THE MOSQUITOFISH[1] AS A BIOLOGICAL CONTROL AGENT AGAINST *CULEX TARSALIS* AND *ANOPHELES FREEBORNI* IN SACRAMENTO VALLEY RICE FIELDS[2]

JAMES B. HOY,[3] ALLAN G. O'BERG [4, 5] AND EUGENE E. KAUFFMAN [4]

ABSTRACT. Eighteen rice fields were stocked with *Gambusia affinis* (Baird & Girard), at six rates, each replicated three times. Stocking rates ranged from 50 to 300 females per acre. Three experimental control fields at 0 fish per acre were also chosen. Mosquito populations, both *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken, were evaluated from mid-June until early September. Population suppression was directly related to stocking rates.

INTRODUCTION. *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken are the two most important mosquito species associated with rice culture in California. Both species are disease vectors, and the latter is also a serious pest species. The tremendous numbers of mosquitoes that can be produced by a single acre of rice and the growing problem of insecticide resistance in the mosquitoes of California are facts that emphasize a need for alternate methods of mosquito control. The severity of the resistance problem is well documented (Wilder and Schaefer, 1970; Womeldorf *et al.*, (1968). Resistant populations of *C. tarsalis* are already found within the rice growing areas of California. Frolli (1968) and Kauffman (1968) clearly stated the increased costs of mosquito control when resistance to the organophosphorus insecticides develops.

The use of *Gambusia affinis* (Baird & Girard), the mosquitofish, for control of mosquitoes in rice fields has been studied by many researchers. Field tests were reported by Geiger and Purdy (1919), Sokolov and Chvaliova (1936), Horsfall (1942), Craven and Steelman (1968), and Hoy and Reed (1970). Although the latter study included six replications each of two stocking rates and controls, no tests using many fields over a large area (with experimental controls) have been reported. With this in mind, we designed an experiment to determine the degree of control at various stocking rates and the variation in effect of stocking among fields. A special effort was made to keep the experimental design consistent with reasonable operational practice.

MATERIALS AND METHODS. Twenty-one fields were stocked at seven rates ranging from 0 to 300 fish per acre. Each stocking rate was replicated three times, with treatments assigned randomly.

All of the fields were within a 6-mile radius of the center of the study area, and within that area, about one-tenth of the land was planted with rice. Other land uses in the area were largely irrigated pasture, orchards, and barley. With respect to rice field problems, the mosquito control program of the immediate past has depended largely on the use of parathion.

The fish (*G. affinis*) used in the study were seined from man-made unmanaged sources such as farm ponds and sewer ponds. Seines with 5.3 x 8-mesh netting were used, and then the fish were sized through 4-mesh screens. Hence, with rare exception, only mature female fish were caught. As the fish were collected, an estimate of the numbers was made by a volumetric method, i.e., filling a clear plastic cylinder with water to a first level and then adding fish to a second level. (That method actually gives a measure of

[1] *Gambusia affinis,* Poeciliidae.

[2] We wish to acknowledge the technical assistance of Mr. Craig Prest, an employee of Sutter-Yuba M.A.D. and the statistical assistance of Dr. Bruce E. Mackey of Biometrical Services, ARS-USDA.

[3] Entomology Research Division, ARS-USDA, 5544 Air Terminal Dr., Fresno, CA 93727.

[4] Sutter-Yuba Mosquito Abatement District, 925 Market St., Yuba City, CA 95991.

[5] Present address: Yuba College, N. Beale Rd. and Linda Ave., Marysville, CA 95901.

biomass rather than true numbers. However, a standardization of biomass is probably more significant than a precise measurement of numbers of fish per acre.)

The fish were trucked to the rice fields in a 500-gallon insulated stainless steel tank. Rather than stocking each paddy, we dumped the fish in batches, at least one batch for each 20 acres. The fields were stocked 4 to 46 days following seeding with a mean elapsed time of 23.6 days. The treatments were assigned randomly without regard to the time after flooding or seeding of the field.

The mosquito production of the experimental fields was judged by dipping for larvae and pupae. During the first evaluation period, each of the two evaluators took 50 dips per field and during the remaining four evaluations each evaluator took 75 dips per field. The samples were concentrated (see Womeldorf *et al.*, 1963) in the field and examined in the laboratory the same day. Larvae and pupae were identified to genus, and larvae to instar. Previous experience had shown that the rice fields of the study area produce primarily *C. tarsalis* and *A. freeborni.* On very rare occasions, single specimens of other anopheline species have been found.

