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INTRODUCTION

The agreement to establish the Central America Malaria Research Station (CAMRS) was signed by the Public Health Service (PHS) and the Agency for International Development (AID) in April 1967. Formal inauguration of the Station took place on February 16, 1968, following initial renovation of three buildings made available rent-free by the Government of El Salvador through the basic Project Agreement of June 2, 1967, between the Ministry of Public Health and Social Welfare and the USAID Mission in El Salvador.

The following additional major renovations and equipment installations were effected during the year:

a. Two climate-simulation cabinets were placed in the insectary, with associated provision of special drainage and electrical lines.

b. A small animal house was constructed for non-human primates and other laboratory animals.

c. Two explosion-proof fume hoods and two electrical ovens were installed with re-wiring as required.

d. A small fireproof building was converted for storage of high-risk chemicals and insecticides. (This building was turned over to CAMRS by the Urban Housing Institute through arrangements by the Ministry of Public Health and Social Welfare.)

e. Modest lunchroom facilities were provided for employees because restaurant facilities are lacking in the suburban location of the Station.

Although the extensive collections in the libraries of the medical and science faculties of the National University of El Salvador have been made available for use by Station technicians, the need for reference material in several specialized fields has required attention. Directly pertinent reprints of malaria and mosquito-associated articles published from 1920 to date have been requested from the respective authors, and a very gratifying response has brought the number of reprints above the 1200 mark. Donations of complete or partial series of 13 journals have been made by individuals and institutions, effectively complementing the present Station subscriptions to a limited list of journals.

EVOLUTION OF THE CAMRS STAFFING PATTERN AND STAFF

The original Participating Agency Service Agreement (PASA) approved a staff of 13 U.S. and 45 national employees, the latter predominantly field personnel (mosquito collectors and blood survey or drug distribution aides). In February 1968 a Mission-wide review of the country staffing level as a part of the "balance of payments" or "BALPA" exercise froze the CAMRS personnel situation, and the Station ended FY 1968 with 4 U.S. technicians and 14 national employees on duty on June 30, 1968.

Early in FY 1969 there was an interagency agreement that established the personnel ceiling of 9 U.S. technicians and 35 national employees. The additional U.S. technicians began arriving at post on August 31, 1968, and the staffing with U.S. employees was completed on January 28, 1969. For the balance of the reporting period, the staff was composed of the chief, one medical epidemiologist, three entomologists, one chemist/wildlife biologist, one public health educator, one administrative officer, and one biological laboratory technician.

During the year consultants on temporary duty at the Station included an administrative officer for two months, an entomologist for five weeks, a statistician for

one week, a parasitologist for two weeks, and an engineer for mosquito source reduction-water management studies for three months.

The ceiling of 35 local employees permitted contracts with additional national personnel. The local national staff increased from 14 on July 1, 1968, to 29 on December 31, 1968, and to 35 by February 1969.

Three microscopist aides of the National Antimalaria Campaign of El Salvador (CNAP) were granted leave by the national program to provide urgently needed services in CAMRS. They were subsequently placed on regular contract status by the Station upon indication that they were no longer essential in the CNAP laboratory.

WORKING RELATIONSHIPS WITH VARIOUS GROUPS

National Organizations

The Ministry of Public Health and Social Welfare of El Salvador actively collaborated with the Station, providing effective liaison with the Urban Housing Institute (IVU) and other governmental agencies with whom contacts were essential during renovation or program developmental activities.

The Director and staff of the CNAP offered indispensable assistance in providing background epidemiological data, in making investigational areas available within limits imposed by the national and regional program commitments, and in cooperative field activities in drug and insecticidal trials.

The Station has cooperated with the Biochemistry Department of the Medical School of the National University of El Salvador in investigation of the prevalence of glucose-6-phosphate dehydrogenase deficiency in populations of coastal areas of the country.

Through arrangements requested by the Ministry of Public Health and Social Welfare, the Air Force of El Salvador furnished excellent airport facilities for the contract plane which carried out the Station's ultra low volume (ULV) spraying trials.

Irrigation specialists of the Ministry of Agriculture and Livestock of El Salvador cooperated fully with the Station's water management consultant in his survey of past, present, and future irrigation-drainage projects in the country.

Cordial, useful relationships were maintained with the National Malaria Eradication Services of Central America and Panama, both directly and through the Work Group for Coordination of Malaria Eradication Programs of Central America and Panama. The Chief, CAMRS, presented research results and outlines of future research programs at the Working Group meetings in Tegucigalpa, Honduras, in August 1968 and April 1969. Specific visits were made to Nicaragua, Honduras, and Panama by CAMRS specialists to discuss larviciding and residual intradomiciliary insecticide problems and possible trials of candidate insecticides. A CAMRS representative participated in the inauguration of the new Servicio Nacional de Erradicacion de la Malaria (SNEM) headquarters building in Costa Rica on October 11, 1968, and three Station technicians observed the Costa Rican collective drug distribution program in April 1969.

The national entomologist of SNEM/Colombia spent the period from April 28 to May 9, 1969, with CAMRS entomology personnel during his observation-training visit.

International Organizations

Effective working relationships were maintained with Pan American Health Organization (PAHO) and United Nations Children's Fund (UNICEF) officials concerned with malaria problems in the region. Preliminary results of CAMRS studies and future program

plans were presented to representatives of these organizations for information and concurrence at the Coordinating Work Group meetings and at meetings in PAHO/Washington.

One CAMRS research entomologist worked with PAHO for one week on anti-vector aspects of an equine encephalitis outbreak in Ecuador (May 2-8, 1969).

Useful contacts were maintained with the director and the agricultural entomologist of the San Salvador-based International Regional Organization of Animal Health and Plant Protection (OIRSA). Possibilities were explored for a CAMRS assessment of the anti-anopheline effects of an OIRSA-sponsored ultra low volume application of a malathion-protein bait combination against Mediterranean fruit flies in Nicaragua. This cooperative study was abandoned upon determination that the fruit fly trial was scheduled for areas with low malaria and anopheline potential.

Embassy and USAID Personnel

Ambassador William G. Bowdler and several Embassy staff members actively encouraged Station developments through personal visits and provision of Mission services. Support by the USAID Director, directly or through the Malaria Coordination Officer, helped resolve many problems. Joint Administrative Services personnel rendered invaluable assistance in many special and routine activities.

All USAID Missions in Central America and in Panama were furnished copies of the FY 1968 Annual Report, of the FY 1969 Work Plans of CAMRS, and of trip reports covering SNEM, Mission, and other contacts made during visits to the respective countries.

One CAMRS research entomologist served as consultant to USAID/Ecuador during the period May 9-20 in connection with the encephalitis outbreak in that country.

PHS Personnel in ROCAP, Central American Countries, and NCDC/Atlanta

Until the NCDC Malaria Eradication Program country advisors left El Salvador (July 1968) and Honduras (December 1968), they greatly facilitated contacts with national and PAHO personnel in those programs.

Exchange of information with the NCDC Malaria Eradication Program's Chief Malaria Advisor in the Regional Office for Central America and Panama (ROCAP) was accomplished through visits, reports, and joint meeting attendance.

The two epidemiologists of the NCDC/MEP Regional Evaluation Office were furnished quarters and limited administrative facilities until that office was closed on August 31, 1968.

CAMRS served as an operational base for, and furnished limited administrative support to, two visiting research scientists of the Biology Section, Technical Development Laboratories (TDL), NCDC, during the period June 10-30, 1969.

Entomologists of the TDL/NCDC in Savannah, Georgia, were furnished a malathion-resistant strain of Anopheles albimanus from El Salvador for colonization efforts. Similar material was made available to an entomologist-geneticist of the University of California.

Support from NCDC has been an essential and encouraging factor in Station development, covering backstopping in many administrative and technical aspects. The extensive resources which can be tapped on short notice continue to constitute one of the strongest investigational features of the Station.

VISITORS TO CAMRS

Eighty-five visitors representing research, training, diplomatic, or commercial interests were received at the Station from July 1, 1968, through June 30, 1969.

DEVELOPMENT OF RESEARCH PROJECTS

In the February 17, 1968, meeting of the Work Group for Coordination of Malaria Eradication Programs of Central America and Panama, the Directors of the several programs expressed priority interest in investigations of:

1. Antimalaria drug combinations with fewer side effects than those already in use;

2. Methods for obtaining increased public acceptance of antimalaria drugs;

3. Larvicides and larvicide application techniques for use in special situations;

4. The possible role of secondary vectors in certain areas (*Anopheles punctimacula* and *A. darlingi*);

5. Aerial application of insecticides for use in special circumstances; and

6. Field application of new residual insecticides against DDT and dieldrin resistant anophelines.

The Coordinating Work Group did not give operational emphasis to such problems as the lack of essential information on the characteristics of local malaria parasite strains nor the comprehensive ecology of the local vector(s), but need for supporting studies on these and other subjects was recognized.

At the invitation of the Coordinating Work Group, the Chief of CAMRS participated in the Group's deliberations in Tegucigalpa in August 1968 and April 1969. Results obtained in ongoing studies were outlined, and CAMRS' Proposed Work Plan for Fiscal Year 1970 was presented to, and endorsed by, the participants.

General and specific reviews of the CAMRS research program were carried out with Malaria Eradication Program/NCDC officials in El Salvador (November 1968 and April 1969) and Atlanta.

The Chief, Research Branch, MEP/NCDC, and the Chief, CAMRS, met with PAHO malaria officials in PAHO/Washington on August 22, 1968, to present CAMRS FY 1969 work plans and to coordinate the Central America malaria research programs of PAHO and NCDC. Following an early March 1969 review of the FY 1970 work plans with Malaria Eradication Program and other NCDC specialists in Atlanta, these plans were furnished PAHO/Washington to facilitate coordination of research with PAHO investigators. The Chief, Research Branch, visited the Station from March 19 to 28, 1969, for final adjustments in the plans and in arrangements for their implementation. CAMRS data on larviciding trials in El Salvador were presented at a Washington meeting (June 30-July 1, 1969) of WHO/PAHO, UNICEF, NCDC, and USAID officials during deliberations concerning the use of OMS-33 or alternative attack measures in problem areas of Central America.

Until the Station complement of U.S. and national employees was completed in January 1969, project development emphasis was largely determined by availability of specialists and auxiliary personnel and of the still-arriving equipment and vehicles.

Investigations during the first semester of the report period were concentrated in El Salvador (an exception was a limited series of larvicidal formulation and dosage trials in Nicaragua). To facilitate the essential cooperation offered by the National Antimalaria Campaign (CNAP) in El Salvador, research projects were implemented (until October 1968) through an ad hoc Coordinating Committee for Malaria Investigations in El Salvador, consisting of the CNAP Director, the Chief of CAMRS, and a research representative of PAHO. Since late 1968 changes in CNAP and local PAHO research leadership, coordination has been accomplished directly with these respective organizations. Valuable informational exchanges have been facilitated by participation of CAMRS, AID, and PAHO advisor personnel in a number of the monthly meetings of senior CNAP staff members.

In the malarious areas of El Salvador where the CNAP has cooperated with CAMRS in chemotherapy, insecticide, or vector ecology investigations, CAMRS specialists and field aides have been in intimate contact with CNAP supervisory and operational personnel.

In the study of local malaria parasite strain characteristics, SNEM/Honduras cooperated closely with CAMRS specialists and a visiting consultant in a search for untreated malaria patients.

During the final quarter of the report period, negotiations were under way with SNEM/Honduras for a cooperative larviciding study in Choluteca, Honduras, and with SNEM/Panama for a study of DDT formulations and dosages in Panama.

VECTOR ECOLOGY STUDIES

Seasonal Breeding of Anophelines - El Salvador, 1968

To determine the year-round breeding places available to, and utilized by, *Anopheles albimanus* and *A. pseudopunctipennis*, larval surveys were conducted during the late rainy season (October 10 - December 11) and during the late dry season (March 3 - May 14). Less specific observations have been made during periods overlapping the rainy/dry and dry/rainy seasons in areas where anophelism is common during both seasons. These observations allow some data-supported generalizations as to (1) the favored seasonal breeding sites of the common *Anopheles* species, (2) more insight into the means of carry-over from one season to another in a given area, (3) the geographic and topographic pattern of breeding on a seasonal basis, and (4) the relative abundance and importance of rainy versus dry season populations.

The dry season population, although geographically restricted to areas with available water, is nevertheless represented by sizable numbers in such areas. The most abundant segment of the dry season population is apparently along the Pacific coastal plain where rivers traverse the plain and empty into estuaries or directly into the ocean. During the dry season the rivers contain pooled water, are open to partial sunlight, and support shallow vegetation. All of these factors favor anopheline breeding in El Salvador. In cases where estuaries (lagoons) are found, the breeding areas are augmented by the estuary and lagoon margins and solid expanses of inundated mangrove swamps. These dry season situations are not uncommon and collectively support anophelism over much of the malarious coastal plain to a far greater extent than might be suspected by casual observation. To illustrate: in one 13-kilometer section of coastal plain east and west of the port city of La Libertad, there are 12 such rivers and three estuaries, all of which support some anopheline breeding during the dry season, and most of them support breeding throughout this period. These breeding sources are sufficiently close to each other to permit geographically uninterrupted breeding throughout this area. Anophelism is assured year-round in such areas by early rains inundating low lying pastures, swamps, roadside ditches, etc., which are habitats that become available by the time rains have flushed the rivers and the estuaries have been opened making these situations unsuitable for mosquito breeding.

In interior areas, there are a sufficient number of lakes and streams to support limited anopheline breeding throughout the dry season. During the rainy season, breeding habitats are greatly increased by accumulated inundations of pastures, ditches, etc., which become available to the already existing mosquito populations. In these areas, it is evident that accumulations of rainy-season waters diminish gradually in some situations, allowing year round breeding since the population has access to alternative permanent pool habitats when the temporary rainy season waters have disappeared.

The dry season larval population, as measured by larval surveys, compares very favorably to rainy season populations measured in the same manner, although the number and extent of the dry season breeding areas are far less than those of the wet season. The data in Table 1 show the results of a dry season survey made between March 3 and May 14, 1968. A total of 6,315 dips from seven coastal rivers resulted in an average of 1.64 larvae per dip. Identification of samples of each collection showed 45.2 percent were A. albimanus and 54.8 percent were A. pseudopunctipennis. A rainy season survey (Table 2) between October 10 and December 11, 1968, involving the same area as well as other sources throughout El Salvador (lakes, ponds, pastures, ditches, etc.) yielded 4,715 larvae in 2,913 dips, or an average of 1.6 larvae per dip; 94.0 percent of samples identified were A. albimanus and 6.0 percent were A. pseudopunctipennis. These data indicate concentrated dry season populations and dispersed rainy season populations. Further investigations into actual densities in terms of malaria suppression activities are needed.

