# DENSITY ESTIMATION AND POPULATION GROWTH OF MOSQUITOFISH (*GAMBUSIA AFFINIS*) IN RICE FIELDS

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ABSTRACT. Mark-release-recapture estimation of population density was performed with mosquitofish in a rice field habitat. Regression analysis showed a relationship between absolute density and mean number of fish per trap. Trap counts were converted to density estimates with data from several fields and growth curves were calculated to describe seasonal growth of mosquitofish populations at three different initial stocking rates. The calculated curves showed a good correspondence to field populations of mosquitofish.

## INTRODUCTION

A common method for control of rice field mosquitoes in California is the introduction of mosquitofish (*Gambusia affinis*) into fields early in the growing season (Fowler 1964; Hoy and Reed 1970, 1971). The population growth and dispersion of mosquitofish allow them to become established predators on mosquito immature stages during the rice season.

Much has been done to determine effects of predation on mosquito species (Davey et al. 1974, Davey and Meisch 1977, Miura et al. 1979, Farley 1980, Stewart et al. 1983), however, information is lacking on population size and growth in absolute terms. According to Reed and Bryant (1974), the use of minnow traps made it possible to monitor population trends of mosquitofish in ricefields and provided good relative estimates. Hoy and O'Grady (1971) used removal trapping to monitor rice field populations of mosquitofish and make absolute population estimates. Relative population or density estimates are useful when it is sufficient to know when one population exceeds another, either in time or space; but comparison on a unit or area basis is not strictly possible. In contrast, absolute density is the measure of the numbers of the organism in a defined area. By adding the attribute of area to the estimate it becomes possible to make comparisons with populations from different areas and to quantify population levels. Determination of organisms per unit area is an important step in the construction of integrated pest management (IPM) models because population levels of different organisms can be compared to one another and linked in calculations of growth and population variation.

The present study was based on a preliminary mark-recapture study by Miura et al. (1982) for developing absolute population estimates of mosquitofish populations. Once these estimates were obtained, we hoped to be able to calculate growth curves that would describe population dynamics.

## MATERIALS AND METHODS

Mosquitofish used in the mark-release-recapture part of this study were obtained from a sewage oxidation lagoon and were captured by minnow traps. The fish were of mixed sizes and were graded for size prior to marking. Fish selected for marking were in the range of 3.0–5.0 cm Standard Length and weighed from 0.75 to 2.0 grams. Marking was known to cause higher mortality rates in smaller fish therefore these were excluded. Marking was with subdermal injection of a fluorescent polystyrene pigment under high pressure. Procedures followed were generally those reported by Vondracek et al. (1980) and Stewart and Miura (1982). After marking, the fish were immediately rinsed with water and placed in aquaria treated with furazone-green (nitrofurazone, furazolidone, methylene blue). Water in the holding tanks was exchanged daily and retreated with furazone-green. Fish were held for one week in the holding aquaria prior to field release during which time they were checked for percent mortality and marking success. Marking success was determined by observing fish in a viewing box equipped with a long wave ultraviolet light source which shone into a glass aquarium. Fish with clearly visible pigment spots were determined to be adequately marked. This same viewing box was used in the field for identifying marked fish from trap collections.

Previous marking studies by Vondracek et al. (1980) and Stewart and Miura (1982), showed that fish could be expected to have clearly visible pigment marks beyond 80 days.

Marked fish were stocked in three separate paddies of an 8 hectare rice field in western Fresno County, Calif. Fish were stocked at approximately 2000 per hectare (800 per acre) in the paddies. The paddy sizes were 0.21, 0.24 and 0.30 hectares and were stocked with 400, 500 and 600 marked fish respectively. Paddy sizes were determined by aerial photographs and ground measurements. Prior to release of marked fish, the entire field was stocked with mosquitofish at 0.22 kg per hectare (approximately 1236 fish per hectare). The unmarked fish were planted in late May 1983 by mosquito abatement personnel. On June 6, 1983, each of the separate paddy weir boxes was screened to prevent fish movement into and out of the paddies. Screening material was pierced metal sheeting with 2 mm diam. holes. This was small enough to prevent passage of all but very small fry. Marked fish were released in the middle and near the ends of each paddy on June 9, 1983.

