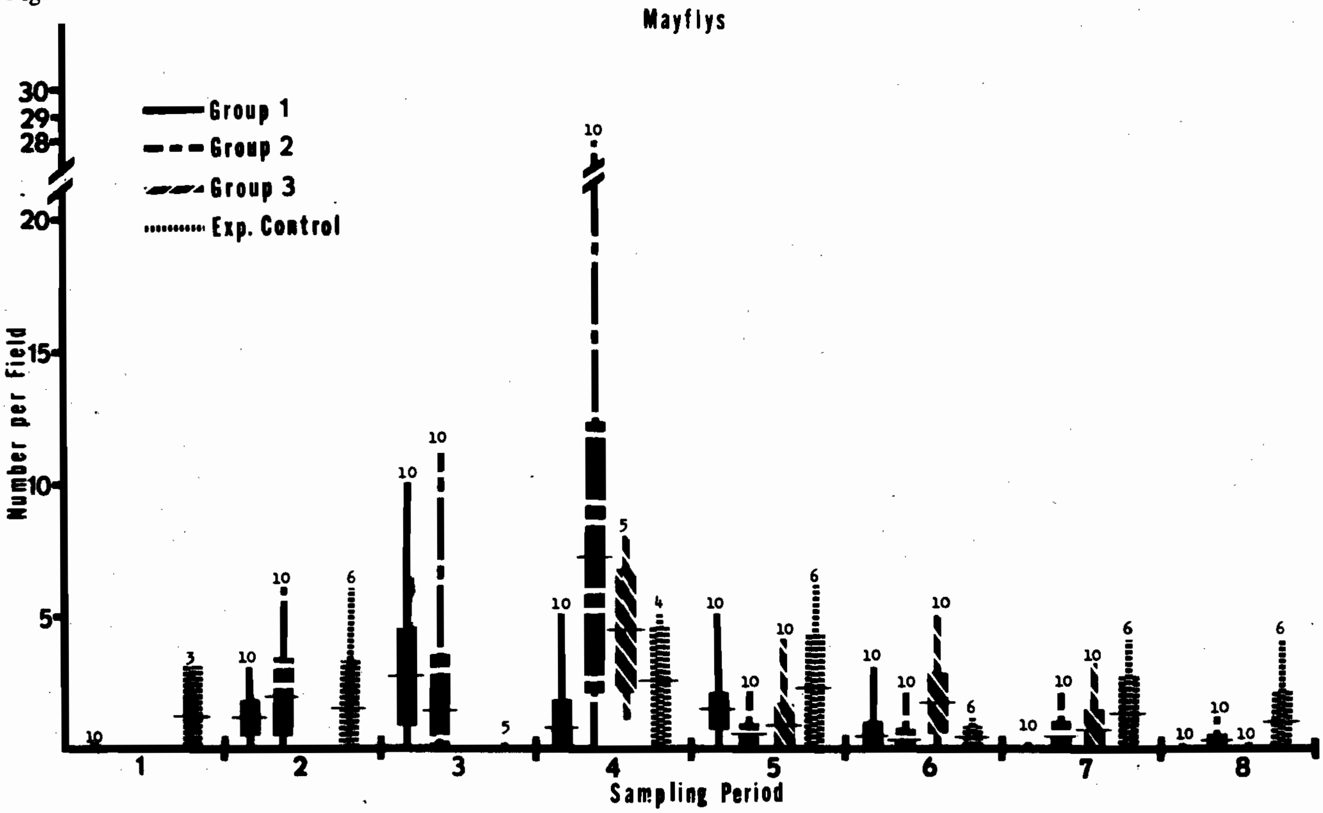
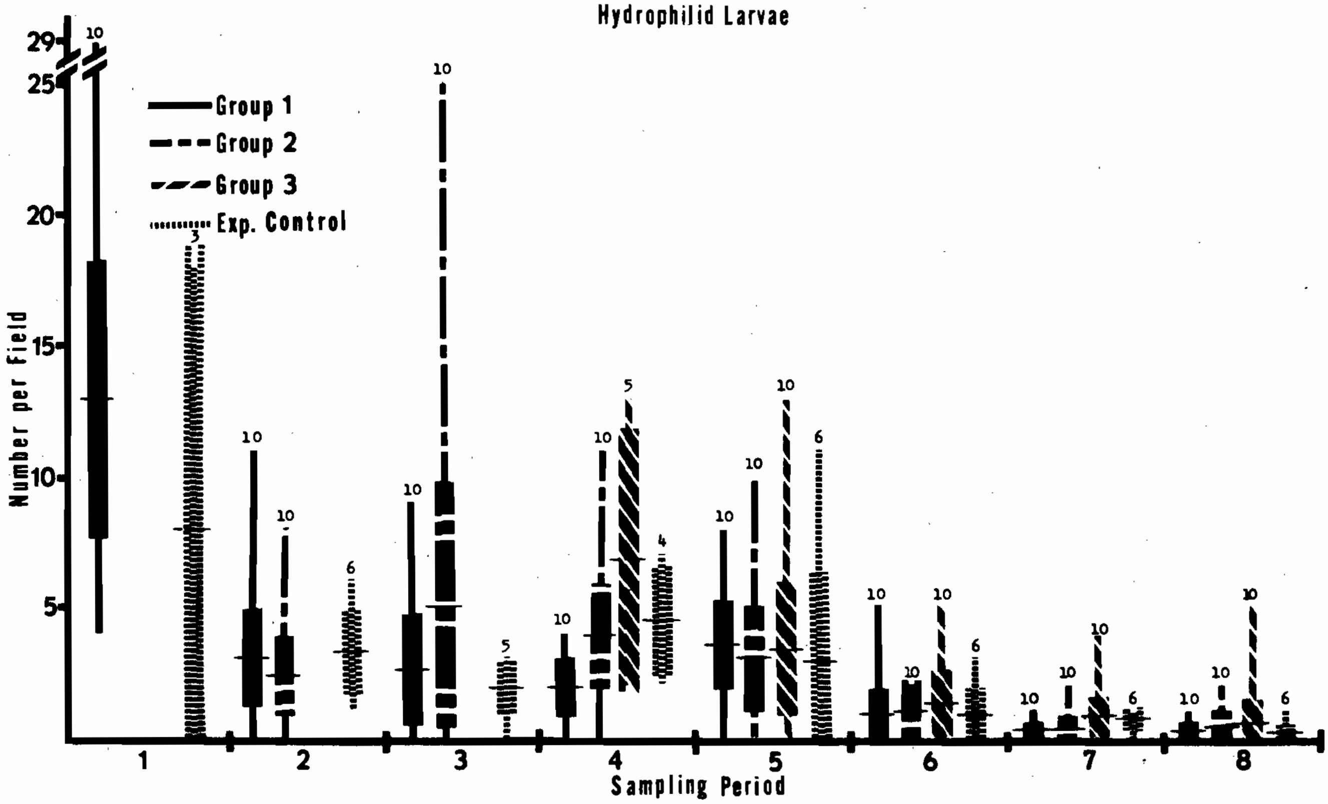


Figure 8.



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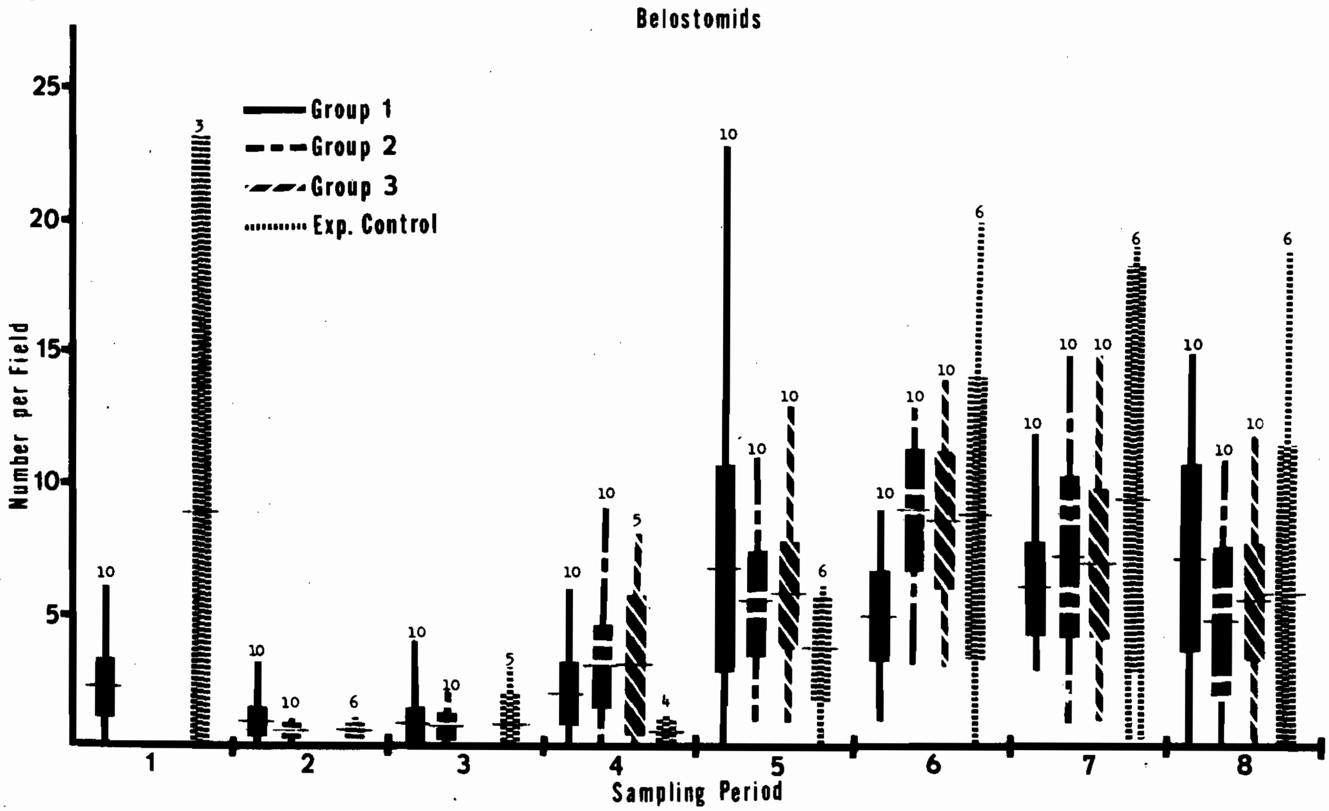


Figure 10.

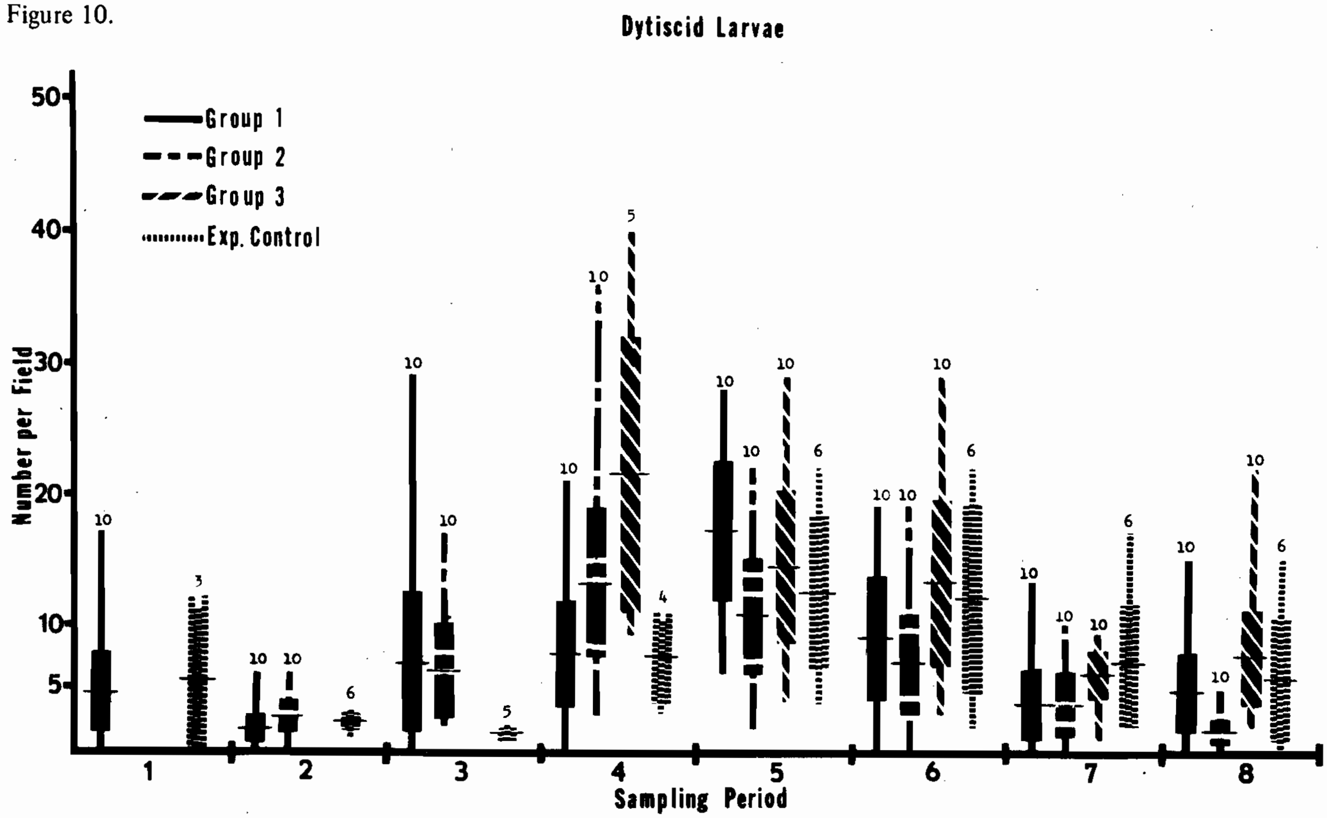
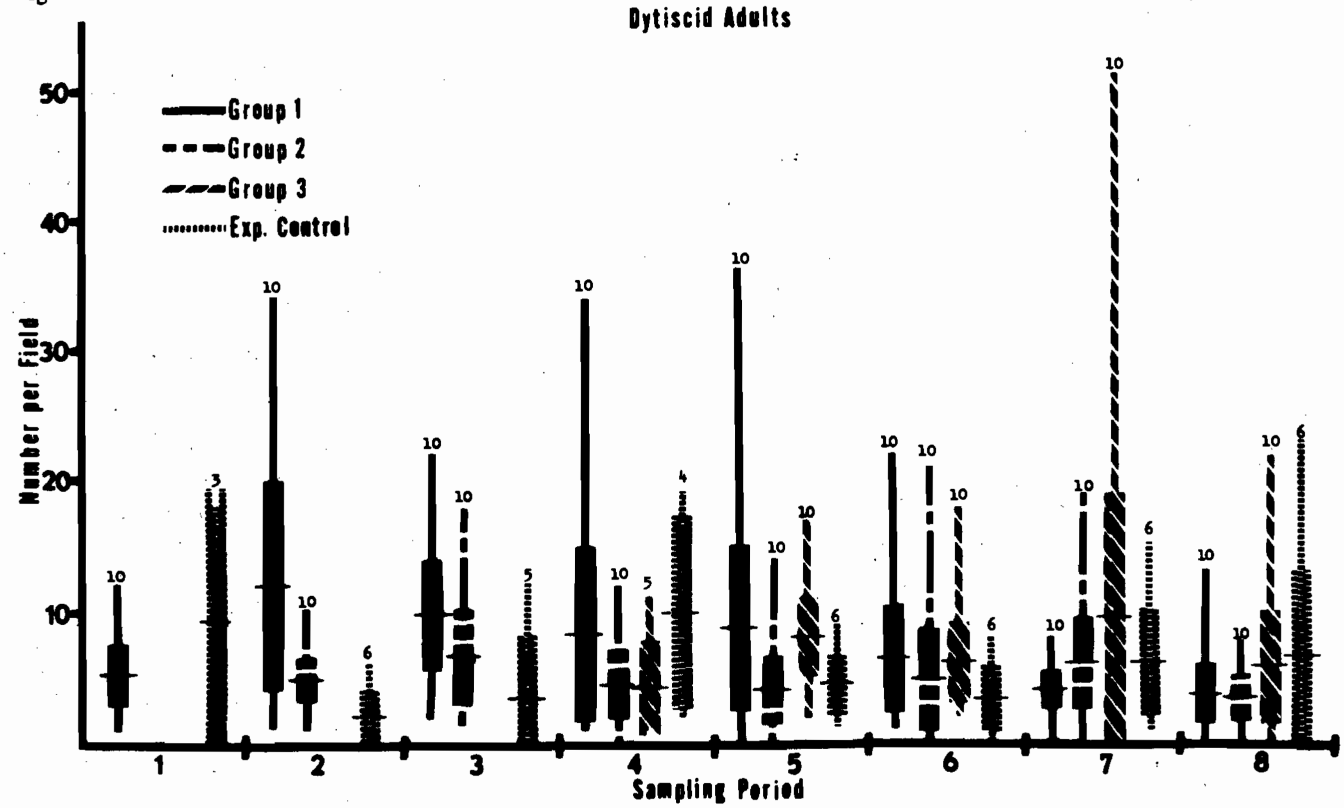


Figure 11.



Population means for hydrophilid beetle adults (Figure 4) were generally higher in the experimental controls than in Group I fields, and significantly higher (.05) during periods 3 and 4. These results do not correlate with the results for hydrophilid larvae which had nearly equal population means in all fields. Ninety percent of the hydrophilid larvae and adults were *Tropisternus lateralis*. Reed and Hoy (1970) found that hydrophilid larvae were not significantly reduced at 200 fish per acre. Ahmed et al. (1970) and Washino and Hokama (1967) reported a very low incidence of *Gambusia* predation of hydrophilid larvae based on gut samples. We are unable to explain the elevated means for the adult hydrophilids in the experimental controls midway through the season.

Chironomid larvae population means (Figure 5) in the experimental control fields were generally higher than populations in the Group I fields, but never reached statistical significance. These results are consistent with results obtained by Bay and Anderson (1966) and Reed and Hoy (1970). This lack of reduction occurs in spite of indications that fish stomachs contain large number of chironomid larvae and pupae (Ahmed et al. 1970 and Washino and Hokama 1967). Hess and Tarzwell (1942) indicate that though *Gambusia* consumes large numbers of chironomids the forage ratios are low. This means that the fish eat relatively large quantities of chironomids only because large quantities are available.

Generally corixids (Figure 6) and mayfly nymphs (Figure 7) also had higher population means in experimental controls than in Group I fields, however, none showed statistical significance. Ahmed et al. (1970) found small numbers of mayflys and corixids in mosquitofish gut samples, and Washino and Hokama (1967) also report small numbers of corixids in gut samples. Reed and Hoy (1970) found slightly higher means in mayfly populations in paddies containing 200 fish per acre and significantly higher (.05 level) in paddies containing 1000 fish per acre.

Hydrophilid larvae (Figure 8), belostomids (Figure 9), and dytiscid larvae (Figure 10) and adults (Figure 11) showed no consistent difference in population means in stocked and non-stocked fields. This indicates that fish have little effect on these organisms. Ahmed et al. (1970) and Washino and Hokama (1967) support this conclusion based on very low numbers of these groups found in mosquitofish gut samples. Reed and Hoy (1970) found significant reduction of hydrophilid larvae in paddies stocked with 1000 fish per acre but not at 200 fish per acre. Belostomids and dytiscids are not mentioned in their paper.

Cladocerans and copepods were not precisely counted during the study due to their tremendous numbers in many of the samples. Instead, their numbers were estimated and assigned symbols: "+" for 1 to 10 per sample, "++" for 10 to 100 per sample and "+++" for numbers greater than 100. No definite conclusions can be drawn from such data, but indications are that cladocerans were collected in nearly equal numbers in both stocked and non-stocked fields during periods 1 through 5. However, during periods 6, 7, and 8, cladocerans were picked up on the order of 0-10 per 10 dip sample in the fish fields and 10-100 per 10 dip sample in the experimental controls. It appears that *Gambusia* do indeed reduce cladoceran populations. This assumption is supported by work done by most of the authors cited above. Copepods on the other hand were seldom found in either stocked or non-stocked fields after period 3. No conclusions can be drawn from the data collected.

In summary, *Gambusia* demonstrated significant deleterious effects on notonectid, damselfly and dragonfly populations in the latter part of fhe season. Population means were lower for hydrophilid adults, chironomids, corixids, and mayflys in fields stocked with fish, but there was no statistical difference at the .05 level. Hydrophilid larvae, belostomids, and dytiscid larvae and adults had nearly equal population means in both stocked fields and experimental controls indicating little effect on these organisms caused by the introduction of *G. affinis*. Copepods and cladocerans were not counted precisely enough for statistical inference, but it appears that cladoceran population means are lower in the presence of fish. Copepod populations were so low in all fields after period 3 that they could not be compared.

In general, population means of all affected invertebrate groups were lowest in Group I fields and highest in the experimental controls. The means for Group II and III fields ranged between the two with Group II fields having a lower mean than Group III. These data indicate a direct correlation between comparative population levels of susceptible invertebrate groups (as prey or other cause) and the post-stocking time of *G. affinis* during the summer months.

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ADAPTATIONS OF *TILAPIA* TO *CULEX* AND CHIRONOMID MIDGE ECOSYSTEMS IN SOUTH CALIFORNIA

E. F. Legner[1] and F. W. Pelsue[2]

Prior to the 1970's three species of African cichlids, *Tilapia hornorum* Trewazas, *T. mossambica* (Peters) and *T. zillii* (Gervais) had existed in isolated colonies in central and south California where they were variously studied for weed and chironomid midge control. Most of the colonies died out in the north due to low winter temperatures, but mixed populations of *T. hornorum* and *T. mossambica* persisted in the comparatively warmer waters of the Colorado Desert in Imperial and Riverside Counties. In 1970 the Southeast and Orange County Mosquito Abatement Districts received permits from the California Department of Fish and Game to secure new cultures of all three species of *Tilapia* from the University of Arizona, Tucson. These cultures were placed in quarantine at the University of California, Riverside where their identity was verified by officials of the California Department of Fish and Game. The new acquisitions were stimulated also by a general increase in magnitude of the aquatic weed problem in the irrigation system of the lower deserts, which was becoming a formidable obstacle to the effective delivery of water to agriculture (Figure 1). Accumulations of dead weeds in the system provided favorable habitats for *Culex tarsalis* breeding (Figure 2).

The first reported plants of *T. zillii* in the Colorado Desert areas were made in 1971 at sites in the Imperial Valley by Department of Fish and Game personnel who reared them at Chino from cultures obtained at Riverside. The fish reproduced in test drains and some weed control effects were observed. Simultaneous encouraging data on mosquito and chironomid midge control by University investigators (Legner and Medved 1972, 1973a, 1973b) were followed by large plants of all three *Tilapia* species in the paved drainage channels and recreational lakes in the south coast basin (Hauser et al. 1976; Legner et al. 1973, 1975). Dissemination of these fish expanded as private and public organizations in California subsequently obtained stocking permits to distribute them for weed and insect control. Results were favorable whenever the minimum stocking density of approximately 400 and 2,000 three-inch fish per surface acre in lakes and running canal waters, respectively, were applied early in the season at tolerable water temperatures in the 65 - 70° F. range. Interference by established predators such as largemouth bass and channel catfish has been definitely observed to reduce *Tilapia* reproduction, especially when stocking rates are less than recommended (Hauser et al. 1976, Legner et al. 1975).

By 1974 *Tilapia* population densities noticeably had increased in most areas where stocking had been made during the three previous years (Figure 3). Aquatic weed control and *Culex* mosquito and chironomid midge reductions were

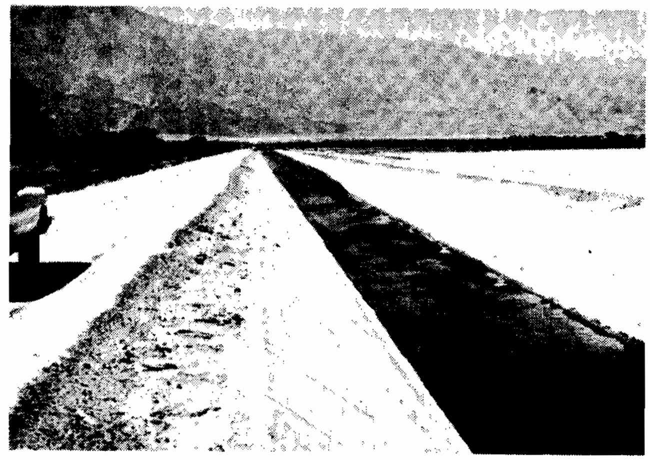

Figure 1.–A paved drainage channel in the Coachella Valley showing a 3-4 foot drop in water level rendered by the mechanical removal of aquatic weeds in a portion of the drainage (foreground). The principal weed species, *Potamogeton pectinatus*, maintains its anchorage on the concrete in the uncleaned section, causing water to cascade down a drop.

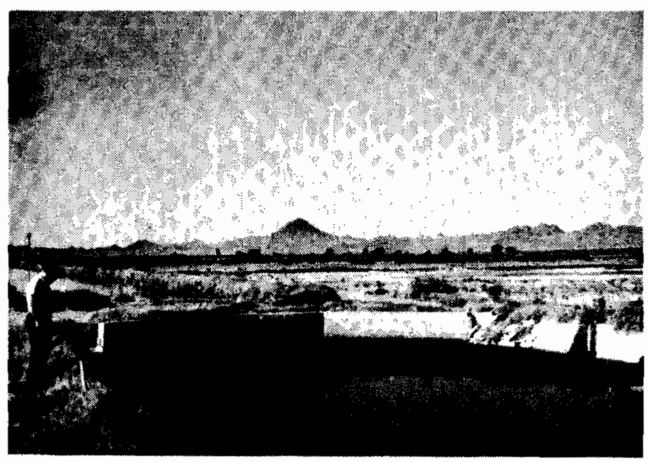

Figure 2.–A water delivery canal in the Palo Verde Valley showing an accumulation of dead aquatic vegetation at a siphon. The ideal habitat for *Culex tarsalis* thus provided often produces densities exceeding 20 mature larvae/400 ml dipper.

obtained. Even partial weed control was recorded from quickly flowing canals in the Imperial and Coachella Valleys, although the slower moving canals and drains were most conducive to *Tilapia* reproduction and subsequent pest control (Hauser et al. 1976, Legner et al. 1975).

One of the most extraordinary adaptations of all was noted in the Coyote Creek of the Los Angeles basin, which had a history of acute chironomid problems (Legner et al. 1975). Although all three *Tilapia* species were stocked in this drainage, apparently only one species, *T. hornorum*, has persisted and now attains extremely dense population

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Figure 3.–Nests of *Tilapia mossambica* constructed in the mud of a drainage channel in the Palo Verde Valley. Note absence of aquatic vegetation in the midst of the relatively high fish population density.

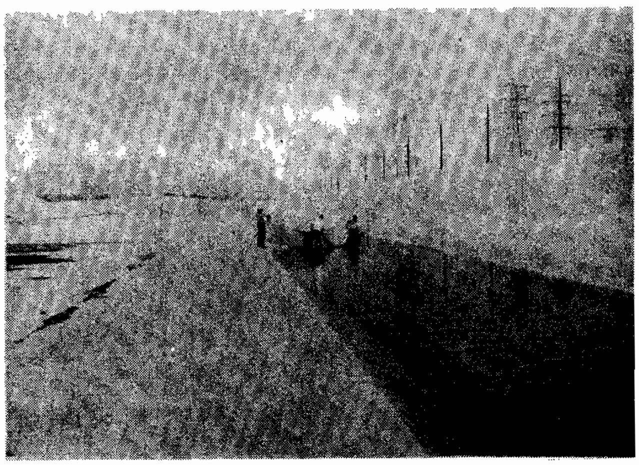

Figure 4.–Seining the Coyote Creek paved channel in Los Angeles for *Tilapia* in November, 1976.

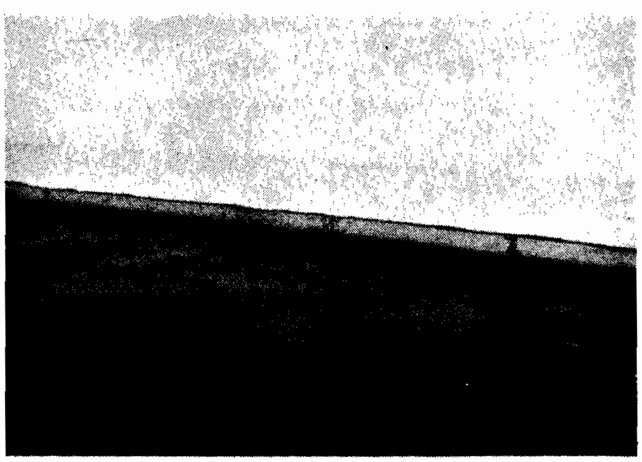

Figure 5.–High density of *Tilapia* exceeding 7 adult fish per ft^2 of water surface in the Coyote Creek, Los Angeles in November, 1976.

Figure 6.–Angling for *Tilapia* with worms in the Coyote Creek, Los Angeles in November 1976.

Figure 7.–Adult *Tilapia* sp. probably *hornorum*, caught on worm bait in the Coyote Creek, Los Angeles, November 1976. Note depressed stomach indicating starvation.

Figure 8.–One seine net catch of *Tilapia* sp. probably *hornorum* from the Coyote Creek, Los Angeles, in November 1976. Note depressed stomachs in some individuals, an apparent starvation syndrome.

densities estimated to exceed 7 adult fish per square-foot of water surface by the month of September (Figures 3-8). Subsequent significant drops in chironomid midge densities in this habitat have now been recorded for two consecutive years, and no chemical treatment has been necessary. The success in this drainage is apparently due indirectly to the presence of a power generating plant located about two miles from the mouth of the Coyote Creek with the ocean. Warm effluent in the 85°F. range is discharged on a year-round basis into the water there, creating a favorable breeding and overwintering habitat for *T. hornorum.* Daily tidal washes of the site favor the persistence of this fish over the other two species which are less interhaline. Although *T. zillii* was originally stocked in the thousands from 1971-1973, it has not been observed to persist. The nesting requirements for rearing young characteristic of this species may also place it at a disadvantage with the mouth-brooding *T. hornorum*, resulting in complete competitive exclusion.

T. hornorum now constitutes a significant fishery in the Coyote Creek, where it is easily caught on worm baits (Figures 6 and 7). Catches average one fish every 2 minutes during summer and autumn, with sizes ranging from 4 to 9 inches (Figure 7). By November the density of *T. hornorum* is usually so great that starvation characteristics are noticeable in all adult individuals, with the depressed stomach appearance being most conspicuous (Figures 7 and 8). The addition of a suitable predatory fish species might improve this fishery as well as expand the catches to include such predators. A more balanced fishery appears to exist in the Salton Sea area where *T. zillii, T. mossambica* and *T. hornorum* coexist in conjunction with several predatory fish species. Catches of *Tilapia* seem to be increasing in that area, and a greater cold water tolerance may have appeared in the *T. zillii* population as some larger individuals (12 inches or more) are now being observed in the Sea during January when the water temperatures plunge to the low 50's F.

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AN INTRODUCTION TO THE NEED OF THREE OPERATIONAL RESEARCH PROJECTS IN THE SUTTER-YUBA MOSQUITO ABATEMENT DISTRICT

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In 1971 great plans were envisioned to provide fantastic results by 1976 in mosquito control in the Sutter-Yuba Mosquito Abatement District. These plans included the hiring of a fisheries biologist, digging fish ponds, fixing oil drums for fish transporation, and arranging for a fish planting crew. Five years of execution of this plan and we haven't come close to fulfillment.

What we have accomplished in this time has been very useful if not absolutely necessary. We've completed work on:

1. Maintenance diet (*Gambusia*)
2. Bird-depredation prevention
3. Waterfiltration of storage tanks
4. Long-haul transportation system
5. Fish stocking rates for summer production of fish
6. Fish weighing device for small numbers of fish
7. Fish stocking rates in rice
8. Economics of fish planting by fixed wing aircraft
9. Effect of temperature on gestation and offspring condition
10. Mosquitofish and bullhead compatibility.

Other interesting studies still in progress:

1. Comparison of foothill (1000′ - 2000′ elevation) and valley fish rearing sites.
2. Semi-covered vs. completely covered fish ponds to prevent Grebe depredation on *Gambusia*.
3. Cage rearing vs. no restriction of movement of mature *Gambusia* females.

These studies have raised the level of enthusiasm of the operators, but not enough to satisfy a question that is persistant in my thoughts. That is, will the field crew be able to cope with the problems that will arise when we start the huge project of planting 100,000 acres of rice to control *Anopheles freeborni* and *Culex tarsalis*?

To assist in the development of the desired philosophy for the work at hand, I decided the District should do more operational research. Four projects were proposed and started in 1976, but only three were completed.

The first project was to investigate ways of modifying the influence of seepage ditches around rice fields. The importance of this ditch is believed to be significant because in May there is very little standing water that is not being treated (if it produces larvae). The rice fields have too much wave action, the orchards are non-producers, the vernal ponds have dried up, therefore the huge numberof untreated seepage ditches are suspected of being very large contributors to that population of mosquitoes that oviposit in the rice fields when the wave action decreases due to rice emergence.

The second project was to determine the cost ratio between chemical and biological control in a roadside-ditch habitat. This project was necessary because field personnel were reluctant to put fish in these sources. They usually explained that such water would dry-up in a month and the fish would die. They failed to realize that the insecticide would last only a few days and the source would have to be retreated.

The third project was to determine the rate of movement of *Gambusia affinis* through a weed-choked ditch. The project was prompted by many miles of this type of habitat that was habitually sprayed with insecticides and by numerous colleagues who expressed confidence that the *Gambusia* would "work" in the source. Numerous literature sources have implied the failure or ineffectiveness of mosquitofish in "weed choked ditches".

The fourth project was to study ways of improving *Gambusia* recovery from roadside areas by habitat modification. The plan was to stock a significant number (5 lb/acre) of fish and have them increase their biomass by reproduction. This biomass was a commodity to be utilized elsewhere. The study was for site-removal improvement, not of reproduction. This project was not completed and will be reinstituted.

The results of these three completed projects are to be published in the 45th Volume of the California Mosquito and Vector Control Proceedings that covers this conference.

THE EFFECTS OF DIFFERENT CONTROL METHODOLOGIES ON MOSQUITO PRODUCTION IN WATER SEEPAGE AREAS IN THE SUTTER-YUBA MOSQUITO ABATEMENT DISTRICT

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INTRODUCTION.—Current land-use practices in California's Sacramento Valley associated with rice culture may provide oviposition sites for *Culex tarsalis* and *Anopheles freeborni* production that are not solely limited to the actual rice paddy area. Specifically, these areas can be seeps or sweat ditches that are created when levees are constructed around the outside perimeter of individual rice fields.

Current rice culture techniques may also initially require a period of flooding, water drawdown and reflooding of the rice paddies. Seeps may serve as reservoirs of continued breeding during the period of sequential flooding, and the adult mosquitoes produced during that time span would then be available to re-establish breeding in nearby or adjacent paddies when the water levels stabilized.

Seeps can also be associated with poorly-sealed irrigation canals, leaking water gates in irrigation systems, or uneven roadside ditches that receive intermittent water, and not be strictly limited to rice fields. However, the majority of the seeps in this District are directly associated with rice fields.

Mosquito production in the seeps, what ever their origin, may not be influenced by applied biocontrol agents or other natural predators in contiguous rice fields because the levees through which the water seeps also act as physical barriers to the predator's movement.

This study was designed to detect the effects of source reduction and biocontrol on mosquito production in seeps of various origins and the comparative cost of both control regimes.

MATERIALS AND METHODS.—Twenty-two sites were selected for study that ranged in size from approximately 100 ft^2 to 12,000+ ft^2. Each site was studied individually to determine which method would be the most appropriate to effect control. Hand tools were used for the source reduction method to either drain the source completely, or lower the water level sufficiently to enhance rapid discharge of the remaining seepage water to decrease its availability as an oviposition site. This was accomplished by cutting channels from the seeps to drainage or roadside ditches that were in close proximity to the sample site.

Mosquitofish, *Gambusia affinis*, were planted at 0.6 pounds per acre (Hoy, Kauffman and O'Berg 1972) in the sites selected for biocontrol. Some of the biocontrol sites were modified with hand tools to enhance rapid movement of the fish through extremely dense vegetation or to areas that were otherwise separated from the main seepage due to irregularities in the ground surface (bifurcated seeps).

Each site was sampled weekly (unless otherwise noted) with a standard dipper to detect mosquito production prior to any actual control work and the results were recorded on individual resume sheets prepared for each site. At the outset of the study, most of the individual sites were weed and/or debris free which allowed the use of a larval concentrator (Husbands 1969). However, as rooted aquatics, terrestrials and algae became more of a deterrent to continued use of the concentrator, samples were then placed in a gussy bucket. The contents were then put in water-filled plastic bags inside 1-gallon ice cream containers and returned to the District. Due to time limitations, not all field samples were separated on the same day as collected. Ten dips were taken at each site on each sampling day from the week of May 10, 1976 to August 31, 1976.

The field samples were individually analyzed for the presence or absence of larvae. If larvae were present, they were removed, killed and placed in separate containers for enumeration and identification and the results transcribed to the resume sheets. All larvae were identified to genera with the aide of a dissecting scope.

RESULTS.—Detailed data are available upon request from the Sutter-Yuba Mosquito Abatement District.

DISCUSSION.—The data collected seem to confirm that there were differences between the pre- and post-treatment larval counts for both biocontrol and source reduction. Unfortunately, pre-control mosquito production levels were not adequately established in all cases to demonstrate that control was obtained. As a result of exceptionally low amounts of rainfall this spring which reduced the availability of adequate irrigation water, and extensive ground preparation, widespread seepages were late developing. Approximately 60 individual sites were inspected prior to actual study site selection in an effort to locate 20 that would meet the design criteria of being active mosquito producers and small enough that one person could reasonably do the work necessary to modify them if required.

Most of the pre-control samples demonstrate the presence of larvae with a "+" when initially checked. If larvae were present and source reduction was selected as the control method of choice for that site, then the work necessary to drain it was carried out.

The data also confirm that there were differences between the mean larvae per dip for biocontrol and source reduction (0.23 versus 0.59 respectively), *Culex* spp. represented 93.24% of all samples collected while *Anopheles* spp. represented only 4.71%. The wide difference in the number collected of each genera may have been a function of either dipping technique or the point where the samples were taken within the site. The high number of *Culex* spp. sampled suggests that seeps can be important reservoirs of mosquito breeding while water levels are stabilizing in the rice paddy areas and perhaps not subject to normal District control techniques.

The higher dip counts for the biocontrol sites may be due to the lag time between the initial fish plantings and their ability to significantly reduce the available larvae. The planting rate selected for this study may not be sufficient to quickly reduce the larvae present in the sites. Future investigators working on sites of this nature may want to consider using a planting rate in excess of 0.6 pounds per acre for faster post-plant control.

The mean time per check for source reduction was 0.33 hours compared to 0.12 hours for biocontrol and the mean number of checks per site was 8.5 for source reduction and 11.1 for biocontrol. What these data suggest is that source reduction work takes longer initially but requires less frequent post-checking than does biocontrol. What the data cannot demonstrate is the wide range of configurations found in seeps that may preclude the use of source reduction as a control technique because of ground surface irregularities, e.g., bifurcated seeps that will not drain without expensive modification.

Consequently, the field man is responsible for making an objective decision concerning the control method that would best be suited for each site on an individual basis. The sole use of biocontrol because it is faster and easier is not justified, as where a source could require multiple fish plantings if the site were subjected to periodic drying and flooding yet could be permanently drained initially.

To develop cost data for this study, the cumulative time attributable to each control technique was extracted with $4.50 per hour used as an arbitrary amount for labor and fringe benefits. Earlier cost analysis work (author 1975) demonstrated a cost of $4.317 per hour for labor and fringe benefits in our District's mosquitofish planting program in rice to which was added an additional 4.5% to obtain the $4.50 figure. The mean cost per pound for the fish was also obtained from that same report.

In this study, no statistically significant difference was found between the mean cost per site for source reduction and biocontrol. The cost figures, particularly biocontrol, were predicted on numerous post-planting checks necessary to demonstrate that the sources had not become breeding sites again because of fish die-off or related cause. Post-control checks should be an integral part of any control program employed in sites of this nature.

In summary, the data demonstrated that in this study both biocontrol and source reduction were effective in reducing larval production. Biocontrol showed higher post-control larval concentrations than source reduction attributable to the lag time between initial planting and subsequent control. The mean cost per site was nearly identical for both methods.

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Table 1.–Cost comparison between BC and SR techniques.

Description of entry	Biocontrol	Source Reduction
A. Number of hours devoted to control technique:	26.77	12.17
B. Cost/hour, including fringe benefits:	$ 4.50	$ 4.50
C. Total labor costs:	$120.465	$54.765
D. Unit cost of planted fish:	$ 1.25 per pound	$ 0
E. Amount of fish planted:	2.6 pounds	0
F. Cost of fish planted:	$ 3.25	$ 0
G. Total costs, labor and materials:	$123.715	$54.765
H. Number of sites treated:	13	6
I. Mean cost per site:	$ 9.517	$ 9.128
J. Number of visits to all sites:	144	51
K. Mean number of visits per site:	11.1	8.5

THE COST OF BIOCONTROL VERSUS CHEMICAL CONTROL IN SELECTED MOSQUITO HABITATS

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INTRODUCTION.–The Sutter-Yuba Mosquito Abatement District has been planting mosquitofish, *Gambusia affinis*, in a wide range of habitat types for a number of years such as railroad borrow pits, vernal ponds and roadside ditches. We feel this program has been particularly sound in light of recent trends of escalating chemical costs, resistance of target species and environmental degradation.

This study was conducted to detect differences, if any, in the actual cost between biocontrol and chemical control of mosquitoes in roadside ditches and/or field drains. The underlying assumption inherent in this study was that both treatments were equally efficacious; however, usually the fish would only have to be planted once whereas chemicals would require application approximately every ten to fourteen days (to coincide with one mosquito life cycle) to provide comparable levels of control.

MATERIALS AND METHODS.–Twenty study sites were selected, ten each in our field foreman areas of West and South Sutter. The actual sites were either roadside ditches or field drains ranging in length from 0.25 miles to 1.0 mile. The length of each site was obtained from either District aerial photos or zone maps. The widths of the sites varied from 2 feet to 10 feet. To minimize the differential in widths of all sites, I considered each to be 8 feet wide,

Table 1.–Resume of all planting and spraying time*, West and South Sutter; May 5 - 18, 1976.**

	Number of Plants/Site	Biocontrol Time	Chemical Control Time	Length of Site (miles)	Acreage (based on 8 ft width)
	1	2.93	3.63	0.5	0.48
	1	2.47	3.42	0.4	0.39
	1	3.02	3.78	0.5	0.48
	1	3.70	3.92	0.5	0.48
	1	3.02	2.80	0.25	0.24
	1	3.38	2.77	0.25	0.24
	1	2.95	2.32	0.3	0.29
	1	3.05	2.57	0.3	0.29
	1	3.12	3.33	0.5	0.48
	1	3.65	3.58	0.5	0.48
Totals:	10	31.29	32.12	4.0	3.85
Means:	-	3.13	3.21	0.4	0.39
	2	6.88	3.50	0.5	0.48
	2	6.30	6.78	1.0	0.96
	2	6.90	3.83	0.5	0.48
	2	6.65	4.18	0.5	0.48
	2	7.93	3.95	0.5	0.48
	2	9.38	5.33	0.5	0.48
	2	7.90	3.45	0.5	0.48
Totals:	7	51.94	31.02	4.0	3.84
Means:	-	7.42	4.43	0.57	0.55
	3	9.53	6.27	0.9	0.87
	3	13.20	7.08	1.0	0.96
	3	11.75	7.21	1.0	0.96
Totals:	3	34.48	20.47	2.9	2.79
Means:	-	11.49	6.82	0.97	0.93

*Time is noted in minutes and tenths of minutes.

**The data above were separated into three categories on the basis of the number of fish plants per site only to demonstrate the differences in time required for single plants vs. multiple plants.

regardless of its actual width, and predicated biomass requirements and equipment limitations on that width.

The simulated chemical applications in each area were conducted by the field foreman from each zone using a Jeep-mounted low-volume, mist blower. The author was responsible for the fish plantings in each of the two zones.

The pesticide choice for this study, for cost data, dosage rates, etc., was Dursban®M, 4 pound material. However, no Dursban was actually applied; instead, the low-volume mist blower used in this study was properly calibrated prior to the test and only water applied to the individual sites. The sites were first sprayed and then planted in the West Sutter sites and that sequence reversed in the South Sutter sites.

All time periods associated with this study were recorded with a stopwatch in seconds and later converted to minutes and tenths of minutes. Recorded times for chemical applications did not include calibration, downtime for equipment failures or driving time between individual sites; but did include blower engine starting time, adjusting the rpm's and actual spraying time.

Fish-planting-time did not include driving times between individual sites, seining the biomass of fish needed for the day's activities and minor functions, but did include driving time between planting sites that required more than one planting due to their length, weighing, planting and closing the fish transporation tank, etc. The fish used in this study were weighed with a water displacement device (author 1976, unpublished) rather than with a tension/compression scale due to the small allotments of fish (0.1 lb +) planted at each of the individual sites.

The total amount of material sprayed at all sites amounted to 3.94 gallons. The "Pesticide Use Information" (Harvey 1976, unpublished) chart lists the amount to mix per gallon (4 lb Dursban in a LV blower) as 4.5 fluid ounces per gallon. With that information, it was calculated that the amount of pesticide that would have been used in the 3.94 gallons as 17.73 fl ounces.

The biomass of fish actually planted during this study amounted to 5.9 pounds – somewhat short of the actual needed biomass of 6.3 pounds (10.48 acres x 0.6 pounds per acre (Hoy, Kauffman and O'Berg 1972).

RESULTS.–For the purpose of this study, the fish cost $1.2553 per pound. This figure was obtained from a 3-year average of the cost per pound for mosquitofish planted in rice (author 1973, 1974, 1975). The cost of Dursban was calculated to be $0.3842 per fluid ounce based on a cost of $49.18 per gallon, including sales tax. The arbitrary labor cost of $4.50 per hour was obtained from the "Rice Planting Analysis" (author 1975, unpublished) which reflected an overall labor and fringe benefit cost of $4.317 per hour and 4.5% was added to that figure to reflect wage increases due to inflation since that time.

Hourly wages can vary tremendously depending upon the category of personnel used to accomplish either control technique. By that is meant only certified technicians or uncertified personnel, with no supervision, can be hired at the prevailing minimum wage to plant fish if District personnel were otherwise involved and not available for biocontrol work. For example: a certified technician's current starting salary is $570.00 per month, or, approximately $3.56 per hour based on a 176 hour work month compared to a minimum salary of $2.50 (as of October 18, 1976).

DISCUSSION.–The times in Table 1 were separated according to the number of fish plantings per site to note any differences between the times for one plant per site, two plants per site or three plants per site. The mean times for the two and three plants per site increased dramatically over the one plant per site due to the driving time required between planting sites at the same individual test site. The cost-benefit ratio, Table 2, is therefore favorable toward chemical control - all plantings considered. However, as the data suggests in Table 1, one plant per site is slightly faster than chemical application. Based on the closeness of the times in that category, it is recommended that only one plant per site be made in those sites less than 0.75 mile in length. That figure is supported by a mosquitofish dispersal study (author 1976b) which reflected a dispersal rate of the fish in a weed and tule infested ditch of 1-2 feet per minute over an hour period. Establishing an operational technique of one plant per site for shorter ditches could establish an even closer economic parity between the two methodologies.

Wastage is another important cost consideration in any program, but especially that of chemical control. In this study, all sites were considered to be 8 feet wide, even though their actual widths varied from 2 feet to 10 feet. Consequently, when a LV mist blower is used on sites that are either 8 feet or 16 feet wide, chemicals are wasted. On a one-time basis this is not intolerable; but this wastage can rapidly add up during the course of a year and become extremely costly.

Conclusions from the results of this study appear straightforward. It costs more initially for biocontrol than it does for chemical control, but only on a one-time basis. If chemicals have to be applied more than once to the same site to achieve acceptable levels of control, there is no way chemical control can compete with biocontrol on an economic basis.

ACKNOWLEDGMENTS.–I would like to thank Jerald F. Harvey, Administrative Assistant, Sutter-Yuba Mosquito Abatement District for his help and advice during the course of this study.

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DISPERSAL RATES OF MOSQUITOFISH THROUGH CATTAILS IN DRAINAGE DITCHES

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INTRODUCTION.–The literature reflects many examples of the alleged inability of mosquitofish, *Gambusia affinis*, to penetrate dense vegetation (Dow 1975; Goma 1966) and subsequently provide acceptable levels of mosquito control.

This study was designed to detect the rate of movement of introduced mosquitofish through cattails, *Typha* sp. and other vegetation in drainage ditches.

MATERIALS AND METHODS.–Two individual sites were selected for study in the West Sutter zone of the Sutter-Yuba Mosquito Abatement District. Both were drainage ditches with heavy vegetative growth present. One of the sites (called the Series I site for ease of data separation) was 2000 feet long, 4 feet wide, encompassed 0.18 acre and had an average water depth of 4 inches. Its vegetation consisted solely of cattails throughout its entire length. The other site was 4000 feet long, averaged 5.5 feet wide, encompassed 0.5 acre, but had water present to a depth of 12 inches. The vegetation present in that site (Series II for identification) consisted of cattails, tules (*Scirpus* spp.) and smartweed (*Polygonum* sp.).

The midway points of both sites, established by measurement, were used as the starting points for locating traps. Five Gee minnow traps were placed at 150 foot intervals above and below the mid point in the Series I site (Figure 1). In the Series II location, however, eight traps were placed above and eight below the mid point, also at 150 foot intervals (Figure 1).

The traps were placed in their respective locations at both sites 66 hours prior to the introduction of the test population to detect or establish baseline populations of resident mosquitofish.

The mid points of both series were also selected as the planting point for the mosquitofish in both locations to detect differences, if any, between upstream and downstream movement rates. The fish movement rate in the Series I site was 60 lineal feet/hour and 600 lineal feet/hour in the Series II site.

An arbitrary planting rate of 20 pounds per acre was selected for both sites to mask trapping results of resident fish if present and to induce fish dispersal by crowding. Thus, 4.5 pounds of fish were planted in the Series I site while the Series II site received 10.0 pounds of fish.

The traps in both series were checked at intervals of 24, 48 and 66 hours prior to the introduction of the test fish. On the day the fish were planted, and subsequent to the actual planting, all minnow traps were checked at 1, 3, 5, 7, 9 and 11 hour intervals. Trapping was discontinued 72 hours after the introduction of the test fish into the Series I site and 173 hours after planting in the Series II site. Interim trap checks were conducted throughout the entire test period.

Each time the traps were checked for the presence or absence of fish, the number and sexual composition were noted and all fish returned to the trap site area. The traps were sampled (checked) during a 15 minute period by two persons each going in opposite directions from the planting point (one upstream and the other downstream). The traps were individually inspected by opening them to count fish and/or to release crawfish, *Procambarus* sp., if present, and were then replaced in the individual trap locations within the ditch.

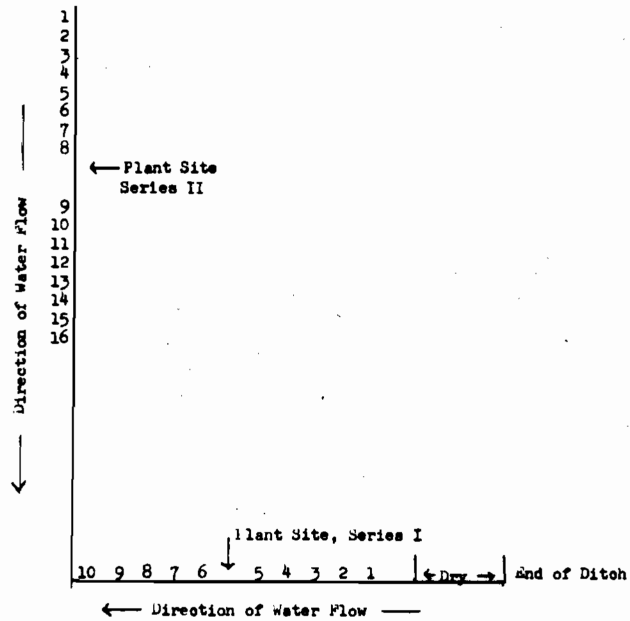

Figure 1.–Relative locations of traps in series I & II sites.

RESULTS.–The results of this study have been recorded in tabular form and are available at the office of the Sutter-Yuba MAD on request.

DISCUSSION.–The data, though not definitive, do permit broad interpretation. For example, the mean dispersal rate of upstream migrants was faster in both series than the mean downstream rate; 2.04 feet/minute versus 1.09 feet/minute for Series I and 1.36 feet/minute versus 0.90 feet/minute in the Series II site. A factor that could have influenced the differing dispersal rates was the wide disparity between the water flow rates in both sites. However, an alternative explanation which may have had a more significant impact on the dispersal rate was the mean depth of the two series.

The data suggest that the fish did not move from the planting sites en masse at a fixed rate of movement. Rather, there appears to have been a small percentage that moved rapidly throughout the length of both sites that were forerunners of the balance of the population that moved more slowly in the sites. The catch data demonstrated that by 27 hours after planting in the Series I site, the remainder of the introduced fish were more or less randomly distributed throughout the ditch, although unevenly. In the Series II site, however, a similar distribution was not observed until some point 31 hours after or prior to 72 hours when the traps were again checked. A comparison of the cumulative

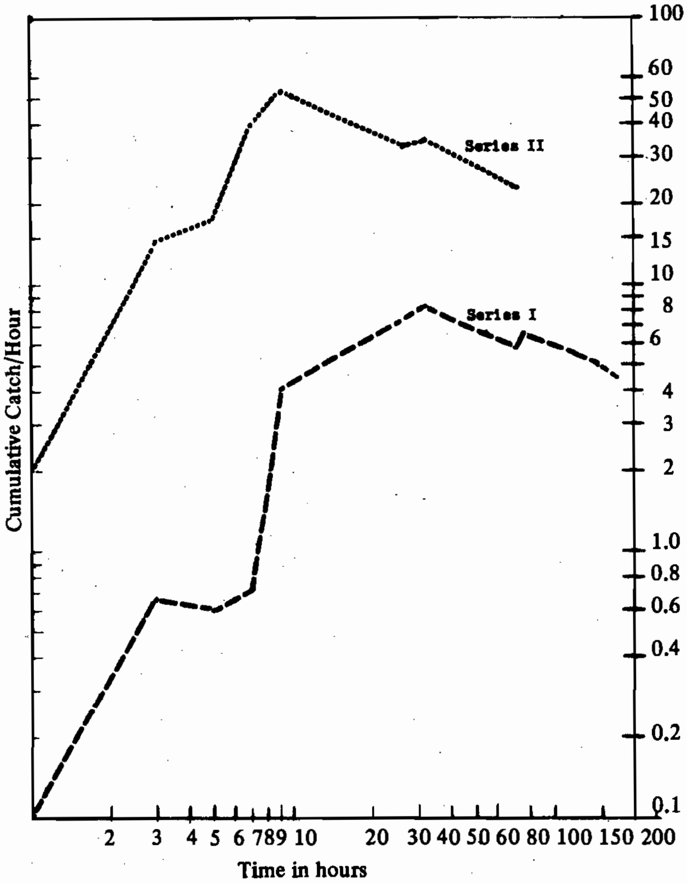

Figure 2.–Cumulative catch/hour of Gambusia affinis from all traps for series I and II, Aug. 20-23, 1976.

catch/cumulative hours post-plant demonstrates an almost identical relationship in the cumulative catch per unit of effort but with one order of magnitude difference between Series I and Series II. The similarity in the cumulative catch per unit of effort suggests that the fish exhibited almost identical movement patterns in both test sites but some intervening factor(s) was responsible for the delay in movement in the Series II site. Undoubtedly the introduced fish were much more vulnerable to trapping in the narrower and shallower Series I site, which could account for the magnitude of difference.

As mentioned earlier in the discussion, differing water depths may have been the single most important factor affecting distribution over time. In this study, movement or dispersal was not a function of higher vegetation density because the density in the Series I site was much greater (100% covered) than in the Series II site (35% open water and 65% covered). Current was not the factor responsible for the greater dispersal rate in Series I as the Series II site had two individual movement rates, greater than the highest found in the Series I site. The water depth would have certainly influenced the density of the fish in the respective water courses and it is felt that the behavioral response to crowding caused them to disperse more rapidly in Site I than in Site II.

The decrease in the catch/unit of effort, after 9 hours in Series I and 32 hours in Series II reflects decreased migration within the sites (Figure 2). Continued sampling after those points in time only monitored sub-populations that had become established in and about the individual trapping sites, and was not a reflection of continued mass movement.

The data strongly demonstrate the mosquitofish's ability to rapidly penetrate dense cattail growth as well as tules and smartweed, at rates of up to 350 lineal feet/hour. To obtain the movement rate of the introduced fish, baseline catch figures were calculated for the pre-plant catch per unit effort for traps in both series. The post-plant movement rates were then calculated from the catch data at the point in time at which they exceeded the pre-plant catch at each site in both series. Post-plant catch/unit effort was greater than preplant catch/unit of effort. The variability of the catch data for each individual trap at both series certainly indicates that trap placement within the sampling area is critical. This problem area could be overcome by using several traps instead of just a few; e.g. more than five.

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A PRELIMINARY FIELD STUDY EMPLOYING *LAGENIDIUM GIGANTEUM*, A FUNGUS, AS A POSSIBLE BIOLOGICAL CONTROL AGENT AGAINST THE PASTURE MOSQUITO *AEDES NIGROMACULIS*

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ABSTRACT

A study site consisting of two side by side checks dammed at both ends was established within a permanent irrigated pasture in Linda, Yuba County, California. Barbed wire fencing was constructed around the study site to keep resident livestock from entering. The study period encompassed four irrigation cycles: 1) August 6th, 2) August 24th, 3) September 14, and 4) October 1st. At each irrigation, well water flooded both checks at the same time for approximately 2½ hours, producing an average water depth of 3 to 4 inches. To determine the mosquito species present and to estimate their density and distribution, dipping samples were taken in both checks two days after irrigation cycle no. 1. Based on the sample variance obtained from this initial sampling, and with the precision needed for accurate assessment of the *Lagenidium* infection rate, it was determined that a minimum of 200 samples per check be taken. At the onset of the second irrigation cycle, one of the checks was inoculated with *Lagenidium* zoospores at the rate of 25 x 10^4 per square foot of water surface. No subsequent inoculations were made. Larval samples in both checks were taken after this and subsequent irrigations when the major portion of the *Ae. nigromaculis* population was in the 2nd instar. Sentinel cages with known numbers of *Cx. tarsalis* and *Ae. nigromaculis* larvae were systematically placed in both checks commencing with the August 24 irrigation. The emerging adult population was sampled by placing 12 metal cylinders (19 inch diam. x 10 inches) over areas of both checks where water was still present when the larvae were in late 4th instar or early pupal stage. The treated check reached a maximum of 45% infection by the second experiment (irrigation 3). The control check was found to be contaminated with *Lagenidium* after inoculation (more than likely the result of small animals crossing back and forth between the two checks). It is interesting to note that the fungus spread rather quickly in the control check and attained a substantial infection rate (32%) by the end of the second experiment. In most cases, adult emergence sampling gave erratic results producing a high sample variance per experiment. This sampling procedure will be improved by using more cylinders (increasing the sample size, n) and locating more precisely the adult emergent habitat. The sentinel mosquitoes produced surprisingly low infection rates. Various reasons can account for these results, but at present they are purely speculative and will be studied further.

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A THREE YEAR STUDY OF THE ECOLOGY OF *LAGENIDIUM* INFECTIONS OF *CULEX TARSALIS* IN CALIFORNIA

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Lagenidium giganteum was recovered in 1976 for the third consecutive year from test sites in Colusa County, California where it had been introduced on an experimental basis 4 years earlier. No subsequent introductions have been made since the initial work of 1972. These sites laid fallow during 1973. Isolations were made in 1974 from native and sentinel mosquito larvae at the site which had had the highest infection rate in 1972. These infections were, however, of low order and erratic in initial laboratory passages. In contrast, high levels of infection were observed in 1975 and 1976. In 1975 the infection persisted throughout the summer until the sites were drained. In 1976, however, the infection was lost in midsummer even though water remained in the ditches until the end of August with the exception of an abnormal dry period which lasted three weeks earlier in the summer. Although infections were isolated again after that dry period, our tentative conclusion was that the premature loss of the fungus could be directly or indirectly attributed to the unusual midseason dry period or by the notable decrease in the numbers of native mosquito larvae.

In conjunction with the Colusa study an attempt was made to determine if *L. giganteum* would survive land management practices associated with rice fields. In 1975 the fungus was introduced into a series of small experimental rice fields. The fungus was successfully recovered in 1976.

We tentatively concluded the following: 1) *L. giganteum* is a highly virulent microbial agent of *Culex tarsalis* larvae, 2) survival of the fungus through seasonal droughts and land management practices associated with rice fields was demonstrated. We were unable to verify the previous hypothesis that high water temperatures and high total salt concentrations are limiting factors.

DEVELOPMENTS IN RESEARCH WITH THE CLEAR LAKE GNAT *CHAOBORUS ASTICTOPUS* IN RELATION TO THE FUNGUS *LAGENIDIUM GIGANTEUM*

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ABSTRACT

The Clear Lake gnat, *Chaoborus astictopus*, a non-biting gnat, causes considerable annoyance and discomfort to residents along the shores of Clear Lake in Lake County, California. Preliminary experiments were conducted to determine whether or not the gnat could be infected with the fungus *Lagenidium giganteum*. A small agricultural pond near Clear Lake was selected as the study site. Nine metal frame tubs with heavy gauge 15 gal. plastic bags for liners were filled with silt and water from the pond. Using an Eckman dredge and a plankton tow net, both late and early instar larvae were collected and 300 added to each of the nine tubs. Six tubs were treated with the fungus while three were left as untreated controls. The six treated tubs were found to be positive for *Lagenidium*. A two inch diameter plexi-glass sectional column was constructed to supply information on the depth to which the gnat larvae penetrate a pond bottom. This information aided in consturction of six, 15 foot plastic columns (five inch diameter), which were sunk approximately 18 inches into the experimental pond's bottom. Collapsible emergence cages with modified light traps were placed over the emersed tubes. Two hundred late and early instar larvae were added to the six tubes; to three of the tubes fungal inoculum was introduced at three dosages: low, 5×10^5 zoospores; medium, 4×10^6 zoospores and high, 15×10^6 zoospores. No difference was observed between the low dose and the untreated tubes, but a clear cut difference was seen between the untreated and the high and medium dose.

LABORATORY INFECTION OF *ANOPHELES FREEBORNI* WITH THE PARASITIC FUNGI, *COELOMOMYCES DODGEI* AND *COELOMOMYCES PUNCTATUS*

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ABSTRACT

Exposure of first-instar larvae of *Anopheles freeborni* in the laboratory to the parasitic fungi, *Coelomomyces dodgei* and *Coelomomyces punctatus*, resulted in mean infection rates (= mortality) of 86 and 90%, respectively. In comparative tests, *A. freeborni* was more susceptible to both fungi than *A. quadrimaculatus*.

INTRODUCTION.–The renewed interest in the development of integrated mosquito control programs has stimulated a substantial research effort directed toward the discovery, evaluation, and development of natural enemies for implementation in mosquito abatement projects (Bay 1974; Chapman 1974). In addition to parasites like the nematode *Romanomermis culicivorax*, and predators such as the notonectid, *Notonecta unifasciata*, and mosquito fish, *Gambusia affinis*, a wide variety of microbial agents including viruses and fungi are receiving considerable attention (Federici 1974; Roberts 1974). Fungi of the genus *Coelomomyces* compose one of the most potentially promising groups of microbial mosquito control agents because of their documented ability to produce high larval mortaltiy rates in the laboratory and field. Additionally, of the wide variety of organisms that occur in the aquatic ecosystems in which mosquitoes breed, the mosquito-specific species of *Coelomomyces* appear to infect only their intermediate copepod hosts and mosquitoes.

Until recently most species of *Coelomomyces* which attacked anophelines were thought to be relatively species specific (Couch and Umphlett 1963). However, in field studies Chapman et al. (1970) and Chapman and Glenn (1972) demonstrated that *Coelomomyces punctatus*, a parasite of *Anopheles quadrimaculatus*, could parasitize *An. bradleyi* and *An. crucians*. Later, Federici et al. (1975) showed that *C. punctatus* could also parasitize the exotic anopheline, *An. stephensi*, but was apparently not able to infect several species of the genera *Aedes*, *Culex*, and *Culiseta*. More recently, Federici and Chapman (1977) demonstrated in laboratory studies that *C. dodgei*, a parasite of *An. crucians*, could infect *An. quadrimaculatus*.

The results of the above studies suggested that the important California anopheline, *An. freeborni*, might be susceptible to *C. dodgei* and *C. punctatus*. The present brief study was undertaken to examine this possibility.

