RELATIVE ABUNDANCE, REMOVAL SAMPLING, AND MARK-RELEASE-RECAPTURE ESTIMATES OF POPULATION SIZE OF *ANOPHELES CULICIFACIES* AND *AN. STEPHENSI* AT DIURNAL RESTING SITES IN RURAL PUNJAB PROVINCE, PAKISTAN

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ABSTRACT. Interrelationships among removal sampling and mark-release-recapture estimates of absolute population size were studied at rural villages in Punjab Province, Pakistan during the spring and premonsoon seasons of 1980, and a model generated relating hand-catch relative abundance to absolute population size. Removal sampling was found to seriously underestimate known numbers of marked adults released in 3 cattle shed resting sites while the mark-release-recapture method using the simple Lincoln index did not differ significantly. A regression formula was generated to correct removal estimates to the actual numbers present and was used to estimate the size of 32 populations in rural Punjab Province. Statistically acceptable estimates were related to hand-catch relative abundance (numbers of mosquitoes mouth-aspirated by 2 collectors in 15 min) by linear regression and provided results comparable to capture-mark-release-recapture estimates at a series of 7 compounds and accurately estimated the numbers of marked adults released in 3 cattle sheds. The relative abundance method was considered suitable to estimate mosquito population size in rural Punjabi villages, from which man-vector contact rates could be calculated.

INTRODUCTION

Accurate and precise estimates of the frequency of man-anopheline contact are critical in epidemiological studies attempting to measure malaria transmission rates and constitute a major component of the models proposed by Macdonald (1953, 1955). Human bait collections provide a direct estimation procedure which seems satisfactory when baits are representative of the entire sleeping population and the vector is largely anthropophagic (see review by Service 1977). However, these direct methods are difficult to apply in rural Pakistan where the vector species, *An. culicifacies* Giles and *An. stephensi* Liston, are largely zoophagic (Reisen and Boreham 1979), feed throughout the night during the transmission seasons (Reisen and Aslamkhan 1978) and where social customs prevent the utilization of certain sex and/or age groups as bait. Attempts to employ indirect methods such as bed nets have also proven unsuccessful in rural areas where villagers sleep near their livestock, because many of the blood-fed females entering the nets had previously fed on bovids (Akiyama 1973).

Recently, we have applied an indirect estimation procedure combining capture-mark-release-recapture methods and precipitin analysis of blood meal hosts, which permitted the calculation of vectorial capacity in one small rural village. However, this procedure was laborious and would be difficult to apply to rural or peri-urban communities large enough to study malaria transmission rates.

Alternatively, Yasuno et al. (1977) have applied a removal sampling method to estimate the numbers of *Cx. quinquefasciatus* Say resting in houses in a large town in India. They proposed to estimate total population size in the town by sampling a representative number of houses, es-

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timating the size of the resting population in each house by removal sampling and then extrapolating their findings to the total number of houses in the town. Considering the difficulty of conducting mark-recapture studies in urban or periurban situations (Lindquist et al. 1967), the removal sampling approach seemed reasonable. The proportion of the females biting on any given night could be estimated from the duration of the gonotrophic cycle and the proportion feeding on man could be estimated from a representative subsample of the freshly-fed females, while the size of the human population and number of houses would be available from census data.

The objectives of the present study were to 1) verify the applicability of the removal sampling method to conditions in rural Punjab Province, 2) relate the results to concurrent mark-release-recapture estimates and 3) develop a model to relate relative abundance estimates to population size.

METHODS AND MATERIALS

DESCRIPTION OF STUDY SITES. All collections were made at cattle sheds in or near the villages of Khano-Harni, Lahore District and Sattoki and Kot Baghicha Singh Walla, Kasur District, Punjab Province, Pakistan during the spring and premonsoon seasons of 1980. These study sites have been routinely sampled for the past 4 to 5 years to monitor temporal changes in anopheline relative abundance as measured by the hand-catch method (the number of specimens mouth-aspirated by a collection team for a specific time period).

POPULATION SIZE ESTIMATION METHODS. As listed by Zippin (1958), the assumptions underlying the application of the removal method of population estimation include: 1) The population must be stationary (or closed), 2) the probability of capture is the same for each individual exposed to capture, and 3) the probability of capture remains constant throughout all catching occasions. In general the collection of adult mosquitoes from diurnal indoor resting sites for timed intervals seemed to fulfill these assumptions. Since the activity of the endophilic anophelines is restricted to night, the resting population sampled would not be subject to additions and deletions, although the degree of exposure to capture (or sampling proportion) may vary (unpublished data). At present we have no data to indicate that sampling probabilities vary between individuals within the same population at the same point in time. The homogeneity of the probability of collection (or the removal rate) over time (or successive sampling occasions) can be tested statistically and if unsatisfactorily variable, the data series may be discarded.

