BIOLOGICAL CONTROL OF *CULEX TARSALIS* IN A CALIFORNIA RICE FIELD

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Each year, nearly 400,000 acres of rice are grown in California. Unless control measures are taken the mosquito production of the rice fields can be very great. The rice is grown within the boundaries of 14 mosquito abatement districts (M.A.D.) where control efforts range from minimal to massive. Some of the districts with the greater rice acreages are least able to afford full control, and, indeed must even ignore those species produced in the rice to concentrate on other species. Of these 14 districts, only three use biological control methods, i.e., the planting of mosquito fish, to any appreciable degree. Sacramento County–Yolo County M.A.D. has been stocking all rice fields within the district with fish for several years. Various reasons have been given for the failure of other districts to adopt this method of control. Twelve years ago Sweetman (1958) in his classic *Principles of Biological Control* stated that "The literature contains numerous enthusiastic statements regarding the effectiveness of top minnows and other fish against mosquitoes, but seldom are data offered to support the enthusiasm." Moreover, a search of recent literature reveals that his sentence could have been written in 1970. We believe that the lack of data and the ease of chemical control explain the failure to accept the method rather than the cost of fish production and distribution.

Fresno Westside M.A.D. has about 17,000 acres of rice within its boundaries and spends about one-third of its budget on the control of rice field mosquitoes. Therefore, about $4.50 per acre is spent to control *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken in the rice fields of this district from mid-May to September. If fish are to be used a basic question that needs to be answered is, "What stocking rate is necessary?" Questions about the cost of producing and distributing fish need to be answered only if a reasonable rate would give satisfactory control for at least part of the mosquito season.

MATERIALS AND METHODS. The study area chosen was a field owned by V. C. Britton Inc. of Firebaugh, Calif., a large producer of rice. The specific field was selected because of its large size, because 1969 was the first year of rice production in this field in the rotation sequence, and because the field was far distant from population centers. The large size was necessary to allow a reasonable replication of plots within the area. The first year of production was preferred in hopes of avoiding weeds. The distance from population centers was necessary because experimental untreated checks were needed.

The experimental design provided a simple comparison of the numbers of mosquito larvae produced in six paddies where no fish were added, the numbers produced in six paddies stocked with 200 fish per acre, and the numbers produced in six paddies stocked with 1000 fish per acre.

The 145-acre field was subdivided into four parts for irrigation, but experimental plots were assigned to only three parts as shown in Fig. 1. Also, only six test paddies were placed in each part. The paddies ranged in size from 0.55 to 4.92 acres and averaged 2.90 acres. The fourth part (D in Fig. 1) served as second control. The paddies were assigned treatments randomly after excluding the first two at the head end of the field, the last

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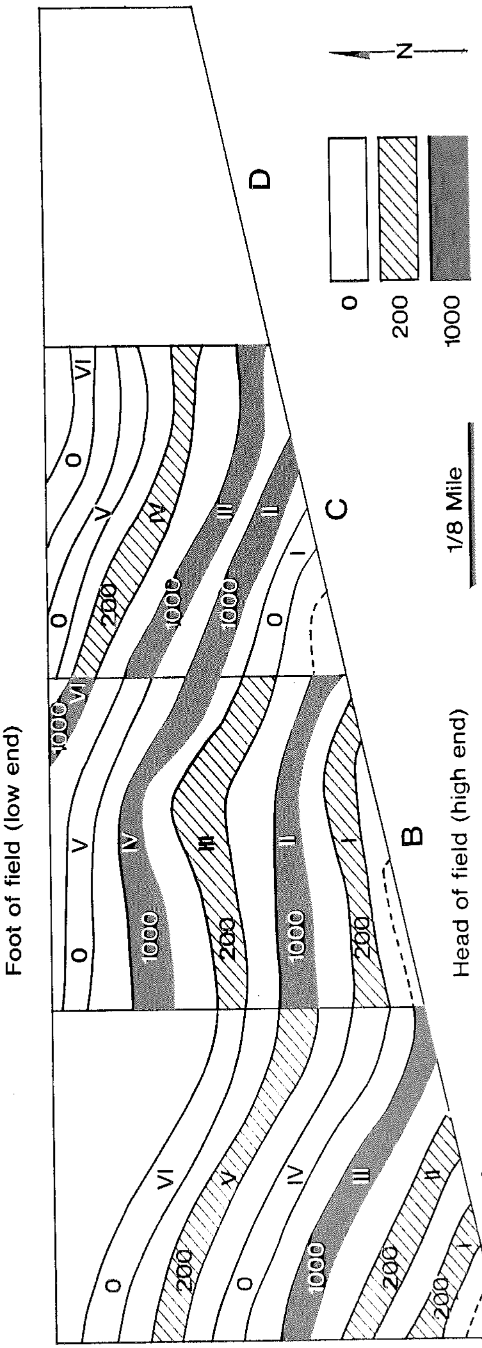

