THE EFFICACY OF MOSQUITOFISH[1] FOR CONTROL OF *CULEX TARSALIS*[2] IN CALIFORNIA RICE FIELDS[3]

JAMES B. HOY[4] AND DAVID E. REED[5, 6]

ABSTRACT. Mosquitofish, *Gambusia affinis* (Baird and Girard) were stocked at two rates (100 and 200 fish per acre) in 15 rice fields. Six other fields served as experimental controls. The mosquito production of the 21 fields was sampled seven times, at two-week intervals. Approximately 99 percent of the specimens collected were *Culex*. Fields stocked with fish had significantly lower production of mosquitoes at both stocking rates. The data were analyzed both by sampling period and stocking rate.

INTRODUCTION. At the Thirty-Seventh Annual Conference of the California Mosquito Control Association, the following statement was made:

> "All associated with mosquito control are aware of the problem posed by mosquito resistance to pesticide chemicals and the rather clear indication that the worst is yet to come. This may be the factor that finally not only conditions us to accept integrated control but forces us into it." (Smith, 1970).

During 1969 we (Hoy and Reed, 1970) conducted in a single rice field a replicated test of the efficacy of mosquitofish for mosquito control. Inasmuch as good control was achieved with a stocking rate of 200 fish per acre we sought answers to the following questions: (a.) How much variation among fields may be expected? (b.) How would reducing the stocking rate to 100 fish per acre affect the degree of mosquito control? (c.) Can mosquitoes be effectively controlled in fields stocked later than June 1?

MATERIALS AND METHODS. The flooding of the rice fields occurs during the last half of April and through May. Occasionally fields are flooded as late as early June, but they are the exceptional cases. Hence, we planned to study three categories of fields, those flooded in early-, mid- or late-season. For each category 7 fields were chosen, 2 as experimental controls, 3 to be stocked with 100 fish per acre, and 2 to be stocked with 200 fish per acre. Thus 21 fields were selected—6 experimental controls, 9 to be stocked at 100/acre and 6 to be stocked at 200/acre. Treatments were assigned to fields at random within each category. All fields within an area roughly 5 miles wide and 15 miles long were eligible for selection unless the field was within 2 miles of Firebaugh or Mendota and unless the acreage was less than 20 or more than 160.

Fish for stocking were seined from local ditches. Using 4-mesh seines ensured that only mature female fish were caught. All stock was measured volumetrically. Restocking of 4 fields was necessary because of draining by the farmer prior to application of an herbicide. In two cases, Fields 11 and 16, part of the stock was obtained from a local bait farmer. Field 17 was stocked entirely from this source (Table 1).

The fields were sampled for mosquitoes by a highly experienced man who was uninformed concerning the treatments that had been applied. Ten paddies were sampled, the 2nd, 3rd, and 4th from the head and the foot of the field and the central 4 paddies. A single U-shaped

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[1] Poecilliidae.

[2] Diptera:Culicidae.

[3] Mention of a pesticide in this paper does not constitute a recommendation of this product by the U. S. Department of Agriculture.

[4] Entomology Research Division, Fresno, Calif. Present address, 1050 San Pablo Ave., Albany, Calif. 94706.

[5] Fresno Westside Mosquito Abatement District, Firebaugh, Calif. 93622.

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TABLE 1.—Treatment assignments (number of fish/acre), seeding dates, and miscellaneous information regarding 21 randomly selected rice fields in Fresno County, Calif., during 1970.

Flooding period[a]	Field No.	Treatment	Seeding date	Stocked post seeding (da.)	Restocking date	Accidental parathion application
1	1	100	Apr 26	12		
	2	100	Apr 27	14		
	3	0	Apr 24	..		
	4	200	May 1	7	June 22	
	5	200	Apr 25	20		
	6	0	Apr 30	..		
	7	100	Apr 21	17		
2	8	100	May 5	16		
	9	0	May 8	..		June 27
	10	200	May 1[b]	20		
	11	100	May 12	9	June 15	
	12	0	May 13	..		
	13	100	May 12	27		
	14	200	May 5	21	June 22	
3	15[c]	0	May 18	..		June 24 and July 16, 29
	16	100	May 9[b]	31	June 15	
	17	200	May 31	16		
	18	100	May 19	21		
	19	0	May 31	..		
	20[c]	100	May 25	21		July 23 and Aug 5
	21[c]	200	May 15	24		June 13 and July 2

[a] The periods may be defined as follows: Period 1, fields flooded prior to May 1; Period 2, fields flooded between May 1 and 14; Period 3, fields flooded after May 15. Note that Field No. 16 was inadvertently classed in Period 3, hence stocking was done in that period.