In the present study 2 collectors sampled adult anophelines resting inside cattle sheds for 5 min intervals. Collections were continued until the numbers collected in each sample markedly decreased relative to the number initially sampled. The number of sampling intervals was thus related to 1) the size of the shed, 2) the dispersion pattern of the resting mosquitoes, and 3) the ability of the collectors to locate the resting mosquitoes. The numbers of adults of each species and sex were then estimated by a least squares fit of the model, $Y_s = p\,N - p(\Sigma y_{s-1})$ where y_s = the number of individuals caught by 2 collectors during sampling interval s, p = the probability of capture or the removal rate, and the term (Σy_{s-1}) = the number of Y_s removed prior to the current sampling occasion. The regression coefficient, P, was tested for departure from 0 by analysis of variance (ANOVA) and the goodness of fit of the model to the data expressed by the coefficient of determination, r^2 (Sokal and Rohlf 1969). When the f value for p was significant ($P < 0.05$) and r^2 approached 1, the probability of capture was assumed to be constant over time.

As listed by Southwood (1966) assumptions underlying the use of mark-release-recapture procedures such as the Lincoln Index which are applicable to mosquitoes include: 1) the marked adults are not affected by being marked and the marks are not lost, 2) the marked mosquitoes become mixed in the population, 3) the probability of capture is independent of mark status, and 4) population is closed or additions and deletions can be measured. Previous studies have indicated that marking with fluorescent dusts does not alter adult longevity (e.g., Reisen and Aslamkhan 1979) or mating behavior (e.g. Baker et al. 1979) and that marked individuals are readily distinguishable for life. When tested in a village situation, marked *An. culicifacies* readily dispersed among collection sites and were considered well mixed with the unmarked population (Reisen et al. 1980a). To date we have no data to indicate that marking or releasing endophilic anophelines alters their probability of capture.

When recaptures were attempted within hours of release, the population was considered closed and the simple Lincoln Index applicable, where N3 = MC/r where N = population size, M = the number released, C = the number collected and r = the number recaptured. When recaptures were attempted over a series of days, Bailey's (1952) modification was applied to account for deletions, where N = (C+1)R/(r+1) where R, the numbers of marked specimens remaining to be sampled, = $(M + R)s_h$ and s_h = constant daily survivorship estimated horizontally by regressing the decline in the numbers of marked specimens recaptured on time after release.

ACCURACY OF METHODS. To assess the accuracy of the removal and mark-recapture estimation methods, known numbers of laboratory-reared *An. culicifacies* and *An. stephensi* females and males were released inside sheds at Khano-Harni on 3 occasions during the premonsoon season of 1980. Release specimens were divided into 2 groups, each marked with a different colored fluorescent dust using procedures described elsewhere (Reisen et al. 1980a). On all occasions released adults were of mixed ages (1 to 6 days) and had been continuously offered 3% sucrose since emergence. For release 3 groups of different ages were offered blood meals on different dates yielding a mixture of unfed, half-gravid and gravid females. All groups were pooled into a single cage and the proportions of each trophic class estimated by examining the abdomens of a subsample of females of each dust color.

Specimens of both colors were concurrently released in late morning and allowed 2 to 3 hr to distribute themselves throughout the shed. Mosquitoes were then collected regardless of mark status following the removal sampling protocol described above. Specimens, kept separate by time interval, were returned to the laboratory, anaesthetized and examined for fluorescent dust using an ultra-violet lamp. For release number 3, the trophic status of the recaptures was also recorded. Since 2 dust colors were used, it was possible to estimate the numbers of group 1 from the ratios of released to recaptured mosquitoes of group 2 using a slight modification of the simple Lincoln Index.

APPLICABILITY OF REMOVAL SAMPLING. To demonstrate the applicability of removal sampling to mosquitoes and conditions in rural Punjab, population size was estimated at a series of cattle sheds following the sampling protocol described above. The numbers collected during the first 15 minutes were compared to estimated population size and a model relating relative abundance and absolute size formulated using least squares analysis.

