

## EFFECTS OF SAMPLING DESIGN ON THE ESTIMATION OF ADULT MOSQUITO ABUNDANCE

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**ABSTRACT.** During 1994–5, *Culex tarsalis* comprised 75% of the 902,643 adult female mosquitoes collected by 63 dry-ice-baited Centers for Disease Control (CDC)-style traps operated biweekly in a uniform sampling grid that covered the southern Coachella Valley, Riverside County, California. The  $\ln(y + 1)$  transformation successfully controlled the variance and normalized the distribution of catch size among trap nights. When tested by analysis of variance, abundance varied significantly among months, years, and trap sites. Although the trap by month interaction was not significant, female distribution changed seasonally as larval habitats shifted from wetlands along the Salton Sea to agriculture to managed duck marshes. Conditional simulations utilized subsets of trap sites to compare sampling designs that required no (uniform, random, and transect designs) or prior (best-estimate and stratified random designs) knowledge of mosquito spatial distribution. All designs provided similar information on population seasonal trends, but a stratified random design provided the most accurate and precise simulation. A uniform trap grid that employed every 2nd trap site subsequently was adopted by the Coachella Valley Mosquito and Vector Control District to provide information on focal changes in abundance indicative of missed or newly created larval habitats or control failures.

**KEY WORDS** *Culex tarsalis*, statistical distributions, sampling designs, conditional simulations, geographical information systems

### INTRODUCTION

Measuring adult mosquito abundance with acceptable accuracy and precision remains a priority in epidemiology, surveillance, and control programs. Because of the vagile nature of adult mosquito populations, abundance typically is measured by a standardized collection protocol and produces data expressed per unit such as number of females per trap night or effort such as numbers per collector hour. These measures are in relative numbers and do not estimate density (numbers per unit area) or absolute size (total mosquitoes, usually estimated by mark–release–recapture methods). Accuracy is important if thresholds of abundance such as counts per trap night are used to make decisions on the intensity of mosquito-borne health risk or the type, focus, and intensity of abatement. Precision is important if estimates are compared over time and/or space. Although many studies have addressed sampling by comparing catch size among different collection methods (Service 1993), few have addressed quantitatively the impact of sampling design on the measurement of abundance; that is, how many samples should be taken how frequently in what type of pattern.

The intensity of sampling usually is dictated by the practical constraints of landscape, access, collection effort, and processing time. Collection sites almost never are distributed in purely random fashion, and therefore do not measure abundance over

large geographic areas that contain both favorable and unfavorable habitats. Sampling frequently is stratified, based on the distribution of the target species (Saugstad et al. 1972), the occurrence of human cases (Szumlas et al. 1996), or other human population attributes such as density and socioeconomic status (Lindsay et al. 1995). The allocation of sampling effort also is made intuitively using a best-estimate approach, where collections are made in areas where they are anticipated to catch large numbers of a target species (Reisen et al. 1996b). Although providing a sensitive indication of change over time, best-estimate sampling provides data restricted to favorable habitats and therefore usually overestimates abundance throughout an area that is a mosaic of favorable and unfavorable habitats. Uniform sampling designs have been utilized infrequently to determine the spatial distribution of a target species (Work et al. 1977a), and are well suited to urban environments, where samples can be blocked spatially and collections made systematically (Reisen et al. 1990). In rural areas where access is limited, samples frequently are taken along transects such as roads or drainage systems (Hayes et al. 1976, Work et al. 1977b) that provide access, but may or may not intersect favorable habitats.

Conditional simulations (or “what if?” analyses) provide one approach to evaluate sampling designs, providing that a large data set is available for retrospective sampling. As part of operational research to develop a geographical information system (GIS) for the Coachella Valley Mosquito and Vector Control District (MVCD) (Lothrop and Reisen 1998), 63 dry-ice-baited traps were operated in a uniform sampling grid throughout the southern Coachella Valley of California. Sampling emphasized *Culex tarsalis* Coquillett, because it is the most abundant mosquito species and the vector of both western