[b] Although the seeding date is probably the most significant date on a season-long basis, fields were assigned to the stocking period on the basis of earliest *known* flooding date for operational reasons.

[c] These fields are not included in the analysis because of the accidental parathion applications on the dates indicated.

transect was made in each paddy, sampling first along a levee, then across the paddy, and finally along the opposite levee. Twenty dips were taken in each paddy and were concentrated with a hand concentrator (Husbands, 1969) in a manner previously described (Hoy and Reed, 1970).

Each field was sampled at 14-day intervals, with a total of 7 sampling periods. A balanced sequence of sampling was designed so that each treatment category was sampled an equal number of times during the three subdivisions of the sampling day. That is, experimental controls and fields stocked with 200 fish per acre (6 each) and the 9 fields stocked at 100 fish per acre were equally divided into early-morning, late-morning, and early-afternoon sampling groups.

Previous experience had shown that mosquito production is negligible prior to advanced development of the rice seedlings. Furthermore, it is during the first third of the rice growing season that the fields are drained for herbicide application. Therefore, the first round of sampling began June 15, with the intent of sampling after reflooding of those fields drained for herbiciding.

In addition to the routine sampling, the junior author sampled each field once during the season, always within 48 hr of the routine sampling. The statistical comparison of his results with the routine collections showed a correlation of 0.896, which is very highly significant—$N=21$.

An effort to assess the sizes of the adult mosquito populations of the various rice fields by means of carbon dioxide traps similar to those of Bellamy and Reeves (1952) yielded very irregular results. The great variation among trap catches within the same field forced us to forego comparisons among fields.

RESULTS AND DISCUSSION. The addition of fish during restocking could be viewed as contributing to an existing population with an end result of an effective stocking rate of twice that intended. However, it should be noted that the contribution was probably more than balanced by the lost time (for reproduction) and actual losses due to drying and bird predation. Furthermore, the effect of stocking, in terms of mosquito control, may well be the work of the first brood of young, hence the young delivered during the first 3 weeks following stocking may be of paramount value. In which case, if the first brood survived draining, the relative importance of the added stock would be slight.

The numbers of *Culex* and *Anopheles* larvae and pupae collected during the comparison sampling by the two evaluators are shown in Table 2. Throughout the season *Culex* vastly outnumbered *Anopheles* in the samples of both evaluators. Since *Anopheles* were so rare in the samples, we have omitted their numbers from further analysis. We feel that presenting an unconfounded set of data that applies to *Culex* (with *Culex tarsalis* Coquillett doubtless the species in at least 99 percent of the cases) best serves the interest of the applied scientist as well as the more esoteric scientist. The rarity of *Anopheles* should be kept in mind when operational strategy is contemplated. Control efforts in Fresno Westside Mosquito Abatement District (M.A.D.) rice fields are directed at *C. tarsalis* to all intents and purposes.

The numbers of *Culex* specimens collected throughout the season are shown in Table 3, arranged by treatment, field, and sampling period. The exclusion of data from sampling periods 1 and 7 is because many fields were drained during those two periods. The early draining was to facilitate weed control and the late draining was in preparation for harvest. One field in each treatment category was excluded from the experimental design because of accidental application of parathion by the M.A.D. airplane. To the best of our knowledge, there were no other pesticide applications in our test fields. Within the county, neither insect pests nor tadpole shrimp are problems for the rice farmers.

The data in Table 3 are the result of 200 dips per field per sampling period. Those fields with 20 or more specimens in the 200 dips would be sprayed with 0.1 lb. parathion per acre under normal procedure. Hence, wherever the number of specimens is 20 or greater, the data are underlined to emphasize that insecticide application would have been made. Of the 25 indicated evaluations of unstocked fields, 17 applications would have been made—68 percent of the cases. Of the 40 indicated evaluations of fields stocked at 100 fish per acre, 9 applications would have been made—22.5 percent of the cases. Of the 25 indicated evaluations of fields stocked at 200 fish per acre, 5 applications would have been made—20 percent of the cases. Also, note that 11 of the 14 hypothetical applications in stocked fields

TABLE 2.—Comparison of the ratio of *Culex* to *Anopheles* specimens arranged by evaluator and sampling period.

