CONTROL OF DARK RICE FIELD MOSQUITO LARVAE, *PSOROPHORA COLUMBIAE* BY MOSQUITOFISH, *GAMBUSIA AFFINIS* AND GREEN SUNFISH, *LEPOMIS CYANELLUS*, IN ARKANSAS RICE FIELDS [1]

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ABSTRACT. Mosquitofish, *Gambusia affinis* (Baird and Girard), green sunfish, *Lepomis cyanellus* Rafinesque, and combinations of both species were evaluated in Arkansas rice plots to ascertain the optimum stocking rate necessary for reduction of dark rice field mosquito larvae, *Psorophora columbiae* (Dyar and Knab). Stocking rates of 2000 mosquitofish/acre produced >90.0% larval reduction. Green sunfish stocked at 480 fish/acre produced >85.0% larval reduction. Green sunfish stocked at heavier rates produced no additional reduction. When both fish species were stocked in combination there appeared to be a slight additive effect. At a stocking rate of 500 mosquitofish and 120 green sunfish/acre, 96.7% larval reduction was obtained which was better than either species stocked alone at all stocking rates. In stocking combinations of both species the benefits of both were obtained. Therefore, it appeared that the combination stockings were the best means of reducing larval populations.

Strict adherence to chemical control has created insecticide resistant mosquito populations in areas such as California, and unpublished data of the 2nd author indicate that such may be the case in Arkansas. To avoid this problem an integrated pest management approach is needed. Fish could form an integral part in such a control program. Davey et al. (1976) reported that the pesticides commonly used in Arkansas rice cultivation produced no adverse effects on fish at recommended field dosages.

The primary emphasis on fish as biological control agents has been with mosquitofish, *Gambusia affinis* (Baird and Girard). There have been many well documented investigations to ascertain the optimum fish stocking rate which effects adequate control of larval mosquitoes in areas other than Arkansas (Craven and Steelman 1968; Hoy and Reed 1970; Hoy and Reed 1971; Hoy et al. 1971; and Hoy et al. 1972). However, in Arkansas the mosquito fauna and methods used in rice cultivation are different. These differences make comparisons with other investigations difficult.

Horsfall (1942) reported larval reduction in 3 ft ^{2} subplots in Arkansas when fish were introduced as larval predators. Davey et al. (1974) reported that mosquitofish reduced larval populations by 100% when stocked at 0.06 fish/ft ^{2}, and green sunfish, *Lepomis cyanellus* Rafinesque effectively reduced larvae by 99.3% when stocked at 0.03 fish/ft ^{2}. Since these 2 fish species were native to Arkansas waters they showed much promise as biological control agents. In a preliminary study in Arkansas rice fields, Meisch and Coombes (1974) reported that stocking rates of 5000 and 2000 mosquitofish/acre produced 94.0 and 87.0% reduction respectively after 48 hr while 400 mosquitofish/acre produced only 52.0% reduction.

The purpose of this investigation was to determine the number of fish necessary to produce adequate control of larvae of the dark rice field mosquito, *Psorophora columbiae* (Dyar and Knab) in rice plots. Mosquitofish, green sunfish and combination stockings of both species were investigated.

MATERIAL AND METHODS. Investigations were conducted in 0.5 acre rice plots at the Rice Branch Experiment Station, Stuttgart, AR. during the summers of 1974, '75, and '76. A similar study was undertaken in 0.25 acre plots at the Southeast Branch Exp. Stn., Kelso, AR. in the summer of 1975. A total of 22 individual stocking rate tests were conducted, 9 using only mosquitofish, 3 using only green sunfish and 10 using various combinations of both species.

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The rice plots were drilled with Starbonnet variety rice at 100 lbs/acre. The herbicide, propanil, was applied at 4 lbs ai/acre. All cultural practices, with the exception of water management, were in accord with rice production in the area. The water management procedures were about the same as those described by Davey et al. (1974). The test plots were drained and allowed to dry 5 days prior to reflooding. To allow maximum hatch of mosquito eggs, plots were allowed to stand 12 hr after reflooding.

The plots used in 1974 were designed so that 1 border, on the high side of the plots, was formed by a combination of Bermudagrass, *Cynodon dactylon* (L) and Barnyardgrass, *Echinochloa crusgalli* (L) extending into the ditch, thus forming a grassy habitat conducive to larval inhabitation. The remaining borders were formed by conventional earthen levees with ditches. The plots used in 1975–76 were of the conventional type having all borders formed by levees.

Immediately prior to fish stocking, mosquito numbers were sampled using a 450 ml capacity enameled dipper attached to a 3 ft wooden handle. The mosquito numbers in the plots tested in 1974 were assessed by taking 40 dips (1 dip/ft) along the grassy margin of the plot. Eighty dips (1 dip/3 ft) were taken in each levee ditch forming the remainder of the plot. One hundred dips (1 dip/3 ft) were taken on the top pan (area where rice plants grow) by diagonally traversing the rice from corner to corner.