Fig. 1.—Map of the study area. Total area 145 acres, 35 acres of which were stocked with fish. The stocking rates and experimental control plots are indicated by 200, 1000, and 0.

at the foot, and every other paddy after the third paddy. Thus, unusual conditions at the head and foot of the field were avoided, and buffer zones between the experimental plots were provided.

After the irrigation boxes had been placed in the levees, screens were placed in the boxes to prevent movement of the stocked fish from one paddy to another. Hardware cloth (4 mesh) served as a screen against floating trash on the high (upstream) side of the box. Eight mesh hardware cloth was placed across the low (downstream) end of the boxes to prevent the fish from moving to the adjacent paddy. During the first 5 days that water came into the field, each screen was checked at 4- to 6-hour intervals to prevent plugging and subsequent breakage of the levees. Daily checking was required during the next 10 days, and nearly that often for the remainder of the experiment.

The fish, *Gambusia affinis* (Baird and Girard), used to stock the paddies were seined from two drainage ditches near the rice field. Because the total number of fish available was difficult to estimate, stocking proceeded in two rounds, first stocking the high rate paddies at 600 per acre and the low rate paddies at the full 200 per acre and then an additional 400 per acre where appropriate. The proper number of fish per paddy was determined by measuring the areas as shown on an aerial photograph. The number of fish was estimated by volume, 275 per dipperful, after the dipper had been calibrated with fish. Also, a representative sample of fish was taken and preserved at the time of calibration. Dissection of 40 females selected randomly from those preserved showed an average of 103.9 eggs or embryos per female. The fish were in excellent condition, large (avg. 37.1 mm standard length), and in almost pure culture. All fish other than *Gambusia affinis* were removed before the stocking. A few males may have been stocked, but the seines used were of ¼ in. mesh so the stocking was done with better than 99 percent mature females because the males and immature females could pass through the seine.

One evaluation of the mosquito populations was made by the junior author (hereafter referred to as evaluator 1) who was, of course, aware of the experimental design. Initially, evaluator 1 took ten dips at the low end of each paddy and ten dips on a transect across each paddy. After the first evaluation, the method was changed to 20 dips on a single transect across each paddy; consequently the estimates of population based on the numbers taken in ten dips from the transect at the first sampling date should be doubled for comparison with subsequent estimates. Each dipperful was poured through a larva concentrator (Husbands, 1969 describes the concentrator) and preserved, so the larvae could be sorted and identified by instar and species in the laboratory.

In addition to the above method of evaluation by evaluator 1, two other samplings were carried out by experienced men who were not informed about the stocking rates in the various paddies. Each dipped for larvae by the method he normally uses in rice fields. Thus, evaluator 2 took three dips at the levee opposite the outlet box, four dips along the low end of the paddy, and three dips along the levee above the outlet box. Evaluator 3 took 20 dips on a transect and poured them through the larva concentrator.

In addition, aquatic light traps similar in design to the one described by Husbands (1967) were tried. Both vertebrate and invertebrate predators entered the traps so, although the killing agent in the traps eventually took effect, the quantitative value of the catches was highly suspect.

Since the rice habitat is changing as the rice develops, conditions in the field at time of dipping can be better understood when they are related to the developmental stage (or phase) of the growing rice. The chronological sequence of conditions in the field and the dates of sampling are shown together in table 1.

TABLE 1.—The chronological sequence of conditions of the rice field studied.

