A LARGE-SCALE FIELD TEST OF *GAMBUSIA AFFINIS* [1] AND CHLORPYRIFOS [2] FOR MOSQUITO CONTROL

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ABSTRACT. Seventy-two rice fields were divided equally into the following four treatment categories: a.) fields stocked with 0.6 lb of fish per acre, b.) fields stocked with 0.2 lb of fish per acre, c.) fields sprayed with 0.0125 lb of chlorpyrifos per acre in mid-June, and d.) experimental control fields. The fields were stocked with mature female *Gambusia affinis* (Baird & Girard) by helicopter and by ground crews. The principal results were: a.) During late June, fields stocked at the lower rate produced significantly more mosquitoes (*Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken) than the other treatments or controls. b.) During late July the fields treated with chlorpyrifos produced significantly more mosquitoes than either the controls or the fields stocked at the higher rate. c.) During late August, the fields treated with chlorpyrifos produced significantly more mosquitoes than the controls or fields stocked with either rate of fish. Evaluation of fish populations showed that by August the stocked fields all had fish present, and only 4 of 36 unstocked fields had detectable fish populations. Speculations are presented that the use of chlorpyrifos and insufficient stocking disrupted invertebrate predator populations.

INTRODUCTION. More than 300,000 acres of rice fields lie in the Sacramento Valley of California. The majority of that acreage has no direct mosquito control effort applied, largely because the cost of chemical control would be so high if a complete program were maintained. However, in nearby San Joaquin Valley, the cost of chemical mosquito control in rice fields was estimated to be $4.50 per acre in 1969 (Hoy and Reed, 1970); in 1970, that figure must have been greatly exceeded as a result of a 25 percent increase in the number of treatments applied, which were no doubt needed. Many mosquito abatement districts cannot afford to spend $4.50 per acre to control *Anopheles freeborni* Aitken and *Culex tarsalis* Coquillett in rice fields. Consequently they concentrate on other mosquito breeding sources. As a result, the so-called irrigated pasture mosquito, *Aedes nigromaculis* (Ludlow), has received heavy insecticidal pressure, with the expected ensuing resistance.

Recent studies have shown that *Gambusia affinis* (Baird & Girard) can be an effective biological control agent in California rice fields. (Hoy *et al.*, 1971; Hoy and Reed, 1970, 1971). The operational aspects of fish distribution and the expected failure rate under operational conditions were not fully determined by these studies. Furthermore, in recent tests by other workers in California, chlorpyrifos was applied from aircraft flying crosswind to obtain swath widths of approximately 660 feet. The obvious reduction of application costs with such wide swaths opens the possibility of treating large, hitherto untreated acreages. Such a method depends on blanket treatment of large areas and lends itself to early season application with the intent of suppressing "brood stock" populations, as opposed to repeated treatments for immediate control. Regardless of the advisability of such strategy, we recognize that this method could become accepted, and that a comparison with the efficacy of the fish is desirable.

Another aspect of an operational test is the relationship of agricultural pest control methods in rice fields to the mosquito control method being tested. In the test area, tadpole shrimp and aquatic weeds (algae and higher plants) are the

[1] Poeciliidae.

[2] Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of the product by the U.S. Department of Agriculture.

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major problems other than mosquitoes. Parathion and copper sulfate are applied for shrimp control. Copper sulfate, molinate, and MCPA are applied for weed control. For at least the immediate future, we must assume that these pest control measures will continue to be implemented. Hence, our experimental design was drawn up so that the efficacy of each mosquito control treatment could be judged in the context of present agricultural practice.