The sampling procedure used in the conventionally constructed plots in 1975–76, except for the 0.25 acre plots, was performed by taking 20 dips (1 dip/18 ft) in the levee ditches forming the short side. There were 25 dips (1 dip/3 ft) taken on the top pan in the manner described above. The 0.25 acre plots were assayed by taking 4 dips (1 dip/5 ft) in each of 2 levee ditches and 4 dips (1 dip/5 ft) on the top pan.

Fish were supplied by the U. S. Department of Interior Federal Fish Farming Exp. Stn. at both Stuttgart and Kelso, AR. Fish were collected from rearing ponds using 0.125 inch mesh 50 ft nylon seine. They were counted and transported to the test plots in 3.0 gal capacity plastic buckets. This was done in the morning to reduce high temperature stress to the fish. Plots were again sampled 24 and 48 hr after fish were released. All mosquitofish were hand sorted and only gravid females were used for stocking purposes. The mean size of these fish was ca 1.5 g and 45.0 mm. The green sunfish used in these tests were immature fish with a mean size of ca 2.0 g and 55.0 mm. The sunfish were not sexed for these investigations.

RESULTS AND DISCUSSION. Mosquitofish stocked in 0.25 acre plots at 1000 fish/acre produced significantly less larval reduction at 24 hr than at 48 hr (Table 1). The 1000 fish/acre stocking rate at 24 hr also produced significantly less larval reduction than 2000 fish/acre at 24 or 48 hr. At 48 hr there was no significant difference between 1000 and 2000 fish/acre. Since

Table 1. Percentage reduction of *Ps. columbiae* larvae in 0.25 rice plots by *G. affinis* at 2 stocking rates 24 and 48 hr after introduction at Kelso, AR. in 1975.

		Post-release			
		24 h		48 h	
Stocking rate fish/acre	Pre-release Mosquito samples (Larvae/dip)	Larvae/dip	% Reduction	Larvae/dip	% Reduction
1,000	7.4	2.4	56.4[a]	1.0	83.8[b]
2,000	4.3	1.2	74.2[b]	0.3	91.9[a]

Numbers flanked by the same letter are not significant at the 0.05 level of probability (Duncan's Multiple Range Test).

the larval period of *Ps. columbiae* required only 4 days under optimum conditions, the ability of 1000 fish/acre to control the larvae effectively required early detection of larvae. If infestations of *Ps. columbiae* were detected when larvae were in early instars (1st or 2nd), 1000 fish/acre provided adequate control. If, however, infestations were not detected until the mosquito larvae were in the later instars (3rd and 4th), the higher stocking rate was necessary in the short time available.

Extremely heavy stockings (5000 and 10,000 fish/acre) were necessary to obtain >99.0% larval reduction using mosquitofish (Table 2). There was very little difference in the percent reduction when stocking rates were 550 or 1200 fish/acre (78.8 and 75.0% respectively). The 2 intermediate stocking rates of 600 and 1000 fish/acre produced 82.6 and 83.8% larval reduction respectively after 48 hr. A stocking rate of either 2000 or 2800 fish/acre was necessary to obtain >90.0% reduction.

In addition to initial or direct reduction when stocked, mosquitofish also afforded the advantage of reproducing over the entire season thus producing later season control of mosquito larvae. The mean gestation period is 23 to 24 days (Krumholz 1948). The mean number of fry may range from 40 (Hildebrand 1921) to 104 (Hoy and Reed 1970). Even when fields were drained to a low level, with water only in the levee ditches, the mosquitofish survived until reflooding (Davey et al. 1974). Upon reflooding the large population of mosquitofish should prey upon larvae which hatched from dry lands which were reinundated. The disadvantage of stocking mosquitofish alone was that to obtain >90.0% reduction heavy stocking rates of 2000 or more fish/acre were required. This would require large numbers of fish to stock large rice acreages.

Green sunfish stocked alone produced a larval reduction of ca 85.0% (Table 3). In contrast to mosquitofish, low stocking rates (480/acre) of green sunfish gave good control. Although they did not produce 90.0% reduction, it appeared that heavier stocking rates did not enhance control.

A disadvantage in stocking green sunfish as opposed to mosquitofish was that no reproduction was obtained by green sunfish over the mosquito season as occurred with mosquitofish. If any mortality occurred these fish were simply lost, and season-long control suffered if mortality was substantial.

When mosquitofish and green sunfish were both stocked in combination it appeared that there was a slight additive ef-

Table 2. Percent reduction of *Ps. columbiae* larvae 24 and 48 hr after introduction of *G. affinis* at various stocking rates in 0.5 and 0.25 acre rice plots at Stuttgart and Kelso, AR. in 1974 and 1975.

