METHODS FOR MEASURING ANOPHELINE DENSITIES IN EL SALVADOR[1]

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ABSTRACT. Various methods for measuring adult densities of *Anopheles albimanus* and *A. pseudopunctipennis* in a coastal malarious area of El Salvador are evaluated from data collected between January 1968 and June 1971. Two New Jersey light traps, operated for a total of 358 trap nights, yielded an average of 93.9 *A. albimanus* per trap night, 96.9 percent of which were females. Collections of *A. pseudopunctipennis* averaged only 0.05 specimens, all females, per trap night. Nighttime collections from two cattle corrals in the same area accounted for an average of 84.4 *A. albimanus* (99.9 percent females) and 1.1 *A. pseudopunctipennis* (100 percent females) in 107 collections. Early evening human bait captures yielded an average of 29.5 *A. albimanus* females and no specimens of *A. pseudopunctipennis* in 23 collections. Daytime collections from natural diurnal resting sites yielded an average of 20.2 *A. albimanus* and 5.8 *A. pseudopunctipennis,* represented by 18.9 percent and 43.7 percent females, respectively. Daytime counts from beneath four bridges in the area collectively averaged 7.2 *A. albimanus* (74.4 percent females) and 9.7 *A. pseudopunctipennis* (63.3 percent females). Early morning captures of anophelines resting on interior surfaces of dwellings yielded an average of 9.4 *A. albimanus* (65.1 percent females) and only a single *A. pseudopunctipennis* female in 72 captures. The relative usefulness of each method is discussed in light of the purpose of the collection.

INTRODUCTION

Pritchard and Pratt (1944) reported on the usefulness of the New Jersey light trap in measuring densities of *Anopheles albimanus* in Puerto Rico. These authors concluded from a series of comparative light trap and animal bait trap collections, that indeed, the former was generally superior in the collection of female *A. albimanus.* Until that time, it had generally been accepted, following the work of Le Prince and Orenstein (1916) in Panama, that *A. albimanus* was not attracted to light. Furthermore, until the present time, notwithstanding the report of Pritchard and Pratt (*ibid.*), the potential for using this device in measuring anophelism, at least in Central America, has not been realized. In part, this is due to the need in ensuing years to measure the dwelling-oriented activities of *A. albimanus* as required by the residual wall spraying regimen of malaria eradication programs in the region. Thus, anophelline density measurements have been restricted essentially to those methods that would measure segments of the vector population considered to be important in evaluating the effectiveness of residual spray deposits.

The most commonly used methods in Central America malaria programs involve the use of human bait in intradomiciliary and peridomiciliary collections; the counting of mosquitoes on interior surfaces of dwellings; and the capture of females from animal concentrations, e.g. cattle corrals, near human habitation. These methods are used to evaluate *Anopheles* propensity for entering, leaving, or resting in human dwellings; for feeding on humans in or near dwellings; and for measuring population levels in the presence of concentrations of domestic animals. While these methods have served definitive purposes, it is not surprising that the light trap and other non-domicile techniques for measuring anopheline densities, *per se,* have been neglected.

Because the research objectives of the Central America Malaria Research Station (CAMRS) require a wider variety of anopheline density measurement techniques than are commonly in use in Cen-

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tral America, it has been necessary to evaluate additional methodology.

Since early 1968, over 2,000 entomological collections have been made in connection with investigative activities of CAMRS. Many of these collections were made for purposes which required a knowledge of overall anopheline densities in a given area, e.g., in the evaluation of area-wide control techniques such as ULV adulticiding or area larviciding. Other collections were made to supply specimens for biological studies of vector populations, such as host preference, longevity, and parasite susceptibility studies. The accumulated data offer an opportunity for a comparative evaluation of methodology for measuring anophelism for specific purposes.

The purpose of this paper is to present data which hopefully will revive interest in the New Jersey light trap for sampling populations of *A. albimanus* and to present data which show the comparative usefulness of this and other techniques in measuring population levels or population segments of both *A. albimanus* and *A. pseudopunctipennis* in El Salvador.