MATERIALS AND METHODS. Four treatments, i.e., fish stocked at a rate of 0.6 lb per acre, fish stocked at a rate of 0.2 lb fish per acre, chlorpyrifos applied at a rate of 0.0125 lb per acre, and experimental controls, were assigned randomly within each of three blocks. Each block contained 11 square miles, 2 square miles of which were stocked with fish at a rate of 0.6 lb per acre. The remaining 9 square miles were equally divided among the remaining two treatments and controls. The subdivisions (1 x 2 mile or 1 x 3 mile) of each block were oriented with the long axis running east and west. The boundaries of the subdivisions were section lines, and in most cases, roads emphasized the division. The entire 33-square mile study area was within the Sutter Basin, which is surrounded by the Sacramento River and the Sutter Bypass, a flood control channel. Elevations were between 20 and 30 feet above sea level. Rice fields account for approximately 50 percent of the land use. Safflower and milo are the other major crops in the area. Between 4 and 12 rice fields were found in each subdivision. Typically, fields were slightly less than 160 acres in size. Only three fields were smaller than 40 acres, and ten fields were larger than 160 acres. In subdivisions with a large number of fields, as many as three fields were randomly eliminated from the study so that each treatment would be replicated 18 times.

The fish used in the experiment were obtained from a variety of sources, some from fortuitous natural populations, some from Sacramento-Yolo M.A.D., and some from a commercial bait dealer. The size of fish stocked varied from 850 to the pound to about 400 to the pound. Although the condition of the stcok was generally good, some batches were almost 100 percent non-gravid. In all cases, approximately 99 percent of the stock were sexually mature females.

Two methods of stocking the fields were used, aerial distribution from a helicopter and surface distribution by truck. The helicopter flew at 5–15 mph at approximately 8 feet above the surface of the water. The stock was carried in the helicopter in plastic bags containing 4 liters of water and 1.5 lb of fish. The bags were held in wooden boxes, 6 per box. As much as 96 pounds of fish were delivered per flight. A 2-man crew poured fish from the bags as the helicopter traversed the rice field. The importance of stocking the intended field dictated that there be a navigator.

Twenty-nine of the 36 fields with fish treatments were stocked in two rounds, the first round approximately 10 days after seeding of the field and the second approximately 20 days later. The seven fields that received the entire stock on one date were stocked approximately three weeks after seeding. In both the aerial and surface stocking, all paddies were stocked, with the occasional exception of the first or last paddy.

Although a large military helicopter was used in the study, an indication of the ease with which fish can be distributed was demonstrated. If the supply of fish is adequate, distribution of more than 200 ponds per hour is quite possible. The operational aspects of stocking both by helicopter and ground crew will be described in detail in another place (Kauffman, ms. in preparation).

The application of chlorpyrifos was made with a PA–18 Super Cub airplane equipped with a 15 teejet system (No. 6 nozzles). The speed of the aircraft was 90 mph, and swath widths were estimated to be 100 feet. This conventional method of application was used rather than the experimental wide-swath method, with the assumption that we were simulating wide-swath at its best. The chlorpyrifos (4 lb emulsifiable in water at 1/2 gal/acre to give 0.0125 lb actual/acre) was applied on 14, 15, and 16 June 1971.

The evaluation of the three methods of mosquito control were based on the results of three periods: 1) late June; 2) late July; 3) late August. These periods roughly corresponded to the peak of the populations of *Culex tarsalis* Coquillett, the major period of overlap of populations of *C. tarsalis* and *Anopheles freeborni* Aitken and the peak of the *A. freeborni* populations respectively (Bailey and Gieke, 1968; Hoy *et al.*, 1971). Each evaluation period was 13 days long and included 6 days of sampling, a 1-day break, and 6 more days of sampling. Six fields were sampled on each day. The choice of fields was at random, with each field sampled once per evaluation period.

Each field was sampled simultaneously by two men (evaluators) who were uninformed with respect to the treatment. The samples were concentrated (see Womeldorf *et al.*, 1963) in the field. During the first two evaluations, each man took 20 dips in the high half of the field and 20 dips in the low half. Hence, four subsamples of 20 dips each were taken and kept over ice until the sample was sorted and counted in the field laboratory. No sample was held for more than 1.5 hours before sorting. Each day after the first two fields had been sampled, the material was taken to the laboratory and processed immediately by a technician. The materials from the third and fourth fields were handled similarly. The material from the last two fields was taken to the laboratory by the evaluators who then helped with the primary sorting and counting of specimens. However, a second search of the material was always done by the laboratory technician. The primary search was sufficiently thorough so that no more than 10 percent of the total number of specimens were recovered during the second search. During the last evaluation period, the samples were increased to 120 dips per field.