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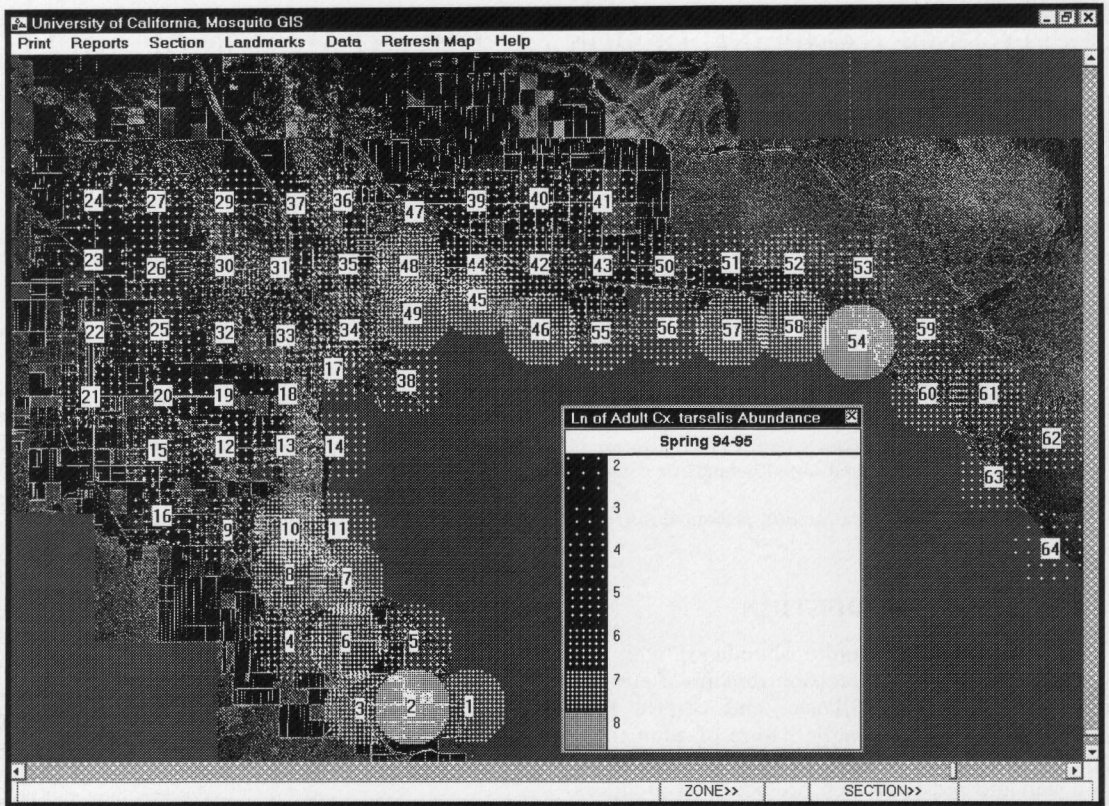


Fig. 1. Maps of the southern Coachella Valley showing the spatial distribution of female *Culex tarsalis* during spring. Data are depicted as dot densities of natural log intervals using the  $\ln(y + 1)$  transformed number of females per trap night per trap per season.

equine encephalomyelitis and St. Louis encephalitis viruses (Reisen et al. 1992), which are endemic in this area (Reisen et al. 1996a). Previous sampling designs used in the Coachella Valley included a best-estimate approach (Reisen et al. 1992) and a series of widely spaced east-west transects (Reisen et al. 1995c). Collectively, analysis of these data indicated that mosquito abundance was temporally bimodal and spatially greatest near salt- and freshwater marshes along the Salton Sea (Reisen et al. 1995a, 1995c).

The current research utilized data on adult mosquito abundance collected at our uniform sampling grid in the Coachella Valley to describe the relationship between the variance and mean and select a transformation most appropriate for least squares analysis, to apply least squares procedures to data collected by the uniform sampling scheme to verify patterns of abundance in time and space previously determined by best-estimate or parallel transect designs, and to utilize conditional simulations to evaluate the ability of different sampling schemes to estimate adult abundance. In these simulations, we assumed that the mean and variance calculated for all traps within our sampling grid adequately estimated the true population abundance values of  $\mu$

and  $\sigma^2$ , respectively, and that the accuracy and precision of various sampling strategies could be ascertained by comparison with these values.

## MATERIALS AND METHODS

Host-seeking female mosquitoes were collected from April 1994 through November 1995 by 63 dry-ice baited Centers for Disease Control (CDC)-style traps (Sudia and Chamberlain 1962) operated at 2-wk intervals without lights from late afternoon to the following morning. Collections were transported alive to the Coachella Valley MVCD where mosquitoes were anesthetized with triethylamine and enumerated by species. Traps were positioned at 1-mi. (1.67-km) intervals at the intersect of township sections throughout a  $10 \times 17$ -mi. ( $16.0 \times 27.4$ -km) area of the southern Coachella Valley, Riverside County, California (Figs. 1–3). The study area was bordered on the south by undeveloped desert and the Salton Sea, on the east by the arid Chocolate Mountains and Mecca Hills, on the west by the Santa Rosa Mountains, and on the north by Highway 195. Highway 195 was an arbitrary boundary, but previous studies indicated that the abundance of *Cx. tarsalis* decreased markedly to

