THE RELATIONSHIP BETWEEN DIPPER COUNTS AND THE ABSOLUTE DENSITY OF *CULEX TARSALIS* LARVAE AND PUPAE IN RICE FIELDS

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ABSTRACT. Three types of experiments were conducted to compare mean dipper counts of *Culex tarsalis* with estimates of absolute density in rice fields. Data from stocked enclosures in rice fields, field population surveys and field populations sampled from enclosures were used to develop linear regression equations describing the relation between counts and population density. There were good correlations ($r^2 \geq 0.93$) for the regressions and the regression lines were similar for third and fourth stage larvae and pupae. Combined with a standardized dipping procedure these data can be used to estimate the absolute population from the mean dipper count.

INTRODUCTION

It is necessary to know the relationship between sample counts and actual densities of the organism being sampled in order to develop reliable population estimates. Relative density measurements can be effectively used for comparative purposes but lack the attribute of quantity per area. The dipper is the most widely used sampling device for immature mosquitoes and it is usually used for estimating relative density.

Knight (1964) reviews methods for larval surveys and points out that relative density estimates are useful whenever it is sufficient to know in what ratio one population exceeds another, either in time or space, when sample methods are uniform. In contrast, absolute density is a count or estimate of all organisms in an area (Andrewartha 1961). Absolute density is seldom measured for mosquito control programs due to the excessive time and effort usually required.

Several studies (Hagstrum 1971, Mogi and Wada 1973, Wada and Mogi 1974) have evaluated the dipper for quantitative purposes in sampling immature mosquitoes and have made some predictions on the use of dipper counts in estimating populations. Wada and Mogi (1974) found that the efficiency of the dipper varies between mosquito species; thus, studies on sampling efficiency should be done for each species in question.

The objective of this study was to develop a method for estimating the absolute population densities of *Culex tarsalis* Coquillett in the rice fields of central California. The general approach was to use the dipper to sample known populations of *Cx. tarsalis* and thus determine the quantitative relationship between counts and immatures per unit of area. Three types of experiments were performed to quantify and relate dipper counts to densities of immatures. One was to stock defined habitat areas with known densities of mosquitoes and then sample to determine efficiency. Second, samples were taken in rice fields and the statistical attributes of the mean counts were compared to counts from stocked "populations." The third was to sample from a defined habitat and then, by a removal method, determine the size of the population. From these studies we hoped to be able to determine absolute density as well as any differences in sampling efficiency between the instars.

MATERIALS AND METHODS

STOCKED ENCLOSURES. The sampling device was a white enameled dipper with a capacity of 473 ml on a 1.5 m wooden handle. Sample enclosures were sheet aluminum cylinders 1.12 m in diameter and 30 cm in height with 1 m^2 surface area. The aluminum sheets used for construction of the cylinders had a pale brown contact paper that adhered to the surface for protection during shipping; this was not removed in order to avoid reflective sides within the enclosures. Twelve cylinders were used on each test date and were placed in a line along the south edge of a rice paddy with one meter spacing (Figure 1). Tests were performed on three dates in 1980 and four dates in 1981. Sampling took place in June, July and August when rice plants ranged in height from 0.4 m to 1 m. The enclosure sites were chosen to represent average rice stand densities with open areas and very weedy places not being used. Areas considered for placement of the enclosures were presampled to avoid natural mosquito populations. Mosquitofish were present in the fields and as much as possible were excluded from the enclosures. Placing the cylinders in the field caused most fish to move away but occasionally a few small fish were enclosed. The cylinders were pushed down into the bottom mud to seal off escape of the mosquitoes which were added.

Laboratory reared *Cx. tarsalis* immatures were separated by stage and counted before being placed in the field enclosures. Each stage was used on each test date and there was no mixing of stages. Six stocking rates were used for fourth stage larvae and five for all others. The rates used and number of replicates are shown in Table 1. Mosquitoes were evenly distributed into the cylinders and 10 to 15 min were allowed to elapse before initiating sampling.