All specimens of the four subsamples were categorized according to genus and instar. The data were recorded as to location in the field and as to evaluator to aid in the analysis for detection of sampling bias.

In mid-August, each field was sampled to find a relative measure of the fish population. On each of 12 consecutive days, eight fields were sampled with 12 minnow traps. Within each field six paddies were sampled with a pair of traps, one with 4-mesh and one with 8-mesh screen. The two traps of each pair were set 5 yards apart. The sites were scattered throughout the field. Both the number and the weight of the fish trapped were recorded.

The statistical analyses, analysis of variance, and product-moment correlation were carried out by Dr. Bruce Mackey (Biometrical Services, Agr. Res. Serv., USDA, Albany, California) with the aid of an IBM 1800 computer. The log (N+1) transformation was applied to all counts of mosquito specimens. The logic of that transformation is well recognized (Snedecor, 1956; Wadley, 1967; Williams 1937.)

Results and Discussion. The combined number of specimens found by the two evaluators is shown in Table 1, ranked from the lowest to the highest counts and arranged by evaluation period and by treatment. A double horizontal line in each column of data indicates the point below which more than 0.2 specimen per dip were found. It should be noted that during the last evaluation period the number of dips per field was increased from 80 to 120 per field; therefore, 24 or more specimens were required to reach 0.2 specimen per dip.

During the June evaluation, the fields stocked at the lower rate exceeded 0.2 specimen per dip in 11 of 18 cases; the control fields exceeded 0.2 specimen per dip in only 5 of 18 cases. The fields stocked at the higher rate exceeded 0.2 specimen per dip in three cases and those treated with chlorpyrifos in four cases.

In July, the control fields exceeded 0.2 specimen per dip in only three cases,

TABLE 1.—Ranked numbers of specimens (*Culex tarsalis* and *Anopheles freeborni*) collected in 18 replicates of each of 4 treatments during 3 evaluation periods. A double horizontal line in each column of data indicates the point below which at least 0.2 specimen per dip were found. A single horizontal line indicates the point below which at least 0.05 specimen per dip were found.

	Total specimens per field at indicated evaluation period											
	June [a]				July [a]				August [b]			
Field rank	0.6 lb. Fish/ acre	0.2 lb. Fish/ acre	Controls	Chlorpyrifos	0.6 lb. Fish/ acre	0.2 lb. Fish/ acre	Controls	Chlorpyrifos	0.6 lb. Fish/ acre	0.2 lb. Fish/ acre	Controls	Chlorpyrifos
1	0	0	0	0	0	0	0	1	0	1	0	0
2	0	0	0	0	0	0	0	2	0	1	0	2
3	0	3	0	0	0	1	0	3	0	1	1	3
4	0	4	0	0	0	1	0	3	0	1	2	3
5	1	6	1	0	0	1	0	4	0	2	3	3
6	1	7	1	0	0	2	0	5	0	2	4	8
7	2	7	6	1	0	2	1	6	1	3	6	9
8	2	18	6	1	0	3	1	6	2	5	6	9
9	6	30	7	4	0	4	1	10	3	5	6	9
10	8	33	10	4	1	4	2	13	4	5	8	10
11	8	35	11	4	2	4	5	13	5	7	8	17
12	8	40	12	7	2	5	7	33	5	7	13	35
13	8	69	15	8	4	6	7	40	7	8	15	37
14	12	77	24	12	4	6	7	51	8	11	15	37
15	12	97	33	23	5	18	12	53	9	11	17	44
16	18	120	49	31	14	68	22	71	16	16	19	58
17	22	163	90	78	33	86	31	150	19	16	20	59
18	56	400	149	200	38	92	43	166	246	27	21	325