MATERIALS AND METHODS

The data reported herein are from a Pacific coastal malarious area extending some 10 kilometers eastward from the Port City of La Libertad which includes anopheline habitat types and populations characteristic of much of El Salvador and other Central American coastal plain situations. This area (Figure 1) has been extensively studied for a 3-year period, and it serves as the basis for comparative data included in this paper. Where pertinent, information from other parts of El Salvador is presented. The map (Figure 1) shows the following collecting points: New Jersey light trap locations at San Diego and Ticuiziapa beaches; diurnal collection points under bridges over Jute, Aquisquillo, Huiza and San Antonio Rivers; cattle corral collecting points at El Coco and Melara; and natural daytime resting sites opposite Ticuiziapa

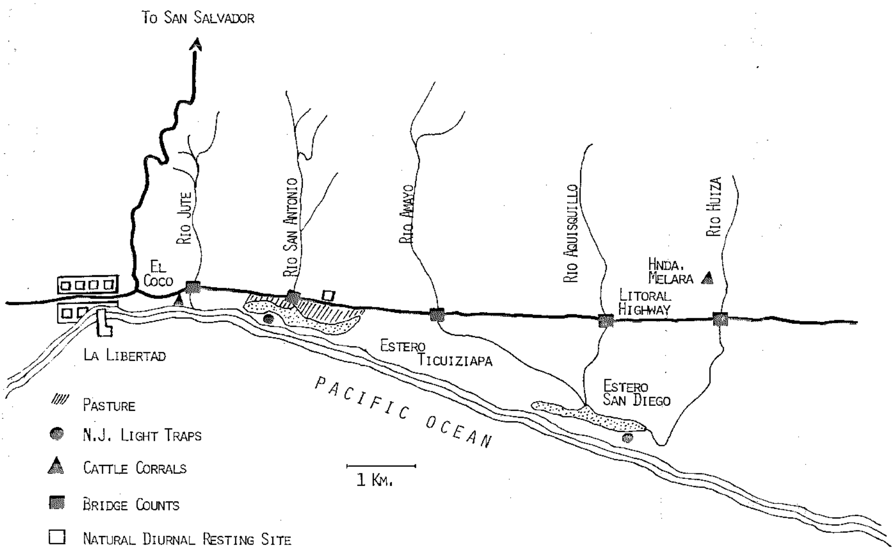

FIG. 1.—Sketch map of La Libertad Area, El Salvador, showing collecting sites.

marsh. In addition to these collection methods, human bait captures and house captures were made at various dwellings throughout the area.

In general, light trap collections were made once weekly between January 1969 and June 1971, and represent a period of 1 trap-night each, between 6:00 p.m. and 6:00 a.m. Diurnal bridge collections were made at irregular intervals between 1969 and 1971, and represent the counting of all adults under a given bridge at a given time during daylight hours. Cattle corral collections were made at irregular intervals between January 1969 and June 1971, and represent all resting adults counted during a given time period, usually 1 hour, shortly after dark. Collections from completely natural resting sites (rock holes, tree holes, animal burrows, etc.) were made at irregular intervals for the same period as cattle corral collections, and represent all adults captured at a given site at a given time during daylight. Human bait and house captures were made on an opportunistic basis at irregular intervals between 1968 and 1971, with the majority of collections being made during 1970–1971. Human bait captures were peridomiciliary and represent 1 man-hour of collecting biting females during the darkness of early evening. House captures represent the collection of all adult mosquitoes found resting on interior surfaces of dwellings shortly after dawn.

In all collections, specimens were counted by species and sex, although only *A. albimanus* and *A. pseudopunctipennis* were recorded from the study area under discussion. The former is considered to be the principal vector of malaria in Central America, and perhaps the only vector in El Salvador, while the vector status of the latter is unknown for Central America.