Fish were captured with modified minnow traps lined with 16 mesh aluminum screening. Traps were placed along five equally spaced lines in each paddy at a density of 62 per hectare and were retrieved and counted after 24 hr. The three paddies used for mark-release-recapture had 12, 15 and 18 traps, respectively, in each. All captured fish were examined under ultraviolet light for fluorescent pigment and then released into the section of the paddy where they were captured.

Absolute population size for each trapping date was calculated for each paddy using the Lincoln Index (Southwood 1978):

$$\hat{N} = an/r$$

where $\hat{N}$ = the estimate of the population size in the study area, n = the total number of individuals captured, a = the total number marked and r = total recaptures. Linear regression analysis of the number of fish captured per trap versus population size was performed, as were analyses for common slopes for regressions from the different paddies (Freese 1980).

Concurrent with the capture-recapture part of the study, three adjacent 9 hectare rice fields were trap monitored weekly. These fields were stocked in May with mosquitofish at three different rates. The standard fish stocking rate by the local mosquito abatement district is approximately 1236 per hectare (500 per acre or 0.2 lbs/acre). Stocking rates per hectare on these fields were 1236, 2471 and 3706. Traps used were modified minnow traps mentioned earlier and were left in the fields for 24 hr before being examined. Fish were counted and returned to the locations where they had been trapped. In 1980 and 1981, trapping of mosquitofish had been carried out on these same fields in western Fresno County using the above described methods.

Trap counts were converted to density estimates using the regression equations derived from mark-recapture data. Growth curves using the logistic equation $dn/dt = rN(k-n/k)$ were calculated with data from fields monitored in 1983. Curves were calculated for each of the three different stocking rates. Predicted growth curve values were compared with measured density estimates using simple linear regression. Validation tests involved forced zero intercept linear regression using predicted growth as the dependent variable.

## RESULTS AND DISCUSSION

Very little fish mortality resulted from either marking procedures or transport of fish to rice fields. Less than 5% of the fish in holding tanks died during the one week holding period. Visual checks of marked fish with ultraviolet light showed that virtually all of them had clearly visible pigment spots prior to release into the ricefields. Delayed transportation induced losses were not assessed.

Absolute population size for each trapping date in each paddy was calculated and is shown in Table 1. On some dates the number of recaptures were insufficient (less than 2) to allow for calculation of population size but this occurred only once for each paddy. Marked fish recapture averaged 1.0% in the three paddies over the course of trapping. The highest per-

Table 1. Absolute population estimates of mosquitofish from capture recapture data.

| Paddy | | Date | | | | | |
|---|---|---|---|---|---|---|---|
| | | June 29 | July 7 | July 14 | July 28 | Aug. 11 | Aug. 25 |
| 1 | No. entire paddy | — | 12,000 | 24,600 | 44,133 | 52,400 | 61,600 |
| | S.E. of paddy estimate | — | 11,784 | 17,210 | 25,269 | 36,819 | 43,307 |
| | No. per ha. | — | 15,122 | 31,001 | 55,617 | 66,035 | 77,629 |
| 2 | No. entire paddy | 7,818 | — | 15,375 | 10,000 | 9,500 | 19,083 |
| | S.E. of paddy estimate | 2,255 | — | 7,531 | 3,220 | 3,055 | 7,642 |
| | No. per ha. | 11,399 | — | 22,414 | 14,579 | 13,850 | 27,821 |
| 3 | No. entire paddy | — | 8,000 | 18,671 | 19,750 | 25,800 | 45,100 |
| | S.E. of paddy estimate | — | 4,608 | 4,477 | 5,646 | 8,092 | 18,321 |
| | No. per ha. | — | 14,628 | 34,139 | 36,114 | 47,053 | 82,467 |

centage of captured marked fish in relation to total catch was 6.4% and the lowest was 0.38%. The percentage of fish recaptured dropped in each paddy over the trapping period.