[a] Eighty dips per field.
[b] One hundred twenty dips per field.

those stocked at 0.6 lb of fish per acre in two cases, and those stocked at 0.2 lb per acre in four cases; the fields treated with chlorpyrifos were excessive in seven cases. Likewise, in August the fields treated with chlorpyrifos were producing mosquitoes in excess of 0.2 specimen per dip in 7 cases of 18, yet the other two treatments, 36 fields in all, had an excess of 0.2 specimen per dip in only two cases. Furthermore, the control fields all had below 0.2 specimen per dip in all 18 cases.

When columns of data for a given treatment are compared in sequence through the three evaluation periods additional information becomes apparent. The numbers of fields over the 0.2 specimen per dip threshold decreased with each period when fish were stocked and also in the control fields. However, when the fields were treated with chlorpyrifos the number of fields with over 0.2 specimen per dip increased from four in June to seven in July and August.

The above statement of the results is rather subjective and revolves around the arbitrary standard of 0.2 specimen per dip. That standard has some relevance, however, in that some mosquito abatement districts use 0.1 specimen per dip as a criterion for control efforts, and others use 0.5 specimen per dip.

The points at which 0.05 specimen per dip was exceeded are also indicated in Table 1 to demonstrate that the trend is similar with other criteria. Although that low number of specimens is no doubt meaningless as a general criterion for control activities, it does show a pattern similar to the 0.2 criterion. The low number of mosquitoes in the Sutter Basin (on a unit of area basis) is shown by the number of control fields that yielded less than 0.05 specimen per dip.

The above results have been presented without regard to the mosquito species involved. The overwhelming abundance of two species, *C. tarsalis* and *A. freeborni* relative to other mosquitoes in the rice fields of Sutter-Yuba M.A.D. was discussed by Hoy *et al.* (1971). During that study in an area approximately 15 miles northeast of the Sutter Basin, very nearly equal numbers of these two species were collected in control fields on a season-long basis. During the present study, the control fields yielded nearly 2.5 times more *C. tarsalis* than *A. freeborni*. However, the seasonal pattern of strong predominance of *C. tarsalis* in June and the reverse in August held good. Both species were found in considerably lower numbers than during the previous study mentioned above; sampling bias seems doubtful since the senior member of the evaluating team was involved in both studies. The overall population was sufficiently low so that a large number of specimens in one collection could mask the general pattern of abundance. This is at least a partial explanation of the predominance of *C. tarsalis* stated above; one sample containing 149 first instar larvae contributed greatly to the difference.

The experimental design used in the study provided for analysis of variance of specimen counts transformed to the log of N+1. The analysis of variance took into account variation among blocks, treatments, ends of fields, and appropriate interactions of these factors (Table 2).

The results of the analysis of variance of the *Culex* and *Anopheles* specimens may be summarized as follows: a.) significant differences among treatments were found in June, July, and August when the *Culex* and *Anopheles* specimens were combined; in June and August when *Culex* specimens alone were analysed; and in July when *Anopheles* specimens were analysed; b.) significant differences among blocks were found in the data for June and August for combined specimens, in the data for June for *Culex* specimens, and in the July data for *Anopheles* specimens; c.) the end of the field sampled was significant only in August and only for combined specimens; d.) pooled block interaction was significant in all analyses except in July for combined specimens and also for *Anopheles* specimens alone.

The analysis points to the variation in the results but does not specify which treatments are different. That relationship is shown in Figure 1, in which the least significant differences at the 5 percent level are indicated by brackets placed around each of the significantly different points. The following relationships are illustrated in the figure: a.) during June, the fields stocked with 0.2 lb of fish per acre produced significantly more mosquitoes than the other fields; b.) during July, the fields treated with chlorpyrifos produced significantly more mosquitoes than either the control fields or the field stocked with 0.6 lb of fish per acre; c.) during August, the fields treated with chlorpyrifos produced significantly more mosquitoes than the controls or treatments in which fields were stocked with fish at either stocking rate.

