BIONOMICS OF LOUISIANA RICELAND MOSQUITO LARVAE I. A COMPARISON OF SAMPLING TECHNIQUES[1]

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ABSTRACT. A study was initiated in 1980 to compare data obtained with a standard larval dipper and a static quadrat device (0.1 m^2) regarding the species composition and seasonal abundance of mosquitoes that inhabit Louisiana rice fields. Emphasis was placed on reducing sample variability to provide accurate and efficient estimates of larval mosquito populations.

Homogeneous trends in population growth were exhibited by both sampling techniques. Statistical analyses were conducted to quantify the relationships between absolute densities (number of preadult mosquitoes/unit area of 0.1 m^2) and dipper sample values for larval populations of *Psorophora columbiae* and *Anopheles crucians*. From these relationships, models were derived to estimate larval densities from mean dipper values.

Statistical comparisons of the data indicated that the area sampler technique was not only less variable and more efficient at detecting larvae at low abundance levels but that more larvae/unit sample and a greater proportion of early instar larvae were collected than with the dipper. The limiting feature of the area sampling device was the amount of tedious labor required to obtain and process collections.

INTRODUCTION

Mosquitoes that inhabit the riceland agroecosystem are among the most likely targets for the application of integrated management schemes since they have adapted themselves to habitats largely under the control of man and are dependent on man and his activities for their continued existence.[3] The development of integrated approaches to riceland mosquito control depends on an ability to detect and quantify biological events as they occur in the aquatic ecosystem under study. In this case, a need exists to develop a scientifically acceptable technique for sampling mosquito larvae in rice fields which simultaneously provides data on the status of predator populations.

Previously, quantitative information regarding the spatial and numerical distribution of riceland mosquito larvae and their interrelationships with predators and selected environmental parameters was estimated from counts with a dipping device, with the assumption that these numbers were proportional to the total larval population (Chambers et al. 1979). Although it is a rapid and economical method for conducting larval surveys, the degree to which the dip sample relates to the actual mosquito population density in a given habitat has not been satisfactorily established for riceland mosquito species.

Boyd (1930) and Goodwin and Eyles (1942) were unable to relate the volume of the dipper to the water surface being surveyed, even with such habitual surface dwelling forms as anopheline larvae. Several investigators (Russell et al. 1945, Horsfall 1946, Belkin 1954, Shemanchuk 1959, Knight 1964 and Farlow et al. 1978) have used static quadrat devices in qualitative surveys and to quantitatively estimate the absolute population densities of the aquatic community under investigation. These were accomplished by relating the surface area of the quadrat measuring device to the surface of the entire breeding area. This method is based on the assumption that mosquito larvae are distributed randomly throughout the habitat. However, unpublished data of Meek and Andis from field studies in Louisiana indicate that mosquito larvae, as well as nontarget macroinvertebrates, are not randomly distributed in the rice field agroecosystem.

The present study compares data obtained with the standard larval dipper and a static quadrat device. The relationship between absolute density and dipper sample values was quantified for estimating the probable population density of the entire community under investigation.

METHODS AND MATERIALS

The monitoring program was conducted weekly in 25 and 29 ha commercial rice fields of Vermilion Parish during 1980 and 1981, re-

[1] This research was conducted as a cooperative effort between the State Agricultural Experiment Stations of Arkansas, California, Louisiana, Mississippi and Texas and the Agricultural Research Service, USDA as part of the USDA/CRS Southern Regional Project S-122 on the Biology, Ecology and Management of Riceland Mosquitoes in the Southern Region.

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spectively. Fields were selected for study on the basis of accessibility, farmer cooperation and history of mosquito productivity. Due to the aggregation of larval mosquito populations, differences in the number of larvae collected among samples often occur and result in biased estimates of population parameters. Because of this variability, a stratified random sampling procedure was devised which effectively reduced the heterogeneity of the larval riceland mosquito populations and increased the precision of the sample estimates of the population means.

Prior to the initial sampling procedure each year, 3 pans (areas planted in rice) within the appropriate field were selected for study at the low, middle and high elevations of the field. Population estimates (Andis and Meek, unpublished data) and the numerical relationship between the 2 larval sampling devices were established by stratifying each pan into 3 nonoverlapping areas; within 1 m of opposing levees (stratum 1), between 1 and 11 m (stratum 2) and at the middle of the pan (stratum 3). Samples were then collected along 3 equidistant transects that extended the length of the field.