RESULTS AND DISCUSSION

The six basic methods used by CAMRS to measure adult anopheline populations in the study area, Figure 1, have included three methods heretofore not extensively used in Central America, viz., New Jersey light traps, diurnal collections from natural resting situations, and diurnal collections from beneath concrete bridges. The remaining three methods (nighttime cattle corral collections, nighttime human bait captures, and early morning captures of mosquitoes resting on surfaces inside of houses) have been more commonly used by malaria programs in the region.

Anopheline density measurements are usually made to determine the activity cycle over a 24-hour period, to determine seasonal fluctuations in numbers, or to measure the effect of control procedures. The following discussion offers comparative results of CAMRS experience with various *Anopheles* collecting methods as related to the objective of the collection.

NEW JERSEY LIGHT TRAPS. A standard New Jersey light trap, equipped with a 60-watt incandescent bulb, was operated at each of two sites. (Ticuiziapa and San Diego Beaches, Figure 1) one night weekly throughout the period of this study. The data of table 1 show a high degree of efficiency for these traps in measuring *A. albimanus* densities, with an average of 148.3 specimens per trap night at San Diego, and 44.5 at Ticuiziapa, with a combined average of 93.9 at both locations. Practically all of the mosquitoes (96.9 percent) were females. On the other hand, the data show the trap to be virtually useless for collecting *A. pseudopunctipennis* with a total of only 17 specimens in 358 collections at the two locations. On one occasion, 1,951 *A. albimanus* were collected in a single trap-night of operation during peak breeding in the nearby San Diego Estuary (June 22, 1970). The trap has been particularly useful in (1) determining activity periods of *A. albimanus;* (2) determining the seasonal fluctuations in numbers of *A. albimanus* in a given vicinity; and (3) rapidly confirming the presence of *A. albimanus* in an area.

The first point, the determination of

TABLE 1.—Summary of anopheline captures by collecting method, La Liberated area of El Salvador, January 1968–June 1971.

	A. albimanus								*A. pseudopunctipennis*						
	No. Coll.	No. Pos.	% Pos.	No. Adults M.	No. Adults F.	No. Adults Total	Ave. No. Per Coll.	% Females	No. Pos.	% Pos.	No. Adults M.	No. Adults F.	No. Adults Total	Ave. No. Per Coll.	% Females
Lt. Trap, San Diego	167	151	90.4	715	24,050	24,765	148.3	97.1%	9	5.3	0	13	13	0.08	100.0%
Lt. Trap, Ticuiziapa	191	170	89.0	326	8,511	8,837	44.5	96.3%	4	2.1	0	4	4	0.02	100.0%
Total, 2 Traps	358	321	89.7	1,041	32,561	33,602	93.9	96.9%	13	3.6	0	17	17	0.05	100.0%
Natural Diurnal Resting	78	62	80.0	1,280	297	1,577	20.2	18.9%	9	11.5	255	198	453	5.8	43.7%
Cattle Corrals															
El Coco	60	50	83.3	0	4,545	4,545	75.7	100.0%	13	21.7	0	48	48	0.8	100.0%
Melara	47	45	95.7	7	4,479	4,486	95.4	99.9%	19	40.4	0	73	73	1.6	100.0%
Total, 2 Corrals	107	95	88.8	7	9,024	9,031	84.4	99.9%	32	30.0	0	121	121	1.1	100.0%
Rio Aquisquillo	34	22	64.7	40	351	391	11.5	89.8%	8	23.5	5	21	26	0.8	80.8%
Rio Huiza	21	15	71.4	53	109	162	7.7	67.3%	18	85.7	270	662	932	44.4	71.0%
Rio Jute	50	41	82.0	104	201	305	6.1	65.9%	45	90.0	262	246	508	10.2	48.4%
Rio San Antonio	47	38	80.9	82	148	230	4.9	64.3%	5	10.6	6	8	14	0.3	57.1%
Total, 4 Rivers	152	116	76.3	279	809	1,088	7.2	74.4%	76	50.0	543	937	1,480	9.7	63.3%
Human Bait	23	18	76.1	0	677	677	29.5	100.0%	0	0	0	0	0	0	0
House Captures	72	29	40.3	236	440	676	9.4	65.1%	1	1.4	0	1	1	–	–

the 24-hour activity pattern, is illustrated by observations that more *A. albimanus* were collected in traps between 6:00 and 9:00 p.m. than during any other 3-hour time period of darkness. This fact agrees with observations made by other collecting methods, e.g., human bait and cattle corral captures, which also indicate an activity period during the early hours after dark. In an earlier report (Breeland, 1972), the 24-hours resting cycle of *A. albimanus* in El Salvador showed that maximum numbers were absent from resting sites during these hours, indicating a reciprocal period of maximum activity.