Each evaluation period was defined as the sampling of 20 fields, Monday through Friday of one week, and the sampling of the 21st field on the following Monday morning. The sampling order was random with respect to the stocking rate and was different for each period. Field evaluations were at 3-week intervals for the second, third and fourth periods and 2-week intervals between the first and second and the fourth and fifth periods.

RESULTS AND DISCUSSION. All immature mosquitoes collected in our study fields were either *Culex* or *Anopheles,* presumably *C. tarsalis* or *A. freeborni. Culex* were most common during the first half of the rice growing season, whereas *Anopheles* were most common during the second half of the season (Table 1). The sharpness of this division is seen both in fields with and fields without fish, but because of the larger numbers of specimens collected in the fields without fish the division is most striking there. This pattern is very similar to that described by Markos and Sherman (1957), and Bailey and Gieke (1968).

Since a few of the fields were not flooded until late May a complete round of evaluation would not have been meaningful until all of the fields had been flooded sufficiently long to allow mosquito production to begin. Hence, the first evaluation period lasted from June 15 through June 22, and by that time the *C. tarsalis* populations were well developed. Since the data are truncated in early season for *C. tarsalis* and in late season for *A. freeborni,* only a rough estimate of the relative sizes of the populations is possible. Furthermore, the differential rates of development of the two species make comparison difficult. Nevertheless, the two species were found in numbers of the same order of magnitude but at two well-defined and different times.

Table 1 shows the numbers of immature mosquitoes found in the various fields, with the experimental control fields at the top of the table and fields with progressively higher stocking rates ranked below.

The relationship between the stocking rate and the total numbers of specimens found is shown in Fig. 1, with fewer mosquitoes at the highest stocking rates. The trend is apparent and there is a significant linear regression. The regression line is calculated, based on the log transformation.

The degree of mosquito control should not be judged only in terms of the *total* mosquito production of the 10 treatment fields. The period of time that we observed the 21 experimental fields ranged from shortly after the beginning of mosquito production until near the time that the rice fields were drained. The degree of success of the various stocking rates changed as the season progressed. In general, the higher stocking rates gave good control of *Culex* continuously from the first evaluation period, whereas the lower stocking rates gave poor control early in the season and improved irregularly as the season progressed.

The data for both species for the three

lower and the three higher stocking rates were lumped for graphic comparison with the experimental controls (Fig. 2). Note the upturn of all three lines at the end of the season. The immature mosquito population of the control fields at the last evaluation was more than 94 percent anopheline in makeup. However, at the time of the fourth evaluation, the populations of the experimental control fields already were made up of a similar proportion of *Anopheles*, i.e. 95 percent. Hence the late season decline of contrast between control fields and experimental fields does not coincide closely with the upsurge in proportion of the anopheline specimens. The significance of this upturn and significance of field to field variation will be discussed jointly following a statement of the degree of variation that occurred.

After the fourth round of evaluation the senior member of the evaluation team was

Table 1.

Numbers of immature mosquitoes found in rice fields, arranged by fish stocking rate, sampling date and genus.