The sampling tools consisted of the standard 400 ml larval dipper and a static quadrat device modified from Farlow et al. (1978) (hereafter referred to as an area sampler) which allowed removal of virtually all aquatic organisms from an area 0.1 m^2. Immature mosquitoes were collected with the area sampler by placing the open-ended area sampler within each site and pressing it firmly into the bottom substrate. All vegetation within the sampler was removed and washed into the unit. The top 2.5 cm of debris and mud was then agitated to facilitate collection of benthic organisms. The entire water sample was immediately removed from the sampler and condensed through a 100 mesh (40.8/cm) bag. The resulting mass of debris and organisms was placed in 95% ethanol, transported to the laboratory, and processed by a salt water flotation technique (Horsfall 1955).

In the case of dipper samples, a standard dipping procedure was adopted to minimize individual sampling differences; in that, all larval dipping was conducted by the same person at each site. On each of the 18 sampling dates 10 larval dips were collected and accumulated adjacent and concurrent to each 0.1 m^2 area sample to allow a direct comparison between the number of larvae collected in a sample of 10 dips and the number of larvae in each 0.1 m^2 area sample.

Statistical analyses were based on a model utilizing one field with 3 pans/field, 3 strata/pan, 5 replicates/stratum, one dipper sample consisting of 10 dips and one area sample/replication for a total of 450 dips and 45 area samples collected/sample date. Data from the dipper and area samples were subjected to an analysis of variance using SAS general linear models procedure for testing the hypothesis that the means obtained from both sampling techniques were equal. Following rejection of this hypothesis, linear regression was used to quantify the relationships between the number of larvae/10 dips and the number/unit area of 0.1 m^2 (SAS 1982). From these relationships, mathematical models were derived for each instar to estimate, with sufficient accuracy, the relative densities per unit area of 0.1 m^2 for given mean dipper values.

RESULTS AND DISCUSSION

Larvae of *Psorophora columbiae* (Dyar and Knab), *Ps. ciliata* (Fabr.), *Anopheles crucians* Wied., *Culex erraticus* (Dyar and Knab), *Cx. salinarius* Coq., *Aedes vexans* (Meigen) and *Uranotaenia sapphirina* (Osten Sacken) were collected from a commercial rice field in 1980. The majority of the species collected were obtained by both sampling techniques. However, the collections of *Ps. columbiae* and *Cx. salinarius* and a single collection of *Ae. vexans* were detected only in area sample collections on 18 March, 15 May and 11 June 1980, respectively. Collections of these species only in area samples can probably be attributed to the greater surface area and volume associated with this 0.1 m^2 device. Thus, it appears that the probability of collecting a single individual of any one species using the area sampler is greater than with the dipper method.

Furthermore, various replicates of the sampling procedure yielded larvae in the area sampler collections whereas the concurrent samples obtained by the dipper method lacked mosquito larvae. For example, *Ps. columbiae* larvae were present in 201 (28%) of the 718 area samples collected and in only 185 (26%) of the dipper samples. *Anopheles crucians* were present in 313 (43.6%) area samples while 264 (36.8%) of the dipper samples contained larvae. *Culex salinarius, Ur. sapphirina* and *Cx. erraticus* were collected in 78 (10.9%), 93 (13.0%) and 23 (3.3%) of the area samples, respectively, while 62 (8.6%), 67 (9.3%) and 17 (2.4%) of the dipper samples were positive for the respective species. This variation in frequency of occurrence was attributed, in part, to the developmental stage of the population sampled. Populations comprised predominantly of early instar larvae (I and II) were less frequently detected with the dipper method than with the area sampler (Meek and Andis, unpublished data). None of the previously mentioned spe-

cies were obtained solely with the dipper method.

The population indices established for preadult mosquitoes collected with the area sampler and dipper methods are presented in Table 1. The trends in abundance (number of individuals/sample of 10 dips) and population density (number of individuals/0.1 m^2) corresponded for all sample dates; although frequently, the mean number/10 dips was significantly less ($P < 0.05$) than the mean number collected using the area sampler. These homogeneous trends in population levels resulted in the establishment of a statistically significant linear relationship ($P < 0.01$) between the number of larvae/10 dips and the number/0.1 m^2, which infers that the dipper method provides a reliable index to the larval population density.