COMPARISON OF RELATIVE ABUNDANCE-POPULATION SIZE MODEL TO MARK-RECAPTURE ESTIMATES. During a series of capture-mark-release-recapture studies to estimate dispersal, losses and additions of *An. culicifacies* populations at the Kot Baghicha Singh Walla area during November 1979, population size was estimated at 7 cattleshed-feedlot compounds over a 10-day period using Bailey's modification of the Lincoln Index (Reisen et al. 1980b). The mean number of mosquitoes collected per 0.5

man hr (M.H.) was then calculated from the total number collected and population size estimated for each compound (or micropopulation), thereby allowing the statistical comparison of the relative-abundance model to mark-recapture estimates.

RESULTS AND DISCUSSION

ACCURACY OF REMOVAL AND MARK-RECAPTURE METHODS. A total of 1784 ♀♀ – 1532 ♂♂ *An. culicifacies* and 1278 ♀♀ – 1273 ♂♂ *An. stephensi* were marked and released inside sheds at Khano-Harni on 30 April and 3 May, and 1 June, respectively (Table 1). Removal estimates of population size (N2) were consistently low and were significantly less than the mean number released (M) when compared by a paired t-test ($t_{(11)} = 16.49$, $P < 0.001$). However, the size of the estimated population (N2) could be used satisfactorily to predict the actual numbers present (M) using a model II regression analysis (Fig. 1). The regression coefficient significantly differed from 0 when tested by ANOVA and $r^2 = .874$ indicating a good fit of the linear model to the data. Although the total number removed (r in Table 1) was consistently greater than 70% of the estimated population size ($N2_j$), rarely were more than 40% of the number released actually recaptured. During the premonsoon season, mosquitoes typically seek suitable mi-

Table 1. Population estimates at cattle sheds at Khano-Harni during 1980 using 3 calculation procedures.

Date	*Anopheles* species	Sex	M[1]	Relative abundance[2] HC	Relative abundance[2] $\hat{N}1$	Removal method[3] w	Removal method[3] P	Removal method[3] r^2	Removal method[3] $\hat{N}2$	Mark-recapture[4] r(%)	Mark-recapture[4] $\hat{N}3$
30 Apr	*culicifacies*	♀♀	481	134	669.6	8	−.425**	.962	165.5	165(34.3)	528.0
		♂♂	329	137	678.8	8	−.503**	.971	160.3	157(47.7)	421.4
		♀♀	490	130	657.2	8	−.531**	.992	151.6	153(31.2)	446.0
		♂♂	489	137	678.8	8	−.364**	.968	177.9	172(35.2)	342.7
3May	*culicifacies*	♀♀	355	21	319.6	10	−.117*	.439	85.3	59(16.6)	229.3
		♂♂	304	26	335.1	10	−.205**	.680	61.4	55(18.1)	272.0
		♀♀	458	33	356.8	10	−.094*	.569	190.8	114(24.9)	685.9
		♂♂	410	23	325.8	10	−.133^{NS}	.329	101.2	74(18.0)	409.0
1 June	*stephensi*	♀♀	597	133	666.5	9	−.189**	.787	283.1	241(40.4)	661.4
		♂♂	599	100	564.3	9	−.177*	.599	248.9	201(33.6)	762.9
		♀♀	678	141	691.2	9	−.210**	.716	307.9	267(39.4)	612.0
		♂♂	674	148	712.9	9	−.217**	.715	293.1	256(38.0)	529.2
		$\bar{\bar{X}}$	479.6	96.9	554.7				185.6	159.5(33.3)	491.7
	± 95% C.I.		83.6	34.2	105.9				52.4	46.9	106.7

[1] M = no. released in each group.

[2] HC = hand catch, total number of each M collected in 0.5 M.H. of aspirator collection effort. $\hat{N}1$ = estimate of population size from relationship between HC and adjusted population size estimated at 3 villages (Fig. 2).

[3] w = number of 5 min. samples taken by 2 collectors, p = sampling probability from the expression $y_s = p\,N2_j - p\,(\sum_{s=1}^{w} y_{s-1})$ with y_s = number collected in each 5 min. sampling period. Fit by least squares and tested for departure from 0 by analysis of variance, **$p < 0.01$, *$.01 < p < .05$, NS $p > .05$ (Sokal and Rohlf, 1969), r^2 = coefficient of determination. $\hat{N}2_j$ = estimated population size at compound j.