A test for common slopes of the regressions of number of fish per hectare (estimated from mark-recapture data) versus mean number of fish captured per trap for the three replicate paddies showed non-significant differences in their slopes. Bartlett's test of homogeneity of variance was applied to the trap data from each of the paddies and showed that trap counts from the three paddies on each date had homogeneous variances. Combined data from the three paddies were used to calculate a single linear regression equation with a correlation coefficient of 0.7606. Using tables from Snedecor and Cochran (1967), the correlation coefficient with 12 degrees of freedom is significant at the 1% level. The linear regression calculated was: $Yi = 6.1139 + 0.0003\ Xi$. Thus, the relative abundance index of mean number of fish per trap shows a significant positive correlation to absolute population size.

Trap counts from 1980, 1981 and 1983 trapping of rice fields were converted to absolute population estimates by the regression equation developed from 1983 mark-recapture trapping. In addition, trap counts made by Reed and Bryant (1975) were converted to population estimates and are shown in Fig. 1. A theoretical growth curve using the logistic equation was calculated and is also shown on Fig. 1. The parameters "k" and $e^x$ were determined empirically and set at 247,097 fish per hectare and 1.75, respectively. Results from Miura et al. (1982) corroborate these estimated parameters.

The 1983 trap counts made from fields stocked at different rates were converted to absolute population estimates and were compared against the theoretical curves (Figs. 2, 3 and 4). Table 2 shows the results of validation tests on predicted versus measured values. Plots of predicted versus measured values showed good correspondence to the zero intercept model chosen. Results showed R-square values in excess of 0.88 for each of the stocking models. F values were significant for each model and the 95% confidence limits for the slope encompassed one. Thus, a one to one fit of predicted to measured can be concluded. Residual plots for the three models are shown in Fig. 5 and show significant deviation ($>|2.0|$) of standardized values from the model for only 2 observations out of a total of 39. Therefore, the measured and predicted values show only 2 cases that are inconsistent with the fit and these two points are in separate stocking rate models. The best fit was to the stocking rate of 2471 per hectare but all models were considered to have good fit to the actual field density measurements.

Table 2. Results of validation tests—forced zero intercept linear regression of predicted versus measured values.

| | Stocking rate per hectare | | |
|---|---|---|---|
| | 1236 | 2471 | 3706 |
| R-Squared | 0.8877 | 0.9589 | 0.8995 |
| Standard error of estimate | 30.0439 | 24.2281 | 39.5053 |
| ANOV | | | |
| F-value | 94.87 | 279.97 | 89.52 |
| Degrees of freedom | 1/12 | 1/12 | 1/10 |
| Regression coefficient | 0.8891 | 1.1131 | 1.0492 |
| 95% confidence interval | | | |
| Lower limit | 0.6902 | 0.9681 | 0.8020 |
| Upper limit | 1.0881 | 1.2580 | 1.2963 |

As Southwood (1978) points out, it is almost impossible to construct a budget or study mortality factors without absolute density estimates of a population. Pest management modeling requires absolute figures to relate the numbers of prey species to predators, pathogens and other nontarget organisms important in control decisions. Further, the standardization of population estimates can aid greatly the combination of population data from different areas.

A usual method for absolute density measurement is some sort of device that samples a unit area of habitat and the organisms found there. Takahashi et al. (1982) used an area sampler to investigate the aquatic fauna in rice fields and found that it did not adequately sample the nekton, including mosquitofish. They found that minnow traps were better for sampling mosquitofish. In this study we used minnow traps and mark-release-recapture to overcome difficulties associated with area samplers. Mark-recapture had the advantage that its accuracy does not depend on an assessment of the number of sampling units in the habitat.