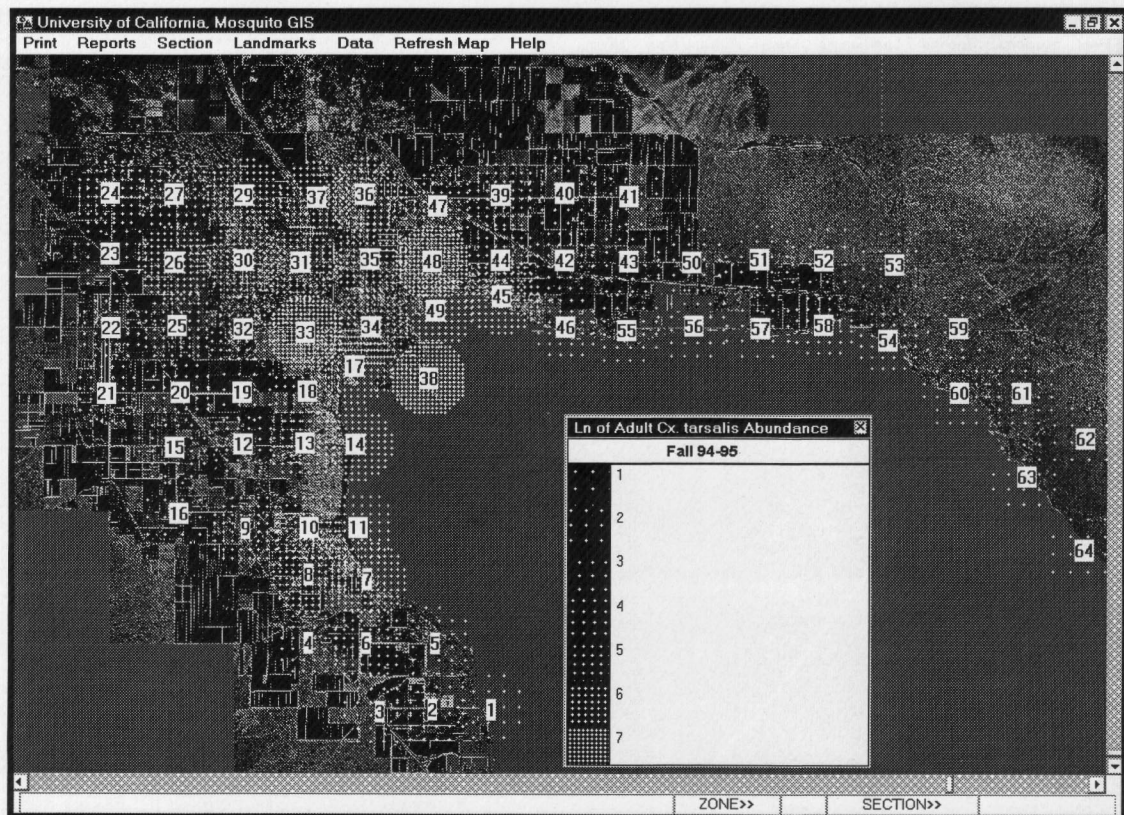


Fig. 2. Map of the southern Coachella Valley showing the spatial distribution of female *Culex tarsalis* during summer. Data are depicted as in Fig. 1.

the north (Reisen et al. 1995a, 1995c) and that few females released along the Salton Sea were recaptured as far north as Highway 195 (Reisen and Lothrop 1995).

The entire data set was used to select a transformation that allowed the variance to vary independently from the mean and that normalized the distribution of catch size among traps. Transformed catch size then was compared over time and space by a general linear model analysis of variance (ANOVA) using NCSS 6.0.21-2 software (Hintze 1996). Trap means within months grouped by posteriori multiple range comparisons depicted changes in population spatial distribution that were displayed using a GIS interface (Lothrop and Reisen 1998). Conditional simulations then were done on the transformed catch of female *Cx. tarsalis* using subsets of traps selected according to sampling designs that either presumed no knowledge of mosquito distribution (transect, uniform, or random arrays) or required information from prior sampling (best-estimate or stratified random designs). Simulation means were considered to be accurate if they fell within the 95% confidence limits (CLs) of the population mean. Precision was indicated by the width of the 95% CLs. Ability to resolve the gen-

eral seasonal pattern was indicated graphically and by correlations between estimates of the monthly mean measured by all traps and the mean measured by the selected subset of traps.

## RESULTS

### Distribution and transformation

A total of 902,643 host-seeking female mosquitoes were collected in 1,972 trap-nights, comprising 12 species in 5 genera (Table 1). *Culex tarsalis* was most abundant (75%), followed by *Psorophora columbiae* (Dyar and Knab) (13%), *Culex erythrorhax* Dyar (6%), and *Aedes vexans* (Meigen) (3%). The ability to exploit a variety of natural and agricultural larval habitats throughout much of the year and disperse widely during host-seeking flights resulted in catch of *Cx. tarsalis* being distributed most evenly among trap sites (i.e., lowest coefficient of variation [CV]). Species with a CV >10 exploited focal and/or ephemeral larval habitats produced in freshwater marshes (e.g., *Anopheles franciscanus* McCracken, filamentous algal mats) or agriculture (e.g., *Ps. columbiae*, flood-irrigated





Fig. 3. Map of the southern Coachella Valley showing the spatial distribution of female *Culex tarsalis* during fall. Data are depicted as in Fig. 1.

pastures and date gardens, and *Ae. vexans*, date and citrus orchards).