TABLE 1

Summary of Dry Season Anopheline Larval Sampling in Coastal Rivers, El Salvador, March 3 - May 14, 1968

River	No. of Collections	Dips			No. Larvae	No. Larvae per Dip	% * albimanus
		Total No.	No. Pos.	% Pos.			
Tunco	9	1500	370	24.7%	1471	0.98	0.9%
Grande	10	1695	737	43.5%	2538	1.50	33.3%
Comasagua	5	240	67	27.9%	294	1.22	0
Chilama	9	1605	594	37.0%	2351	1.46	41.3%
Jute	8	235	---	---	1811	7.70	80.9%
San Antonio	8	290	---	---	806	2.78	89.7%
Aquisquilla	6	750	---	---	1098	1.46	70.0%
T O T A L	55	6315	---	---	10369	1.64	45.2%
1st four only	33	5040	1768	35.1%	---	---	---

* Based on samples of each collection

TABLE 2

Summary of Rainy Season Anopheline Larval Sampling
El Salvador, October 10 - December 11, 1968

Type of Habitat	No. of Collections	Dips			No. Larvae	No. Larvae per Dip	% * albimanus
		Total No.	No. Pos.	% Pos.			
Lakes	9	510	253	49.6%	2876	5.6	100%
Marshes	11	875	136	15.5%	203	0.23	100%
Flooded Pastures	5	661	237	35.9%	931	1.4	79%
Estuaries	7	405	32	7.9%	82	0.2	100%
Miscellaneous: (Ponds, rock holes, roadside ditches, etc.)	7	462	90	19.5%	623	1.3	99%
T O T A L	39	2913	748	25.7%	4715	1.6	94%

* Based on sample of each collection

The great preponderance of A. albimanus during the rainy season and the relative abundance of A. pseudopunctipennis during the dry season should be investigated in terms of vector potential on a seasonal basis. A. pseudopunctipennis seems to be more restricted by habitat requirements than does A. albimanus. The former species has been frequently found in river and inundated dry-land pasture situations but never in estuaries and infrequently in flooded lowland situations. Perhaps a small rainy season population of A. pseudopunctipennis is carried over in the upper reaches of rivers where pooled water is available, but at an altitude too high for A. albimanus. One such situation is known (Hacienda Tepiagua).

Some evidence to indicate rainy season adaptation to newly inundated habitats before rivers are rendered unsuitable by flowing waters is given in Table 3. Larval breeding was found in Río Comasagua as late as June 6, in Ríos Chilama and Grande as late as May 10, and Río Tunco as late as May 14. Furthermore, adult mosquitoes were found under the bridges of Ríos Comasagua and Grande (indicating river breeding) as late as June 17. This would indicate an overlapping population since newly inundated lowland pasture habitats were positive for larvae as early as May 14 (San Antonio and Jute) and had reached heavy densities (9.6 larvae per dip) in the large Ticuiziapa pasture by June 6.

Unfortunately, many of these data were necessarily extracted from those of other studies and certain gaps exist. Nevertheless, the pattern is apparent and more specific studies are planned.

TABLE 3

Summary of Late Dry Season and Early Rainy Season
(Transitional Period) Larval Sampling,
Coastal El Salvador, 1968

River Collections	Date of Collection	No. Dips	No. Pos.	% Pos.	Total Larvae	Avg. No. Larvae/Dip
Comasagua *	May 6	50	35	70%	148	2.96
	June 6	20	4	20%	4	0.2
Chilama	May 10	100	45	45%	118	1.18
Grande **	May 10	100	14	14%	61	0.61
Tunco	May 14	100	12	12%	54	0.54
Pasture Collections						
San Antonio	May 14	50	23	46%	59	1.2
	May 27	100	32	32%	67	0.67
Jute	May 14	50	10	20%	16	0.32
Ticuiziapa	June 6	50	44	88%	488	9.8
	June 17	150	118	79%	432	2.9

* Adults resting under bridge, May 27, June 6, and June 17
** Adults resting under bridge, May 27 and June 17

Relationship of Estuary Lagoon Salinity and Anopheline Breeding

In February 1969, an investigation of the breeding of *Anopheles* species in Estero San Diego in relation to the salinity of the water was initiated. During the rainy season (May - October), the estero is open to the ocean and is subjected to tidal exchange twice every 24 hours. During this rainy period, the large volume of water being discharged into the estero by three rivers is sufficient to keep the estero open. As the rainy season ends, and the volume of water in the rivers decreases, the estero is closed off from the ocean by wave action. The result is a large impounded body of saline water which gradually loses its salinity until the rains come again and the estero is reopened. Figure 1 represents a general outline of the estero and the location of stations used for salinity determinations and larval dipping.

Initially, only nine stations were established in the estero area. Collected water samples were analyzed for total chloride content and the resultant value converted to salinity. Three of the nine stations were positive for the presence of larvae, and at these stations the salinity was 18.00 parts per thousand (ppt) or less. In March, all nine stations were positive for larvae, and the average salinity had dropped from 16.72 ppt to 14.67 ppt. In view of this observation, the number of stations was increased to obtain a better overall view of the situation. The salinity of the

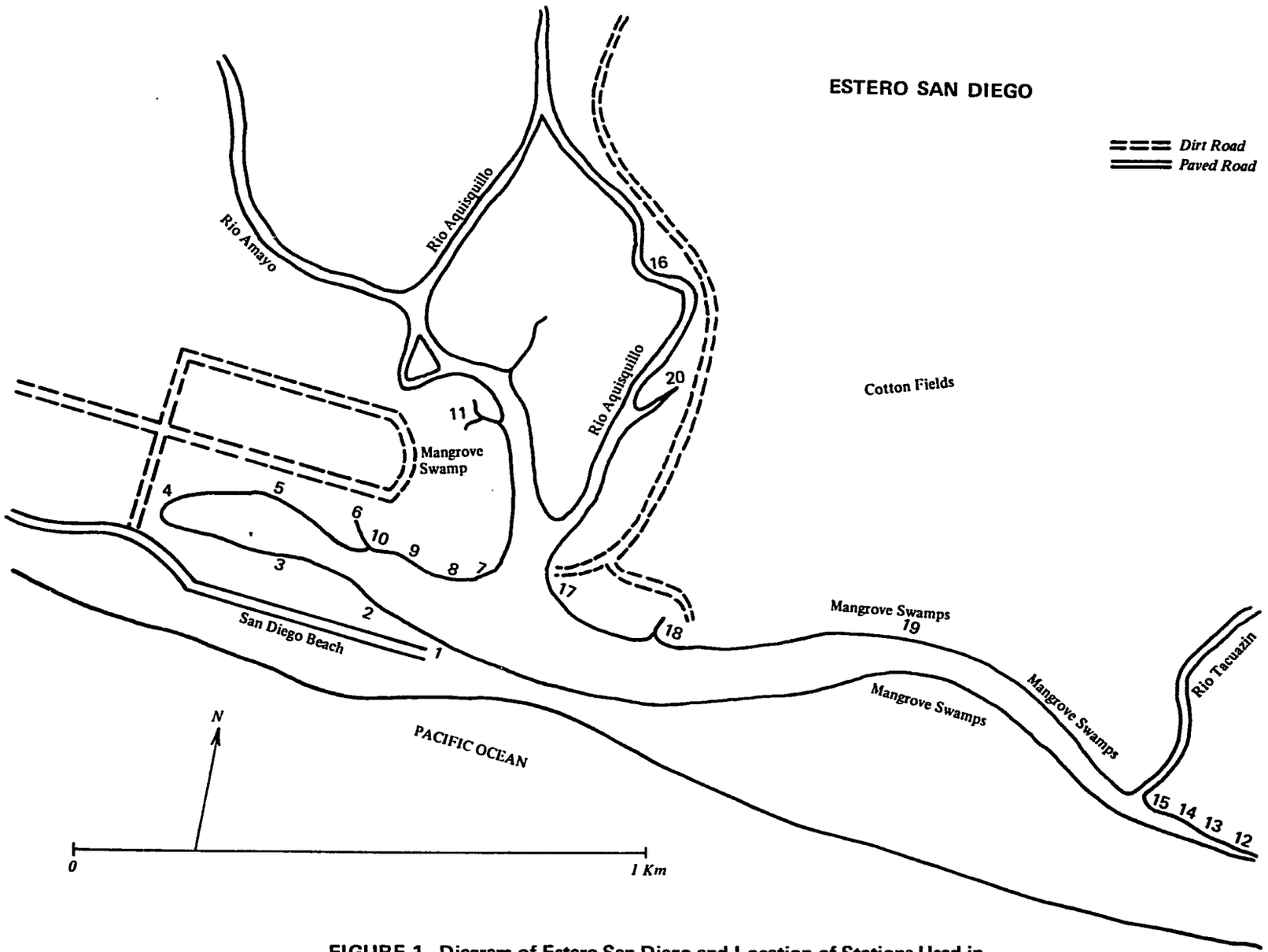


FIGURE 1. Diagram of Estero San Diego and Location of Stations Used in Water Sampling and Larval Dipping.

estero at these stations, and an indication of the presence or absence of larvae during the time from February through June 1969, are given in Table 4.

TABLE 4

Relation of Salinity (in parts per thousand) in Estero San Diego 1/ and Presence of Anopheles Larvae from February Through June 1969

Location 2/	February	March	April	May	June
1	-	13.42(+)	11.41(o)	9.10(+)	27.40(o)
2	-	14.43(+)	12.43(o)	9.70(+)	28.00(o)
3	-	17.41(+)	13.20(+)	11.61(+)	24.80(o)
4	-	17.36(+)	12.21(+)	15.90(+)	11.62(o)
5	-	15.98(+)	12.82(+)	10.61(+)	12.70(o)
6	-	17.12(+)	12.24(+)	9.90(+)	19.10(o)
7	19.66(o)	16.26(+)	11.91(+)	9.21(o)	28.61(o)
8	18.00(+)	13.60(+)	11.73(+)	12.40(+)	28.42(o)
9	-	13.60(+)	11.42(+)	9.01(o)	29.00(o)
10	19.60(o)	15.80(+)	-	-	-
11	15.32(+)	10.20(+)	9.31(+)	6.86(+)	21.61(o)
12	-	16.40(+)	13.10(+)	6.68(+)	0.30(o)
13	22.10(o)	16.20(+)	13.30(+)	-	1.86(o)
14	-	15.70(+)	-	10.04(+)	2.90(o)
15	-	14.24(+)	-	-	26.50(o)
16	3.86(+)	7.36(+)	5.61(+)	3.68(+)	26.80(o)
17	15.96(o)	12.10(+)	10.42(+)	6.50(+)	30.24(o)
18	17.98(o)	14.00(+)	11.00(+)	9.10(+)	26.76(o)
19	17.98(o)	17.60(+)	11.10(+)	11.52(o)	-
20	-	-	7.90(+)	7.79(+)	29.70(o)
Average	16.72	14.67	11.24	9.38	20.90

1/ Salinity based on the total chloride content.
2/ Salinity of ocean water taken at beach = 33.95 parts per thousand. See Figure 1.
(+) = larvae present
(o) = larvae absent

As long as the estero remained closed, there was a continual decrease in salinity accompanied by an observable increase in the number of larvae. In May, an attempt was made to establish what the population index of the Anopheles larvae was in the estero. Figure 2 shows the areas sampled, and Table 5 represents the population found in each. As can be seen by the data, 75 percent of the dips were positive with an average of 11.34 larvae per dip. These findings indicate the area is a most suitable environment for breeding with subsequent development of a large adult population. During the latter part of May the rains became more frequent, and during the first week of June the estero was mechanically opened. As can be seen in Table 4, the salinity increased from an average of 9.38 ppt in May to an average of 20.90 ppt in June. It is estimated that, at low tide, the decrease in water area is approximately 75 percent. No larvae could be found at any of the stations three days after opening the estero. The entire area was, in effect, flushed clean.

TABLE 5

Survey of Anopheles Larvae in Estero San Diego - May 1969

Location*	No. Dips Positive/ No. Dips Taken	% Dips Positive	Larval Instars 1	2	3	4	Total No. Larvae	Avg. No. Larvae/ Dip
A	66/80	82.5	86	103	96	116	401	5.01
B	65/100	65.0	175	47	43	50	315	3.15
C	116/130	89.2	565	453	327	277	1622	12.48
D	81/90	90.0	191	207	196	176	770	8.56
E	167/185	90.3	1228	1345	1323	591	4487	24.25
F	23/60	38.3	61	35	34	26	156	2.60
G	69/145	47.6	83	187	42	43	355	2.45
H	174/215	80.9	911	875	668	836	3290	15.30
TOTAL	761/1005	-	3300	3252	2729	2115	11396	-
AVERAGE	-	75.72	-	-	-	-	-	11.34

* See Figure 2.

The low salinity values at stations 12, 13, and 14 are attributed to the incoming tide pushing the fresh water of the Río Tacuazin back into this area. Although the salinity levels at these three stations at the time of sampling favored breeding, once the tide goes back out, the waters rapidly drain leaving the area void of free surface water. It is anticipated that this study will continue another year to allow a more complete evaluation of these breeding sites. Future studies will include an evaluation of the effect on anopheline breeding of keeping the estero open all year.

During the above study, an interesting observation was made on the habitat of the larvae. Numerous dead leaves were floating partially submerged in the waters throughout the estero. The larvae appeared to use the water film on the top of these as a hiding place from the numerous fish in the water, and on one instance as many as 18 larvae were counted in the water film above one leaf. The species of fish has not yet been determined; however, laboratory tests have shown them to be quite capable of devouring the larvae if given a chance to see and get at them.

Natural Daytime Resting Places of Anophelines

The evaluation of area-wide techniques, e.g., ULV and larviciding, as possible malaria eradication tools has required mosquito indexing methods apart from the more commonly used intradomiciliary and extradomiciliary counts associated with residual spraying of dwellings. This need has resulted in a rather extensive accumulation of data regarding the daytime resting places of both A. albimanus and A. pseudopunctipennis in El Salvador.