MATERIALS AND METHODS.–Mosquito colonies. Adults of *An. quadrimaculatus* and *An. freeborni* were held in 2 ft^3 cages and the females were allowed to engorge on white mice twice a week. Split raisins were placed in the cage for additional nutrition on a weekly basis. For oviposition, paper cups lined with paper toweling and filled with distilled water were placed in the cages for 2 days. The egg containing cups were then removed from the cage and after hatching, approximately 48 hrs later, the larvae were transferred to large shallow containers where they were reared in distilled water on a powdered dog biscuit and brewer's yeast diet (1:1).

In vivo cultures of *C. dodgei* and *C. punctatus*. These two species of fungi have complex life cycles. They alternate obligately between an intermediate copepod host, and a definitive mosquito host. In the laboratory at Riverside, both *C. dodgei* and *C. punctatus* are maintained routinely using *Cyclops vernalis* and *An. quadrimaculatus* as the intermediate and definitive hosts, respectively. The methods used to maintain these in vivo cultures have been described in detail elsewhere (Federici and Roberts 1976; Federici and Chapman 1977). For the sake of brevity only those aspects of the fungal life cycle relevant to mosquito infection, and the procedure for mosquito infection, are described here.

The general life cycle is the same for both *C. dodgei* and *C. punctatus*. In each case, the fungus produces a male or female gametophyte, or in some cases a male and female gametophyte in the copepod. In advanced stages of development, the copepods harboring these gametophytes are easy to recognize because the male gametophyte is bright orange and the female is light amber. These colors are imparted to the copepod. In its ultimate phase, the gametophyte becomes a gametangium and cleaves into thousands of gametes. The gametangium ruptures releasing the gametes and killing the copepod. The gametes escape from the copepod and male and female gametes fuse to form mosquito-infective zygotes. Many details of these complex processes remain to be resolved, but from these observations a simple procedure for infecting mosquito has been developed involving combining patently infected copepods with mosquito larvae in a suitable medium during the above phase of the fungus life cycle.

In the present study, 100 first-instar larvae of *An. freeborni* or *An. quadrimaculatus*, were placed in 50 ml of larval rearing medium in a 8x20x6 cm plastic container along with 12 infected copepods, 4 containing male, 4 containing female, and 4 containing both male and female gametophytes. The copepods and larvae were incubated together at 26° C for 48 hrs, during which mosquito infection occurred. After this period, the larvae were transferred to 1 L of distilled water and reared on the standard larval diet. They were then examined every other day for the presence of sporangia within the hemocoel, definitive evidence of larval infection.

RESULTS.–The results are shown in Table 1. Although these results must be considered preliminary and are only valid for first-instar larvae under the experimental conditions described, *An. freeborni* is apparently more susceptible to *C. dodgei* and *C. punctatus* than *An. quadrimaculatus.*

Table 1.–Results of exposing first-instar larvae of *Anopheles freeborni* and *An. quadrimaculatus* to *Coelomomyces dodgei* and *C. punctatus.*

Replicate	Mosquito Species	% Infection (=Mortality) *C. dodgei*	*C. punctatus*
1	*An. freeborni*	89	99
	An. quadrimaculatus	23	63
2	*An. freeborni*	73	83
	An. quadrimaculatus	56	74
3	*An. freeborni*	96	88
	An. quadrimaculatus	48	52
Mean %	*An. freeborni*	86	90
Infection	*An. quadrimaculatus*	42.3	63

Microscopic examination of infected larvae demonstrated that the sporangia of both *C. dodgei* and *C. punctatus* were typical and could not be distinguished from sporangia of these species produced in *An. quadrimaculatus.* Furthermore, the sporangia released typical meiospores approximately 48 hrs after being flooded with distilled water. Hence, *An. freeborni* is not only highly susceptible to *C. dodgei* and *C. punctatus*, but is a competent host for these species.

DISCUSSION.–The results of this study indicate that *C. dodgei* and *C. punctatus* deserve serious attention as possible biological control agents for *An. freeborni* in California. There are 3 ways in which these species may possibly be used in mosquito abatement programs, two of which are relatively simple and perhaps obtainable in the short-term sense and the third quite complex, most likely requiring a great deal more research and, hence, considered long-term in nature, but possibly providing greater control. In the first case, sporangia could be mass produced and introduced into habitats where *An. freeborni* breeds, along with a suitable intermediate crustacean host if one is not already present. The fungi would then exert their inherent population suppression potential, whatever it may be, with little further input by man. In principle, this is conceptually similar to the introduction of foreign parasites and predators to control introduced or native pests. In the second case, a variation of this technique, copepods could be mass produced, infected, and disseminated, rather than sporangia. Copepods could probably be mass produced using techniques similar to those used to mass produce mosquitofish. Techniques are currently being developed at Riverside to obtain high levels of copepod infection and the initial results look promising. In the third case, without going into great detail, the object would be to mass produce mosquito-infective zygotes by culturing the gametophytes on an artificial medium. The zygotes could then be produced at will and disseminated against larvae of *An. freeborni* using techniques similar to those employed for chemical pesticides.

The development of the above methodologies would have advantages over a chemical in that the materials employed would be very specific, infecting only mosquitoes and an intermediate copepod host. Additionally, these fungi would probably have substantial residual activity and in certain areas, it is likely that they would become permanently established. At present, the main disadvantage of trying to use these agents in mosquito control programs is that the technology necessary for their manipulation has yet to be developed. Furthermore, the environmental impact that would result from their introduction has not been determined. However, considering the advances made in our knowledge of these fungi during the past year, it appears that *C. dodgei* and *C. punctatus* have mosquito control potential and could play a significant role, if developed effectively, in integrated control programs directed toward the abatement of *An. freeborni.*

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COELOMOMYCES PUNCTATUS: THE EFFECT OF HIGH INTERMEDIATE COPEPOD HOST DENSITIES ON LARVAL MOSQUITO INFECTION RATES

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ABSTRACT

The fungus *Coelomomyces punctatus* (Chytridiomyces: Blastocladiales) has a complex life cycle involving an obligate alternation of generations between an intermediate copepod host, *Cyclops vernalis*, and a definitive mosquito host, *Anopheles quadrimaculatus*. Tentatively, sporangia liberated from infected mosquito larvae after death release meiospores which infect copepods. Each meiospore produces either an orange male or light amber female gametophyte. The gametophytes develop into gametangia which subsequently release gametes into the copepod hemocoel. Shortly thereafter, the copepod integument ruptures, possibly as a result of a fungus produced chitinase, and the gametes escape into the surrounding medium where male and female gametes fuse to form mosquito-infective zygotes. The zygotes infect mosquitoes and develop into a sporophyte which produces sporangia, thereby completing the life cycle. During the past year at Riverside, studies have concentrated on identifying the parameters which favor the completion of this life cycle, and therefore, contribute to high rates of copepod and mosquito infection. Three important parameters identified to date are (1) the medium employed in experimental procedures, (2) the developmental stage of the host at the time of infection, first-instar mosquito larvae and copepod nauplii being the most susceptible, and (3) the physiological condition of the copepod during fungal development, particularly as affected by copepod densities and nutrition, e.g., fungal development is retarded at high densities and when copepod nutrition is inadequate. The subject of the present paper is the effect of copepod host densities on gametophyte development, and hence, on larval mosquito infection rates.

From previous studies with *C. punctatus*, it was known that larval mosquito infection rates in experimental containers decreased with time, but could be increased rapidly simply by diluting the experimental medium with an equal amount of water. These experiments were conducted before the copepod, *Cyclops vernalis*, had been identified as an intermediate host for the fungus. With this knowledge, laboratory experiments similar to those conducted previously were performed and special attention was paid to copepod activity in relation to mosquito infection rates.

The results demonstrated that the copepod population underwent a rapid expansion over a period of approximately two to three weeks, after which copepod development was retarded, apparently as a result of overcrowding. Microscopic examination of copepods revealed a small percentage contained gametophytes, most of which were developing slowly. When consecutive groups of larvae were exposed to these dense populations of copepods for 24-hr periods, less than 10% developed infections. However, when the medium was diluted, the larval infection rate climbed to 100% in each group by the fifth day post-dilution. Examination of copepods in the diluted medium demonstrated a rapid development of the gametophytes, with cleavage and liberation of gametes being particularly high from the fourth to sixth days post-dilution. In summary, the population dynamics of the intermediate copepod host have important effects on mosquito infection rates. Further investigation of this phenomenon should provide data which will elucidate the complex of parameters that favor epizootics by these fungi in larval populations and contribute to man's ability to manipulate *Coelomomyces* to his advantage.

COELOMYCIDIUM SIMULII: A FUNGAL PATHOGEN IN LARVAE OF *SIMULIUM VITTATUM* FROM THE COLORADO RIVER

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ABSTRACT

The fungal pathogen *Coelomycidium simulii* Debaisieux (Chytridiomycetes:Chytridiales) has been found in larvae of the blackfly, *Simulium vittatum*, breeding in the Colorado River near Needles, California. The incidence of the disease was low, much less than 1%, although all infected larvae succumbed to the disease. In the early stages of the disease the fungus developed primarily in the larval hemocoel. As the disease progressed the fat body underwent extensive atrophy, and concomitantly, the fungal thalli and sporangia penetrated into the resultant hemocoelic cavities. Zoospores were formed by vesicles in the thalli and sporangia, and were eventually liberated into the hemocoel giving the hemolymph a milky color.

Death usually resulted a few hours after the initiation of zoospore release.

INTRODUCTION.–The fungus, *Coelomycidium simulii* (Chytridiomycetes:Chytridiales) is primarily a parasite of blackfly larvae although there are unconfirmed reports of its occurrence in mosquito larvae (Sherban and Golberg 1971; Kuprijanova and Aksenova 1973) and mayfly nymphs (Weiser 1947). It is widespread and has been reported in many different species of blackflies from Europe (Debaisieux 1920; Loubes and Manier 1974; Weiser and Zizka 1974b), Asia (Levchenko et al. 1974), Africa (Henrard 1930), North America (Jamnback 1973; Weiser and Zizka 1974a), and South America (Jamnback 1973). Debaisieux (1919) published the original description of *C. simulii* as a fungal parasite, but its occurrence in blackfly larvae had been noted earlier by Strickland (1913). Although widespread, its incidence in larval populations is low, usually less than 1%. This fungus has not been successfully transmitted in the laboratory and hence, all of the published studies on the biology and development of the disease have employed field collected specimens. The peculiar development of this fungus, the uncertainty of its taxonomic position, and the fact that once its life cycle is known it may have potential as a biological control agent of blackflies make *C. simulii* of unusual interest.

During the spring of 1975, several larvae of *S. vittatum* collected from the Colorado River were found infected with *C. simulii*. This brief paper reports the initial results of studies on the occurrence and pathology of this strain of *C. simulii* in *S. vittatum*.

MATERIALS AND METHODS.–The healthy and infected larvae used in these investigations were collected from the Colorado River in the vicinity of Needles, California. After collection, they were transferred to water in lidded plastic cups and placed in a polystyrene container on a bed of crushed ice for transportation to the laboratory at Riverside. Observations on the gross pathology of the disease were made by placing diseased and healthy larvae in a small amount of water in a black photographic tray and examining them with a Wild® dissecting microscope at magifications of 12 and 25x. For light microscopy, infected and healthy larvae were fixed in aqueous Bouin's fixative for 24-48 hrs, dehydrated in an ethanol series, and embedded in Paraplast. Serial sections were cut at a thickness of 6μm and subsequently stained with Hamm's (1966) modified azan stain. For electron microscopy larvae were killed and prefixed by placing them in 3% glutaraldehyde in 0.1M cacodylate buffer for 30 minutes. After the pre-fixation, the larvae were dissected into three portions and placed in fresh fixative for two hours. They were then rinsed in buffer, post-fixed in 1% buffered OsO_4, dehydrated, and embedded in Epon-Araldite. Ultrathin sections were cut on a Sorvall MT-2B ultramicrotome using a diamond knife, post-stained with uranyl acetate followed by lead citrate (Venable and Coggeshall 1965), and examined with a Hitachi 12 electron microscope.

RESULTS.–Against the black background of the photographic tray, the fat body tissue of diseased larvae was markedly more opaque than that of healthy specimens. In larvae with advanced infections, a slightly orange to pink discoloration was frequently observed in areas of the fat body heavily penetrated by fungal thalli. Under the dissecting scope, the abdominal cuticle was often translucent, apparently as a result of incomplete sclerotization, and lobate thalli and thin-walled sporangia could be seen beneath these areas. In histological sections of larvae in which the disease was at an early stage of development, pleomorphic thalli and sporangia were observed developing throughout the larval hemocoel (Figure 1). The thalli and sporangia contained both granular and translucent areas. In ultrathin sections of larvae at this stage of disease development the translucent areas were found to consist of large pleomorphic vesicles within which the component structures of flagellated zoospores were formed. The zoospore precursors appeared to assemble at the vesicle membrane and subsequently cleave from the large vesicle, thereby forming a zoospore (Figure 2). The granular areas of the sporangia and thalli consisted of closely packed zoospores.

In larvae with advanced infections, the fat body had greatly atrophied and the fungal thalli had completely invaded the resultant hemocoelic cavities. At this stage, most of the thalli and sporangia were very dense and completely filled with zoospores (Figures 3 and 4). In living infected larvae at this phase many sporangia released their zoospore complement into the hemolymph giving it a milky color

(Figures 5 and 6). In fixed sections these liberated flagellated zoospores were spherical in shape and measured approximately 5 μm in diameter.

Although the incidence of the disease in field-collected larvae was very low, much less than 1%, all larvae brought back to the laboratory and reared eventually succumed to the disease, usually within a few days.

DISCUSSION.--This report represents the first record of *Coelomycidium simulii* in California, although its occurrence here is not surprising given the great number of blackfly species which occur within the state and the apparent ubiquity of *C. simulii*. The widespread occurrence of *C. simulii* raises the question of whether the reports of the disease caused by this fungus represent a single disease caused by a single species, or a group of different diseases with similar characteristics caused by several different related fungi. The limited number of diseased specimens collected from the Colorado River precluded a detailed comparison of this material with the published information available on diseased blackflies collected by other investigators. Yet, even a superficial comparison reveals differences in the structure of the thalli and sporangia reported here and those described by Weiser and Zizka (1947a,b) from *S. latipes*, *S. equinum*, *S. pugetense*, and *Odagmia ornata*. Specifically, these authors demonstrated "Plasmatic bridges" developed between zoospores of *C. simulii* during their formation in the above species. They also demonstrated "free zones" on the periphery of the sporangia. Neither of these types of structures has been observed in *C. simulii* in *S. vittatum*. Ultrastructurally, the *C. simulii* from *S. vittatum* more closely resembles the *C. simulii* studied by Loubes and Manier (1974) in *S. brezzii*, *S. monticola*, *S. aureum*, and *S. variegatum*. In particular, the structure of the sporangia, lacking a distinct "free zone", and the zoospores without "plasmatic" bridges are very similar. The variations observed in *C. simulii* by different investigators have several possible explanations. The fungal specimens studied may be different species of the same type of fungus, or different stages or seasonal forms of the same fungus.

The taxonomic relationship of the organisms described as *C. simulii* here and by other authors will probably only be resolved after the establishment of laboratory cultures of these organisms and comparative studies on the life cycle and development of different isolates is undertaken by several laboratories.

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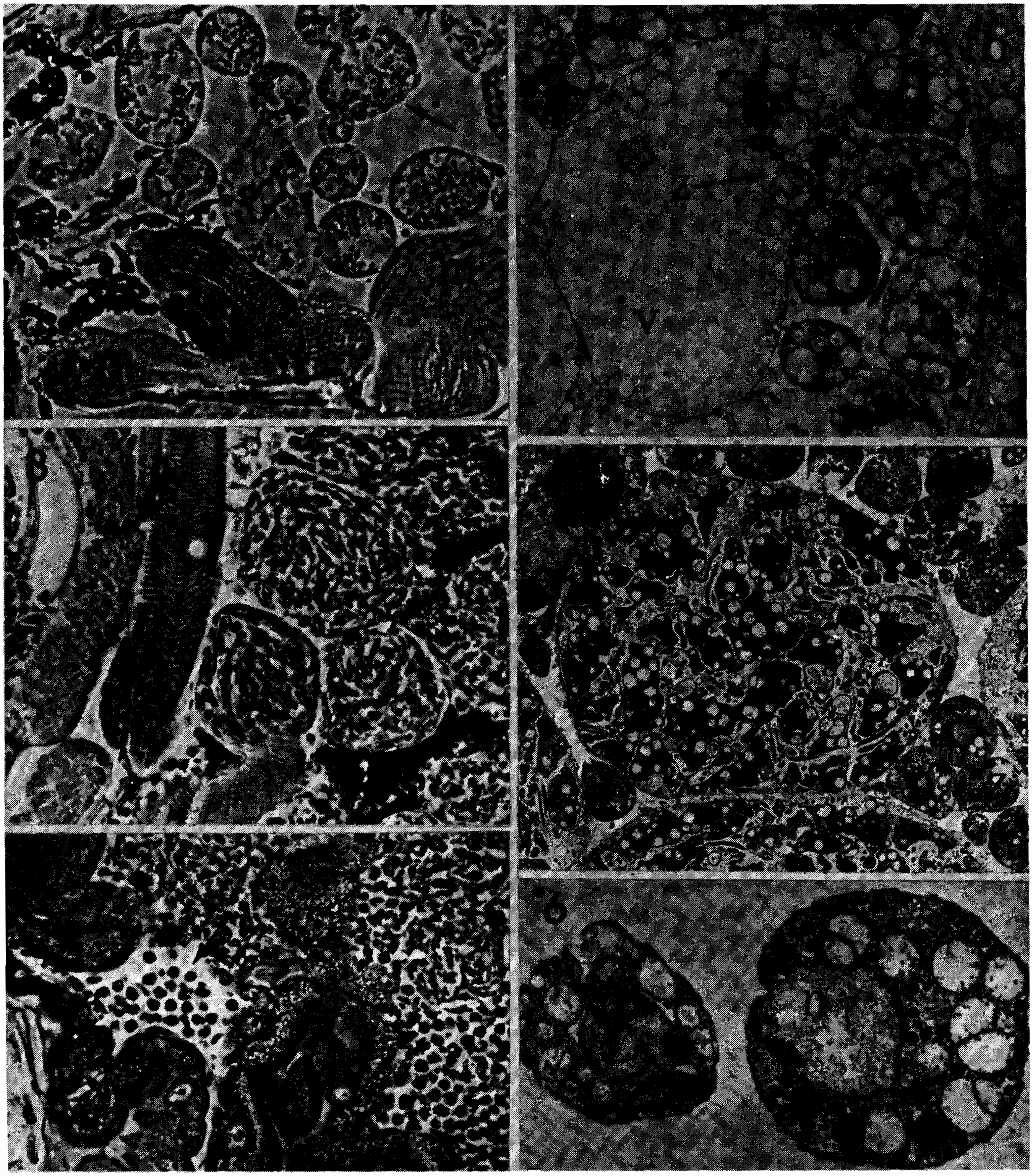

Figures 1-6.—Light and electron micrographs of *Coelomycidium simulii* in *Simulium vittatum*. Fb, Fat body; M, muscle; N, nucleus; T, thallus; V, zoospore-forming vesicle; Z, zoospore.

Figure 1.–Thallus and thin-walled sporangium (arrow) at an early stage of development in the larval hemocoel. The granular areas within the sporangia are mature zoospores. The translucent areas contain large vesicles from which zoospores are formed. 1 μm plastic section, X260.

Figure 2.–Cross section through a portion of a sporangium at a developmental stage similar to the sporangia in Figure 1. A large vesicle is shown in the process of zoospore formation (arrow). The zoospore precursors appear to aggregate at the vesicle periphery after which the complete zoospore is cleaved from the vesicle. Electron micrograph, X5, 200.

Figure 3.–Sporangia in the hemocoel of a larva in which the disease is at an advanced stage of development. The sporangia at this stage are completely filled with mature zoospores. 1 μm plastic section, X500.

Figure 4.–Cross section through a sporangium similar to those shown in Figure 3. Note that this sporangium is completely filled with zoospores and lacks zoospore-forming vesicles. The zoospores surrounding the sporangia have been liberated from other mature sporangia. Electron micrograph, X2, 500.

Figure 5.–Free zoospores (arrow) which have been liberated from sporangia into the larval hemocoel. 1 μm plastic section, X400.

Figure 6.–Section through portions of two zoospores free in the larval hemocoel. Electron micrograph, X11,000.

ISOLATION OF TRANSLOCATION HOMOZGOTES FOR GENETIC CONTROL OF *CULEX TARSALIS*

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ABSTRACT

A pure-breeding line of an autosomal translocation has been isolated. Under current practices of isolating, maintaining and testing translocation stocks, 22 generations had passed before this homozygote line was established. A new method, involving the irradiation of mutant-marked stocks rather than unmarked stocks, has been inaugurated. Such a method should drastically reduce the complexity and duration of testing needed for establishing the homozygote stocks. Translocation homozygotes will be used for generating translocation-heterozygous males that are partially sterile for a genetic control release.

LABORATORY AND FIELD CAGE COMPETITION STUDIES OF TRANSLOCATION-CARRYING MALES IN *CULEX TARSALIS*

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ABSTRACT

Culex tarsalis males from a line carrying a double male-sex-linked heterozygous translocation, T(1;2;3)1A were used in competition studies against normal laboratory and field populations. The complex interchange in this line is transmitted only to males in subsequent generations, and the mean percent hatch of egg rafts fathered by these males is between 25-30%. Laboratory small-cage studies and controlled field studies in modified quonset-hut cages showed that the genetically-altered line at a 1:1 ratio was able to successfully compete with both untreated laboratory and field collected material. In addition, the progeny stemming from matings of the experimental males were able to develop normally under the field environment, and the F_1 males were able to effectively compete to suppress egg hatch in the second generation.

AEDES SIERRENSIS: DETERMINATION OF THE OPTIMAL RADIATION DOSE FOR COMPETITIVE STERILE-MALE CONTROL[1]

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INTRODUCTION.–The feasibility of using radiation to sterilize insect pests and, subsequently, to develop a genetic control program has been established. The control of screw-worm in the southeastern and southwestern United States is the first and best publicized example of this control method. In addition, moderate to good success has been achieved with *Haematobium irritans* in Texas and *Stomoxys calcitrans* in Florida, as well as with a number of agricultural pests (Davidson 1974). However, the radiation-sterilization technique has not found wide use in control of mosquitoes. Only 5 such releases have been made (Table 1) which can be divided into 2 groups. The 1st group was the earlier releases made shortly after the screw-worm eradication in Florida. Their lack of success in inducing sterility into natural populations did much to turn genetic control away from radiation sterilization toward the use of chemosterilants for mosquitoes. These releases used high radiation doses insuring complete sterilization but drastically reduced the mating vigor of the released males. Therefore, the sterility was not carried into the wild population.

The 2nd group consisted of more recent releases with moderate success in inducing sterility in the field. Lower doses of radiation were used. This achieved a compromise in which the released males were not completely sterilized, but were vigorous enough to compete in the field. It is with this background that we are establishing a sterile-male program for control of *Aedes sierrensis*. We are here reporting preliminary results of the sterilization effects of different radiation doses with this species and an approach for evaluating the mating competitiveness of these irradiated males. The goal is to identify the radiation dose that gives the highest sterility while maintaining good mating ability.

MATERIALS AND METHODS.–The strain of *Ae. sierrensis* used originated from larvae collected in Solano County, California in 1974. Insectary conditions were maintained at 24°C, 80% R.H., and 15L:9D light cycle. By trial and error we devised a rearing and radiation schedule for this species that provided uniform growth and good survival with a minimum of effort. Egg papers were placed in a solution of liver powder and dilute tea water for 2 days. This provided 20% hatch. Pans were set up with 150-200 larvae in 1200 ml H_2O with 500 cm^2 surface area. Larval feeding consisted of 0.6 g of ground Purina rat chow every 3rd day. Larval development was completed in 14-16 days and the duration of pupation was 4 days. High mortality was experienced when pupae were collected and held in deionized H_2O. However, when larval rearing water was added, the mortality problem disappeared. Approximately 50-60% of the males emerged on the 18th day and were irradiated. After treatment these males were held for 1 wk before females were introduced. Honey was preferred to sugar cubes as the carbohydrate source. One week later and weekly, thereafter, for the next 3 months an anesthetized mouse was provided. Earlier attempts at bloodfeeding gave sporadic results and were not worth the effort. The following week and weekly, thereafter, egg papers were collected. Allowing 15 days for embryonation (Judson et al. 1966), results from the 1st egg paper were obtained ca. 2 months after the initial hatching. Both 3.8 l and 30x 30 x 30 cm cages were used with no obvious mating or oviposition problem.

A Co-60 source located at the Lawrence Berkeley Laboratory was used. All mosquitoes were 0-24 hr postemerged at the time of irradiation and received a dose rate of ca. 200 R/min. In those experiments requiring an anoxic state,

Table 1.–An historical review of field releases of radiation sterilized male mosquitoes.

Mosquito	Release				Success**
	Location	Date	Dose (krad)	Ratio (I:U)*	
An. quadrimaculatus	Florida	1959-60	12	?	0
Ae. aegypti	Florida	1960-61	11-18	25:1	0
Cx. p. quinquefasciatus	India	1962	7	?	6%
Cx. p. quinquefasciatus	India	1971	6	1:1.5	25%
Cx. p. quinquefasciatus	India	1971	6	5:1	60%

*I = irradiated release males. U = unirradiated wild males.
**The percent reduction in field egg hatch.

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nitrogen was used following the method of Hallinan and Rai (1973).

The sterility effects of the different radiation doses could not be determined by the standard bioassay, i.e. percent egg hatch. Instead, we used percent egg embryonation as the criterion for measuring sterility. This was achieved by placing the egg papers in a sodium chlorite bleaching solution for 12-24 hr (Trpis 1970). Following this treatment eggs with embryos could be readily distinguished from unembryonated eggs (Figure 1).

Lacking genetic markers and/or a convenient method of collecting eggs from single females, we were not able to determine whether an individual mating was with an irradiated or an unirradiated male. A method of calculating male mating competitiveness in sterilization studies with the Mediterranean fruit fly was used (Haisch 1970). This formula was:

$$\underline{e} = \frac{q - f}{n\,(f - p)}$$

and was modified so that:

- q = % embryonation of eggs from unirradiated control cage,
- f = % embryonation of eggs from a competition cage in which irradiated and unirradiated males were placed with unirradiated females,
- p = % embryonation of eggs from the control of unirradiated females mated with irradiated males, and
- n = ratio of irradiated males to unirradiated males in the competition cage (f).

The competition cage was 60 X 60 X 45 cm in dimension and contained 50 irradiated males, 50 unirradiated males, and 50 unirradiated females. Each of the controls contained 25 males and 25 females in a 3.8 l cage.

RESULTS AND DISCUSSION.—In establishing the sterility vs. dose response for *Ae. sierrensis*, 2 atmospheres were used: ambient air and nitrogen (Figure 2). The latter was included because of the documented phenomenon that reduced oxygen tension minimized radiation injury (Patt and Brues 1954). In numerous insect sterile-male programs

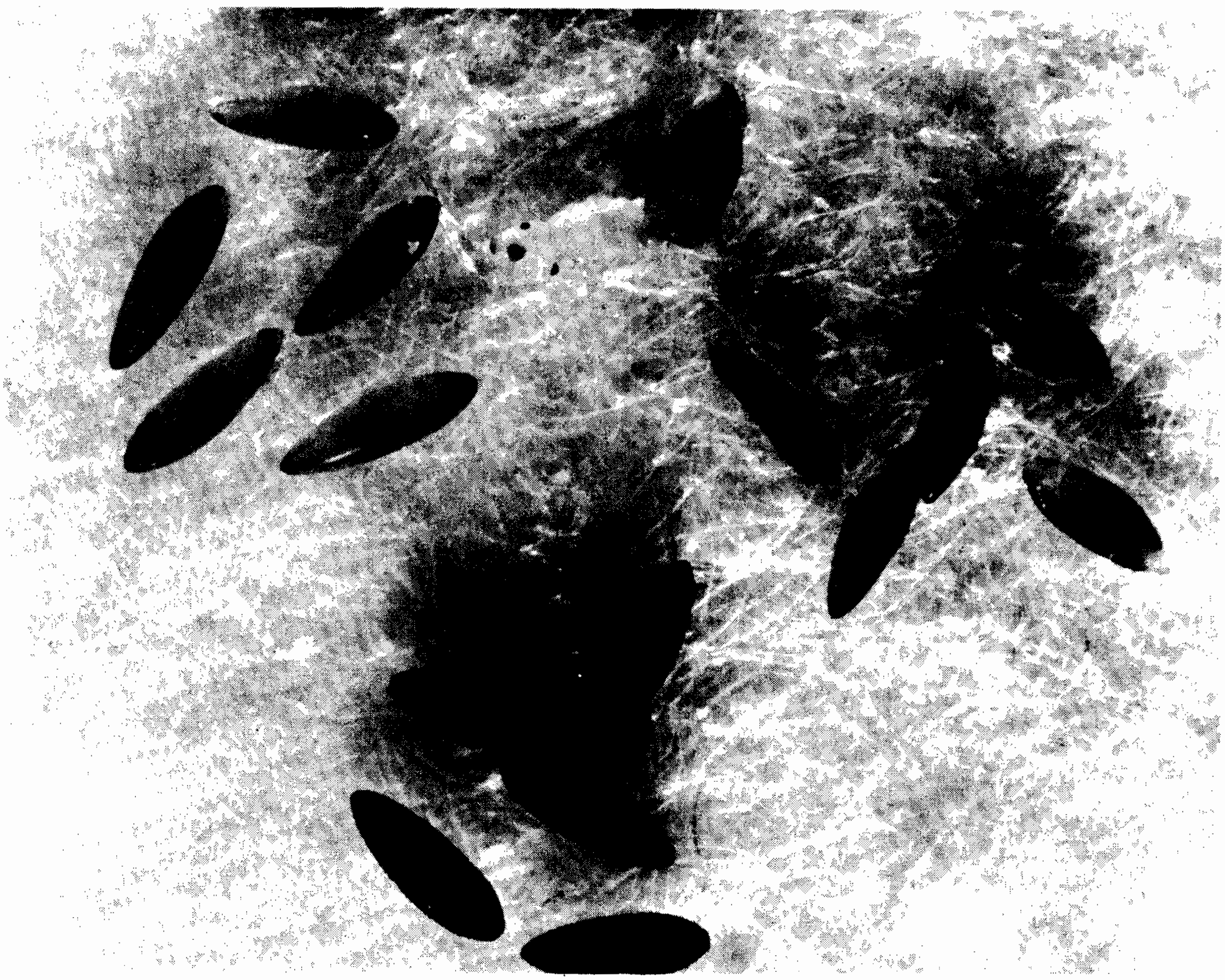

Figure 1.—*Ae. sierrensis* eggs bleached to determine the presence of embryos. Eye-spots and hatching spines are easily detected in bleached eggs on the upper left. The bleached empty eggs on the upper right are unembryonated. The dark eggs in the lower center are left unbleached for comparison.

Table 2.–A comparison of mating competitiveness of *Ae. sierrensis* males irradiated at 5 krad in air and 9 krad in nitrogen.

Dose (krad): Atmosphere	Test ratio Id : Ud : U♀*	Eggs No. examined	Eggs % embryonation	e
0 : air	0 : 1 : 1	1202	75.3	
5 : air	1 : 1 : 1	2543	54.6	0.53
	1 : 0 : 1	1040	15.6	
9 : nitrogen	1 : 1 : 1	4344	51.4	1.01
	1 : 0 : 1	2101	27.8	

*I = irradiated. U = unirradiated.

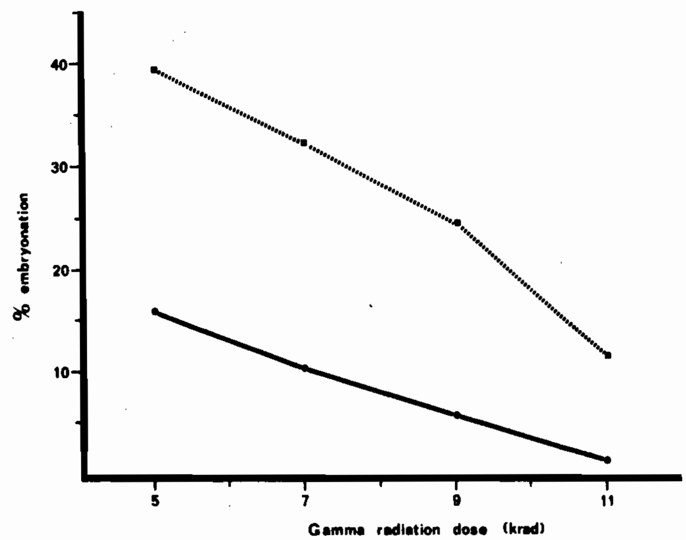

Figure 2.–Sterility response of *Ae. sierrensis* males irradiated at 5, 7, 9, and 11 krad in atmosphere of air (●——●) and nitrogen (■·······■)

it is reported that irradiation in nitrogen produces sexually competitive males (Ashraf et al. 1975). Four gamma radiation doses were used - 5, 7, 9, and 11 krad. The mean number of eggs counted for each dose/atmosphere was 1780 with a range of 1069-2705.

In air, embryonation decreased from 15.8% at 5 krad to 1.9% at 11 krad. The effect at 11 krad may have been greater than that indicated since a portion of the induced dominant lethals will express themselves in later embryonic or larval stages. The sterility curve for irradiation in nitrogen was higher than in air. The embryonation ranged from 39.4% at 5 krad down to 12.0% at 11 krad. This agrees with a similar observation in screw-worm, house fly, and Mediterranean fruit fly programs that irradiation in CO_2 or nitrogen reduces the sterilization effect of a given dose of gamma radiation (Hooper 1971). Therefore, to attain equivalent sterility in the 2 atmospheres it is necessary to irradiate at higher doses in nitrogen than in air. The relationship of 2 doses producing comparable sterilities is termed the "air-nitrogen ratio" (Baldwin and Salthouse 1959). From our preliminary data we are estimating an air-nitrogen ratio of 1.7 for *Ae. sierrensis.* This is reasonably close to the accepted ratio of 2 seen in other insect systems (Hooper 1971).

Having established the preliminary sterility curves for *Ae. sierrensis*, males were irradiated at 5 krad in air and 9 krad in nitrogen for mating competitiveness evaluation (Table 2). Both irradiated controls were similar in sterility to the values predicted in Figure 2. More important, it was demonstrated that in air a dose of 5 krad already reduced male mating vigor by ca. 50%. Probably higher doses of radiation in air will produce still smaller e values. In contrast the higher radiation in nitrogen was not detrimental to male mating competitiveness as evaluated by the present method. Thus it is suggested in this preliminary study that nitrogen provides protection from undesirable radiation side effects, and this agrees with observations made on other insect species.

The reduction in percent embryonation between the unirradiated control and the 2 competitive tests was strikingly similar. However, the strategy to achieve this reduction was different for the 2 tests. Fewer males irradiated with 5 krad in air successfully competed, but those that did delivered a high sterility. Those males irradiated at 9 krad in nitrogen carried a lower sterility but a greater proportion mated.

We are continuing to test other irradiation doses to expand the *Ae. sierrensis* sterility curves from 3 krad to that dose in nitrogen necessary for ca. 1% embryonation. In addition, we are evaluating the mating competitiveness of males irradiated at other doses in search of the optimal dose/atmosphere to be recommended for a sterile-male program.

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ANALYSIS OF FOUR-SITE METHOD FOR TESTING MOSQUITO REPELLENTS[1]

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ABSTRACT

As a part of a program to find a mosquito repellent longer lasting than N,N-diethyl-m-toluamide (deet), the most effective repellent in general use, a four-site testing method using human subjects was developed in order to reduce the number of volunteers necessary for repellent screening. This method was compared to a previously used two-site technique, and no difference was detected between the two methods. Analysis of data also indicated that there was no inter-site or inter-arm variability and that the difference in size of the exposed repellent test site was not significant.

INTRODUCTION.–In efforts to develop a longer-lasting repellent against vector mosquitoes, repellents have been tested on man as a part of the screening program. Mosquito repellent test methods have traditionally employed a single repellent-treated site on each forearm of a test subject, i.e. two-site method (Gilbert et al. 1957, Kurtz et al. 1974). A four-site method was evaluated which (1) reduced the number of volunteers necessary, (2) decreased the number of days to test and (3) eliminated the influence of day-to-day changes in mosquito avidity (Shimmin et al. 1974). This method allowed simultaneous testing of three repellents and deet, the most effective repellent in general use. The current report is a comparison of two-site and four-site application schemes and an analysis of the site variation in the four-site method.

MATERIALS AND METHODS.–In the two-site technique, one 7 x 15 cm test site was drawn on each forearm of five volunteers (10 sites). A predetermined concentration of repellent was applied, spread evenly with a clean glass rod and allowed to dry for fifteen minutes. Adhesive-backed foam pads were placed around the perimeter of each site to prevent site abrasion. Once each hour, the volunteer exposed the repellent-treated site to a cage of 250 female *Aedes aegypti* (L.) mosquitoes for three minutes. If the site received two bites in one test period or in two consecutive test periods, the repellent had failed and that time was recorded as the dry protection time. Testing continued for four days; five different volunteers participated each day (Gilbert et al. 1957).

In the four-site technique, two 7 x 10 cm test sites were drawn on each volunteer's ventral forearm (Shimmin et al. 1974). Repellent application was the same as described for the two-site technique. The applications were rotated so that each repellent was paired with each of the other three repellents at least twice on the same arm of the eight volunteers. The same three-minute biting test was used.

Two groups of four volunteers tested the feasibility of the four-site technique. On the first day group I used the two-site technique while group II used the four-site technique. The groups were reversed on the second day to eliminate subject differences. Volunteers were active-duty military males.

Two repellents were selected for the comparison: deet (N,N-diethyl-m-toluamide, Eastman Chemicals, practical grade) and Rutger's 612 (2-ethyl-1, 3-hexanediol, Eastman Chemicals, practical grade). Repellents were applied at 0.32 mg/cm^2. With the two-site technique, the two repellents appeared an equal number of times on right and left arms. Two additional sites were available on the four-site arms; therefore, two concentrations of an experimental repellent (3,6,6-trioxapentadecan-1-ol (SRI-6), Stanford Research Institute, 96% pure) were selected to accumulate data on the efficacy of this repellent at different concentrations.

The mosquitoes used in the test were *Ae. aegypti* from 6-10 days old, bred so that the eggs hatched in a single 24-hour period. The mosquitoes were kept in constant light to prevent photoperiod effects (Kurtz et al. 1973) and were allowed to feed on sugar-water at will during the test. Approximately 250 female mosquitoes were transferred to the test cage 24 hours prior to the experiment. The test room was kept at 80% relative humidity and 27.8°C.

RESULTS AND DISCUSSION.–In comparing the two-site and four-site techniques, several factors should be considered, including interaction of repellents applied to the same forearm, differences between the upper and lower sites on the same forearm in the four-site method and differences in the protection times against mosquitoes determined by the two methods. Paired comparison of the protection time against mosquitoes revealed no significant difference between deet or Rutger's 612 on a given individual when either two-site or four-site methods were employed (Table 1). Additionally, there was no apparent interaction between repellents applied to the same forearm even though every treatment was paired with every other treatment. Since Khan and Maibach (1972) had previously observed that repellent action against mosquitoes occurred in the air just a few centimeters above the skin, we had not anticipated any observed repellent interaction and our expectations were reaffirmed.

After the four-site technique had been employed for a period of three years as an economical alternative to the two-site technique, mean protection time against mosquitoes afforded by deet with the four-site method was com-

[1]The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

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Table 1.–Protection time in hours for repellents against mosquitoes.

Repellent	Two-site[3] (Mean ± S.D)	Four-site[3] (Mean ± S.D)	t-statistic*
Deet[1]	6.8 ± 2.49	5.9 ± 1.85	0.404
Rutger's 612[1]	3.0 ± 1.41	3.1 ± 0.74	1.323
SRI-6[2]		3.3 ± 3.05	
SRI-6[1]		6.4 ± 4.16	

[1]Repellent concentration of 0.32 mg/cm^2.

[2]Repellent concentration of 0.15 mg/cm^2.

[3]N=8

*t-critical = 2.450; differences are not significant.

Table 2.–Protection time in hours for deet at 0.32 mg/cm^2 against mosquitoes.

Method	N	Mean ± S. D.
4-site	195	6.8 ± 1.88
2-site	39	6.6 ± 2.35

Table 3.–Protection time in hours for deet at 0.32 mg/cm^2 against mosquitoes.

Site	Left (Mean ± S.D.)	Right (Mean ± S. D.)
Wrist	6.5 ± 1.73 (33)	6.9 ± 2.08 (33)
Elbow	7.0 ± 1.81 (34)	6.4 ± 1.96 (32)

pared to the mean protection time obtained when the two-site method had been used in previous years (Table 2). The mean protection times for deet were the same with two test techniques. Hence, the difference in area of the treated site exposed to mosquitoes (70 cm^2 versus 40 cm^2) had little effect on the duration of repellent protection.

The question of inter-site variation between the upper and lower sites on the same forearm, and between the right and left forearms, was resolved by comparing the mean protection times afforded by deet on each site over a period of three years. One concludes from Table 3 that there is no difference in the mean protection time against mosquitoes when the same repellent is applied to the four different test sites. Moreover, there is likewise no difference in the mean protection times determined on the right and left forearms. The latter observation confirms previous studies by Smith et al. (1963) and Kurtz et al. (1973). Since there is no apparent difference in the various test sites, the four-site method can be used in test situations where intersubject differences predominate over the differences between repellents being tested.

In conclusion, the four-site technique provides equivalent mean protection times against mosquitoes compared to a two-site technique used in testing mosquito repellents. The four-site technique reduced the number of volunteers from twenty to eight, decreased the number of test days from four to one, and eliminated the influence of daily changes of mosquito avidity. The four-site technique also has the advantage of each volunteer acting as his own control since deet is always applied to one site. Since we are interested in assessing only which repellents are significantly better than deet, repellents are then analyzed in a simple paired comparison to deet. In addition there appears to be no significant difference in results obtained from either method.

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STUDIES OF REPELLENT FORMULATIONS WITH N,N-diethyl-m-toluamide[1]

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ABSTRACT

Mosquito repellent formulations containing either meta-deet (N,N-diethyl-m-toluamide) or its isomer para-deet (N-N-diethyl-p-toluamide) as active ingredients were developed. Silicone and acrylic polymers and lecithin were incorporated into these formulations. The formulations were evaluated in vivo for protection time against mosquitoes. Representative formulations were evaluated in vitro using an evaporation apparatus. The effect of temperature on evaporation rate was found to be similar for both meta-deet and para-deet. No significant difference was found in protection time against mosquitoes for the two isomers.

INTRODUCTION.–Mosquito repellents are used by civilians and military personnel in situations where the duration of protection against mosquitoes is shortened by sweating, evaporation, abrasion and contact with water (Maibach et al. 1974; Smith et al. 1963; Gabel et al. 1976). Penetration of repellents into the skin represents another limiting factor (Smith et al. 1963). Therefore, mosquito repellents have been formulated to attenuate these avenues of loss while still allowing sufficient repellent evaporation for protection against mosquitoes.

Screening large numbers of formulations for efficacy with human volunteers places a tremendous burden on available resources; therefore, it is desirable to develop in vitro technology for initial formulation screening. This report describes how a repellent evaporation apparatus was used to study various formulations of meta-deet and para-deet with polymers. Results of the invitro evaluation are compared with in vivo tests against mosquitoes.

MATERIALS AND METHODS.–Repellents used in these studies include meta-deet (N,N-diethyl-m-toluamide, practical grade, 95% pure, Eastman Chemicals Co.), para-deet (N,N-diethyl-p-toluamide, practical grade, m.p. 53-55° C), and ^{14}C-labeled analogs of these compounds synthesized for in vitro testing with these formulations. The polymers used were Carboset® 526 and 526M acrylic polymers (B. F. Goodrich Corporation) and Silicone MDX-4-4150B® (Dow Corning Inc.)

Repellents in ethanol solution were applied to two 8 x 15 cm test sites, one on each of the ventral forearms of each subject. Solutions were designed so that a specific weight per unit area of the active repellent ingredient was applied to the skin. Four repellent formulations and deet were applied to the forearms of five subjects for four successive days in a partially balanced incomplete block design (Kurtz et al. 1973). Once each hour following application, the treated arms were covered with plastic sleeves which had 7 x 10 cm holes cut to correspond to the treated sites. The treated site was then exposed in a cage containing 250 female *Aedes aegypti* mosquitoes (7-9 days old). When two bites were received in a three-minute test (or in two successive tests), the repellent was considered to have failed and the time was recorded as the protection time. The mosquitoes were maintained 27.8°C and 80% relative humidity under constant light with sugar solution constantly available.

The in vitro evaporation apparatus used for studying repellent formulations has been described in detail by Spencer et al. (1976). Radio-labeled repellent formulations were applied in ethanol or acetone solution to a scratched aluminum planchet. The disk was then placed in a temperature controlled chamber (32°C) and dry air was passed over the repellent-treated surface and collected in a toluene trap. The amount of radio-labeled repellent evaporating each hour was measured in a liquid scintillation counter (Mark IV, Nuclear Chicago) and was recorded as μg of repellent evaporating each hour for one square centimeter of application ($\mu g/cm^2/hr$).

The temperature dependence of evaporation of the repellent was determined by applying large amounts of the compound (> 2 mg/cm^2) to the surface (0.6cm^2) of the aluminum planchet, thus forming a pool of liquid repellent solution or a smooth surface of the solid. Evaporation rates of the repellent were recorded until a steady state evaporation was reached. The temperature of the evaporation chamber and the air passing over the cup was then changed and steady state evaporation was obtained at each temperature (range: 29.5°C - 37.7°C). The evaporation rate was recorded as a function of temperature for liquid meta-deet, solid powdered para-deet, and liquid para-deet deposited from an ethanolic solution. Since the melting point of the para-deet is low, para-deet applied in solution did not recrystalize to form the solid.

RESULTS AND DISCUSSION.–The evaporation rate and temperature dependence of evaporation are shown in Table 1. Energy of activation or temperature dependence of evaporation rate is a function of the reciprocal of the temperature in Kelvin degrees (Moore 1972). The energy of activation is a measure of the energy necessary to effect the change from liquid to vapor under the conditions in the evaporation chamber. We observed little difference in the evaporation rate of meta-deet and para-deet in the liquid state, which would indicate that there should be little difference in the duration of protection between the two com-

[1]The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

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Table 1.–In Vivo protection times and In Vitro evaporation characteristics of meta-deet and para-deet[1].

Repellent	Evaporation Rate $\mu g/cm^2/h$ at 30°C	Energy of Vaporization (kJ/mole)	In Vivo Protection Time[4]
meta-deet	43.0	80.5	4.7 ± 1.63 (20)
para-deet (1)[2]	39.0	81.9	4.8 ± 1.41 (30)
para-deet (s)[3]	14.7	106.8	NA[5]

[1]Evaporation from scored aluminum planchet, dry air flow 40 ml/min at >2 mg/cm². Values are calculated from evaporation versus reciprocal temperature graphs.

[2]Para-deet applied in ethanol solution.

[3]Pata-deet applied as crystalline solid.

[4]Mean protection time ± standard deviation in hours against *Aedes agypti* mosquitoes at a dose of 0.25 mg/cm². Number of replicates in parenthesis.

[5]Not applicable.

Table 2.–In Vivo protection times and In Vitro evaporation characteristics of meta-deet formulations.

Additive	Additive:Repellent Ratio	Dose[1]	In Vivo Duration[2]	Dose[4]	In Vitro[3] Evap Rate[5]	Duration
none	-	0.25	4.6 ± 1.63 (20)	-		
Carboset® 526	0.5:1	0.25	6.0 ± 2.00 (4)	-		
Carboset® 526	1:1	0.25	4.5 ± 1.92 (4)	0.25	35	6
Carboset® 526	2:1	0.25	5.5 ± 2.52 (4)	0.25	22	5
Carboset® 526	4:1	0.25	3.0 ± 1.63 (4)	0.25	2	0
none	-	0.50	11.3 ± 1.49 (8)	1.00	57	20
Carboset® 526	0.5:1	0.50	18.3 ± 6.14 (8)	1.00	48	27
Carboset® 526M	0.5:1	0.50	14.0 ± 4.52 (8)	-		
Slicone	0.5:1	0.50	8.8 ± 3.01 (8)	1.00	40	41
none	-	0.15	4.43 ± 1.47 (127)	-		
Lecithin	0.5:1	0.15	0.75 ± 0.50 (4)	-		
Lecithin	1:1	0.15	0.50 ± 0.00 (4)	-		
Lecithin	2:1	0.15	0.75 ± 0.50 (4)	-		

[1]Amount of meta-deet applied in milligrams per square centimeter skin surface area.

[2]Mean protection time ± standard deviation in hours against *Aedes agypti* mosquitoes. Number of replicates in parenthesis.

[3]Results from a single trial at 32°C.

[4]Amount of meta-deet applied in milligrams per square centimeter planchet area.

[5]Steady state evaporation rate of repellent at 32°C.

pounds as observed in vivo (Table 1). A markedly lower evaporation rate is associated with solid para-deet which results from the energy required for the solid-liquid transition prior to the liquid-vapor transition, which is reflected in the higher energy of activation observed for the solid para-deet compared to the liquid para-deet or meta-deet (Table 1). The liquid phase rather than the solid phase is the probable form of both meta- and para-deet when applied to the skin in solution.

The energy of activation function can be used to estimate the temperature dependence of the evaporation rate in vivo. Considering the energy of activation from Table 1, one estimates that the rate of evaporation of meta-deet doubles when the temperature increases from 25° to 31°C. Previous work by Khan et al. (1974) has shown that increasing temperature correlates with a decrease in the protection time afforded by meta-deet.

In vitro repellent evaporation rates from formulations estimate only one factor among many (such as skin penetration and repellent-skin lipid interaction) which govern the duration of repellent formulations in vivo. We observed that mixtures of Carboset® and meta-deet did not affect the steady state evaporation rate of meta-deet at an applied dose of one mg/cm² (Table 2). However, a 0.5:1 Carboset: meta-deet formulation (1 mg/cm²) persisted longer in the evaporation chamber (27 h) compared to meta-deet (20 h). When the 0.5:1 formulation was tested in vivo against mosquitoes, the formulation's protection time was longer than the protection time of meta-deet alone (Table 2). If the polymer:meta-deet ratio is increased to 1:1, 2:1 or 4:1 the steady state evaporation rate and the persistence in vitro is reduced (Table 2). These results indicate that higher polymer deet ratios would not lead to increased repellent performance in vivo since sufficient meta-deet cannot evaporate.

Examination of the results of Table 2 indicate superior duration of the silicone formulation over unformulated meta-deet invitro. However, in vivo the duration of silicone formulations is comparable to meta-deet. These results again emphasize the importance of additional factors in in vivo repellent duration.

Lecithin was incorporated into formulations with meta-deet in an attempt to control repellent release and extend protection time; however, these formulations actually resulted in decreased repellent performance (Table 2).

In actual use, wash resistance plays an important role in overall repellent performance. Carboset formulations have been found to give good wash resistance, while the silicone formulation offered little or no protection from wash-off (Kurtz et al. 1973). In addition, consumer cosmetic acceptability complicates overall evaluation of formulations. The specific silicone and Carboset formulations reported here form a tacky, stiff coating when applied to the skin. Hence, the formulatins are cosmetically unacceptable.

In summary, the evaporation apparatus has been used to examine mosquito repellent formulations and the results of these tests have been compared to in vivo testing. Although the formulations provided equal or slightly longer protection times against mosquitoes, abrasion, water resistance and cosmetic acceptability are important additional factors in developing a longer lasting mosquito repellent.

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INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: STATUS OF ORGANOPHOSPHORUS RESISTANCE IN LARVAL *CULEX PIPIENS* SUBSPP., 1976

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California Department of Health

Resistance in *Culex pipiens* subspp. to an organophosphorus (OP) compound was first detected in 1956 when larvae from Kern County were shown to be resistant to malathion (Gjullin, unpublished data). By 1970, the Department of Health, in conjunction with the local mosquito control agencies, had detected widespread malathion resistance in *Cx. pipiens*. Fenthion resistance appeared definite although limited mostly to coastal and southern counties. Insecticide susceptibility levels for *Cx. pipiens* have been previously summarized by Womeldorf et al. (1966, 1968, 1971).

Strains of *Cx. pipiens* from the Kings and San Mateo County Mosquito Abatement Districts (MAD) were colonized at the University of California at Riverside where additional tests were performed and reported upon by Georghiou et al. (1975). Case histories of the development and impact of this resistance on mosquito control have been reported for the Alameda County and San Mateo County MAD's (Dill and Roberts 1975) and the Kings MAD (Stewart 1975).

Mosquito larvae were tested in the California resistance surveillance program (Gillies and Womeldorf 1968). Except where noted, only those data processed with the computerized probit analysis program incorporated into the resistance surveillance program are included in this report.

Levels of less than 0.005 parts per million (ppm) at the population mean lethal concentration (LC_{50}), accompanied by an LC_{90}/LC_{50} ratio of less than two are generally descriptive of susceptible populations for parathion, methyl parathion, fenthion, and chlorpyrifos. For malathion, a susceptible population is indicated by an LC_{50} of less than 0.1 ppm coupled with a ratio of less than two.

The inclusion of an agency does not mean that every *Cx. pipiens* population within the agency boundaries has become resistant, but that some populations have been shown to be resistant. Conversely, the absence of an indication of resistance may mean simply that it has not yet been determined, not that it does not exist.

By 1970, only two agencies, the Northern Salinas Valley and Madera County MADs had larvae in which resistance to all available OP larvicides had been detected (Figure 1). This number escalated to 13 by 1975. This brought the number of mosquito control agencies reporting larval resistance to 28.

Table 1 summarizes highest detected organophosphorus resistance levels to date. Resistance test levels of larval samples from virtually all control agencies tested have surpassed

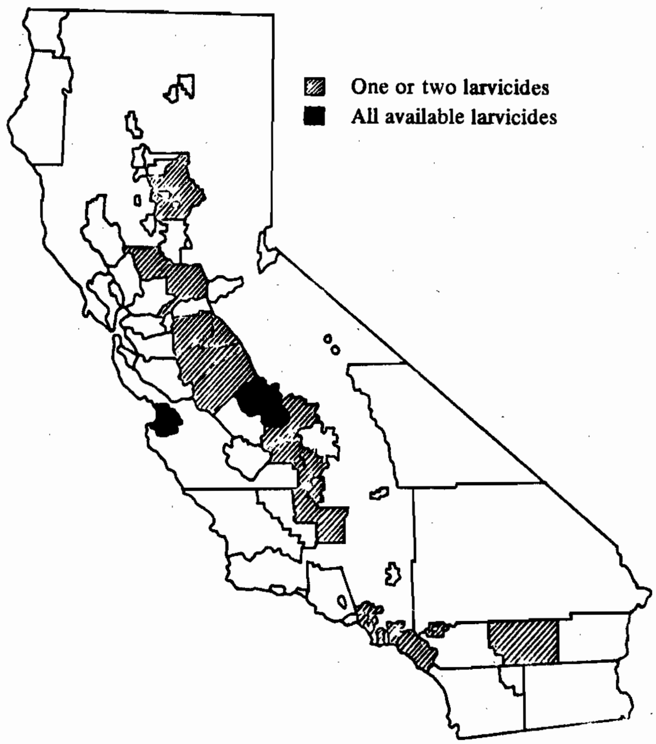

Figure 1a.—Documented organophosphorus resistance in *Culex pipiens* subspp. - - 1970.

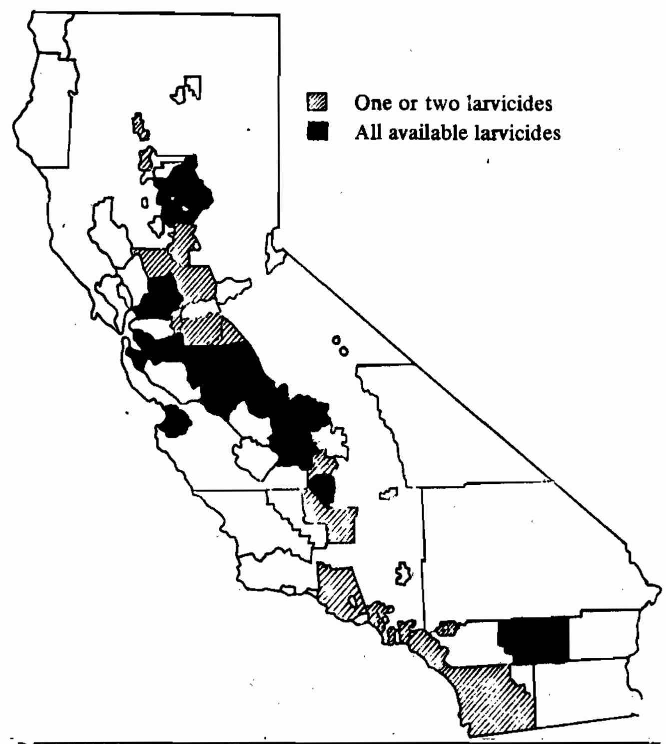

Figure 1b.—Documented organophosphorus resistance in *Culex pipiens* subspp. - - 1975.

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Figure 1c.–California Mosquito Abatement Agencies - - 1976.

the 0.1 ppm susceptibility limits for malathion and have a LC_{90}/LC_{50} ratio greater than two. Figure 2 displays the LC_{50} and LC_{90} levels by district. Since these are plotted on a log scale, the distance between LC_{50} and LC_{90} is a measure of their ratio. The scale is indicated in the legend of the figure.

Occasional high levels of tolerance to parathion and methyl parathion were detected but these findings appear to indicate the upper range of tolerance for the species rather than resistance since the high levels were not generally accompanied by a change in the slope of the log dosage-probit line (Figures 3 and 4). These high levels may be due in part to a cross tolerance due to the use of other organophosphorus insecticides.

Virtually all insecticide resistance tests of fenthion run on the larvae resulted in levels beyond the 0.005 ppm susceptibility threshold. Most populations tested showed increased heterogeneity, which indicates variability within the

Table 1.–Highest laboratory demonstrated level of organophosphorus tolerance in larvae of *Culex pipiens* subspp. through 1976.

	Malathion				Parathion				Methyl Parathion				Fenthion				Dursban			
Control Agency	Year	Slope	LC_{50}-LC_{90}		Year	Slope	LC_{50}-LC_{90}		Year	Slope	LC_{50}-LC_{90}		Year	Slope	LC_{50}-LC_{90}		Year	Slope	LC_{50}-LC_{90}	
Solano Co.	1972	1.62	.16	.19	1969	1.53	.06	.19	1969	1.53	.0042	.0064	1974	2.41	.21	.51	1974	1.90	.04	.077
Diablo Valley	1976	4.77	.10	.48	1976	2.31	.0078	.018					1976	5.57	.014	.078	1976	3.48	.0023	.008
Alameda Co.	1972	2.86	.14	.40									1974	1.64	.039*	.064**	1972	2.85	.021	.060
San Mateo Co.	1975	3.79	.29	1.10									1976	4.05	.23	.94	1973	1.90	.0058	.011
Santa Clara Co.													1976	3.03	.01	.031				
N. Salinas Vy.	1966	1.71	.24	.41	1966	1.87	.0061	.01	1966	1.65	.0065	.011	1966	1.91	.039	.075	1966	2.79	.0037	.010
Goleta Valley													1976	5.25	.057	.30**	1976	7.25	.032	.230
L. A. Co. West	1967	1.60	.086	.14					1972	1.50	.0029	.0043	1967	1.58	.0055	.0087				
Southeast	1966	1.74	.14	.24	1966	1.48	.0039	.0058	1966	1.55	.0046	.0071	1976	2.38	.021	.050**	1966	1.55	.00096	.0015
Compton Creek	1976	1.77	.14	.25	1976	3.23	.0076	.024	1976	1.75	.006	.01	1976	4.31	.011	.049	1976	1.49	.00075	.0011
Orange Co.	1966	2.08	.12	.25	1966	1.97	.0038	.0075	1976	1.87	.0038	.0071	1976	1.87	.0076	.018	1976	2.10	.0009	.0019
Northwest	1976	1.30	.11	.14	1965	1.33	.0034	.0046	1976	1.44	.0041	.0059	1976	1.74	.0064	.011	1969	1.62	.00084	.0014
San Diego Co.					1972	2.25	.0018	.004	1972	1.60	.002	.0032	1972	3.71	.007	.026				
Imperial Co.	1976	1.47	.21	.30	1976	1.47	.0068	.01	1976	1.53	.020	.026	1976	1.33	.020	.026	1976	1.84	.0021	.0039
Coachella Valley	1968	1.84	.14	.26	1971	1.62	.0058	.0093					1965	2.37	.0087	.021	1968	1.42	.002	.0028*
Shasta													1974	14.27	.11*	1.57				
Tehama Co.													1975	3.73	.015	.056	1975	1.64	.0014	.0023
Corning					1965	1.53	.0021	.0032					1965	1.64	.0045	.0074				
Butte Co.	1966	1.93	.11	.21	1972	1.95	.0091	.018					1972	2.40	.017	.041				
Sutter-Yuba	1963	3.20	.079	.25	1963	2.11	.004	.008	1963	2.06	.0032	.0066	1971	3.05	.020*	.061**	1971	4.58	.0012	.0054
Sac.-Yolo													1968	1.64	.0062	.010				
San Joaquin	1976	7.07	.410	2.90	1965	1.30	.0037	.0048	1969	1.55	.0032	.005	1975	24.71	.51	12.60**	1975	2.71	.048	.13
Eastside	1964	2.32	.11	.26	1964	1.67	.0044	.0073	1965	1.48	.0030	.0044	1970	2.14	.006	.013				
Turlock	1975	4.18	.33	1.38	1975	1.47	.03	.044	1966	1.59	.0042	.0066	1975	2.02	.089	.18	1975	2.06	.032	.066
Merced Co.	1975	1.80	.13	.24	1970	1.75	.0021	.0037					1970	1.97	.005	.0097	1975	1.69	.0011	.0018
Madera Co.	1969	1.54	.15	.23	1975	1.68	.0058	.0097	1971	1.56	.0023	.0036	1971	1.64	.0077	.013	1975	2.27	.002	.0045
Fresno	1976	5.06	.54	2.73	1976	4.73	.026	.12	1976	5.30	.038	.20	1976	8.11	.098	.80	1976	9.68	.015	.140
Consolidated	1975	3.01	.58	1.74	1975	4.57	.07	.32	1975	2.54	.047	.12	1975	2.54	.08	.26	1975	2.20	.021	.046
Delta					1965	1.62	.0025	.0041					1976	3.47	.025	.086				
Kings	1974	2.91	.61	1.80	1976	2.25	.027	.061	1976	4.00	.049	.19	1975	2.60	.30	.79	1975	1.80	.047	.084
Tulare	1966	2.17	.18	.39	1966	1.48	.004	.006	1966	1.59	.0032	.0051	1970	1.58	.032	.050				
Delano	1975	2.78	.21	.57	1976	6.63	.0096	.061	1975	4.86	.014	.068	1976	2.72	.016	.043	1975	2.93	.063	.18**
Kern	1966	1.68	.14	.24	1956	1.62	.0062	.010	1966	1.50	.0015	.0023	1966	1.79	.0031	.0056				
Westside	1976	2.04	.30	.60	1976	4.28	.013	.056					1976	4.77	.022	.10				

*M.A.D. Test Results

**Heterogeneity

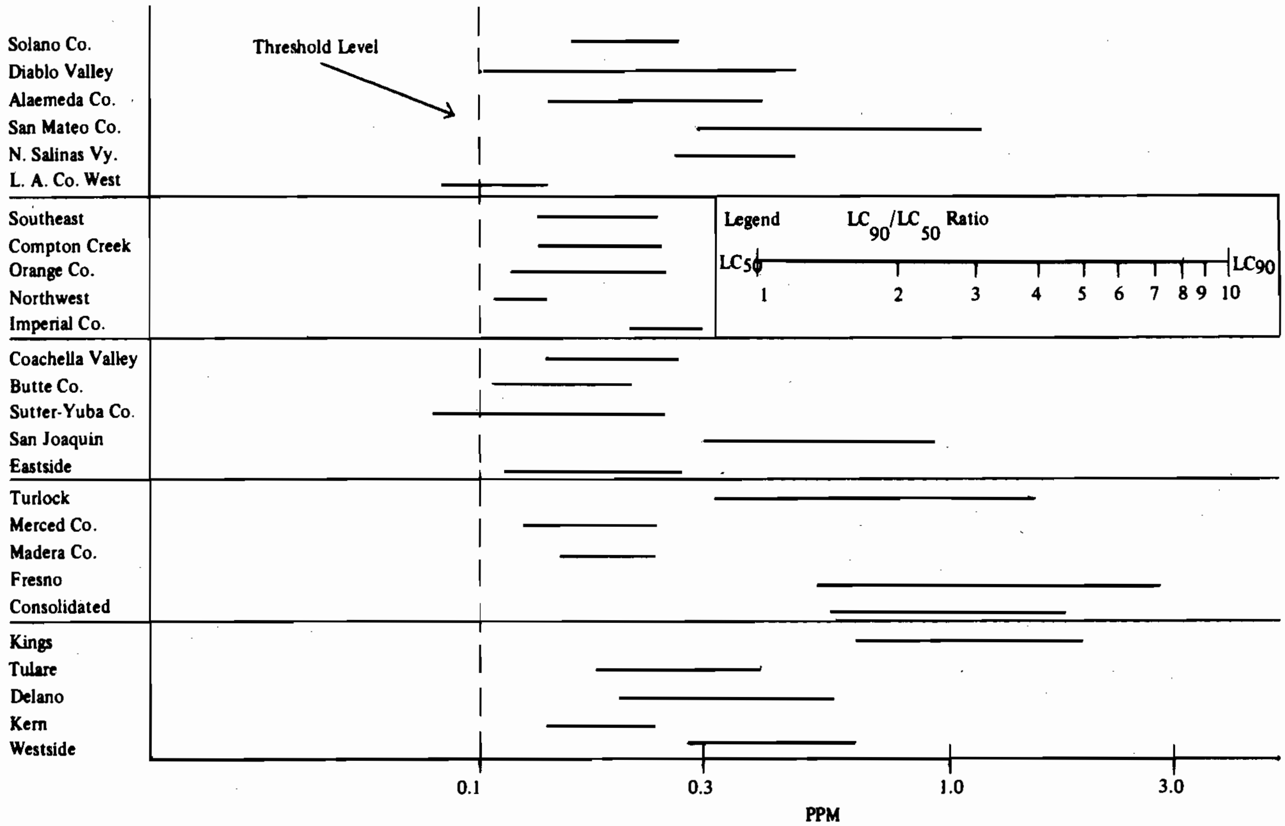

Figure 2.–*Culex pipiens* subspp. larval resistance levels to malathion. Plotted by LC_{50} and LC_{90}.