The data of Figure 2, illustrating the second point, show that the New Jersey light trap at San Diego Beach during 1969 and 1970 accurately reflected the seasonal breeding pattern of larvae in the San Diego estuary. This trap measures adults produced from breeding in the associated estuary, and it is likely that no additional techniques would be required to measure anophelines in this particular situation. Control approaches might be limited to this period of time. The accuracy of the light trap in this instance was sufficiently good in 1970, that a decline from 1,951 specimens on June 22 and 1,127 on June 29 to only 65 on July 6, with even smaller numbers after that date, reflected the fact that the estuary had become open to the sea on June 26. See Figure 3. Experience has shown that when this opening occurs, either by natural or mechanical means, the estuary becomes unsuitable as an anopheline habitat.

On repeated occasions, the use of a single New Jersey light trap has indicated the presence and general level of anopheline populations in an area.

CATTLE CORRAL COLLECTIONS. Cattle corrals, such as those at El Coco and Melara (Figure 1), are common throughout the malarious areas of El Salvador and usually offer a reliable index of anophelism for the locality. Female *A. albimanus* are strongly attracted to the concentration of animals, and *A. pseudopunctipennis* to a lesser degree. In many respects, cattle corral results are similar to those from light trap captures. For example, both depend on nighttime responses to external stimuli, light on the one hand, and animal bait on the other. Both reflect an early evening activity period for *A. albimanus* and, in terms of relative effectiveness, the similarity prevails. Average counts for 107 collections from the two capture sites combined, averaged 84.4 specimens per collection (99.9 percent females) as compared to 93.9 per collection (96.9 percent females) from the two light trap locations (Table 1). The corral at El Coco, in an area of fewer breeding sites than Melara, yielded an average of 75.7 per collection while the Melara corral yielded an average of 95.4 per collection.

Although large numbers of *A. albimanus* are collected in short periods of time in corrals, numbers fluctuate widely with the number of cattle. Specimens collected from cattle corrals serve limited purposes and are used mainly to indicate general levels of anophelism or as a source of specimens for certain types of biological studies, principally bioassays. For example, such collections would be a heavily biased source of specimens for host preference studies.

The corral source has the added disadvantage of requiring the physical presence of collectors during nighttime hours. As seen from the data of Table 1, poor results, an overall average of only 1.1 specimen per collection, were obtained for *A. pseudopunctipennis*.

NATURAL DIURNAL RESTING COLLECTIONS. Early searches in the coastal areas of El Salvador revealed rather extensive use of diurnal natural resting sites, by both *A. albimanus* and *A. pseudopunctipennis*. It soon became apparent that such natural sites as rock crevices, tree cavities, ground holes, etc. were favored daytime resting places of these two species throughout El Salvador. Subsequent spot observations in Nicaragua, Honduras, Costa Rica and Panama indicate similar resting places for *A. albimanus*. While the data of Table 1 show an average of