There is general agreement between the data collected in this study and the preliminary one by Miura et al. (1982). A positive correlation between trap counts and absolute density was found in both studies. One difference we noted was a generally lower capture rate of both marked and unmarked fish in this study. This may partially be explained in a slight difference noticed between the two experimental areas. The field used in the mark-recapture part of this study developed a dense growth of submerged vegetation which may have restricted fish movement.

The growth curves calculated from our data do provide a good fit to data obtained from fish

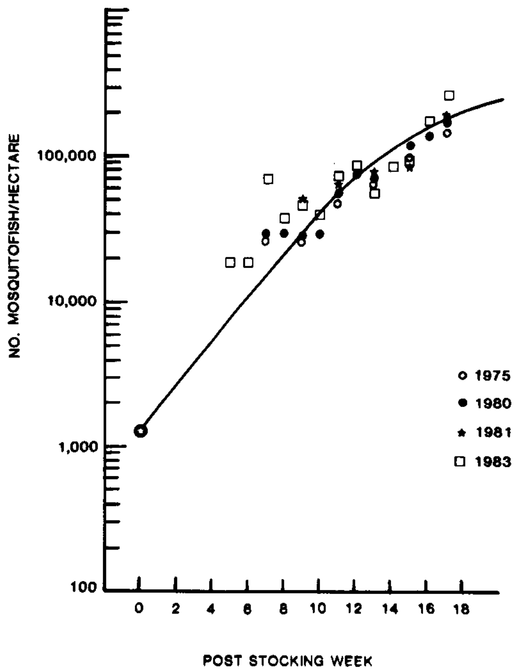


Fig. 1. Comparison of 4 years population growth of mosquitofish in western Fresno County rice fields. The initial stocking rate was approximately 1600 fish per hectare.

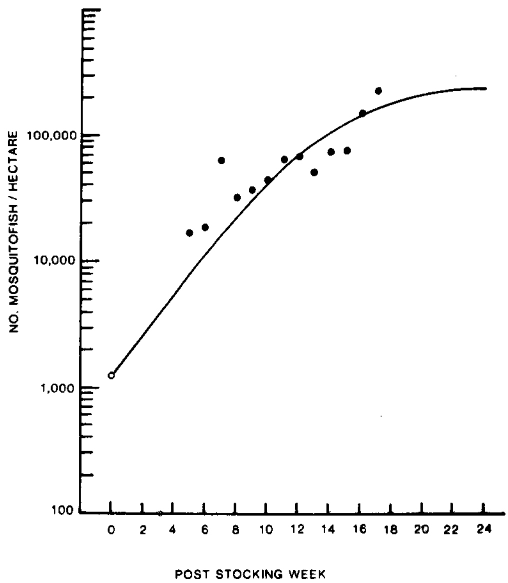


Fig. 2. Theoretical logistic growth curve and absolute population estimates of mosquitofish from a rice field with an initial stocking rate of 1236 fish per hectare.

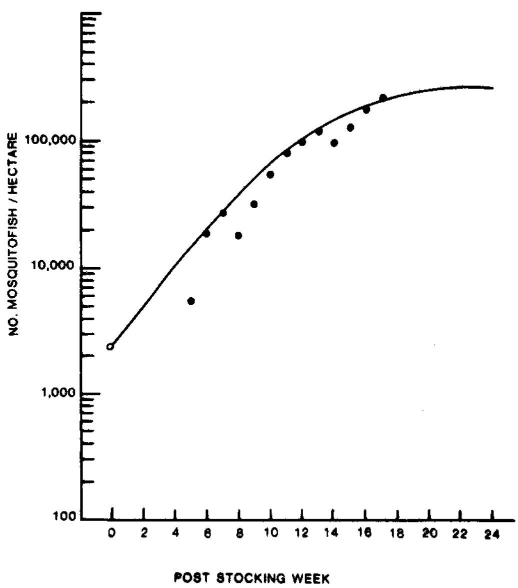


Fig. 3. Theoretical logistic growth curve and absolute population estimates of mosquitofish from a rice field with an initial stocking rate of 2471 fish per hectare.