The statistical relationship between the 2 techniques for sampling larval population of *Ps. columbiae* and *An. crucians*, are presented in Figs. 1 and 2, respectively. These relationships are mathematically described by the general equation, $Y = a\bar{x} + b$, where the dependent variable Y is equal to the regression estimate of the mean number of larvae/10 dips and $\bar{x}$ is the mean number collected/0.1 m^2. The coefficients of determination (r^2) indicated that 77 and 69% of the variation in the numbers of preadult mosquitoes collected/10 dips can be attributed to the variation in the numbers of *Ps. columbiae* and *An. crucians* collected/0.1 m^2, respectively. These statistical analyses quantified the relationship between density and dipper sample values yet, for managerial application, there are 2 limitations to this approach. First, an excessive amount of unexplainable variation existed. Consequently, the abundance data for each species were recalculated and expressed as the mean number of 1st, 2nd, 3rd or 4th-instar larvae collected. Regression analyses performed on these data resulted in more precise conversion factors and the relationships between the 2 sampling methods for each instar of *Ps. columbiae* and *An. crucians* are illustrated in Figs. 3 and 4. It becomes obvious that when the larval population's phase of development is incorporated into the analyses, more precise factors for converting dipper sample values to density are obtained. The second limitation of these regression analyses was that, in this form, estimates of larval density cannot be derived from the dipper sample values. To overcome this problem,

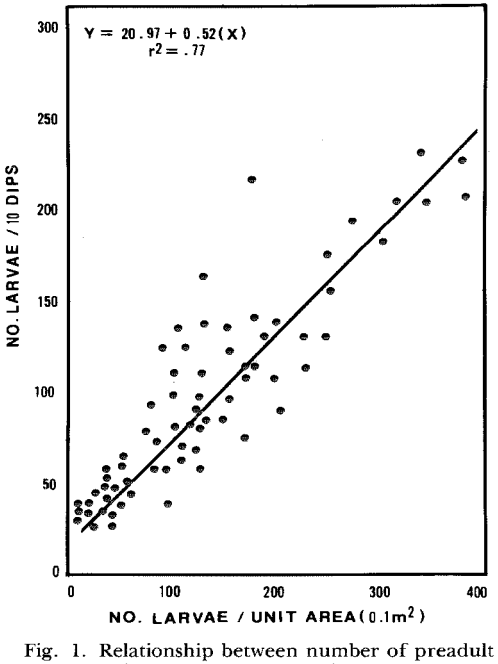

Fig. 1. Relationship between number of preadult *Ps. columbiae*/10 dips and number/area sample (0.1 m^2).

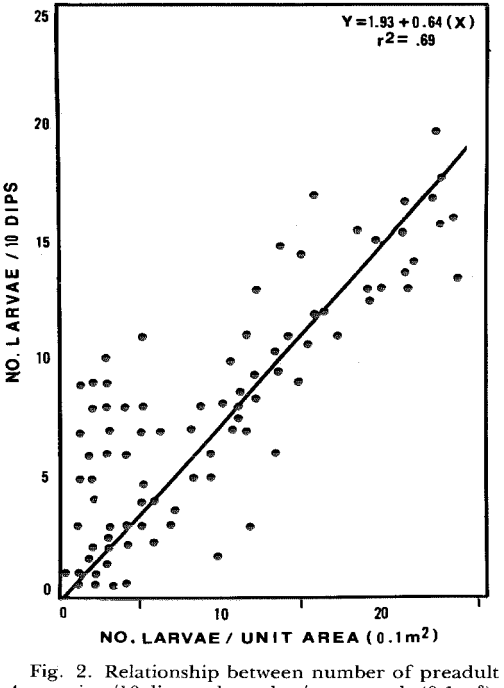

Fig. 2. Relationship between number of preadult *An. crucians*/10 dips and number/area sample (0.1 m^2).