Stocking rate (per acre)	Field number	*Culex* Sampling period[a]						*Anopheles* Sampling period[a]					
		1[b]	2	3	4	5	Total	1	2	3	4	5	Total
0	3	47	3	13	0	2	65	2	0	29	28	82	141
	5	38	99	199	9	13	358	0	4	36	123	127	290
	14	12	100	0	1	1	114	0	0	2	43	46	91
		97	202	212	10	16	537	2	4	67	194	255	522
50	17	19	6	4	0	0	29	0	0	0	7	7	14
	18	22	117	11	0	0	150	1	0	3	6	0	10
	21	152	12	13	1	0	178	1	0	13	10	41	65
		193	135	28	1	0	357	2	0	16	23	48	89
100	6	37	16	1	1	0	55	22	1	6	33	61	123
	8	0	5	4	1	0	10	2	2	7	27	13	51
	11	0	0	1	6	0	7	0	1	4	22	68	95
		37	21	6	8	0	72	24	4	17	82	142	269
150	4	0	2	44	4	3	53	0	6	46	8	43	103
	7	2	1	6	0	0	9	0	0	9	1	10	20
	10	11	266	47	36	1	361	1	8	10	31	40	90
		13	269	97	40	4	423	1	14	65	40	93	213
200	1	0	39	2	0	0	41	0	6	29	1	3	39
	12	36	7	3	0	0	46	3	0	5	0	4	12
	15	8	1	0	0	0	9	1	0	5	3	8	17
		44	47	5	0	0	96	4	6	39	4	15	68
250	16	9	3	6	0	0	18	0	1	4	6	25	36
	19	5	55	21	2	0	83	0	2	12	9	15	38
	20	6	57	4	0	0	67	0	0	6	0	12	18
		20	115	31	2	0	168	0	3	22	15	52	92
300	2	6	0	3	0	0	9	0	0	6	25	40	71
	9	7	9	0	0	0	16	3	4	30	32	44	113
	13	2	0	0	0	0	2	0	0	0	1	28	29
		15	9	3	0	0	27	3	4	36	58	112	213

[a] Periods 1 through 5 began on the respective dates as follows: June 15, June 29, July 20, Aug. 10, Aug. 24.

[b] During Period 1, 100 dips per field, all other periods 150 dips per field.

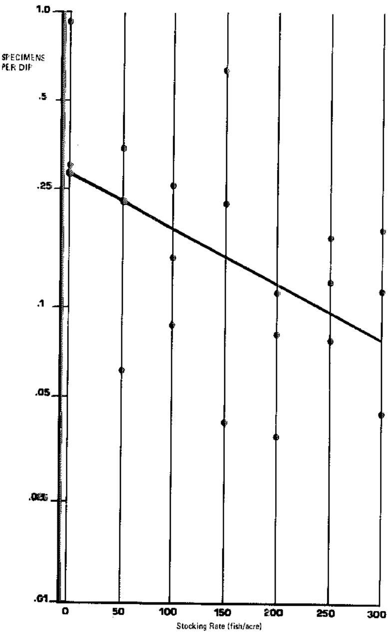

FIG. 1.—The number of specimens per dip relative to fish stocking rate, based on 700 dips per field in 21 fields.

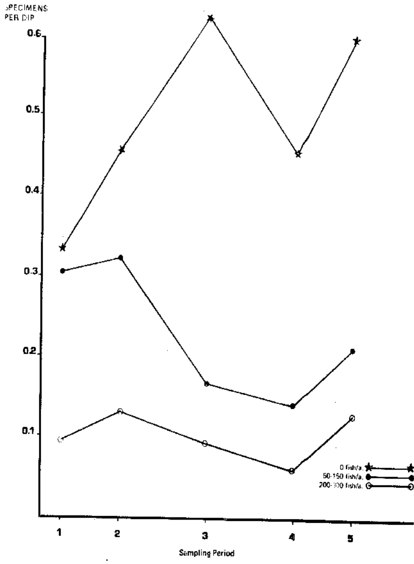

FIG. 2.—The number of specimens per dip relative to combined fish stocking rates at five times during the rice growing season.

asked whether any field stood out as an exception in terms of cultural methods. (At that time the assignments of treatments still had not been disclosed.) His reply was that only one field was exceptional, i.e. Field 10, which had extreme fluctuations of the water level. Reference to Table 1 reveals that of the 18 fields stocked with fish, Field 10 (the third field in the 150 fish per acre category) was not only the field with the greatest deviation from the other members of its category, but also that deviation was in the direction of greater mosquito production. Although the water level varied, the mud was not exposed, and hence floodwater mosquitoes were not provided with oviposition sites.

The variation of results within a particular stocking rate category deserves discussion. We expected that the vagaries of actual stocking rates (by paddies) and the reproductive rates in the various fields and paddies would cause a significant variation in the degree of mosquito control achieved. The variation that we observed is best illustrated in Fig. 1. Note that the slope of the calculated regression line crosses the 0.1 specimen per dip line at a stocking rate of 243 fish per acre. At that stocking rate, based on this study, one would expect approximately half of the fields to produce more than 0.1 specimen per dip. The slope of the line and the degree of variation are indications of how much the stocking rate would have to be increased to have 90% of the stocked fields producing less than 0.1 immature mosquitoes per dip.