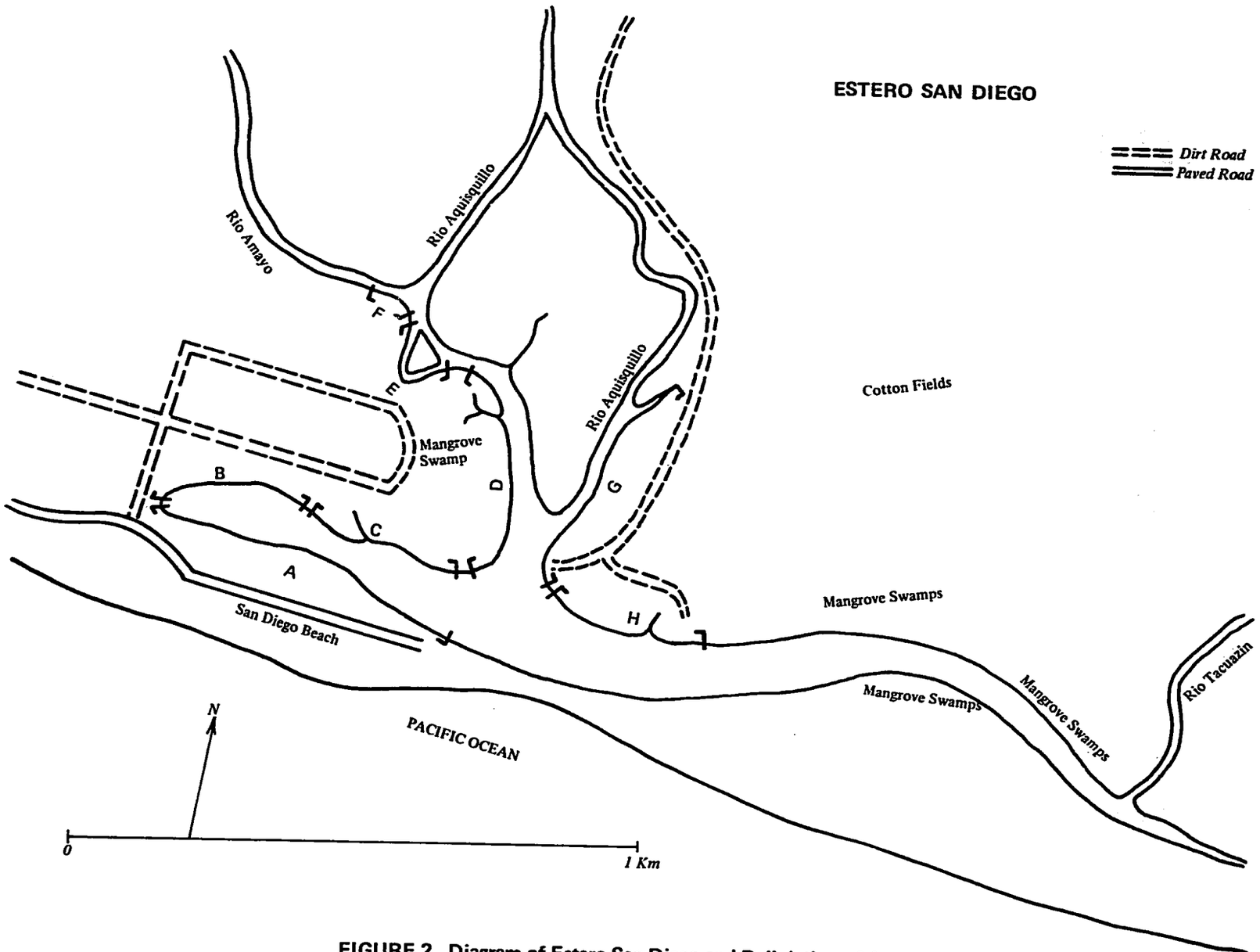


FIGURE 2. Diagram of Estero San Diego and Deliniation of Areas Sampled for Anopheline Larval Population Index.

Since February 1968, a number of observations and collections have been made which indicate that natural diurnal resting sites are not difficult to locate and that such sites offer important anopheline density measurement opportunities. Related observations might help elucidate movement habits of Anopheles, particularly in regard to breeding situations. Favored types of resting sites have been characterized on the basis of observations to date, and this information along with collection data is summarized in Table 6. Large rocks in wooded areas have yielded the best results, followed by rock walls associated with housing or animal shelters, small rocks at ground level in woodlands, lava deposits in shaded areas, tree trunks, and cavities. Miscellaneous sites include such niches as shaded animal burrows and cliffside holes.

Dry Season Survival of Anophelines - Laboratory Studies of Mud as an Oviposition Substrate.

The climate of El Salvador, like that of other Central American countries, is characterized by an extended dry season that usually lasts from November to May. During this period, favorable habitats for both larval and adult mosquitoes are minimal. Floodwater mosquitoes, inhabiting the same areas as anophelines during the rainy season, survive the dry season adversity in the same manner as floodwater mosquitoes survive a winter in North America, i.e., by dormant eggs in the soil. The question arises as to how anophelines survive this period in numbers sufficient to account for rather dramatic increases associated with the advent of the rainy season.

Stone and Reynolds (1939, "Hibernation of Anopheline Eggs in the Tropics." Science 90 (2338): 371-372), reported that in Panama sudden increases in anopheline larvae and adults occurred seven to ten days after the onset of the rainy season. In early June 1968, a large population of anopheline and floodwater aedine larvae was observed coexisting in a coastal pasture in El Salvador flooded in the initial rainy season inundation. Other unconfirmed reports of similar observations are known. In light of these occurrences, and notwithstanding the possibility of such survival mechanisms as aestivation and the fact that some anopheline breeding continues throughout the dry season, it was concluded that further studies on possible dry season survival mechanisms associated with the egg stage of Central American anophelines are warranted. This report is concerned with laboratory aspects of the problem, i.e., the relative suitability of mud as an oviposition substrate and the fate of eggs that are deposited on mud.

To determine the suitability of mud as an oviposition substrate, typically two gravid A. albimanus females from an insectary colony were introduced into lantern globes placed over field mud from a source known to support A. albimanus breeding. The mud was saturated with local, bottled drinking water as is used in the insectary colonies. Care was taken that no free water was present. The females were provided with a sugar water pad on the screened top of the globe. The open bottom of the globe fit snugly inside the rim of a Petri dish. Upon obtaining eggs on mud substrate, the dishes were labeled and served as stock egg supplies for various experiments to determine the fate of eggs under different drying conditions. For experiments requiring large numbers of eggs of a known age or for experiments concerning colony mosquito reaction to mud or other substrates, Petri dishes containing the substrate(s) were introduced into colony cages; the eggs obtained were handled in the same manner as those from globes.

The initial Petri dish experiments indicated that colony albimanus would readily accept field mud as an oviposition substrate. Further experiments with isolated females in lantern globes and with caged colony mosquitoes demonstrated a preference for mud when compared to other substrates, including bottled water, that was being used routinely in colony cages. The data in Table 7 show these results.

TABLE 6

Summary of Captures of Diurnally Resting Adult Anophelines from Natural Situations
El Salvador, February 1968 - June 1969

Type of Site	No. of Coll.	A. albimanus				A. pseudo-punctipennis				Both Species				Comments
		M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.	
Large Rocks. Beneath and between large angled or flat rocks in shaded woodlands or woodland-field ecotones.	43	652	272	924	21.5	203	183	386	9.0	855	455	1310	30.5	Excellent collecting situation--albimanus in more exposed sites; pseudo-punctipennis in the darker and more protected sites.
	2	-	-	7	3.5	-	-	140	70.0	-	-	147	73.5	Mosquitoes not counted by sex.
Rock Walls. Rock, stone, or lava walls near breeding sites, human habitation, or animal concentrations.	12	116	543	659	54.9	0	0	0	0	116	543	659	54.9	Such walls are common in high density situations (around huts, cattle corrals, etc.), a good index situation.
	1	-	-	400	400.0	-	-	0	0	-	-	400	400.0	Mosquitoes not sexed. This concentration represents a 50-foot section of wall near breeding and cattle.
Small Rocks and Tree Trunks. Beneath or among small rocks in shaded areas at ground level and among associated tree roots and trunks.	11	100	317	417	37.9	4	48	52	4.7	104	365	469	42.6	Offers numerous niches in shaded woodlands near breeding areas--albimanus in more exposed, pseudo-punctipennis in more protected niches.
Tree hollows and roots. In cavities of large trees between or among above-ground roots forming cavity-like recesses.	10	31	40	71	7.1	23	10	33	3.3	54	50	104	10.4	A. albimanus in more exposed sites. A. pseudo-punctipennis in more protected sites.

TABLE 6

(Continued)

Type of Site	No. of Coll.	A. albimanus				A. pseudo-punctipennis				Both Species				Comments
		M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.	
Ground Holes. Protected ground holes (leaves and twigs) and associated rocks and tree depressions at ground level in shaded areas.	13	115	166	281	21.6	37	29	66	5.1	152	195	347	26.7	More common in soft ground associated with swamp or lowland conditions, particularly mangrove swamps.
Lava Deposits. Large lava deposits in shaded areas, many large and small recesses.	2	63	189	252	126.0	0	0	0	0	63	189	252	126.0	Offers numerous niches. These lava beds were in banana groves near breeding (200-300 meters). Probably too much light for pseudo-punctipennis.
Large Cliffside. Abrupt cliffside with moderate to heavy vegetative cover. Large and small rock outcroppings, cave-like recesses.	2	9	9	18	9.0	8	3	11	5.5	17	12	29	14.5	A. albimanus in more exposed sites; A. pseudo-punctipennis more protected.
	2	-	-	90	45.0	-	-	106	53.0	-	-	196	98.0	Mosquitoes not sexed; 1 man-hour, each collection.
Tree Trunks and Fallen Logs. Shaded outside of tree trunks and on underside of logs near ground. Shaded areas.	6	24	18	42	7.0	0	0	0	0	24	18	42	7.0	A. albimanus in rather exposed sites (e.g., a rotted stump in open field).
Small Rocks, Shaded Margins of Stream Beds. Beneath or among such rocks.	3	0	12	12	4.0	3	0	3	1.0	3	12	15	5.0	Offers very scattered, isolated resting sites; requires much searching, but probably an important dry season niche.

TABLE 6

(Continued)

Type of Site	No. of Coll.	A. albimanus				A. pseudo-punctipennis				Both Species				Comments
		M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.	
Miscellaneous Sites. Horizontal cliffside cavity behind waterfall.	1	0	0	0	0	16	14	30	30.0	16	14	30	30.0	Very dark, favors pseudopunctipennis.
Base of Century Plant	1	3	14	17	17.0	0	0	0	0	3	14	17	17.0	Probably too exposed for pseudopunctipennis.
Animal Burrow (probably armadillo). Around mouth and in cavity.	1	34	57	91	91.0	0	0	0	0	34	57	91	91.0	A high yield situation with a much greater concentration than other type niches in the same area. Mosquitoes around mouth and in neck of burrow.
Example: A 1-man hour collection from a likely diurnal resting situation 200-300 meters from a breeding habitat. Miscellaneous rocks, tree cavities, etc. (near La Libertad, El Salvador).	1	13	3	16	16.0	54	58	112	112.0	67	61	128	128.0	Represents the type results that one might expect from natural diurnal resting situations during moderate or high density periods.
Summary:														
Sexed collections	106	1160	1640	2800	26.4	348	345	693	6.5	1508	1985	3493	33.0	
Collections not sexed	5	-	-	497	99.4	-	-	246	49.2	-	-	743	148.6	**
All collections - total mosquitoes	111	-	-	3297	29.7	-	-	939	8.5	-	-	4236	38.2	

** The 111 collections yielded 3,297 albimanus adults (77.8%) and 939 (21.2%) pseudopunctipennis adults. The sex ratio of albimanus was 41.4% males and 58.6% females; of pseudopunctipennis 50.2% males and 49.8% females; of both species 43.2% males and 56.8% females. A. pseudopunctipennis showed a decided preference for darker and more protected places than did albimanus. Large and small rocks, rock walls, and scattered rocks in association with ground holes and tree bases offered the favored resting sites to both species.

TABLE 7

Number of Eggs Deposited by A. albimanus Females on Different Substrates in Petri Dishes. July 31, 1968

Dish No.	No. of Females	Substrate*				
		Mud	Water over Mud	Water over Sand	Saturated Sand	Water
1	2	82	54	0	0	0
2	2	86	0	28	0	75
3	2	57	65	0	0	0
4	2	120	0	0	0	68
TOTAL	8	345	119	28	0	143

* Beach sand, field mud, bottled drinking water.

Additional observations (Table 8) showed that colony mosquitoes under laboratory conditions chose mud over water when given a choice and that the source of mud made little difference (Table 9).

TABLE 8

Number of Eggs Deposited by A. albimanus When Offered Mud and Water in Insectary Colony Cages, August 19, 1968

Colony Cage No.	Petri Dish* Containing	
	Mud	Water
R-1	435 (2)**	10 (5)
	360 (1)	46 (9)
R-2	280 (2)	0 (0)
	633 (3)	53 (1)
R-3	212 (0)	25 (6)
	340 (1)	17 (2)
R-4	520 (2)	13 (2)
	350 (3)	11 (2)
S-1	27 (0)	0 (0)
	62 (0)	0 (0)
S-2	0 (0)	0 (1)
	0 (0)	0 (0)
S-3	132 (0)	0 (2)
	0 (0)	0 (0)
TOTAL	3,351 (14)	175 (30)

* Two Petri dishes of mud and two of water in each of seven colony cages.

** Numbers in parentheses indicate dead mosquitoes on the surface of the oviposition dish.

TABLE 9

Number of A. albimanus Eggs Deposited on Field Mud (From a Known Habitat) and Garden Mud (From a Non-anopheline Habitat), August 20, 1968

Cage No.	Field Mud	Garden Mud
R-2*	250	280
	148	135
R-3	137	88
	310	380
R-4	180	310
	1,000	750
TOTAL	2,025	1,943

* Two Petri dishes of each substrate in each of three colony cages.

Having determined that albimanus would deposit its eggs on a mud surface, it remained to determine the fate of such eggs under varying conditions of drying. It was found that:

1. When mud with eggs was allowed to air dry gradually, eggs survived and remained hatchable up to five days after being deposited even though the mud had become crusty or even powder-dry (Table 10).

2. When mud with eggs was allowed to air dry for a time but was re-moistened before a powdery dry state was reached, hatchability was maintained for up to 11 days after the eggs were deposited (Table 10).

3. When mud with eggs was kept saturated (no free water) with a cover on the Petri dish, they remained hatchable for as long as 15 days (Tables 10 and 11), and the larvae literally "exploded" from their shells when inundated (Tables 11 and 12).

4. When eggs obtained on mud on a given day were flooded immediately, the hatching pattern was as though they had been deposited on water, i.e., they hatched two days later and in an irregular manner.

5. When eggs were held 24 hours on mud before being inundated, they hatched on the same day as those flooded immediately, but hatching was more uniform for the lot (Table 12).

6. When eggs were held at least 48 hours on mud, hatching was immediate and was completed within minutes after inundation. This varied with the pre-inundation holding time; Table 12 shows hatching patterns of eggs after being held on mud for varying periods and flooded at 24-hour intervals. Optimum flooding times in terms of rate and completeness of hatch were found to be at 96 or 120 hours after the eggs were deposited.

   Eggs that had been held on mud for 96 hours prior to inundation began hatching within seconds, 50 percent hatching had occurred in a little more than two minutes, hatching was complete for all of the 12 lots within an average time of five minutes, and 99 percent of all eggs handled in this manner hatched.

TABLE 10

Hatching Records of *A. albimanus* Egg Batches Resulting from Inundation of a Portion of the Eggs on Mud at Fixed Intervals after Deposition, August 1968.