Table 1. Comparison of mosquito population indices obtained using 2 larval sampling techniques in Louisiana rice fields.[a]

	Psorophora columbiae		*Anopheles crucians*		*Culex salinarius*		*Culex erraticus*		*Uranotaenia sapphirina*	
1980	Area sampler[a]	Dipper[b]	Area sampler	Dipper	Area sampler	Dipper	Area sampler	Dipper	Area sampler	Dipper
18 Mar	<0.1 (0.1)	0	0	0	0	0	0	0	0	0
20 Mar	0	0	0	0	0	0	0	0	0	0
03 Apr	0	0	1.7 (0.5)	1.1 (0.5)	0	0	0	0	0	0
10 Apr	0	0	1.6 (0.5)	1.4 (0.4)	0	0	0	0	0	0
17 Apr	0	0	1.9 (0.5)	1.7 (0.5)	0	0	0	0	0	0
01 May	0	0	1.4 (0.8)	0.9 (0.4)	0	0	0	0	0	0
07 May	0	0	4.8 (0.9)	4.3 (1.1)	0	0	0	0	0	0
15 May	0.6 (0.3)	<0.1 (0.1)	4.4 (1.1)	3.7 (0.8)	<0.1 (0.1)	0	3.0 (1.4)	0.2 (0.1)	0	0
28 May	0	0	6.8 (1.8)	6.6 (1.7)	6.6 (1.9)	5.7 (1.4)	7.8 (4.5)	0.8 (0.4)	0	0
06 Jun	2.2 (0.5)	1.0 (.03)	9.2 (2.3)	8.1 (2.2)	<0.1 (0.1)	<0.1 (0.1)	5.2 (2.3)	0.6 (0.3)	0	0
11 Jun	10.6 (4.0)	8.6 (4.2)	10.3 (2.2)	8.3 (1.7)	0.9 (0.4)	0.8 (0.4)	12.0 (5.0)	1.3 (0.6)	0.3 (0.1)	<0.1 (0.1)
18 Jun	—	—	—	—	—	—	—	—	—	—
25 Jun	—	—	—	—	—	—	—	—	—	—
02 Jul	—	—	—	—	—	—	—	—	—	—
19 Aug	252.4 (31.9)	146.3 (16.9)	8.8 (2.0)	4.1 (0.9)	1.9 (0.6)	0.7 (0.2)	3.7 (1.4)	0.4 (0.1)	0.8 (0.4)	<0.1 (0.1)
28 Aug	75.8 (5.9)	49.4 (4.0)	2.2 (0.4)	1.4 (0.4)	<0.1 (0.1)	<0.1 (0.1)	17.9 (4.1)	2.4 (0.6)	0.7 (0.4)	0.2 (0.1)
09 Sep	71.3 (3.9)	36.9 (2.2)	6.0 (1.4)	2.4 (0.6)	<0.1 (0.1)	<0.1 (0.1)	0	0	0.9 (0.5)	0.7 (0.4)
17 Sep	0	0	0	0	0	0	0	0	0	0

[a] Mean number of mosquito larvae collected with 0.1 m^2 area sampler and no. larvae/replicate of 10 dips, respectively.

[b] Number in parenthesis is the standard error of the mean.