The arithmetic mean and variance were calculated for female *Cx. tarsalis* catch at each of the 63 trap sites. The variance increased proportionally to the mean number of females collected per trap night (Fig. 4A,  $n = 31-33$ ,  $R^2 = 0.88$ ), and a fre-

quency distribution grouping the number of trap nights by catch size was not normally distributed (Fig. 5A), that is, skewness = 5.02, kurtosis = 37.97, and CV = 2.04. The  $\ln(y + 1)$  transformation controlled the variance (i.e., reduced the CV to 0.46), made the variance independent of the mean (Fig. 4B,  $R^2 = 0.11$ ), and normalized distribution

Table 1. Abundance of host-seeking mosquitoes collected during 1,972 trap-nights in Coachella Valley, 1994-95.<sup>1</sup>

Species	Total	Mean	SE	CV
<i>Anopheles franciscanus</i>	1,032	0.5	6.0	
<i>Anopheles freeborni</i>	1	0.0		12.0
<i>Aedes dorsalis</i>	1,879	1.0	6.7	6.7
<i>Aedes taeniorhynchus</i>	23	0.0		
<i>Aedes vexans</i>	24,937	12.6	190.4	15.1
<i>Psorophora columbiae</i>	115,059	58.3	598.1	10.3
<i>Culiseta inornata</i>	5,929	3.0	17.0	5.7
<i>Culex erythrorhax</i>	52,797	26.8	141.7	5.3
<i>Culex quinquefasciatus</i>	23,201	11.8	50.8	4.3
<i>Culex stigmatosoma</i>	1	0.0		
<i>Culex tarsalis</i>	677,783	343.7	700.5	2.0
<i>Culex thriambus</i>	1	0.0		
Total	902,643	457.7		

<sup>1</sup> SE, standard error; CV, coefficient of variation, = SE/mean.

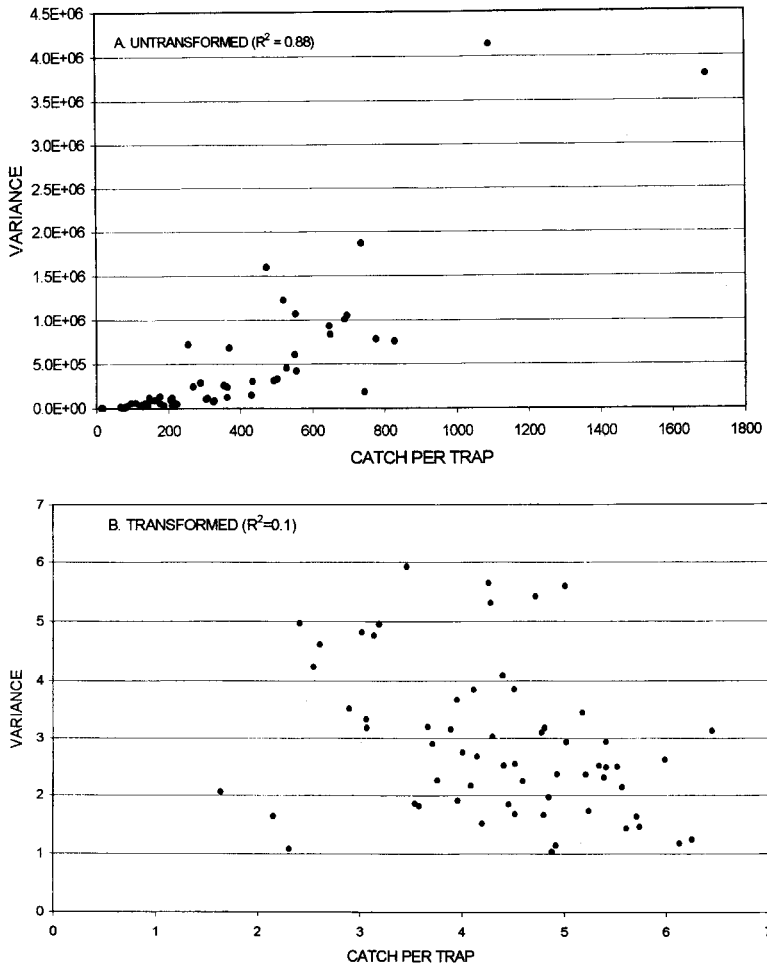


Fig. 4. Variance plotted as function of the mean for each of the 63 trap sites in Coachella Valley ( $n = 30-32$ ). Data were (A) untransformed or (B) transformed by  $\ln(y + 1)$  number of female *Culex tarsalis*.

(Fig. 5B), that is, skewness =  $-0.27$  and kurtosis =  $2.32$ . The  $\sqrt{(y + \frac{1}{2})}$  transformation also has been recommended for count data and was applied for comparison (data not shown). Although the overall variance was reduced ( $s^2 = 25,664$ ,  $CV = 0.93$ ), the variance for each trap remained correlated to the mean ( $R^2 = 0.52$ ), and the distribution, although relatively well centralized (skewness =  $1.83$ ), was not entirely normalized (kurtosis =  $7.01$ ). These statistics indicated that catch data were best transformed by  $\ln(y + 1)$  and that the transformed data could be analyzed by least-squares procedures. Therefore, the geometric or back transformed mean provided an acceptable measure of the central tendency for the data (overall mean =  $76.7$ , upper and lower 95% CL =  $83.9$  and  $70.2$  females per trap per night), even though this value was considerably lower than that for the untransformed ( $343.7$ ,  $312.8-374.6$ ) or backtransformed  $\sqrt{(y + \frac{1}{2})}$  ( $183.4$ ,  $168.7-199.0$ ) data.