Dip samples were taken between 1100 and 1400 hr. The person sampling stood on the north side of the cylinder in order to avoid casting shadows over the water surface. Each cylinder was divided, by marks on the outside, into quadrants which were considered sample zones. The quadrants aided in sampling over the entire enclosed area and reduced bias by the individuals sampling. Samples were taken successively from the different quadrants. Dipping techniques were standardized by the samplers before actual counts were made. The general technique involved a rapid scooping motion with the hand located 1 m from the base of the dipper (Fig. 1). Once a sample was taken, the contents were counted, recorded and returned to the place where taken. Sampling was timed so that at least 3 min elapsed between samples taken from the same enclosure. Each sampler took a minimum of 8 samples from each enclosure on each of the sample dates.

The data collected were analyzed to determine statistical differences between samplers, sample enclosures, sample dates, stocking rates and regression equations for the immature stages. Bartlett's test of homogeneity of vari-

Table 1. Number of replicate enclosures for given densities of *Culex tarsalis* in stocked enclosure studies.

Density no./m²	Immature stage				
	I	II	III	IV	Pupa
10				2	
20	6	6	5	8	6
50	6	6	6	6	6
100	6	6	6	6	6
200	4	4	4	6	4
400	2	3	3	4	4

Fig. 1. Sampling enclosures placed 1 m apart in a rice field.

ance was performed to determine homogeneity between samplers. Additionally, a paired *t*-test was performed to define any significant difference between samplers. An ANOVA and Bartlett's test of homogeneity were performed on the data for the different sample dates over the two years to see if the data could be combined. Data for the mosquito stages at different stocking rates were used in linear regression equations to ascertain fit to a linear relationship. An ANOVA test for common slopes and regressions as described by Freese (1980) was made to determine differences between the slopes for the different mosquito stages.

FIELD SURVEY OF MOSQUITO IMMATURES. Concurrent with studies of stocked populations of mosquitoes we conducted a field sampling program to determine levels of mosquito production and their distribution and dispersal patterns. In 1980 five rice fields were selected for weekly samples. Two fields out of this block of five in western Fresno County, California were used in the first set of tests involving sampling from enclosures. Sample sites were 1 m^2 areas around numbered wooden stakes. Approximately 12 stakes per hectare (5 per acre) were distributed over the study fields for a total of 100 sample sites per field. At each site on each date a dipper sample was taken, the contents identified, counted, recorded and returned. A sample of immature mosquitoes was retained from each weekly sampling for positive identification in the laboratory. More sampling detail is given in Stewart, Schaefer and Miura (1983).

In 1981 one of the fields in this same group was sampled biweekly for mosquitoes using a transect sampling pattern. Immatures were again identified, counted and recorded. Enclosures used for 1981 tests were placed in this field.

Data were analyzed to determine population levels and fit to a mathematical distribution model. The range of immatures found was used to select levels for stocking rates in the enclosure studies.

ENCLOSURE SAMPLING WITH FIELD POPULATIONS. The third part of this study involved a removal method for determining population size from an area previously sampled with the dipper. A large rice field (90 ha) in Kings County, California was sampled for *Cx. tarsalis* during the summer of 1982. This field was found to produce large numbers of mosquitoes. Preliminary sampling revealed areas of greatest production as well as areas devoid of any immatures.

Four aluminum 1 m^2 cylinders (described herein) were placed in the field in a line about 1 m apart. The area chosen was one where presampling and previous sampling showed mosquito immatures to be present. In addition, the cylinders were placed in a typical rice stand area. Some bare spots were present in this field and were avoided in this test. With as little disturbance as possible, the cylinders were placed in the rice field and tightly fitted into the substrate.

Sampling was with a white enameled dipper using the methods previously described. Two people each took 15 dip samples from each of the enclosures. Samples were taken between 1100 and 1300 hr.