Date	Sample Date No.	Condition of Field	Time from Seeding (Days)	Time from Stocking (Days)
May 5–10		Flooded	—12 to —7	—24 to —19
May 17		Seeded	0	—12
May 29		Stocked with fish	12	0
June 23	1	Early tiller	37	25
July 7	2	Late tiller	51	39
July 18		Propanil (herbicide) treatment	62	50
July 21	3	Jointing	65	53
Aug. 4	4	Late boot	79	67
Aug. 15	5	Late bloom	90	78

Commencing with the seeding of the rice field (which in California is done from an airplane after the field has been flooded), the first phase is that of seedling emergence. This phase is followed by tillering, which occurs about 40 to 50 days after seeding, when new shoots develop from one root system. (The number of shoots produced by a rice plant is in inverse proportion to the number of competitive plants around the developing plant). Jointing (jointing of the stems) takes place from 60 to 80 days after seeding and is closely followed by the boot phase, the period when the panicle forms within the plant sheath. Bloom is the term used to describe the next phase, the emergence of the panicle from the protection of its leaf fold, a process that takes about 5 to 7 days and occurs about 90 to 100 days after seeding. Grain formation, with its subphases, is followed by grain maturity and finally by harvest.

Larval dippings in the study area were made at 2-week intervals commencing June 23 and ending August 15. Table 1 summarizes the field condition at the time of each evaluation.

Anopheles freeborni were conspicuously absent during the test in rice surrounding the study area as well as in the study field. Though larvae were plentiful to July and early August 10 miles away in rice within 2 miles of the San Joaquin River, the species was not found in rice fields near the study area until late August. Thus we were able only to evaluate control of *C. tarsalis* in our test.

We want to thank John Britton of V. C. Britton, Inc. for permission to use the rice field, Manuel Rodriguez for help in the coordination of our research with the rice farming operations, and Commander L. A. Tomkins who provided an aerial photograph of the study area. Many of the personnel of the Fresno Westside M.A.D. and the Western Insects Affecting Man and Animals Investigations laboratory lent technical support to the project, especially Keith D. Stewart and Terrell W. Tucker. Also, special thanks are due Lawrence L. Lewallen and Richard C. Husbands of California Dept. of Public Health, who served as experimentally naive evaluators 2 and 3 respectively, and to Bruce Mackey, who gave counsel regarding statistical analysis.

RESULTS. Table 2 gives the total numbers of fourth instar larvae and pupae by evaluator 1. Table 3 shows the numbers of *C. tarsalis* larvae and pupae found collected by evaluator 1. Obviously, paddies without fish produced about 20 times as many larvae and pupae as the paddies stocked with 200 fish per acre. Furthermore, the paddies without fish produced about 95 times as many larvae as paddies stocked with 1000 fish per acre.

The numbers of larvae found within the experimental control paddies and the numbers in six paddies in an adjacent field can be compared by looking at the

TABLE 2.—Numbers of immature *C. tarsalis* collected by evaluator 1 (aware of experimental design) arranged by sampling date and treatment.

Fish/acre	No. collected on indicated sampling date[a] Jun. 23[b]	Jul. 7	Jul. 21	Aug. 4	Aug. 15	Sub-total	Total
	Experimental Plots						
0	0	16	0	0	2	18	
	19	15	18	1	1	54	
	8	4	10	0	0	22	
	0	0	0	0	0	0	
	4	8	15	7	0	34	
	1	27	10	12	9	59	
200	0	0	0	0	0	0	187
	0	0	0	0	1	1	
	1	1	2	0	2	6	
	0	0	0	0	0	0	
	2	1	0	0	0	3	
	0	0	0	0	0	0	
1000	0	0	0	0	2	2	10
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	2
	35	72	55	20	17		199
	Alternate Plots[b]						
0	3	4	6	1	0	14	
	1	0	2	1	0	4	
	17	3	8	2	3	33	
	2	0	2	0	3	7	
	6	3	9	2	5	25	
	0	5	4	9	3	21	
	29	15	31	15	14		104

[a] Twenty dips per paddy except as noted.
[b] Ten dips per paddy.

top six and bottom six lines of data in Table 2. When comparing season totals it should be noted that the sample sizes bear a relationship of 90 dips to 50 dips, favoring the experimental control paddies. That ratio is reflected by the ratio of 187 to 104 specimens found in the two categories respectively.

The results of sampling by the evaluators with no knowledge of the experimental design are given in Table 4. The pattern is similar to that already described. A statistical analysis of the data is presented in the discussion section.

The number of larvae and pupae per paddy was not expected to be a normally distributed statistic; hence, a method of analysis that did not require normally distributed data, the Mann-Whitney U test (Siegel, 1956), was chosen. That judgment was proved well-founded by the end of the study; for example, Table 2 shows the wide range of counts within any given sampling period and category of treatment. Also, many counts were high or low relative to the mean value for that block of data.