Evaluator	Sampling period 1	2	3	4	5	6	All periods
A[a]	29:0	41:0	260:0	32:1	332:2	95:5	789: 8
B	5:0	15:0	417:1	36:0	396:0	230:4	1,094: 5
							1,883:13

[a] The routine evaluator is designated A; the junior author, B.

TABLE 3.—Numbers of *Culex* specimens (larvae and pupae) collected, arranged by treatment, field, and sampling period. Underlining indicates that by present criteria the field would have been treated with parathion.

Fish per acre	Field No.[a]	No. of *Culex* collected in individual sampling period[b, c] 2	3	4	5	6	All periods	Grand total	Field mean	% Control
0	3	69	172	32	71	197	541			
	6	71	90	76	70	250	557			
	9	2	41	5	2	0	50			
	12	49	38	5	1	1	94			
	19	0	23	55	321	545	944	2186	437	
100	1	15	1	0	0	0	16			
	2	18	3	0	0	0	21			
	7	7	33	55	17	85	197			
	8	7	22	3	1	2	35			
	11	39	21	0	0	2	62			
	13	12	5	0	0	0	17			
	16	53	17	8	1	2	81			
	18	60	143	15	0	0	218	647	81	81.5
200	4	39	6	0	0	0	45			
	5	12	0	1	1	0	14			
	10	5	3	0	1	0	9			
	14	78	2	0	1	0	81			
	17	42	150	22	13	9	236	385	77	82.4

[a] Data for fields 15, 20, and 21 were excluded from the analysis because of accidental applications of parathion on the dates indicated in Table 1.

[b] Data from sampling periods 1 and 7 omitted because drained fields reduced the number of replicates below the numbers necessary for valid statistical comparison.

[c] Result of 200 dips, 20/paddy in each of 10 paddies.

would have occurred during the 2nd and 3rd sampling periods, whereas the experimental control fields, as a group, would have required treatment throughout the season.

One might suggest that application of chemical control during a given period would preclude application during the next period. However, during the 1970 season rice fields within Fresno Westside M.A.D. were treated an average of 5.5 times, in other words, about every 2 weeks, from mid-June until late August. This evidence suggests that any given application can be expected to give less than 2 weeks of control. Furthermore, if the insecticide has any lasting effect, the effect should be even greater in the presence of a growing predator population, provided, of course, that the insecticide had no detrimental effects on the predators.

Another aspect of the data in Table 3 is the comparison of treatments on the basis of the mean number of specimens found as a season-long total. Fields averaged 437 specimens where no fish were stocked, 81 specimens where 100 fish per acre were stocked, and 77 specimens where 200 fish per acre were stocked. Converted into terms of percentage control, 81.5 percent control was obtained by stocking of 100 fish per acre and 82.4 percent by stocking of 200 fish per acre. The poor degree of control in the first part of the season is balanced by good control in mid-season and excellent control during the last two evaluation periods.

Just as we were surprised by the very high percentage of control during the last month of the season, we were surprised by the poor control during the initial part of the season. This may be explained in part by having only 5 replicates of unstocked fields. If they as a group tended to be late bloomers, the contrast with stocked fields would shift from slight to very great. Certainly Field 19 was slow in producing, but eventually it produced more specimens than any other field.

The comparisons of the numbers of times that chemical control would have been done, or percentages of control at the two levels of treatment, lack an estimate of the probability that these results could have occurred by chance alone. The Mann-Whitney U test (Siegel, 1956) provides a method of stating a probability that there is no difference. Table 4 shows the probabilities of three comparisons within each sampling period and for the combined data for periods 2 through 6.

When the productions of the unstocked fields were compared with those of fields stocked with 100 fish per acre, the 2nd and 6th periods showed no significant difference. The results during the 5th period were significant at the 1 percent level, and periods 3 and 4 were significant at the 5 percent level. The combined results were also significantly different. When the productions of the unstocked fields were compared with those of fields stocked at 200 fish per acre, all comparisons except that of the 2nd period were significantly different. Yet, comparisons of productions by fields stocked at 100 vs. 200 fish per acre showed no significant differences either by period or with all periods combined.