After dipper sampling was completed, the enclosures were sampled with buckets (7.5 liter capacity), retaining the contents for counting in the lab. As each bucket sample was taken, using a scooping technique, the contents were poured through fine nylon screening to retain the organisms. A set of five buckets was concentrated and was considered one sample. Each concentrated sample was placed in a numbered jar containing 70% ethanol. Six samples were taken from each enclosure for a total of 30 buckets. The samples were counted in the laboratory at a later time to determine the number of each instar present in each sample for each enclosure.

Dipper sampling results were analyzed to determine mean counts for the various instars and the variance associated with the means. An ANOVA test was performed to find any significant differences between samplers and enclosures. The data from the removal method part of the test were analyzed by the methods described in Southwood (1978) to develop population estimates.

RESULTS AND DISCUSSION

STOCKED ENCLOSURES. Table 2 summarizes the mean number of each stage sampled at each density for the combined test dates. There was a good relationship between data collected in 1980 with that from 1981. The tests for homogeneity of variance showed that the variances were homogeneous so that combination of data for the different test dates for both years was possible. Bartlett's test of homogeneity applied to the data collected by the individual samplers showed that the variability encountered in the counts was homogeneous. A paired *t*-test showed that there was no significant difference in the numbers of immatures sampled by the various persons. Data from different sample dates and by the different samplers could be combined and considered as a unit.

For each immature stage there is a corresponding increase in numbers sampled with increasing density. Linear regression analysis showed there was a significant regression of

Table 2. Mean number of *Culex tarsalis* immatures collected for given densities of each stage and linear regression statistics from 1980–81 stocked enclosures.

Density no./m²	Immature stage				
	I	II	III	IV	Pupa
10				0.02	
20	0.08	0.13	0.08	0.13	0.09
50	0.22	0.16	0.25	0.14	0.32
100	0.50	0.28	0.39	0.57	0.54
200	1.01	0.66	0.78	0.99	0.98
400	2.93	0.96	2.29	2.09	1.92
Total no. samples	554	612	660	816	556
slope	0.0075	0.0023	0.0057	0.0053	0.0047
y intercept	−0.2058	0.0831	−0.1260	−0.0290	0.0469
r^2	0.9774	0.9652	0.9711	0.9941	0.9978

density on mean number sampled ($r^2 \geqslant 0.93$). Figure 2 shows the regression lines for each stage with lines for several stages being similar. Results of the ANOVA test for common regressions are shown in Table 3. The tests applied first examined the lines for common slopes and then, if nonsignificant, tested for a difference in the levels of the regression. In all cases if the slopes were not different neither were the levels.

FIELD SURVEY OF IMMATURE MOSQUITOES. The field surveys of rice field populations of *Cx. tarsalis* in 1980 and 1981 revealed that relatively low levels existed in the study fields. The highest mean value of each instar in any one field in 1980 was 0.23, 0.10, 0.07, 0.07 and 0.04, respectively for first stage larvae through pupae. Samples taken in the 1981 field survey showed maximum mean dipper values of 0.10, 0.08, 0.10, 0.02 and 0.02, respectively for first stage larvae through pupae. These fields were considered potential problem fields by the local