As an arbitrary goal for future efforts we have chosen: 90% of the fields achieving 0.1 larvae per dip. At a stocking rate of 300 or 350 fish per acre that goal may well be reached if the fish are stocked immediately after seeding of the field.

Despite the recognized variation among

fields, the various stocking rates can be compared with the control group to determine the significance of differences in the numbers of mosquitoes found. Table 2 shows the probabilities that mosquito production was equal for a variety of comparisons including comparison by genus and by combined stocking rates. As might be expected, the level of statistical significance tended to increase as higher stocking rates were compared with the experimental controls. A less conspicuous quality of Table 2 is that the levels of significance tended to be higher for the comparisons of the *Anopheles* data than the *Culex* data. This should be considered in conjunction with the late-season decline in contrast (Fig. 2) between the mean number of mosquitoes in the control fields and mean numbers in the stocked fields. Whether or not the discrepancy is the result of chance is debatable. However, one alternate hypothesis to that of chance variation could be that in late season the biological control agent has reached a population level that assures control, but simultaneously the habitat has changed sufficiently to shelter a larger portion of the mosquito population than it did during the earlier rounds of evaluation. Thus, the *contrast* between the two categories (stocked fields and controls) is sharp, but the *difference* in population means is not as great as earlier in the season.

With respect to *C. tarsalis,* these results agree well with those reported by Hoy and Reed (1970). In that study very good control was rapidly achieved at the stocking rate of 1000 females per acre. Furthermore, a rate of 200 females per acre resulted in good control. However, the absence of *A. freeborni* in the rice field

TABLE 2.

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

	Experimental controls vs. 1 treatment[a]		Experimental controls vs. similar treatment[b]	
Taxa	Comparison (0/acre vs.)	P	Comparison (0/acre vs.)	P
All species	50/acre	.200		
	100	.050	50–150/acre	n.s.
	150	.200		
	200	.050		
	250	.050	200–300	.01
	300	.050		
Culex	50	.500		
	100	.050	50–150	n.s.
	150	.350		
	200	.050		
	250	.200	200–300	.025
	300	.050		
Anopheles	50	.050		
	100	.200	50–150	.05
	150	.100		
	200	.050		
	250	.050	200–300	.01
	300	.100		

[a] Three control replications compared with 3 experimental replications of each category.
[b] Three control replications compared with 9 experimental replications combined as one category.

studied by Hoy and Reed (1970) [and in that Mosquito Abatement District for all intents and purposes (Reed and Husbands, 1970)] prevents further comparison.

The ultimate measure of a mosquito breeding site is either the number of female mosquitoes that emerge or the number of bites resulting from the production of the site. Since neither measurement is practical at this time we have relied on determination of the number of larvae and pupae found in the water. In the interest of standardization and comparability of this and other studies, we present in Table 3 the distributions of developmental stages found in the three fields where no fish were introduced. (For both *Culex* and *Anopheles* the second instar was the most abundant in the samples.) The amount of time spent in each stadium, differential mortality rates, the time of sampling, and sampling method bias, all contribute to deviation from a 1:1:1:1:1 ratio of the various instars. Therefore, the data in Table 3 represent the net result after the above factors (and perhaps others) have exerted influence.

TABLE 3.

The frequencies of occurrence of developmental stages of mosquitoes in the three experimental control fields.

	Culex					*Anopheles*				
Field	I	II	III	IV	Pupae	I	II	III	IV	Pupae
3	11	29	9	10	6	41	78	8	8	6
5	129	156	39	23	14	64	174	37	12	3
14	31	26	8	14	35	18	62	8	2	1
Total	171	211	56	47	55	123	314	53	22	10
% of total	32	39	10	9	10	24	60	10	4	2

The success of a control method depends on many operational techniques. Following this study we have considered ways of improving upon the methods described in this paper. The large amount of variation in the early-season results might have been reduced if the fish had been stocked sooner than the 4 to 46 day delays described above. Indeed, Sacramento-Yolo County M.A.D. routinely stocks fields at the earliest possible date (J. Fowler, Sacramento-Yolo County M.A.D., personal communication). Our delay was planned, although planned to be an average of 15 days rather than 23.6, with the hypothetical benefit of stocking into fields well along in zooplankton production, thus hopefully avoiding "migration" of the fish. Another assumption made while planning this study was that stocking each paddy was superior to stocking at only one point per field. The method of stocking at one point for each 20 acres (about 6 paddies) of a given field was a compromise demanded by our method of fish transport. That original assumptions stands, but the effect of stocking on less than a paddy by paddy basis is unknown.