TABLE 4.—Probabilities (Mann-Whitney U test), that *Culex* 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 period	Comparisons of stocking rates		
	0 vs. 100 fish per acre	0 vs. 200 fish per acre	100 vs. 200 fish per acre
2	0.467	0.500	0.416
3	0.015	0.048	0.262
4	0.047	0.016	0.362
5	0.005	0.048	0.177
6	0.085	0.048	0.262
Total for 2–6	0.023	0.028	0.416

There are at least two factors that no doubt contributed to the slight difference between the effects of the two stocking rates, one is biological, the other is statistical. Evidence was developed in a parallel study of fish populations that at the two stocking rates used, the numbers of fish in a field reach an asymptote well before mid-season (Hoy and O'Grady, 1971). Therefore, differences in degree of control would only be evident in the first part of the season, if at all. Inspection of Table 3 reveals that during sampling period 3 there was some contrast between the two stocking rates, with respect to the number of fields over the 19 allowable specimens.

The unfortunate loss of 1 replicate of each treatment due to accidental spraying is the statistical factor that made showing a difference less probable.

The question of whether fields stocked later than June 1 can be effectively controlled remains unanswered. All of the 3 fields that were lost due to accidental larviciding were in the 7 fields of the late-flooding period. However, Fields 17 and 18, both of which were in the late-flooding category, were the most productive of those fields stocked with fish. Only 1 of the 10 fields stocked prior to June 8 was highly productive.

CONCLUSIONS. The rice fields within Fresno Westside M.A.D. produce a negligible number of mosquitoes other than *Culex tarsalis*. Where mosquitofish were stocked, fewer fields exceeded the criterion for insecticide application than where no fish were stocked. The contrast between the two stocking rates used was not great on a season-long basis. Future experiments must include special precautions to avoid accidental treatments of experimental plots with larvicides.

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TABANIDAE (DIPTERA) OF KEGONSA STATE PARK, MADISON, WISCONSIN: DISTRIBUTION AND SEASONAL OCCURRENCE AS DETERMINED BY TRAPPING AND NETTING

W. L. GOJMERAC [1] AND E. C. DEVENPORT [2]

INTRODUCTION. The increased demand for camping and other outdoor recreational facilities has led to the development of a number of new parks. In selecting park sites, often insufficient consideration is given to the severity of the nuisance insect problem in spite of the fact that many of the nuisance insects are highly localized in distribution. There may be severe annoyance in a limited area and negligible annoyance in a similar adjacent area.

Such proved to be the case in Kegonsa State Park where deer fly populations were a serious problem in constructing the camp sites. Deer fly populations were of such magnitude as to seriously hinder workmen. Kegonsa State Park is located about 20 miles south of Madison, Wisconsin. It includes open meadow—once farmland and a hardwood-covered hill. Numerous swamps are within a mile radius of the wooded area. Some have been drained and used for agriculture, others are retained in their native marsh condition.

Roberts and Dicke (1958) reported 5 genera, 64 species of Tabanidae as having been collected in Wisconsin. Various trapping devices have been described as useful in capturing large numbers of Tabanidae. Thorsteinson *et al.* (1965) described a trap that attracts Tabanidae. Wilson *et al.* (1966) and DeFoliart and Morris (1967) described traps which employed dry ice as the attractant. Wilson (1968) was able to reduce high populations of Tabanidae by the use of such traps.

This study was designed to determine the distribution of nuisance species of Tabanidae and the feasibility of trapping as a means of reducing the high populations. By our definition, nuisance Tabanidae were those which were captured with an insect net swung over the head while slowly walking through a specific area. Collecting was done on warm sunny days over a specific area in a given time period, so that the numbers collected on specific days and in specific areas were comparable. Collections were made over a 2-year period along a forest path, along a forest road, at the edge of the forest, in the forest, in the meadow and in a marsh. Traps used were similar to those described by Thorsteinson *et al.* (1965), Wilson *et al.* (1966) and DeFoliart and Morris (1967). They were operated for periods varying

[1] Associate Professor, Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.
[2] Formerly Research Assistant, now at the Center for Environmental Studies, Brigham Young University, Provo, Utah 84601.