[4] $r = \sum_{s=1}^{w} y_s$ (percentage recaptured = r/Mx 100), $\hat{N}3(2)$ = size of group 2 estimated by the modified Lincoln Index = M1 (r2)/r1 where M1 and r1 are the numbers in group 1 released and recaptured respectively, and r2 is the number of group 2 recaptured.

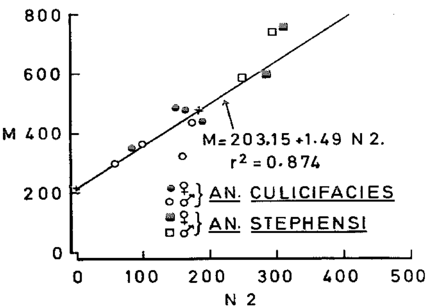

Fig. 1. The numbers of marked *An. culicifacies* and *An. stephensi* females and males released inside cattle sheds at Khano-Harni (M) regressed as a linear function of the removal sampling estimate of absolute population size (N2). r^2 = coefficient of determination.

crohabitats within indoor resting sites, lowering sampling proportions at this time (unpublished data). The number of unfed—half-gravid—gravid *An. stephensi* females of both dust colors recaptured on 1 June were 169–277–62, not significantly different from the expected proportions based on the numbers of each trophic class present in a subsample of the females examined before release, 0.303–0.547–0.149 ($\chi^2_{(2)} = 3.964$, $P > 0.05$). Similarly the overall numbers of females-males recaptured (508–457) did not significantly differ from the proportions of females-males released (0.5–0.5) ($\chi^2_{(1)} = 2.611$, $P > 0.05$). Thus, although a certain proportion of the released mosquitoes was not exposed to capture, the probabilities of recapture were similar regardless of trophic status or sex. Throughout, about ⅔rds of the released population was not exposed to capture, although the numbers collected during each 5 min typically declined sequentially as expected (p in Table 1). These data were presumed representative of unmarked populations.

During each release, the numbers of individuals of each sex in group 1 were estimated from the numbers of the same sex in group 2, and vice versa, using the simple Lincoln Index (N3 in Table 1). The overall mean number estimated by the mark-release-recapture method did not differ significantly from the overall mean number actually released when tested by a paired t-test ($t_{(11)} = 0.26$, $P > 0.05$). Thus, the Lincoln Index provided an accurate method of estimating the number of mosquitoes resting in a cattle shed when the populations were closed (or stationary) and sampled within hours of their release.

APPLICATION OF REMOVAL SAMPLING. On 16 occasions in 1980 the size of indoor resting populations of female and male *An. culicifacies* and *An. stephensi* were estimated by the removal method at 3 villages in rural Punjab (Table 2). Failures to successfully apply removal sampling occurred in 16 of 32 attempts when A) the removal rate between samples was not constant and B) insufficient numbers of specimens were removed during sampling. Variability in the removal rate was expressed as a non-significant probability of capture estimate (p) and a low coefficient of determination, and occurred due to the clumped dispersion pattern of the resting adults inside the cattle shed and the inability of the collectors to locate these mosquito clusters at a constant rate. In newer, well-constructed sheds (such as KH-A) mosquitoes tended to be more readily located than in older, partially broken sheds such as SAT-A. Inadequately low removal rates occurred when mosquito population size was low and few specimens were collected in the initial samples and/or too few samples were taken to sufficiently depress the capture rate (numbers taken per 5 min.). Although collections were all made by 2 man teams selected from a group of 5 collectors with 2 to 5 years experience, the application of the removal method at a selected site was not always consistently successful on different sampling occasions (e.g., nos. 12 and 15, Table 2).

Corrected population size was calculated for 16 occasions when the removal rate significantly differed from 0 (Table

Table 2. Estimated size of *An. stephensi* and *An. culicifacies* populations in cattle sheds at 3 villages in rural Punjab using removal sampling estimation procedures.[1]