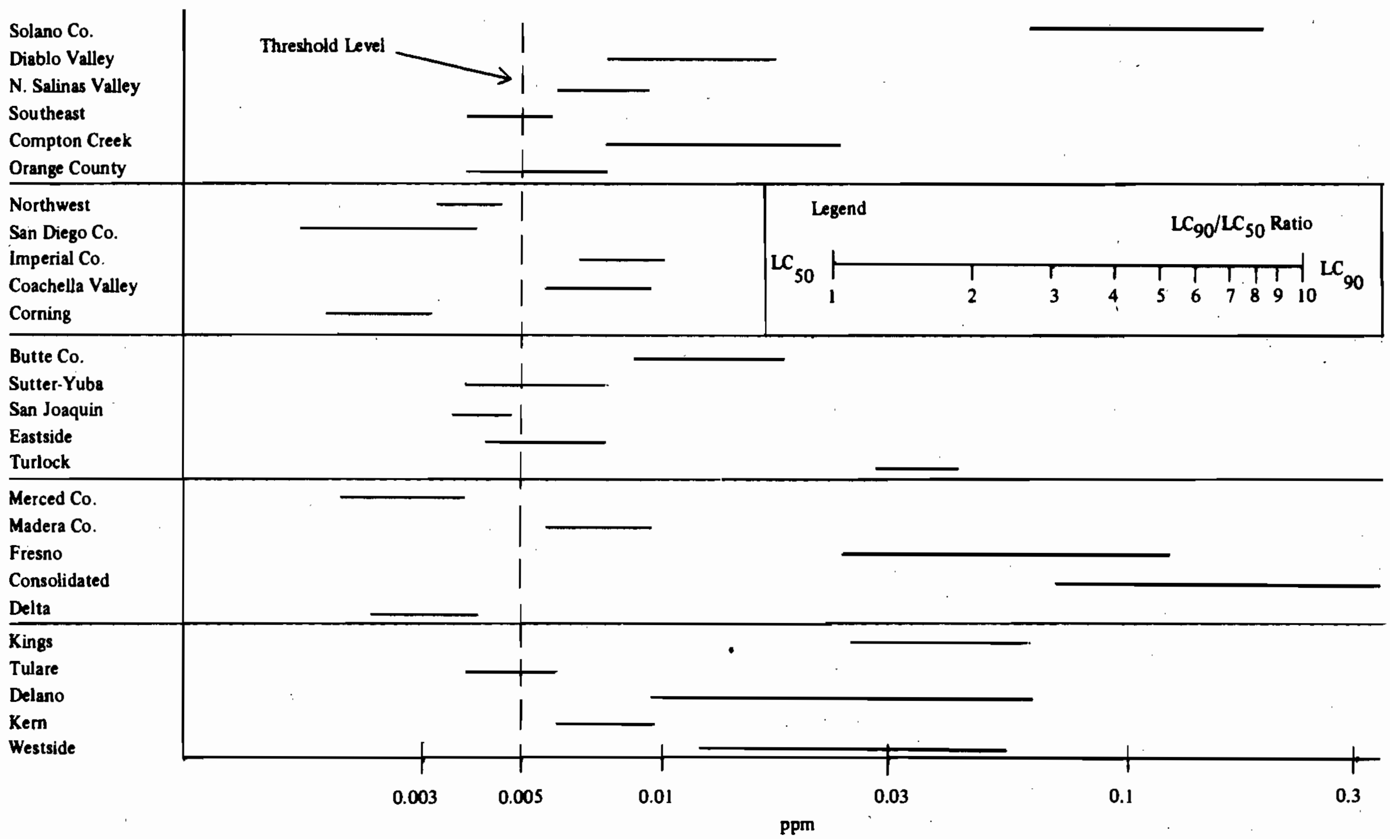

Figure 3.–*Culex pipiens* subspp. larval resistance levels to parathion. Plotted by LC_{50} and LC_{90}.

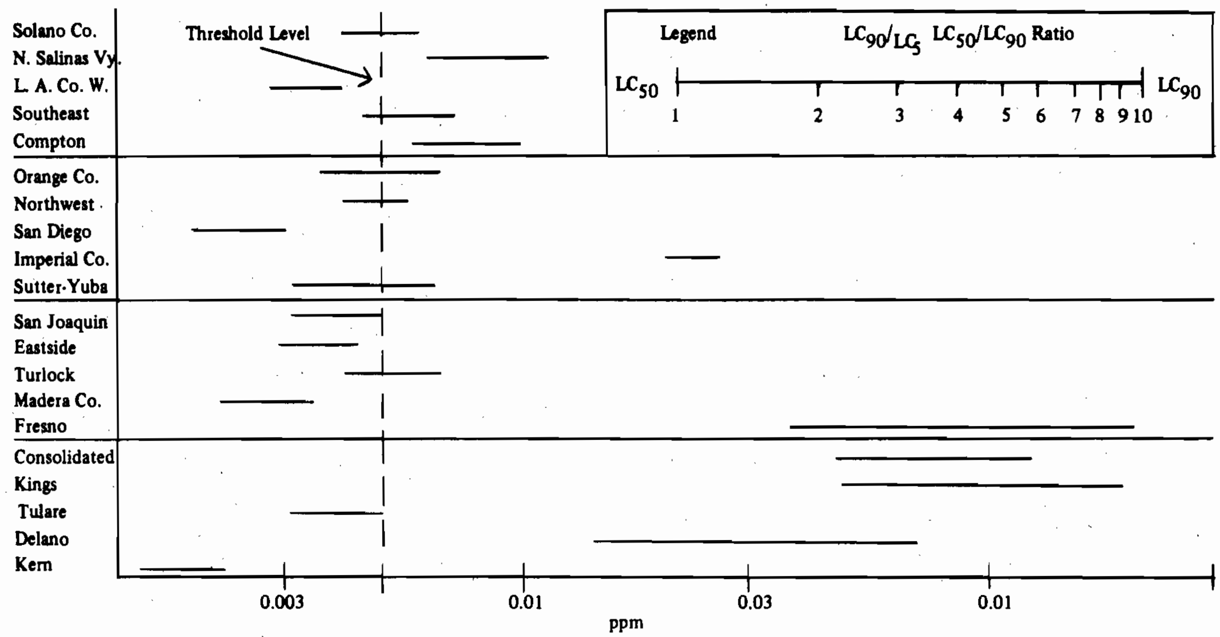

Figure 4.–*Culex pipiens* subspp. larval resistance levels to methyl parathion. Plotted by LC_{50} and LC90.

populations. A final concentration of 0.1 ppm was formerly allowed "on non-crop water areas, under special circumstances of water more than six inches deep and of high organic content". Some larval populations from a few control agencies have surpassed the 0.1 ppm limit and many more are approaching that limit (Figure 3).

Chlorpyrifos continues to be the most used of all the organophosphorus larvicides against *Cx. pipiens* (Figure 6). Resistance levels below the 0.005 ppm susceptibility threshold are evident primarily in the coastal counties of southern California. Most of the remaining areas in the state have larval resistance levels over the 0.005 ppm threshold limit and/or the LC_{90}/LC_{50} ratio is greater than two. Although none of the resistance tests resulted in levels beyond the 0.1 ppm legal limit for highly polluted waters at the time of writing, many are approaching that limit.

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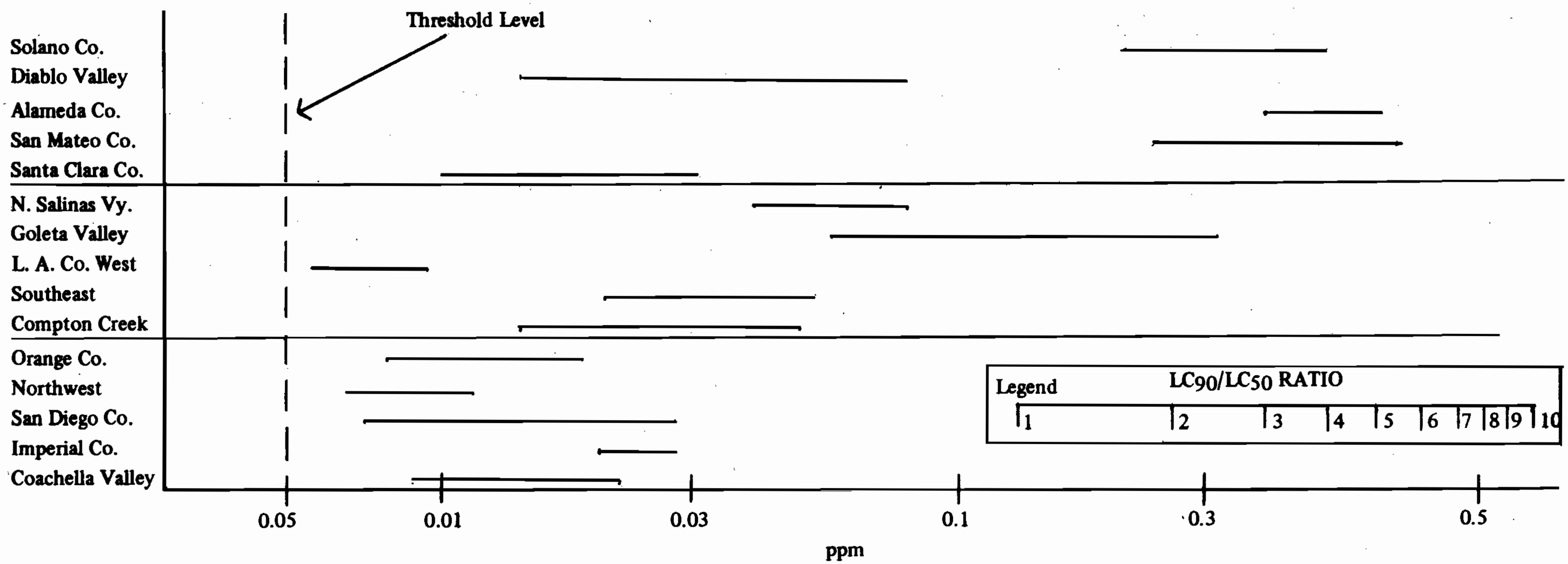

Figure 5A.--*Culex pipiens* subspp. larval resistance levels to fenthion. Plotted by LC_{50} and LC_{90}. Coastal Agencies.

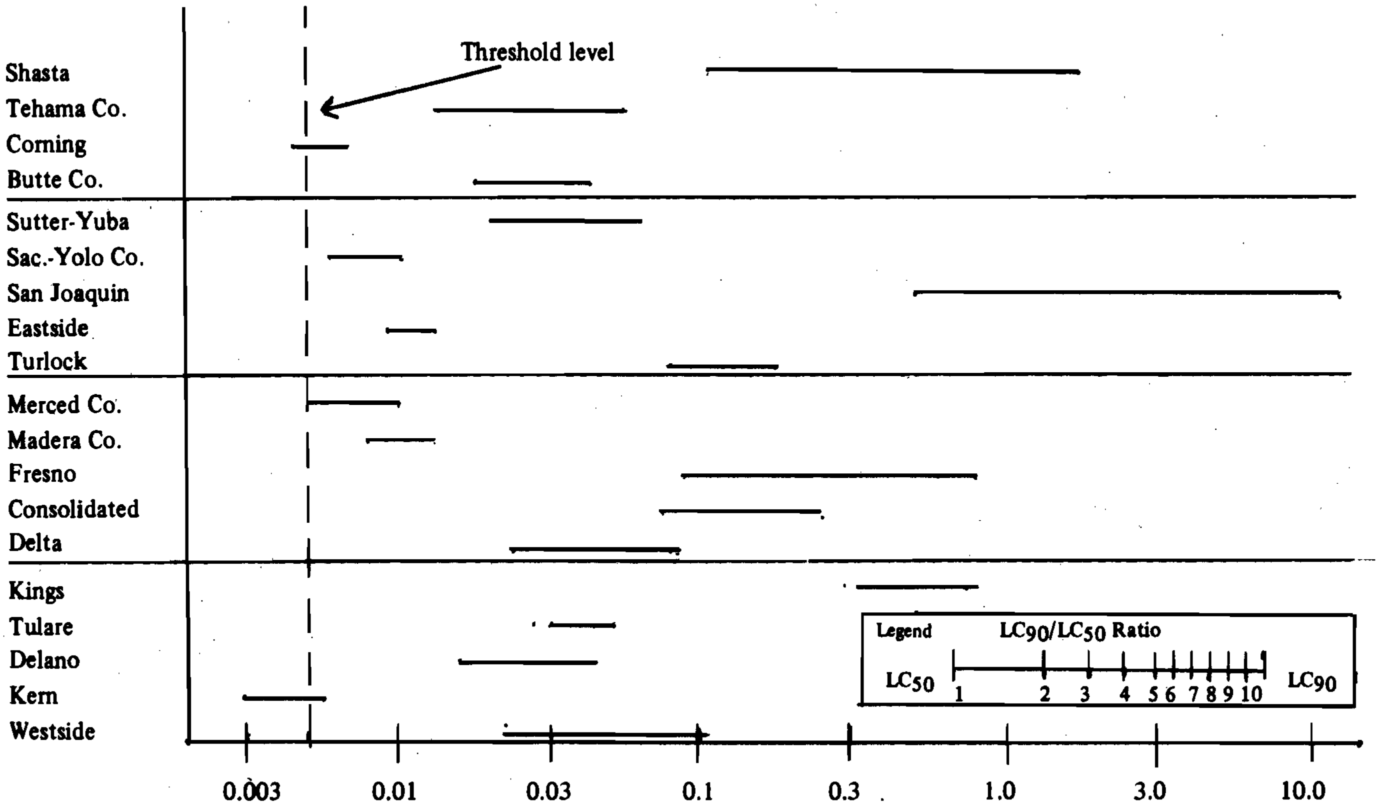

Figure 5B.—Valley agencies. *Culex pipiens* subspp. larval resistance levels to fenthion. Plotted by LC_{50} and LC_{90}.

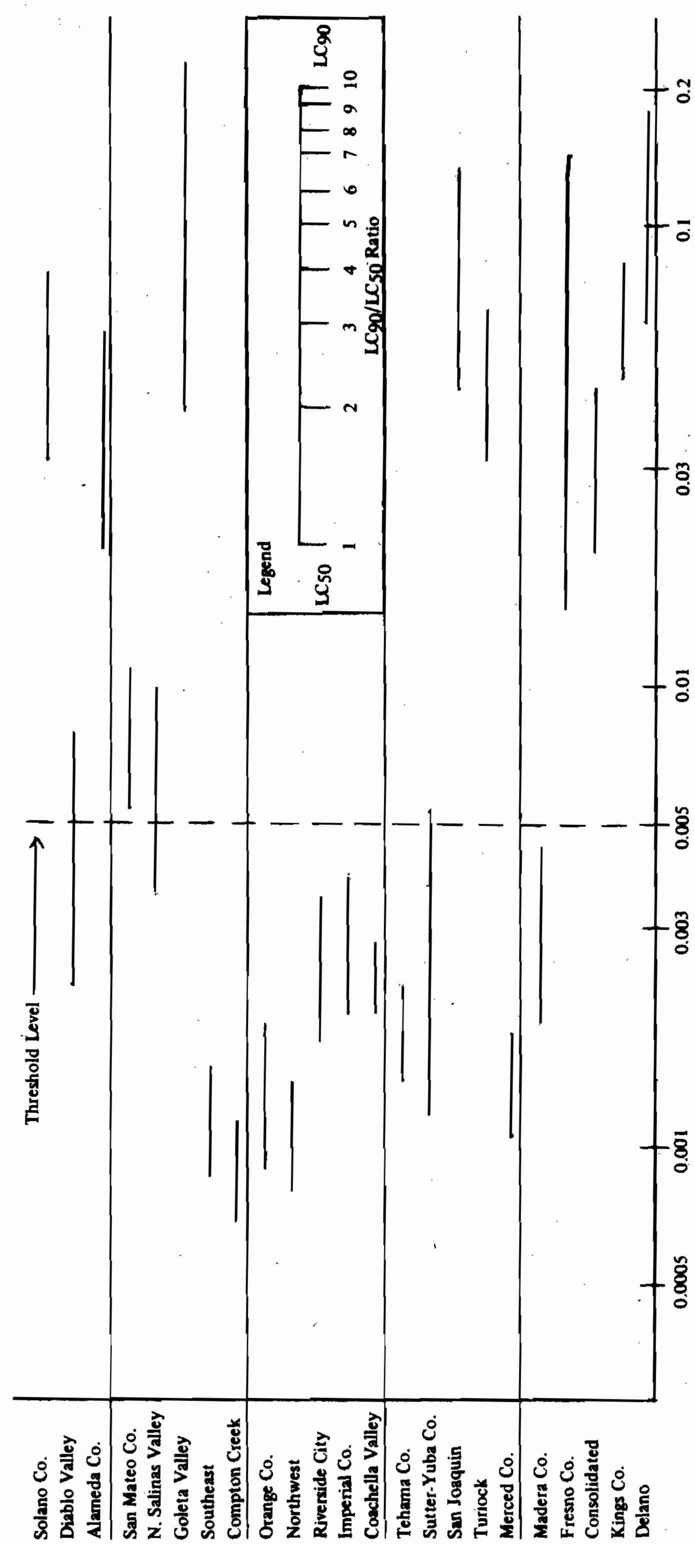

Figure 6.–*Culex pipiens* subspp. larvaal resistance levels to chlorpyrifos. Plotted by LC_{50} and LC_{90}.

SYNERGISM OF CHLORPYRIFOS BY DEF® IN THE CONTROL OF ORGANOPHOSPHORUS RESISTANT *CULEX PIPIENS QUINQUEFASCIATUS* LARVAE, WITH NOTES ON SYNERGISM OF PARATHION AND FENTHION

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ABSTRACT

In 1974 Kings County experienced organophosphorus resistance in *Culex pipiens quinquefasciatus* populations, creating a need for an alternate insecticide to replace chlorpyrifos (Dursban®), the widely used insecticide for this species. Synergising chlorpyrifos with 2.5 ppm DEF® achieved control of *Cx. p. quinquefasciatus* larvae.

INTRODUCTION.–The southern house mosquito, *Cx. p. quinquefasciatus*, has developed resistance to chlorpyrifos in Kings County. This resistance was noted in 1974 and reported by Stewart (1975) and Georghiou et al. (1975a,b). By synergising chlorpyrifos with DEF® (s,s,s, - tributyl phosphorotrithioate), resistance in larvae was almost overcome in the laboratory (Georghiou et al. 1975b). This synergism extended itself to parathion as well as chlorpyrifos. The present paper discusses the field use of DEF as a synergist for chlorpyrifos, and includes notes on the synergism of parathion and fenthion.

MATERIALS AND METHODS.–Larvae were collected from sources to be treated and checked for resistance following the procedure of Gillies and Womeldorf (1968). Resistance ratios were based on comparison with a susceptible strain with an LC_{90} of .001 ppm, exhibited by larvae of a laboratory strain of *Cx. p. quinquefasciatus* maintained by the California Department of Health in Fresno.

Larval population samples were taken with a 450 ml capacity dipper. Single dips were made along the margin of the drain at 3 yard intervals, before and after treatment, as a population index. Counts were taken at 1,3 and 7 days after treatment and weekly thereafter until failure was noted.

The amount of test material required for each drain was calculated using the formula of Steelman et al. (1967). A 3 gal. hand pump sprayer fitted with a .003 straight stream nozzle was used to apply the pesticides. The amount of emulsifiable concentrate required to give the desired concentration in the drain was poured into the sprayer and the sprayer filled to capacity with water. Pesticides were applied to the perimeter and floatage of drains.

Several dairy drains, and a drain ditch near Hanford, were selected for test sites. The Silveria, Matthews, Debrum and Bader dairies each have two drains within 200 feet of each other: each drain served as a test site and was treated once. Single drains on the other dairies were treated once. The Hanford ditch was treated twice.

RESULTS AND DISCUSSION.–It is evident from Table 1 that the Matthews and Silveria dairy drains treated with chlorpyrifos alone showed similar periods of control compared with the adjacent drain treated with DEF and chlorpyrifos. Since the resistance ratios are low for these two dairy drains, and the rates of chlorpyrifos were high, the ineffectiveness of DEF was expected. Synergistic effects apparently were demonstrated in five of the sources treated: the Camara dairy drain, both Debrum dairy drains, and both Bader dairy drains (Table 1). Prior to 1974 *Cx. p. quinquefasciatus* populations were susceptible to chlorpyrifos and usually a control period of approximately one month was maintained per application. For example, the Camara dairy drain was treated twice with chlorpyrifos prior to the synergism test. Neither 0.15 ppm or 0.2 ppm treatment rates achieved control. Treatment with chlorpyrifos and DEF reestablished control, although for only 14 days. This may have been due to the low dose of DEF (1.0 ppm) applied to this drain. The maximal sublethal concentration for DEF, 2.5 ppm, (Georghiou et al. 1975b) was used with 0.1 ppm chlorpyrifos in both Debrum dairy drains and both Bader dairy drains with marked success.

Synergism of 0.1 ppm parathion with 2.5 ppm DEF was not evident in the Giacomazzi dairy drain. When a parathion resistant population of *Cx. p. quinquefasciatus* near this area was checked for synergism with DEF in the laboratory (Georghiou et al. 1975b), a synergism ratio of 18.9 was obtained. This same population showed a 92.3 synergism ratio for chlorpyrifos plus DEF.

The Martella dairy drain was treated with 0.1 ppm fenthion plus 2.5 ppm DEF. The short period of control, 14 days, should not be interpreted to mean a lack of synergism since susceptible populations of *Cx. p. quinquefasciatus* larvae formerly were controlled for approximately this length of time with fenthion in this type of habitat (unpublished Kings Mosquito Abatement District records).

DEF was applied 4 hours prior to the insecticide application in four of the test drains (Table 1). The intent was to inhibit the resistance mechanism prior to the application of the insecticide to which mosquitoes were resistant. There appeared to be no value in applying DEF first and the insecticide later, as shown by the similar control period achieved in the Bader drains compared with the Debrum drains.

Since DEF is a defoliant, care should be taken when applying it. The phytotoxic effects of DEF on weeds were evident in the Hanford ditch; however, when DEF was sprayed on only the water surface of dairy drains no effect was noticed on the surrounding weeds. Since dairy drain water is often used for irrigation, an attempt was made to check the phytotoxic effect of 2.5 ppm DEF treated water on adjacent crops. At three different drains, both of the Debrum drains and the Martella drain, treated water was pour-

Table 1.—Results observed from applications of organophosphorus insecticides and DEF®[1] against OP resistant *Culex pipiens quinquefasciatus* larvae.

Date	Location & Habitat	DEF	Application Rate (ppm) Insecticide	100% Mortality (Days)	LC_{50}-LC_{90}	RR[2]
8-12-75	Hanford drain	1.3	0.17 chlorpyrifos	14	0.001-0.004	4
8-27-75	Hanford drain	2.6	0.34 chlorpyrifos	50	- -	-
10-14-75	Silveria E. dairy drain	0	0.5 chlorpyrifos	40	0.008-0.018	18
8-27-75	Silveria E. dairy drain	2.5	0.5 chlorpyrifos	46	- -	-
10-14-75	Matthews N. dairy drain	0	0.17 chlorpyrifos	30	0.007-0.019	19
10-14-75	Matthews S. dairy drain	1.7	0.17 chlorpyrifos	30	- -	-
9-23-76	Camara Dairy drain	1.0[3]	0.1 chlorpyrifos	14	0.043-0.15	150
10- 5-76	Debrum N. dairy drain	2.5[3]	0.1 chlorpyrifos	28	0.018-0.10	100
10- 5-76	Debrum S. dairy drain	2.5[3]	0.1 chlorpyrifos	28	- -	-
10- 6-76	Bader E. dairy drain	2.5	0.1 chlorpyrifos	25	0.017-0.096	6
10- 6-76	Bader W. dairy drain	2.5	0.1 chlorpyrifos	29+	- -	-
9-10-75	Giacomazzi dairy drain	2.5	0.1 parathion	7	0.025-0.07	-
10- 6-76	Martella dairy drain	2.5[3]	0.1 fenthion	14	0.035-0.18	-

[1]s,s,s - tributyl phosphorotrithioate.

[2]RR = resistance ratio (LC_{90} resistant strain/LC_{90} susceptible strain).

[3]Synergist applied 4 hours in advance of insecticide.

ed over alfalfa plants. There were no noticeable effects on these plants during the 30 day test period. This was a very crude experiment: more work is necessary to determine whether DEF treated water is phytotoxic.

DEF is used extensively in Kings County on cotton. Two major distributors of DEF sold over 35,000 gallons in the County in 1976, and records from past years show similar usage. The effects of this spraying on *Cx. p. quinquefasciatus* populations is not known. Since control was achieved in the drains treated with DEF, agricultural spraying of DEF apparently has not affected the synergism of DEF plus chlorpyrifos.

Dairy drains vary considerably in size. Some of the large dairy drains in Kings County are approximately 1000 feet long by 75 feet wide by 15 feet deep. To treat a drain of this size with DEF at a 2.5 ppm concentration would take approximately 29 gals of 6 lb. AI/gal DEF. The cost of treating such large sources with DEF would, therefore, be a limiting factor (assuming 2.5 ppm DEF is needed). The synergism exhibited in dairy drains by DEF should extend itself to other *Cx. p. quinquefasciatus* sources such as catch basins and septic tanks. Because of their small size, the cost of DEF would be of no consequence.

ACKNOWLEDGMENT.—I would like to thank Dr. George P. Georghiou, University of California at Riverside, for his help in these experiments.

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LARVICIDAL AND ADULTICIDAL TRIALS AGAINST PASTURE MOSQUITOES[1]

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ABSTRACT

No synergistic action was evident with methyl and ethyl parathion mixture (1:1) against the organophosphorus resistant larvae of *Aedes nigromaculis* Ludlow in irrigated pastures. At the total rate of 0.1 lb/A, this mixture failed to control the multi-resistant strain in Tulare County, while good control (90%) was obtained in Kern County where larvae are resistant to methyl parathion but still susceptible to ethyl parathion. More superior results were obtained with ethyl parathion alone than either the methyl alone or the methyl-ethyl mixture.

Dursban (17%) in Golden Bear 1356 petroleum oil controlled adult *Aedes* mosquitoes up to 1 mile downwind when applied as nonthermal aerosol at the rate of 36 oz/min, the vehicle traveling at 5MPH.

INTRODUCTION.–The appearance of resistance in the larval stage of mosquitoes during the past 2 decades has resulted in a critical problem for most mosquito control agencies in California. Due to the development of acquired resistance, several highly active and reasonably priced larvicides have become obsolete or less effective against several species of mosquitoes such as *Aedes nigromaculis* Ludlow, *Culex tarsalis* Coquillett, *Cx. pipiens quinquefasciatus* Say, and *Cx. pipiens pipiens* L. (Georghiou et al. 1975, Gillies et al. 1974). At the present time, some substitute materials have been developed for the control of some of these resistant species with excellent results. Such materials include mosquito larvicidal oils, insect growth regulators (IGRs), aliphatic amines, and synthetic pyrethroids (Darwazeh 1973; Lewallen and Ramke 1974; Mulla and Darwazeh 1976a,b). Utilization of these materials have increased mosquito control operational costs substantially. The rising cost of mosquito control has resulted in the administration of control programs in the populated areas, leaving out large stretches of mosquito breeding sources without intensive and routine control measures. However, adulticides are occasionally applied to prevent adult mosquito flights into the residential areas.

It has been reported that combination of methyl and ethyl parathion has shown good activity against parathion resistant leafhoppers. The current studies were initiated to determine the synergistic effect of currently available and less costly larvicides, ethyl and methyl parathion, against multi-resistant strains of the pasture mosquito *Aedes nigromaculis* Ludlow in irrigated pastures, and to evaluate the performance of chlorpyrifos in petroleum oil as an adulticide applied with a nonthermal ground aerosol generator.

METHODS AND MATERIALS.–Larvicides - - Prior to chemical application, 4th stage larvae were collected from the field test plots, and their susceptibility to methyl and ethyl parathion was determined in the manner as described by Mulla et al (1966). Tests were conducted in irrigated pastures which are known to produce highly resistant *Ae. nigromaculis* larvae in Kern and Tulare counties. In Tulare county, tests were applied in Joe Ramos' pasture, located on Avenue 104 and Road 96, northwest of Pixley. Half a gallon of methyl parathion EC 4 and 0.25 gal of ethyl parathion EC 8 were mixed with 40 gals of water and applied with a Pawnee aircraft at the rate of 1.0 gal of aqueous spray containing 0.1 lb A. I. of both materials per acre. Six swaths (66 ft each) were applied covering 12 checks. Six checks contained 2-3rd stage larvae, while 3-4th stage larvae were present in the others. Four untreated checks in the adjacent field were left untreated as check and contained 3-4th stage larvae. Twenty dips per check were taken prior to and 24 hours after treatment. The same experiment was repeated in John Tosti's pasture, located on Avenue 160 and Road 73, northwest of Tipton.

In Kern County, tests were conducted in Rancho Santa Maria, 2 miles east of Corcoran Highway, on the south side of County Line Road. Materials were applied with a Pawnee Brave aircraft, covering 50 ft per swath. In the first load, one gal of ethyl parathion EC 4 was mixed with 60 gals of water and applied at the rate of 1.5 gal of aqueous spray (0.1 lb AI/acre). Seven swaths were applied to pasture containing 2-3rd stage larvae, and 8 swaths against 3-4th stage larvae. In the second load, the same procedure was utilized in the application of methyl parathion EC 4. In the third load, one gal of each, ethyl and methyl parathion EC 4 were mixed with 120 gal of water and applied at the rate of 1.5 gals of aqueous spray containing 0.05 AI of each material per acre. An entire 80 acre field was treated, and larval assessment was conducted in 8 checks containing 2-3 stage larvae and 8 checks containing 3-4th stage larvae. Ten dips per check were taken prior to and 24 hours after treatment. Two flagmen were utilized, one at each end of the field, to insure line of flight in all tests.

Adulticide - - An 8-M swath was run at 1835, 15minutes before sunset, and ending at 2030, utilizing 19 gallons of material in two loads. Dursban EC 4 was diluted with Golden Bear 1356 larvicidal oil at 1:2 ratio. The Golden Bear 1356 is a commonly used mosquito larvicide, composed of (98%) petroleum oil distillate, (0.5%) aliphatic amine, and (1.5%) inert ingredients. The material was applied with a 4 nozzle cold fogger (Microgen® Model L4-75), transported on the tailgate of a pick-up truck at a speed of 5 mph. Two mph wind prevailed during application. Area treated was heavily infested with adult *Ae. nigromaculis* and were observed swarming in large numbers during application. Prior to treatment, 8 population assessment lines were established with sampling stations at points 0.0, 0.25, 0.50, 0.75 and 1.0 mi. from station prior to and 12

[1]These studies were conducted in cooperation with Kern and Tulare Mosquito Abatement Districts in the southern San Joaquin Valley of California.

hours after treatment. Adulticide application began from Garces and Corcoran Highway, traveling northward for 2 miles to County Line Road, then eastward for 6-mi. to Rowlee Road.

RESULTS AND DISCUSSION.–Larvae collected from test plots in Tulare County were highly resistant to both ethyl and methyl parathion, while larvae obtained from Kern County were equally resistant to methyl parathion, but moderately resistant to ethyl parathion (Table 1).

Table 1.–Susceptibility of *Ae. nigromaculis* larvae to methyl and ethyl parathion.

	(LC_{50}-LC_{90}) ppm	
	Tulare County	Kern County Rancho
Material	Ramos Pasture	Santa Maria
Ethyl Parathion	0.068 - 0.340	0.027 - 0.068
Methyl Parathion	0.180 - 0.680	0.210 - 0.680
Methyl Ethyl Parathion, 1:1	0.070 - 0.500	0.110 - 0.410

At the rate of 0.1 lb/acre, aerial application of ethyl and methyl parathion mixture (0.05 lb/A/AI of each) failed to produce any reduction in test A, while 24 and 36% control was achieved in test B in Tulare County (Table 2).

Table 2.–Aerial application of ethyl and methyl parathion mixture (1:1) for the control of *Ae. nigromaculis* resistant larvae in irrigated pasture at the rate of 0.1 lb/acre (Tulare County).

	Avg. No. of larvae/dip		
Larval Stage	Pre-treat	Post-treat (24 hrs)[1]	(%) Reduction
	Test A, Ramos Pasture		
2-3	7	8	0
3-4	23	25	0
Check	6	20	0
	Test B, Tosti Pasture		
2-3	36	23	36
3-4	25	19	24
Check	22	31	0

[1]24-hrs after treatment, larvae were concentrated in clusters in water (25-50/dip). Prior to treatment, larvae were scattered in the water.

In Kern County, ethyl parathion alone, at 0.1 lb/AI/A, yielded excellent results, producing 97 and 90% reduction in the populations of 2-3rd and 3-4th stage larvae respectively (Table 3). Methyl parathion alone at the same rate produced 82% reduction of the young stages, while only 25% reduction was obtained against the older stages. When both materials were applied together at 0.05 lb/AI/A of each, 90 and 50% reductions were obtained. These findings indicate most mortality was caused primarily by ethyl parathion rather than a synergistic effect of both materials when used together. In the light of these results, ethyl parathion could be utilized operationally in the extensive Rancho Santa Maria holdings in Kern County as an effective mosquito larvicide.

Chlorpyrifos (17%) in Golden Bear petroleum oil yielded excellent results, up to 1-mi. downwind when applied with a nonthermal aerosol generator at the rate of 36 oz/min (Table 4).

These findings indicate that chlorpyrifos in Golden Bear petroleum oil is highly active against adult pasture mosquito *Ae. nigromaculis*, under ideal conditions, at the rate of 0.006 lb/acre, based on the area treated (6 miles x 1 mile) of 3840 acres. Chlorpyrifos concentrate diluted with fuel oil or automotive diesel is likely to produce similar results. These inexpensive petroleum oils should be employed in cold foggers rather than expensive diluents.

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Table 3.—Aerial application of methyl and ethyl parathion for the control of *Ae. nigromaculis* resistant larvae in an irrigated pasture (Kern County)[1].

Material and formulation	Rate lb/A	Larval stage	Avg. no. larvae/dip Pre-treat L	Pre-treat P	Post-treat (24 hrs) L	Post-treat (24 hrs) P	(%) Reduction
Ethyl parathion	0.1	2-3	19	0	0.6	0	97
EC 4		3-4	8	0	1.0	0	87
Methyl parathion	0.1	2-3	40	0	7.0	0	82
EC 4		3-4	24	0	18.0	0	25
Ethyl Methyl	0.1	2-3	3	0	0.3	0.0	90
Parathion Mix 1:1		3-4	7	0	2.8	0.7	50
Check		2-3	12	0	13	0	0

[1]Prior to chemical application, adult population was extremely heavy (50-100), but only 3-5 were observed landing 24-hrs after treatment.

Table 4.—Large scale evaluation of chlorpyrifos (17%) in Golden Bear-1356 petroleum oil for the control of adult mosquitoes (*Ae. nigromaculis*) in irrigated pastures (Kern County)[1].

Rate oz/min	Distance from discharge point (mile)	Avg. no. of adults landing/min Pre-treat	Post-treat[2]	(%) Reduction
36	0.00	128	6	95
	0.25	110	9	92
	0.50	74	36	51
	0.75	321	15	95
	1.00	184	18	90
Check	-	144	104	28

[1]Eight mile swath was run 15-min before sunset, utilizing 19-gal of materials in two loads. Rate of chlorpyrifos, 0.006 lb AI/acre.

[2]12-hrs.

FIELD TRIALS WITH PYDRIN, A SYNTHETIC PYRETHROID, AGAINST *CULEX TARSALIS* AND ITS IMPACT ON NONTARGET ORGANISMS

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Although the efficacy of synthetic pyrethroids as mosquito larvicides and their short-time impact on some nontarget organisms have been reported (Darwazeh and Mulla 1974, Mulla et al. 1975, Mulla and Darwazeh 1976, Miura and Takahashi 1976, Schaefer et al. 1976), more studies concerning factors such as formulation, rate and time of spray, and long-term effects on nontarget organisms are needed.

Pydrin® (SD43775) [Benzeneacetic acid, 4-chloro-alpha-(1-methylethyl)-, cyano (3-phenoxyphenyl)methyl ester] is a synthetic pyrethroid having activity against a broad spectrum of insects. This report presents the results of the field trials with Pydrin against *Culex tarsalis* Coquillett and its impact on nontarget organisms.

STUDY AREA.–Field tests on early-spring *Cx. tarsalis* were conducted in an isolated lateral pool in Yokohl Valley, about 20 miles east of Visalia, California. The pool is one of many situated along Yokohl Creek, an intermittent stream that runs through the valley. The pool is narrow and elongated (285 x 20 ft) with a surface area of about 0.13 acre. Water is supplied by subsurface seepage and remains most of the flow season. Vegetation is scant in the deeper parts but shallow areas were densely covered by patches of spike rush, *Eleocharis* spp., and bermudagrass, *Cynodon dactylon* (L.) Pers.

Field trials on summer populations of *Cx. tarsalis* were studied in small ponds (approx. (ca.) 0.024 acre) at the Tracy experimental plot, located near Buttonwillow, California (about 20 miles northwest of Bakersfield, California). Water for each pond was supplied twice a week from adjacent reservoirs (ca. 0.024 acre) fed by a deep-well. The ponds were connected to the reservoirs by 6 inch diam. pipe, screened (16 x 18 mesh window screen) on one end to prevent movement of larger organisms into the treated ponds or vice versa.

MATERIALS AND METHODS.–Pydrin 2.4 lb/gal EC was utilized in both studies; the required amount of Pydrin for each rate was mixed with ca. 2 gal of water and applied with a 3 gal hand can sprayer. The rate used for early-spring *Cx. tarsalis* control was 0.2 lb AI/acre and for summer populations 0.1 lb AI/acre.

The mosquito species in the Yokohl Valley study site consisted predominantly of immature stages of *Cx. tarsalis* and a few *Culiseta inornata* (Williston). Only *Cx. tarsalis* was collected from the Tracy ponds. Larval population density was censused by taking 10 dips prior to treatment and at indicated intervals during the posttreatment observation period. The chemical impact on nontarget organisms was determined by monitoring changes between pre-and posttreatment populations using techniques described previously (Miura and Takahashi 1976).

During the test period at Yokohl Valley the average daily air temperature varied between 52° to 81°F; lowest and highest temperatures were 37° and 84°F respectively

Table 1.–Effect of Pydrin on nontarget organisms (NTO) in early-spring *Cx. tarsalis* larval habitats. Number of NTO in water samples (2 liters) taken pre- and posttreatment and held in the laboratory for observation (concn 0.2 lb AI/acre).

	Days held in laboratory						
Organisms	0	1	2	4	7	9	11
	Pretreatment						
Cladocerans	269	307	291	305	366	401	609
Copepods	18	62	140	139	257	243	241
Ostracods	56	56	56	56	51	49	45
Chironomid L	9	9	9	9	9	9	9
Mayfly N	7	13	13	8	7	7	7
Beetle L	36[1]						
	Posttreatment						
Cladocerans	134	1	0	0	0	0	
Copepods	15	6	2	2	0	0	
Ostracods	24	17	9	1	0	0	
Chironomid L	7	3	2	1	0	0	
Mayfly N	12	0	0	0	0	0	
Beetle L	16	0	0	0	0	0	

[1]Beetle larvae were taken out from the container to avoid cannibalism.
L = Larvae, N = Nymphs.

(Temp data were taken at the Lemon Cove Station, US Dept. of Commerce, National Climatic Center). The Tracy ponds average daily air temperature varied from 68° to 86° F; lowest and highest temperatures ranged between 55° to 102°F (temp data from the Buttonwillow Station, US Dept. of Commerce, National Climatic Center).

RESULTS AND DISCUSSION.--The Lateral Pool Study - - The effect on immature stages of the mosquitoes, *Cx. tarsalis* and *Cs. inornata* was striking. Prior to treatment the population density was about 3 to 5/dip, after treatment all larvae were killed within 24 hr; pupae survived for 48 hrs or more. Immature mosquito populations did not recover during the entire test period (April 20th to May 10th, 1976) despite continuous oviposition by incoming females. Table 1 shows population changes of planktonic organisms collected before and immediately after treatment and held in the laboratory for observation. Very small mayfly nymphs and beetle larvae were very sensitive to Pydrin; within 24 hours after treatment there was total mortality. Cladocerans, copepods, and small chironomid larvae were also eliminated within 2 to 5 days. In addition to monitoring pre- and posttreatment samples in the laboratory, population changes in the treated fields were periodically checked (Table 2); immature stages of mayflies, corixids, beetles and chironomids were completely eliminated from the treated site; however, crustacean populations recovered after an initial decrease. Turbellarians (*Neorhabdocoela*) and rotifers (*Asplanchna* spp.) were absent in the beginning

Table 2.–Effects of Pydrin on nontarget organisms (NTO) in early-spring *Cx. tarsalis* larval habitats. Number of NTO in field water samples (5 liters) collected at intervals after treatment (concn 0.2 lb AI/acre).

	Field collection day (after treatment)								
Organism	0	1	2	4	7	9	11	14	16
Cladocerans	134	0	1	0	8	15	23	254	620
Copepods	15	3	7	12	14	21	6	7	20
Ostracods	24	6	12	24	113	160	189	937	920
Chiromonid L	7	0	1	0	0	0	0	0	0
Mayfly N	12	0	0	0	0	0	0	0	0
Beetle L	16	0	0	0	0	0	0	0	0
Corixid N	1	0	0	0	0	0	0	0	0
Turbellarians	0	0	8	33	41	24	11	56	10
Rotifers	0	0	1	3	20	26	252	714	8

N = Nymphs, L = Larvae

Table 3.–Effect of Pydrin on nontarget organisms (NTO) in early-spring *Cx. tarsalis* larval habitats. Number of NTO collected by two minnow traps.

	Day after treatment									
Organisms	0	1	2	4	7	9	11	14	16	17
Mayfly N	2	(6)	0	0	0	0	0	0	0	0
Corixid A	4	(6)	1	0	0	0	1	12	15	25
Notonectid A	1	(1)	0	0	0	0	+	+	1	2
Laccophilus sp. A	3	(2)	0	0	0	0	0	+	0	0
Laccophilus sp. L	10	0	0	0	0	0	0	0	0	0
T. lateralis A	3	(11)	0	0	0	0	4	2	2	2
T. lateralis L	2	(1)	0	0	0	0	0	0	0	0
H. triangularis A	0	(1)	0	0	0	0	1	0	0	5
Berosus sp. A	1	(2)	0	0	0	0	0	0	0	+
Rhantus sp. A	0	0	+	1	+	+	2	1	4	4
Beetle L	0	(3)	0	0	0	0	0	0	0	0
Chironomid L	0	(1)	0	0	0	0	0	0	0	0
Mosquitofish	1	(27)	+	+	+	+	+	+	1	0
Tadpoles	3	+	+	+	+	+	2	2	2	16

N = Nymphs, A = Adults, L = Larvae.

(no.) = numbers in parentheses are numbers of moribunds or dead.

T. lateralis = *Tropisternus lateralis*

H. triangularis = *Hydrophilus triangularis*

+ = not trapped but seen in the tested area.

of the test but developed rapidly following treatment.

Effects on free moving large organisms are shown in Table 3. Here again all immature insects were eliminated. Adult insect populations also were eliminated either by poisoning or by dispersal from the treated pool. The pool was eventually reinfested by immigrants. Tadpoles and adult *Rhantus* beetles were severely affected but some of them survived the entire test period. Table 4 shows a list of organisms collected prior to treatment and during the posttreatment period.

The Tracy Pond Study - - Complete control of immature stages of mosquitoes was obtained. Almost immediately after treatment mosquitoes were eliminated for 12 days and required about 15 days to recover to the pretreatment population levels (Table 5). The pond was treated again on the 17th day after the 1st treatment; the mosquito population density was reduced to 0 and no larvae were apparent for a week.

Table 5 also shows the relative abundance of planktonic organisms before and after each of the 2 treatments. Cladocerans were very sensitive; after each treatment their mortality was 100% and recovery took 1 to 2 weeks. Copepod and ostracod populations were affected but never eliminated. Very small mayfly nymphs were very susceptible; after the 1st treatment the population was eliminated and did not recover during the entire test period. Rotifers (*Asplanchna* spp.) were the most tolerant organisms found in the pond. Most of the time their population was absent at the beginning of treatment but bloomed suddenly following treatment and then declined again. This phenomenon was observed at the Yokohl Valley study site (Table 2). Dimilin® (Miura and Takahashi 1976) and chloropyrifos treatment (Hurlbert el al. 1972) against mosquito larvae produced similar results. Hurlbert et al. (1972) reported that "the posttreatment population increase. . . resulted not from a direct stimulation of reproductive physiology by the insecticide but rather from the mortality of predators or competitors. . . [and an] increase in prey." The genus *Asplanchna* is known as predatory rotifers, they feed on other small rotifers, all kinds of metazoa (Pennak 1953), and very small immature insects (Hurlbert et al. 1972).

Table 6 shows the relative abundance of free moving insects in the check and treated ponds. All insects found in the pond were sensitive, especially large sized nymphal mayflies; they were eliminated from the treated pond after the 1st treatment and did not recover during the test period. Adult insects (notonectids and beetles) were eliminated by the chemical, but after a week or more populattions recovered to the pretreatment levels.

Table 4.–Effect of Pydrin on nontarget organisms (NTO) in early-spring *Cx. tarsalis* larval habitats. A list of NTO collected by aquatic nets from the study area.

Organisms	Pretreatment	Posttreatment
Mayfly N *Callibaetis* sp.	+	-
Water striders *Gerris* sp.	+	-
Back swimmers		
Notonecta unifasciata	+	+
Water boatman *Corisella* sp.	+	+
Hesperocorixa laevigata	+	+
Beetles (Adult)		
Agabus sp.	+	-
Rhantus sp.	+	+
T. lateralis	+	+
Helophorus sp.	+	-
Copelatus sp.	+	-
Laccophilus sp.	+	+
Berosus sp.	+	-
H. triangularis	+	+
Deronectes sp.	+	+
Bidessus sp.	+	-
Enochrus sp.	+	-
Hygrotus sp.	+	-
Gyrinus sp.	+	-
Beetles (Larvae)	+	-
Chironomid		
Procladius sp.	+	-
Chironomus stigmaterus	+	-
Shoreflies		
Brachydeutera argentata	+	+
Tadpoles	+	+
Mosquitofish	+	-
Garter snake	+	-

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Table 5.—Effect of Pydrin on nontarget organisms (NTO). Repeated applications on the same pond against *Cx. tarsalis* (Tracy ponds, concn 0.1 lb AI/acre).

Organism	Pretreatment 2	Pretreatment 1	Posttreatment (day) 1	2	4	5	7	8
	1st test (treated on August 1, 1976)							
Cladocerans	52	66	0	0	0	0	0	0
Copepods	61	124	8	13	47	48	289	728
Ostracods	89	135	0	18	7	23	23	534
Mayfly N	11	9	1	0	0	0	0	0
Chironomid L	0	0	16	4	9	8	18	3
Beetle L	2	2	0	0	0	0	0	0
Rotifers	0	0	48	38	398	453	306	156
	2nd test (August 18, 1976)							
Cladocerans	550	526	6	6	0	7	4	66
Copepods	40	28	3	15	17	50	131	77
Ostracods	30	24	10	49	36	25	16	29
Mayfly L	0	0	0	0	0	0	0	0
Chironomid L	10	4	3	14	0	4	0	5
Beetle L	0	2	0	0	1	1	2	1
Rotifers	90	16	46	77	187	320	425	160
Ephydrid L	3	2	0	1	0	1	0	0

Numbers in the table indicate numbers of organisms in daily water samples (5 liters) taken on indicated days.

Table 6.—Effect of Pydrin on nontarget organisms (NTO). Repeated applications on the same pond against *Cx. tarsalis* (Tracy ponds, concn 0.1 lb AI/acre).

Organism	Pre-treatment 1	Posttreatment count (day-after treatment) 2	3	4	5	6	9	10	11	12
	1st application					Check				
Notonectids	56	+	+	+	+	+	+	+	+	27
Hydrophilid A	26	22	17	21	15	8	8	41	7	24
Dytiscid A	9	4	3	20	10	12	6	8	23	13
Beetle L	0	2	1	6	2	0	1	0	1	0
						Treated				
Notonectids	16	0	0	0	0	0	0	0	0	0
Hydrophilid A	12	0	0	0	0	0	0	7	8	13
Dytiscid A	0	0	0	0	0	0	0	0	0	0
Beetle L	0	0	0	0	0	0	0	0	0	0
Mayfly N	28	0	0	0	0	0	0	0	0	0
	2nd application					Check				
Notonectids	5	7	31	11	16	7	11	11	12	
Hydrophilid A	3	3	41	23	2	31	2	6	3	
Dytiscid A	9	22	22	28	21	24	43	34	25	
Beetle L	0	0	0	0	5	12	8	2	6	
						Treated				
Notonectids	3	0	0	0	0	0	0	0	0	
Hydrophilid A	3	0	0	1	2	1	1	3	3	
Dytiscid A	5	0	0	0	0	0	0	0	1	
Beetle L	0	0	0	0	0	5	3	8	9	
Mayfly N	0	0	0	0	0	0	0	0	0	

+ = not counted but recorded as "many".

FIELD EVALUATION OF SD43775, A NEW SYNTHETIC PYRETHROID, FOR MOSQUITO CONTROL

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ABSTRACT

Field tests on the Shell Development Company pyrethroid SD-43775 or Pydrin™ gave excellent control of *Aedes nigromaculis* larvae at 0.05 lb/acre when applied by compressed air sprayer or fixed wing aircraft on pasture and alfalfa. *Culex tarsalis, Cx. peus,* and *Cx. pipiens quinquefasciatus* larvae were controlled at 0.1 lb/acre by compressed air sprayer and aerial applications in alfalfa and irrigated pasture habitats. Compressed air sprayer applications at the rate of 0.25 lb/acre failed to give complete control of *Cx. p. quinquefasciatus* larvae in dairy waste ponds.

The synthetic pyrethroid SD43775 or Pydrin™ was tested on mosquitoes in the laboratory and field by Mulla and Darwazeh (1976) and Schaefer et al. (1976). This report describes an extension of the previous work to develop operational control procedures for field populations of *Aedes nigromaculis, Culex tarsalis, Cx. peus,* and *Cx. pipiens quinquefasciatus* larvae, pupae, and adults. The current studies were conducted to determine efficacy and the optimum rate of application for control of the above species in varying habitats.

MATERIALS AND METHODS.—Portions of pastures with heavy populations of larvae were measured into 1/32 acre plots for compressed air sprayer applications. Pretreatment larval populations were determined by taking 20 random dips within each plot with a 4-inch diameter enamel dipper fitted with a long handle. Suitable aliquots of 2.4 lb/gal emulsifiable concentrate SD43775 [benzeneacetic acid 4-chloro-alpha-(1-methylethyl)-cyano(3-phenoxyphenyl) methyl ester] were placed in 100 ml glass bottles for transport to the field, and emptied into a one gallon stainless steel, compressed air sprayer, then diluted with ½ gallon of water to form the finished spray. The spray was evenly distributed over the surface area of each plot. The spray wand was fitted with an 8004 T-jet® fan nozzle; air pressure was maintained at about 30 psi. Water depth in the plots varied from ¼ inch to 6 inches. Untreated portions of the pasture served as checks.

Complete mortality of larvae occurred within four hours, but most counts were made 18 to 24 hours after treatment. Posttreatment larval counts were based on surviving larvae from 20 random dips made in each plot. Percent mortality was determined by comparing posttreatment counts with pretreatment counts.

Dairy waste ponds were treated and control evaluated in a manner similar to the pasture plots.

Aerial applications were made with a Piper Pawnee fitted with D-8 nozzles and Nos. 45 and 46 swirl plates. The airplane was calibrated to deliver one gallon of finished spray per acre. Areas of treated sources were calculated from Kings Mosquito Abatement District maps. Pre- and posttreatment larval and pupal populations were determined in the same manner as for compressed air sprayer applications.

Adult mosquito populations were determined by a pantleg count method. An observer spent one minute in designated station areas of pastures and recorded the total number of mosquitoes resting on each leg at the end of the designated time.

RESULTS AND DISCUSSION.—Compressed air sprayer applications of SD43775 at 0.05 lb/acre replicated three times yielded 100% mortality of *Ae. nigromaculis* larvae (Table 1), but 0.025 lb/acre was inadequate. Mixed populations of *Cx. p. quinquefasciatus, Cx. peus,* and *Cx. tarsalis* larvae were completely controlled at 0.1 lb/acre (Table 2), but 0.05 lb/acre was inadequate.

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Table 1.—Results obtained with compressed air sprayer applications of SD43775 against *Aedes nigromaculis* larvae in 1/32 acre plots. No mortality was observed in untreated larvae.

Date (1976)	Pasture Location	Dose lb AI/acre	Larval Stages Present	Pre-treatment	24/hr Post-treatment	% Mortality
8/22	Felipe	0.025	3, 4	164	80	50
8/22	Felipe	0.05	3, 4	138	0	100
8/22	Felipe	0.1	3, 4	128	0	100
8/24	Silviera	0.05	3, 4	169	0	100
8/24	Silviera	0.1	3, 4	130	0	100
8/31	Costa	0.025	3, 4	234	6	97
8/31	Costa	0.05	3, 4	328	0	100
8/31	Costa	0.1	3, 4	217	0	100

Table 2.–Results obtained with compressed air sprayer applications of SD43775 against mixed populations of *Culex pipiens quinquefasciatus, Cx. peus* and *Cx. tarsalis* in 1/32 acre plots. No mortality was observed in untreated larvae.

Date (1976)	Pasture Location	Dose lb AI/acre	Larval Stages Present	Pre-treatment	24/hr Post-treatment	% Mortality
9/12	Keith	0.025	1 - 4	137	20	86
9/12	Keith	0.05	1 - 4	224	10	96
9/12	Keith	0.1	1 - 4	165	0	100
9/22	Keith	0.05	1 - 4	114	7	94
9/22	Keith	0.1	1 - 4	106	0	100
9/28	Roach	0.1	4	223	0	100

Table 3.–Results obtained with aircraft applications of SD43775 against immature *Aedes nigromaculis* and *Culex tarsalis.* No mortality was observed in untreated larvae.

(1976)	Location and Habitat	Dosage lb AI/acre	Larval Stages Present	Species	Acres Treated	Pre-Treatment	24/hr Post-Treatment	% Mortality
9/30	Brazil alfalfa	0.1	4, pupae	*Ae. nigromaculis* plus	2	203	0	100
			1 - 4	*Cx. tarsalis*		320	0	100
10/4	Crose pasture	0.05[1]	4	*Ae. nigromaculis*	10	400	0	100
10/4	Perria alfalfa	0.05[1]	4	*Ae. nigromaculis*	3	402	0	100

[1]Finished spray mix prepared by diluting 4-day old 0.1 lb/acre formulation with equal volume of water.

Table 4.–Results obtained with aircraft application of SD43775 against *Aedes nigromaculis* adults on the Crose pasture.

Date (1976)	Dosage lb AI/acre	Station Number	Leg Count		% Reduction
			Pretreatment	18 hr Posttreatment	
10/4	0.05[1]	1	15	4	75
--	--	2	20	3	85
--	--	3	10	0	100

[1]Finished spray mix prepared by diluting 4-day old 0.1 lb/acre formulation with equal volume of water.

The dosage rate of 0.05 lb/acre is an effective dosage on the pupal stage of *Ae. nigromaculis* and 4th instar larvae of *Culex* spp. Except for some oils, currently used insecticides will not control mixed populations of *Aedes* pupae and *Culex* larvae. Since simultaneous larval and pupal control is sometimes desired, this feature offers an operational advantage.

The 0.05 lb/acre aerial dosage applied to the Crose pasture and the Perria alfalfa field was made from excess 0.1 lb/acre spray mixture used on the Brazil alfalfa field four days previously. The solution was kept in a cool, dark place during the four-day period. No deleterious effects on the activity of the compound could be detected. Operationally this is an important point since unused spray is sometimes held in spray tanks until favorable weather conditions allow application.

Table 4 indicates a significant reduction in the adult *Ae. nigromaculis* population at the Crose pasture even though control was not complete. Since only one test was made, further tests are needed to determine the efficacy of SD-43775 applied by aircraft against adult mosquitoes.

Treatment of dairy waste ponds by compressed air sprayer applications of SD43775 at 0.15 lb/acre and 0.25 lb/acre for control of 4th instar *Cx. p. quinquefasciatus* resulted in only about 50% reduction in 24 hours.

Further work must be done to determine the efficacy of SD43775 on adult mosquitoes. Larvicide applications to dairy waste ponds appear to be of doubtful efficacy, but larvicide applications to pasture-type sources at 0.05 and 0.1 lb/acre appear feasible.

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GRANULAR DIMILIN® AS PREHATCH TREATMENT FOR THE CONTROL OF FLOODWATER MOSQUITOES

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ABSTRACT

Granular formulations of the insect growth regulator Dimilin®, were applied as pre-hatch treatments to the breeding sources of 2 floodwater mosquitoes (*Psorophora confinnis* and *Aedes nigromaculis*) prior to flooding. Complete control of the former species was obtained at the rate of 0.01 lb AI/acre, a rate twice as much as needed for complete control of this species at posthatch treatments. At the 0.01 and 0.02 lb AI/acre, only moderate level of control of *Ae. nigromaculis* was obtained. This species requires higher rates of application (probably 0.05 lb/acre) as prehatch treatments.

INTRODUCTION.–Floodwater mosquitoes belonging to the genera *Aedes* and *Psorophora* breed profusely in intermittently irrigated and flooded agricultural fields such as rice, pastures, and others. Eggs of these mosquitoes withstand extreme desiccation and hatch shortly after being flooded with irrigation or rain water. Under desert conditions in southern California, *Ps. confinnis* (L-A) complete larval and pupal development in 3-4 days during June-September. Mosquito breeding sources in the agricultural areas of the lower desert are numerous, producing heavy populations at such a rapid rate that at times these breeding populations go undetected by mosquito control personnel. In addition, large acreages are irrigated at the same time, and mosquito abatement agencies do not possess the necessary manpower or the equipment to larvicide all these fields at the same time. To preclude massive adult emergence under these circumstances, the use of granular formulations of larvicides as prehatch treatment is employed routinely. The granular materials are applied prior to flooding, to breeding sources such as irrigated pastures, alfalfa fields, duck clubs, and others as preventive mosquito control measures.

The (IGR) Dimilin ® or TH-6040 [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] was reported to have high biological activity against mosquitoes (Mulla et al. 1975; Mulla and Darwazeh 1975, 1976; Schaefer et al. 1974, 1975; Steelman et al. 1975) against both susceptible and resistant strains of floodwater mosquitoes in rice fields of Louisiana, and irrigated pastures and alfalfa fields in the Palo Verde Valley and the San Joaquin Valley of California. Based on these studies, Dimilin showed a good potential as a mosquito control agent.

The studies reported here were carried out to evaluate the feasibility of Dimilin granules as a prehatch treatment for the control of floodwater mosquitoes in the Palo Verde and San Joaquin Valleys of California.

METHODS AND MATERIALS.–Granular formulations utilized were obtained from Kern Mosquito Abatement District, Bakersfield, California and Thompson Hayward Chemical co., Kansas City. The Kern granules (1%) were formulated on monterey sand 15/20 mesh, while Thompson Hayward (0.5 and 1.0%) were formulated on Florex 15/30 mesh granules.

To obtain good coverage and to increase bulk, the amount of granules required for each plot were mixed with blank monterey sand. A total of 6 lbs of the mix were applied per plot with a U.S. Borax PCB spreader (Model B), covering the entire plot with 4 swaths. All plots were ¼ mile long, but varied in width. Area of each plot in each test is shown in the tables. Treated areas were irrigated immediately after or within 5-8 hours after treatment. In the Palo Verde Valley, materials were applied at 2-3 rates, utilizing 2 plots per rate and 2 plots left untreated as checks.

Tests were conducted in Rio Rancho alfalfa, located on Defrain and 10th Avenue, and Robinson alfalfa on 10th Avenue, east of Intake Boulevard near the city of Blythe, Palo Verde Valley. Both locations are known to produce high numbers of *Ps. confinnis* larvae, and were used in previous studies (Mulla and Darwazeh 1975). For the control of *Aedes nigromaculis* in irrigated pastures, the formulations were applied at 2 rates, utilizing the same method of application. The tests were conducted in Rancho Santa Maria (Smith Pasture) in Kern County. In all tests, larval populations were assessed 1, 2 and 3 days after flooding, taking 10 dips/plot, and (%) control was determined based on the number of larvae recovered in the treated and untreated checks on the last sampling date (2-3) days after flooding.

In test B in the Palo Verde Valley, surviving larvae on the 3rd day were collected from the treated plots and checks, brought into the laboratory at the University of California, Riverside, for adult emergence assessment by placing 20 larvae in each of 2 cups/treatment. Every two days, dead larvae, pupae and adults were counted and removed, until all organisms either died or emerged as healthy normal adults, and % reduction in emergence was determined, based on the number of adults emerging from each treatment.

RESULTS AND DISCUSSION.–Only moderate control of *Ps. confinnis* larvae was obtained at the rates of 0.0025 and 0.005 lb/A in the Palo Verde Valley (Table 1, Test A). However, complete control was obtained at the higher rate (0.01 lb/A) of Dimilin applied as granules prior to flooding.

[1]The authors express their appreciation and thanks to Mr. Gerald M. Davison, Chief Engineer of the Palo Verde Irrigation District for his cooperation and valuable assistance during the course of these studies.

Table 1.—Evaluation of Dimilin granules applied as prehatch treatment in alfalfa fields against *Ps. confinnis* in the Palo Verde Valley (August-September, 1976)[1].

Formulation	Source	Rate lb/A	Avg. no. larvae/10 dips after flooding (days 1	2	3	(%) control
		Test A				
0.5 G	Thompson Hayward	0.0025	66	54	-	64
PP 178-3	Florex 15/30	0.005	7	34	-	78
Check	-	-	28	152	-	-
		Test B				
1.0 G	Thompson Hayward	0.005	18	10	36	20
PP 354	Florex 15/30	0.010	1	2	0	100
1.0 G	Kern MAD	0.005	8	9	12	73
	Monterey Sand	0.010	8	0	0	100
Check	-	-	33	35	45	-

[1]Plot size, 90 ft. x ¼ mile.

Table 2.—Emergence of *Ps. confinnis* adults resulting from larvae isolated from Dimilin prehatch treatments in alfalfa fields[1] (Palo Verde Valley, August, 1976).

Formulation	Source and Carrier	Rate lb/A	Avg. (%) Cumulative Mort. L	P	A	Reduc. in Emerg. (%)
1.0 G PP 354	Thompson Hayward Florex 15/30	0.005	8	33	18	59
1.0 G	Kern MAD Monterey Sand 15/20	0.005	0	48	3	51
Check	-	-	0	45	0	45

[1]No larvae in plots treated at 0.01 lb/A, see Table 1.

Table 3.—Evaluation of Dimilin granules (1.0 G) as prehatch treatment against *Aedes nigromaculis* in irrigated pasture (Kern County, August, 1976)[1].

Formulation	Source	Rate lb/A	Avg. no. of larvae/10 dips after flooding (days) 1	2	(%) control
1.0 G	Thompson Hayward	0.01	4	15	74
PP 354	Florex 15/30	0.025	7	10	83
Check	-	-	21	58	-

[1]Plot size, 50 ft. x ¼ mile.

At this rate, newly hatched larvae were observed to be dead or in distress one day after flooding, and they were completely eliminated 2 days later (Table 1, Test B). Of those surviving larvae which were collected and isolated from plots treated at the lower rate (0.005 lb/A) for adult emergence, only 41-49% emerged normally. A similar level of emergence (55%) was also obtained from those larvae collected and isolated from the check plots (Table 2). These results indicate that inhibition of emergence (EI) is primarily attributed to weakness of larvae due to transport, rather than to the chemical treatment. Therefore, surviving larvae in the treated plots with the lower rate should reach the adult stage.