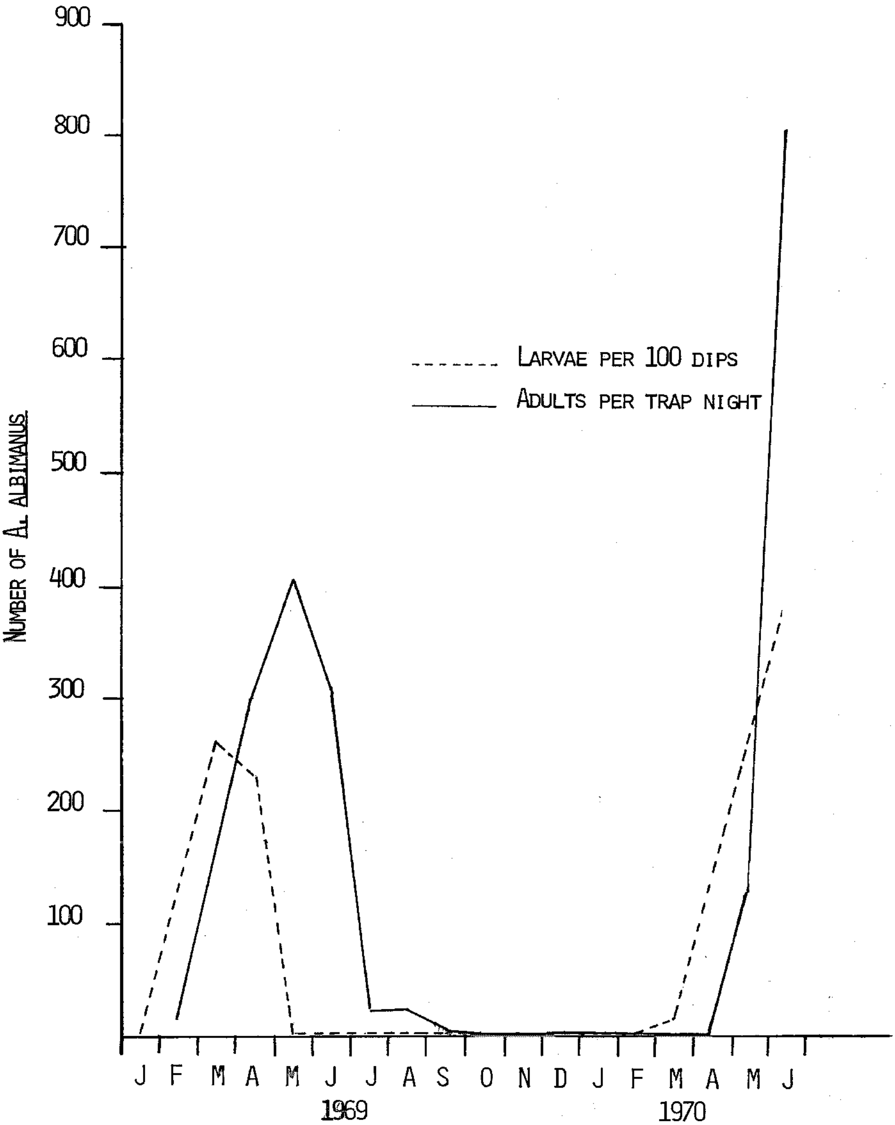

FIG. 2.—Number of *A. albimanus* larvae per 100 dips, Estero San Diego, and number of adult *A. albimanus* per light trap night, San Diego Beach January 1969–June 1970.