Dish No.	No. of Eggs Deposited	Date of Oviposition	Condition of Mud (Exposed to Air)	Date of First Hatching	Date of Last Hatching	No. days Remained Hatchable
S-1	170	Aug. 4	Aug. 6, hard, cracked; Aug. 8, re-moistened	Aug. 6	Aug. 15	11
S-2	102	Aug. 3	flooded immediately (20)*	Aug. 5	Aug. 6	3
			air dried gradually (44)	Aug. 6	Aug. 6	3
			moisture maintained (38)	Aug. 7	Aug. 18	15
S-3	122	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S-4	155	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S-5	350	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S-6	86	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S-7	28	Aug. 3	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	5
S-8	150	Aug. 3	flooded immediately (110)	Aug. 5	Aug. 6	3
			air dried gradually (25)	Aug. 5	Aug. 8	5
			moisture maintained (15)	Aug. 9	Aug. 11	8
S-9	105	Aug. 4	Aug. 6, cracked mud Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S-10	114	Aug. 3	flooded immediately (20)	Aug. 5	Aug. 5	2
			air dried gradually (64)	Aug. 5	Aug. 6	3
			moisture maintained (30)	Aug. 5	Aug. 12	9
S-11	73	Aug. 3	Aug. 6, dry, cracked mud Aug. 8, crusty dry	Aug. 6	Aug. 8	5
R-1	205	Aug. 3	Aug. 6, powdery dry	Aug. 5	Aug. 7	4
R-2	226	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 8	5
R-3	136	Aug. 4	Aug. 6, cracked, dry	no hatch	---	--
R-4	25	Aug. 4	Aug. 6, cracked, dry Aug. 8, crusty	Aug. 6	Aug. 8	4
R-5	165	Aug. 4	Aug. 6, cracked, dry Aug. 8, remoistened	Aug. 6	Aug. 14	10
R-6	200	Aug. 4	Aug. 6, cracked, dry	Aug. 6	Aug. 8	4
R-7	140	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 6	3
R-8	32	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 6	3
R-9	130	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 8	5
R-10	83	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 8	5
R-11	65	Aug. 4	Aug. 6, powdery dry	Aug. 6	Aug. 8	4
R-12	52	Aug. 4	Aug. 6, cracked, dry	Aug. 6	Aug. 8	4

*Numbers in parentheses refer to sub-lots of eggs subjected to distinct treatments.

TABLE 11

Hatching Records of *A. albimanus* Egg Batches Inundated at Fixed Intervals After Being Held on Oviposition Mud in Covered Petri Dishes.

Dish No.	No. of Eggs	Date of Oviposition	Date of First Hatching*	Date of Last Hatching	No. Days Eggs Remained Hatchable
1	108	Aug. 18	Aug. 20	Aug. 28	10
2	92	Aug. 17	Aug. 20	Aug. 28	11
3	63	Aug. 17	Aug. 20	Aug. 28	11
4	78	Aug. 18	Aug. 20	Aug. 28	10
5	91	Aug. 16	Aug. 20	Aug. 28	12
6	34	Aug. 16	Aug. 20	Aug. 23	7
7	127	Aug. 17	Aug. 20	Aug. 28	11
8	65	Aug. 18	Aug. 20	Aug. 28	10
9	None	---	---	---	--
10	138	Aug. 16	Aug. 20	Aug. 28	12
11	206	Aug. 17	Aug. 20	Aug. 28	11
12	84	Aug. 18	Aug. 20	Aug. 28	10
13	45	Aug. 16	Aug. 20	Aug. 28	12
14	150	Aug. 16	Aug. 20	Aug. 28	12
15	None	---	---	---	--
16	95	Aug. 17	Aug. 20	Aug. 28	11
17	142	Aug. 18	Aug. 20	Aug. 28	10

*Hatching occurred in seconds after inundation during first several days and increased to a matter of several minutes toward the final hatching date. Note that no hatching was attempted earlier than 48 hours after oviposition.

TABLE 12

Average Hatching Time in Minutes and Hatching Percentages of 12 Lots of *Anopheles albimanus* Eggs Held on Mud for Varying Periods of Time Prior to Inundation

Hatching Pattern	Pre-Inundation Holding Time							
	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 Hrs.	144 Hrs.	168 Hrs.	>168 Hrs.
Initial Hatch	*	1.3 Min	<1.0 Min	<1.0 Min	<1.0 Min	2.0 Min	2.8 Min	**
50% Hatch		5.5 "	5.7 "	2.3 "	2.8 "	7.6 "	7.6 "	
90% Hatch		21.4 "	31.5 "	4.6 "	6.7 "	15.0 "	16.1 "	
Ultimate Hatch		78.7 "	104 "	5.0 "	7.1 "	26.6 "	18.6 "	
No. Eggs Flooded	325	260	279	266	254	240	240	
No. Eggs Hatched	308	253	258	265	244	128	79	
Percent Hatch	94.8%	97.3%	92.5%	99.0%	96.0%	53.3%	32.8%	

* Eggs held on mud for less than 48 hours behaved as though they were deposited on water, e.g., eggs inundated after being held for 24 hours on mud showed the following hatching pattern after being inundated: initial hatch - 24 hours; 50% hatch - 33 hours; 90% hatch - 48 hours; complete hatch - 54 hours.

** Even after 120 hours on mud (5 days) the hatching pattern became erratic, and a smaller percentage hatched. Hatching was progressively reduced from 32.8% at 168 hours to only 1.7% (4 of 240) after 216 hours (9 days).

TABLE 13

Monthly Pupal Production from Two Colony Cages with Mud and Two with Water as Oviposition Substrate, October 1968 - June 1969.

Month	Mud[1]	Water[2]
October 1968	37,700	23,600
November 1968	23,800	16,100
December 1968	14,000	7,900
January 1969	18,400	8,000
February 1969	28,300	17,100
March 1969 *	5,100	3,700
April 1969 *	3,000	4,300
May 1969	16,200	10,300
June 1969	11,700	6,500
T O T A L	158,200	97,500
Avg. No./Mo.	17,577	10,833

[1]Colony Cages R-1 and R-2

[2]Colony Cages R-3 and R-4

*Colonies reduced by removing mosquitoes for ULV work.

For the 12 lots in the 120-hour group, the average initial hatch was also a matter of seconds, 50 percent hatching had occurred in a little less than three minutes and hatching was complete in 7.1 minutes, with 96 percent of all eggs hatching.

The hatching pattern of eggs held 144 hours or longer more nearly resembled that of eggs held for 48 or 72 hours before inundation (Table 12).

The data are also shown graphically in Figure 3.

ne of the more useful applications of these results is the utility of mud as a sub-trate for insectary colonies. The fact that eggs are apparently more readily eposited on mud than on water, together with their immediate hatchability and ease f handling, facilitates more uniform larval rearing. For nearly a year, mud has een used as an oviposition substrate in two of four colony cages, and water has been sed as the substrate in the remaining two. The "mud-cages" have consistently out-roduced the "water-cages" as measured by daily production of pupae (Table 13).

n summary, A. albimanus females readily deposited their eggs on mud under laboratory onditions. When given a choice of media, the females showed a preference for mud. ome eggs survived for periods of up to 15 days on mud and retained their viability ven though the optimum flooding time was on the fourth or fifth day after deposition n mud. Hatching of eggs after being held on mud for the optimum period occurs within econds of inundation with almost all eggs hatching. A mud substrate for oviposition ffers several advantages over water in maintaining insectary colonies. These results end impetus to further investigation of the possible involvement of egg resistance o desiccation in the dry season survival of albimanus in Central America.

opulation Index Methodology: Design of Collapsible "Red Boxes" for Population ensity Measurement

e quantitative and qualitative measurement of anopheline population densities plays important part in entomologic and epidemiologic aspects of malaria investigations. orkers of the Tennessee Valley Authority in 1939 and 1942 found that empty nail kegs ould be effectively used as artificial resting places for measuring adult Anopheles adrimaculatus populations. An NCDC investigator in 1942 described a box-type rtificial resting place which, as tested in Georgia, proved more attractive to A. adrimaculatus adults than did the other types tested. The device consisted of a 2-inch wooden, cubical box, open on one side, with all interior surfaces painted red black. Boxes of this type, constructed of 1/4-inch plywood, show promise for opheline population measurement in entomologic work carried out by personnel of MRS. However, the space required for transporting necessary numbers of these boxes a truck or station wagon, or in off-road situations, represents a serious dis-vantage in the use of this index method.

mple modifications have been made in the design of the conventional "red box", re-lting in collapsible units which are easily transported and easily assembled for eld use. Eight or more of the collapsed boxes can be transported or stored in the ace required for one of the conventional boxes (Figure 4).

ritability of Anopheles albimanus

r some years, it has been appreciated that the effectiveness of a residual insecti-de in interrupting man-anopheline contact may depend not only on the toxicity of e insecticide when contacted by the mosquitoes, but also on possible irritation of e mosquitoes which may attempt to alight on sprayed surfaces before feeding, after eding, or during a period of daytime repose. Thus, an insecticide which no longer terrupts transmission by killing infective mosquitoes might conceivably still duce transmission if the mosquitoes--through irritation provoked by the insecticide--

FIGURE 3. Average Hatching Rates of 12 Lots of *A. albimanus* Eggs (minimum of 20/lot) When Inundated With Bottled Water After Being Retained on Mud for 48, 72, 96, 120, 144, and 168 Hours.

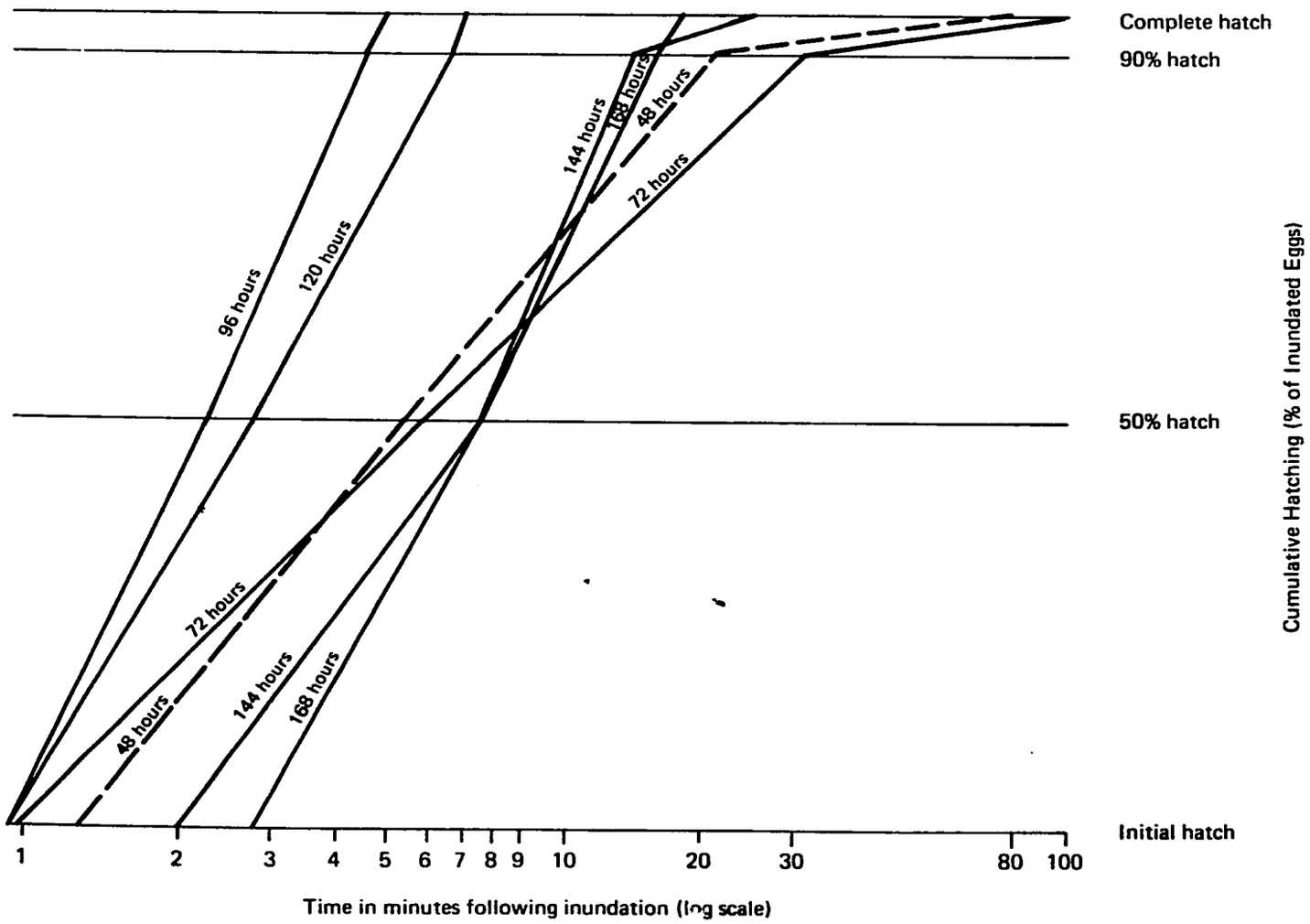


FIGURE 4

Comparison of Eight "Red Boxes" (Rigid Model)
with Eight "Red Boxes" (Collapsible Model)

avoid houses whose walls have been sprayed. Less happily, the irritation produced by an insecticide may result in less-than-lethal contacts by susceptible mosquitoes.

Preliminary tests of local A. albimanus were conducted using the WHO Mosquito Irritability Testing Kit. Mosquitoes from colony or field sources were pre-test-conditioned by holding them individually in tubes for 30 minutes or longer under reduced illumination. Lots of five females were then placed in plastic cones against a translucent substrate of standard WHO DDT-treated paper (2% concentration) or standard control (only Risella oil) paper. After a three-minute adaptation period, numbers of flights made during 15 minutes by the five exposed females and the five control females were counted. Average flights per female were calculated, and the ratio of flights by exposed and control females was calculated. An alternative WHO protocol using single females instead of five-female lots has not been tested.

Table 14 summarizes the average flights per mosquito and the exposed:control ratio of flights of resistant and susceptible mosquitoes from CAMRS colonies and of "wild-caught" mosquitoes from coastal areas near La Libertad, El Salvador.

TABLE 14

Summary of DDT Irritability Tests with Anopheles albimanus
(Using WHO Irritability Test Kit)
(Five Females per Cone)

			Flights				
	Mosqs. Tested		Made by		Avg. per Mosq.		Ratio
Source	Expos.	Contr.	Expos.	Contr.	Expos.	Contr.	Expos:Contr
Colony Metapan "Resistant"	65	35	4,604	238	70.8	6.8	10.4:1
Colony Metapan "Susceptible"	82	55	3,659	693	44.6	12.6	3.5:1
Wild-caught Coastal Areas	138	88	7,158	1,882	51.9	21.4	2.4:1

Tables 15, 16, and 17 summarize the tests made on distinct dates with mosquitoes from the three general sources.

The figures presented in Table 14 mask a great deal of variation evident in Tables 15, 16, and 17, which in turn mask even greater variations obtained in the basic trials with separate lots of five exposed and five control mosquitoes, to the extent that the validity of the testing procedure is questionable. According to observations during the separate trials, the presence of a single hypersensitive or hyperactive mosquito among a lot of five females results in exaggerated activity among the entire lot of mosquitoes in a cone exposure chamber. Additional testing with individual females, although more time-consuming, may give more consistent results.