Dimilin as prehatch treatments at 0.01 and 0.025 lb/A yielded good but not complete control of *Ae. nigromaculis* larvae in irrigated pastures (Table 3). Some newly hatched larvae were observed dead or in distriss 1 and 2 days after flooding. Three days after flooding, the plots dried rapidly, except in small puddles of mud where living larvae and pupae were found in large numbers, but it was not possible to collect or isolate these for adult emergence. It is apparent that prehatch treatments at both rates caused a good deal of larval mortality in the treated plots, as the larval density is much lower in the treated than the untreated plots.

In previous studies evaluating posthatch treatments, *Ps. confinnis* larvae were controlled at much lower rates (0.0025-0.005 lb/A) using several formulations of Dimilin, including granules (Mulla and Darwazeh 1975, 1976). Failure to achieve adequate control at these rates applied as prehatch treatments could be attributed to the high level of dilution of the chemical released into flowing irrigation water.

These findings indicate that complete larval control is not feasible in prehatch treatment at posthatch treatment rates in irrigated fields, and that higher rates will be required. The prehatch treatment rates should be increased 2-4 fold. Even then, the rates of application needed for complete control of *Ps. confinnis* mosquitoes are quite low, not exceeding 0.01 lb/A of effective dosage of Dimilin as prehatch treatment against *Ae. nigromaculis*.

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EVALUATION OF DIMILIN^{TM}, PYDRIN^{TM}, SUMITHION®, RESMETHRIN AND FENETHCARB FOR THE CONTROL OF CALIFORNIA MOSQUITOES

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ABSTRACT

Dimilin^{TM} provides operational control of mosquito larvae at rates of 0.025-0.04 lb AI/acre. Persistence of the active ingredient in field waters or in soil is limited following the application of wettable powder or granular formulations. However, aqueous sprays used to disperse the wettable powder formulations result in persistent and accumulative residues on vegetation. These residues can be largely eliminated using a 1% sand formulation Pydrin^{TM} offers potential as both a larvicide and a pupicide. Sumithion®does not show promise against OP-resistent populations in the San Joaquin Valley. Resmethrin lacked effective adulticidal activity at 0.01 lb AI/acre when applied by aircraft in 1 gal water/acre. A new carbamate, Fenethcarb, shows lower adulticidal activity in field tests than Baygon.

DIMILIN^{TM}.–Dimilin, also known as TH6040 and diflubenzuron is currently being developed as a mosquito larvicide by Thompson-Hayward Chemical Company. It is known that 0.025 lb AI/acre is operationally feasible against *Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar larvae (Schaefer et al. 1975). Multiple aerial applications of 0.02 to 0.04 lb AI/acre, using a 25% wettable powder formulation, resulted in only limited residues in pasture waters (Schaefer and Dupras 1976) and produced no detectable impact on nontarget organisms (Miura and Takahashi 1976). However, these same studies showed that there was a build-up of residues on vegetation, following multiple applications of the wettable powder formulation, and furthermore that these residues did not readily dissipate. Therefore, 1976 studies were aimed at evaluating granular formulations that might penetrate through the canopy of vegetation without leaving undesirable residues.

Comparison of residues following multiple applications of wettable powder and 1% Attaclay granules. - - A pasture (File) in Fresno County was treated 4 times during 1976. One half of the field was treated with the 25% wettable powder formulation to give 0.035 lb AI/gallon of water/acre, while the other half was treated with 1% Attaclay granules (16/30 mesh). The aircraft was calibrated to deliver 3.5 lb of the 1% granules/acre. However, the actual amount dispersed was measured by weighing the contents of the aircraft tank before and after each treatment.

Other tests with the 1% Attaclay granules. - - In order to obtain efficacy data and to collect residue samples, a pasture (Costerisan) in Kern County was treated 3 times at 0.06 to 0.09 lb AI/acre and a pasture (Lowry) in San Joaquin County was treated once at 0.09 lb AI/acre. Table 1 summarized the applications.

Results with the wettable powder and 1% Attaclay granules. - - As found in 1975, the residues on vegetation were high following applications of the wettable powder. The 1% Attaclay granules contained many light particles and these were observed to drift during the application. Post-treatment visual examination revealed that some granules had remained on the vegetation rather than penetrating through the canopy. Analysis of the vegetation revealed that residues were much lower on the portion of the pasture treated with the 1% Attaclay granules, than for that treated with wettable powder, but significant residues still persisted (Schaefer and Dupras 1977). Even after 4 repeated applications of the 1% Attaclay granules, no deleterious impact on nontarget organisms was apparent (Table 2).

Considerable difficulty was involved in applying the granules in both the Kern and San Joaquin County tests. The granules tended to clump as they passed through the slots between the tank and spreader; this resulted in a stoppage of flow. Also, the settings used to obtain a calibrated rate during a given application did not result in the same rate on subsequent application attempts. The Transland Spreader (Model 20180) was used for applying the granules at the File pasture. The spreader allowed application of the desired amounts of the Attaclay granules. Drift in low winds (2-5 mph) was a significant problem as were residues on the vegetation. Vegetation samples from the Costerisan pasture showed significant residue levels (Schaefer and Dupras 1977). Thus, the 1% Attaclay granules do not provide an operational alternative to the use of the wettable powder.

ONE PERCENT SAND GRANULES. These were prepared by mixing 200 lbs of sand (20 mesh) with 1 pint of oil (Golden Bear 1356 or 1313) and then adding 8 lb of the 25% wettable powder and 100 gm of amorphous silica (Hi-Sil 233) followed by careful mixing.

The 1% sand granules were applied 4 times by aircraft (Table 1). A dose of 0.044 lb AI/acre, as well as a later treatment on the same gun club of 0.058 lb AI/acre, gave excellent control of *Culex tarsalis* Coquillett larvae. The effect on nontarget populations was relatively slight (Table 3). These treatments did not produce significant or persistent residues on vegetation. Measurable residues on vegetation were detected immediately after treatment and were apparently due to dust (wettable powder not adhering to the sand) which settled from the aircraft. Application of 0.025 lb AI/acre on a pasture (Cunha) gave 100% control of *Ae. nigromaculis* larvae and while a low residue on vegetation was present 1-hr after treatment, no residues were present in samples after that time. A higher application of 0.074 lb AI/acre at Smith's pasture gave 100% control of *Ae. nigromaculis* and *Ae. melanimon* larvae and no residues on vegetation were found.

Thus, it is possible to formulate the 25% wettable powder of TH6040 onto sand and produce 1% granules which offer operational control of mosquito larvae without pro-

Table 1.–Summary of the 1976 aircraft applications with TH6040.

Field Test No.	Date (1976)	MAD[1]	Location & Habitat		Acres Treated	Formulation	Rate (lb AI/acre)
76-5	7/19	Consolidated	File	Pasture	4	25% WP[2]	0.035
76-5	7/19	Consolidated	File	Pasture	8	1% G(Attaclay)[3]	0.037
76-7	7/30	Kern	Costerisan	Pasture	10	1% G(Attaclay)	0.061
76-8	8/2	Consolidated	File	Pasture	4	25% WP	0.035
76-8	8/2	Consolidated	File	Pasture	8	1% G(Attaclay)	0.041
76-10	8/9	San Joaquin	Lowry	Pasture	3	1% G(Attaclay)	0.094
76-11	8/16	Consolidated	File	Pasture	4	25% WP	0.035
76-11	8/16	Consolidated	File	Pasture	8	1% G(Attaclay)	0.038
76-12	8/20	Kern	Universal	Gun Club	50	1% G(Sand)[4]	0.044
76-15	8/23	Kern	Costerisan	Pasture	20	1% G(Attaclay)	0.070
76-19	8/30	Consolidated	File	Pasture	4	25% WP	0.035
76-19	8/30	Consolidated	File	Pasture	8	1% G(Attaclay)	0.033
76-20	9/9	Kern	Costerisan	Pasture	12	1% G(Attaclay)	0.095
76-21	9/9	Kern	Universal	Gun Club	50	1% G(Sand)	0.058
76-22	9/14	Kern	Smith	Pasture	20	1% G(Sand)	0.074
76-30	9/24	Consolidated	Cunha	Pasture	20	1% G(Sand)	0.025

[1]Mosquito Abatement District.

[2]25% wettable powder (Supplied by Thompson-Hayward Chemical Co.).

[3]1% Attaclay granule, 16/30 mesh (Supplied by Thompson-Hayward Chemical Co.).

[4]1% Sand granule, 20/mesh.

Table 2.–Effects of Dimilin (1% Attaclay granules) on nontarget organisms. Repeated applications on the same field against pasture mosquitoes (F. T. Nos. 76-5, 8, 11 and 19).

Organisms	Application 1st	2nd	3rd	4th
Flatworms	+	+	+	+
Rotifers	+	+	+	+
Nematodes	+	+	+	+
Waterfleas	-	+	+	+
Copepods	-	+	+	+
Seed Shrimps	+	+	+	+
Mayfly nymphs	+	+	+	+
Beetle adults[1]	+	+	+	+
Culicoids larvae	-	+	+	+
Chironomids larvae	-	+	+	+
Oribatid mites	+	+	+	+

[1]*Tropisternus lateralis*, *Thermonectus basillaris*, and *Laccophilus* spp.

+ = Present - = Absent

ducing a residue problem on vegetation. Further studies to develop a "dust-free" sand granule are in progress.

PYDRIN™.–This pyrethroid-type compound, also known as SD43775, was previously shown to have potential as a larvicide against several California mosquitoes (Schaefer et al. 1976). Rates as low as 0.05 lb AI/acre gave good control of mixed larval populations of *Ae. nigromaculis* and *Ae. melanimon* in the field. Laboratory tests also indicated pupicide potential. During 1976, pasture plots (0.05 acre) containing mixed populations of *Ae. nigromaculis* and *Ae. melanimon* pupae were hand-sprayed using a 2.4 lb AI/gal E. C. formulation of Pydrin. Six plots were treated at 0.5, 0.25 and 0.1 AI/acre (each in duplicate). All treatments resulted in 100% pupal mortality. Thus, Pydrin in contrast to most synthetic larvicides, has good potential against pupae.

Treatments of polluted dairy-waste holding ponds with rates of 0.1 and 0.2 lb AI/acre of Pydrin gave incomplete (50-90%) mortality against *Cx. tarsalis* and *Culex pipiens quinquefasciatus* Say populations. All of the treated holding ponds had dense vegetation along the perimeter and large amounts of organic debris were present in the water.

SUMITHION®.–Earlier tests had shown that organophosphorus-resistant strains of *Ae. nigromaculis* were highly cross-resistant to Sumithion (Schaefer and Wilder 1970). However, since Sumithion is projected to be used as a mosquito control agent in some parts of the United States, field evaluations on pasture plots were conducted. A 4 lb AI/gal E. C. formulation of Sumithion was diluted with water and applied by hand-sprayer to 0.05 acre pasture plots containing mixed populations of *Ae. nigromaculis* and *Ae. melanimon* at rates of 0.1, 0.2 and 0.3 lb AI/acre. There was a total mortality of 90% in each case, with only *Ae. nigromaculis* surviving. The lack of increased mortality with increased doses shows that very high cross-resistance is present and that Sumithion would not be effective in operational programs in the San Joaquin Valley.

Table 3.–Effects of Dimilin (1% sand granules) on nontarget organisms: Applied at 0.058 lb AI/acre against *Culex tarsalis* September 9, 1976 (F. T. No. 76-21).

	No. of organisms/4500 ml of water sample				
	Pretreatment	Posttreatment (day)			
Organism	0	0	1	4	6
Water fleas	15	0	0	2	6
Copepods	180	312	0	2	6
Seed Shrimps	1395	1534	850	1025	710
Mayfly Nymphs	105	312	170	105	70
Dragonfly Nymphs	59	95	31	26	12
Damselfly Nymphs	276	57	20	12	6
Beetle adults[1]	3	1	0	4	3
Beetle larvae[2]	5	4	1	5	3
Chironomid larvae	285	394	40	13	120
Shorefly larvae	0	4	0	3	0

1 *Tropisternus lateralis, Thermonectus basillaris* and *Laccophilus* spp.

2 *Tropisternus lateralis* and *Laccophilus* spp.

RESMITHRIN.–This pyrethroid material is a known mosquito adulticide which has been applied by ground fogging equipment with varying degrees of success. It was of interest to determine the potential of using Resmethrin as an adulticide using aerial applications. Because of cost, the manufacturer recommended a rate of 0.005 lb AI/acre. Since the material had not been previously evaluated by aircraft applications, a higher rate of 0.01 lb AI/acre was selected. A 2 lb AI/gal E. C. was diluted with water to give 0.01 lb AI in 1 gal water/acre. Two 40 acre fields in Kings County having high populations of *Ae. nigromaculis* adults were treated (one at 9:35 a.m. and the other at 10:15 a.m.). No knock-down or subsequent reductions of adult numbers was observed. Resmethrin is known to be photo-unstable and the possibility existed that better results might have been obtained by applying the material before sunrise. Therefore, another 20 acre pasture in Tulare County having high numbers of *Ae. nigromaculis* adults was also treated at 0.01 lb AI/gal/acre, but the application was made prior to daybreak. The aerial application was good but still there was no knock-down or subsequent reduction in numbers of adults.

FENETHCARB.–This carbamate [3,5-diethyl-phenyl-N-methyl- carbamate] is currently being developed in Europe, where it has shown potential for adulticiding mosquitoes. Tests in the laboratory (Georghiou and Metcalf 1961) against adult *Cx. p. quinquefasciatus* indicated activity similar to Baygon, as follows:

Compound	LC_{50}	LC_{90}
Fenethcarb	0.051	0.070
Baygon	0.041	0.071

A field test was conducted using an E. C. formulation diluted to give 0.08 lb AI/gal water/acre by aircraft. A 10 acre pasture in Kings County, which had a high leg count (50) of *Ae. nigromaculis* adults, served as the test site. There was some reduction of adults within a few minutes after treatment and the maximum reduction of 65% was observed at 3 hrs post-treatment. As Baygon is used operationally at 0.05 to 0.07 lb AI/acre, Fenethcarb would have to be applied at a much higher rate and the economics of that possibility is doubtful.

SUMMARY.–Of the chemical control agents evaluated during 1976, only two show potential for future operational use. The registration of TH6040 is in progress and hopefully will be accomplished by 1977. Pydrin also continues to show promise. As both of these compounds are also being developed for agricultural markets, the probability that they will be commercially available for mosquito control is enhanced.

ACKNOWLEDGMENT.–The 25% wettable powder and 1% Attaclay formulations of TH6040 were provided by Thompson-Hayward Chemical Company, who also supported this work in part. Research on Pydrin was supported, in part, by Shell Development Company. The assistance and cooperation of personnel from the Consolidated, Kern, Kings, San Joaquin and Tulare Mosquito Abatement Districts allowed these evaluations to be made.

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FIELD TESTING OF METHOPRENE (ALTOSID®) BRIQUETS FOR *CULEX* MOSQUITO CONTROL

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ABSTRACT

Various types of mosquito sources were treated with methoprene (Altosid®) 4% briquets. The major sources treated were septic tank systems, catch basins, swimming pools and irrigation ditches. The average control period was 44 days.

INTRODUCTION.—Methoprene, Altosid® SR-10, has been used since 1973 in Kings County. In 1973 applications were directed towards organophosphorus resistant *Aedes nigromaculis* larvae. In 1974 experiments were conducted in Kings County with Altosid SR-10 to control *Culex tarsalis* larvae. The same treatment rate as applied for *Ae. nigromaculis* (4 oz Altosid SR-10/acre) was used for *Cx. tarsalis.* Altosid was sprayed twice weekly on a seepage area for eight weeks. One hundred percent control was maintained during this period (Lewallen and Stewart unpublished data).

Since *Cx p. quinquefasciatus* populations were found to be OP-resistant in Kings County (Stewart 1975, Georghiou et al. 1975), an alternate class of insecticide was needed. This paper shows the result of experiments with a new formulation of methoprene, Altosid 4% briquets, against *Cx. p. quinquefasciatus*, *Cx. peus* and *Cx. tarsalis* larvae.

MATERIALS AND METHODS.—Various types of mosquito sources were treated with Altosid 4% briquets. Two of these mosquito sources were experimental ponds constructed at the District yard to test the effects of the briquets in water of high organic content. The ponds were earthen holes, 3 ft square by 1 ft deep, lined with vinyl plastic, and filled with water from a local dairy drain. The other areas used for testing are listed in the tables. Untreated checks, not listed in the tables, were observed for emergence of adults. Average check emergence was 90%.

Briquets were hand thrown into all sources. Application rates were based on the manufacturer's label. Non- or low-flow shallow depressions, up to 2 ft in depth, were treated by applying one briquet per hundred square feet. For mosquito sources with water subject to flow, or deeper than 2 ft, the rate of application was one briquet per 75 gal of water. Application rates were changed when control failures were noted.

Treated sources were sampled at weekly intervals. Ten dips were taken with a 450 ml capacity dipper at small sources such as catch basins. Twenty dips were taken per sampling period at larger sources such as dairy drains. All sources treated were sampled for two weeks following operational failure. Three ounces of treated water and pupae were collected and placed in 6 oz wax cups. The number of pupae collected was dependent upon the number of pupae present when the sample was taken.

An 8 oz styrifoam cup, with the bottom replaced by a piece of organdy cloth, served as an emergence chamber. The styrifoam cup was placed over the wax cup. Mortality was based on the number of dead pupae, deformed adults, and adults that could not fly.

RESULTS AND DISCUSSION.—Table 1 shows the control period for each catch basin. As can be seen from this table, a number of catch basins had an increase in water volume. This additional water may have shortened control periods for some basins.

Table 2 shows the percent mortality and control period for mosquito sources with high organic content. Additional water was flowing into all sources except the two ponds at the District yard. When flowing water was found, flows were low, except in the two dairy drains. The large volume of water in the dairy drains made normal treatment rates (one briquet per 75 gals water) impractical. One briquet was placed every three yards of the dairy drain perimeter in Drain 1. Drains 2 and 3 were treated at the rate of one briquet per 2 yard interval and one yard interval respectively. Schaefer and Dupras (1973) checked the persistence of methoprene in different types of water. They found that the material persisted in sewage water as well as in tap, pasture, and pond water. The organic debris which settled to the bottom in sources 1 - 4 probably kept the briquets from releasing enough active ingredient to the surface to provide for mosquito kill. As can be seen from Table 2, control was never achieved in sources 1-4. In contrast, pond 5 (Table 2), which had the briquet suspended above the organic debris, was kept free of mosquitoes for 14 days. Mosquitoes in two septic tank systems were controlled at the normal rate, one briquet per 100 ft^2 of surface area. Additional briquets were needed in three septic tank systems to achieve control.

Table 3 shows the percent mortality and control period for a number of other habitats treated with the briquets. Source 1, a pit surrounding a grain elevator, was treated with four briquets placed at the corners of the pit, since the elevator occupied the center of the pit. The three pools tested were exposed to summer sunlight. Because Schaefer and Wilder (1972) and Schaefer and Dupras (1973) have shown that sunlight reduces the biological activity of methoprene, an additional number of briquets were added over the normal rate to offset the effects of the sunlight.

Table 4 shows the control for a number of treated ditches. Except for Ditch 1, all ditches contained irrigation tail water. Ditch 1 was treated with 27 briquets. Because of the organic debris on the bottom of this ditch a large number of briquets were used. Water depth was about 6

Table 1.–Mortality of *Culex p. quinquefasciatus* larvae in catch basins treated with 4% Altosid® Briquets.

Location	Treatment	Approx. Vol. (Gal.)	No. Briquets Applied	100% M (Days)
1. No. Malone Street, Hanford	6/18/76	20+	1	80
2. So. Malone Street, Hanford	6/18/76	20+	1	75
3. No. Cortner Street, Hanford	6/18/76	12	1	84
4. So. Cortner Street, Hanford	6/18/76	12	1	84
5. NW. Hanna Street, Corcoran	6/21/76	73	1	67
6. NE. Hanna Street, Corcoran	6/21/76	73+	1	23
7. SW. Hanna Street, Corcoran	6/21/76	73+	1	18
8. SE. Hanna Street, Corcoran	6/21/76	73+	1	20
9. So. Josephine Street, Corcoran	6/21/76	73	1	39
10. So. Follet Street, Lemoore	6/25/76	73+	1	49
11. No. Hill Street, Lemoore	6/25/76	73	1	41

+ Additional water added to original volume.

Table 2.–Mortality of *Culex p. quinquefasciatus* larvae in water of high organic content.

Habitat	Location	Treatment Date	Size of Source	No. Briquets Applied Over Normal Rate	No. Briquets Applied	Control (Days)	% M
1. Dairy Drain	5400 Jackson Ave.	7/20/76	485,000 gal.	-(6418)	48	(7)	35%
2. Dairy Drain	6100 14th Ave.	7/21/76	225,000 gal.	-(3340)	60	(7)	25%
3. Drain	11934 19½ Ave.	8/27/76	200 sq. ft.	8	10	(7)	0%
4. Pond	Kings MAD	7/20/76	9 sq. ft.	0	1	(7)	33%
5. Pond	Kings MAD	8/2/76	9 sq. ft.	0	1[2]	(14)	100%
6. Septic Tank	5418 Pueblo Ave.	10/27/76	35 sq. ft.	0	1	(36)	100%
7. Septic Tank	2719 Omaha	7/1/76	24 sq. ft.	1	2	(71)	100%
9a Septic Tank	16900 Jackson Ave.	8/3/76	60 sq. ft.	2	3	(7)	25%
9b Septic Tank	16900 Jackson Ave.	8/20/76	60 sq. ft.	6	7	(50)[1]	100%
10a Septic Tank	7043 Omaha Ave.	6/21/76	80 sq. ft.	2	3	(7)	62%
10b Septic Tank	7043 Omaha Ave.	7/1/76	30 sq. ft.	6	7	(107)	100%

[1]Water source dried.

[2]Briquet suspended 5 inches from water surface.

Table 3.–Mortality of *Culex p. quinquefasciatus* larvae in sources treated with Altosid® 4% Briquets.

1. Elevator Pit Yoder Blvd. 10/27/76

*

Habitat	Location	Treatment Date	Surface Area (Sq. Ft.)	No. Briquets Applied Over Normal Rate	No. Briquets Applied	% M (Days)
1. Elevator Pit	Yoder Blvd.	10/27/76	64	3	4	36
2. Garden Pond[1]	Harris Street	10/13/76	7	0	1	53
3. Pool	12th Avenue	10/4/76	120	4	6	23
4. Pool	Lacey Blvd.	10/6/76	324	2	6	50
5. Pool	White Street	10/13/76	36	1	2	49[2]

[1]Pond had a roof over it, no direct sunlight.

[2]Water source dried.

Table 4.—Mortality of *Culex tarsalis* and *Culex peus* in sources treated with Altosid® Briquets.

Habitat	Location	Treatment Date	Surface Area (Sq. Ft.)	No. Briquets Applied Over Normal Rate	No. Briquets Applied	% M (Days)
1. Ditch	Jackson Avenue	6/22/76	300	24	27	16
2. Ditch[2]	18th Avenue	6/22/76	4,050	-(8)	33	17
3. Ditch	18th Avenue	9/4/76	4,050	1	42	20[1]
4. Ditch	11th Avenue	6/11/76	250	22	25	50

[1]Water source dried.

[2]80% mortality of *Aedes vexans* in addition to the 100% mortality of *Culex tarsalis* and *peus* for 17 days.

inches. The shallow water probably accounts for the difference in control as observed in sources 1-4 (Table 3). Sources 1-4 contained at least 1 ft of water. The amount of briquets needed for control was miscalculated for Ditch 2 (Table 4). The second flooding of this source, Ditch 3, showed that control was achieved when the right dosage was used. Ditch 4 showed alternate periods of flow. In addition to the flowing water, the ditch was narrow and long, 2.5 ft by 100 ft. Because of the two circumstances, an additional number of briquets were used over the normal rate.

It appeared that the rates of application, as specified by the manufacturer controlled *Culex* spp. in several situations. When flowing water, organic debris, sunlight and shape of the source were problems, additional briquets were needed.

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CONTROL OF ADULT *AEDES NIGROMACULIS* (LUDLOW) WITH AEROSOLS OF PYRETHRINS AND SYNTHETIC PYRETHROIDS[1]

I. LABORATORY ASSAYS

Ernst P. Zboray[2] and Gary A. Mount[3]

INTRODUCTION.–Cooperative investigations on the evaluation of pyrethrins and synthetic pyrethroids for use as aerosols against adult *Aedes nigromaculis* (Ludlow) in California were initiated in 1974. Participating were the Insects Affecting Man Research Laboratory, U. S. Department of Agriculture, Gainesville, Florida; the Department of Entomology, University of California at Davis; the Vector and Waste Management Section, California Department of Health; and numerous mosquito abatement agencies.

This paper summarizes three years of laboratory wind tunnel testing during the period 1974 through 1976. The second (Womeldorf and Mount 1977) and third (Washino et al. 1977) papers in the series discuss field evaluations of the effects upon *Ae. nigromaculis* and upon nontarget organisms. The objective of the laboratory assays was to determine the relative toxicity of several potentially useful pyrethrins and synthetic pyrethroids as compared with malathion and propoxur standards.

MATERIALS AND METHODS.–The tests were performed with a wind tunnel system using methods essentially as described by Mount et al. (1976). The adulticides evaluated were synergized pyrethrins and these pyrethroids: tetramethrin; resmethrin; the d-cis isomer of resmethrin; Roussel-Uclaf RU-11679 or bioethanomethrin (5-benzyl-3-furyl methyl trans- (+)-3 (cyclopentyli-denemethyl) -2, 2-dimethylcyclopropanecarboxylate); National Research and Development Corporation NRDC-143 or permethrin (m-phenoxybenzyl cis, trans - (+)-3- (2, 2-dichlorovinyl) -2, 2-dimethylcyclopropanecarboxylate); and NRDC-161 ((S)-[cyano (3-phenoxyphenyl) methyl] cis-(+) -3- (2, 2-dibromoethenyl) -2, 2-dimethylcyclopropanecarboxylate). The synergist was piperonyl butoxide.

Ae. nigromaculis were field collected as 4th instar larvae (sometimes with pupae) and held in laboratory until they were 2- to 4-day old adults. The collections were made from irrigated pastures in the Sacramento County-Yolo County Mosquito Abatement District, the Sutter-Yuba Mosquito Abatement District, and an uncontrolled area located between the Tehama County and Shasta Mosquito Abatement Districts.

Each insecticide treatment was evaluated with 4-8 discriminating concentrations and 6-10 replications of 10-20 (usually 15) female mosquitoes per concentration. Thus, concentration-mortality data for each insecticide were normally based on the treatment of at least 360 mosquitoes. Concentration-mortality data were analyzed with a probit analysis program written according to the procedures given by Finney (1971).

Mosquitoes handled in the same manner as those exposed to the insecticides but exposed only to the acetone solvent averaged 4% mortality at 24 hour posttreatment.

RESULTS AND DISCUSSION.–Table 1 gives the computed LC_{50} and LC_{90} values for the pyrethrins and pyrethroids in order of decreasing toxicity at the LC_{90}. These data were summarized without regard to collection location and year since there was no evidence of resistance to the pyrethrins and pyrethroids. The most toxic pyrethroid, NRDC-161, was 28x and 26x more effective than propoxur and malathion respectively. NRDC-161 has also been outstanding against colonized strains of *Ae. taeniorhynchus* (Wiedemann), *Ae. aegypti* (L.) and *Anopheles quadrimaculatus* Say (Mount, unpublished). Synergized RU-11679 was equally as toxic as NRDC-161 but was only about one-fourth as toxic when tested unsynergized. RU-11679 was potentiated 0-7x by synergism against several other species of mosquitoes (Mount and Pierce 1975). The other pyrethroids and pyrethroid-synergist combinations were 2-7x propoxur and 3-10x malathion. Resmethrin was potentiated about 2x by synergism but tetramethrin was unaffected by the addition of piperonyl butoxide. Synergized resmethrin was equally as toxic as synergized pyrethrins, but when tested unsynergized resmethrin was only half as toxic.

The 1974-75 collections indicated no resistance to propoxur and malathion but the 1976 collections showed an 11x and 5x decrease in susceptibility to propoxur and malathion respectively (Table 1). Some of the fields where the 1976 collections were made have been routinely spray-

Table 1.–Toxicity of pyrethrins and synthetic pyrethroid aerosols to adult female *Aedes nigromaculis* exposed in a wind tunnel. Values in parts per million insecticide only: 95% fiducial limits in parentheses. Synergist, where shown, is piperonyl butoxide at 5 parts to 1 part insecticide.

Adulticide	24 hr LC_{50}	24 hr LC_{90}
Synergized RU-11679	1.9 (1.6-2.3)	7.5 (5.6-6-12)
NRDC-161	1.1 (0.9-1.4	8.4 (6.6-11)
Synergized d-cis resmethrin	3.7 (2.4-5.6)	23 (13-59)
Synergized resmethrin	5.5 (4.4-6.9)	26 (18-47)
RU-11679	5.4 (4.3-6.9)	31(21-53)
Synergized pyrethrin	7.8 (6.3-9.5)	32 (23-47)
Resmethrin	15 (13-18)	56 (41-89)
NRDC-143 (permethrin)	8.6 (6.6-11)	57 (42-91)
Tetramethrin	20 (17-25)	57 (42-87)
Synergized tetramethrin	15 (12-22)	71 (42-190)
Propoxur (1974-75)	56 (47-65)	151 (123-202)
Propoxur (1976)	642 (460-964)	1729 (1186-4220)
Malathion (1974-75)	97 (86-109)	222 (190-272)
Malathion (1976)	897 (316-501)	1079 (787-1915)

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ed with propoxur for several years, and occasional field failures have been reported. However, why the apparent resistance appeared so suddenly is unknown. As to malathion, cross-resistance is a likely possibility since the chemical has not been used in those fields for several years.

ACKNOWLEDGMENTS.—We thank Stanley N. Shikuma and Michael A. Seth, Vector and Waste Management Section, California Department of Health, for their able assistance in conducting the wind tunnel tests.

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CONTROL OF ADULT *AEDES NIGROMACULIS* (LUDLOW) WITH AEROSOLS OF PYRETHRINS AND SYNTHETIC PYRETHROIDS[1]

II. FIELD EVALUATIONS

Don J. Womeldorf[2] and Gary A. Mount[3]

INTRODUCTION.–Cooperative investigations on the evaluations of pyrethrins and synthetic pyrethroids for use as aerosols against adult *Aedes nigromaculis* (Ludlow) in California were initiated in 1974. Participating were the Insects Affecting Man Research Laboratory, U. S. Department of Agriculture, Gainesville, Florida; the Department of Entomology, University of California at Davis; the Vector & Waste Management Section, California Department of Health; and numerous mosquito abatement agencies.

This paper reports upon field evaluations conducted during September of 1974 and August of 1976. The first (Zboray and Mount 1977) and third (Washino et al. 1977) papers in the series discuss laboratory wind tunnel tests against *Ae. nigromaculis* and field evaluations of the effects upon nontarget organisms. The objectives of the field trials were to (1) gain additional information on the feasibility of using ground applied aerosols for the control of adult pasture mosquitoes, and (2) establish minimum effective dosages for pyrethrins and synthetic pyrethroids.

MATERIALS AND METHODS.–Concentrated formulations of synergized pyrethrins, resmethrin, and synergized resmethrin were applied with a truck-mounted ultralow volume (ULV) aerosol generator (Leco HD) against adult mosquitoes (predominantly *Ae. nigromaculis*) in irrigated pastures.

The truck-mounted aerosol generator was driven at a speed of 5 mph for all applications. Nozzle air pressures used were 4-5 psi, depending on flow rate and formulation. A droplet size study indicated that the Leco HD produced a volume median diameter of 15 microns when dispersing 420 ml/min of white mineral oil (Klearol®) at 5 psi nozzle air pressure. This determination was made according to the settlement method described by Mount and Pierce (1972). Insecticide formulation volumes were measured before and after each application to determine the amount applied. Each application was timed with a stopwatch so that actual flow rates could be calculated. Attempted swath widths depended upon pasture size and ranged from 1/8 to 1/2 mile. The dose of each application was computed in lb. AI/acre as follows:

$$\text{lb. AI/acre} = \frac{\text{gal/min} \times \text{lb. AI/gal}}{\text{acre/min}}$$

Basic meteorological measurements were taken at 15 minute intervals before, during, and after each aerosol application. Relative humidity was determined with a sling psychrometer. Temperature determinations were made at 3 and 10 m above the ground with an electronic thermistor thermometer. An anemometer was hand-held at 1.5-2 m to measure wind speed and direction. A stability ratio was computed for each application according to a formula adapted from Haugen et al. (1961) as follows:

$$\text{Stability ratio} = \frac{t_2 - t_1 \quad \times 10^5}{(\bar{u})^2}$$

where

t_2 = temperature C° at 10 m
t_1 = temperature C° at 3 m
$\bar{u}$ = average windspeed (cm/sec).

Assessment of the effects of the aerosol treatment was made by pre and posttreatment landing counts of adult mosquitoes. These counts were usually made by three persons walking the entire width of the target pasture, although in several cases the observations were made by either a single person or as many as five persons. The observations were also made on untreated pastures whenever possible so that control data could be adjusted for natural variations in density. Adjustments were made by using Mulla's formula (Mulla and Darwazeh 1971). Drift and coverage of the aerosols were further assayed with laboratory-reared adult *Culex pipiens quinquefasciatus* Say placed in disposable cages (Townzen and Natvig 1973) and positioned at various distances across each pasture.

The application, meteorological and control data for the pyrethrins and resmethrin aerosols are given in Tables 1 and 2, respectively.

RESULTS.–The results of the 1974 tests with synergized pyrethrins (Table 1) indicated that a dosage rate range of 0.0006-0.001 lb. AI/acre was too low for consistent control. For a ½ mile swath width the 0.001 lb AI/acre rate, requiring an output of 318 ml/min, was near the maximum that could be applied using 5% pyrethrins and an unmodified Leco HD aerosol generator. The two 1976 tests using 12% pyrethrins at 0.006 lb. AI/acre provided 75-76% control of *Ae. nigromaculis*, although the relatively small 8.6 acre pasture was partially reinfested the following day.

Aerosols of resmethrin (Table 2) provided good mosquito control at rates of 0.003 and 0.006 lb. AI/acre in two 1974 tests. Although the rate was increased to 0.02 lb. AI/acre in the 1976 test with resmethrin, somewhat less mosquito control was shown by both kill of caged *Cx. p. quinquefasciatus* positioned at 0.5 ft above ground and

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Table 1.–Pyrethrins + piperonyl butoxide ultra low volume aerosols against adult *Aedes nigromaculis* in irrigated pastures in California.

Date	9/10/74	9/11/74	9/13/74	9/16/74	8/21/76	8/22/76
Hour	2000	0800	1835	1910	2015	1900
Location (MAD)	Butte	Butte	Tehama	Kern	Kings[a]	Kings
Acreage	145	169	168	244	80	8.6
Formulation (%)	5+25	5+25	5+25	5+25	12+60	12+60
Flow rate (ml/min)	318	150	165	264	303	163
Nozzle air pressure (psi)	5	4	4	5	5	4.5
Swath width (miles)	½	½	¼	½	¼	1/8
lb. AI/acre	0.001	0.0006	0.001	0.001	0.006	0.006
Wind speed (kn)	1.4	9.4	1.7	2.3	3.5	2.5
°C at 10m	23.5	20.2	30.3	29.1	25.3	26.9
°C at 3m	25.7	20.3	29.4	28	24.8	26.2
Stability ratio	-42	-0.04	12	7.9	1.5	4.2
Relative humidity (%)	58	55	28	-	62	66
12 hr % kill of caged mosquitoes (3 ft)	89	86	65	71	100	100
12 hr % kill of caged mosquitoes (0.5 ft)	83	29	-	26	79	100
12 hr posttreatment % control of *Ae. nigromaculis*	b	87	c	0	76	d

[a] Alfalfa field instead of grass pasture.

[b] High winds prevented 12 hr count; 60% reduction at 36 hr.

[c] Posttreatment counts indicated less than 50% reduction, but nearby emerging adults were observed at this time.

[d] 28% and 55% at 12 and 18 hr respectively; 75% reduction at ¼ hr posttreatment.

posttreatment reduction of *Ae. nigromaculis*. Two applications of synergized resmethrin (Table 2) at rates of 0.003 and 0.006 lb. AI/acre provided only 45-56% control of *Ae. nigromaculis* despite 100% kills of caged mosquitoes.

DISCUSSIONS AND CONCLUSIONS.—Our field trials did not clearly demonstrate the feasibility of using ground applied aerosols of pyrethrins and resmethrin for the control of adult *Ae. nigromaculis* in pastures. Obviously several of our applications were made with insufficient insecticide rates; however, several other tests with higher rates applied during periods of favorable meteorology failed to provide a high degree of control. We think that two of our tests, 8/21/76 in Table 1 and 8/23/76 in Table 2, suggested a basic reason as to why high percentage controls may be difficult to achieve in some pastures. In both of these tests complete kill of mosquitoes was achieved in cages positioned at 3 ft. above the ground but only 74% and 79% of the mosquitoes were killed in cages held close to the ground and afforded some protection from aerosol contact by vegetative cover. This lower degree of kill coincided with the percentage controls of *Ae. nigromaculis* (69% and 76%). Thus, vegetative height and density appear to be important factors affecting the amount of control obtained with aerosol applications.

Our experience also indicated that successful aerosol applications depend upon several factors. These are (1) adequate insecticide rate, (2) consistent wind velocity and direction to drift the aerosol evenly across the target pasture, (3) temperature inversion to prevent excessive vertical mixing of the aerosol cloud and (4) application during twilight hours when *Ae. nigromaculis* are most active.

Minimum effective insecticide rates were not clearly demonstrated by our investigations. Apparently the variations in meteorological and environmental factors previously mentioned contributed to a poor overall relationship between insecticide rate and mosquito control. The results suggested that at least fair control could be expected at a rate of 0.006 lb. AI/acre with pyrethrins or resmethrin.

In previous tests Sjogren et al. (1973) indicated 59% and 84% control of *Ae. nigromaculis* in ½ mile wide pastures with calculated doses of synergized pyrethrins of 0.003 and 0.01 lb. AI/acre, respectively. Also, Womeldorf et al. (1973) obtained high reductions of this species over a ½ mile swath with only a 0.002 lb. AI/acre rate of synergized pyrethrins. Sjogren et al. (1973) showed 58-80% reductions of *Ae. nigromaculis* with unsynergized resmethrin (SBP-1382) applied at a calculated rate of 0.0025 lb. AI/acre in one mile wide pastures.

One factor in favor of ground aerosol applications is their economic advantage over aircraft sprays. Insecticide rates are usually lower with aerosols and the per-acre cost of application is much lower. Because of this advantage, it may be feasible to consider ground aerosol treatments as an adult mosquito control strategy alternative to aircraft applications.

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Table 2.–Resmethrin and resmethrin + piperonyl butoxide ultra low volume aerosols against adult *Aedes nigromaculis* in irrigated pastures in California.

Date	9/13/74	9/17/74	8/23/76	8/12/76	8/20/76
Hour	2000	1930	1930	2020	1900
Location (MAD)	Tehama	Kings	Kings	Sutter-Yuba	Kings[1]
Acreage	160	560	80	320	80
Formulation (%)	25	25	40	13.3 + 66.7	13.3 + 66.7
Flow-rate (ml/min)	71	291	315	263	272
Nozzle air pressure (psi)	4	4	4.5	5	5
Swath width (miles)	¼	½	¼	½	¼
lb. AI/acre	0.003	0.006	0.02	0.003	0.006
Wind speed (kn)	ca 1	1.2	1.8	4	1
°C at 10m	26.1	30.3	26	27.5	27
°C at 3m	24.8	28.9	25.3	26.5	26.4
Stability ratio	ca 48	36	8.1	2.4	22
Relative humidity	33	-	77	58	71
12 hr % kill of caged mosquitoes (3 ft)	100	100	100	100	100
12 hr % kill of caged mosquitoes (0.5 ft)	98	100	74	-	-
12 hr posttreatment % control of *Ae. nigromaculis*	>98	91	69	56	45

[1]Alfalfa field instead of grass pasture.

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CONTROL OF ADULT *AEDES NIGROMACULIS* (LUDLOW) WITH AEROSOLS OF PYRETHRINS AND SYNTHETIC PYRETHROIDS[1]

III. COMPARISON OF SEVERAL TECHNIQUES OF EVALUATING EFFECTS UPON MOSQUITOES AND NON-TARGET ORGANISMS

R. K. Washino[2], C. K. Fukushima[2], G. A. Mount[3] and D. J. Womeldorf[4]

INTRODUCTION.–Cooperative investigations on the evaluation of pyrethrins and synthetic pyrethroids for use as aerosols against adult *Aedes nigromaculis* (Ludlow) in California were initiated in 1974. Participating were the Insects Affecting Man Research Laboratory, U. S. Department of Agriculture, Gainesville, Florida; the Department of Entomology, University of California at Davis; the Vector and Waste Management Section, California Department of Health; and numerous mosquito abatement agencies.

The first paper in the series (Zboray and Mount 1977) summarizes three years of laboratory wind tunnel testing during the period 1974 through 1976. The second (Womeldorf and Mount 1977) in the series discusses field evaluation of the effects upon *Ae. nigromaculis*.

This paper reports on the field evaluation of the effects upon nontarget organisms. In addition, observations pertaining to the use of the deZulueta 24-ft^2 net collection to further monitor the adult mosquito population is noted.

MATERIALS AND METHODS.–The procedure for taking pre- and post-treatment landing counts of adult mosquitoes was previously described (Womeldorf and Mount 1977). The deZulueta 24-ft^2 net collections, used in a previous evaluation (Lusk et al. 1976) were utilized in test sites by taking 5 to 6 "set" collections, usually on a transect through the irrigated pasture or alfalfa field. A specific collection was initiated by placing a net 8 ft x 4 ft x 6 ft over a given surface area. Smoke from a bee smoker was used to force arthropods resting at ground level, usually on the pasture grass and entrapped within the cage area, to fly or crawl to the upper areas of the net where they were mechanically aspirated and transferred to a 1-pint carton cage. The material was subsequently returned to the laboratory for identification and counting.

The 16 ft^2 paper sheets in wooden frames to monitor the actual knockdown of mosquitoes and nontarget organisms as used in the 1977 trials (Lusk et al. 1976) were also utilized in urban situations, and in some of the early pasture trials.

RESULTS.–DeZulueta net collections were made in 8 untreated fields: in irrigated pastures and 2 in alfalfa fields. Results of the collection are summarized in Table 1. The variety of insects other than mosquitoes from these collections is expressed in Table 2. The landing rate counts from 12 untreated fields are summarized in Table 3. In at least 4 of the above fields, assessment of the aerosol treatment was made so that both a pre- and post-treatment deZulueta net collection was made in these instances (Table 4). The landing rate counts from the same four fields are summarized in Womeldorf and Mount (1977).

Table 1.–Number of *Aedes nigromaculis* from deZulueta net collection in untreated irrigated pastures and alfalfa fields, Sutter and Kings Counties, California, 1976.

Field No.	No. Counts per Field	deZulueta collection No. *Ae. nigromaculis* range	♀♀ per count $\bar{x}$
1	6	2-32	11.8
2	6	0-4	2.2
3	6	0.6	3.3
4	5	0-6	2.8
5	6	8-31	16.3
6	6	0-10	4.5
7	6	0-31	10.3
8	5	2-14	7.0

Table 2.–Insect families from deZulueta net collections, Sutter and Kings Counties, California, 1976.

Order	No. families
Ephemeroptera	1
Odonata	2
Orthoptera	3
Hemiptera	5
Homoptera	4
Neuroptera	1
Coleoptera	5
Lepidoptera	5
Diptera	14
Hymenoptera	11
Total	51

In test sites 1 and 3 (Table 4), the number of *Aedes nigromaculis* ♀♀ was greater in post-treatment than the pretreatment deZulueta net collections. This trend is not consistent with the 28% reduction in Test Site 1 (Womeldorf and Mount 1977: Table 1, Kings 8/22/76) and 56% reduc-

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tion in Test Site 3 (Womeldorf and Mount 1977: Table 3, Sutter-Yuba, 8/12/76) in 12 hrs post-treatment estimates based on landing rate counts. Reduction in the post-treatment population in Test Sites 2 and 4 (Table 4) were consistent with the landing rate counts (Womeldorf and Mount 1977: Table 1, Kings, 8/21/76 and Table 2, Kings, 8/20/76). The *Ae. nigromaculis* population from the deZulueta net collection was of such low order that assessing the effect of the aerosol treatment appeared unreliable. The same can be concluded with the data on both the Miridae and Chrysomelidae. Sufficient Cicadellidae however, were collected and with sufficient consistency so that the reduction of this insect in Test Sites 1, 2 and 3 appears to be real. An increase was noted in Test Site 4.

DISCUSSION.—Much of the emphasis on environmental impact assessment of mosquito control activity in California has been in delineating the effects of chemical larvicides on aquatic nontarget organisms. With greater reliance on chemical adulticiding agents in many local programs in recent years, there is rising concern that little or no information exists on the impact of some of the nonthermal aerosol applications of the newer adulticides on target and terrestrial nontarget organisms in California. In assessing the effects of nonthermal aerosol applications of an adulticide on target and nontarget organisms in an urban area, the deZulueta net collection and 16 ft^2 paper sheet in a wooden frame for knockdown estimates were among the several methods utilized in an attempt to fulfill the need for additional sampling of population changes in terrestrial insects (Lusk et al. 1976). In these observations the chief advantage of utilizing these methods was in sampling a wider variety of nontarget organisms than by the use of any other methods. In particular, the knockdown sheets provided a method of sampling only those arthropods directly affected by the aerosol treatment without expending major efforts in sampling and processing arthropods unaffected in an acute fashion by the treatment. The disadvantage was, however, that repeated applications with a reference or standard chemical agent would be necessary before an acceptable baseline estimate could be made of any insect population in the area. In 1976, the same knockdown sheet assessment was repeated in an urban and rural situation, but the results yielded moderately to considerably less numbers or variety of insects than the previous year. Whether or not this could be attributed solely to the differential effects of chlorpyrifos (Lusk et al. 1976) or pyrethroids remains to be seen.

Table 3.—Number of *Aedes nigromaculis* adult ♀♀ in landing counts in untreated irrigated pastures and alfalfa fields, Kings County, California, 1976.

Field No.	No. Counts per field	Landing Count No. *Ae. nigromaculis* range	Landing Count per count $\bar{x}$
1	72	0-26	4.2
2	61	0-31	6.7
3	52	0-21	7.1
4	48	0-30	9.8
5	29	0-80	32.9
6	24	0-80	26.2
7	74	0-8	1.7
8	72	0-18	7.5
9	22	0-110	54.0
10	70	0-125	19.1
11	22	0-93	55.2
12	71	0-9	3.0

Table 4.—Mean number of *Aedes nigromaculis* ♀♀ and 3 nontarget insects per deZulueta 24 ft^2 collection in 4 fields, prior to and within 24 hours of aerosol treatment, Sutter and Kings Counties, California 1977.

	$\bar{x}$ number collected and ± S. E. (n-6/test site)							
	Test Site 1		Test Site 2		Test Site 3		Test Site 4	
Insect	pre-	post-	pre-	post-	pre-	post-	pre-	post-
Ae. nigromaculis	2.0 ± 7.1	11.8 ± 5.4	2.2 ± .7	0.5 ± .2	26.3 ± 5.2	44.8 ± 8.4	3.2 ± 1.6	1.7 ± .7
Cicadellidae	18.3 ± 6.2	12.7 ± 12.7	57.5 ± 12.7	30.3 ± 9.7	5.2 ± 1.2	1.0 ± .5	13.0 ± 8.9	23.2 ± 7.6
Miridae	0.8 ± .8	4.5 ± 2.3	18.8 ± 1.8	10.7 ± 2.1	0.0	0.0	2.2 ± 1.0	2.3 ± 1.2
Chrysomelidae	0.3 ± .21	0.0	1.0 ± .4	1.5 ± 1.0	0.2 ± .2	0.0	1.8 ± .7	4.5 ± 1.6

In comparison to the landing rate count, the deZulueta net collection did not yield consistant estimates of *Ae. nigromaculis* adult females. Whether or not the inconsistency can be attributed at least partially to differences in physiological state and/or age composition of the population sampled by the two methods is a subject area for further trials. If for nothing else, the deZulueta method appeared to offer some promise in assessing nontarget insects for further evaluation. An obvious need exists for greater sampling replicates as indicated by the high standard error values in the treatment areas (Table 4).

In summary, our observations indicated that deZulueta net collections in irrigated pastures can include a good number of mosquitoes and other insects. One advantage in assessing mosquitoes by this technique was that it included males, and presumably females in a physiological state other than only those seeking blood meals. Counts per field will probably have to be increased considerably, however, to obtain meaningful estimates. For purposes of assessing the insecticide impact in the test sites, the results were not entirely consistent with the conclusions based on landing rate counts. Pre- and post-treatment estimation of the nontarget insects did not show consistent reduction in all sites, but the data suggested impact in some of the sites. Since no other comparable method of assessment of the insect fauna is presently available, further collection trials with the de-Zulueta net seem to be in order.

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OVERCROWDING FACTORS OF MOSQUITO LARVAE – LARVICIDAL ACTIVITY OF SUBSTITUTED ALKANOIC ACIDS AND THEIR ESTERS

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To develop novel chemicals for mosquito control, our research efforts have been focused on the chemistry and biology of natural products involved in the ecology of mosquitoes. Our objectives are to study the chemical ecology of mosquitoes, to elucidate chemical compounds that regulate population density of mosquitoes, and to exploit the potential of some ecological chemicals as selective, biodegradable, and relatively safe mosquito-control agents.

One aspect of these studies relates to a group of self-regulating chemicals known as the overcrowding factors of mosquito larvae. These chemicals are secreted by older larvae of mosquitoes in response to overcrowding pressures. These chemical agents induce mortality in younger larvae, thus reducing the population pressure (Ikeshoji and Mulla 1974a, b). The overall effect is for regulating the population below the saturation point to avoid extinction of the species (Hwang and Mulla 1976a).

Our efforts have been directed toward the elucidation of structure-activity relationship of these chemical factors. Chemical compounds with possible biological activity are designed, synthesized, and evaluated in these studies. In addition to various analogues of the overcrowding factors previously investigated (Ikeshoji and Mulla 1974b; Hwang et al. 1974a, b, 1976a, b; Hwang and Mulla 1975, 1976a, b; Hwang 1976), we have further synthesized and evaluated two new series of analogues to obtain more active compounds. They are: 3-methylalkanoic acids, 2-haloalkanoic acids, and their methyl, ethyl, and isopropyl esters. Some of these new compounds show good larvicidal activity.

2-HALOALKANOIC ACIDS AND THEIR METHYL, ETHYL, AND ISOPROPYL ESTERS.–In studying the relationship of structural modifications to biological activity of these ecological chemicals, we previously reported that substitution of the methyl group in 2-methylalkanoic acids for a bromine atom resulted in obtaining more active 2-bromoalkanoic acids (Hwang 1976). Thus, 2-methyloctadecanoic acid had an LC_{50} greater than 25 ppm, whereas its bromo analogue, 2-bromooctadecanoic acid had an LC_{50} of 0.6 ppm. We also reported the synthesis and evaluation of 2-bromoalkanoic acids from C-10 to C-22 and their methyl esters (Hwang 1976, Hwang and Mulla 1976b).

To expand this aspect of research, we have synthesized some 2-haloalkanoic acids and their derivatives. They include 2-chlorooctadecanoic acid (X=Cl, R=H), 2-bromooctadecanoic acid (X=Br, R=H), 2-iodooctadecanoic acid (X=I, R=H), and their methyl (X=Cl, Br, or I; R-CH_3), ethyl (X=Cl, Br, or I; R=C_2H_5), and isopropyl [X=Cl, Br, or I; R=$CH(CH_3)_2$] esters.

$$CH_3(CH_2)_{15}CH(X)CO_2R$$

X=Cl, Br, I R=CH_3, C_2H_5, $CH(CH_3)_2$

These halogen-substituted carboxylic acids and esters were synthesized as follows. Octadecanoic acid was allowed to react with an excess of thionyl chloride to give octadecanoyl chloride which upon halogenation with N-chlorosuccinimide, N-bromosuccinimide, or molecular iodine yielded 2-chloro-, 2 bromo-, or 2-iodooctadecanoyl chloride. The acid chlorides were subjected to solvolysis with water, methanol, ethanol, or isopropyl alcohol to give 2-halooctadecanoic acids, their methyl, ethyl, and isopropyl esters.

First-instar larvae of *Culex pipiens quinquefasciatus* Say were used in assessing the biological activity of these 2-halogen-substituted alkanoic acids and esters. The bioassay procedure was previously reported (Hwang et al. 1974a, b). The biological activity of the test compounds was expressed in terms of lethal concentrations in ppm inhibiting the emergence of 50 and 90% of the population (LC_{50} and LC_{90}).

The bioassay tests showed that the chloro- and bromo-substituted carboxylic acids and esters were more active than the iodo-substituted analogues, and that the acids were more active than the esters. 2-Chlorooctadecanoic acid and 2-bromooctadecanoic acid were the most active. LC_{50} and LC_{90} being less than 1 and 1-2 ppm, respectively. Ethyl and isopropyl 2-iodooctadecanoates were the least active. The rest of the acids and esters evaluated possessed moderate activity.

3-METHYLALKANOIC ACIDS AND THEIR METHYL, ETHYL, AND ISOPROPYL ESTERS.–In the course of investigating the structure-activity relationship of the overcrowding factors of mosquito larvae, we found that 3-methyloctadecanoic acid showed a high level of larvicidal activity (Ikeshoji and Mulla 1974b, Hwang et al. 1974a). Two homologues of this acid, 3-methylheptadecanoic acid and 3-methylnonadecanoic acid, also exhibited the same level of activity, and methyl esters of these three acids showed considerable activity but were less active than their corresponding acids (Hwang 1976).

In order to fully expore the structure-activity relationship of this series of compounds, we synthesized 3-methylalkanoic acids having carbon atoms from 11 to 20 in the main chain. They are: 3-methyltridecanoic acid (n=9, R=H), 3-methyltetradecanoic acid (n=10, R=H), 3-methylpentadecanoic acid (n=11, R=H), 3-methylhexadecanoic acid (n=12, R=H), 3-methylheptadecanoic acid (n=13, R=H), 3-methyloctadecanoic acid (n=14, R=H), 3-methyl=nonadecanoic acid (n=15, R=H), and 3-methyleicosanoic acid (n=16, R=H). Methyl (R=CH_3), ethyl (R=C_2H_5), and isopropyl [R=$CH(CH_3)_2$] esters of these 3-methylalkanoic acids were also prepared.

$$CH_3(CH_2)_nCH(CH_3)CH_2CO_2R$$

n= 9, 10, 11, 12, 13, 14, 15, 16

R= CH_3, C_2H_5, $CH(CH_3)_2$

These acids and esters were synthesized from various 1-alkenes ranging from 1-dodecene to 1-nonadecene. The synthetic procedure was reported elsewhere (Hwang 1976). Briefly, oxymercuration of 1-alkenes with mercuric acetate gave oxymercurials which on demercuration with sodium borohydride yielded 2-alkanols. Tosylation of the secondary alcohols and subsequent substitution reaction of the resulting tosylates with sodium bromide produced 2-bromoalkanes. Malonic ester condensation of the tosylates or the bromides yielded substituted malonic esters which were hydrolized and decarboxylated to give the desired 3-methylalkanoic acids. Solvolysis of 3-methylalkanoyl chloride in methanol, ethanol, or isopropyl alcohol gave methyl, ethyl, or isopropyl 3-methylalkanoates.

These compounds were evaluated against first-instars of *Cx. p. quinquefasciatus* until adult emergence. The biological evaluation showed that the activity of these acids generally increased as the chain length of the compounds increased. Hence, 3-methylheptadecanoic acid, 3-methyloctadecanoic acid, 3-methylnonadecanoic acid, and 3-methyleicosanoic acid were more active than their lower homologues. These four acids exhibited an LC_{50} and an LC_{90} at 0.3-0.5 and 0.4-0.6 ppm, respectively.

The esters are generally less active than their corresponding acids. The activity of the esters having the same acid moiety gradually decreased as the alcohol moiety of the esters changed from methyl, ethyl, to isopropyl. For example, the activity decreased in the order of 3-methyloctadecanoic acid, methyl 3-methyloctadecanoate, ethyl 3-methyloctadecanoate, and isopropyl 3-methyoctadecanoate. The activity of the esters having the same alcohol moiety generally increased as the chain length of the acid moiety of the esters increased. For instance, methyl 3-methylheptadecanoate was more active than methyl 3-methylhexadecanoate, and isopropyl 3-methylnonadecanoate was more active than isopropyl 3- methyloctadecanoate. Of these esters studied, methyl 3-methyloctadecanoate, methyl, ethyl, and isopropyl 3-methylnonadecanoates, and methyl, ethyl, and isopropyl 3-methyleicosanoates exhibited the highest level of activity with an LC_{50} less than 1 ppm.

CONCLUSION.—The activity of 2-haloalkanoic acids and esters is influenced by the halogens attached to the acid moiety. The activity of 3-methylalkanoic acids and esters were directly proportional to the chain length of the acids.

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CEMETERY MOSQUITOES AND THEIR CONTROL WITH ORGANOPHOSPHORUS LARVICIDES AND THE INSECT GROWTH REGULATOR METHOPRENE

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ABSTRACT

The magnitude and extent of the mosquito problem in a large urban cemetery in Los Angeles County was studied. Three species of mosquitoes, *Culex pues, Cx. p. quinquefasciatus*, and *Culiseta incidens*, propagated throughout the year in flower vases containing stagnant water. Vases with or without flowers, but containing fresh water, did not produce mosquitoes.

The standard method of control by applying sprays by boom sprayer or a mist blower (using temephos at 0.188 lb in 50 gal water/acre) was found ineffective. Increasing the rate to 0.2 lb/acre yielded good control, but for only 2 weeks. Chlorpyrifos at 0.2 lb/acre in 50 gal water yielded excellent control for 1-2 months in the vases.

Placement of 10 g methoprene charcoal briquettes (containing 4% active) produced excellent inhibition of emergence of adults for over 5 months. It is very likely that smaller briquettes will yield similar results. Slow-release formulations of methoprene are specific and safe to use, and will provide practical control of mosquitoes in cemeteries where source reduction measures cannot be implemented.

INTRODUCTION.–Flower vases in cemetery plots, if not emptied regularly or turned after removal of wilted flowers, breed heavy populations of mosquitoes. In California, Kelley (1941) focused attention on mosquito production in cemetery vases in Alameda County, and Aarons (1948) and Schanafelt (1969) studied various chemical treatments for the control of cemetery mosquitoes. Most of the methods evaluated were found to be costly or impractical. Lewis and Christensen (1973) evaluated the placement of grids in vases containing water and achieved excellent control of one species of mosquitoes. However, due to fluctuating level of water, placement of grids to break water surface does not seem to provide practical control measures in large cemetery establishments. Moreover, during placement of flowers, grids are removed from the vases anyway.

During 1975, Dr. Jack Hazelrigg, Entomologist of the Southeast Mosquito Abatement District, Los Angeles County, made an assessment of mosquito production and their control in Rose Hills Memorial Park, the largest cemetery development in one location. As mentioned in Hazelrigg's unpublished report, the larviciding program (using aqueous sprays of temephos at 0.1 lb/acre as complete coverage treatment) practiced by the cemetery management was found ineffective. Therefore, the current studies were initiated to assess mosquito breeding in cemetery vases and to investigate the efficacy of source reduction, larvicides, and slow-release insect growth regulators (IGRs) for the control of mosquitoes. This paper presents information on the efficacy of chemical control measures applied to flower vases in a large urban cemetery in Los Angeles County, California.

EXTENT AND MAGNITUDE OF MOSQUITO PRODUCTION.–Practically all grave sites in a cemetery are provided with flower vases (capacity about 1 qt). These are made of alloy metals, sheet metal, or other impermeable materials. The vases are placed in concrete or other types of sleeves in the ground. The top of the vase is flush with the ground surface so that equipment for mowing, cleaning, and spraying can be driven over the top. Visitors to the sites empty and wash the vases, fill them with fresh water and place therein plastic or natural flowers. Sprinkler irrigation provides a constant source of water and keeps the vases filled at all times. The natural flowers wilt within a week and are removed by the management. In some cemeteries, the vases are turned upside down, thus eliminating further need for mosquito control. This practice, however, cannot be employed where durable plastic flowers are placed in the vases, or during busy visiting season such as from April to June during which Easter, Memorial Day, Mother's and Father's Day fall.

The sequence of events, as a rule, is as follows: On visitation of the sites and placement of flowers, the vases are taken out, washed, refilled with fresh water, then the flowers inserted and the vases placed back in the sleeves. Within a week or so, the wilted flowers (not the plastic) are removed. The water in the vases remain fresh for about 2 weeks, and during this period, there is no oviposition by mosquitoes. By the 3rd week, the water becomes quite stagnant and attrative to ovipositing females. Therefore, frequent visitation of grave sites actually results in suppression of mosquito populations.

A survey of 7 cemeteries in Los Angeles and Riverside Counties showed various management practices for vases, flower removal and mosquito control techniques. Some remove the wilted flowers and let the vases accumulate water to harbor mosquitoes, while others routinely turn vases after removal of wilted flowers. In some cemeteries, the design of the vase and sleeve is such that when the vase is upright it sticks up 4-6 inches above the ground, interfering with the mowers and other equipment. When turned over into their sleeves, the vases are flush with the ground. Therefore, these type of vases have to be turned after flower removal in order for the mowers and other equipment to drive over the lawns.

Management of some cemeteries feel that turning down of vases is desirable from the standpoint of safety and aesthetics. Upright vases in sleeves can catch heels and thus lead to the falling of visitors. Also, vases with debris and grass clippings not only support mosquito breeding, but also contain live earthworms and other animals which are unsightly to visitors and produce bad odors. For these reasons, the vases are turned upside down routinely.

Various species of mosquitoes breed in cemetery flower vases. The most common ones are: *Cx. p. quinquefasciatus*, *Cx. peus*, *Culiseta incidens* and possibly *Cx. tarsalis*. Most cemetery vases breed mosquitoes on a year-round basis with heavy production occurring from March to November. The extent of mosquito positive vases varies depending on the location and time of the year, but ranges anywhere from 20-100% positive from a few to more than 200/vase.

Current chemical control techniques consist of the application of larvicides (temephos or lindane) or oil. The materials are applied as area-wide sprays or as squirts into individual vases. These treatments are believed to yield control for 3-4 months, and therefore, treatment of vases is scheduled on a cycle of 90-120 days.

We initiated comprehensive studies on the extent and magnitude of mosquito problems in Rose Hills Memorial Park, Whittier, Los Angeles County, where intensive mosquito breeding was found to occur in most vases. Present control techniques were also evaluated, and we confirmed Dr. Hazelrigg's findings. In addition, source reduction measures, as well as chemical control strategies and efficacies, were evaluated.

In this cemetery, 500 out of 2700 acres are developed as needed in the future. There are about 200 thousand grave sites with 190 thousand vases. The cemetery is located in an urban habitat, and many residential and business establishments are developed on sites immediately adjacent to the cemetery.

CHEMICAL CONTROL.–1. Larval Susceptibility.--For an efficient mosquito larviciding program, it is essential to know larval susceptibility to available larvicides. This information will aid in the selection of the most effective material for operational programs. The three predominant mosquito species present were collected from four locations, including flat grounds, high steep hills, and new and old lawns. Larval bioassays were conducted in the laboratory using temephos, chlorpyrifos and fenthion.

Mosquito larvae were collected from each lawn separately and transported to Riverside in an ice chest. Stock solutions (1%) of technical grade materials were prepared in acetone, and serial dilutions of each material were prepared as needed. Ten 4th-stage larvae were placed in 4 oz. treated dixie cups containing 100 ml of tap water, and the required amount of toxicant solution was added (0.1-1.0 ml/cup), and (%) mortality was determined after 24 hours of exposure. Each concentration was run in duplicate, and 3-4 concentrations were utilized in each test. Three to five tests were conducted per material against each species, and 2 checks were used along with each test. The % mortality at different concentrations was plotted on 3 cycle log-probit paper, and LC_{50} - LC_{90} in ppm were determined from a line fitted visually through the points.

Cx. p. quinquefasciatus (Say) was found to be susceptible to temephos, chlorpyrifos, but less susceptible to fenthion (Table 1). Similar results were obtained against 4th-stage larvae of *C. peus* Spieser, while *Culiseta incidens* (Thomson) was more tolerant to temephos and fenthion, but somewhat susceptible to chlorpyrifos. Fourth stage larvae of this species are larger, therefore, the LC_{50} - LC_{90} values were higher.

2. Field Evaluation of Organophosphorous Larvicides.--The effectiveness of temephos as used for mosquito control during the past several years in this cemetery was assessed

Table 1.–Susceptibility of mosquito larvae breeding in cemetery vases to organophosphorus larvicides (Rose Hills Memorial Park, 1976).