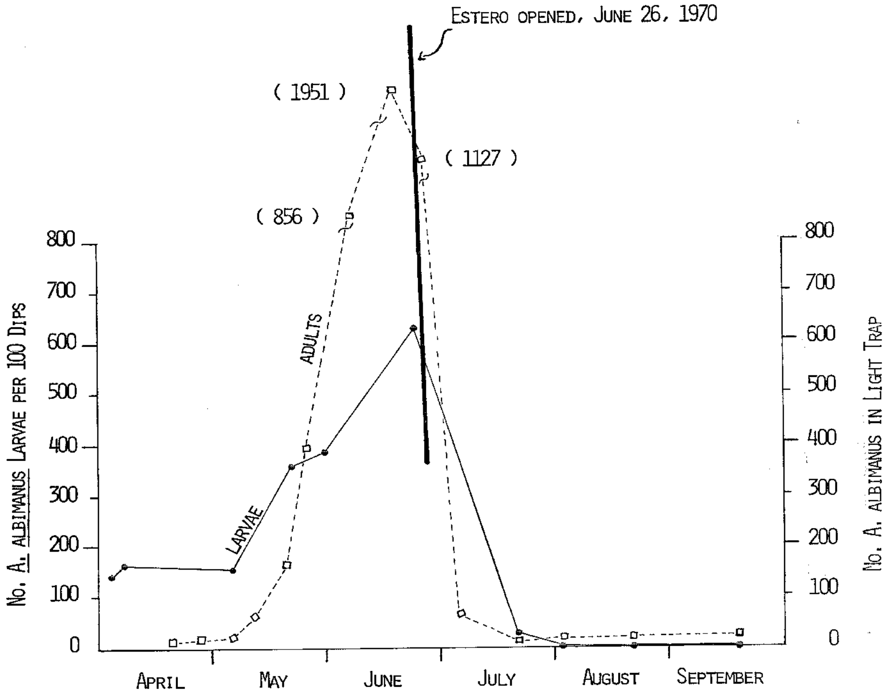

Fig. 3.—Density of *Anopheles albimanus* larvae in Estero San Diego and adults in San Diego Beach N. J. light trap, 1970.

only 20.2 and 5.8 specimens per collection, respectively, for *A. albimanus* and *A. pseudopunctipennis* from natural sites in the area under discussion, country-wide studies showed an average of 42.4 and 12.9 respectively, in 181 collections.

Daytime density measurements for certain purposes are highly desirable. The collection of specimens from such sites for host preference studies, for example, would seem to result in less bias than collections from night host-specific situations such as cattle corrals, human bait or human dwellings. Also, the percentage of males, 81.1 percent for *A. albimanus* and 56.3 percent for *A. pseudopunctipennis* (Table 1) would indicate close proximity to the breeding area, and the average number of *A. pseudopunctipennis* of 5.8 per collection is the highest of any method for collecting this species except for the similar method of daytime collections from beneath bridges.

In general, counts for *A. albimanus* from natural diurnal resting places near breeding areas reflect adult populations equally as well as some nighttime methods. For density measurements, considering the added cost and drain on manpower of night captures, plus the effect of climatic conditions such as wind, rain, and moon phase on *A. albimanus* during the nighttime activity period, it is probable that daytime counts of resting mosquitoes in protected niches might be even more useful as a population index. Experience has shown that when *A. albimanus* is found in a cattle corral during a night-capture period, it is correspondingly present in daytime niches in the same vicinity and much more conveniently counted. For example, crevices in a lava wall near the stable at Lake Jocotal have repeatedly yielded hundreds of specimens of *A. albimanus* which could be easily counted during any period of daylight in a matter of 20 to 30 minutes.

Diurnal counts have been particularly useful in evaluating the effectiveness of area-wide larviciding and adulticiding projects; as a source of specimens for host preference and longevity studies; and for the determination of population densities. In addition, the determination of the diel resting pattern over a 24-hour period involved the counting of mosquitoes in given resting places at 1-hour intervals over a 24-hour activity pattern by constructing a reciprocal curve.

DIURNAL BRIDGE COLLECTIONS. Diurnal collections from beneath bridges offer a convenient method of measuring populations of adults produced in the rivers below. Since such breeding is limited to the dry season months (December–June), it is easily determined that the bridges essentially reflect only dry season river breeding. The data of Table 1 show an average of 7.2 *A. albimanus* per collection from the combined counts from the four suitable bridges in the area, and an average of 9.7 *A. pseudopunctipennis* from the same sources. The bridge counts represent the single best source of specimens for *A. pseudopunctipennis,* which, in the coastal areas, is a dry-season mosquito. The pooled, algae-laden water of the rivers is the preferred habitat of the species. The data (Table 1) further show higher counts of *A. albimanus* in Rios Aquisquillo and San Antonio than *A. pseudopunctipennis,* and higher counts of the latter species in Rios Huiza and Jute. This accurately reflects breeding revealed by larval sampling in Rio San Antonio, Rio Huiza and Rio Jute. However, Rio Aquisquillo yields a higher percentage of *A. pseudopunctipennis* larvae than *A. albimanus.* This departure can be explained by the fact that the bridge over Rio Aquisquillo is higher and permits more light than the other bridges, a condition which discourages its use by *A. pseudopunctipennis* as a resting site.