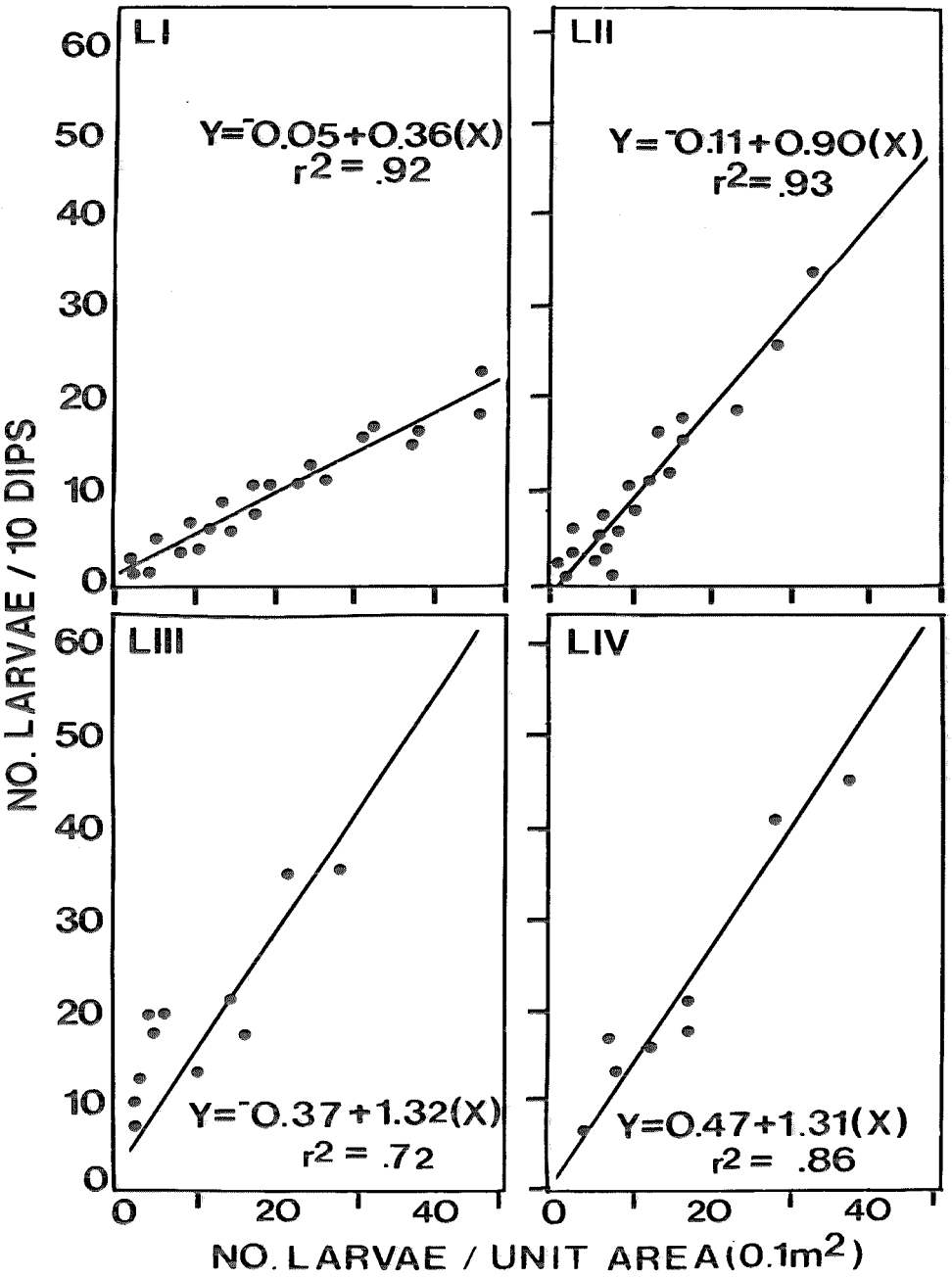

Fig. 3. Relationship between number of individual instar (I–IV) *Ps. columbiae*/10 dips and number/area sample (0.1 m²).