Location	LC_{50} - LC_{90} (ppm) Fenthion	Temephos	Chlorpyrifos
	Culex pipiens quinquefasciatus		
Lakeside Gardens	.017-.032	.0015-.0025	.0009-.0018
Pinewood Gardens	.012-.030	.0014-.0023	.0010-.0017
Lilac Lawn	.015-.029	.0010-.0030	.0008-.0015
Terrace of Hope	.025-.065	.0020-.0065	.0020-.0035
Terrace of Light	.014-.027	.0023-.0040	.0010-.0015
Sky Church	.012-.025	.0012-.0022	.0008-.0015
Average	.016-.035	.0016-.0030	.0010-.002
	Culex peus		
Lakeside Gardens	.025-.045	.0024-.0035	.0012-.0022
Pinewood Gardens	.020-.035	.0020-.0030	.0012-.0023
Lilac Lawn	.025-.060	.0080-.0150	.0010-.0015
Terrace of Hope	.025-.060	.0030-.0040	.0008-.0010
Average	.026-.05	.0038-.007	.001 -.0017
	Culiseta incidens		
Lakeside Gardens	.045-.070	.014-.025	.0023-.0045
Pinewood Gardens	.035-.060	.012-.021	.0020-.0040
Lilac Lawn	.022-.038	.008-.015	.0025-.0050
Terrace of Hope	.050-.085	.010-.022	.0030-.0044
Terrace of Light	.033-.052	.009-.016	.0049-.0070
Sky Church	.025-.044	.015-.025	.0037-.0080
Average	.034-.058	.011-.021	.0031-.0055

in the manner employed by the personnel of the cemetery. Along with temephos, chlorpyrifos was also evaluated.

In the first test, temephos EC 4 at the rate of 5 fl. oz (=0.188 lb/A) in 100 gal of water/acre applied by boom sprayer (standard treatment used in this cemetery), yielded complete control of mosquito larvae in the flower vases initially, but the level of control was very low 2 weeks posttreatment. Chlorpyrifos EC 2 was less effective and yielded mediocre reduction in the population at the rate of 4 fl. oz (=0.063 lb/A) in 100 gal of water/acre. Control with each material lasted for less than 2 weeks (Table 2).

In the second test, both materials were applied at the same rate as in the previous test, but using a mist blower. Again, temephos was more effective, achieving 89 percent reduction in the population, compared to 27 percent with chlorpyrifos one week after treatment. The boom sprayer seemed to be more effective than the mist blower, but none of the treatments produced desired level of suppression.

When chlorpyrifos and temephos were applied at about equal rates, both materials produced excellent control. At the rate of 0.13 lb/A, temephos was effective for about 3 weeks, while chlorpyrifos gave excellent control for 30 days (Table 3).

In another test, temephos at a higher rate (0.2 lb/A) produced poorer control of larvae, lasting for only 2 weeks or so. Chlorpyrifos was more stable, yielding excellent control of larvae for more than 54 days (Table 4). This material reduced numbers of larvae to a very low level, and pupae were almost absent for the entire posttreatment period.

From the data obtained, it seems that the old sprayer (Hardie Spray Rig) is more effective in making the applications than the newly acquired sprayer (Myer Mighty Mist)

Table 2.–Evaluation of various mosquito larvicides and application equipment for the control of mosquito larvae in cemetery flower vases.

Material and formulation	Rate oz. EC/A	(%) of vases breeding and avg. no. of larvae/vase[1]										
		Pre-treatment			Post-treatment (days)							
					7				14			
		w	w/o	L/V	w	w/o	L/V	(%R)	w	w/o	L/V	(%R)
Test 1, July 1976 (Boom Sprayer)												
Chlorpyrifos EC 2	4	100	0	83	29	71	14	83	41	29	29	65
Temephos EC 4	6	94	6	57	3	97	0.3	100	61	39	25	27
Check	-	87	13	45	83	17	49	0	88	12	50	0
Test 2, July 1976 (Mighty Mist)												
Chlorpyrifos EC 2	4	78	22	41	35	65	30	27	65	35	51	0
Temephos EC 4	6	94	6	55	23	77	6	89	70	30	27	52
Check	-	87	13	51	97	3	80	0	100	0	72	0

[1]W= (%) vases with larvae, W/O= (%) vases without larvae, L/V= average no. larvae/vase, (%R) = % reduction.

Table 3.–Efficacy and longevity of larvicides in controlling mosquito larvae in cemetery flower vases.

Post-treat (days)	Avg. no. of larvae and pupae/vase								
	Chlorpyrifos 0.1 lb/A			Temephos 0.13 lb/A			Check		
	L	P	(%R)	L	P	(%R)	L	P	(%R)
Pre-treat	36	5	-	44	4	-	33	3	-
5	0	0	100	4	0	97	32	5	0
19	5	0	95	5	0	95	39	1	0
26	6	1	87	24	1	48	54	3	0
30	8	1	79	22	3	48	34	4	0
44	18	2	51	23	2	48	38	3	0

Table 4.–Efficacy and longevity of larvicides in controlling mosquito larvae in cemetery flower vases.

Post-treat (days)	Avg. no. of larvae and pupae/vase								
	Chlorpyrifos 0.2 lb/A			Temephos 0.2 lb/A			Check		
	L	P	(%R)	L	P	(%R)	L	P	(%R)
Pre-treat	27	3	-	33	3	-	35	4	-
5	1	0	97	3	1	89	40	3	0
12	2	0	94	8	2	73	36	3	0
26	1	0	97	20	1	42	38	4	0
40	0.3	0.1	99	19	1	44	45	6	0
54	3	0.1	91	19	1	44	37	2	0

for mosquito larviciding purposes in the cemetery. However, improvements in the old sprayer and method of application are needed for greater efficiency. The present system is extremely slow and requires 1 full day to treat 2-3 lawns.

It is apparent that with the present system of equipment, materials and dosages, it is not feasible to bring the mosquito larval population under control in all breeding sites in a large cemetery establishment. Frequent treatments with temephos at biweekly intervals and chlorpyrifos treat-

Table 5.–Evaluation of methoprene (4%) briquettes against mosquito larvae breeding in flower vases in cemetery plots (1976).

Post-treat (days)	Avg. (%) cumulative mortality of stadia and inhibition of emergence in isolated populations obtained from indicated locations[1]											
	Terrace of Light			Vets Memorial			Sky Church			Check		
	L	P	(%EI)	L	P	(%EI)	L	P	(%EI)	L	P	(%EI)
5	13	84	97	13	78	100	14	84	98	16	12	28
12	11	89	100	15	85	100	9	91	100	7	12	19
24	15	85	100	7	90	97	0	91	100	14	5	19
42	16	83	99	28	71	99	21	75	96	11	23	34
56	6	56	62	9	88	97	5	93	98	11	57	68
70	22	58	83	4	72	77	10	89	99	4	11	17
85	9	90	99	4	93	100	15	81	96	10	9	20
100	6	93	99	9	61	71	4	81	88	13	26	39
115	15	65	80	5	95	100	11	89	100	15	7	23
130	9	90	99	5	95	100	5	94	99	4	2	6
145	6	93	100	46	54	100	6	89	95	3	22	25

[1]25 vases treated in each location. For evaluation, larvae collected from 4 treated and 2 untreated vases from each location. Each sample run in 2 replicates for inhibition of emergence. Only 4th-instar larvae were employed. Slight mortality (1-2%) occurred in adults also.

ments at bimonthly intervals have to be made. This, however, is not practical or possible at the present time in a large establishment such as Rose Hills Memorial Park. To reduce the frequency of applications, a slow-release formulation of the IGR methoprene was studied.

3. Slow-Release Methoprene Briquettes.--Briquette formulations of the insect growth regulator methoprene have been shown to persist in water and yield long-lasting control of mosquitoes. To evaluate this material, one 10-g briquette (containing 4% methoprene) was placed in each vase and the inhibition of adult emergence monitored over a 5-month period. These inhibited mosquito adult emergence for more than 145 days. Most of mortality occurred in the pupal stage when treated larvae were isolated in the laboratory from 3 different locations (Table 5). It should be noted that inhibition of adult emergence was quite high in the checks at times. This is probably due to the transfer of larvae to tap water from that of the vases in laboratory. It is thus shown that methoprene briquettes provide long-lasting control of mosquitoes, and it is possible that only 1/4-1/5 quantity of one briquette or small briquettes might be needed to provide the needed longevity.

On each sampling date, treated vases were inspected and were disregarded when fresh flowers were observed in the vases. The number of vases disregarded increased gradually, and the experiment was discontinued after 158 days when the extent of treated vases (still containing briquette material) declined to 32% in Veterans Memorial and Terrace of Light, and 56% in Sky Church Memorial (Table 6).

As can be seen, the rate of attrition of briquettes or other slow-release formulations will depend on the rate of visitation. The higher the rate of visitation, the higher the rate of attrition and loss of slow-release formulations. Even with this problem, the slow-release formulations of an innocuous material such as methoprene seems to provide excellent long-lasting control of larvae in flower vases. This method of control is both economical and practical, as the vases can be treated at the time of flower removal.

Table 6.–Prevalence of methoprene briquettes in treated vases (1976).

Post-treat (days)	Proportion (%) of treated and untreated vases examined after treatment (days)					
	Terrace of Light		Vets Memorial		Sky Church	
	w	w/o	w	w/o	w	w/o
Treat	100	0	100	0	100	0
5	96	4	92	8	100	0
14	84	16	80	20	100	0
23	72	28	80	20	100	0
38	72	28	68	32	100	0
52	72	28	68	32	100	0
67	72	28	64	36	96	4
80	72	28	60	40	96	4
94	72	28	56	44	92	8
108	64	36	56	44	60	40
122	64	36	52	48	60	40
136	56	44	48	52	52	48
158	32	68	32	68	44	56

w: with briquettes
w/o: without briquettes

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SALT MARSH RESTORATION IN ALAMEDA COUNTY AND ITS IMPACT ON MOSQUITO CONTROL

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Mosquito control in Alameda County offers many challenges, perhaps none so interesting and ironic as those posed by the salt marshes. Indeed, the District owes its existence to the salt marshes. The District was formed on the basis of a petition by some 30,000 residents submitted to the Alameda County Board of Supervisors in 1930. The petition was stimulated by hordes of viciously biting mosquitoes that made outdoor living very uncomfortable at times. The mosquitoes were primarily two species, *Aedes dorsalis*, the salt marsh mosquito and *Aedes squamiger* the winter salt marsh mosquito. It is not surprising then that the Districts original view of the salt marsh was as an enemy. For the first 40 years of the District, open marshes were simply to be ditched, drained or diked. Reclamation of the marsh, as diking was called at that time, was supported by the District as conducive to mosquito control practices. Open marshes that became land fill sites, salt ponds, or dry farm lands were not only beneficial to the local economy, but also beneficial to mosquito control efforts. Of approximately 12,000 acres of salt marsh that existed in Alameda County in 1930, only about 2300 acres remain. (U. S. Fish and Wildlife Service, 1976; Hayward Shoreline Planning Agency, 1973). Much of the remaining marsh is scattered throughout the county in strips and patches on the outside of levees. Salt ponds have now replaced about 5,600 acres of open salt marsh and dry-diked areas comprise another 4,100 acres.

RETURN OF THE SALT MARSH.--The salt marsh in the San Francisco Bay Area has recently been defined by the public and various public agencies as valuable and worthy of preservation. The public has become aware of the variety of plants and animals, especially bird life, that inhabit the salt marsh. The following quote is illustrative of the publics "modern view" of salt marshes: " . . . salt marsh along the Hayward Shoreline occupy a prominent place in the overall environmental picture. . ." "They support a wealth of interrelated - - and sometimes specially adapted -- organisms that range from inconspicuous algae growing on pickleweed stems to graceful Marsh Hawks soaring overhead." "They have served as a part of a special 'evolutionary laboratory' that today provides sanctuary for several rare and endangered species." "And their luxuriant swaths of cordgrass have helped earn the salt marsh their position as the most productive type of natural vegetation in North America." "For the ecological reasons outlined above, salt marshes should be given very high environmental priority in any plan for the use of bay shore lands". (Hayward Area Shoreline Planning Agency, 1973).

"CREATIVE" ECOLOGY.–In 1973 the trend toward elimination of salt marshes was reversed in Alameda County with the advent of a pioneer project to create a salt marsh by the United States Corps of Engineers. The corps, in conjunction with a dredging operation at the mouth of Alameda Creek, created a salt marsh of 150 acres. The material from the dredge operation was piped to an old salt pond that lay parallel to the creek. Unfortunately, the District was not contacted by the corps to provide recommendations to prevent mosquito problems. The local flood district asked us to contact the corps during the project, however, and the District was able to provide hurried and partially effective recommendations. The result was a series of mistakes that could have been avoided in the planning stages:

1) More dredge material was placed on the site than anticipated. One hundred and twelve acres of the 150 acre site turned out to be above mean high water. The result was 112 acres of potential mosquito producing area with very little of the highly desired cordgrass that grows in the lower elevation of the marsh.

2) The inboard levee system was not upgraded to withstand tidal inundation. When the outboard levee adjacent to the bay was breached to allow tidal inundations, the inboard levee leaked and flooded an adjacent reclaimed marsh thereby creating additional mosquito problems.

3) The dredge material was allowed to subside and crack in the upper elevations of the marsh. When the levee was breached tidal water was trapped in the cracks, and with the expected invasion of pickleweed, the cracked ground may be expected to provide an ideal larval habitat for salt marsh mosquitoes.

4) A topographical survey was not made to detect low spots that would trap and hold hight tidal waters. Therefore no ditches were planned to promote circulation and thereby prevent mosquito production.

5) The final mistake was caused by the corps breaching the outboard levee before needed corrections could be made. Unfortunately, our District was not contacted before the levee was breached.

"CORRECTIVE" Ecology.–The marsh restoration project, from the point of view of our District, had tremendous potential for the production of salt marsh mosquitoes. The District contacted the Coastal Region of the California Mosquito Control Association and the State Vector and Waste Management Section to ask for their assistance in the matter. With their technical assistance, the District recommended remedial action to the corps. Fortunately the corps had equipment available on the site for their cordgrass planting program. The corps disked the cracked ground and, based upon an engineering survey by Reuben Junkert of the State Vector and Waste Management Section, the corps also excavated ditches from the main slough to low areas in the upper reaches of the marsh. The ditches serve to increase water circulation and allow the entrance of predator fish.

Subsequent to the marsh restoration project the Coastal Region of the California Mosquito Control Association has prepared a document titled "Standard Recommendations to Prevent Mosquito Problems with Salt Marsh Restoration Projects in the San Francisco Bay Area" (Coastal Region, California Mosquito Control Assocition, 1976). The recommendations were considered necessary since many agencies have plans for the restoration of salt marshes in the Bay Area.

THE FUTURE.–Salt marshes will be returning to Alameda County over the next decade. Specific and general plans have been made for the creation of some 2,000 acres of salt marsh within the county, increasing the total acreage from the current 2,300 acres of open marsh to as much as 4,300 acres. The marshes, for the most part would be created by reverting dry-diked land back to marsh land. The marshes are planned by the San Francisco Bay National Wildlife Refuge, and the Hayward Area Shoreline Planning Agency. They are fostered by the policies of regulatory agencies including the Corps of Engineers, the California Department of Fish and Game, the Bay Conservation and Development Commission and possibly the San Francisco Bay Region Water Quality Control Board. The requirement by regulatory agencies of "mitigation measures" before permitting development appears to be a catalyst to the creation of salt marshes as mitigation in shoreline development.

It is essential that our agency provide input early in the planning stages of marsh restoration projects. The staff of the planning agencies should recognize that marsh restoration is not simply breaching levees, but requires sophisticated planning, site preparation and long-term maintenance to avoid mosquito problems. The alternative to proper planning is expensive remedial action for mosquito control purposes after the marsh has been created. The methods available to remedy the problem at that time may well be limited and inimical to the goals of the planners.

The District has involved itself whenever possible in the planning of marshes. We believe the planning agencies have found the expertise of the staff valuable beyond that of just mosquito prevention. The District makes recommendations based upon open marsh "recirculation systems" as described in the Coastal Region's Recommendations. Field observations by vector biologists suggest the recirculation method of mosquito control is not only compatible with the marsh wildlife, but may even enhance the productivity of the marsh. The District needs solid scientific evidence, however. For this reason, the District is in support of a research project proposed by the Coastal Region to study the impact of recirculation ditches in the salt marshes. We believe the research would provide data to support the compatibility of recirculation ditches with desireable ecological objectives and would provide our agency with a strong position in the planning process.

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THE EVALUATION OF GYPSUM SOIL SUPPLEMENTS AND SULFUROUS ACID WATER TREATMENT FOR IMPROVING WATER PENETRATION ON ALKALI PASTURES

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ABSTRACT

A total of 300 tons of gypsum was applied at rates of 5 to 15 tons/acre to pastures having a serious history of mosquito breeding. The irrigation water of another such field was treated with sulfurous acid on every irrigation for two consecutive years. Over 1900 quantitative analyses were made to monitor possible chemical changes of the soil. The fields were observed and sampled for up to six years after treatment. No apparent improvements occurred as a result of these treatments and serious mosquito production was not reduced.

INTRODUCTION.–Irrigated pastures have long been recognized as a tool for reclaiming alkali soils. Kelley (1951) states, "From the foregoing information it follows that an irrigated pasture can also be utilized as a means of reclaiming alkali soil by including in the seed mixture species of plants that are fairly tolerant of alkali. This can be done either in conjunction with chemical treatment or without chemical treatment if the alkali conditions are not too severe. By irrigating the pasture freely, the required chemical reactions will take place gradually. After a few years in pasture, during which time some economic return will be obtained from the land, other kinds of crops can be grown. In this way the cost of reclamation need be only minimal". Because of a history of difficulties in controlling the irrigated pasture mosquito, *Aedes nigromaculis* (Ludlow) in alkali soil areas of the San Joaquin Valley, a study was conducted on the possible effects of alkaline pasture water on the stability of mosquito larvicides (Schaefer and Dupras 1969); they stated, "We conclude that water quality of San Joaquin Valley alkaline pastures does not have any practical effect on the performance of organophosphorus larvicides. However, it is apparent that there is a significant relationship between alkali pastures and control difficulties. The alkali pastures in question are located on soils which have poor drainage; these lands are difficult to farm and, therefore, are usually maintained as permanent pastures rather than being rotated to other higher return crops, as occurs in other areas. The continued presence of pastures results in a given area being treated very frequently, season after season, for the control of *Ae. nigromaculis*. We believe that this (selection pressure) explains why insecticide resistance first appears in these alkali areas and that insecticide resistance is the significant cause of control difficulties". As organophosphorus resistance in *Ae. nigromaculis* worsened, it was imperative to explore noninsecticidal means of reducing mosquito numbers (Schaefer 1970).

One positive approach seemed to be the possible use of soil amendments to reduce the length of time water stands on irrigated fields, hopefully to a lesser time than that required for mosquito development. Secondly, the use of soil amendments might allow a shorter time interval for reclamation and thereby allow more rapid rotation to a higher return crop.

Kelley (1951) reviewed several methods for improving the alkali soils having a high exchangeable Na+ content (as occurs in many areas of the San Joaquin Valley): (1) Gypsum ($CaS0_4$) can be applied and then with considerable water Ca^{++} will exchange with Na^+ and the latter can be removed by leaching. The physical condition of the soil for plant growth and tillage operations would be considerably improved. (2) Sulfur can be applied to alkali soils having $CaC0_3$ present. The gradual oxidation of S to H_2S0_4, by sulfur-oxiding bacteria, in the presence of $CaC0_3$ will allow for an exchange of Ca^{++} onto the clay as expressed by the following equation: $H_2S0_4 + 2\ CaC0_3 + 4\ Na\ clay \Rightarrow 2\ Ca\ clay + Na_2S0_4 + NaHC0_3$. (3) Sulfuric acid can be applied directly to achieve the results as in (2), if $CaC0_3$ is present.

These methods were employed by Overstreet et al. (1951) in an attempt to reclaim an alkali soil of the Fresno soil series (Fresno County). The test area had been planted in cotton and the yield had been poor. Small plots (15 x 300 feet each) were treated with gypsum at 10, H_2S0_4 at 5.7, and sulfur at 1.86 tons/acre. Each plot was replicated six times and the area was seeded with a mixture of pasture plants. For two years after treatment the yields of grasses from the sulfur-treated plots were not significantly higher than for the untreated plots; the yields from the H_2S0_4 treated plots were higher than those treated with gypsum or sulfur. Apparently the two year period was not long enough to allow the oxidation of the sulfur. In 1955, Overstreet et al. reported, for the plots described above, that after five years following treatment the plots that had received no amendments were gradually reclaimed through leaching alone and that by this time little difference, in yield was apparent between any of the plots. In this second study (Overstreet et al. 1955) an attempt was made to reclaim soil of the Hacienda series in Kings County. Small plots (30 x 145 feet) were treated with gypsum at 4.22 and 12.47 tons/acre and with H_2S0_4 at 3.59 and at 6.95 tons/acre. Each treatment was made in triplicate and the plots were planted with alfalfa. The area was swamp irrigated and the plots were observed for over three years. The authors concluded that the H_2S0_4 treatments were significantly more effective than the gypsum treatments, and that reclamation of the untreated plots was considerably slower than for treated plots. Also, there was no difference in yields between the high versus low gypsum treatments or between the high and low acid treatements, which suggested that those used might have been equally effective.

Since the potential of sulfur treatments appeared to be very slow and since field applications of H_2SO_4 had been discontinued (because of cost and hazards) it appeared that gypsum applications offered the most realistic potential for improving irrigated pastures having a distinct history of mosquito breeding problems. Consultations with personnel of the Tulare Mosquito Abatement District and the Delta Vector Control District and with cooperative land owners led to the selection of test fields to receive gypsum treatments. The objective of this study was to treat plots with various rates of gypsum and then to make observations on water standing time and mosquito production in successive irrigation seasons; further evidence of changes were followed by chemical analysis of the soil.

Also, because a commercial process of generating sulfurous acid was available, direct acidification of irrigation water was evaluated as means of improving soil water penetration and hopefully reducing water standing time.

MATERIALS AND METHODS.—After careful consideration of numerous possible test fields, three pastures which had an established history of excessive mosquito production were selected for treatment with gypsum [Toaste, Sanchez and Grant] and one similar pasture was selected to be treated with sulfurous acid [Avila].

Descriptions of these fields and the methods used to treat and evaluate the effects are as follows:

1. Toaste pasture - This 16 acre field is located approximately 9 miles south of Tulare, California [T21S-R-24ES20-SW1/4]. The soil series is Traver sandy loam and serious mosquito production has been a perennial problem on this pasture.

   During May 1969 the field was ripped to a depth of about 24 inches and then disced. The field was divided into 24 plots (0.67 acres each) and then gypsum was applied at rates of 0 (2 plots), 5 (3 plots), 9 (7 plots), and 15 (2 plots) tons/acre. The field was redisced, irrigated and seeded with Coastal Bermuda (NK-37) at 15 lbs/acre by aircraft. The field was irrigated using well water.

   Pretreatment soil cores (1.75 x 6 inches) were taken from each plot just prior to the application of gypsum and posttreatment samples were collected at the ends of the 1969, 1970, 1971 and 1974 irrigation seasons. At each of these sampling intervals 3 soil cores were taken at random on each plot from the upper (0-6 inches) and lower (6-12 inches) soil depths.

2. Sanchez pasture - This 24 acre pasture is located approximately 8 miles south of Tulare, California [T21S-R24E-S8-NE1/4], the soil series is Pond Loam with a moderate alkali content, and it has been a severe mosquito producing site.

   During May 1969 it was ripped to a depth of about 24 inches and disced. It contained 20 irrigation checks (40 x 1320 feet or 1.21 acres each) and each was utilized as a plot. Gypsum treatments were assigned at random and 6 plots were treated with 5 tons/acre, 4 with 10 tons/acre, 2 with 15 tons/acre and 8 were not treated. The field was redisced, irrigated and seeded with Coastal Bermuda (NK-37) at 15 lbs/acre by aircraft. Irrigations utilized ditch water.

   Pretreatement soil samples were taken from each check just before gypsum applications and posttreatment samples were collected at the ends of the 1969, 1970, 1971 and 1974 irrigation seasons. At each of these intervals 3 soil cores were taken at random at 3 different locations on each plot from both the upper (0-6 inches) and lower (6-12 inches) soil depths.

3. Grant pasture - This 18 acre field is 3 miles northeast of Goshen, California [T18S-R23E-S1-NW1/4] the soil series is Fresno fine sandy loam with strong alkali content. This field had not previously been farmed and was part of a larger field undergoing reclamation.

   During August 1971 the field was ripped to about a 24 inch depth and leveled. Irrigation borders were made to give 4 checks (60 x 2600 feet each or 3.58 acres each). Two of the checks were treated with gypsum, 1 with 5 tons/acre and 1 with 10 tons/acre; the remaining 2 large plots were utilized as controls. The field was reseeded with a mixture of pasture grasses. Well water was used for irrigation.

   Pretreatment soil samples were taken before gypsum application from each check and posttreatment samples were taken at the ends of the 1971, 1972, 1973 and 1974 irrigation seasons.

   Triplicate soil cores were taken at random and pooled at 5 different sampling locations on each check from both the upper (0-6 inches) and lower (6-12 inches) soil depths.

4. Avila pasture - This 40 acre pasture is located 4 miles west of Goshen, California [T18S-R23E-S22-SE1/4]. The soil series is Fresno fine sandy loam with a moderate alkali content. This field is representative of one of the worst mosquito producers encountered in this area.

   In November 1970 the field was ripped to a depth of about 24 inches, it was disced and reseeded with a mixture of pasture grasses. A commercial sulfurous acid generator was used to treat the irrigation water each time the field was irrigated (every 10 to 14 days) from May through October 1971 and again throughout the same period of 1972. The sulfur burn rate varied from an initial high of 24 lbs/hr to the rate generally used of 15 lbs/hr. Ionic content of the well water before and after acidification was determined.

   Soil samples were taken from 3 different locations on each of 5 irrigation checks, providing representative samples from all parts of the field. At each location 3 soil cores were collected at random and pooled from both the upper (0-6 inches) and lower (6-12 inches) depths. Soil samples were taken before the treatments started and at the ends of the 1971 and 1972 irrigation seasons (end of acid treatments) and again after the 1974 irrigation season.

Gypsum - Treatments were contracted with commercial applicators.

Posttreatment Observation - For each field described above, observations on the extent of mosquito production, post-irrigation water standing times, and production of forage were made following the treatment and until the end of the 1976 irrigation season. Aerial photographs were periodically taken of each field to monitor possible changes.

Analysis of Soil Samples - The soil samples were taken into the laboratory, mixed and placed on paper to air-dry at room temperature until moisture was constant (about 5%). The soil was then ground in a Wiley mill and passed through a 60-mesh Tyler screen. Each sample was then analyzed for pH and electrical conductivity and both water saturation extracts (to show exchangeable ionic content) were prepared and each was analyzed for Cl^-, Na^+, Mg^{++} and Ca^{++} as follows:

pH - The pH was determined on a 1:2.5 (V/V) soil-water suspension on a Corning Model 12 pH meter.

Electrical Conductivity - Twenty grams of soil were added to 40 mls of distilled water; the samples were shaken vigorously for a few minutes and allowed to stand for 1 hour. After settling, the liquid was poured into the conductivity cell and measured on a Lab-Line CF-Soil Lectro MHO-Meter.

Preparation of Saturation Extracts - A saturated paste was made by adding distilled water to 100 gm of soil until the soil would not take any more water without puddling; after standing 24 hours, the soil was filtered through a Buchner funnel using Watman No. 42 filter paper. The filtrate was collected and then stored in capped tubes.

Cl^- in Saturation Extracts - Chloride ion concentration was determined on the undiluted saturation extract using an Orion chloride probe on a Corning Model 12 pH meter.

Na^+, Ca^{++}, Mg^{++} in Saturation Extracts - After adequate dilution, the concentrations of these ions were determined using a Perkin-Elmer Model 290 atomic absorption spectrophotometer.

Preparation of Ammonium Chloride Extracts - Five grams of soil were placed in a filter tube and leached with 1N ammonium chloride to give a final volume of 500 mls.

Na^+, Ca^{++}, Mg^{++} in Ammonium Chloride Extracts - The ammonium chloride extracts were diluted and the concentrations of these ions were determined by atomic absorption spectroscopy.

Water Analysis - Samples of the irrigation water from each field were analyzed using the same methods as described above: $CO_3^=$ and HCO_3^- concentrations were determined on 100 ml water samples by titration with .05 N HCL.

RESULTS.—Approximately 1900 quantitative chemical measurements were made during the period of this study. These data were compared by t-tests (5% level) of the means and of the paired differences between untreated and treated means. Because of the voluminous amount of raw data, it is not practical for publication but it will be held on file at Fresno for interested persons. General results for each field are as follows:

Toaste pasture - The well water is alkaline and is high in Na^+ (pH 8.1, Na^+ 1.4, Ca^{++} 0.9 and Mg^{++} 0.4 meq/L). Following application of gypsum there were significant increases in Ca^{++} in the water saturation extracts at the 9 and 15 tons/acre rates but these increases were no longer apparent after 3 years. There was no evidence of increases in exchangeable Ca^{++} (ammonium chloride extract). At the end of 5 years there was no chemical evidence that the ionic content was improved from the pretreatment levels. The field was maintained as a pasture throughout the study period.

There was no apparent reduction in water standing time on any of the plots, there was no improvement in the amounts or quality of vegetation, nor was there any reduction in the mosquito breeding problem during or at the end of the study. Water continued to stand long enough after each irrigation to allow both *Ae. nigromaculis* and *Culex tarsalis* Coquillett to complete their developmental cycles.

Sanchez pasture - The ditch water used for irrigations was highly alkaline and high in Na^+ (pH 9.0, Na^+ 1.7, Ca^{++} 0.4 and Mg^{++} 0.2 meq/L). There were significant increases in Ca^{++} in the water saturation extracts from plots treated with 10 and those treated with 15 tons gypsum/acre but not for those treated at the 5 ton/acre rate; these increases were apparent only in the 0-6 inch depth for up to 2 years after treatment, but were not apparent at 3 and 5 years after treatment. The exchangeable Ca^{++} showed the same pattern. There was no decline in Na^{++} in the water saturation or NH_4Cl extracts and thus no evidence of sodium leaching; this, however, may have been masked by the input of Na^+ from the irrigation water. There were no other significant changes in the chemical measurements which were monitored.

During 1974 the Sanchez pasture was redisced and planted in field corn. The numbers and heights of corn plants showed extreme variation across the area of treated plots, but there was no correlation that could be found between plant height or density and rate of gypsum treatment.

Grant pasture - The well water is alkaline (pH 8.2, Na^+ 1.3, Ca^{++} 0.3 and Mg^{++} 0.3 meq/L), similar to that for the other fields. Following gypsum treatments there were significant increases in Ca^{++} in the water saturation extracts of the 0-6 inch soil samples but not in the 6-12 inch samples; however, at 2 and 4 years after treatment there were no significant differences in Ca^{++} of the water saturation extracts. No increases of exchangeable Ca^{++} were observed and there were no significant reductions of Na^+ even after 4 years. There were no improvements of forage production between treated and untreated plots, nor any differences in mosquito production.

Summary of Gypsum Treatments - It must be emphasized again that the pastures utilized for the gypsum experiments are representative of very poor soil conditions. The quality of the irrigation water can adversely affect reclamation. While the total salt concentration of these irrigation waters is relatively low, they do not supply enough Ca^{++} or Mg^{++}. Reclamation is especially difficult on these fields because of low subsoil permeability. The information is still important because such fields exist over large areas of the San Joaquin Valley, their assessed valuations are low and they produce pasture mosquitoes which are a serious problem for mosquito abatement districts. It is now apparent that adding Ca^{++} to these soils by gypsum applications of up to 15 tons/acre does not solve the problem. Such treatments show no sign of Na^+ leaching from the upper soil layers, which may partly be due to the continual addition of Na^+ from the irrigation water.

Avila pasture - Since the irrigation water was acidified at the well, the entire 40 acres were treated. Statistical comparisons had to be made to the pretreatment samples, since there were no concurrent untreated samples. Analysis of the well water before and after sulfurous acid treatment is shown in Table 1. Acidification eliminates carbonates and

Table 1.—Analysis of Avila well water before and after treatment with sulfurous acid.

	Untreated	Acid-treated
pH	9.50	4.10
EC[1]	0.14	0.20
Na^{+}[2]	1.18	1.21
Ca^{++}	0.04	0.01
Mg^{++}	0.002	0.002
Cl^{-}	0.59	— —
$CO_3^{=}$	0.75	N.D.[3]
HCO_3^{-}	1.35	N.D.

[1]EC=Electrical conductivity in millimhos per centimeter.

[2]Ionic contents in milliequivalents per liter.

[3]N.C.= not detected.

bicarbonates changing the water from a calcium precipitating to a calcium dissolving fluid. Proof of the latter is obtained by analysis of the irrigation water at 50 foot intervals during irrigation (Table 2). It is apparent that the acidic irrigation water is titrated as it moves down the field, that Ca^{++} is dissolved and free Na^{+} greatly increases.

Consecutive treatment of the irrigation water with sulfurous acid produced significant chemical effects. The pH of the soil significantly lowered from about 10 to about 8.5 by 1974 (both 0-6 inch and 6-12 inch depths). There was no significant increase in exchangeable Ca^{++} at the end of the 1st and 2nd year of irrigation with acidification but there was little, if any, difference after 2 additional years of irrigation without acidification. There was a big increase of Na^{+} in the water saturation extracts following treatments and by 1974 there was a reduction of exchangeable Na^{+} in both soil levels.

Unfortunately, there was no reduction in the water standing time, nor reduction of mosquito breeding. Also, production of pasture grasses was very spotty and after 2 years in pasture the field was redisced and planted with Sudan grass; the yield was so low that only small patches, ca. 15% of the field, survived. An attempt the next year to grow alfalfa also resulted in a similar failure of plant response. Therefore, acidification, which cost about $10/acre/year in 1972, did not reduce the problem under these very difficult soil conditions and the field continues to be a low yield pasture which produces mosquitoes regularly during the irrigation season.

DISCUSSION.—In each case no practical improvements resulted from treatments of gypsum at 5, 10, or 15 tons/acre or from 2 successive years of acidification of the water used for each irrigation. The question arises as to why these results are so negative and yet Overstreet et al. (1951, 1955) were able to show improvements of alkali soils in the San Joaquin Valley. The answer apparently is that Overstreet et al. worked with much better soil conditions initially; they worked with fields that had been farmed – one field was described as having given a poor yield of cotton. The fields described in this study were never cultivated because of their poor quality and their use as pastures did not provide reclamation so that higher crops could be grown.

Unfortunately, in some cases of such difficult conditions the annual cost for mosquito control is greater than the return obtained by the grower. This is, of course, ridiculous, but what else can be done? One possibility would be to evaluate the application of even larger amounts of soil amendments, including gypsum, acid and manure, or other organic byproducts; however, the economics of making such large investments in such poor conditions are not inviting and consideration must be given to retiring such land from production and making reclamation investments in better soils. But how can a mosquito abatement district effect retirement of such properties? It could either rent or buy and retire production on such lands but such action

Table 2.—Qualitative and quantitative changes in acid-treated irrigation water[1] during irrigation (ionic contents in milliequivalents per liter, EC in millimhos per cm).

Distance from irrigation ditch (feet)	pH	EC	HCO_3^{-}	Na^{+}	Ca^{++}	Mg^{++}	Cl^{-}	$CO_3^{=}$
0	4.20	0.43	0	1.043	0.100	0.010	0.037	N.D.[2]
50	4.30	0.38	0	0.999	0.125	0.016	0.034	N.D.
100	4.90	0.35	0	0.957	0.195	0.039	0.082	N.D.
150	4.70	0.32	0	0.783	0.215	0.046	0.048	N.D.
200	4.90	0.33	0	1.043	0.229	0.046	0.062	N.D.
250	5.00	0.32	0.25	1.087	0.394	0.085	0.056	N.D.
300	4.90	0.35	0.25	1.130	0.434	0.102	0.124	N.D.
350	5.10	0.35	0.40	1.261	0.419	0.131	0.180	N.D.
400	5.40	0.35	0.40	1.391	0.409	0.125	0.180	N.D.
450	5.60	0.39	0.55	1.521	0.400	0.151	0.395	N.D.
478	6.00	0.45	0.60	1.739	0.519	0.174	0.733	N.D.

[1]Samples collected 3.5 hours after irrigation was started on this check and had progressed 478 feet from the ditch.

[2]N.D. = not detected.

would cause it to become owner or lessor of all sub-standard properties, which few trustees would accept. Thus, it seems that the only reasonable approach is to take a positive attitude and attempt to cooperate in source reduction programs, provide educational assistance through farm advisors and provide mosquito control (at cost or without cost depending on policy) when it is apparent that serious attempts to resolve the problem are being made. However, in situations where the owners have lost incentive after fighting very difficult water and land quality obstacles and thereafter apply minimal management effort which results in mosquito production causing either nuisance or health hazards, a mosquito abatement district's only alternative becomes use of the legal process. In dealing with the most difficult of lands on which management is being attempted, source reduction is not necessarily a solution, in contrast to those who preach to its simple answer for mosquito control!

ACKNOWLEDGMENTS.–These studies were supported, in part, by funds from the Tulare Mosquito Abatement District and the Delta Vector Control District. Valley Nitrogen Producers donated and delivered 150 tons of gypsum for treatment of the Sanchez pasture. Fry Aviation Industries, Tulare, California donated aerial application of seed. Chemsoil Corporation of Bakersfield, California donated the use of one sulfurous acid generator for two irrigation seasons. The fine cooperation of Mr. Keith Evans and Mr. John Toaste for use of the Toaste pasture, Mr. Julian Sanchez for use of the Sanchez pasture, Mr. Allen Grant for use of the Grant pasture and for providing 50 tons of gypsum, and of Mr. Joseph Avila for use of the Avila pasture and who provided the sulfur for operation of the sulfurous acid generator, are gratefully acknowledged. Numerous hours of work in support of these studies were provided by personnel and heavy equipment from the Delta Vector Control District and the Tulare Mosquito Abatement District. The technical advice of Dr. D. W. Henderson, Department of Water Sciences and Engineering, University of California at Davis, during the early phase of these studies is also acknowledged.

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OVERWINTERING BIOLOGY OF *CULEX (NEOCULEX) BOHARTI* BROOKMAN AND REEVES IN NORTHERN CALIFORNIA: A PROGRESS REPORT

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Due to the increasing evidence of the euryphilic nature of some diseases (Burton et al. 1966; Adams et al. 1970; Hoff and Trainer 1973), their biocenosis is realized as a more complex phenomenon. With this increase in complexity, there is also an increase in the number of species involved, and the subsequent sum total of host relationships usually requires ecological information on species formerly unexamined. The possible involvement with disease transmission of implicated species cannot be assessed until their biology is understood.

The isolation of western equine encephalomyelitis virus from several vertebrate ectotherms has focused interest on their possible role in arbovirus ecology (Gebhardt et al. 1964; Spalatin et al. 1964; Burton et al. 1966; McLintock et al. 1967; Bowen 1977). The involvement of ectothermal vertebrates as hosts of the virus has indirectly implicated certain mosquito species considered to be both amphibian and reptilian feeders as possible maintenance cycle vectors (Murphy et al. 1967; Edman 1974; Templis 1975). However, species of the subgenus *Neoculex*, in regard to North American fauna, have not been examined in terms of possible involvement with virus transmission and the role they may play in the maintenance cycle of western encephalitis.

In the fall of 1975 an investigation was initiated to determine specific aspects of the biology of the California species of the subgenus *Neoculex*. The winter absence of adult *Culex boharti* Brookman and Reeves, even from overwintering resting sites where sympatrically occurring *Culex apicalis* Adams is readily collected, apparently suggests a possible variation in the normal overwintering biology of temperate western North American *Culex* (Carpenter and La Casse 1955).

Since no specific adult trapping method has been developed for members of the subgenus *Neoculex*, quantification of adult populations must be inferred from data gathered from immature collections. The objective of this study was to determine the overwintering biology of *Culex boharti* by monitoring a larval population throughout the year.

METHODS.—The study area was located in a rural district five miles east of Roseville, California, in the lower foothill woodland plant community. In the fall of 1975, six larval habitats were selected within a quarter mile radius. Sampling stations consisted of (1) one dredger pond, (2) two vernal ponds, and (3) two temporary streams. Two stations were selected on the dredger pond (approximately 200′ by 222′), where the dominate vegetation consisted of *Azolla filiculoides*, *Polygonum* sp., *Lemna* sp. and *Typha latifolia*. The presence of *Ondatra zibethica* and *Rana catesbeiana* suggested a permanent pond situation even though the pond dried up in early August during 1976. The complete drying of the pond might have been due to the unseasonally low rainfall in that year. Both vernal ponds, one approximately 12′ by 40′ and the other approximately 12′ by 90′, were formed in conjunction with dredger tailings. Major flora representatives of the vernal ponds were *Typha latifolia* and *Carex* sp. The final two stations were located in temporary streams, one an overflow area approximately 20′ by 60′ where the dominate plants consisted of *Rumex* sp., *Xanthium* sp., *Carex* sp. and *Hordeum* sp. and the other a stream impoundment approximately 14′ by 72′ created by a restricted outflow, and the dominate flora consisted of *Lemna* sp.

Bi-weekly samples were taken as soon as possible after the stations were flooded in the fall and continued until the stations dried up in late spring or early summer. Three 20 dip samples were taken for each station on all sampling dates, or until drying precluded sampling. All larvae and pupae collected were counted and classified as to larval instars and pupae.

Water quality measurements consisted of conductivity and pH readings taken from each 20-dip sample on each collection date. Water temperature was measured with a maximum-minimum thermometer over a 14-day period at each station. Climatological data on atmospheric temperature and precipitation was obtained from the U. S. Weather Bureau Station at Folsom Dam, Sacramento County.

RESULTS AND DISCUSSION.—*Culex boharti* larvae were present throughout the fall, winter and spring of 1975-76 (Figure 1). During this period larvae were not present in some habitats, but present in others (Figure 2). The increasing ratio of later to earlier instars of *Cx. boharti* throughout fall and early winter collections (Figure 3), indicated a decrease in oviposition during that time period. However, the continued presence of small numbers of 1st instar larvae in the collections until mid-December indicated that oviposition continued throughout fall and early

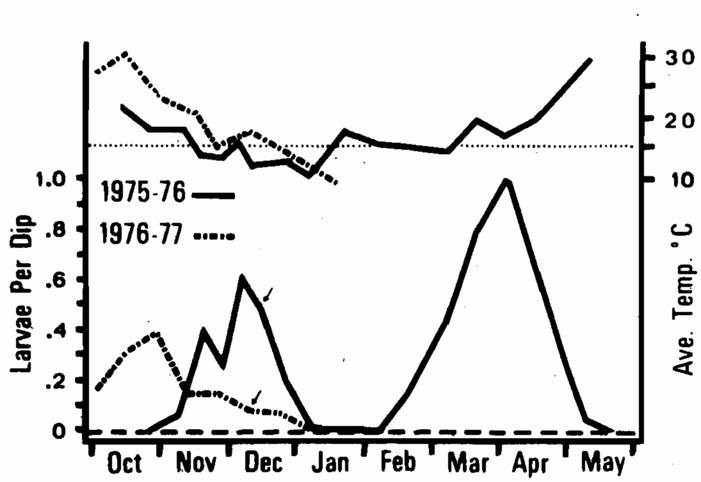

Figure 1.—Average number of *Culex boharti* larvae per dip 1975-76. Arrows indicate last collection of 1st instar larvae in December.

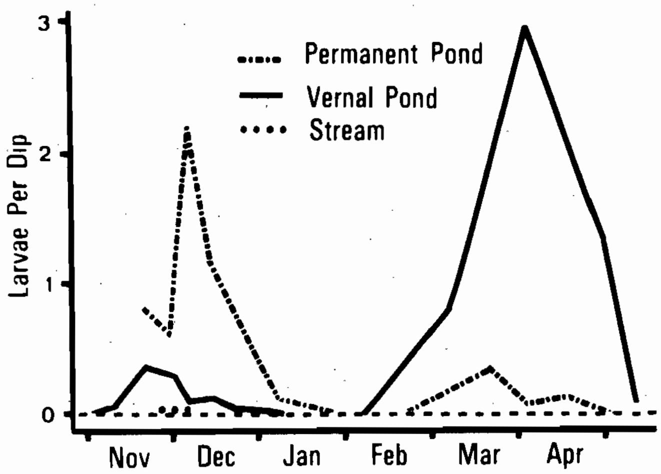

Figure 2.–Average number of *Culex boharti* per dip in selected larval habitats 1975-76.

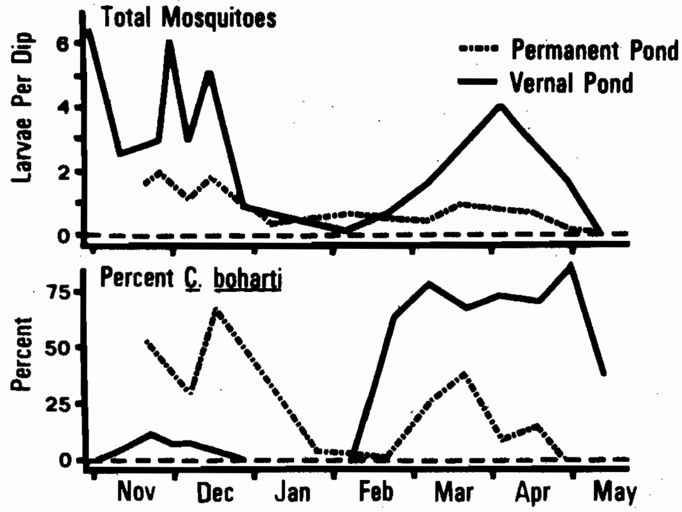

Figure 4.–*Culex boharti* as percent of total mosquito collection 1975-76.

winter. The increasing short day conditions between September and mid-December does not apparently initiate diapause in *Cx. boharti* as it does in *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken in the Central Valley of California (Harwood and Halfhill 1964; Depner and Harwood 1966; Washino et al. 1971). The presence of larval *Cx. boharti* during the winter months perhaps indicates that overwintering is accomplished by (1) diapausing larvae (2) slowly developing larvae from late fall generation or by (3) quiescing adults that have emerged in late fall and winter. An increase in oviposition was shown by the late winter presence of a substantial number of early instar larvae. Larval collections in mid-March to April indicated a balance state between oviposition and emergence (Figure 3). The observed decline in the late spring population may be a result of the drying of principle ovipositional sites.

Larval populations showed habitat seasonal reversals; those larval habitats which supported the largest popula-

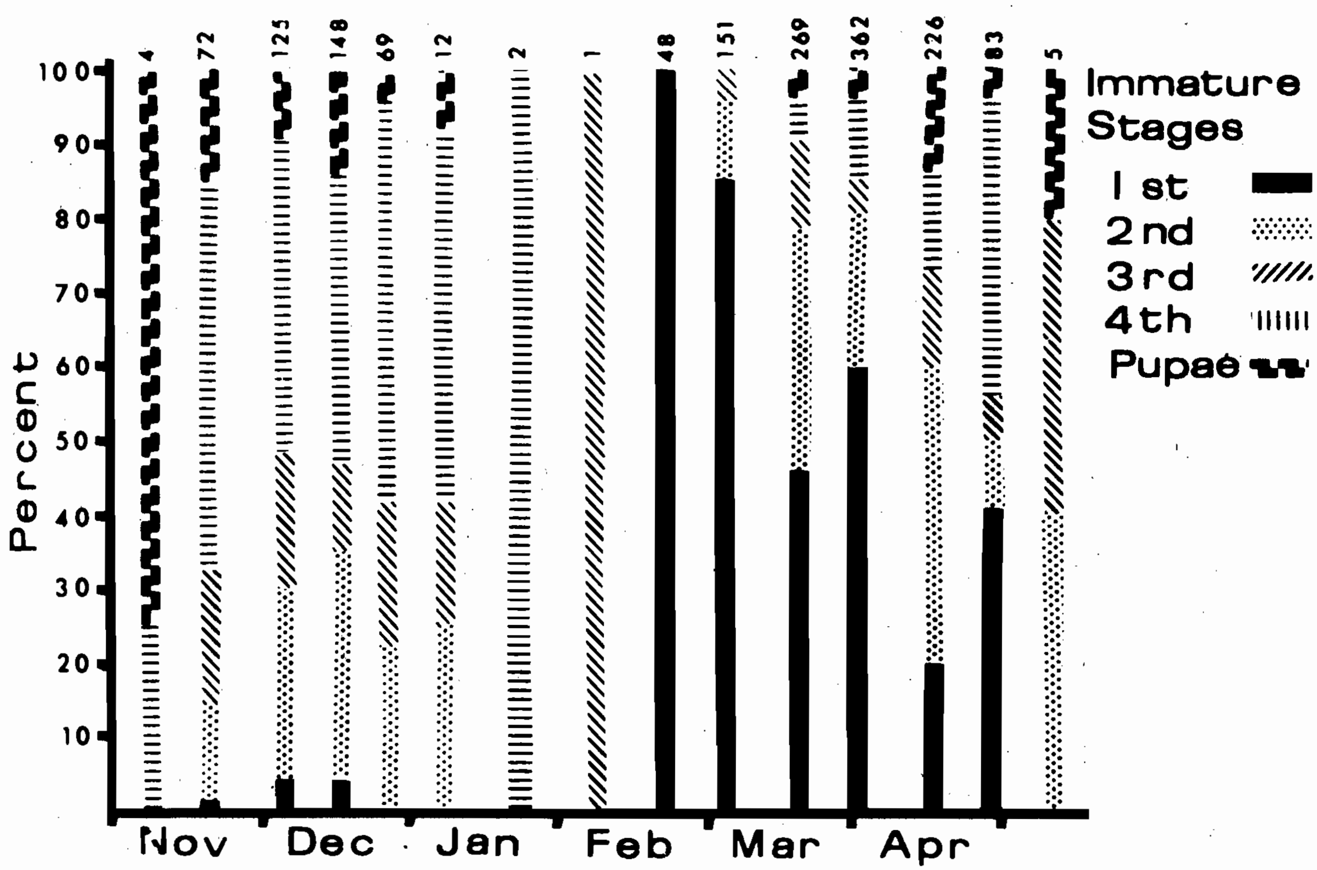

Figure 3.–Age profile of immature *Culex boharti* 1975-76.

tions in the fall did not support the largest populations in the spring (Figure 2). To determine if the seasonal population changes in *Cx. boharti* in the pond situations was due to changes in culicid production, the total numbers of mosquitoes collected were tabulated in the lotic environments (Figure 4). The total mosquito production between fall and spring in the pond habitats was nearly equal, supporting the premise that the *Cx. boharti* population variation was not due to habitat nutritional changes between the seasons. This assumption would be supported if resource partitioning was not a major factor between mosquito species occupying the same larval habitat. The change in percent composition was probably due to changes in ovipositional stimulus, mortality rates or host availability.

The disappearance of the 1st instar *Cx. boharti* in December, demonstrated for both years, seems to have been correlated with a drop in the mean daily temperature to approximately 15°C. (Figure 1). The effect perhaps resulted in an induced adult quiescence or a similar pattern in available host activity. Initial host preference studies indicated that *Cx. boharti* feeds predominately on reptiles (unpublished date). Field observations on associated lepidosaurian populations indicated that *Thamnophis elegans*, *Thamnophis sirtalis* and *Sceloporus occidentalis* were the dominate reptilian species observed in the study area. Stewart (1965) determined that *Thamnophis sirtalis* and *Thamnophis ordinoides* have a voluntary minimum temperature between 10 and 17°C. *Sceloporus occidentalis* enters dormancy during October and November (Garrick 1972). The disappearance of 1st instar larvae, which occurred after the mean daily maximum temperature fell below approximately 15°C., in conjunction with the effect of the thermal dormancy limitations on host populations, is a probable explanation of the cessation of oviposition during late December to mid-February. The more obvious explanation, adult quiescence, cannot be determined until field and laboratory studies can be conducted. The absence of large numbers of immature stages of *Cx. boharti* from late-December to January indicates that larval diapause is not the overwintering mechanism.

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FALL AND WINTER POPULATIONS OF MOSQUITOES SAMPLED BY DRY ICE BAITED CDC MINIATURE LIGHT TRAPS IN CENTRAL CALIFORNIA

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INTRODUCTION.—As part of a two year study on the biology of *Culiseta inornata* (Williston) in Central California (Meyer and Washino 1976) fall and winter populations of associated mosquito species were monitored by CDC miniature light trap baited with 5 lbs. of dry-ice. Other than *C. inornata* and *Culex tarsalis* Coquillett (Hayes et al. 1958) fall and winter activity of mosquitoes measured by supplemental light traps has not been reported. In most studies (Bellamy and Reeves 1963; Washino and Bailey 1970) collections of adult mosquitoes were those taken from either artificial or natural shelters. To determine the activity and abundance of mosquito species associated with discrete habitats in Central California the CDC miniature light trap was selected for (1) portability and ease of operation (Sudia and Chamberlain 1962) and (2) necessity for live-trapping adults from which physiological-age parameters could be determined. Considering the influence of air temperature on adult activity (Bidlengmayer 1970) dry ice was supplied to increase mosquito response to trap selectivity and assure moderate sample sizes. Thus, population trends could be attributed to either changes in temperature or shifts in the dominance of the principle species being collected.

Materials and Methods.—From October through January, 1975-76 and 1976-77, mosquito populations associated with Suisuin Marsh, Central Valley, and Sierra Foothill habitats were sampled by CDC minature light traps baited with 5 lbs of dry ice. Traps were operated during this period for one night every two weeks at each location (Table 1). Adults were sequestered in standard CDC mesh bags attached to the bottom of each trap. A nylon screen cone was fitted to the base of each trap to prevent escape in case of either trap or battery failure. Collections were evaluated for total number and species for all mosquitoes collected.

Table 1.—Location and classification (U=urban, R=rural) of light trap stations sampled biweekly for one night only.

Location	Date	
Suisuin Marsh (Coast)	1975 - 76	1976 - 77
Grizzly Island (R)	+	+
Joice Island (R)	+	+
Fairfield (U)	+	+
Sacramento Valley (Valley)		
Dixon (R)	+	+
Sutter (U) (R)	+	0
Knights Landing (U) (R)	0	+
Sierra Foothill (Foothill)		
Folsom (R)	+	+
Roseville (R)	+	+
Fair Oaks (R)	+	+

Each location was designated as either urban (U) or rural (R) (Table 1), depending upon location and ecological characteristics of the surrounding area. Two locations were used to determine mosquito diversity and activity for rural areas while only one location was chosen for urban areas. Valley collections from Sutter, Sutter County, were taken during the 1975-76 season only. Surveillance was discontinued in 1976-77 with 2 alternative locations selected at Knights Landing.

RESULTS AND DISCUSSION.—In two seasons of trap operation a total of 12,918 mosquitoes representing 15 species were collected in dry ice baited CDC minature light traps operated at all locations (Table 2). Coastal collections, principally the Joice and Grizzly Island components, accounted for more than 84% of the total number of mosquitoes collected. Both *Culex tarsalis* and *Cs. inornata* represented 72% of the total number for all species collected, *Cx. tarsalis* (36.9%) and *Cs. inornata* (35.7%).

Considering species diversity, more species (13) were collected from foothill habitats than from either valley (9) or coastal (7) locations. Five species of *Culex* collected in the Sierra foothill (Table 2) represented the largest assemblage of congenerics. Four species of *Aedes* and three species for both *Anopheles* and *Culiseta* contributed to the remaining species recovered.

Table 2.—Total number and species of mosquitoes (male and female) collected in dry ice baited CDC traps operated in each region of Central California, 1975-77.

Species	Coast		Valley		Foothill	
	number collected					
Anopheles	(R)	(U)	(R)	(U)	(R)	(U)
An. franciscanus	0	1	1	0	0	0
An. freeborni	1	0	30	17	6	0
An. punctipennis	0	0	0	0	2	0
Culiseta						
Cs. incidens	0	0	0	0	10	4
Cs. inornata	3926	94	450	33	111	11
Cs. particeps	0	0	0	0	18	0
Aedes						
Ae. dorsalis	1707	1	0	0	0	0
Ae. melanimon	0	0	35	18	2	0
Ae. nigromaculis	0	0	1	0	0	1
Ae. vexans	0	0	1	1	702	2
Culex						
Cx. erythrothorax	456	0	5	0	137	0
Cx. peus	0	0	0	0	85	13
Cx. pipiens	0	33	72	1	90	6
Cx. tarsalis	4652	35	19	6	50	6
Cx. thriambus	0	0	0	0	66	0
Total	10742	164	614	76	1279	43

The average number of mosquitoes collected at urban and rural stations for both seasons is illustrated in Figure 1. Irrespective of region, significantly higher numbers of mosquitoes were collected at rural locations by comparison to the total recorded for urban locations. Both urban and rural collections from coastal areas were substantually higher than those from either valley or foothill regions. In relation to seasonality and temperature, population trends in all areas showed a continual decline from October to January. The observed decreases in mosquitoes collected, which began in late November and early December (Meyer and Washino 1976), were probably attributed to decreasing air temperatures recorded for each location. Bidlingmayer (1967) showed that trap efficiency relative to the number of mosquitoes collected was directly correlated to both air temperature and relative humidity conditions coincident with the period of trap operation. Similarly, the results of this study indicate that activity in mosquitoes associated with all three regions declined abruptly with the onset of colder temperatures in December. During the month of January partial warming occurred. However, the higher air temperatures and increased activity were observed only for the Suisuin Marsh populations, principally *Cs. inornata* (Figure 2). Apparently mosquito activity at both foothill and valley locations was not affected. Temperatures, however, were noticably cooler in both regions than in the Suisuin Marsh during January.

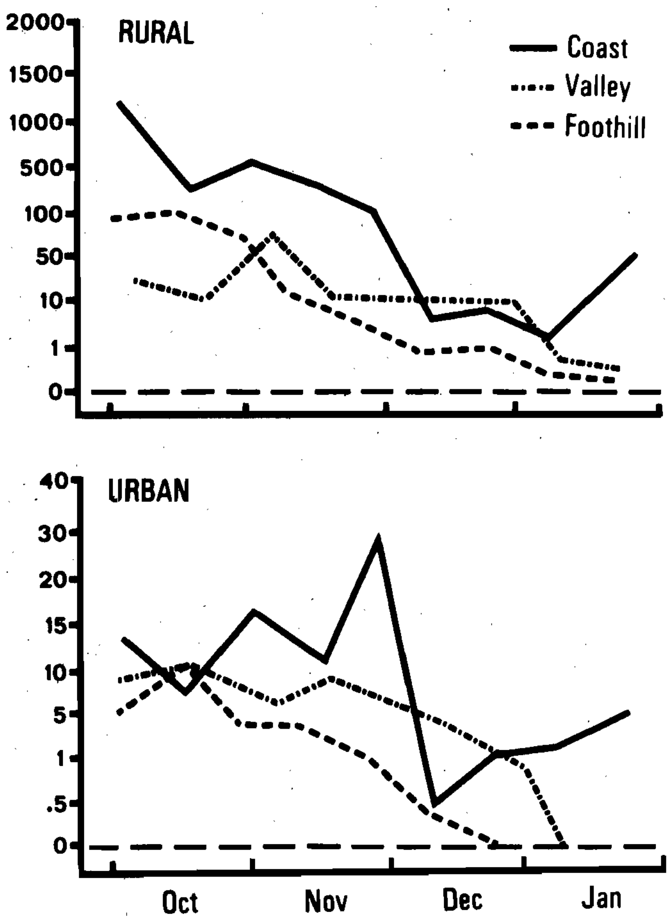

Figure 1.–Average number of mosquitoes (male and female) collected at urban and rural locations for coast, valley and foothill regions, 1975-77.

The collections from both Joice and Grizzly Islands, which were significantly higher than in the remaining two regions, were possibly attributed to a combination of 2 major factors, (1) temperatures at the time of trap operation, implications of which have been previously discussed and (2) the continued presence of an extensive larval breeding source (s) from which large adult populations could be produced. In relation to the latter, fall flooded game ponds, which cover a substantial portion of both islands, provided the principle source for the adult population being sampled. Equivalant areas of breeding habitat were not present in valley or foothill areas where traps were being operated. Accordingly, average collection size, prior to December, was noticably smaller in these areas.

Drought conditions which have persisted in northern California for the 1975-76 and 1976-77 season may have significantly influenced the breeding potential (Winter-Spring) in both valley and foothill areas. Most foothill larval habitats, which are produced under conditions of normal rainfall, were not flooded or only partially so during both seasons. Breeding was restricted to water sources available primarily in lowland drainage areas and man-made empoundments. Valley habitats, to a certain degree, are less vulnerable to drought conditions considering wide spread irrigation practices in most areas. However, many dry field

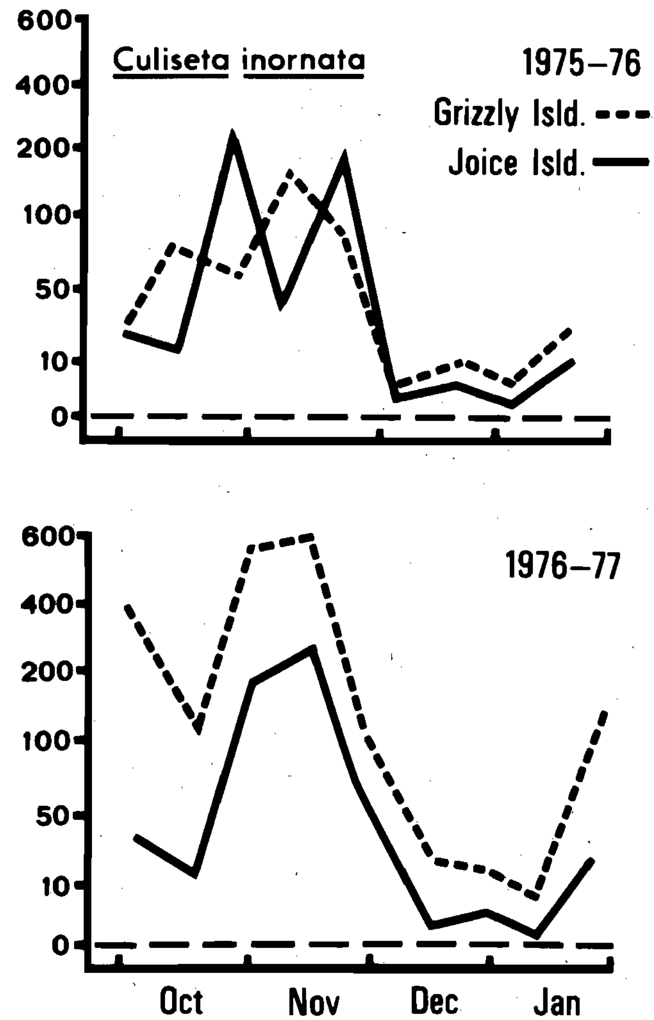

Figure 2.–Total number of *Culiseta inornata* collected at Suisuin Marsh locations for 1975-76 and 1976-77.

swales were observed that normally contained water in years prior to this study.

Collections were grouped by month and the percentage contribution for each species calculated. The maximum number of monthly collections for both seasons from rural locations on Suisuin Marsh ranged from 10 (October and November) to 8 (December and January). A total of 12 collections for October and 8 for each remaining month were taken at locations in the Sierra foothill. The total number of urban collections averaged 4 for all locations with the exception of the 6 collections taken both seasons at Fair Oaks.

Coastal Populations: (Suisuin Marsh-Fairfield): During the month of October *Cx. tarsalis* (58.0%) was the dominant species collected (Table 3). For 1976-77 a marked reduction in the total number of females collected (1279) was observed relative to the 3372 females collected the previous year. A similar pattern was shown for *Culex erythrothorax* Dyar. Early flooding of principle breeding sources followed by aerial (ULV) applications of pesticide to control *Aedes dorsalis* (Meigen) and an increase in salinity as a result of drought conditions, may have contributed to the reduction of larval populations for each species. Numbers of *Ae. dorsalis* and *Cs. inornata* did not differ appreciably for each season. There was, however, a marked increase in the number of *Cs. inornata* collected on Grizzly Island (Figure 2). Activity of *Ae. dorsalis* and *Cx. tarsalis* declined sharply from October to November for each season. Both species were not recovered in CDC traps operated during December and January. *Cs. inornata* was the dominant species collected from November (83.3%) to January (99.5%). For November 1976 one *Anopheles freeborni* Aitken (0.5%) and one *Cx. erythrothorax* (0.5%) in January 1976 were collected in the CDC trap operated on Grizzly Island.

Both *Culex pipiens* Linnaeus (40.5%) and *Cx. tarsalis* (39.0%) accounted for the largest portion of collections at Fairfield for October (Table 3). Activity for each species

Table 3.–Mosquito species collected at coastal locations during 1975-77, and expressed as the percent of the total number collected for each set of monthly collections.