Stocking rate fish/acre	Pre-release Mosquito samples (Larvae/dip)	Post-release 24 h Larvae/dip	Post-release 24 h % Reduction	Post-release 48 h Larvae/dip	Post-release 48 h % Reduction
550	0.52	0.18	65.4	0.11	78.8
600	3.74	1.18	68.4	0.65	82.6
1000*	7.40	2.40	58.1	1.00	83.8
1200	0.24	0.16	33.3	0.06	75.0
2000*	4.30	1.20	71.6	0.30	92.4
2000	12.44	1.55	87.5	1.00	91.9
2800	3.19	2.20	31.1	0.30	90.3
5000*	5.00	0.05	99.0	0.00	100.0
10000*	5.50	0.09	98.5	0.00	100.0

* Tests conducted in 0.25 acre plots.

Table 3. Percentage reduction of *Ps. columbiae* larvae 24 and 48 hr after introduction of *L. cyanellus* at various stocking rates in 0.5 acre rice plots at Stuttgart, AR. in 1975.

Stocking rate fish/acre	Pre-release Mosquito samples (Larvae/dip)	Post-release 24 h Larvae/dip	24 h % Reduction	48 h Larvae/dip	48 h % Reduction
480	3.76	1.08	66.9	0.47	85.6
830	1.56	0.71	54.7	0.22	85.6
1200	4.36	1.78	59.4	0.64	85.3

fect not produced by either fish alone (Table 4). In all instances except 1 (600 mosquitofish and 200 green sunfish) the percent reduction of mosquito larvae exceeded 90.0% At the lowest combination stocking rate of 500 mosquitofish and 120 green sunfish/ acre, 96.7% larval reduction when stocked alone, in combination a 4-fold reduction of the stocking rate of each species alone produced better results. It appeared that only by stocking at the maximum combination of 800 mosquitofish and 300, 400 or 500 green sunfish/acre could 99.0 to 100.0% reduction be expected. This seemed insignificant for the number of extra fish needed to increase reduction by 4.0%.

In using the combination stockings the benefits of both species were obtained. Green sunfish produced better control at lower stocking rates and mosquitofish reproduced and enhanced late season control. It appeared from these investigations that the combination stocking of both species was the best means of controlling larval mosquitoes.

In all instances regardless of single or combination stockings it was observed that 48 hr was necessary to attain maximum reduction. The fish required at least 24 hr to acclimate to the new surroundings and to begin actively seeking out larvae. It appeared that the combination stocking rates (Table 4) produced the best larval reduction within 24 hr. Green sunfish produced ca 60.0% reduction in 24 hr (Table 3). In 2 instances mosquitofish gave only ca 30.0% reduction at 24 hr, indicating that it may

Table 4. Percent reduction of *Ps. columbiae* larvae 24 and 48 hr after introduction of *G. affinis* and *L. cyanellus* in combination at various stocking rates in 0.5 acre rice plots at Stuttgart, AR. in 1976.

Stocking rate fish/acre *G. affinis*	Stocking rate fish/acre *L. cyanellus*	Pre-release Mosquito samples (Larvae/dip)	Post-release 24 h Larvae/dip	24 h % Reduction	48 h Larvae/dip	48 h % Reduction
500	120	0.70	0.11	85.0	0.03	96.7
500	160	2.44	0.75	69.1	0.21	92.0
500	200	4.34	0.68	84.3	0.35	92.4
600	200	2.59	0.96	62.7	0.51	80.5
600	250	2.84	0.55	80.5	0.21	93.0
600	300	2.22	0.28	87.3	0.11	94.7
600	600	1.54	0.54	65.0	0.13	91.5
800	300	1.18	0.08	93.0	0.00	100.0
800	400	3.56	0.21	94.1	0.01	99.6
800	500	1.34	0.16	87.7	0.01	99.1

require longer for them to become acclimatized (Table 2).

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SYMPOSIUM: MEDICAL ENTOMOLOGY CENTENARY

A symposium commemorating Manson's discovery of mosquito transmission of bancroftian filariasis is being convened in London, November 23–25, 1977 under the sponsorship of the Royal Society of Tropical Medicine and Hygiene. Dr. M. W. Service of the Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA will be glad to furnish details upon request. In addition to Dr. Service, many other distinguished medical entomologists will participate. Mosquito workers will be interested especially in presentations by P. E. C. Manson-Bahr, G. S. Nelson, M. T. Gillies, A. J. Haddow, W. W. Macdonald, J. D. Gillett, P. C. C. Garnham, J. R. Busvine, G. Davidson, T. Lepes, and L. Molineaux.