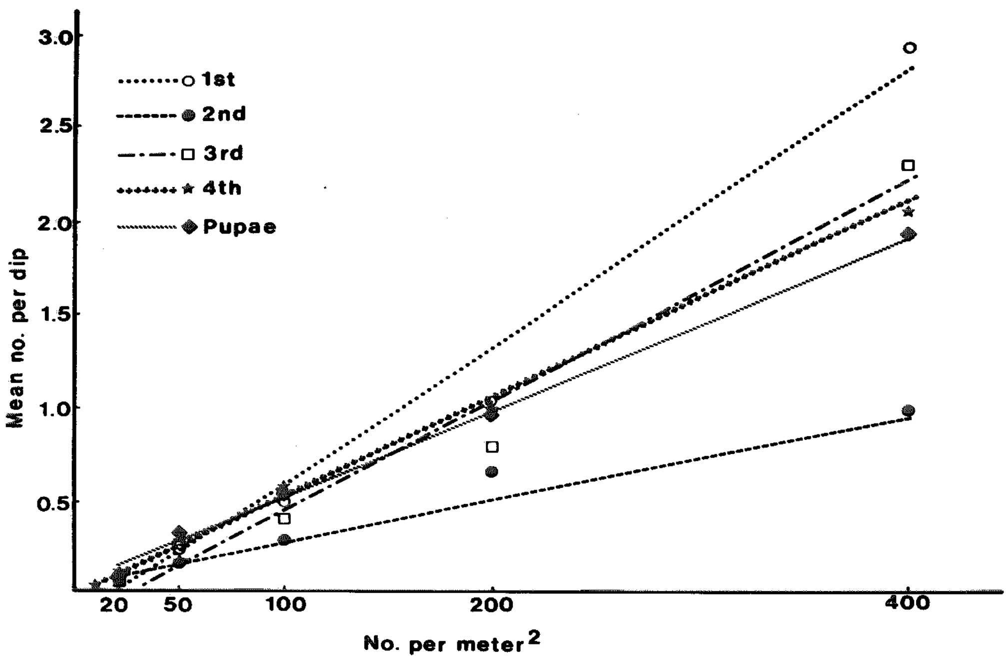

Fig. 2. Regressions of mean dipper count versus known densities of the immature stages of *Cx. tarsalis*.

Table. 3. Test of common slopes for the different immature stages.

Stage	I	II	III	IV	Pupa
I	—	**			*
II		—	**	**	**
III			—		
IV				—	
Pupa					—

* Difference at 0.05 level.
** Difference at 0.01 level.

mosquito control district and normal control measures would have been implemented when the mosquito levels exceeded a set mean value of 0.10 per dip.

Density levels used in the stocked enclosures were selected to reflect field population levels; thus we included low stocking rates of 10 and 20 per m^2 as well as the much higher rates of 200 and 400 per m^2.

Data on four of the fields sampled in 1980 were analyzed for a fit to known dispersal patterns. Following the methods of Bliss and Owen (1958) we found the data fit the negative binomial dispersion pattern. This followed results of Mackey and Hoy (1978) who also found a negative binomial pattern for *Cx. tarsalis* in rice fields of western Fresno County. Values for the dispersal constant and Chi-square tests are shown in Stewart et al. (1983).

Comparison of the field data to that from the stocked enclosures is difficult for a number of reasons. Even though the mosquito species are the same there may be some differences in ability to collect between the two sites. Secondly, and more importantly, the densities of mosquitoes in the field may be different from those from the stocked enclosures. Since mean and variance are related (Taylor et al. 1978) and tend to increase together it would be important in any comparison to use similar mean values. The data for the combined enclosure studies of 1980, 1981 for all stages were compared to one field in the series studied in 1980. The mean value of the overall enclosure study data was very high when compared to field data from any of the fields so the data for the lowest two stocking rates of 10 and 20 per m^2 were compared to that from one of the fields. Bartlett's test of homogeneity of variance indicated that the variances from the enclosures stocked at the lowest densities were homogeneous with that from one of the field survey fields. An ANOVA and F-test showed that the means were significantly different (however, not highly significant) but since the variances were similar, a comparison can be made between stocked enclosures at certain densities and field populations.