Rural (Grizzly and Joice Islands)	% of monthly collection (no. collected)			
Species	Oct. (7389)	Nov. (3083)	Dec. (61)	Jan. (192)
Aedes dorsalis				
Aedes dorsalis	22.0	11.3	0.0	0.0
Anopheles freeborni	0.0	0.02	0.0	0.0
Culex erythrothorax	2.8	0.5	0.0	0.5
Culex tarsalis	58.0	4.8	0.0	0.0
Culiseta inornata	17.5	83.3	100.0	99.5
Urban (Fairfield)	Oct. (54)	Nov. (97)	Dec. (3)	Jan. (11)
Aedes dorsalis	0.0	1.0	0.0	0.0
Anopheles franciscanus	1.5	0.0	0.0	0.0
Culex pipiens	40.5	11.0	0.0	9.0
Culex tarsalis	39.0	12.0	0.0	18.0
Culiseta inornata	19.5	76.0	100.0	73.0

declined in November with no recoveries shown for December. Of the total 11 adults collected during January, 2 female *Cx. tarsalis* (1976) and one *Cx. pipiens* (1977) were collected. The remaining adults consisted of *Cs. inornata.* These recoveries were consistant with the warmer air temperatures experienced on the marsh. November collections were larger (97) than those recorded for October (54). The contribution of *Cs. inornata* increased from 19.5% in October to 76.0% for November. Three female *Cs. inornata* accounted for the total number of mosquitoes collected in December for both seasons; only 9 females were collected in January. One *Anopheles franciscanus* McCracken was collected during October 1975.

Valley Collections: (Dixon-Knights Landing-Sutter): Although *Cx. pipiens* represented 33.5% of the number of mosquitoes collected at rural locations for October (Table 4), the value is the result of a single collection (i.e., 71 females) taken at Dixon. Disregarding the number collected for that sampling period, *Cx. pipiens* accounted for less than 2.0% of the collections taken at the remaining 2 rural locations.

The total number of mosquitoes collected from October through November increased from 156 to 362 respectively. The number of *Cs. inornata* recoveries at both Dixon and Sutter locations, which accounted for 86% of all species collected for November, increased dramatically and was consistant for both seasons. The primary source for the Dixon component (*Cs. inornata*), which also contributed to producing the *Cx. pipiens* collected during October 1976, consisted of a series of large oxidation ponds which were located approximately 1 mile SW of the Dixon location. Fewer *Cs. inornata* were collected at the rural station located at Knights Landing.

Prior to the onset of colder nightly temperatures characteristic for December, the species which contributed principally to October collections were *Ae. melanimon* (13.5%), *An. freeborni* (12.4%) and *Cx. tarsalis* (15.5%). By November the activity for these species declined dramatically. Of the three species, none were collected in CDC traps operated in December and January at all valley locations for both seasons.

Results from valley urban collections are shown in Table 4. Female *Ae. melanimon* Dyar (40.5%) comprised the principle portion of October collections taken at Sutter, 1975. The apparent abundance of this species in early season collections was probably attributed to the presence of irrigated pasture habitats located to the south and east of Sutter. *Ae. melanimon* activity declined significantly in November with no recoveries present in either December or January collections. *An. freeborni* (24.3%), *Cx. tarsalis* (10.8%), and *Cs. inornata* (21.6%) contributed to the remaining component of codominant species collected in October. Of the total number of *An. freeborni* and *Cs. inornata* collected, slightly more females were taken at Knights Landing. Unlike *Cx. tarsalis*, activity for the *An. freeborni* remained high during November (Table 3). Neither species was taken in collections after November for both seasons. *Cs. inornata* was the dominant species collected in both November (55.2%) and December (100.0%) with no recoveries recorded for either season in January.

Foothill Populations: (Fair Oaks-Folsom-Roseville): Results for species collected at all foothill locations is shown in Table 5. During October and November *Ae.*

Table 4.–Mosquito species collected at Valley locations during 1975-77, and expressed as the percent of the total number collected for each set of monthly collections.

Rural (Dixon-Knights Landing-Sutter)				
	% of monthly collections (no. collected)			
Species	Oct. (156)	Nov. (362)	Dec. (94)	Jan. (5)
Aedes melanimon	13.5	8.0	0.0	0.0
Aedes nigromaculis	0.9	0.0	0.0	0.0
Aedes vexans	0.0	0.5	0.0	0.0
Anopheles freeborni	12.4	4.6	0.0	0.0
Culex erythrothorax	3.5	0.2	0.0	0.0
Culex pipiens	33.5	0.2	0.0	0.0
Culex tarsalis	15.5	0.5	0.0	0.0
Culiseta inornata	21.5	86.0	100.0	100.0
Urban (Knights Landing-Sutter)				
	Oct. (37)	Nov. (29)	Dec. (9)	Jan. (0)
Aedes melanimon	40.5	10.3	0.0	0.0
Anopheles freeborni	24.3	27.5	0.0	0.0
Culex pipiens	2.7	0.0	0.0	0.0
Culex tarsalis	10.8	6.9	0.0	0.0
Culiseta inornata	21.6	55.2	100.0	0.0

vexans was the principle species collected at both rural locations. Female *Ae. vexans* accounted for 41.5% and 39.5% of the total number collected for each month respectively. Remaining species consisted of *Cx. erythrothorax* (12.8-21.8%), *Cx. tarsalis* (13.7-0.0%), and *Cs. inornata* (10.5-21.8%). A ten fold decrease in the total number of mosquitoes collected occurred between October (1132) and November (113). The proportion of *Ae. vexans* and *Culex peus* Speiser remained relatively unchanged. There was, however, a two fold increase observed in the percentage of *Cx. erythrothorax* collected (Table 5). The flight activity of this species is perhaps less restricted by lower temperatures than that observed for the remaining species. With the exception of *Cs. inornata*, no activity was recorded for the remaining twelve species for either December or January. Accordingly, the effect of temperature on adult activity was most pronounced in the foothill region.

Rains which occurred during early September, 1976, resulted in the flooding of most temporary (vernal) breeding sources that are not usually flooded until normal precipitation patterns begin in October and November. As a result larvae of 11 species were recovered from one woodland vernal pond located near Roseville. Species consisted of both spring and summer, and fall and winter active forms. By comparison to 1975, the number of *Ae. vexans*, *Cx. erythrothorax* and *Cx. thriambus* Dyar collected in CDC traps operated at Roseville during 1976 were significantly higher. During the fall, 1976, the number of *Ae. vexans* collected at Folsom was substantually lower than recorded for the previous year. Flooding which occurred along Willow Creek in October, 1975, but not during 1976, had probably created flood water conditions favorable for *Ae. vexans* development.

Table 5.–Mosquito species collected at Foothill locations during 1975-77, and expressed as the percent of the total number collected for each set of monthly collections.

Rural (Folsom-Roseville)				
	% of monthly collections (no. collected)			
Species	Oct. (1132)	Nov. (113)	Dec. (9)	Jan. (4)
Aedes melanimon	1.3	0.0	0.0	0.0
Aedes vexans	41.5	39.5	0.0	0.0
Anopheles freeborni	1.1	2.0	0.0	0.0
Anopheles punctipennis	0.1	2.0	0.0	0.0
Culex erythrothorax	12.8	21.8	0.0	0.0
Culex peus	8.5	9.5	0.0	0.0
Culex pipiens	4.8	2.3	0.0	0.0
Culex tarsalis	13.7	0.0	0.0	0.0
Culex thriambus	4.0	0.0	0.0	0.0
Culiseta incidens	0.8	1.8	0.0	0.0
Culiseta inornata	10.5	21.8	100.0	100.0
Culiseta particeps	1.8	0.8	0.0	0.0
Urban (Fair Oaks)				
	Oct. (35)	Nov. (8)	Dec. (1)	Jan. (0)
Aedes nigromaculis	3.0	0.0	0.0	0.0
Aedes vexans	6.5	25.0	0.0	0.0
Culex peus	34.5	0.0	0.0	0.0
Culex pipiens	12.5	0.0	0.0	0.0
Culex tarsalis	18.0	0.0	0.0	0.0
Culiseta incidens	8.5	12.5	0.0	0.0
Culiseta inornata	18.0	62.5	100.0	0.0

Relative to both phenological patterns and species diversity in mosquito communities associated with discrete habitats in the Sierra foothill, the number of species (13) recorded from collections at rural locations, especially Roseville, reflected the observed diversity in major larval microhabitats. Principle breeding sources consisted of (1) temporary and permanent ponds (woodland and field), (2) dredger ponds, (3) streams with negligeable flow, (4) tree holes, and (5) rock holes. Disregarding tree and rock hole habitats, variations in the above were affected by the amount of exposure to direct sunlight and physiognomy of associated aquatic plant communities. In conjunction with the species collected in CDC traps, larval surveys being conducted concurrently have shown that an additional 9 species were found associated with the above larval habitats, *Aedes bicristatus* Thurman and Winkler, *Ae. nigromaculis*, *Ae. increpitus* Dyar, *Ae. sierrensis* (Ludlow), *Anopheles franciscanus* McCracken, *Coquillettidia (=Mansonia) perturbans* (Walker), *Culex apicalis* Adams, *Cx. boharti* Brookman and Reeves, and *Orthopodomyia signifera* Coquillett. Temporal differences in seasonal activity and low temperatures during times of trap operation are possible reasons explaining the absence of these species in the adult collections.

A total of 44 mosquitoes were collected in 18 nights of trap operation at Fair Oaks (Table 5). Of these 35 were recovered during October for both seasons. Female *Cx. peus* (34.5%) comprised the major portion of all species (7) taken for that month. Both *Cx. tarsalis* and *Cs. inornata*, 18.0% each, accounted for the next largest component of species collected. By November the number collected totaled 8 of which 5 were *Cs. inornata*, one *Cx. incidens* and two *Ae. vexans*. One female *Cs. inornata* was collected in December. No mosquitoes were recovered in the 4 collections taken in January.

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SOME OBSERVATIONS ON LIGHT TRAP PLACEMENT IN SALT LAKE COUNTY, UTAH

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Managers and entomologists of mosquito abatement districts are aware that the placement of light traps has a great effect on the catch. Sites can be selected so as to catch a minimum number of mosquitoes and thus impress trustees and the public with the efficiency of the control program. Sites can also be selected that will catch maximum numbers of mosquitoes, and these can be used to impress on the field workers how much more needs to be done. Ideally, sites should be selected which would reflect accurately mosquito populations, changes in them throughout the season and throughout the area being sampled. Unfortunately the ideal is hard to realize and we are often, if not always, unable to be certain exactly what our light trap data means. This paper reports some of the problems the South Salt Lake County Mosquito Abatement District has had in evaluating light trap data and how small changes in location to apparently equally suitable sites causes great changes in the catches.

The first light traps operated in what is now the South Salt Lake County Mosquito Abatement District were set out by Don Rees and me in 1952, the year before control operations were to start in the district. The purpose was to obtain data on adult mosquito populations before control. In 1952, three trap locations were used. The same trap locations were used in 1953 and the data compared. Dr. Rees (1954) concluded that the light traps indicated control procedures were responsible for a 98% reduction in the mosquito population.

These three traps were placed more or less in a straight line from north to south close to the Jordan River where many of the mosquito problems were located, but also were placed close to populated areas; specifically the cities of Murray, Midvale and South Jordan. At the time, we recognized that more traps were needed; but neither the traps nor the manpower were available.

Since 1953 control has been greatly improved, and all three of the original light trap sites have been abandoned because they take too few mosquitoes. The number of sites now used is 11, and the final sites were selected over a period of years to catch the greatest number of mosquitoes selected possible in the areas where the nuisance of mosquitoes would be most important. During the process of trap site selection, a number of sites were selected that were used for a few years and then abandoned because too few mosquitoes were collected. Some of the sites collected fair numbers of mosquitoes at first but called attention to a control problem which was resolved and the catches were reduced to almost zero.

Of the 11 light trap sites now used, two (Traps 1 and 7) take many more mosquitoes than is expected considering their location and the level of control. Trap 11 also takes a relatively high number of mosquitoes, but this is expected because the trap is located near a chicken coop at the edge of an extensive over-irrigated pasture. The average here is high because of small control failures near the trap. These are usually discovered and corrected after the trap takes an unusually high number in a night.

Table 1 summarizes light trap collection data for the district from 1973 through 1976.

In studying the data from traps 1 and 7, it was apparent that many more males than females were taken and that they were mostly *Culex tarsalis*. Intensive inspection around the traps failed to explain the relatively high numbers. Residents of the area and particularly the owners of the houses where the traps were located were questioned. Mr. Ray Demke, owner of the house where trap 7 is located, reported that no mosquitoes were seen during the year and stated that several lawn parties were held at night with people being in the immediate vincinity of the trap and no mosquitoes were noticed.

Frank Peterson, owner of the house where trap 1 is located, did report some nuisance during the year but the mosquitoes he described were *Aedes dorsalis*. Only two of

Table 1.—Average number of mosquitoes caught per trap per night 1973 - 1976.

	1976		1975		1974		1973	
Trap Number	Total	Female	Total	Female	Total	Female	Total	Female
1	21.9	4.9	11.5	4.0	30.6	6.3	42.4	9.5
2	0.8	0.2	2.4	0.3	0.6	0.1	2.5	0.6
3	3.0	1.3	3.8	1.6	2.8	1.0	5.8	2.5
4	1.7	0.8	4.3	1.6	4.3	1.3	2.2	0.9
5	4.4	0.9	1.2	0.4	0.3	0.1	1.0	0.3
6	4.4	1.9	5.4	2.5	3.8	1.9	4.9	2.6
7	22.6	6.3	21.2	5.9	26.2	7.7	27.6	11.8
8	0.4	0.2	0.4	0.2	0.2	0.1	0.2	0.1
9	5.6	2.0	10.3	4.0	6.3	2.2	9.8	3.2
10	6.2	4.5	2.7	1.8	3.0	1.8	5.2	3.5
11	16.7	5.5	12.9	7.3	9.9	5.2	8.2	4.1
All Traps	8.0	2.6	6.9	2.7	8.1	2.4	10.0	3.6

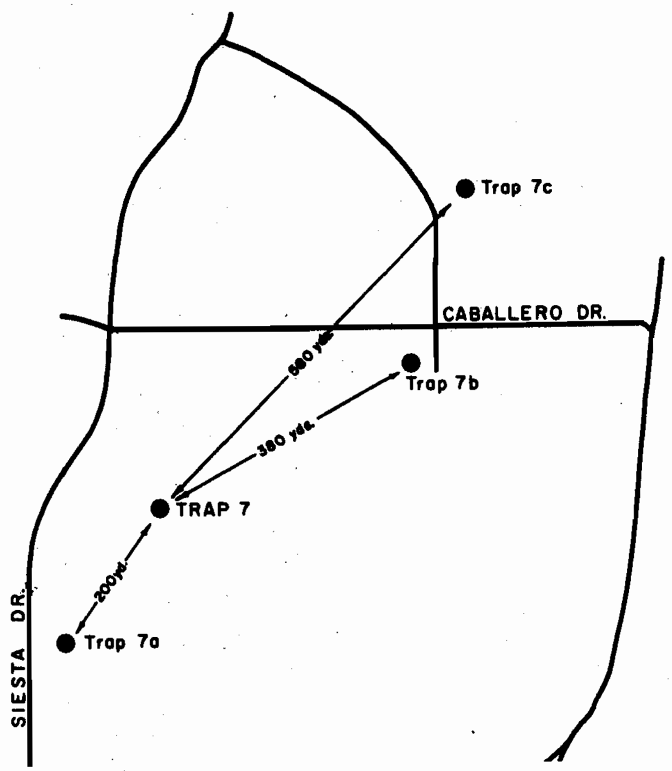

Figure 1.–Location of alternate light trap sights around trap 7.

these were taken in the trap during the year, but other evidence indicated they were more numerous than the catch would indicated.

The indication from light trap catches at traps 1 and 7, intensive inspection in the areas and the comments of residents were that the catches in the trap did not reflect total mosquito populations in the way that other trap sites did; and that the trap locations might be particularly suited to collecting mosquitoes, particularly males.

To test this hypothesis, three additional light trap sites were selected close to traps 1 and 7 in situations as nearly identical as possible to the original sites. Two of the three additional sites around trap 1 encountered problems and had to be abandoned. A trap was operated at the other site and numbered as 1A. It was 400 yards from trap 1 in a situation that appeared to be identical to trap 1. All three additional traps were operated near trap 7 (Figure 1) and were labeled 7A, 7B and 7C. Before control started an estimate was made as to how the catches would compare with trap 7. It was felt that 7A should catch as many as 7, 7B should catch more and 7C less.

The actual results are shown in Table 2. The catches in all of the new locations were dramatically lower. Also in all the new locations more males were taken than females, but the percentage of the catch that was male was not as high as at the original sites.

Why the sites 1 and 7 are especially attracitve to mosquitoes, particularly males, is unknown. Both sites have cultivated flowers. Nielsen and Stireman (1976) have shown mosquitoes are attracted to flowers. This may be a factor, but other sites also have flowers near them.

SUMMARY AND CONCLUSIONS.–At the present time, it is not possible to tell in all cases which sites are suitable to place traps so that catches will reflect populations accurately.

The traps in the South Salt Lake County MAD were placed, where, in the judgement of the professional staff, the catch would reflect populations. Yet in two of 11 light trap site collections, it did not appear to do so and changes of location of only a few hundred yards reduced catches dramatically, usually more than 90%. The new sites appeared to the professional staff to be as suitable as the original.

In the district it would be possible to reduce the average number of mosquitoes taken per trap per night 60-65% simply by moving traps 1 and 7 a few hundred yards and eliminating trap 11. This may impress trustees but would not provide needed information. More studies are needed to determine exactly what light trap catches mean and which sites are best.

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Table 2.–Species trapped - 1976 - (48 nights).

	Trap No. 1		Trap No. 1A		Trap No. 7		Trap No. 7A		Trap No. 7B		Trap No. 7C	
	M	F	M	F	M	F	M	F	M	F	M	F
Aedes dorsalis	2	11	2	2	0	0	0	0	2	0	0	0
Aedes nigromaculis	0	1	0	0	0	0	0	0	0	0	0	0
Aedes vexans	0	0	0	0	40	6	2	0	0	0	0	0
Culex erythrothorax	0	0	0	0	0	0	0	0	0	0	0	0
Culex pipiens	21	33	3	5	154	106	31	28	7	16	9	13
Culex tarsalis	709	132	56	13	567	27	63	11	23	3	11	0
Culiseta inornata	84	56	0	6	17	145	1	22	1	3	1	2
Culiseta incidens	0	1	0	0	4	14	0	1	0	0	1	0
Others	0	0	0	0	4	2	1	1	0	0	0	0
	816	234	61	26	786	300	98	63	33	22	22	15
Totals	1050		87		1086		161		55		37	
% Reduction from Original Site			92%				85%		95%		96.6%	

POSTAESTIVAL ADULT ACTIVITY IN *CULISETA INORNATA* (WILLISTON) IN RELATION TO LARVAL BREEDING AND CHANGES IN SOIL TEMPERATURE[1]

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In the Coachella Valley of southern California, adult populations of *Culiseta inornata* (Williston) peak during the late fall and winter months (Apperson et al. 1974). Subsidence of general activity occurs prior to the onset of hot summers wherein maximum daily air temperatures average 40°C during June to September. During early fall, *Cs. inornata* reappears in light trap collections as postaestivating adult females (Barnard unpublished observations). Through the summer months, aboveground resting sites, capable of providing long-term relief from the pervasive effects of summer heat, do not exist. Since hibernation of *Cs. inornata* is known to take place in tunnels and mammalian burrows at more northerly latitudes (Shemanchuk 1959, 1965), it seems probable that this species aestivates at southern latitudes in similar but relatively cool underground habitats.

The effects of soil temperature fluctuation on the activity of dormant insects, particularly the relationship between soil temperature inversion and the appearance of posthibernating individuals, has been studied for *Cs. inornata* (Shemanchuk 1965), *Culex tarsalis* (Bennington et al. 1958), and other insect species (McColloch and Hayes 1923; Holmquist 1928; Mail 1930). To assess the relationship between the fall recrudesence of general activity in *Cs. inornata* and soil temperature changes, the latter were monitored beginning 7/10/76, and light trap collections for adults, and larval sampling for immatures was instituted beginning 8/25/76. The object of this study was to document the fall appearance of postaestival adult *Cs. inornata* in relation to changes in soil temperature and in the absence of breeding activity by this species.

METHODS AND MATERIALS.—The LaQuinta area in the Coachella Valley of California annually exhibits high levels of adult activity in the early fall, and was therefore selected as the study site. Soil temperature readings were taken using a Cole-Parmer® thermistor-thermometer (Model 8510-20) with thermistor probes buried at 30 cm, 90 cm, and 180 cm depths in a location protected by a dense overstory of tamarisk trees (*Tamarisk pentandra* Pallas). The initial excavation was made using a 180 cm soil sampling tube and hammer. Three thermistor probes were attached to wooden dowel, placed into the hole and then covered with earth. To protect against vandalism, all probe leads were concealed within a 0.5 liter can (with removable lid) buried just below ground level.

Soil temperatures were recorded every 7-10 days by locating the buried probe-lead can and connecting each probe to a thermistor input socket. Surface soil temperature was taken simultaneously by inserting a free probe into the soil 2.5 cm below ground level for 10 minutes. All temperature readings were taken at approximately 0800 h.

Previous studies (Barnard unpublished observations) have shown that, for *Cs. inornata*, collecting efficiency of the New Jersey light trap can be increased by increasing trap light-intensity. As a result, New Jersey light traps employing 100W bulbs were set out at the LaQuinta site beginning in mid-August 1976. Four such light traps were operated, one each at distances of 35 m due east, 22 m due south, 30 m southwest, and 800 m due north of the soil temperature monitoring site. To supplement light trapping to the north of the study area, four CO_2-baited CDC minature light traps were run 2 nights per week along an east-west directed transect located 35 m to the north of the temperature monitoring site.

All discernible breeding sites were sampled weekly for larvae of *Cs. inornata* between 8/25/76 and 10/25/76. For logistical reasons, regular sampling was restricted to the La Quinta area (bounded by mountains on 3 sides) and to within a radius of 1600 m of the temperature monitoring

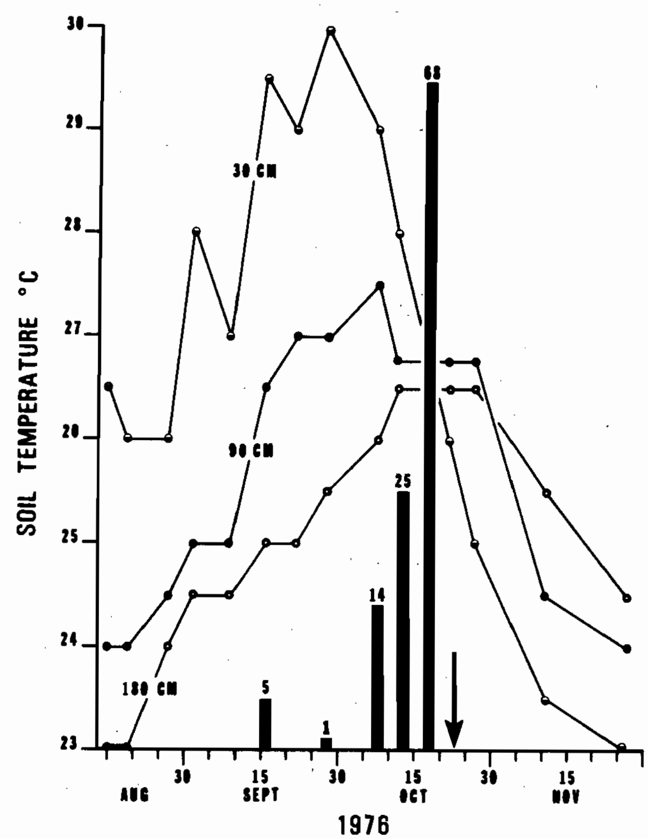

Figure 1.—Soil temperatures recorded from 3 depths, La Quinta, California. Solid bars represent total postaestivating *Cs. inornata* adult females collected per trap-week from 4 New Jersey light traps. Arrow indicates point at which 1st fall generation adults began to emerge from breeding sites.

[1]This study was made possible in part through facilities provided by the Coachella Valley Mosquito Abatement District, Thermal, California, and through monies obtained from the Chancellor's Patent Fund, University of California.

site and light trap locations. Breeding sites beyond this distance were sampled fortuitously, and adult activity was monitored valley-wide by Coachella Valley Mosquito Abatement District light traps.

RESULTS AND DISCUSSION.–Soil temperature trends, light trap collections, and the occurrence of larval breeding during the late summer-fall of 1976 are shown in Figure 1. Soil temperatures at all levels rose steadily throughout the summer. As would be expected, temperatures at the 180 cm depth were the most stable, changing no more than ±1.0°C during any 14-day period. Temperatures at the 90 cm level were also quite stable, but changed more rapidly than a the preceeding depth (± 2.0°C over a 14 day period), while temperatures at the 30 cm depth were the least stable, fluctuating by as much as 3°C over a 2 week period. Temperatures at all depths peaked during the early part of October, and during the week of 10/12 the 30 cm and 90 cm depth temperatures inverted, the former becoming cooler than the latter. Similarly, during the week of 10/26 soil temperatures at the 30 cm through 90 cm level fell below those measured at the 180 cm level.

Intensive larval sampling in the LaQuinta area revealed the absence of *Cs. inornata* immatures between 8/25 and 10/19 despite the widespread availability of breeding sites. Breeding was first detected on 10/19 with the observation of first and second instar larvae in standing water in date groves 100 m due east of the temperature monitoring site and 65 m due east of one of the New Jersey light traps. One week later, 10/26, this same site had 3rd and 4th instar larvae and pupae, and the assumption was made that some first fall generation adults of *Cs. inornata* had emerged.

Light trap collections were made between 8/25 and 10/26. CDC minature light traps were unsuccessful in the collection of *Cs. inornata* and were discontinued after 10/12 in lieu of collections made from the more efficient New Jersey traps. The nonexistance of male *Cs. inornata* in any of the light trap collections during the course of this study is noteworthy since light trap records of the Coachella Valley Mosquito Abatement District reveal such absence to be an annually reoccurring phenomenon, i.e. males are not taken in the early fall until after breeding is established, and emergence of the 1st generation has occurred.

The first adult females of *Cs. inornata* were collected by light traps in the La Quinta area during the weeks of 9/15 and 9/28, but the appearance of these individuals bears no relation to the soil temperature changes observed some weeks later. The most likely explanation for their occurrence is based upon a probable soil temperature inversion, induced temporarily and at scattered locations in the study area by both increasing temperatures at the 90 cm depth and the sudden depression of 30 cm depth temperatures; the latter having occurred as a result of heavy rains from two tropical storms visiting the area on 9/10-13 and 9/24.

In the nearly two week period between 10/7 and 10/19, the numbers of female *Cs. inornata* collected by light trap increased steadily. This activity correlates well with the lowering of surface and subsurface temperatures and the subsequent deepening of the cool layer down to the 90 cm depth by the week of 10/19. On this basis, it is theorized that soil temperature inversions such as were observed at this site stimulate aestivating adults to renewed activity during the late summer each year.

Table 1 shows the numbers of adult *Cs. inornata* collected between 9/15 and 10/19 including gonotrophic status and oviparity of females. As is apparent from this data, postaestivating individuals occur as both gravid and empty females, with the latter category represented by parous individuals only. These data compare favorably with those determined for posthiberning *Cs. inornata* in Weld Co., Colorado (Dow et al. 1976) in which postdormant females consisted of both gravid and nongravid-parous individuals.

In conclusion, the significance of the preceeding observations can be summarized as follows: 1) Soil temperatures increased slowly throughout the course of the summer of 1976, and inversion of temperatures at different soil depths occurred over a short period of time once average daily air temperatures began to fall. 2) Postaestivating adults of *Cs. inornata* were collected by New Jersey light trap as early as 9/15/76. 3) Greatest postaestivating adult activity, as measured by these same means, occurred simultaneously with an inversion of soil temperatures at the 30 cm and 90 cm depths. 4) Larval breeding occurred beginning 10/18 and 1st fall generation adults did not appear until a week or so later.

In our studies, adult *Cs. inornata* have been observed resting at entrances to mammalian burrows in areas adjacent to the study site described in this paper. It thus appears likely that these same sites may be among those utilized by *Cs. inornata* for purposes of aestivation. In addition, a wide spectrum of mammalian species inhabit the Coachella Valley area, including coyote, striped skunk, kit fox, wood rat, 2 species of rabbits, some or all of whose burrows may provide potential aestivation sites for adult *Cs. inornata* during the summer months.

Table 1.–Postaestivating adult female *Cs. inornata* collected from 4 continuously operating New Jersey light traps 9/15/76 to 10/19/76, La Quinta, California.

Date	No. collected	Empty	Blooded	Gravid	Oviparity[1] Parous	Nulliparous
9/15/76	5	5	0	0	5	0
9/22/76	0	-	-	-	-	-
9/28/76	1	0	0	1	-	-
10/2/76	1	2	0	12	2	0
10/7/76	25	5	0	20	3	0
10/19/76	68	21	0	47	18	0

[1]Discrepancies between numbers collected and numbers dissected for oviparity determination are a result of specimen damage by light trap.

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THE PERSISTENCE OF MINOR MOSQUITO SPECIES IN SOUTH SALT LAKE COUNTY

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The South Salt Lake County Mosquito Abatement District (MAD) includes 217 square miles in the southern two-thirds of the County. The human population in the District is largely urbanized, but there is a considerable amount of land still devoted to farming. The eastern side of the District is bounded by the Wasatch Mountains which supply the water for the valley, and from which several mountain streams, some large and some small, issue into the valley. The valley is drained by the Jordan River, which runs northward and empties into the Great Salt Lake. Most of the mosquito sources are small, usually less than one acre, but all are close to human habitation. There are approximately 2000 sources in the District, which are inspected weekly from March 1 to September 30, and treated when necessary.

The District has conducted an ongoing detailed larval survey since 1956. Data collected include pool type, water source, depth, percent shade and source of shade, size of pool, larval instar and density, percent emergent vegetation, air and water temperature, time of day, and a representative sample of larvae collected for later identification from each positive inspection. This data is recorded at the time of inspection and takes little added time. The data is transferred to sheets for compilation by the University of Utah Computer Center. Some results have been reported elsewhere.

The major objectives of the larval survey are to determine seasonal histories of larval populations in the District, to determine the relative importance of each species, to obtain information that will aid in predicting unusual increases in larval populations, to determine trends in mosquito larval populations, and to obtain more knowledge of larval habitats preferred by each species (Graham 1959a).

The sources are located throughout the District, often along stream sides, and particularly along the sides of the Jordan River. Much of the problem is related to the remnants of farming activity. Dispersed pieces of farming land are scattered among urban housing developments, with water rights that are frequently adequate to irrigate the original large farm acreage. The users of these areas are no longer full-time farmers and are consequently lax in their control of the more than ample supply of water.

The nature of the District is such that a high level of control is demanded because all sources are close to people. The nature of the control problem and the funding for it makes a high level of control not only possible but readily achieved with a vigorous, well-supervised program.

The mosquito control program is primarily concerned with five major species of mosquitoes which account for more than 98% of the mosquito production. These species are *Aedes dorsalis, Ae. vexans, Culex tarsalis, Cx. pipiens,* and *Culiseta inornata.* In addition to the five major species, 11 minor species have been found. These are *Ae. flavescens, Ae. cataphylla, Ae. spencerii, Ae. fitchii, Ae. niphadopsis, Ae. campestris, Ae. nigromaculis, Ae. increpitus, Culiseta incidens, Culex erythrothorax* and *Anopheles freeborni.*

The relative abundance of these minor species in the larval survey is shown in Table 1, in which the species are listed in order of abundance. When they are found as larvae they are often found with one or another of the five major species and are not usually a large component of the larval population in the site. For the most part, the minor species do not create nuisances and do not constitute a problem for the District. They are, however, controlled along with the major species and are subjected to intensive control pressure.

Of the minor species only *Ae. increpitus* and *Ae. nigromaculis* cause nuisances. *Ae. increpitus* is usually found along streams near the mountains in heavy vegetation where inspection and control is difficult. *Ae. nigromaculis* is found in open pastures. Larval populations of both species

Table 1.—Yearly totals of pools found to contain larvae of minor mosquito species in South Salt Lake County, Utah.

Species	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	Total
Aedes flavescens	0	0	0	0	0	0	0	0	0	0	1	0	1
Aedes cataphylla	0	0	0	1	0	1	1	1	1	0	0	2	7
Aedes spencerii	0	0	1	1	0	2	3	1	4	0	0	0	12
Culiseta incidens	0	0	0	6	1	2	0	0	2	3	1	1	16
Aedes fitchii	1	0	0	5	5	3	8	3	3	5	1	0	34
Aedes niphadopsis	11	5	0	3	5	3	1	19	7	9	51	12	126
Aedes campestris	2	3	4	3	2	6	14	12	13	21	24	28	132
Culex erythrothorax	14	14	3	11	41	9	11	20	4	7	5	12	151
Aedes nigromaculis	11	5	7	5	2	25	19	31	17	8	35	29	194
Anopheles freeborni	58	39	52	55	52	33	13	25	7	10	19	21	384
Aedes increpitus	11	17	5	11	9	49	21	25	44	51	98	79	420
Totals	108	83	72	101	117	133	91	137	102	114	235	184	1477

are relatively dense and when a source is missed by inspectors or sprayers, the nuisance can be significant.

Ae. nigromaculis has caused concern in Utah out of proportion to its numbers primarily because of California's experience with this species and because it will occasionally move considerable distances under Utah conditions (Graham and Collett 1972). Concern has been expressed that this species would increase in numbers as it has in California, but according to Graham (1959b) it was apparent that no major change in populations was apt to occur and none has.

Ae. freeborni as larvae are found more often than *Ae. nigromaculis* but the densities are low and the species has not been reported as a nuisance for at least 15 years. There were expectations that this species might be eliminated in the District by intensive control pressure (Graham et al. 1958), but by 1962 Havertz and Minson had determined that the population fluctuated and was not apt to disappear because of control measures.

Ae. niphadopsis, *Ae. campestris* and *Cx. erythrothorax*, over the period reported here, are roughly equal in numbers of larval sources. *Ae. niphadopsis* appears too early in the season to be a nuisance to man and is not abundant in any event. *Ae. campestris* is not usually noticed as a nuisance but is found with *Ae. dorsalis* and may be confused with it in superficial field observations. *Cx. erythrothorax* is a vicious biter but is noticed only rarely, as when one goes into areas of cattails (*Typha* sp.) or sedges (*Scirpus* sp.).

Ae. flavescens was collected in 1975 as a single larva. This species had not been collected in the District since its origin in 1953, and according to Don M. Rees (personal communication) had not been collected in the County in more than 30 years.

The other minor species are so rare that they are not noted in the field but only found in unusual instances in collections identified in the laboratory, but they do persist in spite of intensive control pressure.

The District operates 11 light traps each year on Monday and Thursday nights from May 1 to October 15. During the period of the larval survey reported here, the light traps collected 6 of the 11 minor species. These were *Ae. campestris, Ae. increpitus, Ae. nigromaculis, An. freeborni, Cx. erythrothorax* and *Cs. inornata.* In this 12-year period only one female *Ae. campestris* was taken. None of the minor species has been taken every year.

The correspondence of larval data to light trap data is not close but in general the more often larvae of a species are found in the survey, the more often adults are taken in traps. To obtain light trap data as good as the larval data would require a much larger number of traps than is practical.

Ae. niphadopsis is found more often than some minor species in larval surveys but has not been taken in light traps because the adults are mostly gone by the time the traps are operated.

Cx. erythrothorax larval sites have been remarkably constant throughout the survey with only 1969 being much different when 41 sources were found. The other 11 years averaged 10 positive sites. Light trap collections of *Cx. erythrothorax* were up in 1969 (43 adults were taken), but they were also up in 1967 (44) and 1968 (81). In all years the catch was almost completely females yet in 1975 and 1976, with larval sites positive for *Cx. erythrothorax* being essentially the same as 1967 and 1968, none of this species was taken in the traps.

SUMMARY AND CONCLUSIONS.–A study of adult and larval populations of mosquitoes in South Salt Lake County from 1965 to 1976 inclusive shows five species to be of major importance. The situation in the District is such that intensive control pressure can, and in fact must, be exerted on all larval populations. In spite of intensive control pressure throughout the mosquito producing season, species which are rare continue to persist and in some cases have apparently even increased in number. It appears that chemical control measures will not eradicate even minor species, and only ecological changes that destroy the habitat will eliminate mosquito species in Salt Lake County.

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CURRENT STATUS OF *AEDES VEXANS* IN SOUTH SALT LAKE COUNTY, UTAH

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In 1967 we (Minson and Graham) reported that *Aedes vexans* (Meigen) populations in Salt Lake County, Utah, had shown progressive increases from 1963 through 1966. The increase was reported in terms of larval populations because data on adult populations remaining after control measures have been applied are not adequate for determining changes accurately. In addition techniques have been developed for measuring changes in larval populations in the area that are more precise than those for measuring adult populations. Increases in population reported at the time were in the number of pools producing this species, in the number of times individual pools contained larvae, and as an increase in distribution in the county into areas not typical of the larval habitat of *Ae. vexans* in Utah. We also found (1968) that this increase continued through 1967.

Intensive larval surveys in which a sample of larvae is taken from every positive source for laboratory identification have continued in the county. The present study considers the data through 1976. For convenience some of the data in the figures have been consolidated.

Population of *Ae. vexans* as measured by the number of positive larval sources continued to rise until 1970 when a total of 1022 was found. This compares with an average of less than 80 positive larval sources for the first five years of the survey. *Aedes dorsalis* (Meigen) and *Ae. vexans* are both floodwater species and share similar habitats. However, *Ae. dorsalis* populations have not shown similar changes. Figure 1 compares average larval populations of *Ae. dorsalis* and *Ae. vexans* for two periods: the first 11 years, 1956-66, and the second of 10 years, 1967-76. While *Ae. dorsalis* populations stayed almost the same in both periods, the increase in *Ae. vexans* was large. After 1970, larval populations of *Ae. vexans* dropped but still remained high when compared with the first 10 years of the study. In 1976 the number of sources dropped to 374 which is almost 150 below the average of the five previous years but still higher than any of the first 11 years of the study. To us this appears to be a fluctuation rather than a trend but future studies will determine this.

The changes in *Ae. vexans* populations based on 5-year periods from the beginning of the study in 1956 through 1975 are shown in Figure 2.

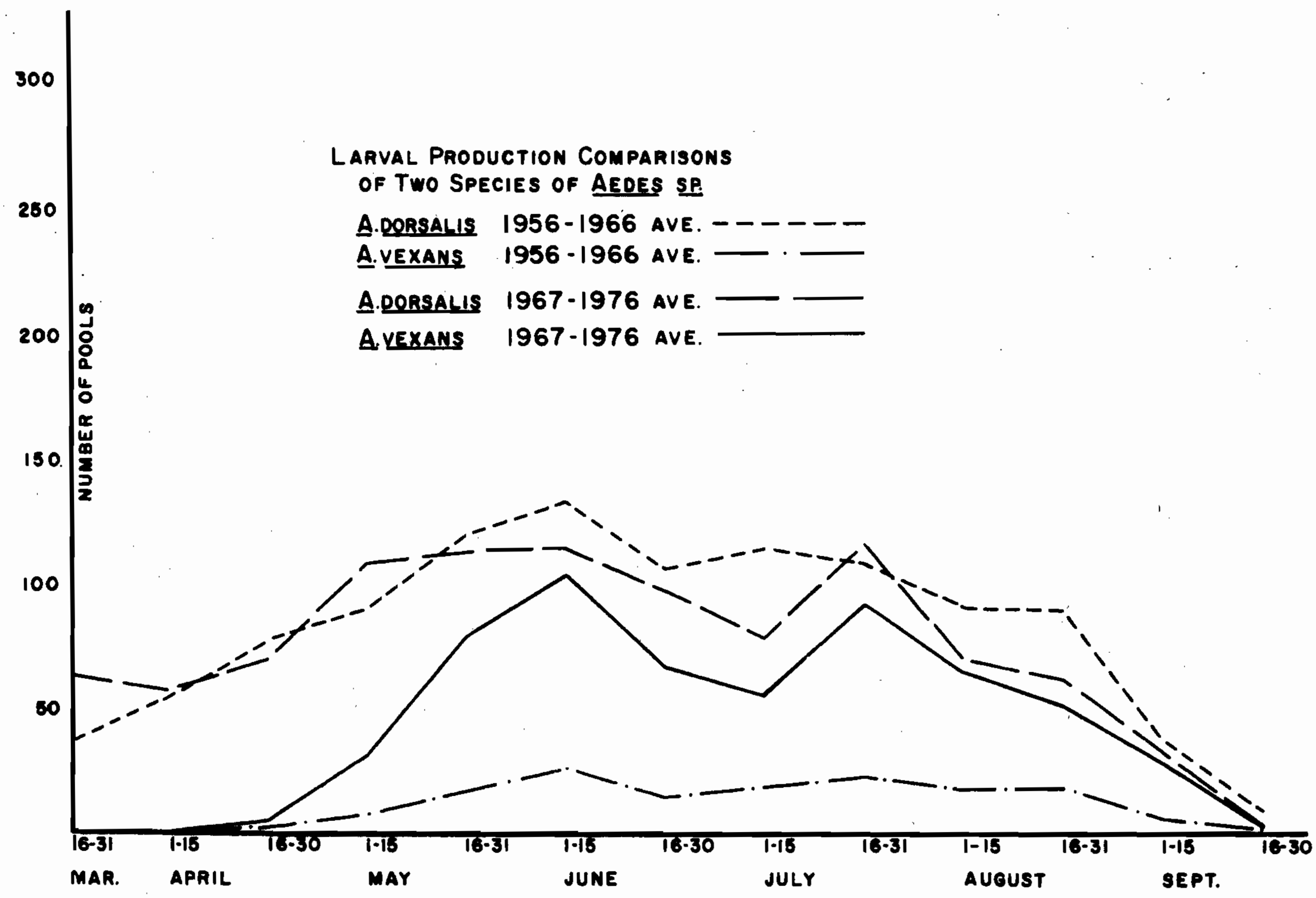

Figure 1.—Larval production of two species: *Ae. vexans* and *Ae. dorsalis* comparing a ten-year and an eleven-year span.

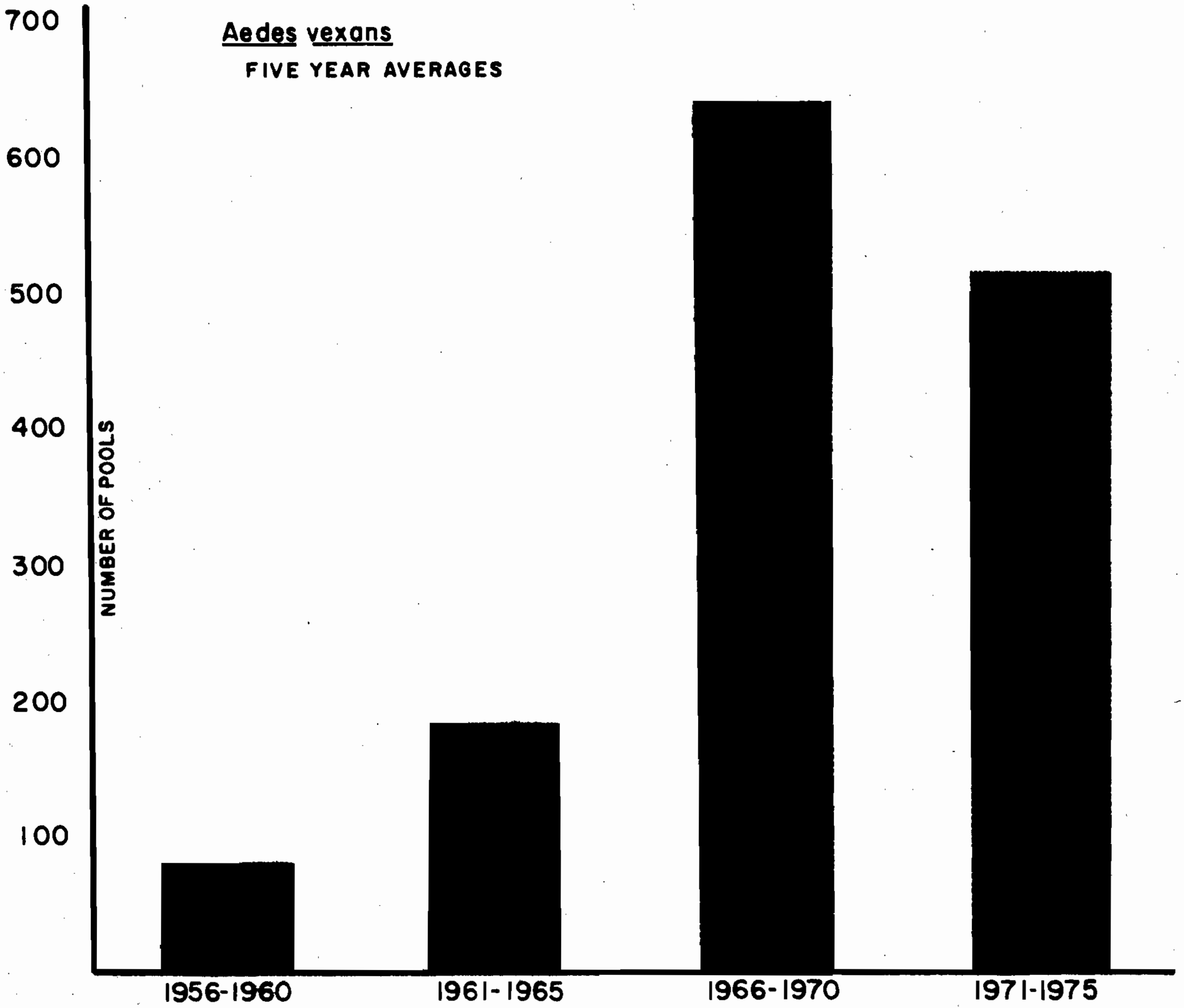

Figure 2.–Comparison of the number of positive sources for *Ae. vexans* divided into 5-year periods from 1956-1975.

Changes in the distribution of *Ae. vexans* larval habitats are shown in Figure 3. *Ae. vexans* was reported as abundant in Salt Lake County before the present study started (Rees 1939). Graham et al. (1958) reported it had become much less abundant and would be only a minor nuisance if left uncontrolled. The reason for the change given was that urbanization in the area had greatly reduced stream-side sources which were the primary larval habitat of the species. When the increase occurred beginning in 1963, the mosquito spread from shaded stream-side habitats to open pastures which were typically sources of *Ae. dorsalis*. L. T. Nielsen (per. com.) reports being able to find *Ae. vexans* in such larval habitats before the increase occurred and this has also been observed in the larval survey program. The obvious change has been in the abundance of the species in this kind of habitat.

Graham and Bradley (1962) noted that one factor determining mosquito populations was the number of times a species could occupy a larval habitat without other mosquito species being present. The change in this important factor over the years is shown in Figure 4. The years 1959, 1967, and 1970 were chosen because they each signified a particular point in the study time frame. The data in 1959 is adequate to use to compare the initial population of *Ae. vexans*; 1967 shows the year in which the comparisons are equal, and 1970 represents the high year of production to date.

As the number of positive pools increased for *Ae. vexans*, it became possible to detect relationships of populations of this species to *Ae. dorsalis* (Figure 1). *Ae. vexans* larvae always appear later in the season and are never as abundant as *Ae. dorsalis*. *Ae. vexans* reaches its first larval population peak the first half of June at which time it is almost equal to *Ae. dorsalis*. For the rest of the season, populations of the two species are close and both reach another peak the last half of July.

Bradley and Graham (1973) investigated the tendencies of five common species of mosquitoes to inhabit the same or different larval habitats. Of the ten possible combinations of two species of mosquitoes occupying one habitat, only the combination of *Ae. dorsalis* and *Ae. vexans* showed a positive correlation. This pattern would indicate that

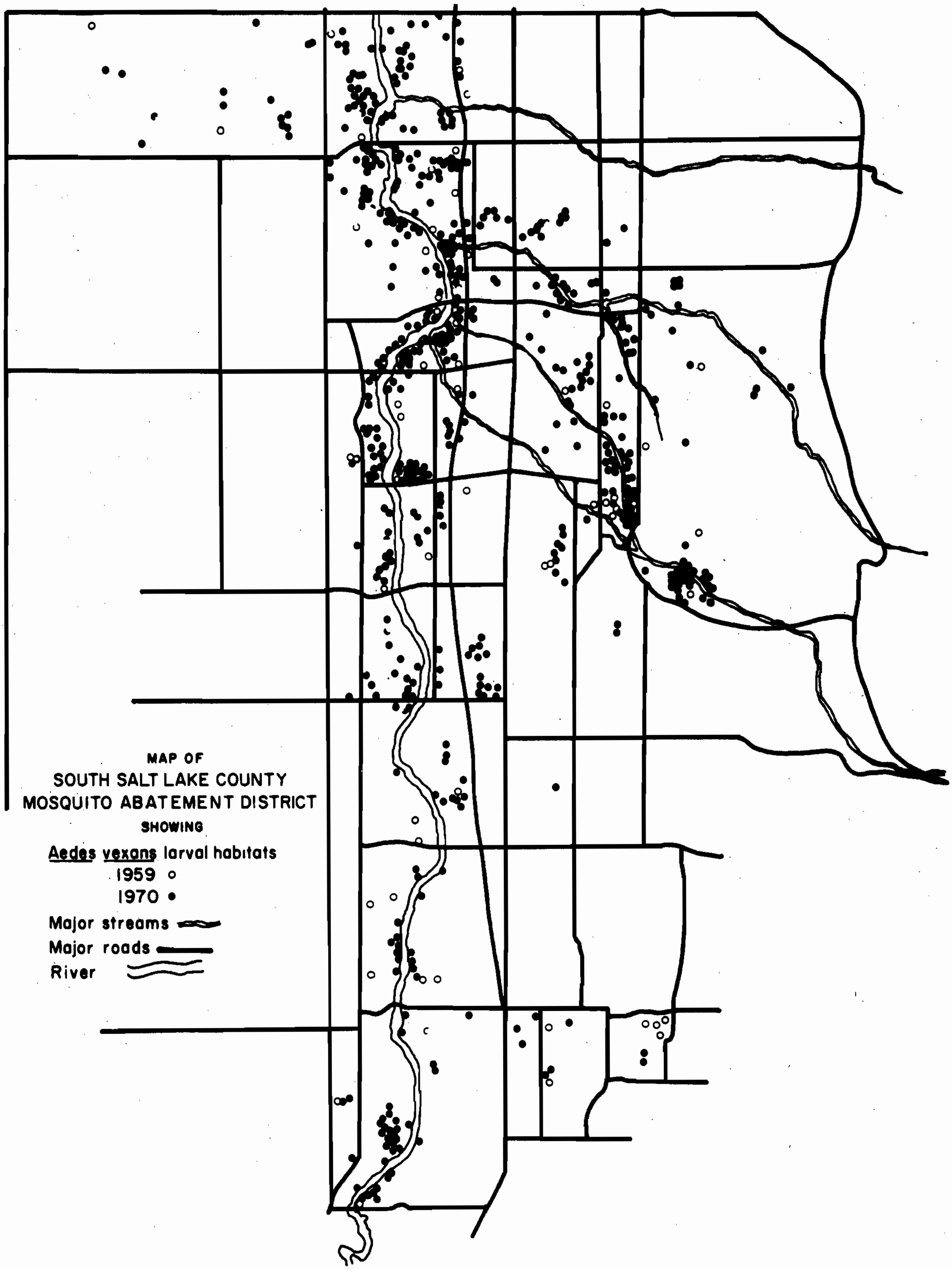

Figure 3.–A diagrammatic map comparing larval habitats of *Ae. vexans* during the year 1959 with the year 1970.

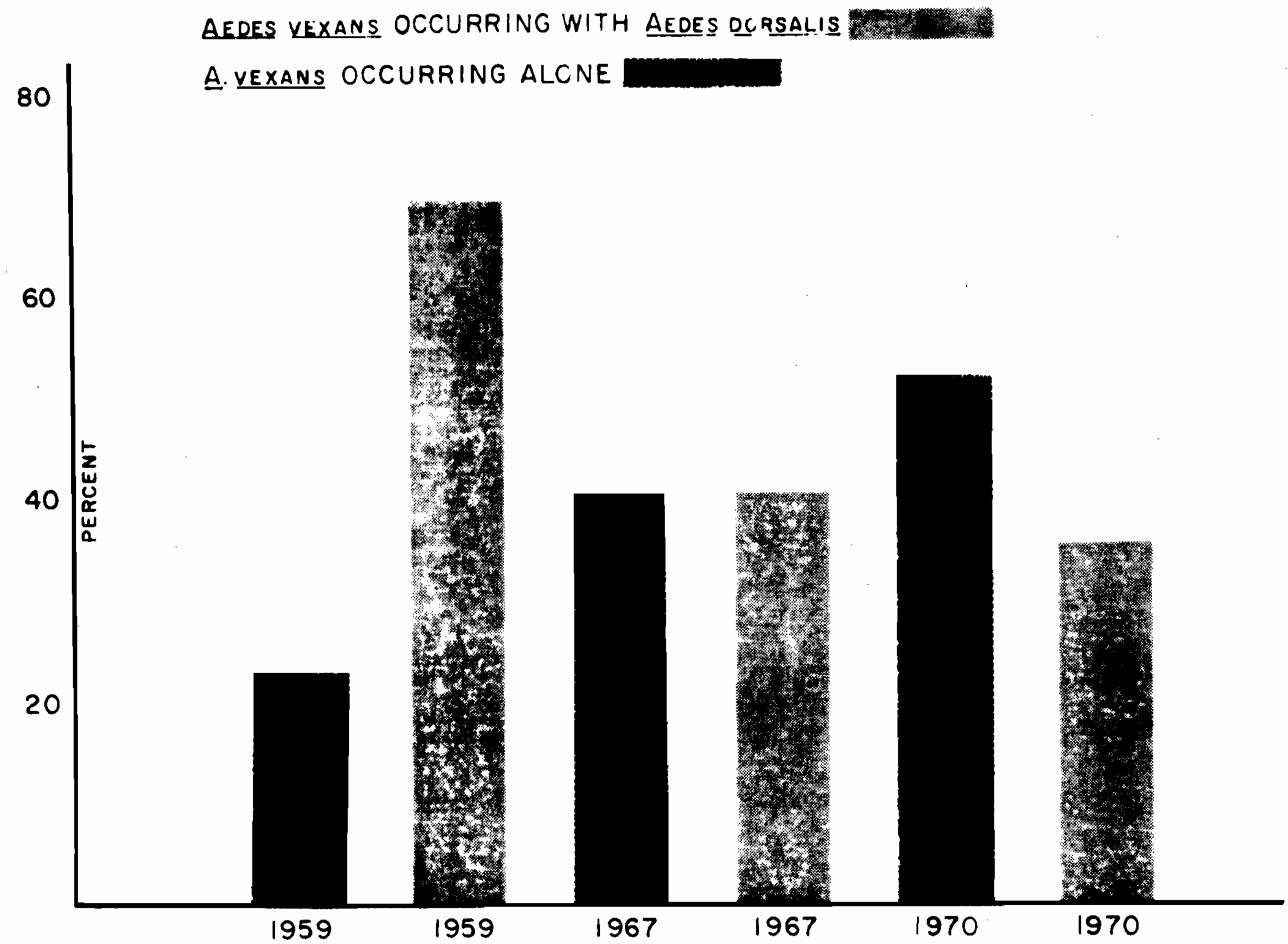

Figure 4.—Comparison of percentages of producing areas for *Ae. vexans* occurring with *Aedes dorsalis* compared with *Ae. vexans* occurring alone.

they occupy similar habitats and are affected by the same conditions once *Ae. vexans* populations get started.

SUMMARY AND CONCLUSIONS'—A study of mosquito larval populations in South Salt Lake County, Utah, from 1956 through 1976, shows that *Ae. vexans* populations increased greatly from the first 5-year period to 1970 when the population of this species was 13 times as great as at the first of the study. Other species did not show such an increase. After the peak year of 1970, the population has apparently stabilized at about five times the original population and appears to have reached a plateau at this higher level. At the start of the study the species' larval habitat was mostly along wooded stream-sides. The increase in the population occurred as a result of the species increasing its larval habitat by being able to occupy open pastures in much greater numbers.

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MOSQUITOES OF BIG MORONGO OASIS, SAN BERNARDINO COUNTY, CALIFORNIA, AND THEIR PUBLIC HEALTH SIGNIFICANCE

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INTRODUCTION.–Morongo Valley is located in the Little San Bernardino Mountains, about fifteen miles north of Palm Springs. Earth movements along the Morongo Valley fault have forced underground water to the surface to form a desert oasis. The available water in the form of two open ponds, a marsh and a small stream, has allowed many plants and animals to exist there.

We know encephalitis is present in the bird population around the Needles area of the Colorado River and since these birds are migratory, we think that some of them use the Morongo Valley Oasis as a stopping point in their migratory path flight up through the San Bernardino Valley and the rest of California.

Since over 235 species of migratory and residential birds visit Morongo Valley Wildlife Preserve throughout the year, we hypothesized that if the correct mosquitoes were present along with the migratory bird population, we could expect to find encephalitis viruses. If we could find them at this locality, and if the mosquitoes were present, we would hope that by monitoring the mosquitoes at the Morongo Valley Wildlife Preserve we could intercept and predict the introduction of encephalitis virus to the San Bernardino Valley and the major population centers of our County. In order to test this hypothesis a plan was proposed to sample the mosquito population several times during the year using CDC miniature light traps baited with dry ice.

PROCEDURES.–Five traps were placed in various locations around the Oasis. Trap 1 was placed in a thick growth of cattails and bulrushes; Trap 2 was placed in a willow tree beside two large ponds and the edge of a clearing; Trap 3 was placed in a tree beside a boggy marsh in one of the lower ponds; Trap 4 was placed approximately 150 meters from the ponds in a dry ravine in order to test the flight range of the mosquitoes that were caught; and Trap 5 was placed midway in a dense marshy area where water approximately 5 cm. deep flows across the surface of the ground covering an area approximately 25 meters wide at this point.

FINDINGS.–Trap 1 - consistently caught the majority of *Culex erythrothorax* Dyar, a species known to inhabit cattail and bulrush vegetation. *Cx. erythrothorax* is a strong and persistent biter. It is the primary nuisance mosquito in the area. Chew and Gunstream (1970) suggest that light traps are not effective in capturing this species; however, the addition of dry ice makes the CDC trap an efficient collecting device.

Trap 2 - was the next closest to the cattail vegetation and also collected many *Cx. erythrothorax*.

Trap 3 - collected a variety of mosquitoes due to its location on the edge of several eco-systems.

Trap 4 - located in the ravine, collected a large majority of *Culiseta* species. *Culiseta* are presumed to be strong flyers and to venture farther than the other mosquitoes from the aquatic habitat.

Trap 5 - in the marsh collected a variety of mosquitoes throughout the year.

SPECIES DISTRIBUTIONS IN TIME.–*Cx. erythrothorax* was by far the most abundant mosquito comprising 91% of the sample population. This species dominance was consistant in each month of the year, lowest during February and increasing to a peak in October then dropping rapidly with the onset of cold weather. The population of this species closely follows the growth cycle of the cattails with which it is associated.

Culiseta inornata (Williston), normally a cool weather mosquito, exhibited an unexpected peak during June but was most abundant in a dramatic peak during November, following the die-off of *Cx. erythrothorax*. It persisted throughout the year.

Culiseta particeps (Adams), the third most abundant species, showed a remarkable consistency throughout the

Table 1.–Summary of presence and frequency of mosquito species.

Species	Totals	% of Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Culex erythrothorax	8912	90.86	198	12		34	1993	1429		1961		2472	746	67
Culiseta inornata	418	4.26	32	5		8	1	40		1		56	258	17
Culiseta particeps	383	3.90	52	11		18	37	45		62		39	97	22
Anopheles freeborni	67	0.68	1				27	6		30		1	3	
Culiseta incidens	13	0.13						4				4	5	
Culex tarsalis	8	0.08						5		1		1		1
Anopheles franciscanus	7	0.07					1	1		3			2	
Totals	9808		283	28		60	2059	1530		2058		2573	1111	107

year, occurring in numbers equal to at least 11% of its peak in November.

Anopheles freeborni (Aitken) had early and late summer peaks and like the following species was not active during the winter. *Culiseta incidens* (Thomson) was present in small numbers in summer and fall. Eight *Culex tarsalis* (Coquillett) were collected during the last half of the year and *Anopheles pseudopunctipennis franciscanus* (McCracken) showed a similar pattern.

Morongo Valley Wildlife Preserve is also interesting because it contains a naturally occurring population of mosquitoes that has never been influenced by the introduction of insecticides. The mosquito fish, *Gambusia affinis* (Baird and Girard), was introduced a few years ago into the two large clear ponds on the wildlife preserve. This is the only control measure that has been exerted against any of the mosquitoes. *Gambusia* is effective in those areas where it can reach; however, much of the preserve is marshy and not suited for fish.

CONCLUSION.–The disease vectors *Culex pipiens quinquefasciatus* Say and *Cx. tarsalis* were conspicuously absent. The few individuals of *Cx. tarsalis* collected indicate either that a low population was maintained throughout the year or that these mosquitoes sporadically flew in from other areas. Because of the virtual absence of these two species we can conclude that there is minimal risk of contacting or spreading arbovirus encephalitis from the Morongo Valley Wildlife Preserve.

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MOSQUITO PROBLEMS IN A DEVELOPING BAYSIDE COMMUNITY

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A community constructed on bay fill creates unique mosquito control problems. Foster City, California, is located on Brewer Island at the west anchorage of the San Mateo-Hayward Bridge, approximately eight miles south of the San Francisco International Airport. The City is constructed on reclaimed salt evaporator ponds (Leslie Salt Co.), filled bay marsh and abandoned farm lands.

The City has a central lagoon system which receives catch basin runoff water. These lagoon waters theoretically circulate through the system and then are discharged into San Francisco Bay. This circulation system precludes the use of heavy larviciding oils and organophosphorus compounds that may have a deleterious affect on the biota as well as the aesthetics of the lagoon system. The system can fluctuate from a low of 97 ft to a high of 99.2 ft (based on 1929 U. S. Coast and Geodetic survey with 100.0 elevation equaling mean high tide level). The water inlet at the southeast of the City is from Belmont Cove an arm of San Francisco Bay. The desired water level is maintained by use of a float system both at the inlet and discharge points. Tide gates with three 42 inch pipes allow water to move into the lagoon at high tide, and two 48 inch evacuation lines are utilized at the north side of the City to discharge waters back into the bay. This system is designed to handle a one-in-one-hundred year storm flood condition. Winter water levels are maintained at 97.5 ft while the summer levels fluctuate from 98.5 to 99 ft. elevation. The catch basin-storm drain lines elevation grade from 97 ft to a maximum at the 100 ft elevation.

These lagoon levels create a condition of very poor water exchange within the storm drain system and thus make mosquito control efforts extremely difficult.

Some of the areas of the island that were hydraulically filled (approximately 18,000,000 cubic yards), as well as dry fill over old slough beds and salt ponds, are showing signs of soil subsidence. It is this dynamic condition which has created havoc with the systems' designed to be static, such as the storm drain lines.

METHODS USED TO SUPPRESS MOSQUITO POPULATIONS IN A BAYSIDE COMMUNITY

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Later this afternoon, a paper will be presented dealing with the formation and subsequent development of Foster City, a Bayside community. At this time I would like to present to you a review of our mosquito control efforts in this developing area. Early control efforts prior to development of this community were handled with DDT, malathion and later by fenthion. As this area was altered, we experienced a change in the species present with *Aedes dorsalis* and *Ae. squamiger* being replaced by *Culex pipiens.*

The uniqueness of this area, surrounded by Bay water and interwoven with canals that drain to a central lagoon and ultimately empty into San Francisco Bay, has compelled the San Mateo County Mosquito Abatement District to be ever-vigilant. Since all run-off water eventually finds it way into the canals and lagoons of the City, we are marked with a system of significantly marginal water quality. Except for isolated locations, the catch basin lines are filled with, and replenished by, fresh water run-off year around.

Catch basin inspection frequently is hampered by the assortment of basin covers with which we have to cope. The conventional grate-type cover that can be opened easily, occurs only in limited numbers. The most common cover is a round concrete plate. The newest cover is a metal lid bolted to the base with a trash bar over the curb opening. Sampling in the area where the new basins have been installed is virtually impossible.

Initial efforts to effect a control of the *Culex pipiens* population in catch basins was handled by the mosquito abatement's work-horse insecticide, fenthion. Early control successes in 1964 were followed by an ever increasing number of areas that showed rapidly deteriorating levels of control. In 1973, the recognition of the onset of resistance in the *Cx. pipiens* population prompted us to conduct an area-wide bio-assay evaluation. This has since become a standard part of our program. The threshold level of potential failure for *Cx. pipiens* established by the California Bureau of Vector Control was an LC_{50} of .005 ppm. We found the LC_{50} of fenthion for the population ranged from .18 to .32 ppm, far above the recognized threshold.

Since it became obvious that fenthion was no longer of use as one of our control tools, we were anxious to explore other control or suppressive methods. The results of the tests are as follows:

Mid 1974 - Evaluation of light oils

GB 1010®, GB 1111®, GB 1356®, and Flit MLO® were tested in catch basins. Flit MLO, although more effective than the other oils, did not provide an adequate level of control. Frequently, an inspection of the catch basins revealed the water surface was completely covered with garden litter and garbage, which precludes an effective insecticidal penetration.