TABLE 15

Irritability of Anopheles albimanus ("Resistant" Colony) Females to 2% DDT Papers Using Five Females per Cone in WHO Irritability Test

Date	Source	Mosqs. Tested		Flights Made by		Flights Avg.per Mosq.		Ratio
		Expos.	Contr.	Expos.	Contr.	Expos.	Contr.	Expos:Contr
*12/13/68	PAHO "Resistant"	5	5	261	85	52.2	17.0	3.1:1
* 1/5/69	PAHO "Resistant" (R-2)	5	5	212	63	42.4	12.6	3.4:1
** 1/21/69	PAHO "Resistant" (R-3)	40	10	3,056	52	76.4	5.2	14.7:1
* 2/2/69	PAHO "Resistant" (R-3)	10	10	820	14	82.0	1.4	58.6:1
** 5/8/69	PAHO "Resistant"	5	5	255	24	51.0	4.8	10.6:1

* Fed on guinea pig during afternoon of previous day.
** Recently engorged.

TABLE 16

Irritability of Anopheles albimanus ("Susceptible" Colony) Females to 2% DDT Papers Using Five Females per Cone in WHO Irritability Test

Date	Source	Mosqs. Tested		Flights Made by		Flights Avg.per Mosq.		Ratio
		Expos.	Contr.	Expos.	Contr.	Expos.	Contr.	Expos:Contr
*12/12/68	PAHO Metapan	5	5	209	0	41.8	0.0	---
*12/19/68	CAMRS Metapan	5	5	427	8	85.4	1.6	53.4:1
*12/19/68	PAHO Metapan (S-2)	5	5	281	55	56.2	11.0	5.1:1
* 1/5/69	PAHO Metapan (S-3)	5	5	327	262	65.4	52.4	1.2:1
** 1/20/69	PAHO Metapan (S-1)	38	10	1,338	7	35.2	0.7	50.3:1
** 1/28/69	PAHO Metapan (S-1)	15	10	712	178	47.5	17.8	26.7:1
** 1/28/69	PAHO Metapan (S-2)	9	15	365	183	40.6	12.2	3.3:1

* Fed on guinea pig during afternoon of previous day.
** Recently engorged.

TABLE 17

Irritability of Anopheles albimanus ("Wild-Caught") to 2% DDT Papers Using Five Females per Cone in WHO Irritability Test

Date	Source (all from area La Libertad)	Mosqs. Tested		Flights Made by		Flights Avg.per Mosq.		Ratio
		Expos.	Contr.	Expos.	Contr.	Expos.	Contr.	Expos:Contr
1/17/69	El Coco (Stable)	35	15	1,758	216	50.2	14.4	3.5:1
1/22/69	El Coco (Stable)	35	10	1,721	282	49.2	28.2	1.7:1
1/22/69	Sta.Emilia (Stable)	5	10	281	36	56.2	3.6	15.6:1
1/25/69	Sta.Emilia (Stable)	9	9	593	204	65.9	22.7	2.9:1
1/25/69	El Coco (Stable)	14	14	539	271	38.5	19.4	2.0:1
1/29/69	El Coco (Stable)	15	10	784	328	52.3	32.8	1.6:1
1/29/69	Potrero de Tiqu. (Corral)	15	10	785	279	52.3	27.9	1.9:1
2/12/69	El Coco	10	10	697	266	69.7	26.6	2.6:1

Note: All specimens were collected in afternoon (under bridges) or in the evening (stables) and held overnight on sugar-water.

Detection of Minute Quantities of Insecticide on Mosquito Tarsi

An experiment was designed to establish whether the amount of p,p'-DDT adsorbed on the tarsi of mosquitoes could be determined. Three groups of 20 A. albimanus were placed in separate cylinders lined with WHO test papers impregnated with 0%, 2%, and 4% p,p'-DDT in Risella oil. The mosquitoes were held for one hour, removed, immobilized and the tarsi removed. The tarsi were then extracted with hexane, the solvent decanted and concentrated to a desired volume. Analysis was by electron capture gas chromatography.

A peak was detected in all three groups of mosquitoes which corresponded to p,p'-DDT. Considering the amount of chemical present in the mosquitoes exposed to the 0% papers as representative of a possible previous exposure for the group as a whole, this quantity was then subtracted from that amount found in the mosquitoes exposed to the 2% and 4% test papers. The results were 2.34 ppm for those on the 2% papers and 7.94 ppm for those on the 4% paper.

Since this represents only a single test, and the absence of suitable chemicals prevented a clean-up procedure or confirmation by thin layer chromatography, these data should be considered as tentative only. Future work on the problem is planned.

VECTOR CONTROL STUDIES

Exterior Applications of Residual Insecticides to Houses versus Conventional Interior Treatments

The study of using an outside treatment of candidate insecticides on rural houses was begun in a portion of the Village of Zapotal where a group of houses is located along the shores of a small lagoon. The houses are of mixed construction; poles, thatch palm, and other plant products are used. The houses lie in a line along the lagoon, and all had recently been treated inside routinely by the CNAP with DDT at $2g/m^2$. Two were selected for outside treatment with Dursban at $2g/m^2$; and, at a distance of approximately 400 meters, two others were chosen to be treated with DDT at $2g/m^2$. In between these houses, another was chosed as a "control." The outside treatment was done by an experienced CAMRS employee on June 18, 1969. The exterior wall surfaces were treated from the top of the wall including the eaves to the ground.

1. Pretreatment study.

   a. Bioassay of outside pole walls. On May 21, the outside pole walls were bioassayed using a special chamber developed for this purpose. Two chambers were used to a side, one in the upper half of the wall and the other in the lower half. Ten to 15 blood-fed females of the El Jocatal strain were used per chamber. These tests were made during the day and at night with a 60-minute exposure. The results are summarized below:

House No.	% Mort. at 24 hours Daylight	Evening	
504	23	6	(Upper half of walls)
	0	15	(Lower half of walls)
502	38	14	
	8	4	
507B	25	3	
	0	3	

   Note: The outside of the houses under the eaves is routinely treated by CNAP when spraying the house with DDT.

   b. Early evening captures. Captures in the early evening were made, based on one man per house capturing from his exposed legs, during a period of two hours outside and one hour inside with the following results:

House No.	Date	Number of bites per hour Peri	Intra
504	May 29	14.5	35.0
	June 5	45.0	30.0
502	May 29	8.0	2.0
	June 5	11.0	12.0
507B	May 29	.5	0
	June 5	10.5	5.0

   c. Early morning captures. On June 6, early morning house searches for albimanus were also conducted prior to treatment, with approximately 15 minutes being spent per house searching for live mosquitoes and five minutes for dead ones, with the following results:

House No.	Total Per House	B/F	Gravid	N/F
504	5	1	4	0
501	6	0	3	3
502	9	0	7	2
507B	1	0	1	0
507A	14	2	10	2

d. Pretreatment all night captures. All-night captures were made with one man per house. Each man started outside, 1800 to 2000 hours, and then went inside until 0600. A man was present for supervising and checking during the night to see that all capturers were awake. The results are summarized below:

House No.	Total Captured	Avg. bites per man per hour		Avg. bites per man for two houses	
		Peri	Intra	Peri	Intra
504	196	24	15	-	-
501	225	58	11	35	6
502	38	11	2		
507A	137	11	12	11	6
507B	33	12	1		

2. Post-treatment study.

a. Post-treatment all night captures. These captures were made one week after treatment using the same houses as before. Due to the marked differences noted between some baits, the baits for this capture were used in the same houses where they had worked previously. The same routine was used as to peri- and intra-domiciliary capture with one exception, the 15 minutes of the capture before 0600 were used by each man in making an inside search of the houses to find live or dead albimanus. The results are shown below:

House No.	Type of outside treatment	Total No. Captured	Avg. bites per man per house		Avg. bites for both treat. houses		Total No. found resting in morning
			Peri	Intra	Peri	Intra	
504	None (Control)	125	41	4	-	-	5
501	Dursban 2g/m^2	262	74	12	49	7	25
502	" "	74	25	3			
507A	DDT 2g/m^2	100	20	6	28	5	4
507B	" "	117	36	5			

b. Post-treatment bioassay tests. The surfaces of houses 504 (control), 502, and 507B were bioassayed on June 27, one week after treatment. For the treated huts two cones were used per side of each house, one up and one down. The local strain of mosquitoes captured in the corral of Zapotal were used. The exposure period was 60 minutes. As the tests were done during the day, the chambers were covered with several pieces of paper toweling and a black cloth. The results of this test are shown below:

House No.	Treatment	Wk	Percent mortality at 24 hrs. Upper portion	Lower portion	Paper control
504	None	-	2		0
502	Dursban	1	100	81	
507B	DDT	1	22	2	

Based on the pretreatment captures and bioassay tests as compared with those of post-treatment and of the control house, these preliminary results of the outside treatments were not too encouraging.

Ultra Low Volume (ULV) Aerial Application of Insecticides

Two separate but integrated projects involving the ULV aerial application of malathion against Anopheles mosquitoes were conducted during the dry season, February-May 1969.

Project I was concerned with the evaluation of the ULV technique as an antimalaria tool through vector control in a 3,000 acre coastal plain near La Libertad (see map, Figure 5). The plan called for the ULV aerial application of malathion at the rate of three fluid ounces per acre over the entire area at weekly intervals for three successive weeks with the aim of reducing the existing mosquito population to near zero. This set of treatments was to be followed by successive treatments at 17- or 18-day intervals with the aim of killing potentially infective females before sporozoites could be developed. The first five treatments (February 18, 25; March 3, 20; April 8) were conducted on schedule, but the La Libertad area was abandoned after the fifth treatment in favor of an area near Acajutla (see map, Figure 6) where albimanus had been shown to be more susceptible to malathion. The approach of three treatments at weekly intervals was repeated (April 17, 24; May 5) in the Acajutla area since the La Libertad trials had not clearly shown significant reductions of the anopheline population.

Project II was designed to protect a large segment of the population against malaria infection during the critical Holy Week period (March 28-April 6). During this week, thousands of persons visit the beaches along the coast of western El Salvador, thereby becoming subject to acquiring malaria infections. It was hoped that some subsequent epidemiologic readout might be obtained by comparing new cases from the eastern coast of El Salvador (untreated) to those from the western coast (treated). Meanwhile, only a limited entomologic evaluation was possible. The treatments were carried out as scheduled on March 20, 29, and April 7, 1969 (see map, Figure 6).

A summary of all ten ULV treatments comprising both projects is as follows:

Treatment I. February 18, 1969. La Libertad, 3,000 acre triangle, first of three successive weekly treatments to reduce the Anopheles population. Three fluid ounces of technical malathion were applied per acre at early dawn in good weather, with an average of 24.1 droplets per square inch being obtained on malathion-sensitive dye cards. A discrepancy was noted between adult mortality in cages and larval mortality in ice cream cartons (higher mortality among larvae than adults even though adults were targeted) as related to droplet distribution on adjacent dye cards. It

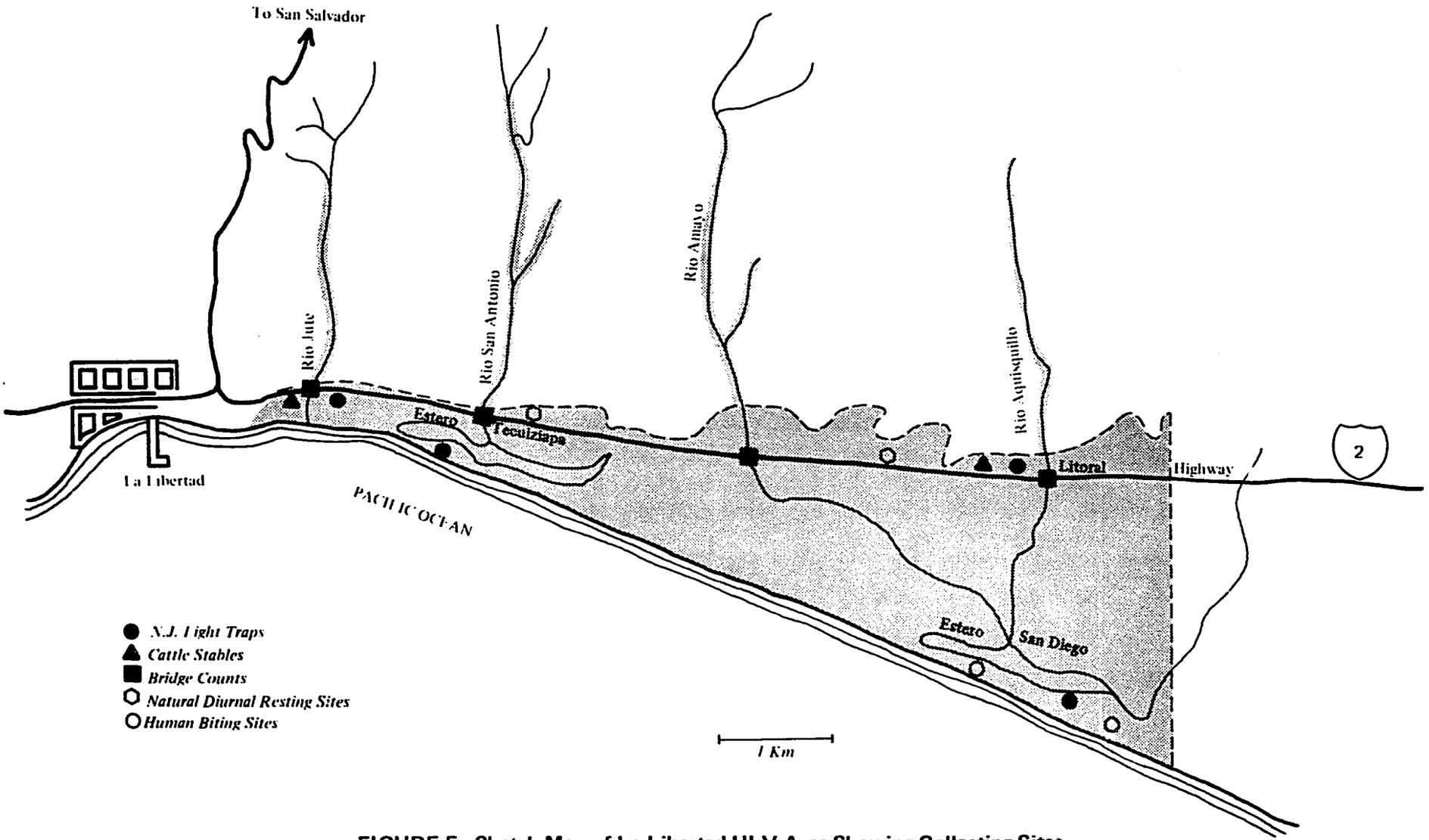
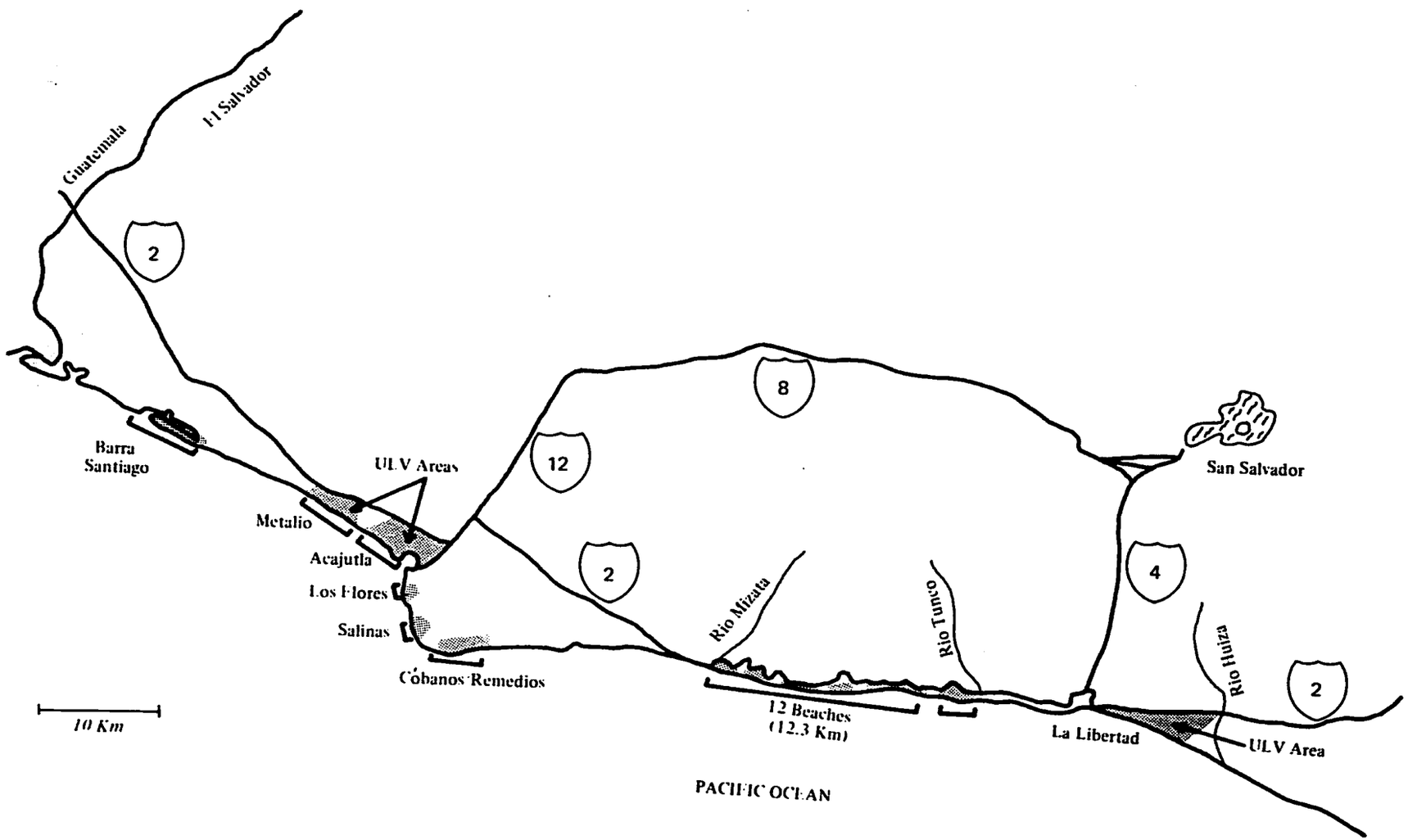