Bridge collections have served essentially the same purposes as natural resting sites, offering good sources of mosquitoes for host preference and longevity studies, and serving as the most dependable source of *A. pseudopunctipennis* during the dry season. Additionally, bridge counts were especially useful indices of control results in connection with dry season larviciding of river breeding sources in 1969 and with evaluation of aerial applications of ultra low volume (ULV) malathion to the general area in 1968 and 1969.

HUMAN BAIT COLLECTIONS. Collecting anophelines attempting to bite humans is a realistic approach to measuring some of the more important aspects of anopheline behavior in relation to malaria transmission. This method has been used by CAMRS in connection with ULV studies and as a source of specimens for longevity studies and for studies on susceptibility to malaria parasites. During the ULV trials, it was discovered that biting collections around concentrations of light, particularly around isolated gasoline service stations, offer a good indexing situation because of the positive response of *A. albimanus* to combined effect of light and bait sources. As a general indexing tool, the data of Table 1 show that, in the area studied, human bait captures, with an average of 29.5 *A. albimanus,* did not compare favorably with the other nighttime methods of light traps (93.9 per collection) and stable captures (84.4 per collection). However these were somewhat better than the daytime methods of natural resting (20.2) and bridge counts (7.2). On the other hand the two daytime methods produced an average of 5.8 and 9.7 *A. pseudopunctipennis,* while the human bait captures produced no specimens of this species. All CAMRS human bait collections were peridomiciliary, in close proximity to houses.

EARLY MORNING HOUSE CAPTURES. For the time and effort expended, this has been the least productive of the density measurement methods used by CAMRS. The counts are generally made shortly after dawn by collecting mosquitoes resting on surfaces inside houses. However, our experience indicates that satisfactory counts can be made later in the day, at least in the more tightly constructed dwellings. Such collections yielded an average of only 9.4 *A. albimanus* per collection, 34.9 percent of which were males. Only a single specimen of *A. pseudopunctipennis* was collected among the 72 collections. Additionally, only 29 of the 72 collections (40.3 percent) were positive for mosquitoes. This compares with a range of positive collections between 76.1 percent and 95.7 percent for other methods. It is perhaps reasonable to suspect that residual wall deposits of insecticide and the excito-repellency factor might affect this method. Also, houses vary to a great degree in structure and attractiveness to mosquitoes. In loosely constructed houses, there is a greater tendency for mosquitoes to leave the house in search of more suitable shelter. In many instances, more specimens have been counted on lower exterior wall surfaces and beneath eaves than on interior surfaces of the same house during early morning hours. In general, intradomiciliary house counts serve very limited purposes and their use should be accompanied by a thorough understanding of certain behavioristic characteristics of the mosquito species. A case in point can be made from CAMRS host preference studies which show that less than 20 percent of blooded *A. albimanus* collected from inside houses during early morning hours have fed on humans, an indication that such mosquitoes must have already fed on an animal other than man before entering the house. Thus, the house must have been chosen mainly for shelter rather than reflecting an endophilic behavior pattern. Otherwise, other animal sources must have been in the houses, which might indicate stronger zoophilic than anthropophilic tendencies, further complicating the picture.

SEASONAL FACTORS. The data of Table 2 show that the area represented in this study is essentially one predominated by dry season mosquito populations. While this may be due, in part, to the effect of cotton spraying on adults in the area between July and December, it seems more likely that the general prevalence of adults during the dry season reflects the predominance of breeding habitats typical of dry season mosquito production. For example, the main sources of dry season breeding are estuaries, rivers, irrigated pastures, and permanent bodies of fresh water. Dominating this area are two rather large estuaries, several rivers, and a large irrigated pasture. There are no permanent bodies of fresh water and the only typical rainy season habitat is the Ticuiziapa marsh which serves as a favorable habitat only during early rainy season in June and July, shortly after inundation. Later in the season, after July, the water in this marsh becomes polluted and unsuitable for anopheline breeding, resulting from accumulated cattle droppings during the dry season.