March 21, 1975 - Flush basins with Bay water

We considered that a salt water exchange in the catch basin lines (15 inches to 18 inches in diameter at the distal end increasing to 54 inches diameter at the lagoon), a total distance of 1400 feet from the Bay to the lagoon, could sufficiently disrupt and eliminate the *Cx. pipiens* population. The Foster City Street Department volunteered their equipment and manpower. Thirty thousand gallons of Bay water (sp. gr. 1.0135) were pumped into the line by a single 340 gallon/minute pump. Dye was introduced at the first basin and, 17 seconds later, was detected at the next catch basin, 300 feet from the point of introduction. Dyed water was not detected beyond that point.

June 10, 1975 - Second flush with Bay water

With the assistance of personnel from Foster City, a second attempt was made to flush the basins. The same procedure was followed except three 340 gallon/minute pumps were utilized. Pre-flush specific gravity readings were made and the presence or absence of mosquito larvae was recorded. Approximately 200,000 gallons of Bay water was introduced within a three hour period. Shortly after completion of the test, the water in the head basin was virtually fresh. Twenty-four hours after, specific gravity readings were made at several locations in the line. Water in all the basins exhibited specific gravity readings near to fresh water, indicating an incursion of fresh water from another source. Such unusual findings indicated to us a possible separation in the line. At meetings with Street Department personnel, our suspicions of line separations were confirmed.

August 14, 1975 to January 21, 1976 - Introduction of mosquito fish in the basins

Method: One line of 16 pre-selected basins was stocked with *Gambusia affinis*. Approximately two dozen mature fish were placed into each basin. Eleven basins were breeding and subsequently treated with Flit MLO prior to planting fish. Five basins had no breeding while one basin was covered with crankcase oil. Five months after planting the *Gambusia affinis*, some fish were noted to have survived in a single basin.

Conclusion: Fish may prove to be a successful tool if used in larger numbers and where basins are relatively clean.

March 17, 1976 - Pyrocide Intermediate 57® and Flit MLO® (1/3 oz. Pyrocide + 5 gal. Flit)

Throughout the City, the adult mosquito population in the catch basin system continued to be abundant and

Table 1.—Altosid Briquet® Tests.

Test Sites	Treatment	Duration
Foster City	1st	5 wks
	2nd.	7 wks
	3rd	6 wks
San Mateo Park	1st	7 wks
	2nd.	8 wks
San Mateo-Sunnybrae	1st	7 wks
	3rd	10 wks

troublesome. Four basins were selected and pre-treatment counts were made. An isolated area of 106 basins was selected for treatment with the Pyrocide-Flit mixture. The material was blown into the catch basin openings for a two second duration with a KWH model TT no. 60 back-pack mistblower. The experiment was terminated after 17 days when large adult numbers were noted in two of the four sampling basins.

Conclusion: Although of short duration, the combination adulticide and larvicide mixture was very effective.

April 21, 1976 - Shell No Pest Strips (20% Vapona) in basins

Both full and half strips were suspended in basins approximately 12 inches above the water level. The recommended air space is 1000 cu. ft/strip - the basins average 64 cu. ft. Little air exhange is noted within the basins. The life expenctancy of the No Pest Strip is considered to be four months, but we experienced failure within five weeks in an area of less than 10% the recommended air space.

Conclusion: The material and labor costs associated with the No Pest Strips made the item uneconomical for their period of effective control.

May 11, 1976 - Addition of salt (Na Cl) to the catch basins

A bio-assay analysis indicated the addition of approximately 15 pounds of salt to a catch basin should produce a 100% mortality in the *Culex pipiens* larval population.

Both fine and rock salt was evaluated.

After introduction of salt into the basins, the water was thoroughly agitated to give a uniform distribution. After both the 7th and the 14th day samples failed to exhibit any reduction in the larval population, the test was terminated.

Conclusion: None could be drawn.

June 11, 1976 to November 1, 1976 - Altosid Briquets® in basins

Material: Zoecon Corporation supplied to the San Mateo County Mosquito Abatement District experimental material to be tested in catch basins. The briquets consisted of Altosid ® impregnated in charcoal and each briquet formed into cylinders 1 inch in diameter and 1 inch long.

Test areas: Two areas were selected for evaluation of the briquets - Foster City and San Mateo. Prior to selection, each basin was opened and sampled in order to determine the larval density. Upon selection each basin was marked and treated with one 400 mg. Altosid Briquet. The Zoecon Corporation estimated the life of the slow release briquet to be approximately 30 days.

Chemical Evaluation: Basins in each test location were sampled randomly in order to obtain a cross section of the population. An effort was made to obtain a total of 50 pupae from the treated basins and a similar number from check basins in areas adjacent to the test sites. In some instances, we were required to accept a lesser number of pupae. Samples were taken three and seven days after treatment. Thereafter, samples were taken weekly.

The effectiveness of the chemical was measured by counting emergent adults in each sample. Failure of the material in the laboratory was signified when the emergent adult population rose sharply from a low suppressed level. When this occurred the entire area was retreated.

Table 2.—Altosid Briquet (400 mg.) evaluation in catch basins. Adult emergence (%).

		Weeks									
Applications	Pre-treat	1	2	3	4	5	6	7	8	9	10
Foster City											
No. 1. treated	96	36	22	23	62	38					
No. 2. treated	38	22	50	58	34	30	55	28			
treated- breeding					94	96	74	90			
No. 3 treated	28	30	46	10	45	18	50				
treated-breeding	90	29	0	88	-	59	59				
San Mateo Park											
No. 1 treated	100	4	4	0	4	14	26	42			
No. 2 treated	42	12	0	18	34	8	6	16	21		
San Mateo-Sunnybrae											
No. 1 treated	97	10	0	2	2	-	40	43			
No. 2 treated	43	9	20	16	2	0	10	6	-	-	59

In the Foster City test site, one treated catch basin reacted as if no treatment had occurred, thus providing a picture of poor control for the entire test site. During the first week after the single basin was separated from the test, we noted emergence in the treated-breeding basin at 94% while the remaining basins had 34% emergence. (Table 2) This particular catch basin displayed erratic results throughout the entire test program. We referred to this basin as treated-breeding.

Water samples from the single basin and one sample from the ine basin test site were submitted to the San Francisco Water Company for evaluation. The results are shown in Table 3.

Table 3.—Water quality evaluation for test basins.

	Treated	Treated Breeding
pH	7.24	6.67
Electrical conductivity	501	2801 micromhos
Alkalinity	114	466 ppm
Hardness	118	580 ppm
Chlorides	95	560 ppm

The unusually high readings in the treated-breeding basin may be indicative of an intrusion of brackish water through a separation in the catch basin line.

Conclusion: Our data dealing with the area of Altosid Briquets, especially in areas of highly organic water, has been very encouraging. The period of effective population suppression of 8-10 weeks is considerably longer than that suggested by the Zoecon Corporation. (Table 1.)

In summary, it is readily apparent that regardless of the intended control practice of the District, whether it be chemical or biological, neither method will perform to its utmost unless basins are maintained in a debris-free manner. By keeping the residents of the district informed as to what, why, and when we do what we do, their cooperation in avoiding debris accumulations may be attained.

ENTOMOLOGICAL EVALUATIONS FOLLOWING TROPICAL STORM KATHLEEN, IMPERIAL COUNTY, 1976

Harvey I. Magy[1], Donald L. Rohe[1], Claire V. Thomas[2], Moise B. Mizrahi[3], Robert Miller[4], and Roberta J. Bebout[1]

Following Tropical Storm Kathleen, which struck Imperial County on September 11, 1976, an evaluation of the storm's effects on the mosquito population of the Imperial Valley was made by cooperating local, state and federal agencies, coordinated by the California State Health Department. The storm dumped three inches of rain within a few hours, causing flash floods in the western desert part of the valley causing death, and extensive damage to buildings in the small desert town of Ocotillo, and cut main highways and railroads. The runoff water from the barren hills and mountains broke through the Westside Main Canal flooding the land west of the New River, which is a major drainage channel (see map, Figure 1), and also flooding the plains of the New River.

An aerial inspection of the Imperial Valley five days after the storm revealed widespread flooding of agricultural lands, overflowing agricultural drains, water impoundments in normally dry desert swales, stranded impoundments of desert runoff water adjoining levees, and extensive ponding inside cattle feedlots. Within the cities of El Centro, Holtville, and Imperial there was extensive flooding of backyards and storm drains. There were probably numerous collections of water in backyards, flower pots, buckets, tires, fish ponds, and other containers. All of these sources can produce mosquito vectors of St. Louis Encephalitis (SLE), such as *Culex tarsalis* and *Culex pipiens quinquefasciatus* complex, with the latter more often found in urban sources. For purposes of this report, the latter species will be referred to as *Culex pipiens*.

Within ten days of the tropical storm, there were additional scattered storms in the valley creating pools of water in previously unaffected areas. These storms replenished pools of water that were drying up, thus perpetuating and extending the mosquito problem, adding to the threat of an SLE disease outbreak.

Mosquito control and surveillance activities by the Imperial County Health Department were rapidly augmented by equipment and personnel of the California State Health Department, the San Diego County Health Department, the U. S. Naval Vector Control Section at Alameda, the Coachella Valley Mosquito Abatement District and the Southeast Mosquito Abatement District.

DISEASE CONSIDERATIONS.—Imperial County has had a continuing history of arboviral disease in humans and horses for at least twenty years prior to 1973 (Olson 1977). With more intensive study since 1971, arboviruses were recovered almost yearly in mosquitoes as reported by Work (1974), Emmons et al. (1973, 1974, 1975, 1976), and Madon (1973). There was ample evidence to alert public health officials about a real threat of SLE to humans in 1976. In June, 1976, SLE virus was recovered by a State Health Department survey team from pools of mosquitoes near the towns of Calexico, Seeley and Brawley (Emmons 1977) which are located adjacent to the New River. Webb et al. (1977) reported extensive SLE virus activity in *Cx. tarsalis* mosquito pools in June, July and August at collecting sites in a special arbovirology study area along the New River extending from the Mexican border 10 miles north towards Seeley.

During the course of this evaluation following Tropical Storm Kathleen, apprehension was heightened when word was received on September 18 that a San Diego woman had apparently acquired SLE while fishing near the Westside Main Canal several weeks prior to the storm. Her husband subsequently developed a positive titer for SLE, although without clinical symptoms (Emmons 1977).

In light of the 1975 outbreak of SLE in 22 states of the United States in which *Cx. pipiens* was considered to be the prime vector there was concern about the transferrence of the disease to *Cx. pipiens* which was known to have occurred in significantly large numbers in the urban-agricultural areas of the Imperial Valley (Magy et al. 1976). Bown and Work (1973) also demonstrated significant SLE infection of *Cx. pipiens* along the Mexican border in the Alamo River.

OBJECTIVES AND METHODS.—Entomological evaluations of the mosquito population following the storm were made: (1) To determine the effect of the storm on the mosquito population and the number of complaints directed to the Imperial County Health Department. (2) To determine the severity of the threat of *Cx. pipiens* as a potential vector of SLE in the agricultural and urban areas of Imperial Valley. (3) To determine if there was a SLE virus movement from the New River into farming and urbanized areas to be demonstrated by means of recovering SLE virus in adult female *Cx. tarsalis* and *Cx. pipiens* mosquitoes.

METHODS.—1. Larval surveys were made by the Imperial County Health Department throughout the urban and adjacent farming areas of the western and southern part of Imperial Valley from September 18 until the conclusion of the treatment program in November. This was done in conjunction with a program of mapping sources to assist in directing control operations. Specimens were identified to species and tabulated by place and type of source for ecological evaluations.

2. A CDC-carbon dioxide (CDC-CO_2) light trap was placed in each of thirteen semiurban and agricultural sites and collected several times a week from September 27 through November 4. (See map, Figure 1). Female *Cx. pipiens* and *Cx. tarsalis* were made up into pools of a maxi-

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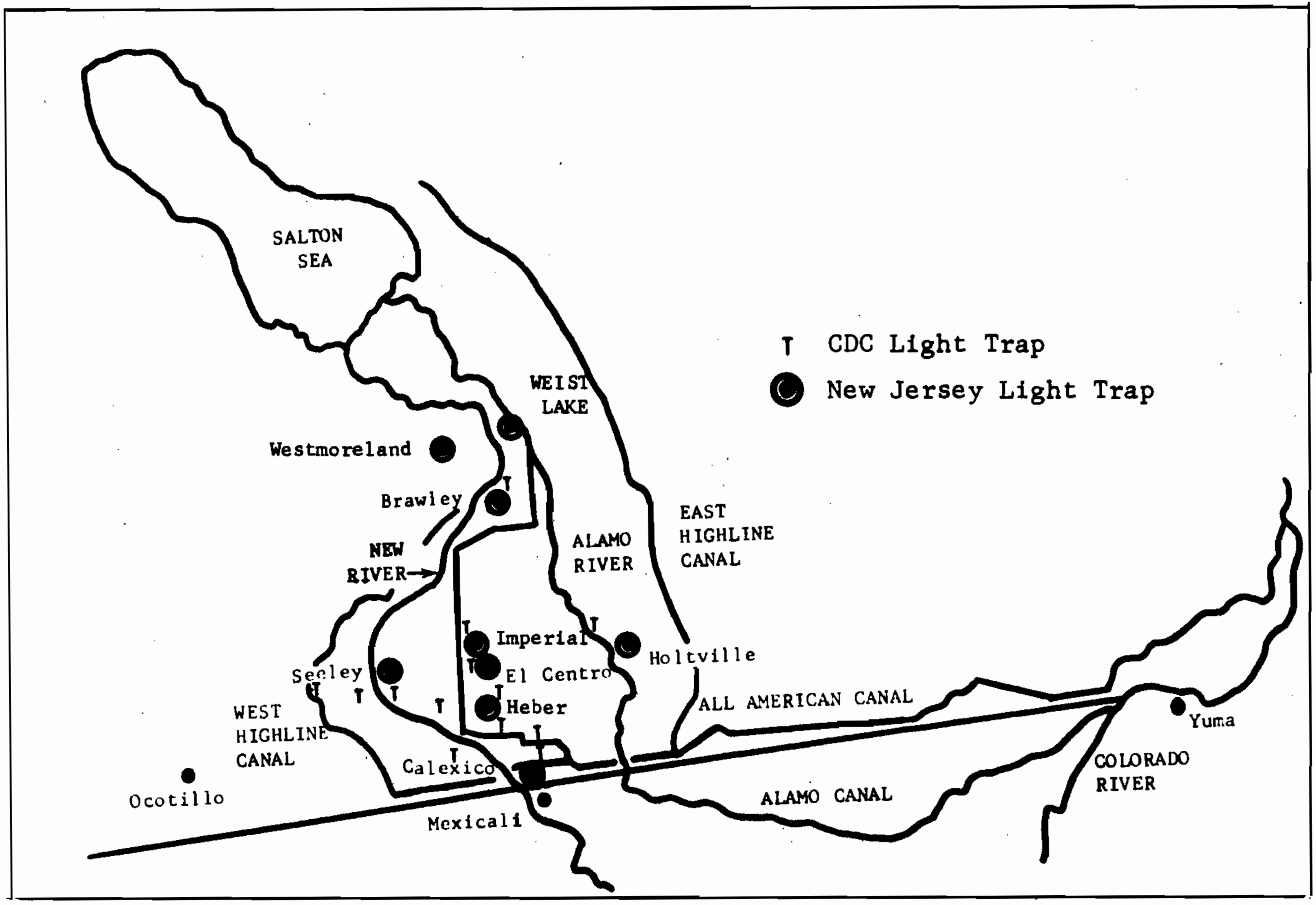

Figure 1.–Adult mosquito survey Imperial Valley, 1976.

mum of 50 females per pool, frozen and shipped to the State Department of Health laboratory for virus determination.

3. Female adult mosquito counts made from March through December, 1976 from nine New Jersey light traps, one in each of nine communities (See map,Figure 1), were compiled and analyzed for seasonal population fluctuations and the possible effect of temperature and humidity, as it might reflect SLE virus transmission and mosquito complaints.

RESULTS AND DISCUSSION.–The summary of the mosquito collections of nine New Jersey light trap sites in urban areas of the valley is given in Figure 2, which also graphically depicts the rainfall as reported by the Imperial Irrigation District at Imperial. This also demonstrates the typical bimodal peak previously reported by Magy et al. (1976) with a summer slump except for one high weekly collection in August. As anticipated *Cx. pipiens* had a low count. The high *Cx. tarsalis* population in the late spring and early summer peak is consistent with the subsequent recoveries of SLE positive *Cx. tarsalis* pools in June, July and August (Work 1977). Following the tropical storm and subsequent rains *Cx. tarsalis* numbers increased. It is of interest that all the peaks as described were above the average ten per trap night which has been suggested by Reeves (1968) as the numbers of female *Cx. tarsalis* adults that seem to be required for transmitting arboviruses in California.

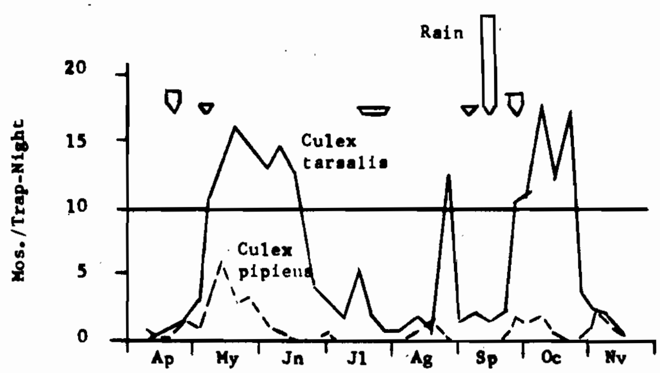

Figure 2.–New Jersey light trap collections and rain. Imperial Valley, 1976.

When comparing the occurrence of *Psorophora columbii* with complaints prior to, during, and after Tropical Storm Kathleen as shown in Figure 3, it is noted that prior to the storm the August high of *Psorophora* brought a moderate number of complaints, but following the storm the complaints more than tripled within several weeks. However, the actual number of *Psorophora* collected did not triple and was much lower than the prestorm peak in August. This might be explained by examining the data from New Jersey light traps at El Centro when plotted with temperature and humidity (Figure 4). For two weeks following the storm the humidity remained high and the temperature

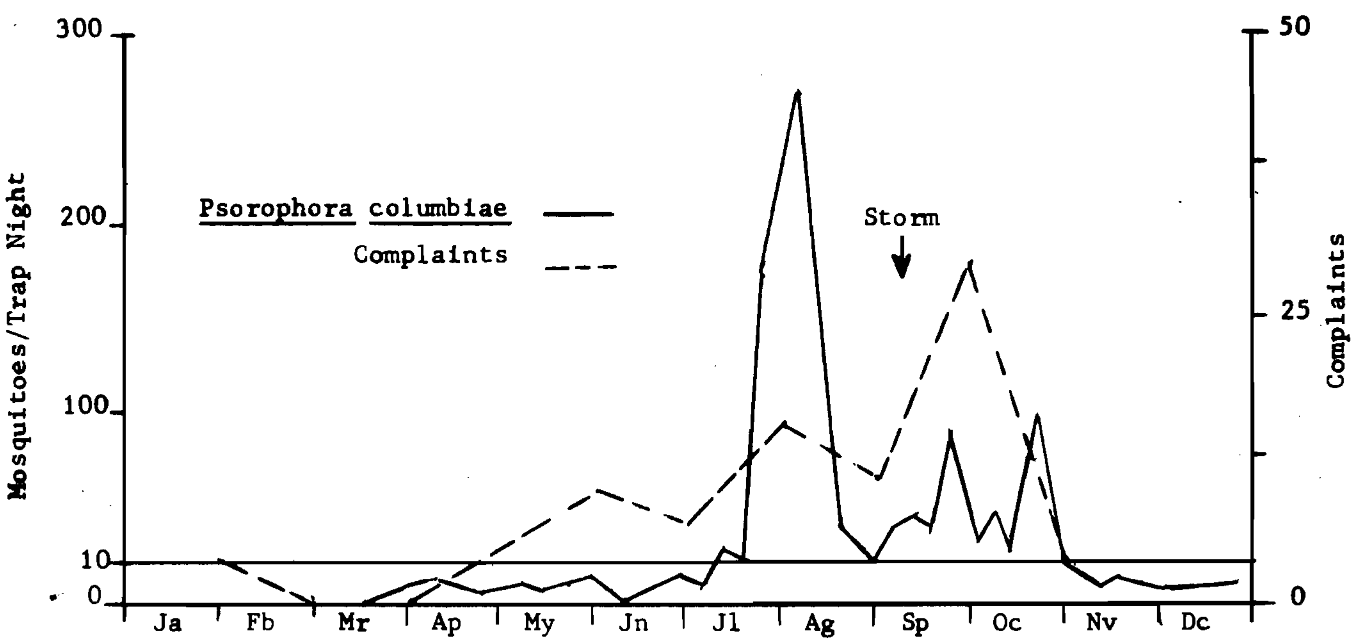

Figure 3.–*Psorophora columbiae* (collected by N. J. light trap at El Centro) and weather (temperature data Imperial FAA; humidity 6 day average reading @ 8:00 a.m., Imperial Irrigation District, Imperial), El Centro, 1976.

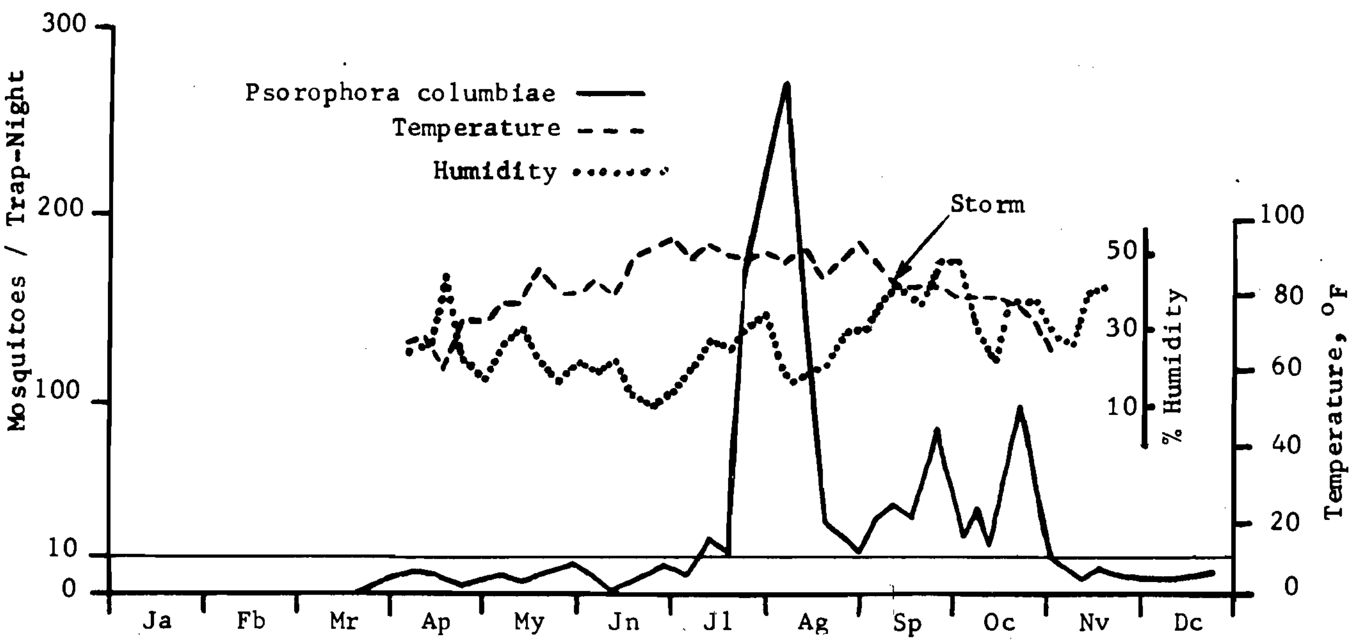

Figure 4.–Mosquitoes vs. complaints, El Centro, 1976.

dropped from the summer highs of the 90°F + to the median temperatures of 80°F (Figure 3.), probably resulting in more favorable biting conditions and accounting for an increase in complaints directed to the health department. However, some of the complaints were probably due to the subsequent emergence of *Cx. tarsalis* and *Cx. pipiens* which were widespread throughout the urban areas and urban fringe farmlands where the New Jersey light traps were placed.

Positive larval collections of *Cx. tarsalis* and *Cx. pipiens* obtained in a five week period in late September were compared to adult females collected by New Jersey light traps and CDC-CO_2 light traps (Table 1). This comparison indicates that the ratio of *Cx. tarsalis* to *Cx. pipiens* were about two to one in the larval collections with a ratio of 15 to 1 with the New Jersey light traps and over 31 to 1 with the CDC light traps. This strongly suggests that the existing light trap collecting procedures may not truly reflect the amount of *Cx. pipiens* that may be produced in an area where SLE is of great concern. The amount of *Cx. pipiens* breeding in this urbanized-agricultural area would indeed have posed a more significant threat had SLE virus entered the eco-system than suggested by the adult numbers collected by New Jersey or CDC light traps. It should be

Table 1.–Collections of *Cx. tarsalis* and *Cx. pipiens* September 1976, Imperial County.

Collection	Species *Cx. tarsalis*	*Cx. pipiens*	Ratio Ct:Cp
Larval Sept. 27 - Oct. 2	144	68	2.1:1
9 N.J. light traps Sept. 27 - Oct. 22 (226 trap nights)	3,338	228	14.8:1
13 CDC-CO_2 light trap Sept. 28 - Oct. 23 (130 trap nights)	20,381	655	31:1

pointed out that the ratio of two to one of *Cx. tarsalis* to *Cx. pipiens* larval collections reflects collections in the largely agricultural area near urban areas. It is possible that if these sources were more urban as they were in 1954 according to Magy et al. (1976) the ratio of *Cx. tarsalis* to *Cx. pipiens* might be almost one to one. It is noteworthy that attempts to collect *Cx. pipiens* in resting stations in culverts, under bridges, garages, etc. were not successful during this period.

The mosquito collections of 13 CDC-CO_2 light traps that were placed (See map, Figure 1.) in the urban and semiagricultural areas of the valley that were set up within a week after the storm are given in Table 2. By four weeks following the storm *Cx. tarsalis* numbers peaked tenfold with SLE virus being recovered from one pool the first week in October. Shortly after, the numbers declined rapidly through early November. The numbers of *Cx. pipiens* pooled remained low as expected because of poor attraction to light traps as discussed in this paper. One *Cx. tarsalis* pool positive for Turlock Virus was also collected in late October.

To account for the low numbers of mosquito pools positive for SLE, it is possible that the storm destroyed large numbers of infected mosquitoes and dispersed the avian reservoir of SLE thus interfering with the cycle of disease transmission. Also, an intensive mosquito larval and adult control program mounted by the Imperial County Health Department and augmented by other agencies (Thomas 1977) probably had a significant impact on interfering with SLE transmission.

SUMMARY AND CONCLUSIONS.–1. Extensive mosquito breeding conditions were created in Imperial Valley following Tropical Storm Kathleen. *Cx. pipiens* and *Cx. tarsalis* larvae were found in large numbers in urban and agricultural areas that are normally dry in September and October. The initial increase of mosquito biting complaints to the health department was probably due to the more favorable humidity and temperature for biting mosquitoes, with *Psorophora columbii* probably being the principal biting mosquito at that time, while *Cx. tarsalis* and *Cx. pipiens* had a more important role several weeks following the storm.

2. The large numbers of *Cx. tarsalis* and *Cx. pipiens* larval sources in rural and semiurban areas adjacent to the New River, an area of significantly high recovery of SLE virus in *Cx. tarsalis* in the summer, was a real threat to the inhabitants of the area. *Cx. pipiens*, a good biter of man could conceivably pick up the virus from suspect avian hosts and transmit it to man. This threat was confirmed by the increasing numbers of adult females of these species collected in New Jersey and CDC-CO_2 mosquito light traps through September, October and early November.

3. The low level of SLE virus in mosquito pools following the storm was possibly due to the dispersal of suspect avian hosts and infected mosquitoes during the storm, but also due to the very intensive adult and larval mosquito control effort launched by the Imperial County Health Department.

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Table 2.–Mosquitoes collected from CDC-CO_2 baited light traps at 13 sites in Imperial Valley, 1976.

Date	Trap Nights	*Cx. pipiens* ♀ Pools	Avg/Trap Night	*Cx. tarsalis* ♀ Pools	Avg/Trap Night	Virus Recovered
Sept. 22-24	29	11	3.6	31	10.2	
Sept. 28-29	26	20	4.7	43	54.4	
Oct. 2-3	26	9	6.8	48	84.8	1 SLE (near Seeley) c.t. 1 Turlock " " c.t.
Oct. 5-6	26	15	15.8	68	126.4	
Oct. 12-13	26	15	12.2	55	89.4	
Oct. 22-23	26	10	8.0	20	23.0	
Nov. 3-4	26	27	13.2	37	5.0	

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DEVELOPMENTS IN IMPERIAL COUNTY FOLLOWING TROPICAL STORM KATHLEEN IN SEPTEMBER 1976

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On September 10, 1976 tropical storm Kathleen struck Southern California causing extensive damage in Imperial and Riverside counties. The storm deposited eight inches of rain in the mountains west of Imperial Valley causing flash floods in the valley. The western side of Imperial Valley was inundated wiping out part of the town of Ocotillo. The Westside Main Canal spilled over into miles of agricultural land. Localized rainfall in Imperial Valley swamped water removal systems simply because the capacity could not accommodate the input.

During the week following the storm Imperial County Health Department received as many complaints about mosquitoes as had been registered in the previous 10 weeks beginning in July, usually a season of high humidity and high mosquito activity. High humidity and somewhat cooler temperatures resulting from the storm greatly increased mosquito activity.

Psorophora confinnis populations in the Valley are always large in the late summer. Increased human biting activity caused extreme annoyance. Rainfall in the center of the Valley a week earlier may have been partly responsible for an increased *P. confinnis* population. September did exhibit an unusual precipitation pattern. Rain fell in the Valley on seven different days with many areas receiving in excess of a year's average within the last two weeks of the month. In fact, the whole year's rainfall was highly unusual, especially at the northern end of the Valley where Brawley, Calipatria and Niland received about two and a half years' worth of rain, almost all in September, October, and November.

The first work day after Kathleen the regular mosquito control staff of the Imperial County Health Department increased efforts to treat known problem areas. This staff consists of two vector control specialists operating two International Scouts equipped for larviciding and adulticiding, under the supervision of one vector biologist. Imperial County contains 500,000 acres of irrigated land in addition to non-irrigated mosquito producing acres along the Colorado River where the primary use is for recreational purposes. The total Imperial County population is only 83,000. Of the seven incorporated cities and eight unincorporated communities, only three have more than four thousand residents.

As the mosquito complaints increased, it was realized that the scope of the problem was greater than anticipated. It became obvious that the available equipment and manpower were grossly inadequate. Sanitarians were pressed into service as larval source locaters and equipment operators. This freed the trucks for almost around the clock service, as long as the equipment was operable. Attempts were also made to obtain additional equipment. Captain George Stains, manager of Coachella Valley MAD, offered invaluable advice. He also loaned Imperial County a Jeep equipped with an oil sprayer for larviciding. Still later he provided an ultra-low volume cold fogger.

Frank Pelsue, manager of Southeast MAD, provided a Scout equipped with a new Microgen ULV unit. Within a week following the storm this equipment was in use by the mosquito control personnel. Our request to our neighbor, San Diego County, resulted in assignment of both men and equipment. They had just received information that a San Diego woman was hospitalized for St. Louis Encephalitis. There was substantial evidence that she had been infected during one of several fishing trips to the west side of the Imperial Valley earlier in the summer (Emmons 1977). Many thousands of San Diego residents routinely visit Imperial County each weekend. Jerry Quick, Chief of Environmental Health and Sanitation, San Diego County Health Department, responded immediately with help which consisted of Moise Mizrahi, their vector ecologist, and two mosquito control operators with a truck and oil larvicidal equipment. They augmented our operations for three full weeks.

A request was also extended to the Navy for help from their vector control unit at Alameda, California. Captain Chew, Commanding Officer of the National Parachute Test Range near El Centro, requested assistance to protect the base personnel. Although it took a full week of negotiations after our initial request, permission was finally granted to send down three men, two trucks, and four ULV units. Lt. Robert Miller, Entomologist, Harold U'ren, Master Mechanic, and Chief Norman Stewart were the men who were sent.

The City of El Centro released one of their service equipment operators to do adulticiding. The Imperial County Manpower Services office also supplied five men to do larviciding and source location.

Southern California Regional Supervisor Harvey Magy mobilized expert vector control personnel of the California State Department of Health to maintain close surveillance of virus activity, to help with source location, and to provide advice and other assistance. Including summer helpers, eight persons participated at various times. The increased surveillance which was conducted was closely coordinated with the work being done by the UCLA research team so that the resulting data would not be redundant.

Aerial spraying of either larvicide or adulticide was first considered within a few days following the storm. Because of the nature of the mosquito sources which were mainly agricultural, covering vast areas, and because of the widely scattered human population, the idea was rejected at that time as too costly and inappropriate.

Beginning the first Monday following the storm and continuing for six weeks thereafter, a seven day a week fogging and larviciding schedule was maintained, fogging both morning and evening when weather and equipment permitted. Larviciding was accomplished almost totally with Flit MLO, and adulticiding was done with Resmethrin, Pyrenone, Dursban and Malathion, choice determined mainly by availability. Quantities used are listed in Table 1.

Table 1.– Chemical usage following Tropical Storm Kathleen, Sept. 10, 1976 to Nov. 30, 1976.

Chemical	Gallons
Flit MLO	700
SBP-1382 (Resmethrin)	93
Pyrenone	115
Dursban	30
Malathion	30

With a budget allocation for the 1976-77 fiscal year of $10,600 for chemicals and equipment, it did not take long for this increased work load and expanded crew to use up that much money. The Imperial County Board of Supervisors granted a request for an additional $15,000, which proved to be sufficient to cover the cost of the chemicals actually used but was insufficient to allow for an aerial spray program which was still under consideration.

Once the area was declared in a state of emergency and then a federal disaster area it was anticipated that funds would become available for mosquito control. This did not turn out to be the case. There seemed to be a good deal of confusion on the part of all concerned as to whether funds could be made available for this purpose.

At first the problem consisted of showing that the status of the mosquito population and disease threat were directly due to the storm. If data from previous seasons had been available this would have been a simple matter. Unfortunately, owing to the fact that the mosquito control program itself was less than two years old and no vector biologist had been employed during the corresponding period the previous year, such information was unavailable. It seemed that it should be intuitively obvious that the storms had greatly increased mosquito production and disease transmission potential, but intuition is not a sound scientific basis from which to draw conclusions.

During the third week following Kathleen, Dr. Robert Taylor, CDC, Atlanta, was invited in by Mr. Terry House of FDAA to make an evaluation of the situation in regard to a potential encephalitis epidemic. After he had taken a flight over the valley to observe the extent of the remaining standing water, and been fully informed of the events that had transpired, of the situation that had existed prior to the storm, and of the current circumstances, he came to the conclusion that there was insufficient potential for an epidemic to warrant spending any emergency money on it at all. His conclusions were in direct conflict with those of Harvey Magy and other California State Health Department personnel, and with Dr. Telford Work, as well as with opinions of members of our own Health Department. However, since the OES discounted all other opinions and went only by Dr. Taylor's, no funds were made available for mosquito control. This effectively ruled out any large scale aerial spray program. It also had the effect of curtailing the length of time that the Navy personnel could remain.

So far we have not received one penny of the emergency relief money that was made available. As far as we have been able to determine, we are not going to receive any. This lack of concern about disease prevention in the face of a clearly defined potential for disease transmission was most disheartening. In effect and in fact they said, "Show us bodies and we'll give you money." This attitude on the part of persons supposedly engaged in the practice of preventive medicine was appalling.

There is definite need for better planning and training of public service personnel at the state and county levels as to their responsibilities in the event of an emergency. Also, there should be clear guidelines written and distributed to all who need them explaining how to fill out all the forms that are required in order to avoid delays and wasted time when time is most precious.

In the Imperial Valley, mosquito populations and complaints remained high until mid-November. Spray operations were continued, to the extent that available personnel and equipment permitted, until that time. No additional cases of encephalitis were reported. We feel that our efforts significantly reduced mosquito populations and reduced the potential for disease transmission.

EYE GNATS PEST AND PLAGUE OF MANKIND

"THE FRIENDLY COACHELLA VALLEY SALUTE"

Mir S. Mulla[1] and George S. Stains[2]

As we sit in this plush meeting room or gaze from the top of the lofty San Jacinto Mountains, there lies before us a fertile and sunny-warm valley, the Coachella Valley which once was no more than a picturesque piece of the Sonoran Desert. Sand dunes and wind-swept hills added to the beauty of this deep valley.

It was not until the turn of the 20th century that man began to tap the subsurface water resources and to cultivate the sand dunes, putting the desert into date gardens, vegetable and field crops. Within a few years, tiny little insects known then as "buzz gnats" but most commonly called "eye gnats", began to pester humans and transmit pathogenic organisms to man and domestic animals. Since the date palm culture and eye gnats appeared on the scene at almost the same time, it was commonly believed that eye gnats were introduced along with date palm trees from N. Africa. This notion as we know it now is false, because there are no *Hippelates* eye gnats present in N. Africa. Moreover, eye gnat plagues were reported from Florida as early as 1895 (Schwarz 1895) and the same year Townsend described our major pest species *Hippelates collusor* as *Oscinus collusor* from Cabo San Lucas, Baja California. We know now that the eye gnats were here in California long before the introduction of date palm culture. Farming and establishment of golf courses provided ideal breeding sources and harborage grounds for these gnats. Due to man's own activity, the eye gnats have found a haven in this valley, especially after transport of water from the Colorado River in the mid 1940's which brought about phenomenal increase in farming and recreation. To drive the persistent pesky gnats away, children, adults and people of all walks of life wave their hands and arms in self defense and, thus perform the "Friendly Coachella Valley Salute".

What are the eye gnats and what do they do? The eye gnats belong to the genus *Hippelates* (Family Chloropidae, Order Diptera), they are veritable pests of man and vectors of pathogenic organisms to man and animals in southwestern, southern, and southeastern states and elsewhere. This genus, according to Sabrosky (1941), is neotropic in origin with ecological homologs occurring in the oriental region. There are between 30-40 species in the genus *Hippelates* which are highly pestiferous to man and higher animals. These insects feed frequently and have a predilection for feeding on the moist secretions of the eyes, ears, mouth, nose, face, and other exposed portions of their hosts. Due to the intermittent but persistent visitation by these insects, they cause a high level of physical and mental discomfort for humans residing, working, or pursuing leisure and recreation in eye gnat infested areas. The most common type of reaction exhibited by humans to eye gnat annoyance is "they (the eye gnats) drive me crazy". One cannot visualize the extent of physical and mental discomfort unless one has lived in eye gnat infested locations or has experienced attacks by these insects.

Aside from being highly pestiferous, the eye gnats are proven vectors of several causal agents pathogenic to man and animals. The spirochaete (*Treponema pertinue*) producing yaws in man has been found in motile state in the gut of *Hippelates* eye gnats (Cuellar 1941, Kumm 1935, Kumm and Turner 1936, Kumm et al. 1935). Other spirochaetal organisms causing mal del pinto were transmitted to volunteer humans through the agency of *Hippelates* eye gnats (Blanco and Parra 1941). Circumstantial evidence gathered in California (Dawson 1960, Schneider 1927), in Georgia (Bengston 1933, Dow and Hines 1957), Texas (Davis and Pittman 1950), and other countries (Vargas 1941) incriminates the members of this genus as possible vectors of Koch-Weeks bacillus causing acute conjunctivitis in humans. More recently, Taplin et al. (1967) considered *Hippelates flavipes* Loew to be the most probable vector of staphylococcal infections of man in Panama. Bassett (1967) reported *Hippelates peruvanus* Becker to be the most persistent feeder on impetiginous skin lesions in Trinidad and recovered β-haemolytic streptococci from these gnats, thus incriminating them in the dissemination of acute nephritis.

The most important species of eye gnats affecting human health and quality of living in the United States are: *Hippelates collusor* (Townsend), *H. dorsalis* Loew, and *H. impressus* (Becker) which prevail in large numbers in the southwestern states. *H. collusor* is by far the most abundant and important species in the southwest and Mexico. In the south central states, *H. pusio* Loew and *H. dorsalis* are quite important. The southeastern states are plagued with *H. pusio* and *H. pallipes*, where humans are attacked by myriads of these gnats in the warmer months.

Considering the distribution, seasonal abundance (8-9 months/year), and pestiferous nature and vector potential of eye gnats in the United States, one can point to *H. collusor* and *H. pusio* as the real problem species in terms of the intensity of their attacks and voraciousness with which they feed on human hosts. These two species are quite distinct from each other biologically and ecologically, but based on morphological features, they have been at times called the same species. Sabrosky (1941), however, gave them specific or subspecific status. Although these two species are very important from the standpoint of their widespread distribution and anthropophilic behavior, this does not imply that other species may not be equally important in certain situations.

Even though eye gnats pose a serious threat to human health and well-being in the infested areas, no practical and economical control measures were at hand. With the advent of synthetic insecticides, a moderate level of suppression was achieved for a short time by the application of soil insecticides to the breeding sources of eye gnats (Dow and Willis 1959, Mulla 1960a, b, 1961, Mulla et al. 1960, Tinkham 1951, 1953). However, it was within a short time that eye gnats developed a high level of resistance to most of the chlorinated hydrocarbon insecticides in California (Georg-

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hiou and Mulla 1961, Mulla 1962). By 1962-63, the Coachella Valley Mosquito Abatement District in California, on recommendations of the senior author, ceased all larvicidal programs using organochlorine insecticides.

As a substitute to persistent chemicals for eye gnat control, habitat management provided a partial solution to the eye gnat problem in southern California (Mulla 1962, 1963). This practice, consisting of weed and cover crop control in perennial crops where eye gnats breed profusely in the soil, could not be adopted in all the breeding sources in a given area. For example, vegetation control in numerous breeding sources, such as golf courses, household lawns and flower beds, and annual crops, could not be expedited. These breeding sources also contribute to the production of *Hippelates* eye gnats in southern California and elsewhere.

Research on the infestation and propagation of natural enemies of eye gnats and their release of these in southern California did not produce any noticeable effects on the equilibrium level of eye gnat populations in this region (Bay and Legner 1964, Bay et al. 1964, Legner and Bay 1964, 1965, Legner et al. 1966). Ultra-low volume applications of quick-knockdown insecticides, such as dichlorvos, naled, and others, although causing mortality in caged eye gnats, did not produce any reduction in field populations of eye gnats in North Carolina and California (Axtell and Edwards 1970, Mulla et al. 1969 unpublished data). Chemical repellents have been studied, but no suitable safe and effective compounds have been found (Axtell 1967, Mulla 1963c).

It thus appears that the eye gnats, posing an important public health menace, have escaped all control measures and methodologies studied thus far. The quality of living, labor efficiency on the farms and construction projects, pursuit of outdoor recreations, and the enjoyment of outdoor living are severely affected by the presence of hordes of eye gnats in infested areas.

How long have the gnats been a problem in the Valley and elsewhere? Florida was plagued a few years earlier than California (Schwarz 1895). In talking with the old timers in the Coachella Valley, gnats became pestiferous sometimes during the 1908-1910 period. By 1913 they prevailed in pestiferous proportions, but it was not until the 1920's that the gnats became a public menace and were believed to be involved in the transmission of pink eye or "gnat sore eye" in the Coachella and Imperial Valleys where schools had to be closed on account of pink eye epidemics (Schneider 1927). The gnats became so intolerable that the community requested help from the University of California. W. B. Herms and his student, R. W. Burgess, initiated studies in 1926-1930 on *Hippelates* eye gnats, using an adobe house built for them as their laboratory (Herms 1926, 1928, Herms and Burgess 1930). The United States Congress appropriated an annual emergency budget of $12,000 (equal to some $120,000 now) for investigating the gnat problem in this Valley. D. C. Parman and D. G. Hall from the U. S. Department of Agriculture joined the research group from the University. This team developed a great deal of fundamental information regarding the biology, ecology, and feeding behavior of the gnats (Burgess 1935, 1951). Breeding sources were determined, life history was studied, and a large scale bait trapping program employing chopped liver with urea was initiated in the early 1930's (Parman 1932). By this time, the Coachella Valley Mosquito Abatement District, organized in 1928 specifically to abate eye gnats, was in full operation.

During this period, the total farmland was no more than 10,000 acres as compared to some 65,000 acres today (Figure 1 and Table 1). There were no golf courses, motels and resort establishments of the type one sees in the Valley today. At the present time, there are more than 43 country clubs and golf courses (Figure 2), and many more are scheduled for development in this Valley, providing ideal breeding and harborage habitats for the gnats and presenting optimum conditions for encounter between eye gnats and their hosts, man and domestic animals. Additionally, the influx of city folks into the Valley from near and afar, and the phenomenal increase in permanent resident population during the past 25 years, have given a different dimension to the gnat problem. This tremendous growth of farming, the development of golf courses and country clubs, and the great influx of tourists and visitors since 1950 into the Coachella Valley, has immensely increased the problem of pestiferous eye gnats.

Limited bait trapping continued to be the major abatement practice up to the early 1950's. However, with the advent of residual organochlorine insecticides, soil larviciding was initiated using aldrin at the rate of 1.5-2.0 lb AI/acre. This material was disked into the soil and it was believed that one treatment provided control of eye gnats for 1-2

Table 1.–Five year average of crop acreage in Coachella Valley.

	Acreage of[1]				Total
Year	Citrus	Deciduous	Truck	Field	average
1935	2,700	5,100	3,900	2,500	14,200
1940	2,800	5,700	4,100	2,600	15,200
1945	2,700	6,700	6,800	2,000	18,200
1950	2,500	10,500	8,800	5,500	27,300
1955	3,500	13,800	11,100	16,400	44,800
1960	9,400	16,200	12,200	15,400	53,200
1965	14,800	16,600	14,800	14,000	60,200
1970	18,000	14,000	13,900	11,200	57,100
1975	19,900	11,700	14,200	11,400	57,200
1976	19,200	11,800	18,400	12,900	62,300

[1]Citrus includes oranges, grapefruit, tangerines and lemons. Decidious fruits include dates, grapes, peaches, figs, and olives. Truck crops include all vegetables. Field crops include alfalfa, cotton, sugar beets, and pastures.

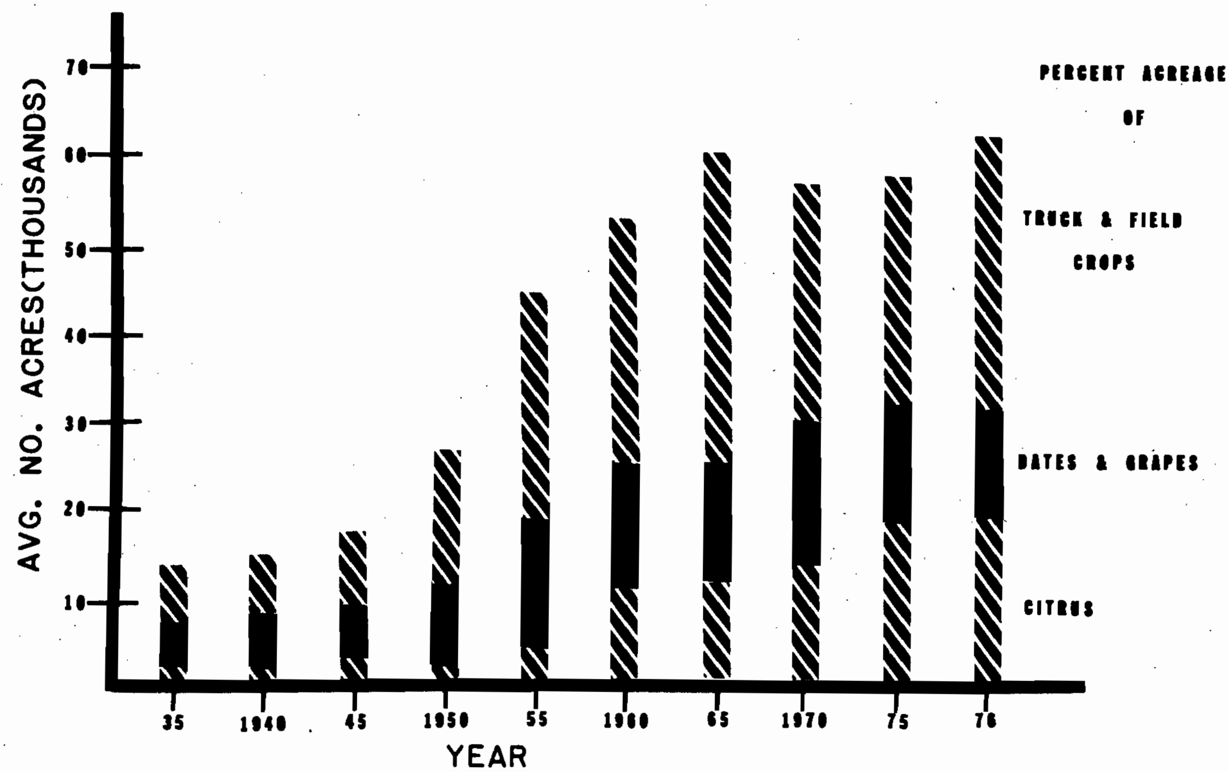

Figure 1.–Acreage of farmland irrigated and tilled since 1935 in the Coachella Valley of southern California, where the eye gnat *Hippelates collusor* breeds profusely in the cultivated soil.

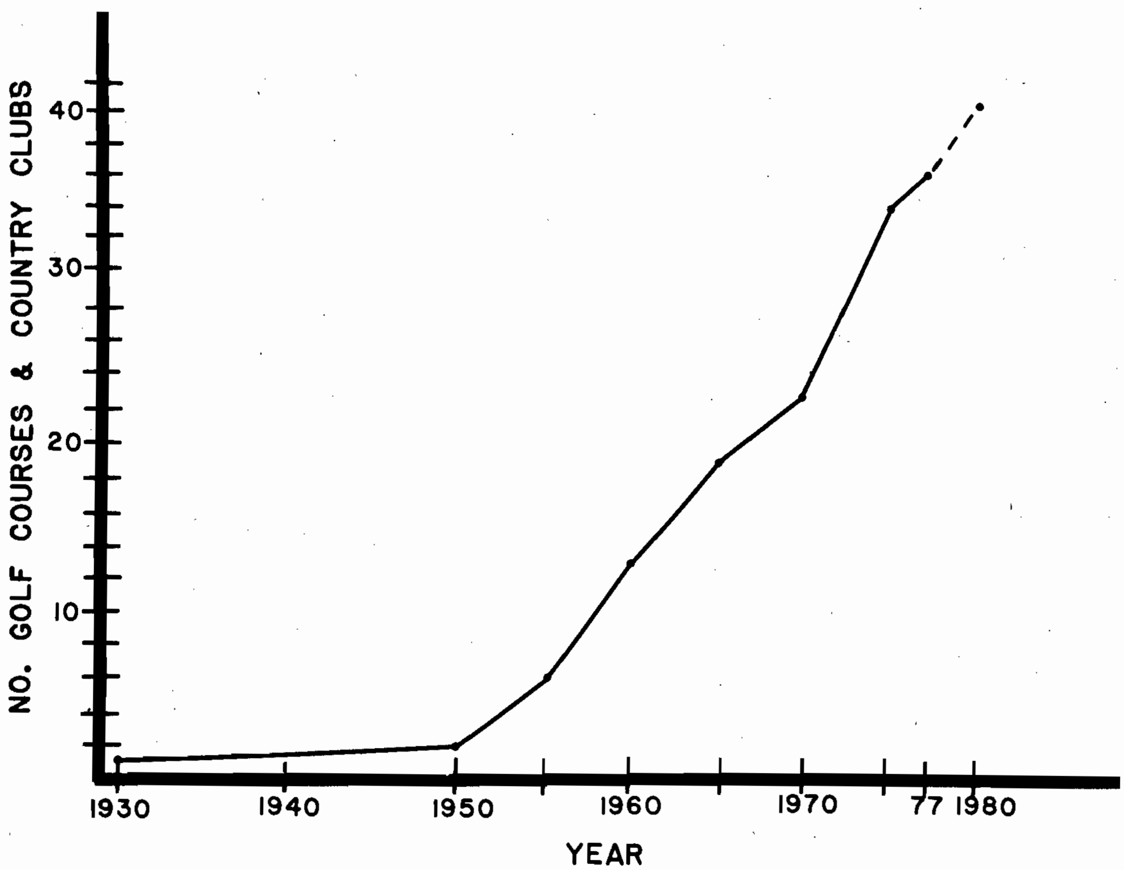

Figure 2.–The growth and development of golf courses and country clubs in the Coachella Valley of southern California. *H. collusor*, a pestiferous gnat, breeds and rests in these habitats, and annoys people seeking outdoor leisure and recreation.

years. Eye gnat populations were markedly suppressed by the application of new organochlorine and organophosphorus insecticides to crops where eye gnats propagated and prevailed. Notwithstanding this, heavy populations of gnats reappeared in 1954-56. In 1956, the senior author, with assistance from the Coachella Valley Mosquito Abatement District, was given the responsibility to study the *Hippelates* eye gnat problem. The successful colonization of eye gnats in the laboratory (Mulla 1962b) made it possible to study their resistance to insecticides (Mulla 1962a). They were found highly resistant to aldrin, dieldrin, and others. The use of these compounds was discontinued in 1958.

DDT still seemed somewhat effective, but even this material at rates as high as 20 lb/acre did not produce a high level of control. Its use was discontinued in 1962-63. At this time, some organophosphorus insecticides were employed as residual soil larvicides. Their use was also terminated by 1965-66, as development of resistance to soil larvicides was quite rapid.

Since no effective insecticides were at hand for eye gnat control, farm management practicies such as noncultivation, or cultivation of ground that is dryish, and the application of weed oils to cover-crops and crop residues 2-2-5 days prior to disking provided some degree of solution. These practicies, however, were costly and could not be implemented over the entire breeding and harborage areas.

With the encouragement and assistance of the Coachella Valley Mosquito Abatement District, a new research program on the development of chemical attractants and their formulations was initiated in 1968. Dr. Y.-S. Hwang, an organic chemist, joined the research group of the senior author at the Department of Entomology, University of California, Riverside, and started to isolate and identify the chemical nature of attractants produced on putrefaction of whole chicken egg suspension in water.

The use of chemical attractants and the development of their formulations were the only practical option left for the management of eye gnat populations. As described earlier, eye gnats are attracted to a variety of putrefying proteins, among which fermented aqueous suspension of chicken whole-egg powder was found to be highly attractive (Mulla et al. 1960b, c). Some physico-chemical factors influencing the attractancy of the fermented aqueous suspension and its distillate were studied (Hwang and Mulla 1973 a). As a result of these studies, an attractive material, prepared by lyophilizing the fermented aqueous suspension of whole-egg powder, was developed against eye gnats, and its efficacy was determined (Mulla et al. 1973, Mulla 1973). Various attractive formulations containing the putrefied freeze-dried egg powder and toxicants were formulated and evaluated (Mulla and Axelrod 1974a, b). They produced good attractancy, kill, and knockdown of the eye gnats. These baits, however, are quite variable in efficacy and have a number of disadvantages in area-wide control programs.

During the course of our investigations on the chemical nature of attractants emanated from fermented aqueous suspensions of whole-egg powder, oleic and linoleic acids were isolated and identified from the fermented whole-egg slurry. Since these two unsaturated fatty acids per se were not attractive but enhanced the attractancy of the main attractants present in the water-soluble fraction of the distillate, they were designated as coattractants (Hwang and Mulla 1971). The water-soluble fraction showed weak attractancy against the eye gnats; therefore, it was concluded that the main attractants existed in this fraction. From the water-soluble fraction, a crystalline residue possessing weak attractancy was isolated (Hwang and Mulla 1973a, b). All of the chemicals required for a high level of attractancy against eye gnats were finally isolated from fermented egg and chemically identified (Hwang et al. 1975).

Progress in research on the chemical nature of volatile attractants (produced on putrefaction and probably the same volatiles that are emanated from the human body) has been significant. It is known now that 5 chemicals in proper proportions are needed to produce highly bioactive attractant formulations for the eye gnats (Mulla et al. 1977). The compounds and their concentrations are:

Trimethylamine hydrochloride	2.5%
Ammonium sulfate	4.0%
Indole	0.2%
Linoleic acid	1.0%
n-Butyric acid	2.0%
Inert (fishmeal)	90.3%

n-Butyric acid has a bad odor and is not to be used where odor is a problem. This formulation, when placed on damp ground or substrate, liberates gaseous trimethylamine and ammonia and the other attractants over a period of a week or so. The formulation, prior to application, is mixed with a dichlorvos-sugar bait (1:1). The mixture is applied as spot treatments (2-5 g) about 50-60 meters apart. Gnats attracted by the formulation are killed within seconds after they come in the vicinity of the attractant-toxicant formulation. This new formulation containing chemical attractants and toxicant is applied to most of the breeding and harborage grounds in the Coachella Valley. Complete coverage is not at hand as yet. The program has had great success in the residential and recreational establishments where the attractants were applied. Agricultural breeding sources with moderate eye gnat production have also shown declines in populations following initiation of the program. However, eye gnat populations in heavy producing areas have not been materially depressed as yet. In these habitats, eye gnat population is so high that the present system of coverage and spot treatments has not produced desired effects.

Research is underway to introduce improvements into the program and to develop more effective and long-lasting formulations of the chemical attractants. Toxicants with stability and high activity are being investigated. The physiology and attraction behavior of the target insect will also be investigated, which hopefully will lead to more efficient use of chemical attractants against eye gnats.

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POPULATION DYNAMICS OF CHIRONOMID MIDGE LARVAE IN WESTLAKE, CALIFORNIA

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ABSTRACT

Population trends of chironomid midge larvae in a manmade lake (Westlake, California) were studied during 1972-1975. Westlake, having 160 surface acres, 10-12 ft deep with 9 miles of shoreline, is located 40 miles west of Los Angeles, California, at an elevation of 870 ft above sea level. This lake was filled and developed during 1967-69.

Chironomid midge larval density was assessed by taking benthic samples at 14 stations in the lake. Larvae were segregated into the subfamilies Chironominae and Tanypodinae.

Over the 4-year period, 2 significant changes have occurred in the midge fauna of this lake. First, the number of larvae steadily declined from a yearly average of 39.1/sample (156/ft^2) in 1972 to 13.8 (55/ft^2) in 1975. The second change involved a switch in composition of the 2 subfamilies from 17% Chironominae and 83% Tanypodinae in 1972 to 79% Chironominae and 21% Tanypodinae in 1975. These changes are probably natural phenomena taking place in response to changes in the quality of water and physicochemical conditions at the lake bottom.

INTRODUCTION.–The number of residential-recreational lakes developed in southern California is rapidly increasing. These lakes, soon after filling, experience weed, phytoplankton, and nuisance midge problems. These lakes are generally shallow, and drainage from both streets and lawns introduces large quantities of nutrients and organic material into the aquatic system. The introduction of nutrients and the resulting phytoplankton growth is particuarly favorable for the production of nuisance chironomid midges, as phytoplankton constitute an important food item for midge larvae. As the lakes become more productive and the lower water strata undergo periods of oxygen reduction and increases in metabolic products of microbial decomposition, the number of animals adapted to these conditions decreases markedly. The resultant absence of competition from many groups of aquatic animals, plus the ability to survive low oxygen conditions which may exist for extended periods during the warmer months, has allowed chironomid midges to exploit these benthic habitats in shallow man-made lakes.

During the summer, large numbers of chironomid midges emerge from these lakes, becoming a nuisance due to their mating swarms, and invading residential areas that have been constructed along the shorelines. They also create an economic problem for commercial and industrial facilities which may be located nearby. Chemical and cultural control measures have been used in the past to keep populations at a tolerable level.

Problems created by chironomid midges in southern California and measures employed to control them have previously been reported (Mulla et al. 1971, 1974, 1975). The studies reported here were initiated in 1972 at Westlake, California as part of a 4-year study to gather information on seasonal population trends. This information might be useful in future lake management programs and in timing needed insecticide treatments.

METHODS AND MATERIALS.–Westlake (Figure 1) was formed by the construction of a concrete-covered earth-filled dam in 1966, and the lake was filled to capacity in 1967. The water level is maintained by underground springs, surface run-off, and two wells. In the center of the lake there is an island. To increase the total shoreline for lakefront housing and businesses, fingers or inlets of water

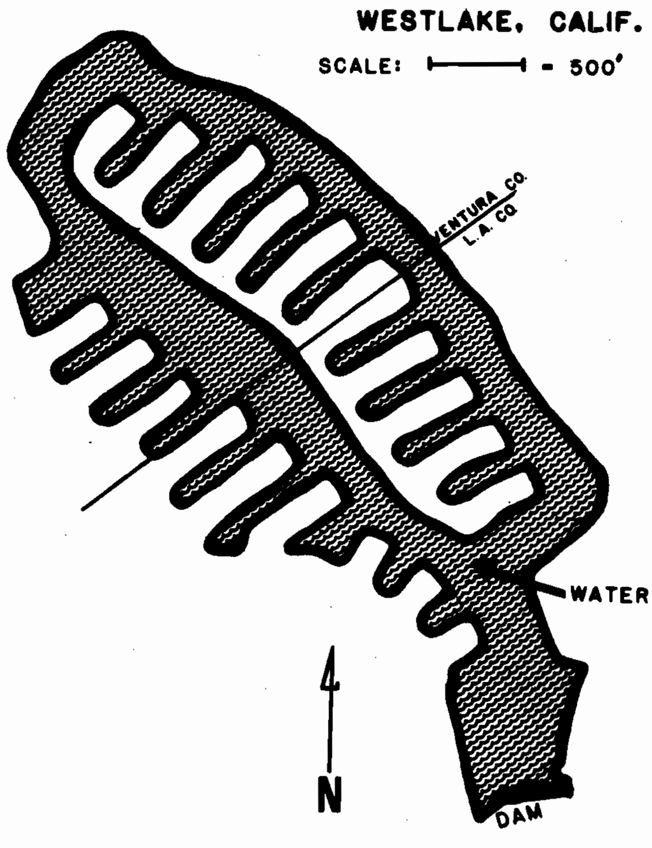

Figure 1.–Map of Westlake, California.

extend into the island and the mainland from the two main channels of the lake. The average width of these fingers is 90 ft and the average length 750 ft.

The total surface area of the lake is 160 acres, and the lake has 9 miles of shoreline which is now totally developed with residential, commerical, and recreational facilities. The average depth of the lake is 12 ft in the main channels and 8 ft in the fingers. The bottom substrate is mud throughout most of the lake, but in certain small areas the substrates range from gravel to sand. Westlake lies at an elevation of 870′ above sea level and is located 40 miles west of downtown Los Angeles, California, and 12 miles inland from the Pacific Ocean.

Sporadic larval samples were taken in Westlake during the years 1968-71, but during 1972, a program was developed to collect more complete information on seasonal population trends. In January, 1972, 14 sampling stations around the lake were established and larval samples were taken at these sites at regular intervals through December, 1975. Water temperature, dissolved oxygen, and pH were measured at the surface and monitored during 1972 and 1973, while in 1974 and 1975, only water temperature was monitored.

For sampling the benthic larval chironomid midges, an Ekman dredge (6x6x2 in.) was used. Each sample was transferred to a plastic bucket and mixed thoroughly with water. The sample was then sieved through a 52-mesh screen (Mulla et al. 1971), transferred to a pint plastic container, and placed on ice for transportation to the laboratory. The larvae were floated using procedures developed previously (Mulla et al. 1971). After segregation into the 2 subfamilies, Chironominae and Tanypodinae, the larvae were counted using a 5X magnifying lamp.

RESULTS AND DISCUSSION.—The species of adult midges collected from Westlake include the following tanypodine species: *Procladius sublettei* Roback, *Procladius freemani* Sublette, *Psilotanypus bellus* (Loew), *Tanypus punctipennis* Meigen, and *Tanypus nubifer* Coquillett (=*grodhausi* Sublette). Adult chironomine species collected were: *Chironomus frommeri* Atchley and Martin, *Chironomus decorus* group (=*attenuatus* group), *Dicrotendipes californicus* (Joh.), *Cryptochironomus darbyi* (Sublette), *Parachironomus tenuicaudatus* (Malloch), *Cladotanytarsus viridiventris* (Malloch), and *Tanytarsus dendyi* Sublette.

The population trends of larvae in the benthos are presented in Figure 2, and insecticide treatments made during the course of this study to reduce larval chironomids are also indicated. Treatments were made when counts were between 40-50 larvae per sample and midges were emerging, In both the 1972 and 1973 treatments of chlorpyrifos (Dursban®), 1500 lbs of the 2% granule formulation (.19 lbs AI/surface acre) was used and in the 1974 temephos (Abate®) treatment, 1600 lbs of the 2% granule formulation (.20 lbs AI/surface acre) was used. The efficacy of these treatments varied greatly with regard to the degree of suppression and length of activity as is illustrated by larval population trends following treatments. Except for the second chlorpyrifos treatment made during 1972, all treatments yielded a satisfactory degree of control. The inactivity of the second chlorpyrifos treatment of 1972 remains unexplained.