Finally, our stock was from varied sources. That fact would assure that some fields were stocked with the best fish available, yet others were stocked with less than the best. Hence, our results would be less than the best possible. Again we point out that, with respect to the stocking technique, this study was executed in a manner not greatly different from expected operational routine. Thus refinements of technique would perhaps have given even better results. Development of a reliable source of healthy fish is imperative for full efficiency of mosquito control with *Gambusia*.

SUMMARY AND CONCLUSIONS. In Sutter-Yuba Mosquito Abatement District 18 rice fields were stocked with mature female *Gambusia affinis*, the mosquitofish, at six rates, each replicated three times. Stocking rates ranged from 50 fish per acre to 300 fish per acre at increments of 50 fish per acre. Three experimental control fields were also chosen. At approximately equal intervals throughout the rice growing season the fields were sampled five times by two skilled evaluators, who were unaware of the stocking rates. During the first evaluation each man took 50 dips in each field. During the four subsequent evaluations each took 75 dips in each field. The immature mosquitoes found were classified according to genus and instar.

The seasonal distributions of immature *Culex* and *Anopheles* were determined to be sharply different. The overall degree of reduction of *Culex* populations where fish were stocked was good, but individual fields varied greatly. The overall control of *Anopheles* was less striking, but the reliability of effect was greater than with *Culex*. Methods for improving upon the degree of control are discussed.

From this study, we have concluded that reasonable mosquito control in California rice fields can be achieved by early season stocking of approximately 300 mature *Gambusia affinis* per acre.

Literature Cited

Bailey, S. F. and Gieke, P. A. 1968. A study of the effect of water temperatures on rice field mosquito development. Proc. Calif. Mosquito Control Assoc. 36:53–61.

Craven, B. R. and Steelman, C. D. 1968. Studies on a biological and a chemical method of controlling the dark rice field mosquito in Louisiana. J. Econ. Entomol. 61:1333–1336.

Frolli, R. F. 1968. The effects of insecticide resistance on Kings County Mosquito Abatement District. Proc. Calif. Mosquito Control Assoc. 36:89–90.

Geiger, J. C. and Purdy, W. C. 1919. Experimental mosquito control in rice-fields. J. Amer. Med. Assoc. 72:774–779.

Horsfall, W. R. 1942. Biology and control of mosquitoes in the rice area. Univ. Arkansas Agr. Exp. Sta. Bull. 427, pp. 40–41.

Hoy, J. B. and Reed, D. E. 1970. Biological control of *Culex tarsalis* in a California rice field. Mosq. News 30:222–230.

Kauffman, E. E. 1968. The effects of insecticide resistance on Sutter-Yuba Mosquito Abatement District. Proc. Calif. Mosquito Control Assoc. 36:91.

Markos, B. G. and Sherman, E. J. 1957. Additional studies on the distribution of mosquito larvae and pupae within a rice field check. Mosq. News 17:40–43.

Reed, D. E. and Husbands, R. C. 1970. Integration of larval surveillance techniques in the operational program of the Fresno Westside Mosquito Abatement District. Proc. Calif. Mosquito Control Assoc. 37:98–101.

Sokolov, N. P. and Chvaliova, M. A. 1936. Nutrition of *Gambusia affinis* on the rice fields of Turkestan. J. Animal Ecol. 5:390–395.

Wilder, W. W. and Schaefer, C. H. 1970. Organophosphorus-resistance levels in adults and larvae of the pasture mosquito, *Aedes nigromaculis* (Ludlow), in the San Joaquin Valley of California. Proc. Calif. Mosquito Control Assoc. 37:64–67.

Womeldorf, D. J., Gillies, P. A. and Holton, J. R. 1963. An improved mosquito larvae concentrator. Mosq. News 23:351–352.

Womeldorf, D. J., Gillies, P. A. and White, K. E. 1968. Present status of insecticide resistance in California mosquito larvae. Proc. Calif. Mosq. Control Assoc. 36:81–84.