No.	Date (1980)	Village-Shed	Sex	HC	w	P	r^2	$\hat{N}_2$	X%	$\hat{N}\hat{2}$
			An. stephensi							
1	18 Mar	KH − A	♀♀	355	6	−.232**	.990	665.6	79.0	1194.6
			♂♂	182	6	−.175**	.969	393.8	70.1	789.6
2[a]		KH − B	♀♀	117	6	$-.201^{NS}$	.361	212.2	78.2	NE
			♂♂	124	6	$-.239^{NS}$	.588	208.7	81.9	NE
3		KH − D	♀♀	382	6	−.322**	.972	587.5	88.3	1078.3
			♂♂	146	6	−.423**	.952	222.4	95.3	534.4
4[b]		KH − E	♀♀	166	6	$-.104^{NS}$	.210	645.0	47.8	NE
			♂♂	92	6	$-.034^{NS}$	.221	939.0	19.6	NE
5[b]	23 Mar	SAT − A	♀♀	85	10	$-.055^{NS}$	.339	460.1	44.6	NE
			♂♂	28	10	$+.007^{NS}$	.005	−1447.4	NE	NE
6	25 Mar	KH − A	♀♀	202	10	−.158**	.841	492.0	82.1	936.0
			♂♂	36	10	−.102*	.482	128.7	66.1	394.9
7		KH − D	♀♀	378	14	−.121**	.811	1125.7	83.2	1880.0
			♂♂	43	14	−.119**	.667	125.2	83.1	389.6
8[a]	30 Mar	SAT − A	♀♀	38	8	−.175**	.897	105.8	75.6	360.7
			♂♂	18	8	$-.102^{NS}$	.194	68.5	55.5	NE
9[a]	1 Apr	KH − A	♀♀	74	10	−.120*	.549	228.2	73.4	543.1
			♂♂	33	10	$-.126^{NS}$	.411	89.9	75.7	NE
10		KH − D	♀♀	237	13	−.168**	.896	624.7	90.3	1133.7
			♂♂	41	13	−.151**	.571	131.9	86.4	399.6
			An. culicifacies							
11[a, b]	22 Mar	KB − B	♀♀	61	5	$-.345^{NS}$	.885	83.8	72.8	NE
			♂♂	17	5	$+.092^{NS}$	.779	−33.3	NE	NE
12[a, b]		KB − C	♀♀	49	6	$-.114^{NS}$	.384	146.1	54.1	NE
			♂♂	27	6	$-.100^{NS}$	.218	85.6	51.4	NE
13[b]	23 Mar	SAT − A	♀♀	38	10	$-.099^{NS}$	.277	141.0	64.5	NE
			♂♂	10	10	$+.085^{NS}$	.116	−18.3	NE	NE
14	30 Mar	SAT − A	♀♀	54	8	−.238**	.762	101.3	86.9	354.0
			♂♂	24	8	$-.283^{NS}$	.497	46.3	86.4	NE
15	31 Mar	KB − C	♀♀	90	8	−.149*	.575	283.6	60.3	625.6
			♂♂	70	8	−.157**	.846	176.3	74.9	465.8
16[a]	1 Apr	KB − D	♀♀	58	7	$-.089^{NS}$	.427	180.8	54.2	NE
			♂♂	69	7	−.230**	.727	143.0	83.9	416.2

[1] Column heading abbreviations: No. = estimate number; a = not acceptable due to variability in the removal rate; b = not acceptable due to low sampling percentage. village—shed: KH = Khano-Harni, Lahore District; SAT = Sattoki and KB = Kot Baghicha Singh Walla, Kasur District, Punjab Province. HC = hand-catch, number of mosquitoes collected by 2 men using mouth aspirators for 15 minutes. w = number of 5 min. samples taken; p = sampling probability in regression model $y_s = p\,N2 + p\,(\sum_{s=1}^{w} y_{s-1})$ where y_s = No. mosquitoes collected by 2 men in 5 min. and N2 = estimated population size when $y_s = 0$; p tested by analysis of variance, $^{**}p < 0.01$, $^{*}0.01 < p < .05$, NS $p > 0.05$ (Sokal and Rohlf, 1969). r^2 = coefficient of determination (Sokal and Rohlf, 1969). X%, Sampling proportion, $= \sum_{s=1}^{w} y_s / N2$. $\hat{N}\hat{2}$, corrected population size, $= 203.15 + 1.49$ N2.

2) using the relationship between the removal estimate of population size and actual numbers present (Fig. 1). Hand-catch relative abundance (numbers collected by 2 men in 15 min or 0.5 M.H.) was considered a good predictor of corrected population size over the range of values encountered (Fig. 2). The regression coefficient significantly differed from 0 when tested by ANOVA and r^2 approached 0.85. In agreement, mean population size (N1) estimated from the numbers of individuals collected during 0.5 M.H. at Khano-Harni was not significantly different from the mean numbers of specimens released, applying a paired t-test ($t_{(11)} = 1.956$, $P > 0.05$) (Table 1).