### Dynamics in time and space

When analyzed by ANOVA, the transformed catch of *Cx. tarsalis* females varied significantly among trap sites ( $F = 20.9$ ,  $df = 62$ ,  $1,899$ ,  $P < 0.001$ ), months ( $F = 123.8$ ,  $df = 9$ ,  $1,899$ ,  $P < 0.001$ ), and years ( $F = 30.6$ ,  $df = 1$ ,  $1,899$ ,  $P < 0.001$ ). Overall, abundance was greatest at trap sites 54 ( $709.5$  females per trap night), 7 ( $522.2$ ), and 48 ( $456.1$ ) at wetland habitats near the Salton Sea (Figs. 1-3). Variability among trap sites was significant (Fig. 6), but contributed only 11.9% to the total sum of the squares in the ANOVA. In contrast, variability among months explained 70.1%, with catch size peaking in spring with the rise of the Salton Sea, declining in summer as the marshes dried, and then increasing slightly during fall when managed duck marshes were flooded for migratory waterfowl (Fig. 7). In Coachella Valley, females emerging in October and November enter a repro-

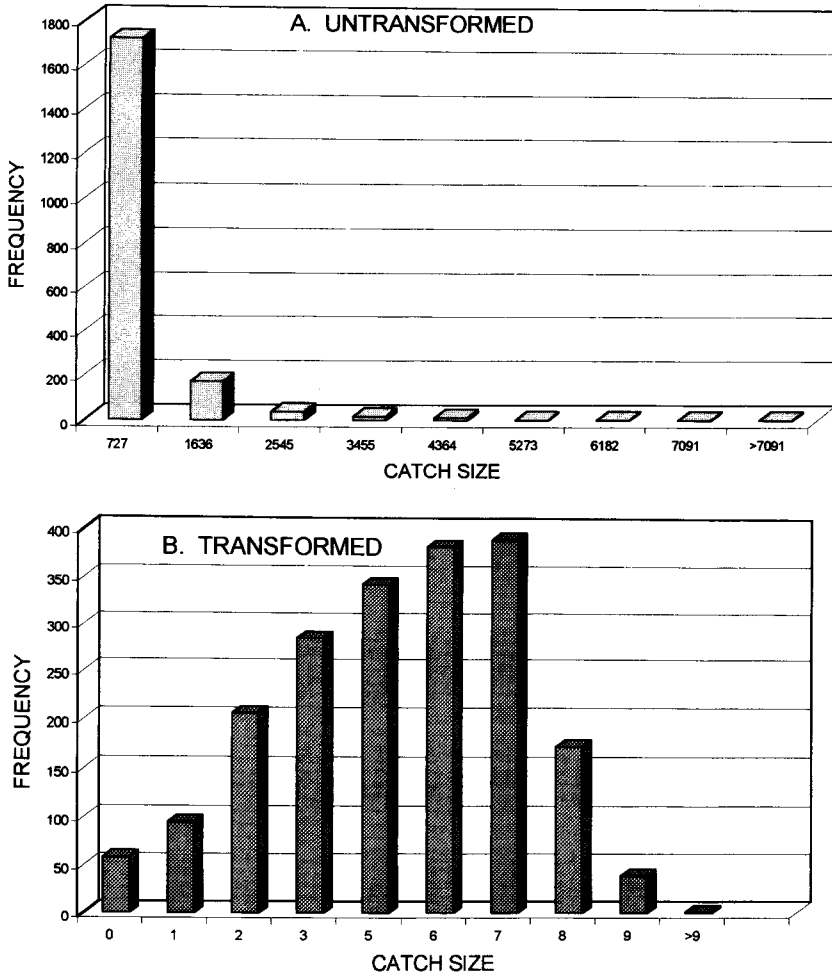


Fig. 5. Frequency distribution of trap nights ( $n = 1,972$ ) plotted as a function of female *Culex tarsalis* catch size grouped into 9 size classes for (A) untransformed and (B) transformed by  $\ln(y + 1)$  data.

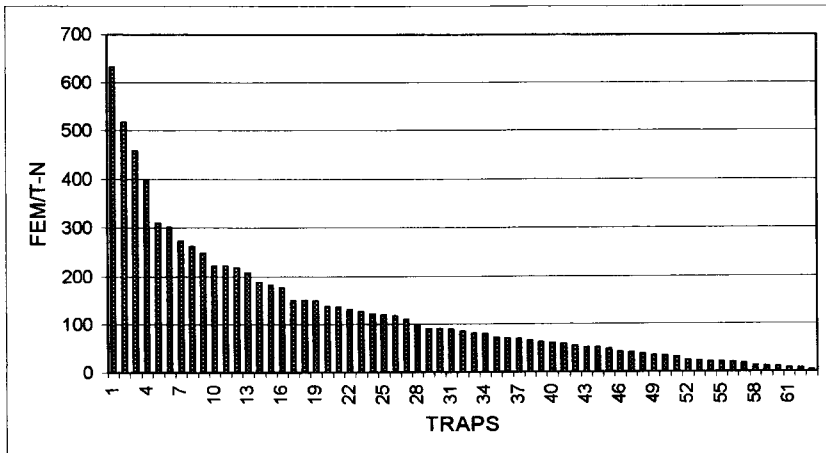


Fig. 6. Geometric mean number of female *Culex tarsalis* per trap night for each of the 63 traps. Note: x axis numbers are ranks in abundance and not trap site designation.