FIGURE 5. Sketch Map of La Libertad ULV Area Showing Collecting Sites. Treated Areas Stippled.

FIGURE 6. Sketch Map of Western Coastal El Salvador Showing La Libertad ULV Area, and Littoral ULV Areas in Stippling.



was surmised that aluminum cages might be interfering with droplet penetration through the cage wire.

Treatment II. February 25, 1969. La Libertad, 3,000 acre triangle, second of three successive weekly treatments. Three fluid ounces were applied per acre at early dawn in good weather, with an average of 22.6 droplets per square inch. Dye cards placed inside aluminum cages received an average on only 7.7 droplets per square inch as compared to 22.6 droplets per square inch on uncaged cards (20 caged and 20 uncaged exposed cards). It was concluded that the aluminum screening must be interfering with droplet penetration inside the cages.

Treatment III. March 3, 1969. La Libertad, 3,000 acre triangle, third of three successive weekly treatments. Three fluid ounces were applied per acre under marginal wind conditions at early evening, with an average of only 4.0 droplets per square inch. Special tests to find a more suitable screening material than aluminum resulted in 16-mesh galvanized and 20-mesh nylon cages giving droplet penetration essentially equal to that of unscreened cards. All subsequent tests were conducted using new cages made of galvanized wire.

Treatment IV. March 20, 1969. La Libertad, 3,000 acre triangle, first of the 17-18-day treatments applied with the objective of killing potentially infective (sporozoite-bearing) mosquitoes. Three fluid ounces were applied per acre at early dawn in good weather, with an average of 21.2 droplets per square inch.

Treatment IV-A. In addition to the above treatment (IV) was the first of the Holy Week treatments involving 6,400 additional acres of discontinuous beach between La Libertad and the Guatemala border (see map, Figure 6 and Table 18).

Treatment V. March 29, 1969. Second Holy Week treatment of beaches, 6,400 acres, was made with three fluid ounces per acre at early dawn in good weather (Table 18). Additional tests were conducted with the galvanized cages.

Treatment VI. April 7, 1969. Third Holy Week treatment of beaches, 6,400 acres, was made with three fluid ounces per acre at early dawn in good weather (Table 18).

Treatment VII. April 8, 1969. La Libertad triangle, second of 17-18-day treatments. Six fluid ounces were applied per acre at early dawn in good weather with an average of 50.7 droplets per square inch. Resistance of the La Libertad population of albimanus to malathion resulted in this trial with six ounces. After this treatment, it was decided to try a set of three weekly treatments in an area near Acajutla where tests had shown albimanus to be more susceptible to malathion.

Treatment VIII. April 17, 1969. Acajutla near Hacienda Atalaya and airport (see map, Figure 6), 3,000 acres, first of three successive weekly treatments designed to reduce existing mosquito population. Three fluid ounces were applied per acre at early dawn in good weather with an average of 46.0 droplets per square inch.

Treatment IX. April 24, 1969. Second of three Acajutla treatments. Three fluid ounces were applied per acre at early dawn in good weather with an average of 21.6 droplets per square inch.

Treatment X. May 5, 1969. Third of three Acajutla treatments, 3,000 acres. Three fluid ounces were applied per acre at early evening in good weather with an average of 13.3 droplets per square inch.

All treatments were by a C-47 aircraft (Registration N6650) flying at 160 m.p.h. at an altitude of 125 feet and were evaluated physically by the use of malathion-sensitive oil red dye cards (4" x 5") for measuring droplet distribution and entomologically by various combinations of indices including caged mosquitoes, human biting counts, night

TABLE 18

Summary of ULV Aerial Applications of Malathion Against *Anopheles albimanus*
Coastal Beaches of Western El Salvador, Holy Week Protection, 1969

(Littoral beach areas between Río Zunzal and Guatemala border with high visitor populations during holy week were treated at strategic time intervals to reduce malaria transmission potential. Zunzal Beaches - 2.0 km., Zunzal to Mizata discontinuous - 12.3 km., Remedios/Cóbanos Beach - 2.0 km., Salinis Beach - 1.0 km., Las Flores Beach - 1.0 km., Acajutla Beach - 4.0 km., Metalío Beach - 6.0 km., Barra de Santiago - 6.0 km.... total 34.3 km. of discontinuous beach treated to a depth of 0.5 km. inward from beach area (6,400 acres). See Map, Fig. 6.)

Treatment No. and Dosage	Date & Time	Avg. No. Droplets per Sq. In.	% Mortality Caged, wild-caught females	Comments
				- Time & logistics limitations did not permit detailed counts of the wild population - however, it was hoped that a subsequent epidemiological read out comparing coastal Western El Salvador (treated) to coastal Eastern El Salvador (untreated) might be possible.
IV-A 3 oz./acre	Mar. 20 7:00 AM- 8:30 AM	Litoral - 7.6 Remedios - 9.9 Acajutla - 3.3 Metalío - 8.9 Santiago - 10.2 Total - 8.0	*San Diego - 74.4% (21.2 droplets per sq. in.)	Excellent coverage at San Diego Beach, La Libertad, which was scheduled to receive regular treatment; small beach areas of less than 0.5 km. not hit well; beaches less than 2.0 km. hit moderately well; beaches more than 2 km. (except Acajutla) well hit. Overall coverage of 8.0 droplets per square inch among such discontinuous beaches should be considered good.
V 3 oz./acre	Mar. 29 6:00 AM- 7:30 AM	Mizata - 0.04 Remedios - 8.6 Acajutla - 6.0 Metalío - 8.8 Santiago - 23.4 Total - 11.0	No Data	Improved treatment in all areas. Heavily visited beaches pretty well hit (Remedios - 8.6 droplets/in^2, Acajutla - 6.0, Metalío - 8.8, Santiago - 23.4).
VI 3 oz./acre	Apr. 7 5:45 AM- 7:15 AM	Mizata - 0.31 Remedios - 14.1 Acajutla - 2.8 Metalío - 4.4 Total - 4.9	Acajutla 52% Metalío 48.7%	Overall treatment poor, Acajutla (2.8 droplets per in^2) and Metalio (4.4 droplets per in^2) particularly poor despite favorable weather conditions. However, even with low droplet distribution, mortalities among caged mosquitoes were around 50 percent.

* La Libertad area only. Mosquitoes intended for use in Metalío and Acajutla had excess mortality due to poor handling.

animal stable counts, light traps, diurnal resting counts, etc. Data from all treatments are summarized in accompanying tables (Holy Week trials, Table 18; La Libertad trials, Tables 19-21; and Acajutla trials, Table 22).

Results:

1. Distribution and Penetration of Droplets

Based on data from the preliminary ULV trials in 1968, it was anticipated that an average distribution of 10 droplets of ULV malathion per square inch, as measured by oil red dye cards, would result in albimanus mortalities approaching 100 percent in the 1969 target areas. Thus, the contract for the trials specified an average droplet distribution of no less than 15.0 droplets per square inch as measured by the dye cards. Actually obtained in the block-treated areas were droplet distributions (average per square inch) of 24.1, 22.6, 4.0 (windy), 21.2, and 50.7 (6 oz./acre) in the La Libertad trials, and 46.0, 21.6, and 13.3 (delayed count) in the Acajutla trials. These counts compare with distributions (average per square inch) of 5.5, 11.3, and 16.8 obtained during the 1968 treatments. Thus, it must be concluded that droplet distribution for the 1969 trials was excellent and generally far exceeded expectations.

The rather protected niches of albimanus daytime resting places require not only good distribution of droplets, but good penetration through various types of protective cover. Thus, index stations including caged mosquitoes and dye cards were always placed under varying conditions of exposure. The data of Table 23 are typical and show that all 40 sites received droplets and in many cases more than the minimum number (10 per square inch) considered adequate for effective kill. On the other hand, the data show considerable variation between stations (range 40 to 2,080 droplets per card, average 482.8). Even in exposed sites, there is much variation, notwithstanding that 31 of 40 dye cards received at least 100 droplets. Many of the stations were deliberately placed in hard-to-reach places; and, all in all, penetration through heavy canopy, under bridges, etc., as well as the area-wide distribution of the insecticide, was impressive.

2. Droplet Distribution and Mosquito Mortality

It was evident after the first several treatments in the La Libertad area that little correlation existed between mortality of caged female albimanus and droplet distribution on adjacent dye cards, certainly not in the range of 10-15 droplets per square inch. The data of Table 23 show, for example, that stations 1, 9, 11, and 17 all received more than 40 droplets per square inch, yet mosquito mortality at these sites was 80.0, 81.8, 56.2, and 61.0 percent, respectively; on the other hand, stations 23, 30, 35, and 38 showed 100 percent mortalities with droplet distributions of 23.5, 16.9, 12.5, and 7.2, respectively.

During the trials it was found that albimanus in the La Libertad area were resistant to malathion, and the trials were continued in the Acajutla area. In the latter area, there was a better correlation between caged mosquito kill and adjacent dye card droplet counts, but still not in the range of 10-15 droplets per square inch. The data of Table 24 illustrate this point. Despite better correlation in the Acajutla area studies, the point was never reached during the 1969 trials when one could consistently equate droplet distribution with mosquito mortality in adjacent cages. However, the overall data require more study, and when the factors of resistance, wild populations versus insectary specimens, overall area insecticidal distribution, etc. are weighed in proper perspective perhaps a better correlation may become evident.

TABLE 19

Summary of ULV Aerial Applications of Malathion Against Adult *Anopheles albimanus*
La Libertad Trials, El Salvador, 1969

(3,000 acres of coastal plain. Three successive treatments at weekly intervals to reduce existing population. Two subsequent treatments at 17/18-day intervals to destroy *A. albimanus* females before infective sporozoites could be developed.)

Treatment No.	Date & Time	Dosage (oz/acre) and Meteorological Conditions	Avg. No. Droplets per in.^{2}	PRE- AND POST-TREATMENT COUNTS AND PERCENT REDUCTION						
				* Caged Adults	Wild Population Indices**					
					[1]Human Biting	[2]Animal Stables	[3]Light Traps	[4]Natural Daytime Resting	[5]Daytime Resting (Bridges)	Total Wild Population Indices
I	Feb. 18 7:05 AM- 7:45 AM	3 oz. GOOD	24.1	714-226 68.3% (40 cages, colony females)	--	16-0 100%	26-50 0%	Insuf. Nos.	3-7 0%	45-57 0%
II	Feb. 18 7:00 AM- 7:36 AM	3 oz. GOOD	22.6	397-222 44.1% (20 cages, colony females)	--	54-1 98.3%	49-10 80%	Insuf. Nos.	15-4 73%	118-15 96%
III	Mar. 3 5:25 PM- 6:05 PM	3 oz. WINDY	4.0	--	--	12-6 50%	54-19 65%	Insuf. Nos.	7-3 57%	73-28 62%
IV	Mar. 20 6:00 AM- 6:35 AM	3 oz. GOOD	21.2	168-43 74.4% (20 cages, colony females)	--	61-100 0%	296-214 28%	Insuf. Nos.	22-73 0%	379-387 0%
VII	Apr. 8 6:05 AM- 7:05 AM	6 oz. GOOD	50.7	444-311 30.0% (23 cages, wild-caught females)	495-380 23%	6-65 0%	586-221 62%	144-111 23%	107-84 21%	1338-861 36%

* Caged females were exposed to treatment under varying degrees of vegetative or artificial cover, subsequently removed from cages and transferred to clean holding cups 45 minutes after treatment had terminated. Mortality counts were made 24 hours thereafter. Control mortality negligible.

