SAMPLING AND DISTRIBUTION OF *ANOPHELES QUADRIMACULATUS* IMMATURES IN RICE FIELDS

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ABSTRACT. Dipper samples of *Anopheles quadrimaculatus* immatures from stocked enclosures in Arkansas rice fields were used to develop regression equations relating dipper sample counts to absolute density. Confidence limits were developed for mean number of immatures collected at each density and stadia, including combined stadia. These data can be used to estimate absolute density from mean dipper count. Distribution of rice field immatures approximated but did not fit the Poisson distribution. Sample size was calculated for 10, 25 and 50% of the true mean, at various levels of Type I and II error. A sample size of N = 6,424 was necessary to detect differences within 10% of the true mean, with 5 and 10% probability of Type I and II error, respectively.

INTRODUCTION

The most commonly used sampling device for mosquito immatures is a 350–450 ml dipper. Nagamine et al. (1979), define an efficient sampling tool as one that produces a desired effect with minimal effort; and an effective sampling tool as one capable of performing a task. The dipper represents a compromise between an efficient and effective sampling tool. Dipper sample values are useful for comparing populations in similar habitats but provide no direct estimate of absolute density.

Area samplers represent an alternative to dipper samples and have been demonstrated to be highly effective in estimating population densities of mosquito immatures in rice fields (Goodwin and Eyles 1942, Andis et al. 1983, Stewart and Schaefer 1983). The principal advantage of the area sampler is that results can be expressed as density (Andis et al. 1983). The principal disadvantage of the area sampler is the time involved in assessing a population of mosquito immatures when large areas such as rice fields are being examined.

In addition to density, dipper counts are almost invariably related to other factors. However, Stewart and Schaefer (1983) demonstrated a linear relationship between mean dipper count and absolute density of *Culex tarsalis* Coquillett and used this to estimate absolute density. Croset et al. (1976) examined mark-recapture, removal and dipping methods for estimating culicid mosquito populations and found numbers of mosquitoes sampled by the three methods were within 20% of each other. Andis et al. (1983) established statistically significant linear relationships between the number of *Anopheles crucians* Wiedemann and *Psorophoro columbiae* (Dyar and Knab) larvae/10 dips and the number/0.1 m^2. They concluded that the dipper method provided a reliable index to the population density for the two species.

Service (1976) pointed out the need to evaluate the efficiency of dipper sampling for each mosquito species within their habitats. Andis et al. (1984) stated that type of larval distribution, degree of aggregation, and permanence of groups all must be known to accurately estimate density. The relationship of dipper sample values and absolute density and distribution of rice field *Anopheles quadrimaculatus* Say have not been established. Thus, an objective of this study was to relate number of *An. quadrimaculatus* immatures/dip from a standardized dipping method to absolute density.

All immature stages were monitored to determine if observed low frequencies of later stadia, particularly pupae, were due to actual density differences or behavioral differences enabling these stages to react more rapidly to disturbance or to otherwise avoid capture. Distribution of rice field *An. quadrimaculatus* immatures was determined and sample size calculated for accurate estimates of the true mean of the population.

MATERIALS AND METHODS

Sampling and estimation of absolute density. Investigations were conducted in selected rice fields near Stuttgart, Arkansas (Arkansas and Prairie counties) during the summers of 1983–85.

Anopheles quadrimaculatus immatures were reared from a natural population using techniques described by Dame et al. (1978) and Bailey et al. (1980). When sufficient numbers of the desired immature stage were available, larvae and/or pupae were removed from rearing trays using 100 mesh nylon fabric along with 100/120 ml of the rearing solution and placed in 240 ml waxed paper cartons. Approximately 200 larvae or pupae were placed in each carton, transported to the field and placed in 1 m diam enclosures (0.72 m^2) constructed of sheet aluminum which were ca. 30 cm in height. Stewart

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and Schaefer (1983) noted the necessity of nonreflective surfaces within such enclosures, therefore aged enclosures which were not reflective were used.

Each enclosure had two 1.5 cm diam openings below the water line with 100 mesh aluminum screen covering each opening to allow for movement of water between the enclosure and rice field. Enclosures were placed in rice fields a minimum of 2 hr prior to sampling. Location of enclosures was standardized for water depth and plant density (mean of 224 ± 30 stems/0.72 m^2). Enclosures were pushed into the soil to prevent escape of immature mosquitoes. Enclosures were located within 2 km of the rearing facility, so that handling time and stress on immatures during transport was minimized. Locations of enclosures within fields were initially sampled by dipping (ca. 100 dips) to insure that no indigenous larvae were present.