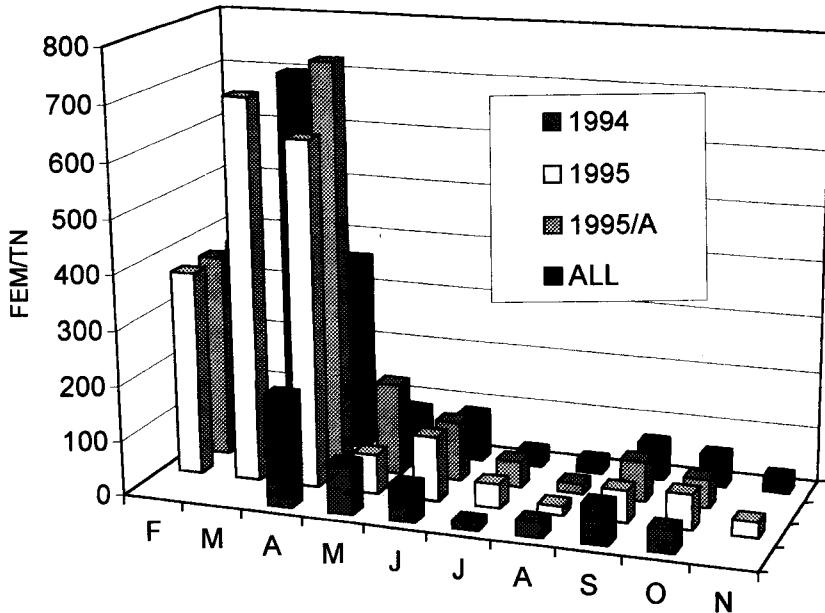


Fig. 7. Geometric mean number of female *Culex tarsalis* per trap night plotted by month for 1994, 1995, alternate (4-wk intervals) occasions during 1995 (1995/A), and both years combined.

ductive diapause that is terminated in late December (Reisen et al. 1995b), and therefore sampling was suspended during December 1994 and January 1995.

Data were grouped by seasons (Table 2), and depicted spatially using our GIS to show seasonal changes in the distribution of host-seeking females (Figs. 1–3). Even though the interaction terms in the 3-way ANOVA accounted for <1% of the total sum of the squares, the distribution of *Cx. tarsalis* catch among trap sites varied among seasons as production shifted from wetlands along the shore of the Salton Sea (Fig. 1) to scattered agriculture sites (Fig. 2) to managed marshes mostly along the Whitewater channel (Fig. 3). Comparable production from widely distributed wetland sources resulted in a lower CV during spring than during summer and fall, when larval habitats were restricted spatially. Although traps with the greatest catch generally were distributed near productive larval sites, field inspection revealed that greatest catch

frequently was clustered at traps adjacent to upland orchards or tamarisk. This was especially noticeable during spring on the west side of the study area, when most larval habitats were associated with new surface pools formed by the rise in the Salton Sea, but catch was greatest in traps located approximately 1 km inland (Fig. 1).

**Conditional simulations**

Five sampling strategies were evaluated for their accuracy and precision in estimating the population mean of all traps (Table 3) and for their ability to resolve the pattern of temporal abundance (Fig. 8).

*Random:* Eight trap sites (representing ca. 1/8th or 13% of the total) were selected randomly during each of 3 trials (Table 3). The trial 1 mean fell below the lower limit of the 95% CL; however, the 95% CL overlapped the population CL, indicating that these means were not significantly different ( $P > 0.05$ ). Reducing sample size from 63 to 8 traps

Table 2. Seasonal changes in abundance and dispersion of female *Culex tarsalis*<sup>1</sup>.

Data	Spring	Summer	Fall
No. trap nights	674	750	548
Mean	5.46	3.57	4.07
Geometric mean	234.1	24.5	57.6
95% CL	5.32–5.59	3.44–3.70	3.91–4.23
CV	0.32	0.51	0.46
Highest 5 traps <sup>2</sup>	54, 2, 57, 58, 10	54, 45, 7, 44, 60	38, 48, 33, 45, 34

<sup>1</sup> Abundance transformed by  $\ln(y + 1)$ ; geometric mean back transformed as  $e^y - 1$ ; CL, confidence limit; CV, coefficient of variation, mean/SE.

<sup>2</sup> Traps with significantly greatest counts during time period when tested by Newman–Keuls multiple range test ( $P > 0.05$ ).