Note that the control fields were producing most heavily during June. Also the trend of mosquito production was similar to the control fields, but at a lower level, in those fields stocked with 0.6 lb of fish per acre.

When the species makeup of the three significantly different points is compared with the makeup of the control fields, we find that the *Culex* to *Anopheles* ratio was similar in both treatments in June, with 35 times as many *Culex* as *Anopheles*. However, in July, there were only 2.5 times as many *Culex* as *Anopheles* in the control fields but 13 times as many in the chlorpyrifos-treated fields. The

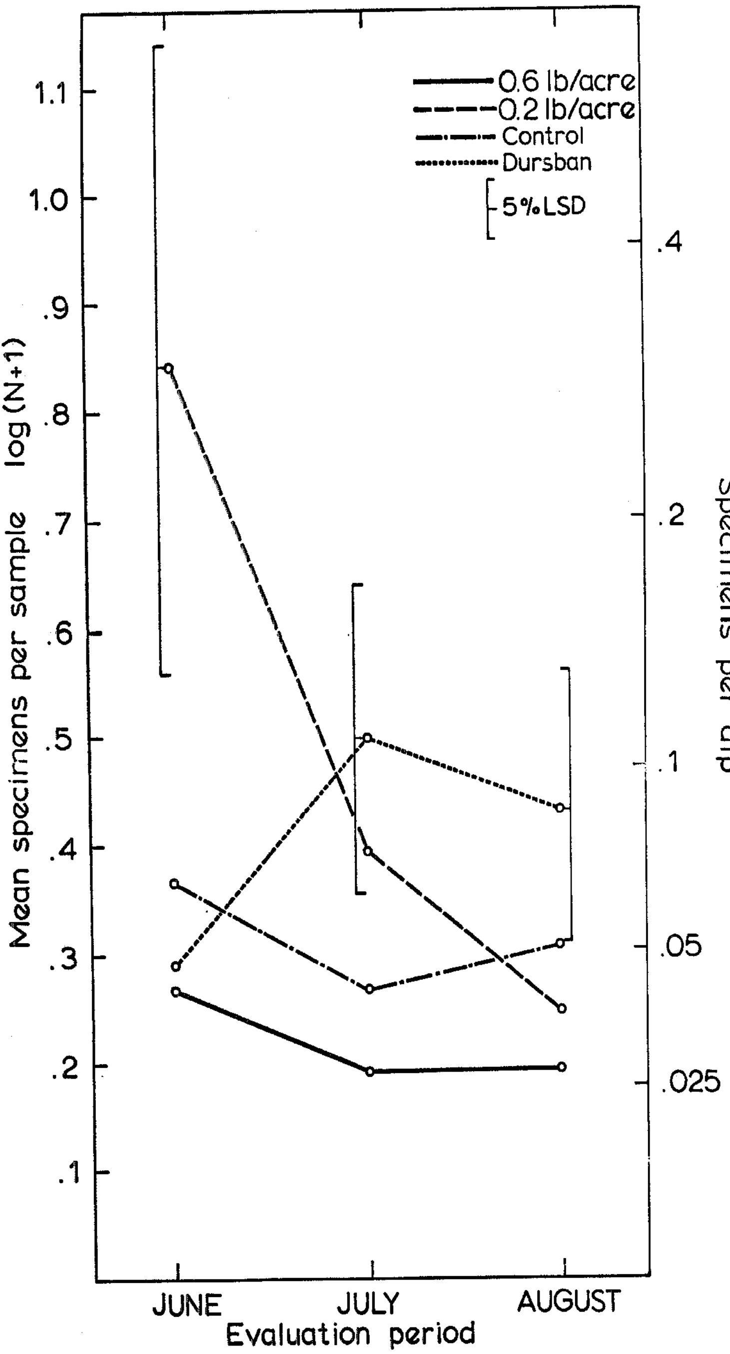

FIG. 1.—Numbers of specimens found in rice fields during three evaluation periods. Each treatment was replicated 18 times and 4 subsamples were taken within each replicate; therefore each point represents 72 subsamples. A total of 20,160 dips is thus represented.