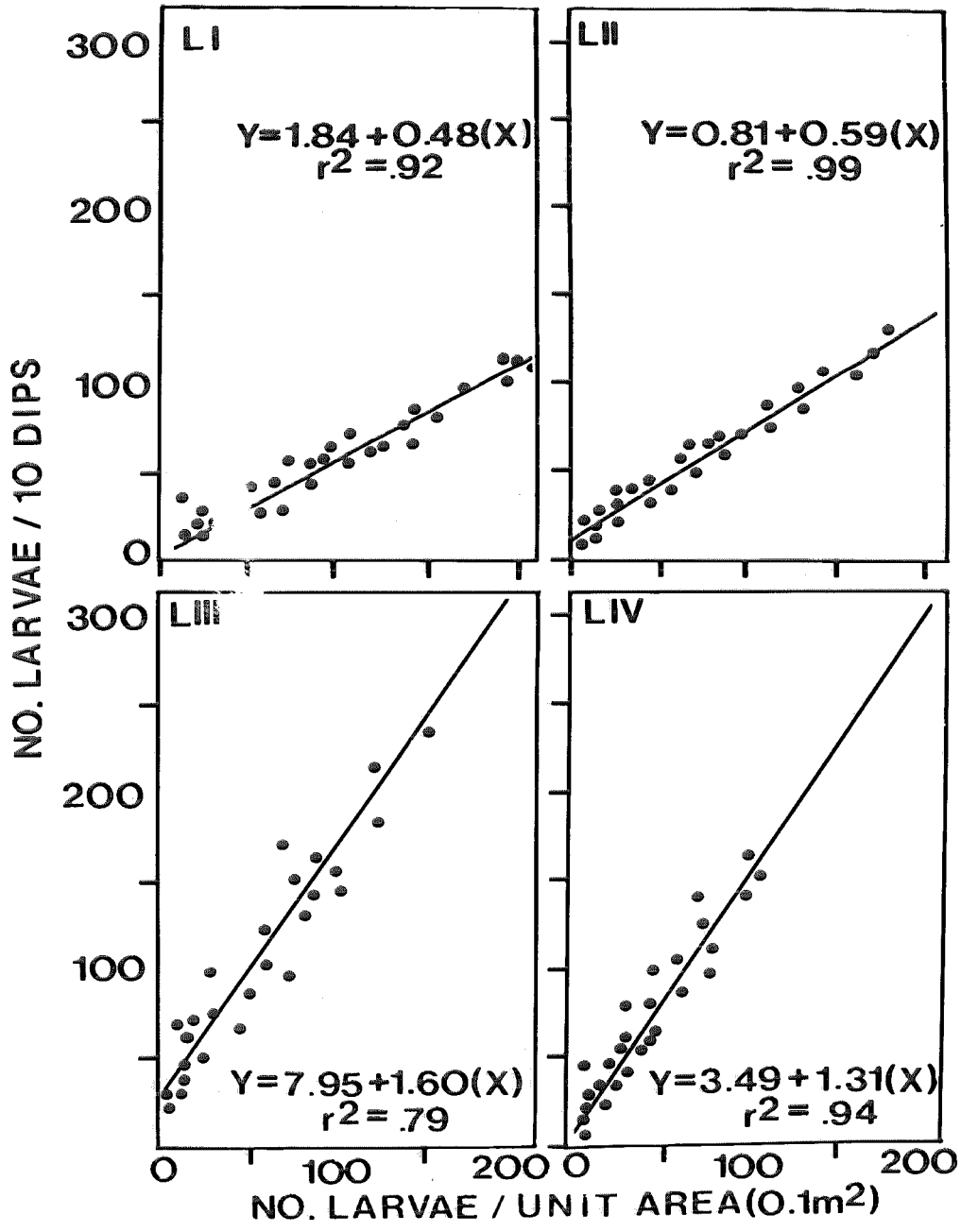

Fig. 4. Relationship between number of individual instar (I–IV) *An. crucians*/10 dips and number/area sample (0.1 m^2).

mathematical models were derived from the regression analyses to estimate the density (number of larvae/0.1 m²) of the predominant preadult rice field mosquito species from the mean number of larvae collected/10 dips. The predictive models presented in Table 2 were derived for each larval instar to obtain the most precise population estimates and should be useful to those involved in planning control strategies for integrated riceland mosquito management programs by enabling the estimation of mosquito density from larval surveys.

Statistical comparisons indicate that qualitatively, the area sampler was not only more precise at detecting species in low levels of abundance but that quantitatively, more larvae/unit sample and a greater proportion of early instar larvae were collected than with the dipper. The latter may be attributed, in part, to the vertical limitations of the dipper and to behavioral differences of larval instars. An additional advantage of the area sampler is illustrated for *Ps. columbiae* and *An. crucians* in Figs. 5 and 6, respectively. The population means, in individuals/area sample of 0.1 m² and in individuals/10 dips, were plotted against the coefficients of variation for all species; the latter index is used because the means and variances were not independent. The coefficient of variation tends to be lower for the area sample

Table 2. Predictive models for the calculation of larval *Ps. columbiae* and *An. crucians* density from dipper sample values.

Species	Instar	Predictive model[a]	$1-r^2$ [b]
Ps. columbiae	I	X = −0.27 + 1.92 (Y)	0.08
	II	X = −0.93 + 1.69 (Y)	0.01
	III	X = 1.70 + 0.49 (Y)	0.21
	IV	X = −1.63 + 0.72 (Y)	0.06
	I–IV[c]	X = −16.29 + 1.48 (Y)	0.23
An. crucians	I	X = 1.11 + 2.56 (Y)	0.08
	II	X = 0.63 + 1.04 (Y)	0.07
	III	X = 1.33 + 0.55 (Y)	0.28
	IV	X = 0.14 + 0.66 (Y)	0.14
	I–IV[c]	X = 1.20 + 1.08 (Y)	0.31

[a] X = number of larvae/unit area of 0.1 m².
Y = mean number of larvae/10 dips.
[b] Amount of unexplainable variation.
[c] All larval instars combined.