Table 3. Comparisons among condition simulations of 5 sampling strategies to estimate the mean abundance per trap night.<sup>1</sup>

Sampling strategy	Traps	N	Transformed ln (y + 1)			Back transformed			(U - L)
			Mean	L95%CL	U95%CL	Mean	L95%CL	U95%CL	
All traps	63	1,972	4.353	4.264	4.441	76.7	70.1	83.9	13.8
Random 10%									
Trial 1	8	252	4.192	3.941	4.444	65.2	50.5	84.1	33.6
Trial 2	8	254	4.269	4.036	4.502	70.5	55.6	89.2	33.6
Trial 3	8	224	4.408	4.137	4.679	81.1	61.6	106.7	45.0
Uniform									
50% traps	34	1,059	4.590	4.474	4.706	97.5	86.7	109.6	22.9
25% traps	17	520	4.403	4.233	4.573	80.7	67.9	95.8	27.9
13% traps	9	288	4.603	4.371	4.836	98.8	78.1	125.0	46.8
8% traps	5	160	3.945	3.613	4.277	50.7	36.1	71.0	34.9
5% traps	3	99	4.451	4.040	4.861	84.7	55.8	128.2	72.3
Transect									
Highway 111	6	193	4.073	3.764	4.382	57.7	42.1	79.0	36.9
Highway 86	5	159	3.915	3.628	4.203	49.1	36.6	65.9	29.2
Best estimate	9	290	5.014	4.779	5.248	149.5	118.0	189.2	71.2
Stratified random									
Trial 1	11	341	4.419	4.205	4.632	82.0	66.0	101.7	35.7
Trial 2	11	349	4.012	3.795	4.229	54.3	43.5	67.6	24.2
Trial 3	11	351	4.221	4.008	4.434	67.1	54.0	83.3	29.2

<sup>1</sup> Traps, number of trap sites; N, number of trap nights; L95%CL and U95%CL are the lower and upper 95% confidence limits.

markedly reduced precision and the breadth of the confidence intervals ranged from 2.4 to 3.3 times greater than the population value.

*Uniform:* Progressively decreasing the number of traps included in the uniform sampling scheme

increased the distance between adjacent traps. As expected, precision decreased as a function of the number of traps included and therefore sample size (Table 3). Only 1 of the 5 estimated means (25%) fell within the 95% confidence interval of the pop-

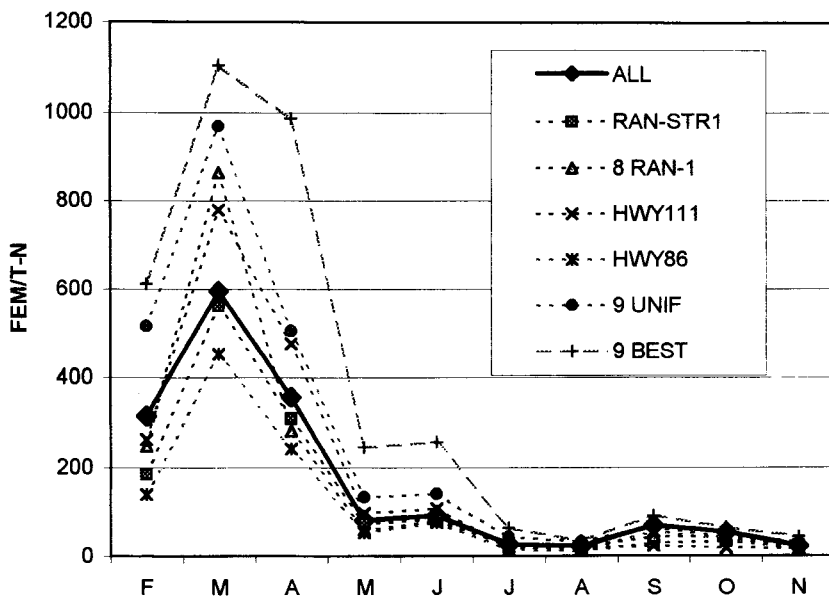


Fig. 8. Geometric mean number of female *Culex tarsalis* per trap night plotted by month for all traps (All), stratified random sample 1 (RAN-STR1), 8 random traps selected in trial 1 (8 RAN-1), transect along Highway 111 (HWY111), transect along Highway 86 (HWY86), 9 uniform traps (9 UNIF), and 9 traps having the greatest catch (9 BEST).



ulation mean. Two means (50 and 8%) were significantly ( $P < 0.05$ ) greater and less than the population mean, respectively; that is, the CLs did not overlap.

**Transect:** To simulate transect sampling, 6 and 5 traps located along state highways 111 and 86, respectively, were selected for analysis (Fig. 2). Traps along Highway 111 produced a mean that did not differ significantly ( $P > 0.05$ ) from the population mean, but traps along Highway 86 collected significantly fewer female *Cx. tarsalis* than the overall mean (Table 3). Highway 111 transected agricultural habitat near the town of Mecca, but then paralleled the shore of the Salton Sea to the east. In contrast, Highway 86 remained upland from the western shore and transected habitats with lower trap counts than average.