Table 5 shows low probabilities for comparisons between 0 and 200 fish per acre, and very low probabilities result from the comparisons of 0 fish per acre to 1,000 fish per acre. Therefore, in general, the alternate hypothesis is accepted. Comparisons made between 200 and 1,000 fish per acre showed no significant difference at the 5 percent level of confidence.

Table 3.—Numbers of fourth-instar larval and pupal *C. tarsalis* collected by evaluator 1, arranged by sampling period, and plot treatment.

Fish per acre	No. collected on indicated sampling date[a]					Sub-total	Total
	June 23[b]	July 7	July 21	Aug. 4	Aug. 15		
0	0	0	0	0	1	1	
	4	9	12	0	0	25	
	0	2	3	0	0	5	
	0	0	0	0	0	0	
	0	3	7	4	0	14	
	0	18	8	0	0	26	71
200	0	0	0	0	0	0	
	0	0	0	0	1	1	
	0	0	2	0	0	2	
	0	0	0	0	0	0	
	1	0	0	0	0	1	
	0	0	0	0	0	0	4
1000	0	0	0	0	1	1	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	1
							76

[a] Twenty dips per paddy except as noted.
[b] Ten dips per paddy.

The total count for the season for each paddy is perhaps the best single indication of the effect of the treatment. These comparisons are found at the bottom of Table 5. Clearly, there was a difference between unstocked paddies and those stocked with 200 fish per acre. The 1 percent level of significance was approached in that comparison, and, the results of the comparison of unstocked paddies with those stocked with 1,000 fish per acre was well below the 1 percent level of significance. The same levels of significance were reached when only the fourth instar larvae and pupae were considered (Table 6). The similarity between the two tables indicates that the fish exert pressure on all instars, not just early or late stages of development.

When the results of individual sampling periods are considered, additional information is discovered. In general, the differences between treatment levels was great during the first sampling period, greater during the second and third periods, moderate during the fourth period, and slight during the fifth period.

Discussion. It is interesting to compare the results of the dipping by evaluator 1 who knew the experimental design with those obtained by evaluators 2 and 3 who did not (Table 4). Overall, almost identical results were obtained by evaluators 1 and 2 at the first sampling date; also, though evaluator 1 found significantly more larvae than evaluator 3 in the third sampling period, the patterns of distribution were quite similar. Note that during both periods 1 and 3, evaluators 2 and 3 found greater differences between treatments than did evaluator 1 (Table 5).

The degeneration of contrast between treatments at the end of the season may be attributed to the low larval population levels and perhaps to the maturation of any *Gambusia* that had escaped the stocked paddies and invaded the control paddies and begun to reproduce. Such *Gambusia* would have made the results of stocking less striking because these fish

TABLE 4.—Numbers of immature *C. tarsalis* collected arranged by sampling date, evaluator, and treatment.

Fish per acre	No. collected by indicated evaluator on June 23[a] and July 21[b] — June 23: Evaluator 1	June 23: Evaluator 2	July 21: Evaluator 1	July 21: Evaluator 3
0	0	7	0	0
	19	6	18	5
	8	13	10	2
	0	0	0	1
	4	2	15	7
	1	3	10	4
	32	31	53	19
200	0	0	0	0
	0	0	0	0
	1	0	2	0
	0	0	0	0
	2	0	0	0
	0	4	0	0
	3	4	2	0
1000	0	0	0	2
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	2

[a] Ten dips per paddy.
[b] Twenty dips per paddy.

TABLE 5.—Probabilities (Mann-Whitney U test), that mosquito production was equal at two conditions. Results significant at the 5 percent level of confidence underlined once, those significant at the 1 percent level underlined twice.

Sampling date	Evaluator	Comparisons of stocking rates: 0 vs 200 fish/acre	0 vs 1000 fish/acre	200 vs 1000 fish/acre
June 23	1	.0721	$\underline{.0110}$	.0694
June 23	2	$\underline{.0162}$	$\underline{\underline{.0037}}$	.1587
July 7	1	$\underline{\underline{.0099}}$	$\underline{\underline{.0037}}$	.0694
July 21	1	$\underline{.0244}$	$\underline{.0110}$	.1587
July 21	3	$\underline{\underline{.0037}}$	$\underline{\underline{.0082}}$	.1587[a]
August 4	1	$\underline{.0294}$	$\underline{.0294}$	....[b]
August 15	1	.2358	.1251	.2981
Total season	1	$\underline{.0139}$	$\underline{\underline{.0066}}$	.1075

[a] The null hypothesis for this comparison: Paddies stocked with 1000 fish per acre produce no more mosquitoes than those with 200 fish per acre. The hypothesis was accepted.
[b] All samples were negative.