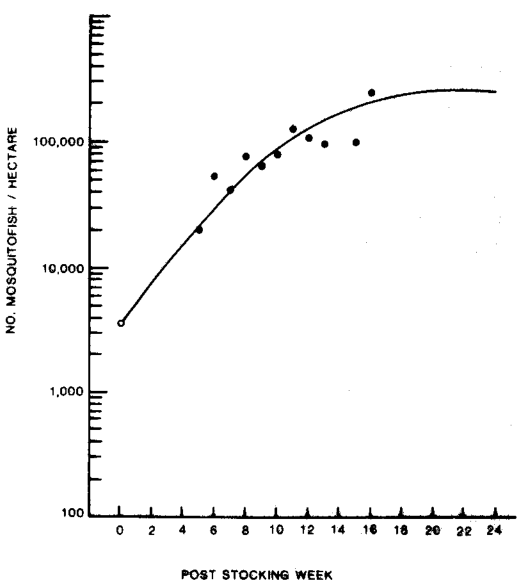


Fig. 4. Theoretical logistic growth curve and absolute population estimates of mosquitofish from a rice field with an initial stocking rate of 3706 fish per hectare.

trapping in other fields in this area of Fresno County, Calif. (Reed and Bryant 1975). Differences due to variations in habitat may affect the regression of trap counts versus absolute density. Other variable criteria such as climate, food supply and biotic factors also influence the growth of populations.

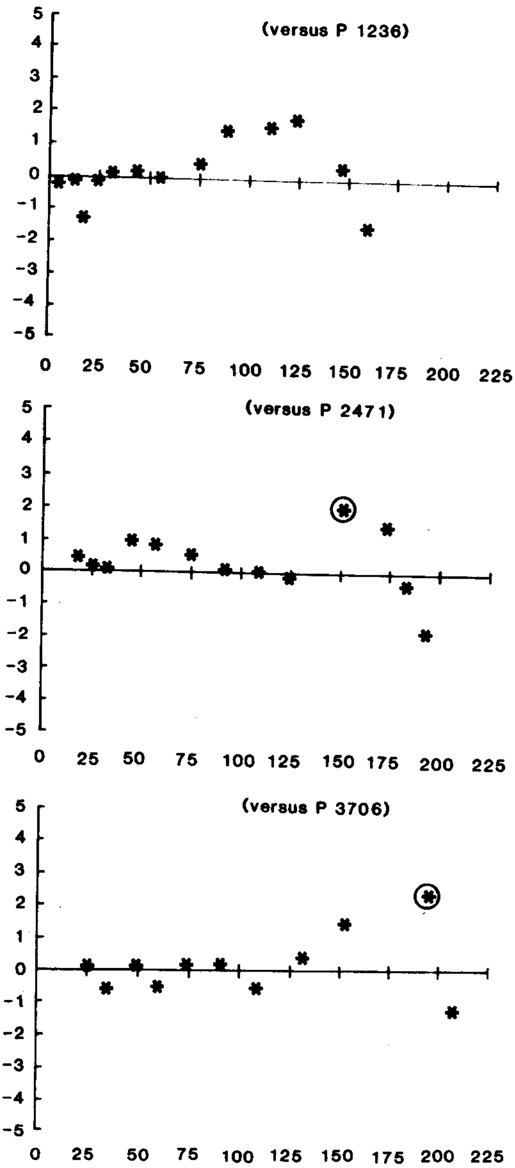


Fig. 5. Validation tests—standardized residuals from zero intercept regression of predicted versus measured population estimates for three different stocking rate models. Predicted values are coded by dividing by 100. Circled points represent values with higher than normal residuals.

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