Immatures were uniformly distributed throughout the enclosures and allowed to acclimate and disperse for ca. 1 hr before sampling. Each immature stage of *An. quadrimaculatus* was stocked in enclosures at the following densities: 10, 50, 100, 200, 500, 1,000 and 1,500 immatures/0.72 m^2 enclosure.

Each enclosure was divided into a center site and four quadrants exclusive of the center site for a total of five sampling sites per enclosure. Each site within an enclosure was sampled two times for a total of 10 samples per enclosure. Four persons, experienced in sampling *An. quadrimaculatus* larvae, sampled an enclosure with 350 ml plastic dippers (Clarke Mosquito Control Service and Supplies, Roselle, IL) fitted to 1 m wooden handles. A minimum 3 min waiting period between samples allowed for acclimation of immatures. Each enclosure was consecutively sampled by one sampler before replications within a single enclosure were made. Persons taking the samples faced the sun to minimize shadow disturbance of the larvae. Dipping was accomplished by gradually submerging the dipper against rice stems and allowing surrounding surface water to drain into the dipper (Knight 1964). Sampling in such a manner maximized encounters with *An. quadrimaculatus* immatures (Sandoski et al. 1986). After recording number of immatures collected, samples were returned to the respective site within the enclosure. Number of immatures/10 dips/sampler/enclosure was totalled. Coefficients of variation were calculated for each sampler. Sample size was consistent with sampling reported by Stewart and Schaefer (1983).

Data were subjected to analysis of variance (ANOVA). Means were separated by Duncan's Multiple Range Test ($P = 0.05$) (Duncan 1955). Data for each stadium and for all stadia combined were subjected to regression analysis (Anonymous 1978), and predictive equations to estimate absolute density of rice field *An. quadrimaculatus* based upon number of larvae/10 dips were derived using the least squares method.

Distribution and sample size determination. Indigenous populations of *An. quadrimaculatus* immatures were sampled to provide data necessary to determine distribution and calculate sample size to accurately estimate immature populations. Fields were selected on the basis of adequate numbers of *An. quadrimaculatus* immatures (at least 0.5 immatures/dip) and uniformity of rice stand. Plots within the same field were separated by a minimum of 150 m. Sampling of indigenous populations was conducted as described by Sandoski et al. (1986). Stratification of sampling was not necessary because Stark and Meisch (1984) observed no difference between the number of *An. quadrimaculatus* captured in ditch and pan areas.

Taylor's (1965) index of aggregation, 'b', was utilized in determining distribution of rice field immatures. Taylor's 'b' was chosen since it is species constant and is applicable to samples collected from different populations at different times (Taylor 1965, 1970). Taylor's 'b' was calculated based upon transformed data [log(X + 1)]. The index also provides a precise transformation (z), where $z = x^{1-0.5b}$ (Taylor et al. 1978). The distribution suggested by Taylor's index of aggregation was tested for goodness-of-fit in the manner described by Southwood (1978). Sample size was calculated as described by Cochran and Cox (1957) for various percentages within the true mean (10, 25 and 50%) and various levels of Type I and Type II error.

RESULTS AND DISCUSSION

Sampling and estimation of absolute density. Significant differences ($P = 0.05$) in mean number of immatures/10 dips for samplers were observed across all densities and stadia (Table 1). Coefficients of variation by sampler was not significantly different ($P > 0.05$). While means differed among samplers, variability did not differ significantly and combined sampler data were used for subsequent analyses.

Significant differences in mean number of immatures/10 dips for stage, density, and a significant interaction between stage and density were detected (ANOVA, $P < 0.05$) (Table 2). Across all densities and samplers the mean number of fourth instar larvae and pupae was significantly greater than the mean number of second and third instar larvae. Number of first instar larvae was intermediate, being significantly different from second instar larvae but not signif-

icantly different from pupae, third or fourth instar larvae.

No significant differences were observed across all stadia and samplers between means of densities for 10, 50, 100 and 200 immatures/0.72 m^2. Significant differences were observed between means of density for 500, 1,000 and 1,500 immatures/0.72 m^2. As stage and density of immatures/0.72 m^2 increased, number of immatures recovered increased.