ENCLOSURE SAMPLING WITH FIELD POPULATIONS. The mean number of immatures per sample and number of mosquitoes per m^2 are shown in Table 4. From the data it was found that the populations in all four of the enclosures were not statistically different and so each enclosure could be considered a replicate density. This is not surprising because the enclosures were placed around an intact mosquito source. Tests of homogeneity of variance showed that samples taken from each of the enclosures had homogeneous variances and that there was homogeneity in the samples taken by the two samplers. An ANOVA and F-test showed that there was no difference in the numbers of each stage collected by each sampler and no difference in numbers of each stage in the different enclosures. The information presented in Table 4 thus shows only one mean sample value for each stage as well as only one density estimate value for each stage.

Table 4. Mean number of *Culex tarsalis* immatures sampled and densities estimated by removal collecting.

Immature stage	Mean per sample	No. per m^2
I	0.29 ± 0.16	93.67 ± 9.91
II	1.23 ± 0.27	124.69 ± 6.34
III	1.10 ± 0.23	181.79 ± 20.09
IV	0.49 ± 0.14	59.87 ± 11.65

Removal collecting estimates of numbers of *Cx. tarsalis* per m^2 enclosure are seen in Table 4. Since data from the dipper samples showed that we were sampling from the same population, the numbers per sample collected in the removal collecting were combined to give one estimate per immature stage. These mean values for number per sample and number per m^2 were added to the data from the stocked enclosure tests to determine if the regressions would be affected. Table 5 shows the regression statistics for the combined data. As no pupae were sampled or collected during either the dipper estimate part of the test or in the removal collecting, data for this stage are not presented.

Table 5. Regression statistics for combined data of stocked enclosures and enclosure sampling of field populations of *Culex tarsalis*.

	Immature stage			
	I	II	III	IV
slope	0.0076	0.0021	0.0058	0.0052
y intercept	−0.2554	0.2593	−0.1027	0.0127
r^2	0.9729	0.3960	0.9636	0.9833

The slopes for the four stages shown are not statistically different from those in the stocked enclosure tests, however there is some difference in the regression equation r^2 value for 2nd stage larvae. The regression lines for all but the 2nd remain essentially the same with r^2 values almost as high as those presented in Table 2. Except for 2nd stage larvae there is good correspondence between data for stocked enclosures as well as that from enclosures containing field populations.

Mosquito populations outside of the enclosures were sampled extensively for 3 weeks prior to and 4 weeks after the enclosure part of the test. On the day of the enclosure test, 50 samples taken in the area around the enclosures showed that the populations consisted of the same stages found within and that pupae were absent. Samples taken over 4 days (2 before the day of enclosure sampling and 2 days after) showed that the percentage of each stage present in the field was close to that found in the enclosures. For larval stages one through four respectively, the percentages for enclosure sampled mosquitoes versus field sampled mosquitoes were: 20.4–23.7, 27.1–29.7, 39.6–31.2, 12.9–15.4. In addition, mosquito immature sample data from the area of the field where the enclosures were placed showed a homogeneity in variance when compared to enclosure data.

SUMMARY AND CONCLUSIONS

Wada and Mogi (1974) concluded that the dipper could provide good estimates of the absolute density of mosquito larvae but species have to be considered separately. Chubachi (1976) found that the efficiency of the dipper varies with the type of environment where the samples are taken. In this study, for *Cx. tarsalis* immatures in rice fields, there is a linear relationship between dipper counts and the absolute density in enclosures. Comparison between variances in sampled populations outside of the enclosures and those within showed that at similar densities or with similar mean values, the variances are homogeneous. A comparison between samples taken from confined immatures (enclosures) and those in a field thus seems possible. Sampling of a field population of *Cx. tarsalis* from an enclosure showed that the data obtained were consistent with that from stocked enclosures.

The linear relationship between mean dipper count and absolute density can be used to convert to estimates of the absolute population. By use of the regression equations developed, the mean dipper counts which would normally be considered only relative counts, can be used to estimate the absolute density. Table 6 shows the estimated numbers of *Cx. tarsalis* per hectare corresponding to various mean dipper counts. Based on the results of the test for common slopes in Table 3, stages III, IV and pupae are considered to have common regression lines and are included together in Table 6.