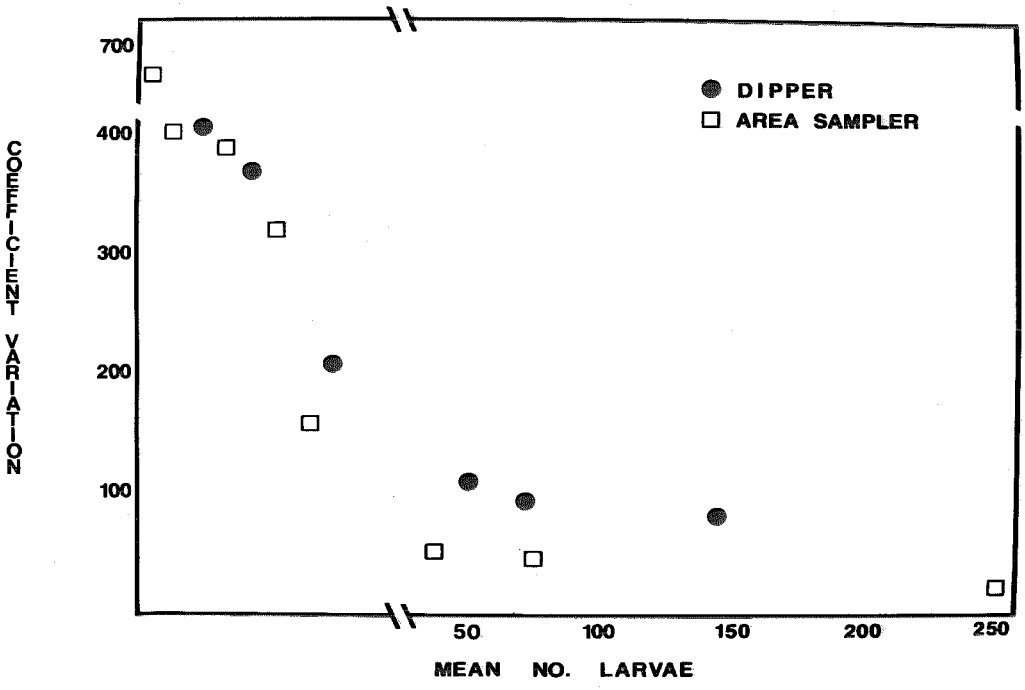

Fig. 5. Comparison of variability of area sampler and dipper methods for estimating relative abundance of *Ps. columbiae*.