Table 2.—Analysis of variance of mosquito specimens collected in 72 rice fields in Sutter County, California, during 1971.

		Mean Square log (N+1)							
		All Specimens			*Culex*			*Anopheles*	
Source	d.f.	June	July	Aug.	June	July	Aug.	July	Aug.
Blocks	2	1.742*	.461	.595*	2.317*	.492	.265	.292*	.187
Treatments	3	4.628*	1.263*	.981*	6.212*	1.538	1.471*	.364*	.439
End of field	1	3.422	.839	1.383*	5.072	.746	.517	.275	.496
Treat. x End	3	.090	.390	.418	.195	.630	.331	.029	.099
Pooled block interaction [a]	14	1.102*	.290	.289*	1.514*	.508*	.277*	.077	.163*
Residual [b]	264	.213	.184	.147	.318	.268	.093	.089	.090

* Significant at the =5% level.
[a] Treatments, end of field, and treatments x end of field interactions with blocks. Used as an error term for these sources.
[b] Includes nonsignificant evaluators and interactions as well as 4–10 replicates and interactions. Used to test blocks and block interactions.

difference was caused by an increase of *Culex* in the chlorpyrifos-treated fields, not by a decrease in the *Anopheles* in the control fields. In August, the same pattern was observed, but to a much greater degree. In the control fields, *Culex* specimens were only 0.04 times as abundant as *Anopheles;* in the chlorpyrifos-treated fields, *Culex* specimens were 3.8 times more abundant than *Anopheles.* Again, the difference was the result of an excess of *Culex* in the chlorpyrifos-treated fields rather than a deficit of *Anopheles* in the control fields. On a season-long basis, the pattern for *Culex* was an exaggerated version of that shown in Figure 1, with the high yield of specimens occurring in the fields with 0.2 lb of fish per acre during June and in the field treated with chlorpyrifos during July and August.

The variation among blocks was significant in June and August when combined specimens were analysed. Although the effect is presumed to be real, no explanation is obvious. The large study area cut across many properties and areas of grower influence. The block effect, though unexplained, is assignable to a category outside of chance variation.

During August, a significant difference in the numbers of specimens collected in the high and low ends of the field was observed. This effect seemed to be associated with two of the four treatments, but it did not reach the 5 percent level of significance. The direction of the effect is for greater numbers of specimens at the low end of the field. Of 72 fields, 39 had a majority of specimens in the low end, 20 had a majority in the high end, and 13 had equal numbers in both ends. In many of the latter categories, no specimens were found. Of the 21 fields with 0.1 specimen or more per dip, 14 had a majority of specimens in the low end.

Although the maximum number of *Anopheles* specimens were collected in August and although during that month the *Culex* specimens were largely from those fields treated with chlorpyrifos, the effect of the end of the field was demonstrated in terms of the total mosquito production. The effect was most evident in the 18 fields treated with chlorpyrifos, with 13 having a majority of specimens in the low end and only 3 having a majority in the high end.

For each evaluation period, the numbers of specimens collected by the senior member of the evaluation team were correlated with those of the junior member on the site-by-site basis, i.e., one comparison for each end of each field (Table 3). The analysis was made for genera

TABLE 3.—Correlation coefficients for comparisons between the numbers of specimens collected by the two evaluators arranged by evaluation periods and types of specimens.

	Evaluation Period		
	June	July	August
Culex	.71***	.74***	.94***
Anopheles	.21*	.45***	.45***
All specimens	.71***	.77***	.70***

* Significant at the 5% level.
*** Significant at the 0.1% level.

and for the combined numbers of specimens. With one exception, all comparisons showed a positive correlation that was significant at the 0.1 percent level. The exceptional case was significant at the 5 percent level and was also positive, although it resulted from the very low numbers of *Anopheles* found in June. Because of the strong agreement between the samples of the two evaluators, in the analysis of variance their samples were treated as replicates within each end of each field.