** All post-treatment counts were taken in the same situation during the same time periods as were the pretreatment counts. [1]San Diego Beach; [2]Sitio El Coco; [3]Sum of 4 traps: Ticuiziapa Beach, San Diego Beach, Sitio El Coco, Hacienda San Diego; [4]Ticuiziapa Swamp; [5]Sum of 3 bridges: Río Jute, Río San Antonio, Río Aquisquilla.

TABLE 20

Summary of ULV Applications of Malathion Against *A. albimanus* Larvae in Rivers
La Libertad Trials, 1969

Treatment No. and Data	Survey Data	PRE- AND POST-TREATMENT NUMBERS AND PERCENT REDUCTION BY RIVER														
		Río Jute			Río Amayo			Río Aquisquilla			All Rivers			Control-Río Huiza		
		Pre	Post	% Red.	Pre	Post	% Red.	Pre	Post	% Red.	Pre	Post	% Red.	Pre	Post	% Red.
I																
Feb. 18	No. Dips	200	200		100	100		200	200		500	500		200	200	
AM	No. Pos.	78	47		9	4		24	8		111	59		68	88	
3 oz./acre	% Pos.	39	23.5		9.0	4.0		12.0	4.0		22.2	11.8		34	44	
24.1 droplets	No. Larvae	393	156	60.3	24	8	66.7	84	13	84.5	501	177	64.7	255	336	0
per sq. in.	Larvae/Dip	2.0	0.8		0.2	.08		4.2	0.7		1.0	.35		1.3	1.7	
II																
Feb. 25	No. Dips	200	200		100	100		200	200		500	500		200	200	
AM	No. Pos.	86	87		5	7		9	6		100	100		88	75	
3 oz./acre	% Pos.	43	43.5		5.0	7.0		4.5	3.0		20.0	20.0		44.0	37.5	
22.6 droplets	No. Larvae	459	669	0	32	12	62.5	16	13	18.8	507	694	0	336	265	21.1
per sq. in.	Larvae/Dip	2.3	3.3		0.3	0.12		.08	.07		1.0	1.4		1.7	1.3	
III																
Mar. 3	No. Dips	200	200		100	100		200	200		500	500		200	200	
PM	No. Pos.	111	98		4	12		14	36		129	146		75	37	
3 oz./acre	% Pos.	55.5	49		4.0	12.0		7.0	18.0		25.8	29.2		37.5	18.5	
4.0 droplets	No. Larvae	1294	1208	6.6	10	14	0	31	63	0	1335	1285	3.7	265	93	64.9
per sq. in.	Larvae/Dip	6.5	6.0		0.1	0.14		.16	0.3		2.7	2.6		1.3	0.5	
IV																
Mar. 20	No. Dips	200	200		100	100		200	200		500	500		200	200	
AM	No. Pos.	119	124		9	4		41	34		169	162		65	45	
3 oz./acre	% Pos.	59.5	62.0		9.0	4.0		20.5	17.0		33.8	32.4		32.5	22.5	
21.2 droplets	No. Larvae	1196	1134	5.2	16	16	0	80	87	0	1292	1237	4.3	192	171	10.9
per sq. in.	Larvae/Dip	6.0	5.7		0.16	0.16		0.4	0.44		2.6	2.5		1.0	0.86	
VII																
Apr. 8	No. Dips	200	200		100	100		200	200		500	500		200	200	
AM	No. Pos.	104	51		5	0		40	34		149	85		50	26	
6 oz./acre	% Pos.	52	25.5		5.0	0		20.0	17.0		29.8	17.0		25.0	13.0	
50.7 droplets	No. Larvae	949	306	67.8	16	0	100	109	92	15.6	1074	398	62.9	159	44	72.3
per sq. in.	Larvae/Dip	4.7	1.5		0.16	0		0.55	0.46		2.1	0.8		0.8	0.22	

TABLE 21

Summary of ULV Applications of Malathion Against *Anopheles albimanus* Larvae in Estuaries
La Libertad Trials, El Salvador, 1969

Treatment No. Date and Time	Dosage and Meteorological Conditions	Avg. No. Droplets per in.^{2}	Confined Larvae		ESTUARY					
					TICUIZIAPA			SAN DIEGO		
					Pre-Treatment	Post-Treatment	% Reduction	Pre-Treatment	Post-Treatment	% Reduction
I Feb. 18 7:05 AM- 7:45 AM	3 oz. GOOD	24.1	786-164 79.1% (40 containers, colony larvae)	No. Dips No. Pos. % Pos. No. Larvae Larvae/Dip	200 61 30.5% 201 1.0	200 14 7.0% 24 0.12	88.1%	200 56 28.0% 194 1.0	200 53 26.5% 346 1.7	0
II Feb. 25 7:00 AM- 7:36 AM	3 oz. GOOD	22.6	--	No. Dips No. Pos. % Pos. No. Larvae Larvae/Dip	200 14 7.0% 24 0.12	200 6 3.0% 22 0.11	8.3%	200 53 26.5% 346 1.7	200 13 6.5% 120 0.6	65.3%
III Mar. 3 5:25 PM- 6:05 PM	3 oz. WINDY	4.0	--	No. Dips No. Pos. % Pos. No. Larvae Larvae/Dip	200 6 3.0% 22 0.11	200 14 7.0% 26 0.13	0	200 13 6.5% 120 0.6	200 98 49.0% 417 2.1	0
IV Mar. 20 6:00 AM- 6:35 AM	3 oz. GOOD	21.2	--	No. Dips No. Pos. % Pos. No. Larvae Larvae/Dip	200 43 21.5% 102 0.5	200 25 12.5% 227 1.1	0	200 90 45.0% 459 2.3	200 102 51.0% 818 4.1	0
VII Apr. 8 6:05 AM- 7:05 AM	6 oz. GOOD	50.7	--	No. Dips No. Pos. % Pos. No. Larvae Larvae/Dip	200 60 30.0% 132 0.66	200 33 16.5% 72 0.36	45.5%	200 92 46.0% 790 4.0	200 34 17.0% 121 0.6	84.7%

TABLE 22

Summary of ULV Aerial Applications of Malathion Against Anopheles albimanus Adults

Acajutla Trials, El Salvador, 1969

(3,000 acres of Coastal plain. Three successive treatments at close intervals to reduce existing population in an area with mosquitoes apparently more susceptible to malathion than those in the La Libertad area.)

Treatment No.	Date & Time	Dosage (oz/acre) and Meteoro-logical Conditions	Avg. No. Droplets per sq. in.	PRE- AND POST-TREATMENT COUNTS AND PERCENT REDUCTION					
				Caged Females*	Wild Population Indices**				
					[1]Human Biting	[2]Animal Stables	[3]Natural Daytime Resting	[4]Light Traps	Total Wild Population Indices
VIII	Apr. 17	3 oz.	46.0	556 - 193	80-51	1280-824	129-38	215-67	1704-980
	6:15 AM-6:45 AM	GOOD		65.3% (30 cages wild-caught females)	36.3%	35.6%	70.5%	68.8%	42.5%
IX	Apr. 24	3 oz.	21.6	549 - 154	76-54	1805-1044	121-65	227-115	2229-1278
	6:25 AM-6:55 AM	GOOD		71.9% (30 cages wild-caught females)	28.9%	42.2%	46.3%	49.3%	42.7%
X	May 5	3 oz.	13.3	--	181-81	1728-1918	211-66	491-346	2611-2411
	6:30 PM-7:10 PM	GOOD			55.2%	0%	68.7%	29.5%	7.7%

* Caged females were exposed to treatment under varying degrees of vegetative or artificial cover, subsequently removed from cages and transferred to clean holding cups 45 minutes after treatment had terminated. Mortality counts were made 24 hours thereafter. Control mortality negligible.

** All post-treatment counts were taken in the same situations during the same time periods as were the pretreatment counts. [1]Three gasoline stations near Acajutla within treatment area; [2]Hnda. San Antonio, Hnda. Atalaya; [3]situations near animal stables; [4]sum of two traps, Hnda. San Antonio and Hnda. Atalaya.

TABLE 23

Distribution of Malathion Droplets on Oil Red Dye Cards, Treatment I, February 18, 1969

Site No.	Description of Site	No. of Malathion Droplets Per Card	Per in^2
1	Río Jute, under Bridge, North side	928	46.4
2	" " " " " "	487	24.3
3	" " " " South side	80	4.0
4	" " " " " "	102	5.1
5	Río San Antonio, under Bridge, North side	40	2.0
6	" " " " " " "	191	9.5
7	" " " " " South side	254	12.7
8	" " " " " " "	101	5.0
9	Río Amayo, under Bridge, North side	946	47.3
10	" " " " " "	309	15.5
11	Río Amayo, West River Margin, under tall trees, loose canopy	1,024	51.2
12	Río Amayo, East River Margin, under low dense canopy	232	11.6
13	Río Aquisquilla, under Bridge, SW corner	752	37.6
14	" " " " South side	66	3.3
15	" " " " " "	85	4.3
16	" " " " " "	40	2.0
17	Under double canopy, near house	912	45.6
18	Under low shrubs, near house	600	30.0
19	Under thick bamboo, fence-row	61	3.0
20	Under double canopy, fence-row	71	3.6
21	Under heavy broadleaf tree canopy	174	8.7
22	Under light roadside canopy	1,084	54.2
23	Under dense roadside canopy	470	23.5
24	On vacant beach lot in light tall grass	1,500	75.0
25	Exposed site	2,080	104.0
26	Under moderate broadleaf canopy	716	35.8
27	Under dense, low canopy	121	6.0
28	Exposed site	1,180	59.0
29	Under moderate, low canopy	932	46.6
30	Under heavy cover near palm tree	339	16.9
31	In yard, under heavy cover	400	20.0
32	Under small bush	836	41.8
33	Exposed site	600	30.0
34	Under thatch roof, heavy cover, open on sides	90	4.5
35	Heavy, under double layer canopy	250	12.5
36	Among light tree cover near building	634	31.7
37	Exposed, side of small bush	266	13.3
38	Under heavy leaf cover	144	7.2
39	Under dense cover in estuary	130	6.5
40	Under dense cover in estuary	87	4.4
TOTAL		19,314	
Avg. No. Droplets per square inch		---	24.1
Avg. No. Droplets per card		482.9	---

TABLE 24

Distribution of Malathion Droplets on Oil Red Dye Cards (4" x 5") and 24-hour Mortality of Caged Wild-caught Anopheles albimanus Females ULV Treatment IX. April 24, 1969

Site No.*	No. Droplets		24-hour Mortality		
	Per Card	Per Square Inch	Alive	Dead	% Mortality
1	826	41.3	0	19	100.0
2	954	47.7	4	12	75.0
3	1,166	58.3	0	19	100.0
4	1,372	68.6	0	20	100.0
5	690	34.5	1	17	94.4
6	516	25.8	4	11	73.3
7	340	17.0	11	9	45.0
8	1,084	54.2	0	20	100.0
9	2,852	142.6	0	19	100.0
10	432	21.6	6	12	66.6

* Site Nos. 1-10, Hacienda San Antonio.

3. Three Ounces versus Six Ounces per Acre

Treatment VII of the La Libertad trials (Table 19) was conducted using six ounces of malathion per acre, and the measured distribution of 50.7 droplets per square inch was approximately double that of the three-ounce treatments (I, II, and IV). All of these were early morning treatments of the same area and should give some insight into the relative value of a six-ounce treatment. When compared to three-ounce treatments in terms of caged mosquito mortality, the results were better with three ounces (68.3%, 44.1%, 74.4% versus 30.0%). However, the difference between the use of colony mosquitoes in the three-ounce treatments versus the use of wild-caught mosquitoes in the six-ounce treatment is a consideration. The remainder of the data from wild population indices is inconclusive. The six-ounce treatment showed an overall reduction of 36 percent among the wild population indices as compared to 0, 96 percent, and 0 in the three-ounce treatments I, II, and IV, respectively. From these data, no real advantage of a six-ounce treatment over the three-ounce treatments has been shown. Since mosquitoes in the area were malathion-resistant, it is significant that no appreciable difference was made by doubling the dosage of insecticide.

4. Morning versus Evening Treatments

The data of Table 22 (Treatments VIII, IX, and X) from the Acajutla area do not show that the evening treatment (No. X) was superior to the morning treatments of the same area (Nos. VIII and IX). In the overall wild population indices, a 7.7 percent reduction was measured in the evening treatment as compared to 42.5 percent and 42.7 percent in the morning treatments. It should be noted, however, that the evening treatment did not include periods of darkness, and it is unlikely that resting mosquitoes were in niches different than those of early morning.

5. Effect of Malathion Resistance

Possible malathion resistance became suspect during the La Libertad trials and later tests confirmed the presence of resistance in the La Libertad population. Further tests indicated relative susceptibility in the Acajutla population. The effect of this difference can be seen by comparing data from Table 25 (La Libertad, Treatment VII, six ounces per acre) with those of Table 26 (Acajutla, Treatment IX, three ounces per acre). In the La Libertad area, 80 percent mortality of wild-caught caged albimanus was exceeded in only one of 23 stations and mortalities greater than 90 percent were never reached. Also, a droplet distribution of less than 50 per square inch resulted in no mortality above 40 percent. By contrast, in the Acajutla area, a droplet distribution of greater than 50 droplets per square inch resulted in 100 percent mortality of caged albimanus; distributions between 11 and 50 per square inch resulted in proportionate mortalities, and in cases of less than 10 droplets per square inch, mortalities between 31 and 95 percent were noted. In general, in La Libertad it required fifty-plus droplets per square inch to equal mortalities resulting from less than ten droplets per square inch in the Acajutla area.

TABLE 25

ULV Droplet Distribution as Related to Mortality
of Caged, Wild-caught albimanus Females
Treatment VII, La Libertad Trials, 1969.
(Six Ounces Malation per Acre)

	No. of Stations with Indicated Number of Droplets per square inch						
% Mortality	<10	11-20	21-30	31-40	41-50	50+	Total
0 - 10	2	1	0	2	1	1	7
11 - 20	0	1	0	1	0	0	2
21 - 30	1	2	1	0	0	2	6
31 - 40	0	1	0	0	0	1	2
41 - 50	0	0	0	0	0	2	2
51 - 60	0	0	0	0	0	0	0
61 - 70	0	0	0	0	0	1	1
71 - 80	0	0	0	0	0	2	2
81 - 90	0	0	0	0	0	1	1
91 - 99	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
TOTAL	3	5	1	3	1	10	23

TABLE 26

ULV Droplet Distribution as Related to Mortality of Caged, Wild-caught albimanus Females Treatment IX, Acajutla Trials, 1969. (Three Ounces Malathion per Acre)

% Mortality	No. of Stations with Indicated Number of Droplets per square inch						
	<10	11-20	21-30	31-40	41-50	50+	Total
0 - 10	0	0	0	0	0	0	0
11 - 20	0	0	0	0	0	0	0
21 - 30	0	0	0	0	0	0	0
31 - 40	1	0	0	0	0	0	1
41 - 50	4	1	0	0	0	0	5
51 - 60	2	1	0	0	0	0	3
61 - 70	1	1	1	0	0	0	3
71 - 80	2	0	1	0	1	0	4
81 - 90	4	1	0	0	0	0	5
91 - 99	2	1	0	1	0	0	4
100	0	0	0	0	1	4	5
TOTAL	16	5	2	1	2	4	30

6. Monitoring of Insecticidal Residues Following ULV Applications

A total of 13 samples was collected in the ULV study area prior to malathion treatment. These samples included water from the four rivers within the area, water from four different locations in Estero Ticuiziapa, foliage from two cattle pastures and a sugar cane field, and chloroform rinsings from some rock outcroppings known to be natural resting sites of Anopheles.