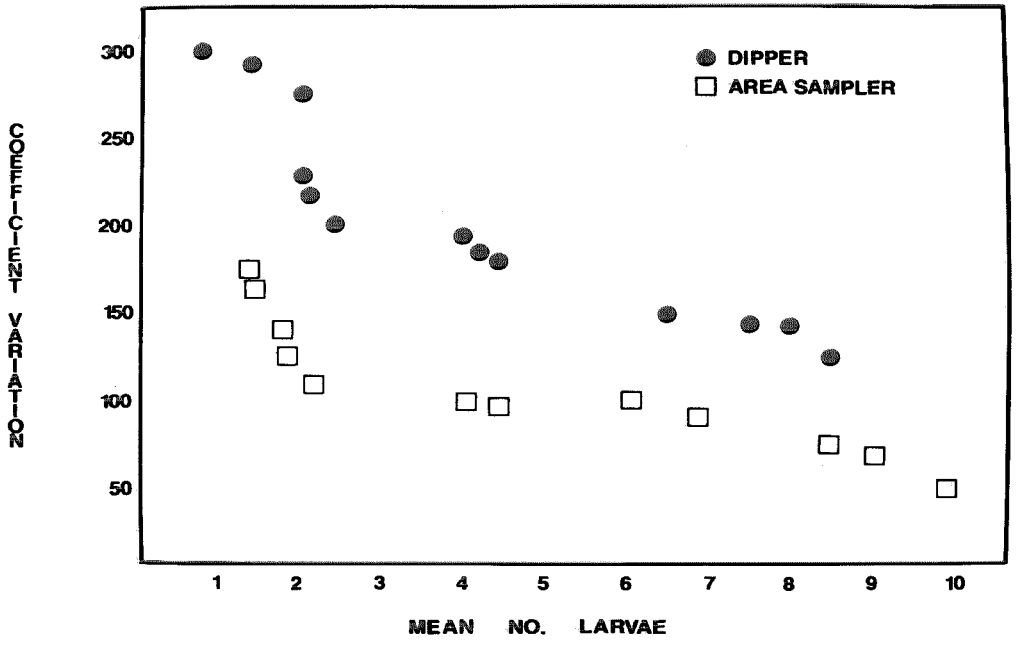

Fig. 6. Comparison of variability of area sampler and dipper methods for estimating relative abundance of *An. crucians*.

method and decreases as the population density increase, for all species collected. The area sampler was also more useful in dense vegetation and the number of individuals escaping was small if not negligible. However, the principal advantage of the area sampler is that the results can be stated as density whereas the dipper method cannot be used to make density measurements. Almost invariably the number of larvae/dip is related to other factors in addition to density. For example, with the area sampler, more opportunity exists to collect larvae (especially early instars) that are disturbed at the surface by the approach of the collector. Furthermore, from the estimates of density it is possible to calculate the probable population of the entire community under investigation (Andis and Meek, unpublished data).

The limiting feature of an area sampling device is the amount of tedious labor required to obtain and process collections.[4] However, the negative aspect of time efficiency may be overcome by the positive aspect of precision when sampling for mosquito larvae. Additionally, in an integrated management program, the ability of the area sampler to simultaneously provide qualitative and quantitative data regarding the nontarget aquatic invertebrates (Takahashi et al. 1982) must be taken into consideration. For this device to be accepted as an efficient sampling technique in a riceland mosquito management program, further emphasis must be placed on decreasing the handling time of area samples without decreasing precision of the sample estimates.

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THE HOST-FEEDING PATTERNS OF *CULEX QUINQUEFASCIATUS* IN MISSISSIPPI[1]

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ABSTRACT. Blood-feeding patterns of *Culex quinquefasciatus*, analyzed by a simple percentage calculation of the total number of blood-meals that had been derived from a specific host, indicated a preference for avian hosts. Percent avian feedings decreased from 70.3% to 59.6% from June through August 1976. The trend reversed by October when 77.5% of the blood-meals were avian. Similarly, during the period May through July 1977, the avian preference ranged from 96.3% in May to 57.2% in July.

INTRODUCTION

In recent years St. Louis encephalitis (SLE) has occurred in epidemic proportions in Mississippi and other states. St. Louis encephalitis is known to be a cyclic disease within the bird-mosquito complex, with occasional involvement of humans. In Mississippi it has been generally accepted that *Culex quinquefasciatus* Say is responsible for the transmission of SLE to man, since SLE virus positive mosquito populations have been isolated. Tempelis et al. (1967), found that 96% of engorged *Cx. quinquefasciatus* had fed on birds. However, Suyomoto et al. (1973) determined that only 56% of *Cx. quinquefasciatus*, in the southern United States, had utilized an avian host. *Culex tarsalis* Coq., a known vector of SLE in the western United States, shifts from bird hosts during the spring and early summer, to mammals during the mid- and late summer months (Tempelis 1975). This trend was reversed in the fall with a return to birds as the preferred host. As variable feeding patterns have been demonstrated for *Cx. quinquefasciatus* (Hess et al. 1968), the objective of this investigation was to determine the blood-feeding patterns for this species during the summer months.

MATERIALS AND METHODS

Two study areas were selected in Oktibbeha County, Mississippi, one urban and the other rural. The urban study area was within the city limits of Starkville (east central Mississippi), and the rural area consisted of the campus, Plant Research Center and the Animal Research Center of Mississippi State University (MSU). Six resting stations were selected randomly within each of the study areas. Typical resting stations consisted of culverts, garages, animal shelters, bridges and livestock barns. Engorged specimens were collected three times weekly from 15 March to 15 October 1976 and 15 March to 31 July 1977. The resting stations

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