Fish traps in each of the 72 study fields gave a rough estimate of the various fish populations in mid-August. The results of trapping are shown in Table 4. The number of fields in which fish were found was 40. All stocked fields yielded fish as did three of the control fields and one of the chlorpyrifos-treated fields. One of the control fields yielded more fish than the average for the stocked fields. The other two control fields and the chlorpyrifos-treated field had very low numbers of fish, 34, 33, and 3, respectively. Of the 36 stocked fields, 5 yielded fewer than 35 fish, and 6 yielded more than 1100 fish. We believe that this great variation is largely the result of the limited number of traps (12) per field and the great variation in the trap catches within any field. There seemed to be relatively little difference between the average number of fish per field for the two stocking rates. An even smaller relative difference was seen when the average weights (biomasses) of fish caught were compared. The higher stocking rate was associated with the larger number of fish per field; the lower stocking rate was associated with the greater biomass per field.

In certain fields, the number of invertebrate predators was strikingly apparent when the fish traps were tended. The interaction between these invertebrates and the fish in the traps contributed additional variation to the observed trap catches. However, the trap data clearly demonstrate that the fields of the study area would not have had effective fish populations had they not been stocked. The one control field with a large fish population almost certainly was stocked accidentally.

March and Metcalf (1949) reported the failure of benzene hexachloride for fly control in southern California shortly after DDT resistance had been found. Dieldrin, then an experimental compound, also was shown by them (on the basis of laboratory tests) to be less than fully effective against a southern California strain of flies. Since 1949, the problem of insecticide resistance in California has become widespread; it is reported for several species of mosquitoes,

Table 4.—Summary of fish trapping data, treatment, the range of the numbers of fish caught, the mean number per field, and the biomass per field.

Treatment	No. of fields[a]	Range of no. trapped	Mean no. per field	Mean weight per field (in grams)
Controls (0/acre)	18 (3)	0–778	47	21
300 Fish/acre	18 (18)	12–1362	527	157
100 Fish/acre	18 (18)	9–2453	428	168
Chlorpyrifos	18 (1)	0–3	<1	<1

[a] The number of fields in which fish were found is shown in parentheses.

and includes resistance to the organophosphorous insecticides as well as to the chlorinated hydrocarbons. The rapidly spreading resistance to the organophosphorous insecticides has made chemical mosquito control impossible in some areas and prohibitively expensive in others (Frolli, 1971; Womeldorf *et al.*, 1971).

The prospect of an encephalitis epidemic was clearly stated by Dr. William C. Reeves (1970) when he said, "I have every reason to expect epidemics of mosquito-borne viruses in the future unless new methods and materials are found to suppress the insecticide-resistant *C. tarsalis* population." Since that time, the 1971 VEE epidemic in Mexico and Texas has sharpened the point on that prediction.

Ferguson *et al.* (1966) suggested that the high tolerance of *G. affinis* to chlorpyrifos made that compound suitable for combination with the fish for mosquito control. In 1971 chlorpyrifos was authorized for application to rice fields in California at 0.025 pound per acre. Our initial experimental design included the combination of chlorpyrifos and *Gambusia affinis* as one of three alternate methods of controlling rice-field mosquitoes. However, an inadequate supply of fish forced us to omit stocking of fish into those fields scheduled for chlorpyrifos treatment. Hence, the experimental treatment that had been planned to be a single application of chlorpyrifos combined with 0.2 lb fish per acre became a unilateral chemical application similar to the blanket treatment to reduce the mosquito brood stock that has been proposed by others.

The results of the June field evaluations showed a statistically significant outbreak of mosquitoes (almost exclusively *C. tarsalis*) in those fields stocked with 0.2 lb of fish per acre. These results were surprising but became much less so as the results of the July evaluation became available.