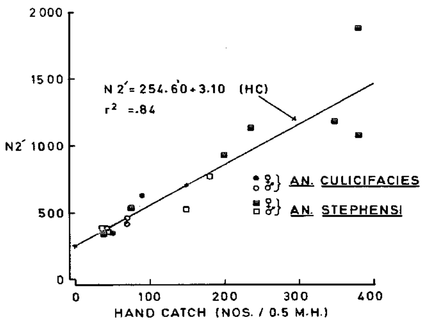

Fig. 2. Adjusted removal sampling estimates of absolute population size (N2′) regressed as a linear function of hand-catch relative abundance (HC = number of mosquitoes mouth-aspirated by 2 collectors in 15 minutes) for female and male *An. culicifacies* and *An. stephensi* from 3 collection sites on 16 occasions. (Only statistically acceptable estimates included, Table 2.)

COMPARISON BETWEEN MARK-RECAPTURE AND RELATIVE ABUNDANCE ESTIMATES. During November 1979, capture mark-release-recapture estimates of absolute population size were calculated for 7 cattle-shed micropopulations of *An. culicifacies* females and males in an agrarian setting near Kot Baghicha Singh Walla (Reisen et al. 1980b). Mosquitoes were sampled by 2 collectors using mouth aspirators for 15 min. per room from a total of 12 of the 16 possible rooms in the 7 compounds. The numbers of mosquitoes collected per 0.5 M.H. were calculated and used to estimate the numbers of females and males per room in each compound. The numbers per room were then multiplied by the number of rooms/compound and the population size at each compound calculated (Table 3). Mean relative abundance estimates of absolute female and male population size for the entire study area did not differ significantly ($P > 0.05$) from calculated capture-mark-release-recapture estimates when compared by 2-factor randomized complete block design ANOVA. The female population was significantly larger than the male population, and micropopulations significantly varied among compounds. Both estimates were positively correlated over micropopulations (r: females = .686, males = .794, $P < 0.05$), indicating that although somewhat variable both N1 and N2 provided related estimates of size which were of a comparable order of magnitude.

In conclusion, estimates of absolute size calculated from the relative abundance model agreed well with more laboriously determined mark-recapture estimates at Kot Baghicha (Table 3) and accurately estimated the numbers of mosquitoes released on 3 occasions at Khano-Harni (Table 1). Although further verification of these encouraging results are required for different sized populations measured at different times of the year, it appeared that relative abundance or hand-catch data could be used to measure man-vector contact in rural Punjabi villages. In practice, the mean number of mosquitoes per 0.5 M.H. can be determined from timed hand-catch collections conducted in a sample of all available resting sites in the village and this value entered into the regression model to estimate the numbers of mosquitoes resting within the average room. By multiplying by the total number of rooms in the entire village, the absolute size of the female anopheline population could be calculated for each species.

Table 3. Relative abundance ($\hat{N}1$) and capture-mark-release-recapture ($\hat{N}3$) estimates of absolute population size at 7 cattle sheds near Kot Baghicha Singh Walla in November 1979.

Shed No.	No. rooms sampled	HC[1]	Total No. rooms	$\hat{N}1$	$\hat{N}3$
1 + 1a	2	40.1–17.9	5	2017.5–1550.2	1799.2–1365.2
2 + 3a + 3b	3	35.3–18.7	4	1455.7–1249.8	2307.6–1051.9
4	1	48.8–21.3	1	405.7– 320.6	872.9– 644.6
5	1	28.0–21.3	1	341.3– 320.6	461.6– 587.8
6	3	83.3–68.0	5	2562.9–2326.0	2912.7–2908.5
7	1	89.6–64.0	1	532.1– 452.8	1303.2–1138.2
8	1	96.5–31.6	4	2213.8–1409.9	3863.1– 666.2
	12		16 = $\overline{X}$	1361.3–1090.0	1931.5–1194.6
		Group mean	=	1225.6	1563.1

[1] HC = hand catch, No. ♀♀–♂♂ / 2 collectors / 15 min.

The present data indicated that the uncorrected removal sampling estimates of population size were spuriously low during the late spring and premonsoon seasons when sampling proportions were typically low (unpublished data). Better results may be attained during the cooler and more humid seasons, when mosquitoes rest more in the open and sampling proportions are correspondingly higher. Further observations are therefore suggested before the removal sampling method is totally abandoned as a means of estimating anopheline population size.

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