**Best estimate:** Catch at the 9 traps with the greatest overall means produced a simulation mean that was significantly ( $P < 0.05$ ) greater than population mean and had the widest CLs among sampling designs with comparable numbers of traps (Table 3). Increased variability here reflected the magnitude of change in temporal abundance (Fig. 8).

**Stratified random:** If the general pattern of abundance is known, a stratified random sample should provide the best estimate of abundance. In the current simulation, we randomly selected sets of traps positioned within 1-mi. strata extending inland from the shore of the Salton Sea, because previous research had shown that catch size decreased as a function of trap distance from these wetlands (Reisen et al. 1995c). Because most traps were positioned within 2 mi. of the shore (Figs. 1–3), the number of traps within strata was not equal; that is, 3 were along the shore, 3 were 1 mi. inland, 2 each were at 2 and 3 mi., and 1 was at 4 mi. Trials 2 and 3 produced means below the lower 95% CL of the population mean, and the CLs of trial 2 did not overlap that of the population CL (Table 3).

Estimates of monthly female abundance for representative sampling strategies were plotted in Fig. 8 to depict how well they delineated seasonal trends in abundance. All curves basically were similar in shape with vernal maxima, and monthly means from all designs were correlated significantly with the means from all traps ( $r > 0.95$ ,  $df = 8$ ,  $P < 0.001$ ). However, the amplitude of the March peak varied from a geometric mean of 450 females per trap night for traps along Highway 86 to 1,103 females per trap night for the 9 traps with the greatest abundance. The best estimate traps also produced a curve that remained high during April after the other estimates had declined markedly.

Although not designed to investigate the frequency of trap operation needed to generate seasonal profiles, our data showed the importance of historical databases in evaluating the amplitude of season peaks. Geometric monthly means from all traps were compared to monthly means from traps operated in 1994 alone, in 1995 alone, and at al-

ternate or 4-wk intervals in 1995 (Fig. 7). Again the greatest differences were seen in the amplitude of the vernal maxima, with abundance in 1995 markedly greater than in 1994.

## DISCUSSION

The function  $\ln(y + 1)$  was most suited to transform catch data before least squares analysis, because it adequately normalized the distribution and controlled the variance (i.e., making it independent of the mean). Back-transformed or geometric means consistently provided more conservative estimates than arithmetic or back-transformed  $\sqrt{(y + 1)}$  means; however, geometric means were centralized and less influenced by very high or low counts.

The sampling effort (number of traps) required to provide an appropriate data set increased with the complexity of the information required. The bimodal seasonal abundance pattern was depicted adequately by all sampling designs (even those with as few as 3 traps), and monthly means from all simulations were correlated temporally with the population trend delineated by all traps. However, the magnitude and duration of the vernal peak and the estimate of abundance were influenced significantly by both the number of traps and their pattern of deployment. Positioning traps at locations known to produce high counts (best estimate samples) significantly overestimated the rate of population increase and the level of abundance. In Coachella Valley, these traps were positioned within or adjacent to wetland habitats and were  $>7$  km distance from the nearest concentration of humans at the town of Mecca. Therefore, although best-estimate sampling may have reflected vector abundance at arbovirus foci along the Salton Sea, the intensity of human–vector contact would be overestimated. This information could be critical if the rate of vector population growth and mean abundance levels were used to determine the intensity of mosquito control or the issuance of a medical alert.

Random or transect deployment of traps seemed to be a “hit or miss” procedure. If the traps fortuitously sampled a representative cross section of favorable and unfavorable habitats, then the data accurately measured overall abundance. However, if no or inadequate preliminary sampling was done and the true population value was unknown, then the accuracy of these samples would be unknown and may over- or underestimate true abundance, even though adequately delineating population phenology. Information on population distribution in relation to habitat features can be exploited by stratified random designs. Previous studies in Coachella Valley (Reisen et al. 1995c) determined that trap catch decreased as a function of distance from the Salton Sea, and this information was used to stratify the location of trap sites, producing the most accurate estimates of abundance.

In addition to the rate of change and shape of

the seasonal abundance curve, mosquito control agencies are interested in changes in the spatial pattern of abundance to detect new or overlooked larval habitats or detect control failures. A uniform distribution of traps providing complete geographic coverage would seem necessary to meet this objective. The number of traps required would depend heavily upon the heterogeneity of the environment and the vagility of the target mosquito. *Culex tarsalis* is highly dispersive in Coachella Valley, with mean cohort dispersal from breeding sites averaging approximately 1 km per day (Reisen and Lothrop 1995). Therefore, we have settled on a 2-mi. interval between adjacent traps (i.e., 50% uniform sampling of the current grid) as being optimum to monitor the abundance of *Cx. tarsalis* for our GIS (Lothrop and Reisen 1998). Host-seeking females readily traverse these distances (Reisen and Lothrop 1995), and traps seem to detect localized increases in abundance within the surrounding sections (Lothrop and Reisen 1999).

The current research emphasized the spatial aspects of mosquito sampling. Additional studies will determine the frequency of sampling required to delineate seasonal curves. Research also is needed to decide which microhabitats are best for trap placement to provide the most sensitive indication of population abundance.

#### ACKNOWLEDGMENTS

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