Regression equations were developed for number of immatures/10 dips at the varying densities investigated (Table 3). Unexplained variability in excess of that reported by Andis et al. (1983) for *An. crucians* was observed in the present study in the relationship between number of immatures/10 immatures/10 dips and density of *An. quadrimaculatus*/0.72 m^2. When abundance data for each immature stage were subjected to regression analysis, better fits were obtained from predictive equations for each immature stage except second instar larvae. Coefficients of determination (r^2) ranged from 0.43 for second instar larvae to 0.90 for pupae. Regression equations were solved to estimate the number of immatures/0.72 m^2 from mean number of immatures/10 dips at each of the various densities. These estimates are presented in Table 2.

These data are in contrast to the findings of Chambers et al. (1979). They estimated absolute density of rice field *An. quadrimaculatus* to be 1,341 larvae/ha @ 0.0154 larvae/dip. This estimate was based upon the correlation of surface area of the dipper with total surface area of the sample site. The predictive equation presented here for combined stadia estimates a population of 23,333 immatures/ha, a value 17× greater than that of Chambers et al. (1979). Goodwin and Eyles (1942) stated that attempts to relate dipper capacity to water surface had been unsatisfactory.

These data support the hypothesis that low

Table 1. Overall mean and coefficient of variation for samplers of *Anopheles quadrimaculatus* immatures/10 dips from 0.72 m^2 rice field enclosures containing various densities and stadia of immatures.[1]

Sampler	Mean no./10 dips[2]	Coefficient of variation
A	32.7a	115.7a
A	18.1b	159.9a
C	27.8a	152.6a
D	18.9b	138.3a

[1] Stocking densities of 10, 50, 100, 200, 500, 1,000, 1,500, per 0.72 m^2 for each stadia (n = 1,400).

[2] Means in columns not followed by the same letter are significantly different ($P \leq 0.05$); Duncan's (1955) multiple range test.

Table 3. Regression equations for immature *Anopheles quadrimaculatus* density from dipper sample values.

Stadia	Predictive number of immatures/0.70 dips	$(1 - r^2)^b$
I	Y = 0.50 + 0.05 (X)[a]	0.40
II	Y = 3.43 + 0.02 (X)	0.57
III	Y = 4.39 + 0.03 (X)	0.35
IV	Y = −3.43 + 0.07 (X)	0.27
P	Y = −4.53 + 0.08 (X)	0.10
All stages	Y = 0.07 + 0.05 (X)	0.41

[a] X = number of immatures/0.7 m^2; Y = number of immatures/10 dips.

[b] Amount of unexplainable variation.

Table 2. Mean number and 95% confidence limits of *Anopheles quadrimaculatus* immatures/10 dips and density estimates of immatures/0.72 m^2 from rice field enclosures containing various densities of immatures.

	No. of immatures/0.72 m^2[3,4]							
Stadia	10	50	100	200	500	1,000	1,500	Mean
I-P[1,2]	2.2 ± 1.2D (41.6)	2.8 ± 1.4D (55.6)	6.4 ± 1.8D (125.6)	9.6 ± 2.2D (189.6)	23.6 ± 5.4C (470.6)	47.2 ± 14.4B (941.6)	79.0 ± 23.5A (1,579.6)	
I	1.3 ± 1.2l (16.0)	1.5 ± 0.4l (20.0)	5.5 ± 2.9kl (100.0)	9.0 ± 4.2kl (170.0)	27.5 ± 23.0ghij (540.0)	61.0 ± 47.9c (1,210.0)	77.0 ± 32.5b (1,530.0)	26.4AB
II	1.3 ± 1.0l (−106.5)	5.8 ± 4.0kl (118.5)	5.8 ± 2.6kl (118.5)	5.8 ± 3.6kl (118.5)	13.0 ± 4.2k (478.5)	36.3 ± 11.5efg (1,643.5)	32.3 ± 10.3fghi (1,443.5)	14.5C
III	3.5 ± 5.1kl (−29.7)	1.5 ± 1.2l (−96.3)	7.0 ± 5.3kl (87.0)	11.0 ± 5.1kl (220.3)	25.8 ± 7.9hij (713.7)	35.3 ± 10.9fgh (1,030.3)	40.8 ± 9.5ef (1,213.7)	17.5BC
IV	3.8 ± 2.0kl (103.3)	2.8 ± 1.6kl (89.0)	5.8 ± 3.2kl (131.9)	10.0 ± 4.4kl (191.9)	28.5 ± 1.2ghij (456.1)	46.5 ± 10.5de (713.3)	120.3 ± 39.8a (1,767.6)	31.2A
P	1.0 ± 1.0l (69.1)	2.8 ± 1.8kl (91.6)	8.0 ± 3.4kl (156.6)	10.5 ± 3.9kl (187.9)	23.3 ± 7.3ij (347.9)	55.3 ± 25.5cd (747.9)	125.0 ± 3.7a (1,619.1)	32.3A

[1] All immature stages combined.