SUMMARY

Entomological studies conducted by the Central America Malaria Research Station between January 1968 and June 1971 in a coastal malarious area extending 10 kilometers east of the port and city of La Libertad, afford the opportunity to compare various methods used to measure anophelism in the area. The New Jersey light trap was superior to other collecting methods in average numbers of *A. albimanus* with 93.9 per collection, 96.9 percent of which were females, and was particularly useful in measuring seasonal fluctuations, in determining the nocturnal activity peak, and in detecting the presence of the species in a given locality. This method was inadequate for measuring *A. pseudopunctipennis* populations. Collections from cattle corrals in the area yielded an average of 84.4 specimens of *A. albimanus* per collection, 99.9 percent of which were females. This method

TABLE 2.—Average number of *Anopheles albimanus* adults per collection by months, La Liberated, El Salvador Jan. 1968–June 1971

Type Collection	Dry Season						Rainy Season						Comments
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Light Traps (167 collections)	3	9	80	233	268	541	47	30	10	4	3	5	Two traps (San Diego and Ticuiziapa) measure adults from estuary and pasture breeding, dry season sources.
Stable Captures (107 collections)	64	42	31	140	189	368	119	111	1	45	6	128	Two stables (El Coco and Melara) measure adults from rivers, marshes and irrigated pastures. Low counts Sept.–Nov. could be partially due to cotton spraying.
Natural Resting (78 collections)	36	6	13	32	112	81	11	1	4	<1	2	14	Area of Ticuiziapa measures marsh and estuary breeding, dry season sources.
Bridge Shelters (152 collections)	8	21	23	37	38	11	<1	0	0	0	0	0	Four rivers (Aquisquillo, Jute, Huiza and San Antonio), measure dry season river breeding.
House Resting (72 collections)	22	13	4	4	23	6	21	15	19	4	<1	0	Measures only that segment of population entering houses; individual houses vary in attractiveness to mosquitoes.
Human Bait (23 collections)	..	61	..	11	50	210	9	..	..	..	..	..	Opportunistic collections, but May–June peak is consistent with same peak shown by other methods.

compares favorably with the light trap, but requires the physical presence of collectors during nighttime hours and yields few specimens of *A. pseudopunctipennis* (average of 1.1 per collection. Diurnal collections from natural resting sites yielded an average of 20.2 *A. albimanus* per collection and 5.8 *A. pseudopunctipennis* per collection. The percentage of males, 71.1 percent and 56.3 percent, respectively, for the two species, was the highest for any method used. Collections from beneath bridges accurately measured breeding in the associated rivers. Such collections yielded an average of F.2 *A. albimanus* (74.4 percent females) and 9.7 *A. pseudopunctipennis* (63.3 percent females) per collection. Such sites are particularly useful for collecting mosquitoes for host preference and longevity studies, as a source of *A. pseudopunctipennis,* and for evaluating control projects involving larval abatement in the rivers and nearby breeding sites.

Peridomiciliary human bait captures yielded an average of 29.5 *A. albimanus* per collection and no specimens of *A. pseudopunctipennis*. This method, along with that of inside house captures, which accounted for an average of 9.4 *A. albimanus* (65.1 percent females), and only a single *A. pseudopunctipennis* female, measures only a segment of the anopheline population, i.e., that portion attracted to a specific human bait source or disposed to enter particular houses at a given time. Such collections are erratic, but highly useful if properly understood and related to specific measurement purposes.

The data, for the area under study, show a predominance of anopheline densities during the dry season. This is believed to be due to a dominance of dry season habitats in the area rather than the influence of cotton spraying.

This comparative appraisal of methodology for measuring anophelism in a malarious area of coastal El Salvador should aid workers concerned with the problem in other areas and situations within the range of *A. albimanus* and *A. pseudopunctipennis*.

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