Table 6.—Probabilities (Mann-Whitney U test) that production of fourth-instar *C. tarsalis* larvae and pupae was equal at two conditions. Results significant at the 5 percent level of confidence are underlined once; those significant at the 1 percent level are underlined twice.

Sampling date	Comparisons of stocking rates: 0 vs 200 fish/acre	0 vs 1000 fish/acre	200 vs 1000 fish/acre
June 23 [a]	...	...	...
July 7	.0110	.0110	... [b]
July 21	.0250	.0110	.1587
August 4 [a]	...	...	...
August 15 [a]	...	...	...
Total season	.0294	.0082	.1056

[a] No tests made because of very low numbers in all categories.
[b] All samples were negative.

failed to help in their assigned plot and depressed larval populations in the control plots.

Our test did not reproduce the conditions that would exist if *Gambusia* were used for mosquito control in rice fields. For example, and most significant, individual paddies were screened off and only alternate paddies were stocked. If the fish normally tend to concentrate in a particular part of a field, the screens counteracted that tendency and gave us better control than could be expected normally. However, if any significant movement of mature fish had occurred, we would have found large numbers of fish trapped in the irrigation boxes, particularly in plots stocked with 1,000 fish per acre. That did not occur. Also, the screening may have restricted the entry of a fish population with the irrigation water. However, carp were observed throughout the field despite the screening. They either passed through the screens as fry or jumped the screens and/or levees.

The use of alternate paddies would have tended to produce a dilution of the effective stocking rates if there had been significant movement of newborn fish through the screens and into neighboring paddies. The net result would have been to make the larval counts somewhat higher than would be expected if fish had been stocked in all paddies. However, such escapees would have had to pass through two irrigation boxes to reach another experimental plot. Furthermore, of the six experimental control plots, invasion could only have occurred from either side of two, and from one side only of three (See Fig. 1). Therefore, the probability that the escapees would have had a significant influence on the experimental control paddies seems to be slight.

The distribution and movement of *Gambusia* within a rice field may be major factors in the effectiveness of this control method. The necessary stocking rate for a high level of control can be judged precisely and fairly if, and only if, experimental testing is done in both an operationally feasible and biologically reasonable manner. We believe our test demonstrated that in individual paddies the fish were effective.

Horsfall (1942) reduced mosquito production by placing *Gambusia affinis* in 3-foot square enclosures in a rice field; his stocking rates were 4,840 and 9,680 per acre. Sokolov and Chvaliova (1936) found that a density of 9,293 *Gambusia* per acre (inferred to be their stocking rate) reduced the production of anophelines by approximately 90 percent. Thus, the density of fish used in these early studies and that used in the present study differed greatly, yet the desired effect was achieved with stocking rates of 1,000 fish per acre or less. In fact, in terms of percentage control, a stocking rate of only 200 fish per acre resulted in 95 percent control on a season-long basis.

In our view, the major obstacle to widespread use of *Gambusia* for mosquito control in California rice fields is the limited numbers of fish available in May and early June. That obstacle plus the lack of valid information about the method's success: failure ratio has prevented adoption of fish for control.

SUMMARY AND CONCLUSIONS. *Gambusia affinis* were stocked in six rice paddies at 200 mature females per acre, and in six paddies at 1,000 per acre. An additional six unstocked paddies within the study area were used as experimental controls. Also, six unstocked paddies in an adjacent field were evaluated. The populations of larval *Culex tarsalis* were evaluated five times, at 2-week intervals, from late June until mid-August.

On the basis of sampling by one person of the three types of paddies, 94 percent of the mosquito larvae were in the unstocked paddies, 5 percent were found in paddies stocked with 200 fish per acre, and 1 percent were found in paddies stocked with 1,000 fish per acre. Statistical analysis, by evaluation periods as well as on a season-long basis showed that larval populations were significantly lower where fish had been stocked, except on the last evaluation period when larval populations were quite low, thus making chance variation a more important factor.

We conclude that the stocking of the rice paddies with *Gambusia affinis* was responsible for suppressing mosquito larval populations in the rice paddies. Furthermore, we conclude that a testing program comparing fields rather than paddies is justified.

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