Table 6. Estimated numbers of *Culex tarsalis* immatures based on regression statistics of 1980–81 enclosure studies.

Stage	No./dip	No./hectare
	0.10	407,700
I	0.50	941,400
	1.00	1,608,400
	5.00	6,944,712
	0.10	29,100
II	0.50	1,984,600
	1.00	4,429,000
	5.00	23,984,418
	0.10[a]	250,104
III, IV, Pupa	0.50	1,013,951
	1.00	1,968,759
	5.00	9,607,228

[a] Cumulative total for all 3 stages.

In our studies with stocked enclosures, second-stage larvae showed a tendency toward a different regression line from the other stages. When field data from 1982 counts were compared to stocked enclosure data only the second-stage larvae showed noncorrespondence. The reasons for this difference are not clear; however, for control purposes the later stages are more significant. The combining of counts of the later three immature stages has several important advantages. These later stages are much easier to see and count in dipper samples and represent the portion of the population most likely to reach maturity. A combination of stages also makes it easier on field sampling personnel by allowing only one cumulative count to be kept instead of separate ones for each stage.

The absolute density estimates obtained from the regression equations applied to field counts of *Cx. tarsalis* can be applied to modeling of mosquito populations in rice fields. Relative density estimates are difficult to compare even with other relative densities of mosquito populations. Absolute density can be used to relate the numbers of mosquitoes to predators, pathogenic organisms and nontarget organisms important in control programs.

ACKNOWLEDGMENT

This work was funded, in part, by EPA Cooperative Agreement No. CR-806771-01-1. The cooperation of personnel of the Fresno Westside and Kings County Mosquito Abatement Districts is gratefully acknowledged.

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ABUNDANCE AND SEASONAL DISTRIBUTION OF TABANIDAE IN A TEMPERATE AND IN A SUBARCTIC LOCALITY OF QUÉBEC

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ABSTRACT. The seasonal distribution of adult Tabanidae was studied in 2 Québec localities for 2 years: Trois-Rivières, located in the temperate zone (46°20′N) and the Lake Delorme area, in the high-subarctic (55°00′N). Of the 43 species collected in southern Québec, *Chrysops aestuans* was the dominant species, followed by *Hybomitra pechumani, H. lasiophthalma, C. frigidus* and *H. zonalis.* The flight period began in mid-May with the greatest abundance from mid-June to mid-July. Five phenological groups were distinguished by first appearance of the species and their respective abundance peaks. In the Lake Delorme area, 20 species were identified of which 12 were also present in the southernmost area. *Hybomitra arpadi* was the dominant species followed by *C. furcatus, C. excitans* and *H. zonalis.* The flight period began on July 8 with the most activity during the third week of July.

During 1977 and 1978, an adult survey was conducted in 2 localities of Québec Province, one located in the temperate zone, at Trois-Rivières (46°20′N), the other in the high-subarctic life zone (Ducruc et al. 1976), in the Lake Delorme area (55°00′N) along the Caniapiscau River. The objective of the study was to compare the phenology of Tabanidae in 2 climatically different areas, and to emphasize the activity patterns of adult Tabanidae.

MATERIAL AND METHODS

Trois-Rivières is located in the temperate life zone occupied by the deciduous forest formation of eastern North America, characterizing the Great-Lakes-St-Lawrence bioclimatic zone (Munroe 1956). More precisely, this is the climax hardwood forest dominated by *Acer saccharum* (Grandtner 1966), covering the southern part of Québec from approximately the 45th to 47th parallel. According to Treidl (1978), the mean annual temperature is 4.9°C (1941–70).

Lake Delorme area is included in the subarctic zone dominated by black spruce forests (Ducruc et al. 1976). The mean annual temperature is about −3°C (Richard 1978). Ecologists distinguish 3 subzones based on vegetation. The studied area is at the northern limit of the