As a group, the fields treated with chlorpyrifos in mid-June were heavy producers of mosquitoes in July and August. The differences between these fields and the less productive control fields were statistically significant during both months. This striking case of pest resurgence emphasized the importance of the invertebrate predator population. The mosquito population of the chlorpyrifos-treated fields did not simply return from the depressed level that immediately followed spraying to a level comparable to that in the control fields; it increased until it was several times greater than the controls. The reproductive rate of the mosquitoes, long recognized as well adapted to exploit opportune moments, undoubtedly exceeded that of the recolonizing invertebrate predators.

The speculative nature of our comments regarding the effect of the chlorpyrifos on the invertebrate predators reflects the limited published information available concerning nontarget organisms in general. Hurlbert *et al.* (1970) report perhaps the most complete study, though it was not done in the rice field habitat. In another paper, Hurlbert (1969) reported that the application of chlorpyrifos stimulated hatching of tadpole shrimp, a side effect that would have a negative economic value in rice fields. An inconclusive report of the impact of chlorpyrifos on certain rice field nontarget organisms was published in a trade journal (Washino *et al.*, 1968).

The degree that the mosquito population was depressed immediately following the chlorpyrifos application was not clearly established, nor was it intended to be, because the experiment was designed to find the value of the various control methods on a long-term basis. However, it should be pointed out that Steelman and Poche (1970) got 90 percent control of *Psorophora confinnis* (Lynch Arribálzaga) by using Dursban at a rate of 0.0125 lb per acre in a granular formulation on fertilizer. Our evaluations in June were made throughout a period 5 to 19 days posttreatment. At that time control was only about 40 percent. That degree of control does not seem unreasonable when one considers that Steelman and Poche found 10 percent of their population surviving immediately following application; and with the short generation time of *C. tarsalis,* the resurgence was probably well underway by late June.

Notonectidae are seasonally abundant in rice fields, but none of the California studies mentioned provide data regarding that family. The notonectids have been shown by extensive laboratory studies to be voracious mosquito predators (Ellis and Borden, 1970); and disruption of notonectid populations may be the basis for both the outbreaks where few fish were stocked, and the resurgence where the chlorpyrifos was applied.

We suspect that the outbreak of mosquitoes in June in fields stocked with fish at the lower rate was caused by a disruption in the invertebrate predator population by a fish population not large enough to be effective against the mosquitoes but sufficiently large to affect adversely the invertebrate predators. The assumed importance of the invertebrate predators in our study area is also based on the relatively low numbers of mosquitoes produced in the control fields.

During 1971, another mosquito abatement district in California stocked approximately 2300 acres of rice fields with *G. affinis* at a rate of slightly less than 0.12 lb per acre. Light trap counts in that district showed *C. tarsalis* populations to be above average in the rice growing areas (personal communication, D. E. Reed). However, the stocked acreage represented only 14 percent of the rice acreage in the district. Furthermore, with increased resistance to the organophosphorous insecticides, a general gain in the size of the *C. tarsalis* population could be expected. Perhaps the resistance did not contribute to the increase in the population, so much as the low stocking rate. In light of our June evaluation results, that possibility should be given consideration.

The potential for causing an extraordinary population of *C. tarsalis* either by stocking inadequate amounts of fish or through "one shot" insecticide applications has serious aspects in terms of epidemiology. Inappropriate control measures could contribute to the development of an epidemic.

In conclusion, we found relatively low numbers of mosquitoes developing in the undisturbed control fields of the Sutter Basin. Also, disturbing factors (fish stocked at too low a rate and insecticide application) produced statistically significant increases in mosquito production. Finally, where fish were stocked at 0.6 lb per acre, mosquito production was lower than in the control fields, but the difference was not significant at the 5 percent level. The poor contrast between these treatments is attributed to the impact of a reasonably effective invertebrate predator population. Valuable estimates of the efficiencies of aerial and surface stocking methods were gained.

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