[2] Means in columns or rows not followed by the same upper case letter are significantly different ($P = 0.05$); Duncan's (1955) multiple range test.

[3] Values in parentheses are regression estimates of density (number of immatures/0.72 m^2) from equations in Table 3 based upon mean number of immatures/10 dips.

[4] Means in columns and rows not followed by the same lower case letter are significantly different ($P = 0.05$); Duncan's multiple range test.

populations of fourth instar larvae and pupae, as observed by Stark and Meisch (1984), are due to high mortality of early immature stages. These data suggest that dipper samples cannot detect differences in *An. quadrimaculatus* immature populations of ≤200 immatures/0.72 m^2. This is critical in that 200 immatures/0.72 m^2 corresponds with a mean of 9.55 immatures/10 dips. Although values of 9.55 immatures/10 dips are often observed for *An. quadrimaculatus* in the Stuttgart, AR vicinity, values <9.55 immatures/10 dips are typical (Meisch et al. 1982).

Distribution and sample size determination. Distribution of *An. quadrimaculatus* immatures in a rice field environment was examined. Regression analysis deriving Taylor's index of aggregation 'b' revealed that slope (b = 0.821) was significantly different from 0, but not significantly different from one (*t*-test, a = 0.05). An aggregation index with a value of one suggested the data fit a Poisson distribution. Fit of the data to the Poisson distribution was tested using two methods. First, the index of dispersal (I_D = 4976.7) was found to lie outside the χ^2 limits 0.95 and 0.05 (degrees of freedom = 3,245). The χ^2 (χ^2 = 5,005) goodness of fit test rejected the null hypothesis ($\chi^2_{0.05,9 \text{ d.f.} = 16.9}$), that the data fit the Poisson distribution. The distribution approximated but did not fit the Poisson distribution. The resultant transformation provided by Taylor's 'b' was $z = x^{0.59}$; 'z' representing the transformed value and 'x' representing the frequency observation. This transformation is similar to the transformation recommended for the Poisson distribution, $x^{0.5}$ (square root) (Southwood 1978). Data should be transformed for analysis of density estimates only if it is determined that conditions for statistical tests are violated.

Sample size estimates were calculated using the method of Cochran and Cox (1957), which assumes random (Poisson) distribution. The method includes Type I and II errors. The "true difference desired to detect" (d) was arbitrarily set at 10, 25 and 50% of the observed mean (0.6195 immatures/dip), corresponding with 0.062, 0.155 and 0.310, respectively (Table 4). Sample size of N = 6,424 was required to detect differences within 10% of the true mean, with 5 and 10% probability of Type I and II error, respectively. As percentage of the true mean and probability of Type I and II error increase, resultant sample size is decreased. While sample sizes of the magnitude necessary to provide statistically accurate estimates are prohibitive in terms of applicability in immature mosquito surveys, alternatives such as sequential sampling programs are prohibited due to the lack of fit to the negative binomial distribution.

Table 4. Sample size estimates (No. of dips) for rice field *Anopheles quadrimaculatus* immatures at 10, 25, and 50% of the true mean, various levels of Type I and Type II errors.[1]

	Type II β error					
	0.10			0.20		
	% of mean			% of mean		
Type I α	10	25	50	10	25	50
error	0.062	0.155	0.310	0.062	0.155	0.310
0.05	6,424	1,028	257	5,196	831	208
0.10	5,351	856	214	4,235	678	169
0.20	4,235	678	169	3,249	520	130

[1] Cochran and Cox (1957). Mean = 0.6195, standard error = 0.975. df = 3,245.

Estimates of absolute density for *An. quadrimaculatus* immatures in rice fields can be based upon dipper sample values using regression equations and confidence intervals established by sampling known densities of immatures in enclosures. Sample sizes necessary to accurately estimate the mean of indigenous populations are prohibitively high.

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