Larval Biology.—Larvae of the subfamily Chironominae build tubes in which they live on or in the substrate. These tubes or cases consist of substrate particles which are bonded together by silk-like threads secreted by the salivary glands. Feeding takes place at the substrate surface. Chironominae larvae are microphagous, consuming mainly phytoplankton and detritus.

Tanypodine midges inhabiting Westlake are also bottom dwelling, but in contrast to the Chironominae, are atubiculous. These larvae are free swimming and generally move freely over the substrate surface. The mouthparts of this group are strong and modified for a carnivorous diet, and according to Morgan (1949), fellow members of the family make up the majority of their diet. Among carnivor-

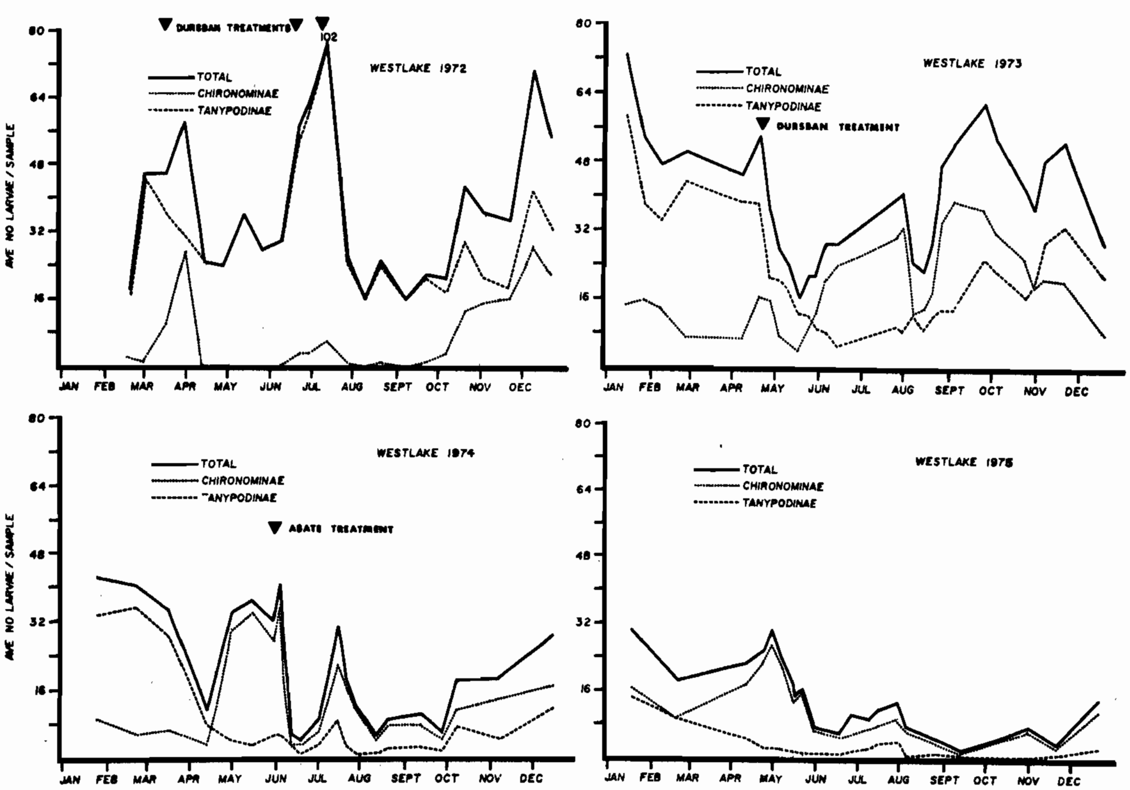

Figure 2.—Chironomid larval population trends in Westlake, California during 1972-1975.

ous tanypodine larvae, the young (first instars) are largely detrivores (Kajak and Dusage 1970), but beyond the second instar, they shift to feeding on chironomid larvae, ostracods, and other small crustacca, and oligochaetes. Although tanypodine larvae are predaceous, their diet is most likely variable, depending on prey accessibility.

Larval Population Trends.–The population dynamics of larval chironomid midges collected in the Ekman samples during the four year study are presented in Figure 2, and the physical parameters measured during the same period are shown in Figure 3.

In Westlake, midges overwinter in the larval stage in a state of inactivity, induced by lower temperatures and reduced primary production which start in the fall. Although larval populations may be high during the winter (Figure 2, 1972-73), generally, no adult emergence takes place. Some emergence, however, occurs in the winter months during limited periods of warm weather when the lake's water temperature increases substantially.

Chironomid emergence usually begins in the spring and adult midges are present until October or November. If a particular year is characterized by a synchronous emergence (sudden increase in temperatures for 2-3 weeks) of the overwintering population, the larval population may exhibit a decline as illustrated in 1974 in Figure 2. If the emergence of overwintering midges is asynchronous, young larvae of the new year may balance the numbers of those midges emerging from the overwintering population, thus precluding a drastic decline in the larval population.

Density of chrionomid larval populations is governed by weather, physicochemical factors, and predation by other organisms. Tanypodine larvae feed on chironomine larvae, and fish also are important predators that feed on larvae of both subfamilies.

In studies on a eutrophic lake in Indiana, Gerking (1962) showed tht midge larvae constituted 45% of the diet of the bluegill sunfish, *Lepomis macrochirus* Rafinesque and that an estimated 50% of the midge production during the summer months was consumed by this fish. Although the effect of *Lepomis* predation has not been studied in Westlake, this species makes up the major portion of the Westlake fish fauna. Other Westlake fish species include: largemouth bass (*Micropterus salmoides*), black crappie (*Pomoxis nigromaculatus*), channel catfish *Ictalurus punctatus*), bullhead catfish (*Ictalurus melas*), carp (*Cyprinus carpio*), and mosquito fish (*Gambusia affinis*).

It is very interesting to note the switch in benthic fauna of midge larvae sampled in Westlake during 1972-75 (Figure 4). In 1972, the tanypodine fauna was dominant, but by 1975, tanypodines had declined and were replaced by a chironomine fauna. This switch in fauna, as illustrated by Figure 5, is significant in view of its implications for lake management. In 1975, no insecticide treatments were necessary due to low larval populations (below 40-50 larvae per sample) and the resulting low emergence. It is difficult to pinpoint the precise causes of the larval decline and the compositional switch, but it may be explained by the process of eutrophication.

The term "eutrophication" refers to the addition of nutrients. Eutrophication is a natural process that occurs at varying rates in all lakes. In Westlake and other man-make lakes, this "natural" process has been greatly accelerated by the addition of nutrients to the aquatic system. These nutrients are mostly in the form of fertilizers from lawns and street drains. Increasing the nutrient supply of the lake increases the production of phytoplankton. This may result in a developing oxygen deficit for the benthos with prolonged periods of benthic oxygen deprivation. Within the limits of autochthonus productivity, the production of benthic organisms increases with greater eutrophication. Under these conditions, food is generally abundant and oxygen becomes the limiting factor.

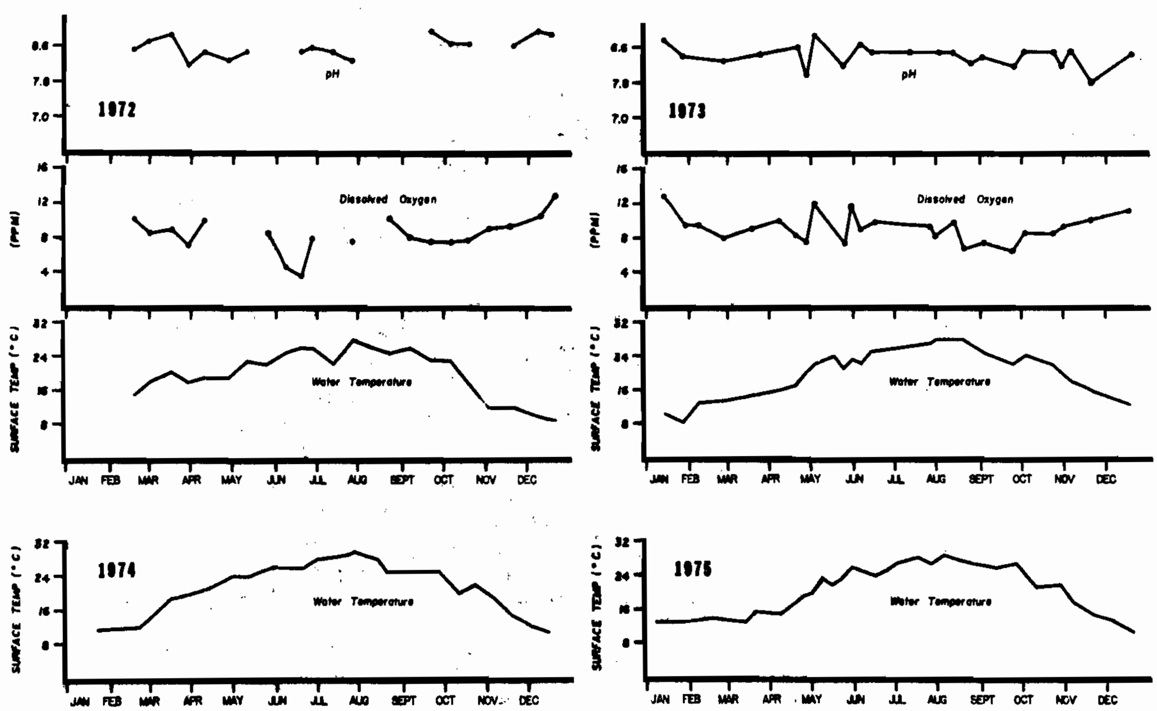

Figure 3.–Physical factors measured in Westlake, California during 1972-1975.

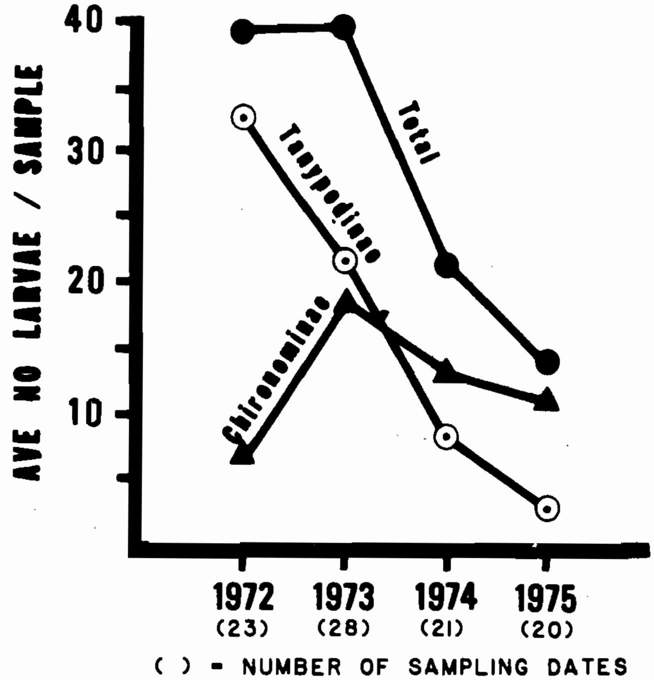

Figure 4.–Changes in relative abundance of midge larvae during 1972-75 in Westlake, California.

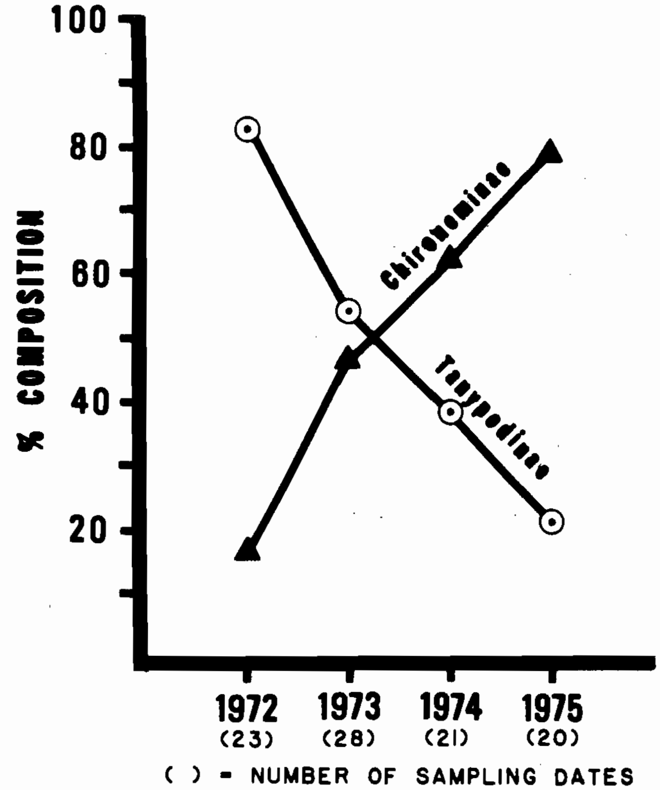

Figure 5.–Changes in relative abundance of the subfamilies Chironominae and Tanypodinae in Westlake, California, during 1972-1975, expressed as a percentage of the total number of specimens collected in each year.

Larvae in the subfamily Chironominae possess hemoglobin in their blood which allows them to function at low oxygen concentrations and for periods of time to survive anaerobic conditions.

Tanypodine larvae are limited in distribution by low oxygen concentrations which are known to occur at the bottom during certain times in the summer. To survive short anaerobic periods periodically occurring at the bottom, the highly motile tanypodine larvae may swim to a higher strata of water where the concentration of oxygen is probably higher (Oliver 1971), but they do not possess hemoglobin which would allow them to survive in prolonged near anaerobic conditions at the bottom. As can be seen from Figure 2, except for 1972, tanypodine larvae poulations for any given year are greater in the fall, winter, and spring since higher concentrations of oxygen at the bottom are available to the larvae during these periods as compared to the summer. In 1972, oxygen concentrations apparently were not a limiting factor and tanypodine larval populations were high throughout the summer.

With further increases in eutrophication, as well as prolongation of the period of oxygen reduction and associated chemical changes, the rates of respiratory activity of the adapted benthic animals may be reduced. If the organic loading becomes so great that the conditions at the mud-water interface are intolerable to the most adapted fauna, as occurs in extremely hypereutrophic lakes, productivity of the animals decreases. Oligochaete annelids are one of the few groups of benthic fauna adapted to these most extreme conditions. Although the scope of this study did not permit quantitative studies on oligochaete populations, large numbers of these organisms were collected in benthic samples throughout the course of the study.

The extent and nature of eutrophication in Westlake are not precisely known at this time. Although there has been a reduction in absolute numbers of larvae taken during the study period as shown in Figure 4, the lake productivity in terms of biomass has probably not declined since most chironomine larvae are substantially larger and heavier than tanypodine larvae. Most likely, the lake is reaching some equilibrium state with regard to chironomid production, but further studies will be necessary to monitor the population levels in the coming years.

ACKNOWLEDGMENTS.–The encouragement and assistance of Mr. Bo Bigelow, Mr. Don Danyko, and Mr. Bert Dell are acknowledged. Dr. James Sublette of Eastern New Mexico University, Portales, New Mexico, confirmed identification of midges.

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BITING FLIES IN THE LOWER COLORADO RIVER BASIN. II. ADULT ACTIVITIES OF THE BLACKFLY, *SIMULIUM VITTATUM* ZETTERSTEDT (DIPTERA:SIMULIIDAE)

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ABSTRACT

Studies were conducted throughout the summer of 1974 and then periodically until November, 1976 on the adult activity of *Simulium vittatum* Zetterstedt in the Mohave Valley of the lower Colorado River Basin.

During the summer months, flies did not disperse more than ¼ mile from the river. Resting flies showed a preference for shoreline vegetation, especially salt cedar. During the cooler months, *S. vittatum* adults dispersed as far as 30 miles from the river. No definite preference for any particular resting substrate was observed during cooler weather.

Two peaks of female host seeking activity, one in the early morning before sunrise and the other in the early evening after sunset, were observed during the summer. Flies were most active between light intensities of 1 to 1000 ft candles. Very little activity was observed during the summer heat, after sunrise and before sunset. As the temperature declined in the fall, the two activity periods lengthened until they eventually coalesced. Low temperatures and high winds were the main inhibitory factors governing female activity during the cooler months.

Male swarming activity was influenced by the same factors that governed female behavior. In the summer, the densest swarms occurred in the evening at light intensities of 1.5-70 ft candles. In the cooler months when temperature was sufficiently high and wind velocity was low, swarming in loose aggregations took place throughout the day.

INTRODUCTION.–In any area inhabited by a number of *Simulium* spp., wide variations in adult behavior may be observed. Bradbury and Bennett (1974) reported a spectrum of color preferences by different species of host-seeking adult females. Anderson and DeFoliart (1961) reported different hosts preferred by several blackfly species. The literature is replete with other examples.

Variations in the behavior of a single species may be observed in different environments. *Simulium vittatum* Zetterstedt has one of the widest geographical and ecological distributions of the Simuliidae in the western hemisphere. It is found throughout Canada (Shewell 1958) and the continental United States (Stains and Knowlton 1943) including Alaska (Stone 1952). It feeds on a variety of hosts. Under certain conditions, it bites man readily (Rees and Peterson 1953) or only occasionally (Mulla and Lacey 1976), but is reported to prefer large ungulates, especially cattle and horses (Peterson 1956, Anderson and DeFoliart 1961). It is mainly a non-biting pest of man, causing discomfort by swarming about the face and ears (Hocking and Pickering 1954, Mulla and Lacey 1976).

Few studies have been conducted on *Simulium* spp. in the arid southwestern United States. This paper presents information on the adult stage of *S. vittatum* in the Mohave Valley area of Arizona, California and Nevada, with emphasis on resting sites, pestiferousness and swarming behavior. The study was carried out during the summer of 1974 and sporadically thereafter until November 1976.

METHODS AND MATERIALS.–Resting Sites. - - Initially, several sites and shoreline plants were sampled from Davis Dam to Park Moabi, California on both sides of the Colorado River to determine optimal resting sites. Adult flies were sampled by taking 5 sweeps of the vegetation with a 12-inch insect sweep net. Once a week in mid-morning (9-10 a.m.), resting behavior was studied at a site just below Davis Dam. Collected flies were counted, sexed, and their numbers reported per sweep. Since the summer heat restricted fly populations to shoreline vegetation, samples were taken only from shoreline to not more than 50 ft inland from the water. A variety of plants on which the flies rest were initially sampled; however, preliminary studies revealed a noticeable preference for salt cedar, *Tamarix pentandra* Pell, so thereafter, sampling was restricted to this plant. Standarized winter sampling for resting adults was omitted, due to the extremely dispersed population and brevity of our study visits.

Female Fly Pestiferousness. - - In our study, due to the lack of actual landing and biting by the host-seeking female flies, flies that came within one and a half feet of the sampler's head over a sixty second period were collected with an insect net. In quantitative (standardized) sampling, the sampler remained inside an automobile prior to the sampling period and betwen individual samples. Non-standardized samples were also taken where the sampler was continuously exposed to flies to provide an index of what an exposed fisherman, camper, or worker typically encounters.

At the beginning of the study, several sites along the river were sampled both qualitatively and quantitatively for host-seeking female flies. Since the area just below Davis Dam was found to have the largest population of these flies, a standardized weekly sampling program was carried out there. When possible, sampling was carried out over the entire range of the activity period, both morning and early evening during the summer of 1974. Samples were taken every 15 minutes from 0530 hrs, just before light until activity ceased soon after sunrise in the morning and from just before sunset until dark at approximately 2000 hrs. The same host bait was used for all quantitative pestiferous samplings.

Incident light intensity, wind velocity, and ambient air temperature were measured just prior to each sampling with a Gossen® Luna Pro light meter, Dwyer® wind meter, and °C thermometer, respectively.

Male Swarms and Mating Activity. - - During the summer of 1974, male swarming activity was measured concurrently with sampling of female flies in the Davis Dam area weekly, both early in the morning and evening. Quantitative sampling of swarms was conducted by sweeping through the swarm with a 12 in. insect net. Flies collected were counted and checked for the presence of females; light, temperature, and wind velocity were measured in the manner described above. The majority of the information gathered on male swarming behavior was qualitative, since repeated sampling of even large swarms resulted in dissipation of the swarm.

Observations were made not only at the Davis Dam site, but also at Laughlin and South Point, Nevada, and at Bullhead City, Arizona.

Information was also gathered on oviposition activity and nectar food sources.

RESULTS AND DISCUSSION.–Resting Sites. - - Samples taken between Davis Dam and a point 33 miles downstream showed that shoreline vegetation near the Dam harbored the densest population of *S. vittatum* adults. More flies were taken between sunrise and 10 a.m. than were taken later during the day. In the afternoons, flies moved down from the vegetation to the moist rocks, especially when the water level was low.

In 25 samplings of salt cedar and arrowweed (*Pluchea sericea*) at various sites and times at the shoreline, an 11 to 1 preference for salt cedar over arrowweed was found (1549 to 145). These two plants make up the majority of streamside flora.

In addition to preferring shoreline salt cedar, insects chose the shaded side (Arizona, AM) of this plant facing the river over the unshaded side facing the land 103 to 4 (25 to 1) in 2 samples. Salt cedar is preferred as a summer resting site because of its high transpiration rate which provides optimum humidity for resting adults.

Adult *S. vittatum* also showed a heavy preference for Mesquite (*Prosopis juliflora*) when it was present, but the numbers of this plant were relatively low along the shoreline. In addition, it proved virtually impossible to sample with an insect net because it is thorny.

Samples taken at a high population density (30 Aug) from shoreline and at distances inland yielded the following results: 366 flies/sweep from the shaded shoreline *Tamarix*; 217 flies/sweep from shaded *Tamarix* 30 ft from shore; 69 flies/sweep from shaded *Tamarix* 40 ft from shore. At a lower population density (5 Sept), sampling at the same site and time of day from the same vegetation yielded the following results: 31 flies/sweep at shoreline; 1 fly/sweep at 30 ft inward; 0 flies/sweep at 40 ft inward. The negative correlation (mean r= -0.97) between resting fly density and distance from the river is likely to be a function of decreased humidity and increased temperature. Man-made structures at the shoreline also provided suitable resting sites for the flies. Boats, docks, and the cement walls of the dam forebay and spillways harbored resting flies. Densities of resting flies became great enough at peak populations to obscure the surface below them.

Flight activity is initiated at these sites and from vegetation disturbed by contact with man or waves from passing boats.

The heaviest resting populations were generally found near the densest larval populations. Two of the most populated areas were Davis Dam and Riviera Beach, Arizona. Conversely, the portions of the river with scant larval populations yielded few resting adults in the nearby vegetation. Park Moabi, California, and the Havasu Wildlife Reserve, Arizona, harbored only a few of these flies. Results of quantitative sampling at Davis Dam from August 6 to September 21, 1974 during the period of 9-10 AM are shown in Figure 1. Although adult *S. vittatum* emerged continuously, definite peaks in population density were apparent. Resting female flies in this region of the river outnumbered males 1.29 to 1.

The summer heat restricted both lateral and vertical dispersal of *S. vittatum* from the river and in the vegetation along the banks (see Table 1 for temperatures).

Flies removed from the vegetative protection and exposed to sunlight in average summer afternoon temperatures died within minutes, and usually in less than 60 seconds.

Strong winds occasionally altered resting population densities by driving flies to a lower position on the substrate. Winds during the summer months, however, were generally mild and from the south. High winds in spring, fall, and winter, undoubtedly facilitated the dispersal of

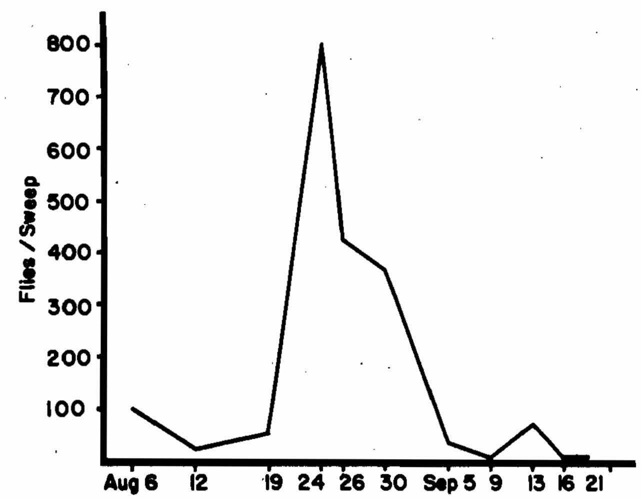

Figure 1.–Population density of adult *S. vittatum* sampled from resting sites in salt cedar at Davis Dam, Colorado River (samples taken between 9-10 a.m., Aug. - Sept. 1974).

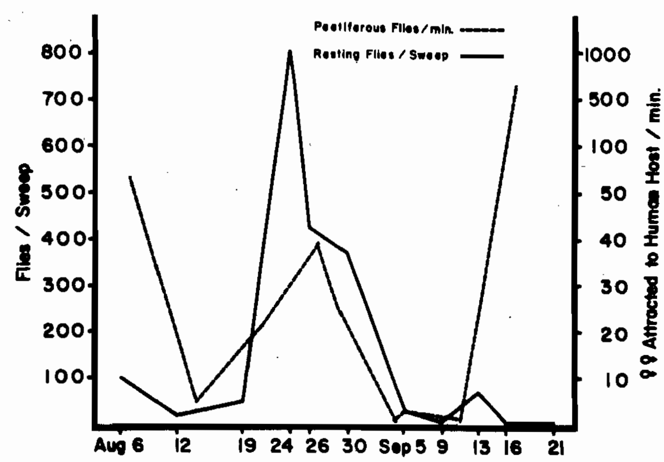

Figure 2.–Pestiferous (attracted to humans) and resting (in tamarisks) populations of *S. vittatum* along the Colorado River below Davis Dam (Aug. - Sept. 1974).

adult flies 20-30 miles from the river. No such dispersal was observed in the summer.

Late fall and winter resting populations proved more difficult to quantitate; they were more dispersed, but they were found in greater numbers along the shoreline vegetation. No definite preference for any one type of resting substrate was evident.

Female Pestiferousness. - - The peak numbers of female flies attracted to human bait during the summer period (standardized sampling) are graphed in Figure 2. The actual number of host-seeking female *S. vittatum* may be considerably higher under nonstandardized conditions (host continuously exposed). Although up to 645 were netted/min (Sept. 16, 1974) with the standardized method, 1256 flies/min were taken during same general period when the sampler was continuously exposed. From mid-August until September 9, 1974, the density of host-seeking females roughly paralleled the density of resting flies (Figure 2). A parallel of the two densities was not observed prior to or after this period.

While many factors influence the pestiferous behavior of female *S. vittatum* in the summer on the Colorado River, our studies indicate that light intensity, temperature and relative humidity exert the major influences. Winds 6 mph or higher reduce activity.

Temperatures low enough to inhibit adult activity were not observed during the summer months. High temperatures inhibited pestiferous activity considerably, but activity usually ceased shortly after light intensities of 1000 ft candles were reached and before temperatures above 27°C were reached. Early evening temperatures over 35°C did not inhibit the female flies.

We believe that light exerts the greatest influence on the host-seeking activity of the females during the summer months. Prior to sunrise, the temperature changed very gradually as the light intensity increased geometrically. In a few cases, morning air temperature declined as the light intensity increased. This was due to the change in wind direction (coming from the river rather than from the desert). Sometimes the reverse occurred during the evening samplings. In both cases, the reverse trend in temperatures as related to light intensity did not affect female activity appreciably.

The graphical expression of female attraction to human bait as related to light intensity is shown in Figure 3. Periods of moderate to high (Aug. 7) and low to moderate (Aug. 21) pestiferous activity are shown. The early morning and evening peaks of activity, as shown in this Figure, were apparent throughout the summer. The range of light intensities during a full period of activity was generally between 1 ft candle to 1000 ft candles, similar to the findings of Shogaki and Yoshida (1956). In their study, the biting activity of *S. nacojapi* was influenced by an optimal illumination of ca. 100-1800 ft. candles.

As late summer temperatures dropped to a range of 21°C/37.2°C maximum for the period of September 11-21, pestiferous activity persisted past the 1000 ft candle light intensity in early and mid-morning. Also during this period, the range of host-seeking activity was extended from shoreline to approximately ½ mile inland. As temperatures declined further in early fall, the two peaks of activity became extended and eventually coalesced. Figure 4 depicts a typical activity pattern when above-minimum temperatures are reached and wind velocity is not sufficiently high to inhibit host-seeking activity. Light appears to exert less influence on the late fall, winter and early spring activity pattern. Activity during this and other sampling periods throughout the cooler months increased sharply after sunrise with a subsequent rise in temperature. The late afternoon decline in host-seeking females (Figure 4) was probably due to an increase in wind velocity (7-9 mph) and not to an increase in light intensity or temperature, which remained fairly stable.

In addition to minimum temperature, high wind was observed to be another major factor controlling the activity of pestiferous female flies. Table 2 presents data obtained the day after that shown in Figure 4. The increase in wind velocity clearly inhibited the host-seeking activity of *S. vittatum*. The activity in wind-sheltered areas was comparable to that observed the previous day. Throughout the winter and until mid-spring, high winds are not uncommon in the Mohave Valley area. Wind was found to be a major inhibitor of *Simulium* host-seeking activities in studies conducted by Guttman (1972).

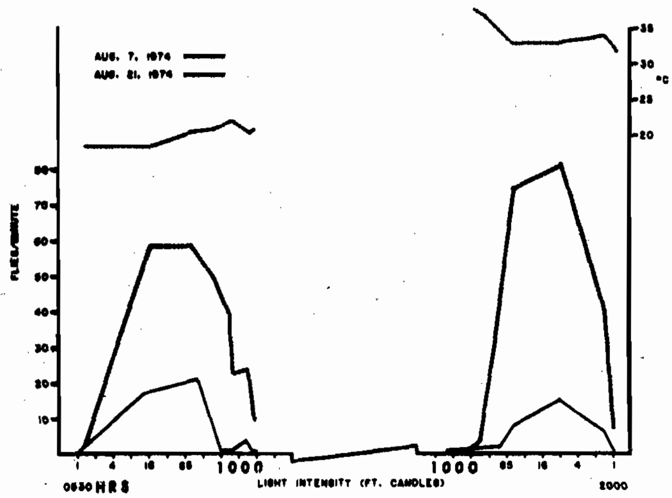

Figure 3.—Summer activity patterns of female *S. vittatum* taken from human host at Davis Dam, Colorado River.

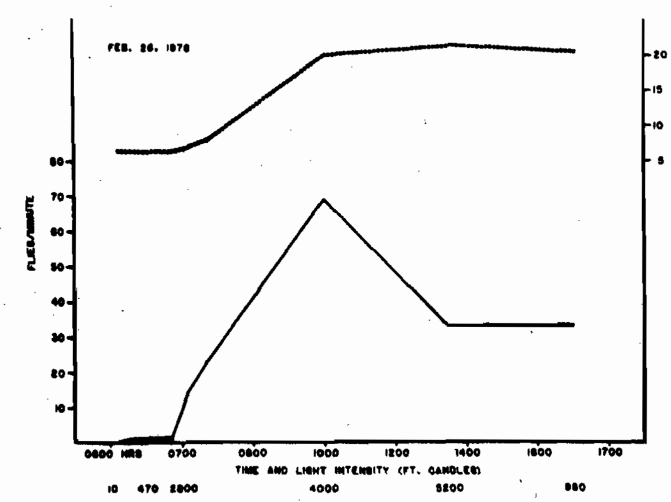

Figure 4.—Winter activity pattern of female *S. vittatum* taken from human host at Davis Dam, Colorado River.

Table 1.–Temperatures in the Mohave Valley during July 1st-September 11, 1974.

Month	Avg. low (°C)	Avg. high (°C)	Peak temp. (°C)
July	26.0	42	48.3
Aug.	20.5	42	45.0
Sept.	25.0	42	44.4

Humans are occasionally bitten in the Mohave Valley, but equines and bovines are readily attacked (Mulla and Lacey 1976). Owing to the paucity of large mammals in the valley and the large number of flies that are unable to obtain a blood-meal, it is likely that *S. vittatum* is autogenous in this area. Autogeny was proved for this species under laboratory conditions by Wu (1931) and Davies and Peterson (1956).

In addition to the bloodmeals taken by the female flies, male *S. vittatum* have been observed taking nectar from salt cedar blossoms. Arrowweed blooms probably contribute a source of carbohydrate earlier in the summer and throughout the spring. Shoreline mesquite blooms throughout the summer and probably provides localized nectar sources. Although female flies were not observed taking nectar, it is probable that they also exhibit this behavior. During the act of taking blood meals, female *S. vittatum* were observed emitting a droplet of clear fluid–possibly nectar–from the anus. Several other nectar sources were reported for this species by Davies and Peterson (1956).

Table 2.–Attraction of *S. vittatum* to humans in high winds and sheltered areas at Davis Dam, Mohave Valley (February 27, 1976).

Wind Velocity (mph)	Time	Temp. (°C)	Flies/ min
10-15	0645	14.0	0
0- 3*	0647	14.0	35
9-13	0715	14.5	0
0- 4*	0717	14.5	85
8- 9	0930	16.5	0
0- 3*	0932	16.5	52
7-15	1115	20.5	0
0- 3*	1117	20.5	4

*Protected from wind.

Male Swarms. - - Formation of the male swarm is governed by several physical factors similar to those that initiate and influence female host-seeking activity. Although male swarming activity occurs during the same general time as female pestiferous activity, males are more numerous and active in early evening than they are in the morning.

In the summer, male swarms formed just before or soon after sunset (1845-1915 hr). At first they are diffuse aggregations of flies starting at shoreline and extending approximately 15 ft inland. Vertically, the flies may occupy a space 4 to 30 ft above the ground. They are all oriented into the wind. If there is no breeze, the flies face the direction of the last gust detected. At this time, the flight of the individual male is a slow bouncing motion, usually with movement of less than a few centimeters in any direction. The initial swarm density was 1 to 3 males per sweep. As the evening progressed (15 to 30 min after the initial aggregation of male swarms was formed), the swarms became larger, occupying a space from shoreline to approximately 50 ft inland. At this time, small individual swarms began to condense over markers (bushes, people, vehicles). Light intensity during this clumping period ranged from 75 to 115 ft candles. Male activity increased considerably.

The sex recognition behavior was similar to that cited by Peterson (1962) in that the males collided with one another constantly during the course of their bouncing flight pattern within the swarm. As male encountered male, the repulsion was instantaneous, with a few observed exceptions where males actually paired and dropped from the swarm. Our observations differ from those of Peterson's with regard to male-female coupling in that the paired flies did not fly vertically out of the swarm, but dropped to the ground or water. Upon hitting the substrate, the female actively walked around with the male passively being carried in copula. In general, coupling lasted not more than 30 seconds. Pairs that landed on the water floated in copula for a few seconds before returning to the air separately. Swarm density during this period (1915-1930 hr) ranged from 2 to 10 flies per sweep and averaged 5 flies per sweep.

From light intensities of 70 to 1.5 ft candles (1930-2000 hrs), the swarms became even denser. Males became increasingly more active and mated pairs were formed in their greatest numbers. Females were detected in the swarms at from 0-5% during this period. The largest and densest swarms yielded as many as 133 flies per sweep. At light intensities of less than 1 ft. candle, swarms began to dissipate as finite entities, although many flies could be observed readily in flight after dark by illuminating the area. Upon illumination, however, the flies were attracted to the lights.

Morning swarms followed a similar pattern, but in reverse order, and were much sparser than evening swams. Contrasted to female host-seeking behavior, male swarming is initiated slightly earlier in the morning and occasionally in the evening. Also, it ends later--ca. 5 min--in the evening and is less affected by wind velocities of 6 mph or lower.

Male flies actively fight wind gusts of 6 to 8 mph to stay over their markers, but the swarm often condenses and lowers in wind. Wind velocities higher than 8 mph begin to inhibit swarming.

As males generally show preference for markers on which to fix their swarm at shoreline, they become an annoyance factor at the boating docks and many other locations. Passengers disembarking from the shuttle ferries or boats are inundated with several thousand flies during peak populations. To worsen the situation, the flies actively orient on moving markers and passengers accumulate flies as they proceed along the dock ramps.

During the cooler months, males aggregate in loose swarms, going as high as 20-30 ft above ground in the absence of wind. Mating was observed to take place in the middle of the day and literally thousands of pairs were observed to drop from the loose swarms to the ground. These types of swarms do not occur in the hot summer months.

A nonhost-seeking type of pestiferousness was observed during the hours of darkness in the warmer months when *S. vittatum* males and females, as well as chironomid midges, were attracted to indoor incandescent lighting. The numbers of flies attracted corresponded roughly to the population dynamics of the resting adults. During peak populations, flies attracted to artificial lighting became quite bothersome, interfering with cooking and other indoor activities near lighting.

Oviposition. - - Throughout the summer, female flies were observed dipping their abdomens into the surface of the water during flight in a manner similar to that described by Davies and Peterson (1956) and even alighting on the surface of the river. It was suspected that this may have been a possible ovipositional behavior. In cooler weather, moist rocks at the shoreline and objects trailing in the current (dock ropes, etc.) were observed as oviposition sites. Often, several females were observed ovipositing at the same site prior to and shortly after sunset. Their egg masses frequently were several layers thick at these locations. Females found alive and dead adhering to the sticky egg masses were not uncommon. Upon dissection, many of the dead females were found with well developed eggs still in the abdomen. This observation was also made by Wu (1931).

Oviposition by *S. vittatum* in lentic water was observed by Davies and Peterson (1956). Indirect evidence for this activity was the discovery of larvae in the darkness of the water chamber of a turbine at Davis Dam below Lake Mohave. The turbine had been under repair for 2 months and only a small trickle of water ran through it from the placid forebay above.

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PREVALENCE OF NUISANCE MIDGES[1] ON PREMISES ADJACENT TO THE SANTA ANA RIVER SPREADING SYSTEM[2]

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In urban and suburban areas of southern California, shallow and open flood control channels (collecting domestic and industrial waste water and storm water), man-made residential-recreational lakes, sewage oxidation and percolation ponds, and shallow water spreading structures (used to percolate and replenish subsurface water) are some of the typical aquatic habitats which produce large numbers of nuisance chironomid midges. These midges, although nonbiting, pose pest problems to humans residing near their breeding sources because they swarm and rest in and around nearby residential and industrial structures during the daytime. At night, the adult midges are attracted to indoor and outdoor lights. The nuisance and economic aspects of the varied problems caused by adult midges in California were reported by Anderson et al. (1965), Grodhaus (1963), and Mulla (1974).

Investigations were initiated in June 1974, on the seasonal cycle, abundance, and control of the nuisance midge fauna in the Santa Ana River Spreading System in Orange County. The progress of this study was reported in several papers (Ali and Mulla 1975, 1976a, b, 1977a, b). This paper presents information gathered during these studies on the species composition and abundance of adult midges in and around various premises situated adjacent to the water spreading system. Such basic information is necessary for developing practical criteria for the control of nuisance midges.

MATERIALS AND METHODS.–The Santa Ana River Spreading System is described elsewhere (Ali and Mulla 1976a). It covers ca. 300 ha of water spreading grounds and consists of a number of shallow basins and a flood control channel extending over an 11-km long area in Anaheim, Orange County, California. Along most of its length, this spreading system is lined with small and large business establishments, residences, and also a hospital (Canyon Hospital) ca. 30-50 m away from the water's edge (Ali and Mulla 1975). People residing or working in this area experience discomfort and suffer economic loss due to the heavy outbreaks of adult midge swarms. The midges invade manufacturing facilities, swarm inside and get imbedded in paint finishes, plastics and other manufactured goods.

To study the prevalence of adult midges, New Jersey light traps were used to attract the adults during the night. These traps were fitted with 40 watt bulbs and were equipped with automatic time switch, adjusted as needed to operate the traps from sunset to sunrise. A total of 3 traps (about 1 km apart from one another) were used in the area between Imperial Highway and Riverside Freeway (Ali and Mulla 1976a). One of these traps was located on the premises of Field Headquarters of the Orange County Water District, one in the Canyon Hospital, and the other in a small cement factory. Each trap was hung 1-2 m above the ground.

Midge collections from each trap were made at weekly intervals from April 1975 to June 1976. Surface temperature of stagnant water (in spreading basins) and slowly moving water (in the flood control channel) at the time of adult samplings (usually between 8 to 10 a.m.) also was measured. The daily maximum and minimum air temperature data were obtained from records of the Orange County Vector Control District's permanent weather station located in the area.

In the laboratory, adult midges were segregated into genera (S. I. Frommer of Dept. of Ent., University of California at Riverside confirmed identification of some of the adult midges.) by using Darby's keys (Darby 1962) and counted. Samples containing large numbers of adults had to be subsampled by weight. However, at least ¼ portion of large samples was segregated to genus and counted.

RESULTS AND DISCUSSION.–Although in the available time it was not possible to process, examine and identify to species all weekly catches of adult midges, a representative number of adults of the monthly collected genera was processed according to Borror et al. (1976) for microscopic examination of the male genitalia. The following species of adult midges were recognized in the light trap collections from April 1975 to June 1976: *Chironomus decorus* Johannsen, *Chironomus frommeri* Atchley and Martin, *Tanytarsus* n. sp. 2 and 3 after Sublette (Darby 1962), *Tanytarsus dendyi* Sublette, *Procladius sublettei* Roback, *Procladius freemani* Sublette, *Cricotopus bicinctus* (Meigen), *Cricotopus sylvestris* (Fabr.), *Dicrotendipes californicus* (Johannsen), *Paralauterborniella subcincta* (Townes), *Cryptochironomus fulvus* (Johannsen), and *Cryptochironomus chaetoala* Sublette. Adults of *Pentaneura* sp., *Tanypus* sp., *Psectrotanypus* sp., *Polypedilum* sp., and *Tribelos* sp. did not occur in the specimens examined, although their larvae were found in small numbers in the spreading system (Ali and Mulla 1975).

Among the species of midges listed above, only adults of *Tanytarsus* spp., *Cricotopus* spp., *Chironomus* spp., and *Procladius* spp., in that order, were of quantitative importance. Seasonal abundance of adults of these 4 genera and the total midge adults are shown in Figure 1, along with air and water temperature records. The water temperature represents the daytime mean of a minimum of 5 readings taken in different spreading structures at the time of adult collections, usually between 8-10 a.m. The air temperature represents the monthly mean temperature in the area, derived from the daily minimum and maximum air temperatures.

[1]Diptera:Chironomidae.

[2]These studies were partially supported by the Orange County Water and Mosquito Abatement Districts, California.

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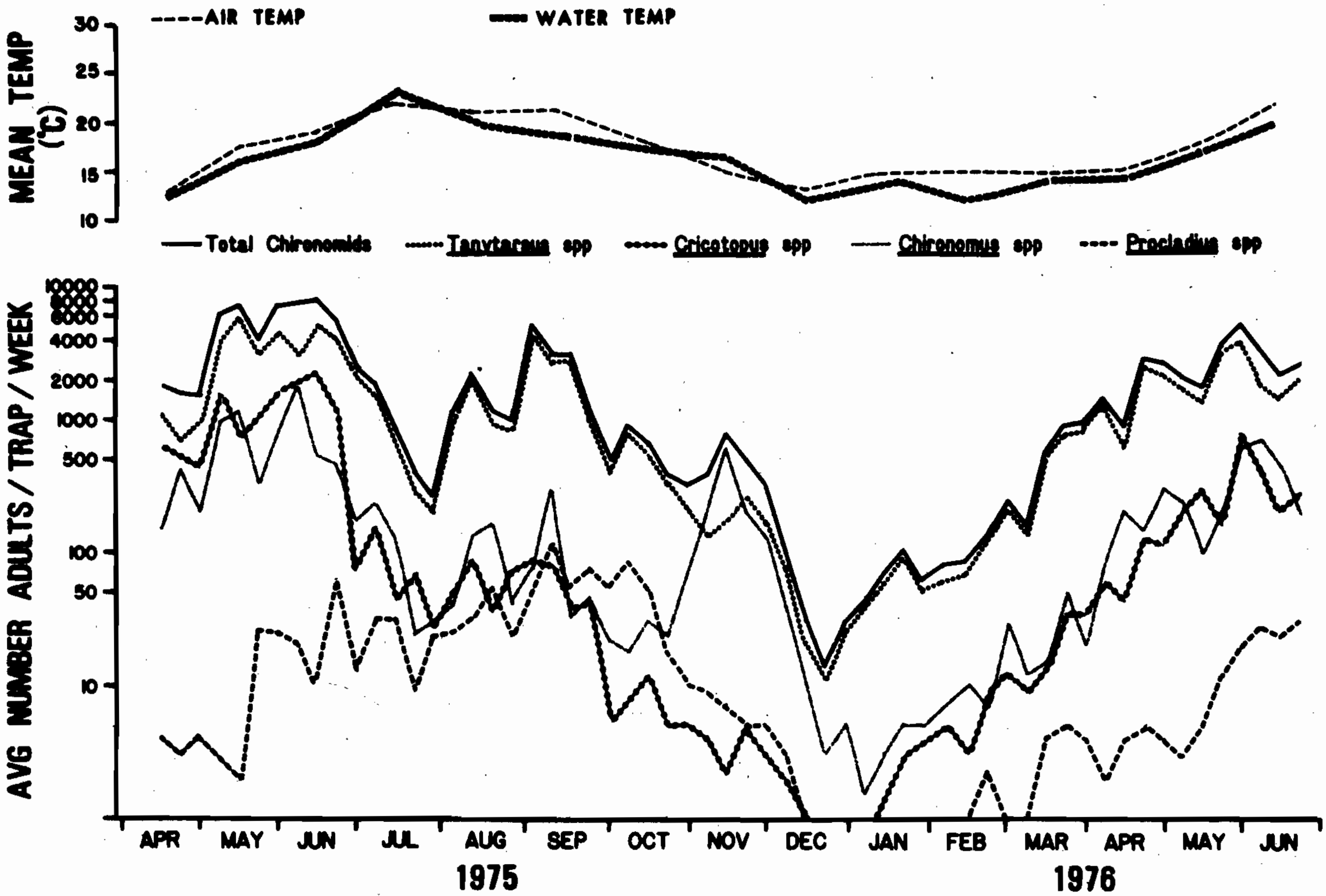

Figure 1.–Seasonal abundance of adult nuisance midges in the vicinity of the Santa Ana River spreading system in Orange County, California.

It can be seen from Figure 1 that adults of *Tanytarsus* spp. [predominantly *Tanytarsus* n. sp. 3 after Sublette (Darby 1962)] were present year-around and were numerically dominant throughout the year. *Chironomus* spp. (mostly *C. decorus*) were also present throughout the study period, but their numbers in weekly collections were much lower than those of *Tanytarsus* spp. (Figure 1). Adults of *Cricotopus* spp. (predominantly *C. bicinctus*) prevailed in large numbers (500-4000/trap/week) only in the months of May and June, while maximum numbers (50-115/trap/week) of *Procladius* spp. (predominantly *P. sublettei*) occurred from August to October. *Cricotopus* spp., trapped in low numbers during the winter months, were absent from the area during December-January, while *Procladius* spp. were absent in traps from mid December until the first week of February (Figure 1).

Adults of *Tanytarsus* spp. formed 72%; *Chironomus* spp. 11%; *Cricotopus* spp. 15%; *Procladius* spp. 1%; and the other midge genera 1% of the total chironomid adults collected from April 1975 to June 1976. The monthly composition of *Tanytarsus* spp. ranged from 37-92%, while that of *Chironomus* spp. 4-61%; *Cricotopus* spp. 1-31%, and *Procladius* spp. 0-7% of the total adults trapped each month. Several sharp peaks of *Tanytarsus* spp. were noticed between May-June and August-September when their average weekly numbers ranged from 3000-6000/trap (Figure 1). *Tanytarsus* spp. declined in the months of July and early August, and also during the winter months (November to February) while their average number/trap ranged from 11-250/week.

Chironomus spp. were most abundant once during May-June and then again in November. During these months, their average numbers increased up to 1100/trap/week (Figure 1). Between December and March, adults of *Chironomus* spp. did not exceed 50/trap in the weekly catches. Overall, the total midge adults were most abundant in the study area during the months of May-June and August-September when the populations in weekly catches reached up to 8000/trap (Figure 1). Adults of *Dicrotendipes californicus*, *Paralauterborniella subcincta*, and *Cryptochironomus* spp. (not shown in Figure 1) were taken only in small numbers from April to October.

Figure 1. also shows that the abundance of the 4 midge genera was seasonally variable. Larger numbers of adults occurring from April to November, in general, coincided with higher air and water temperatures during these months. Similarly, the decline in adult populations during winter months (December to February) was influenced by the decreasing air and water temperatures. The reason for lower numbers collected from July to August of 1975 and April to June 1976, may have been due to the implementation of field trials during these periods on chemical control of midge larvae in some spreading structures in the area (Ali and Mulla 1976a, 1977a). Also, the periodic drying and flooding of the spreading structures (Ali and Mulla 1975, 1977b) might have reduced breeding sources in the area at these times.

These studies show that *Tanytarsus* spp., *Cricotopus* spp., *Chironomus* spp., and *Procladius* spp. are the 4 main groups of midges prevailing in large numbers in and around residential, business, and industrial premises along the Santa Ana River spreading system. The quantitative composition of some of these groups did not necessarily correspond with their larval abundance in benthic mud of various spreading structures during the same time period (Ali and Mulla 1976a, b, 1977a, b). This was especially true for *Cricotopus* spp. Larvae of *Cricotopus* spp. at times were not as common in the benthic mud in the spreading structures (flood control channel or spreading basins) as were their adults in the light trap catches, and exactly the opposite was true for *Procladius* spp. Adults of *Procladius* spp. either remain very close to the water (i.e. their site of emergence) or perhaps are not as much attracted to light as do the other midge groups. The abundance of *Cricotopus* spp. adults in light traps at times when their larvae were not so abundant in the spreading structures can be attributed to the higher larval densities of *Cricotopus* spp. in the upstream areas of the main river than in the spreading structures and the down-stream transfer of their pupae in the drift (Ali and Mulla, unpublished observations). The river water carries these pupae to the spreading structures where the adults emerge.

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SPATIAL DISTRIBUTION PATTERNS AND SAMPLING VARIABILITY IN THE BENTHIC CHIRONOMID FAUNA OF CLEAR LAKE, CALIFORNIA

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INTRODUCTION.–The benthic dipteran fauna of Clear Lake, California has received considerable attention from aquatic biologists over the past several decades, mainly because of nuisance problems created by the nonbiting chaoborid gnat *Chaoborus astictopus* Dyar and Shannon (Prine et al. 1975). The purpose of the present study is to examine spatial distribution patterns and their influence on sampling variability in the benthic midge population of the Oaks Arm of Clear Lake. Several investigators have identified substrate preferences as inducing non-randomness in distributional patterns in lotic ecosystems (Scott 1958, Ulfstrand 1967, Allan 1975). However, the distributional patterns that occur within an area of uniform substrate, such as found in the profundal habitats of lentic environments, have not been examined in detail.

Alley and Anderson (1968) examined the spatial distribution patterns of Lake Michigan macrobenthos and reported that larval chironomids were randomly distributed in an area of sand-detritus substrate at a depth of 17 m. Likewise, Ricker (1952) reported a random distribution for larval chironomids in Cultus Lake, British Columbia. However, both studies examined the spatial distributions of the benthos at the family, rather than at the species level. Paterson and Fernando (1971) reported non-random distributions for several species of chironomids, as well as for the family, in shallow water habitats (0.5 - 3.0 m in depth) of several Canadian lakes. They suggested that the random distributions reported in previous studies may actually have been non-random patterns if the chironomids had been examined at the species level. Paterson and Fernando (1971) identified habitat heterogeneity of the shallow water environments as the primary factor contributing to non-randomness in distribution.

The failure to examine midge larvae at the species level in previous studies is primarily caused by difficulties in identifying larval chironomids to the species level. However, underlying species-specific distributional patterns may be masked when larvae of different species are combined into a single taxon. The larval stages of Clear Lake midges are relatively well known and species level identifications were usually possible for fourth instar individuals. Therefore, in this study distributional patterns for both species and family level classifications were compared.

MATERIALS AND METHODS.–Clear Lake is a shallow, highly eutrophic natural lake of approximately 16,200 ha and an average depth of about 6.5 m (Cook 1967). The Oaks Arm is located at the eastern end of the lake and is approximately 7 km by 2.5 km at its widest point and averages about 11 m in depth (Goldman and Wetzel 1963).

A total of 136 quantitative samples were collected from the Oaks Arm over five sampling dates from June 21, 1976 to October 7, 1976. Two large series of samples (n>30) from single sites were taken on July 13 and October 7 in order to determine conformance of the benthic species to various spatial distribution models (Table 1). Both sites were located in the profundal area near the geographical center of the arm. The sites were characterized by a uniform silt and mud substrate as determined by Wentworth Scale measurements. Samples were collected with an Ekman grab sampler (0.004 m^3) at a depth of 12m. Any grabs which failed to fill the sampler to capacity with sediment were discarded. Each sample was sieved in the field with a U.S. Sieve No. 35 (10 meshes per cm), retaining larvae with head capsules larger than 0.5 mm in diameter, which were primarily fourth (last) instar chironomids. Some late third instar larvae were also extracted but those were not treated in the analysis. Samples were preserved in 5% formalin.

The binomial family of distributions, which describes the dispersion of organisms in space, served as the model for the distributional patterns displayed by the benthic larvae. Random distributions are described by a Poisson series of numbers, which requires that there is equal probability of an organism occurring at any point in space:

$$P(\chi) = e^{-\lambda} \frac{\lambda^{\chi}}{\chi!}$$

where $P(\chi)$ is the probability of χ individuals in a sampling unit, λ is the mean of the population and e is the base of natural (Napierian) logarithms (Elliot 1971).

Clumped, or contagious, distributions are described by negative binomial or logarithmic distributions. The exponent "k" of the negative binomial distribution is calculated for each taxon using the maximum likelihood equation (Southwood 1966). Also calculated is the index of dispersion "I", the variance to mean ratio.

Sample size statistics are calculated to quantify the number of samples required to obtain a sample mean within a specified error of the estimated population mean with a chosen probability level. The sample size "N" is calculated by the formula:

$$N = \left(\frac{t \cdot s}{D \cdot \bar{x}} \right)^2$$

where $\bar{x}$ is the arithmetic mean, s is the standard deviation, D is the desired level of accuracy (as a decimel) and t is the t-statistic for the chosen probability level and n-1 degrees of freedom (Southwood 1966).

RESULTS.–Fauna - - The zoobenthos if the Oaks Arm consisted of Chironomidae and Chaoboridae (Diptera) (Table 1), various crustaceans and high densities of oligochaetes. Individuals of *Procladius* (Chironomidae) could not be identified but at least three species, *P. freemani* Sublette, *P. subletti* Robach and *P. bellus* (Loew), are known to coexist in Clear Lake (G. Grodhaus, pers. comm.). *Chironomus* (Chironomidae) larvae in the fourth instar could be dis-

Table 1.–Descriptive statistics, results of Kolmogorov-Smirnov and chi-square goodness of fit tests for a Poisson series, and sample size statistics for Chironomidae and Chaoboridae of the Oaks Arm (α = .05 for all tests). I=index of dispersion; k=clumping statistic, $\to\infty$ = approaching infinity; N=samples size statistic; D=specified error of the mean.

Statistic	*Chaoborus astictopus* July	*Chaoborus astictopus* Oct.	*Chironomus plumosus* July	*Chironomus plumosus* Oct.	*Chironomus frommeri* July	*Chironomus frommeri* Oct.	*Chironomus* spp. July	*Chironomus* spp. Oct.	*Procladius* spp. July	*Procladius* spp. Oct.	Total Benthos July	Total Benthos Oct.
$\bar{X} \pm S\bar{x}$	0.33 ±0.13	0.10 ±0.056	10.20 ±0.66	0.50 ±0.16	16.13 ±0.89	0.10 ±0.056	26.33 ±1.12	0.60 ±0.16	23.70 ±1.24	1.13 ±0.21	50.37 ±1.80	1.93 ±0.23
s^2	0.51	0.09	13.13	0.74	23.64	0.09	37.47	0.80	47.04	1.29	100.79	1.65
I	1.52	0.93	1.29	1.48	1.46	0.93	1.42	1.33	1.98	1.14	2.00	0.85
k	0.63	$\to\infty$	$\to\infty$	0.70	$\to\infty$	$\to\infty$	$\to\infty$	1.28	$\to\infty$	8.95	$\to\infty$	$\to\infty$
Kolmogorov-Smirnov:												
critical value	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
test statistic (d_{max})	0.68	0.87	0.08	0.57	0.11	0.87	0.06	0.52	0.12	0.29	0.20	0.26
Chi-square:												
critical value	3.84	3.84	16.92	3.84	16.92	3.84	23.68	3.84	22.36	7.81	28.87	9.49
test statistic (X^2p)	0.77	0.06	3.57	1.56	13.33	0.06	6.51	1.29	7.33	0.75	21.45	0.88
p-value	p>.04	p>.80	p>.90	p>.20	p>.15	p>.90	p>.95	p>.25	p>.85	p>.85	p>.25	p>.90
N (D=.20)	469	931	13	296	10	931	6	220	9	102	4	45

tinguished to species by characters of the head capsule and two species, *C. plumosus* (Linnaeus) and *C. frommeri* Atchley and Martin, were the predominant representatives of the genus in the Oaks Arm (Table 1).

Spatial Distribution - - Spatial distribution patterns of the Oaks Arm benthic fauna were examined for July; and October samples for specific, generic and family taxonomic groupings. The distributional patterns of the benthic midges in July were tested for agreement with Poisson series of numbers, or random distributional patterns, by various statistical tests including the chi-square goodness of fit test (Elliot 1971) and the Kolmogorov-Smirnov test (Skal and Rohlf 1969) (Table 1). The distributional patterns of all chironomid taxa in July conformed to Poisson series of numbers, which indicated random distributions for the species *C. plumosus* and *C. frommeri*, the genera *Procladius* and *Chironomus* and for the total midge benthos (Figure 1, Table 1). Two dispersion coefficients, the index of dispersion, I, and the exponent K also indicated random distributions for the chironomid taxa. Approximation to unity for the index I implies a random distribution as does a value of k greater than 8.0. Maximum contagion (i.e., a logarithmic series) would be indicated at k equals zero (Southwood 1966).

In October a younger age class structure was present in the chironomid community, which resulted in a greater number of early instar larvae and fewer fourth instar larvae being collected (Table 1). The genera *Chironomus* and *Procladius* displayed spatial patterns approaching logarithmic distributions due to the frequency of zero counts being recorded for the samples and the consequent skewing of the distributions (Figure 1). The chi-square test for goodness of fit to a Poisson series indicated that those genera and total midge benthos could be randomly distributed in October (Table 1). However, the Kolmogorov-Smirnov test for agreement with a Poisson series indicated that those distributions were non-random. Again, the low densities of those taxa and of the species *C. plumosus* and *C. frommeri* may be responsible for this indication of contagious distribution in October as measured by the Kolmogorov-Smirnov test, which is more sensitive at smaller sample sizes (n<100). The Clear Lake gnat, *Chaoborus astictopus*, was present in very low densities throughout the sampling period (Table1) and also displayed a spatial pattern approaching a logarithmic distribution. Unlike the chironomid benthos, no distinct difference in age class structure was detected between July and October by our sampling technique.

The number of samples required to produce an estimated mean within 20% of the population mean with 95% probability, a range suggested by Elliot (1971) as appropriate for benthic sampling, was calculated for each taxon (Table 1). In July the required sample size among the chironomid benthos varied from N=4 for the total benthos to N=13 for *C. plumosus*. In contrast, the required sample size for October varied from N=45 for the total benthos to N=931 for *C. frommeri*. Extremely large numbers of samples would have been needed to estimate the mean of *Chaoborus astictopus* during either sampling date (N=469 - 931).

DISCUSSION.–Substrate heterogeneity has been identified as contributing to non-randomness in the distribution of zoobenthos in running water environments (Scott 1958, Ulfstrand 1967, Egglishaw 1969, Barber and Kevern 1973). The relatively uniform silt and mud substrate of the Oaks Arm of Clear Lake may indicate that random distributional patterns for the zoobenthos would be expected due to the reduction in microenvironmental variation. Contagious distributions of Chironomidae, as noted for shallow water benthos (Paterson and Fernando 1971), might indicate one or both of two things: 1) microenvironmental variation resulting in aggregation in preferred habitat, or 2) a behavioral component to aggregation. In the Oaks Arm, aggregation due to microenvironmental variation appears to be lacking.

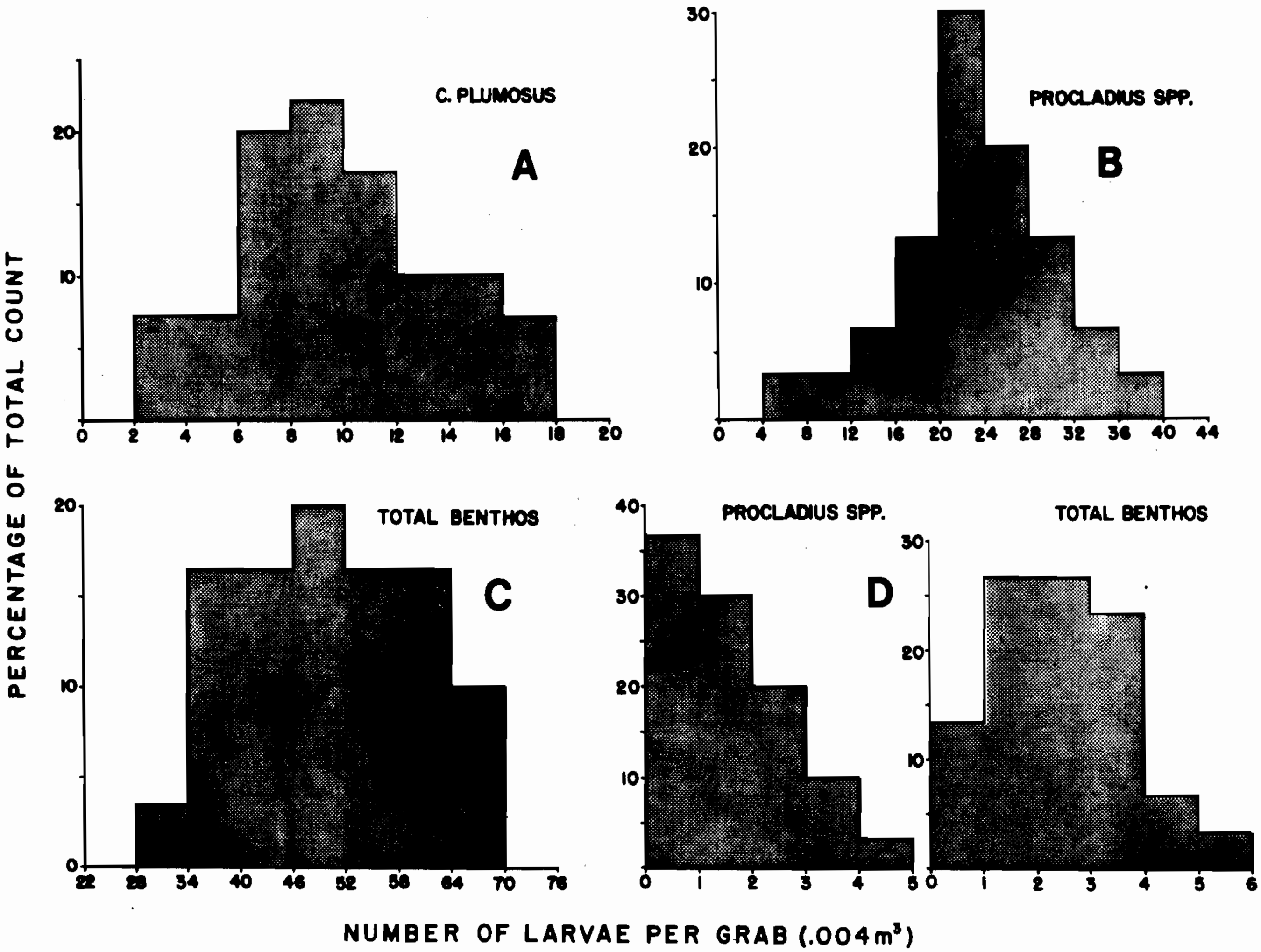

Figure 1.—Frequency distributions for selected species of Chironomidae in the Oaks Arm of Clear Lake. A, B, C = July distributions; D = October distributions.

No information is available to test the second possibility but the random distributional patterns suggest that behavior is not a major factor influencing the spatial patterns within the benthic community of the Oaks Arm.

The contagious distributions exhibited by *C. astictopus* throughout the sampling period and by the chironomid benthos in October were apparently produced by the changing age class structure of the community, which is a function of the phenology of those species. Some interesting phenological patterns or spatial distributions might emerge with more intensive sampling of the zoobenthos. An alternative method to increasing the sample size during those periods would include increasing the dimensions of the sampling unit to include larger numbers of late instar larvae, thus reducing the number of zero counts, or decreasing the mesh size of the seive to retain earlier instar larvae. It is evident from this study that a benthic sampling regime or procedure that is appropriate during one part of a species' life cycle may not be suitable during other periods.

The accuracy of quantitative benthic sampling and the reduction of sampling variability may be largely dependent on substrate heterogeneity. Undoubtedly, this may only be one of a multitude of extrinsic and intrinsic factors that influence the spatial distribution of a population. However, the establishment of the spatial distribution pattern of a species coupled with its correlation with a significant factor such as substrate can provide the basis for a statistically sound and representative sampling program.

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