Following the early morning application of the chemical, a similar sampling pattern was followed, with the collections representing 2-4, 24, 48, and 144 hours after application. This sequence was repeated following the second early morning treatment. The third application was made in the early evening, and samples were taken starting the following morning which represent 16, 40, 64, and 88 hours after treatment.

All foliage samples were frozen for future analysis. The chloroform rinsings were stored in the cold for future clean up and analysis. All water samples were processed immediately, and the extracts concentrated and stored in the cold for future analysis.

The late arrival of some of the analytical equipment had permitted the analysis of only a part of these samples by the end of the reporting period. A description of the water sampling areas is given in Table 27 and the residue values, as determined by electron capture gas chromatography, are expressed in parts per billion (ppb) in Table 28.

TABLE 27

Description of Water Sampling Sites in ULV Study Area

Estero Ticuiziapa	
Station 1	Ditch drainage connected to estero, canopy on three sides.
2	Ditch drainage connected to estero, canopy on two sides.
3	Open water, no canopy nor surrounding vegetation.
4	Open water, no canopy but surrounded by small trees.
Río El Jute	Small pool, running water, partial canopy.
Río San Antonio	Small pool, running water, beneath bridge.
Río Aquisquilla	Large pool, running water, partial canopy.
Río Amayo	Small pool, stagnant, partial canopy.

TABLE 28

Residue Values, Expressed in ppb, of Malathion in Various Aquatic Stations Following ULV Application 1/

Location	Control	Hrs. after 1st Application				Hrs. after 2nd Application				Hrs. after 3rd Application 2/			
		2-4	24	48	144	2-4	24	48	144	16	48	64	88
Estero Ticuiziapa													
Station 1	0	0	0	0	0	8	2	0	0	4	2	1	0
2	0	27	3	0	0	25	3	2	0	1	1	0	0
3	0	90	13	6	0	50	6	2	0	9	4	2	0
4	0	82	17	6	0	57	11	4	0	11	6	2	0
Río El Jute	0	18	3	0	0	6	3	0	0	0	0	0	0
Río San Antonio	0	8	0	0	0	0	0	0	0	8	3	0	0
Río Aquisquilla	0	96	0	0	0	12	1	0	0	4	1	0	0
Río Amayo 3/	0	8	0	0	0	10	0	0	0	-	-	-	-

1/ Rate of application = 3 oz./acre.
2/ 3rd application made in early evening.
3/ Omitted from sampling sequence due to insufficient water.

Since three of the rivers had flowing water in them, it is concluded that the major portion of the chemical was quickly carried away. In general, Estero Ticuiziapa had the greater concentration and degree of persistence of the compound. This might be attributed to: (1) the estero proper is quite devoid of a cover canopy allowing for good deposition of the chemical, (2) the water was not open to the ocean and was considered as impounded, and (3) one of the rivers in the treatment area discharges into the estero. Although this area had the greatest concentration, it was only at the ppb level, a fact attributed to dilution. Persistence of the chemical was recorded 48 hours after treatment but not after 144 hours.

Entomologic data associated with these three treatments showed some significant reductions in the mosquito larval population in some of these breeding areas (Tables 20 and 21).

7. Efficacy of ULV Spraying for Anopheles Control

Results in the block-treated areas of La Libertad (Tables 19-21) and Acajutla (Table 22) show a great amount of inconsistency and little effect in terms of significantly reducing the natural mosquito populations in the respective areas. There was somewhat more consistency in the Acajutla data (susceptible population), but even here a high confidence level was not established. Although a complete evaluation of all ULV data remains to be made, a preliminary assessment shows no evidence from these studies sufficiently strong to recommend the ULV technique as a routine operational vector control measure in antimalaria programs.

On the other hand, it would be remiss not to work at solving the problems that stand in the way of developing this technique, for it has been demonstrated that the system can deliver insecticide in a manner and form that permit maximum distribution and penetration with a minimum amount of material over wide areas of difficult terrain. It would seem that two problems are evident: (1) the resting habits of albimanus are such that no daytime chemical treatment will reach this species in all of its protected niches, but ULV seems to have the best chance on an areawide basis, and (2) the insecticide resistance problem is apparent, but certainly with increasing availability of chemicals and improvements in spraying systems this can be overcome.

There are several possible solutions to the problem of reaching the protected mosquitoes. One would be utilization of predawn or night treatments. In many areas low-level nighttime flying would be entirely possible with proper marking. This was not possible in the El Salvador trials because of the proximity of mountainous terrain. The "high-low" technique also might offer possibilities. Another is an agitation of the resting mosquitoes either immediately before or during the ULV treatment. Perhaps a smoke or agitating chemical could be put down immediately before treatment or an additive such as small amounts of pyrethrum could serve this purpose at the time of treatment.

In any event, reasonable efforts should be made to develop the ULV technique as a usable tool for special situations in the vector control aspects of antimalaria programs.

Thermal Aerosol Fogging

Since thermal aerosol fogging might serve as an effective means of rapidly eliminating infected mosquitoes in a restricted, heavily populated area undergoing a malaria outbreak, limited studies were made from June to October 1968 and in May 1969 on the effects of fogging on the adult A. albimanus populations in the villages of Jocotal and La Curruncha near Lake Jocotal. Evaluations were made of malathion, fenthion, and naled in diesel oil dispersed with a Dyna Fog 150 thermal aerosol apparatus between 7:00 and 8:00 p.m.

Jocotal consisted of 34 houses with pole walls and thatched or tile roofs, and La Curruncha contained 35 houses of similar construction. The marshy edges of Lake Jocotal provided an almost constant source of adult mosquitoes for test purposes.

Evaluations were based on pretreatment and post-treatment counts of adult mosquitoes resting within the houses and in "red boxes" placed at strategic locations within the villages and on the 24-hour mortalities of mosquitoes exposed in small screen cages placed inside the houses during the aerosol applications. The red box counts were made beginning at 7:00 a.m. with the house counts following immediately thereafter and usually terminating by 10:00 a.m. The pretreatment counts were made on the mornings of the days when the aerosol treatments were applied in the evenings. The post-treatment counts were made on the mornings immediately following the evening aerosol applications.

It must be borne in mind that the effects of a thermal aerosol application are achieved only at the time the insecticidal fog is suspended in the air and there is no residual activity from this kind of treatment. In making the post-treatment counts, it was impossible to differentiate between a mosquito that had survived the treatment and one that had migrated into the treated area after the fog had dissipated. Since continuous movement of the adult mosquitoes was undoubtedly occurring, it is not surprising that 100 percent reductions were not achieved. Significant reductions were noted by the evaluation methods employed, indicating an effect from the treatments.

The malathion and fenthion formulations contained 2 ml. of Tenac additive per gallon of finished material, and the naled formulation contained 30 ml. of Ortho additive per gallon of finished material.

The results of three tests with each candidate insecticide are shown below:

Insecticide	Dosage	Volume of Finished Spray Applied	Dial Setting	Average 24-hour % Reduction		
				Red Box	House	Cage
Fenthion (8 lb./gal. E.C.)	3 fl. oz./ gal. diesel	2 gal.	4	79	82*	94
Malathion (Technical)	6 fl. oz./ gal. diesel	2 gal.	6	79	--	75
Naled (Dibrom-14)	1 fl. oz./ gal. diesel	3 gal.	4	60*	82*	97*

* Based on 2 replicates only.

In addition to the three evaluation measures indicated above, the two naled tests were also evaluated by pre- and post-treatment human bait captures, NCDC miniature light traps, and counts of resting adult mosquitoes in surrounding natural resting places. The average, 24-hour percent reductions effected by the naled treatments as measured by each of these methods were 90 percent, 90 percent, and 56 percent, respectively.

Evaluation of Candidate Larvicides in Field Plot Tests

In El Salvador during the dry season (November-May), anopheline breeding is restricted primarily to river and lake margins, permanent swamps, and irrigated pastures. During this season, the sources are sufficiently restricted to allow for practical application of mosquito larvicides as a control method in many areas. To obtain information on effective dosages of insecticides that might be used in this manner, small plot tests were conducted.

Plots 1/8 acre in size were used for all tests. For river systems, the average width of the stream was determined and linear measuresments made to provide 1/8 acre of surface area. Although breeding was restricted to quiet marginal waters, all stream water surfaces were sprayed to ensure even coverage. For lake margins and swampy areas, quadrangular plots were measured and marked with stakes.

The finished spray was made by adding aliquots of 4 lb./gal. emulsion concentrate formulations to water to provide dosages ranging from 0.0025 to 0.05 lb./acre. The finished spray was applied with two-gallon Hudson X-Pert sprayers pumped to about 30 psi and fitted with an 8002 Tee-jet fan nozzle on the spray wand. A standard volume of eight gallons of finished spray per acre was used.

Pretreatment and 24-hour post-treatment larval populations of all stages were determined by using a 5-inch diameter dipper for making 20 dips at random in each plot.

The results obtained in the small plot tests in still water in marshy areas and in the river margins are shown in Tables 29 and 30, respectively.

TABLE 29

Field Plot Tests with Larvicides Applied Manually in Still-water Marshes in El Salvador Against *A. albimanus*

Insecticide	No. Tests	Dosage of Toxicant per acre (lbs.)	Average 24-hour Percent Reductions in Larval Populations	Range in Percent Reductions
Abate	1	0.005	81.0	-
Abate	5	0.01	95.6	86-100
Abate	5	0.02	99.7	98.4-100
Abate	3	0.04	96.1	88.4-100
Fenthion	2	0.005	85.5	79-92
Fenthion	3	0.01	100	-
Fenthion	1	0.02	100	-
Dursban	1	0.0025	72.0	-
Dursban	3	0.005	97.7	94-100
Dursban	4	0.01	100	-
Dursban	2	0.02	100	-
Dursban	1	0.04	100	-

TABLE 30

Field Plot Tests with Larvicides Applied Manually to River Margins in El Salvador Against *A. albimanus*

Insecticide	No. Tests	Dosage of Toxicant per acre (lbs.)	Average 24-hour Percent Reductions in Larval Populations	Range in Percent Reductions
Abate	3	0.002	78	76-80
Abate	2	0.005	85	74-96
Abate	1	0.01	96	-
Abate	2	0.02	98	98-98.6
Abate	1	0.04	99	-
Abate	2	0.05	100	-
Fenthion	1	0.005	98	-
Fenthion	1	0.01	100	-
Dursban	1	0.005	96	-
Dursban	1	0.01	100	-

Evaluation of Larviciding During the Dry Season in a Rural Area

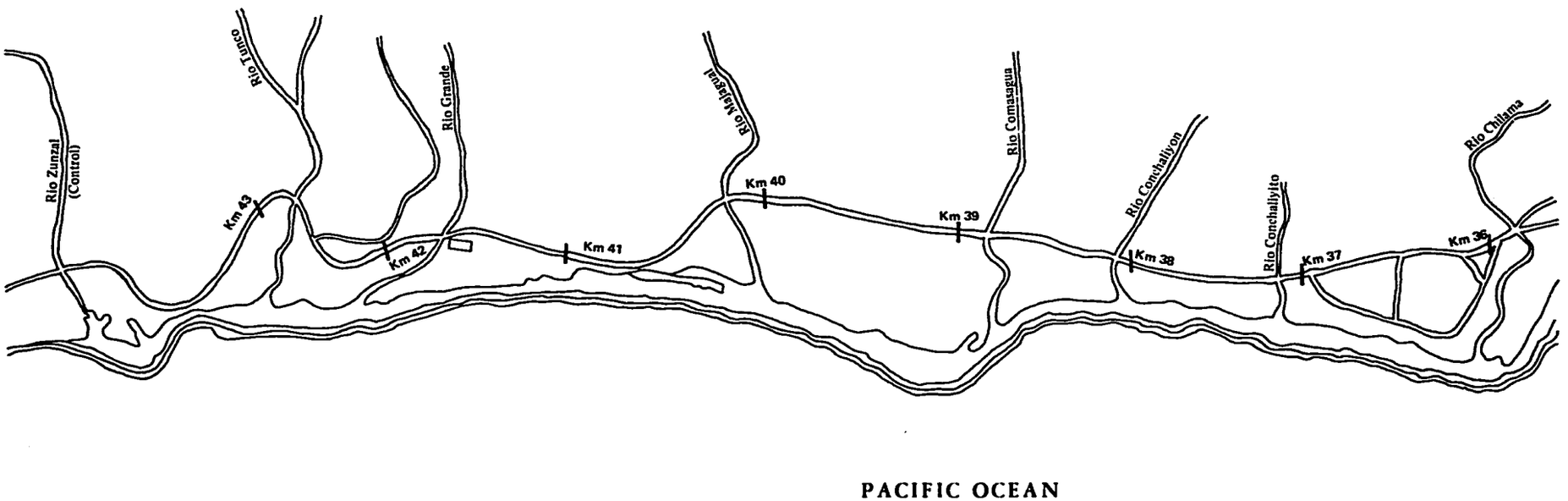
The rivers in Central America are used by the rural populace for laundering clothes, bathing, drinking, and general household purposes. In view of this, and because of its very low mammalian toxicity, Abate was selected for a large area larviciding study during the dry season in El Salvador. Abate 4 lb./gal. emulsion concentrate diluted with water to form the finished spray was applied to seven river systems, an irrigated pasture, and two estuaries in the coastal area of El Salvador west of La Libertad (Figure 7) from January 20 to May 30, 1969. From January 20 to April 20 the dosage was maintained at 0.02 lb./acre, and from April 20 to May 30 0.04 lb./acre was applied.

All rivers within the study area were sprayed from a point approximately two kms. north of the littoral highway to the ocean. The finished spray was applied with three gallon (2 gallon operational capacity) Hudson X-Pert sprayers pumped to about 30 psi and fitted with an 8002 Tee-jet fan nozzle on the spray wand. The volume applied was equivalent to eight gal./acre.

Since there is no residual activity from Abate applied in the formulation used, a schedule of weekly larval inspection and treatment was maintained. On large rivers, it was most practical to have one man inspecting for larvae ahead of a two-man spray crew stationed one on each side of the river. In this manner, spraying proceeded straight down the river without the spraymen needing to pass back and forth across the river and retrace their steps.

Adult mosquito populations within the area being larvicided were determined by bridge counts (Table 31) and stable captures (Table 32) in the evening. When the larviciding operations were carried out properly, the adult populations were kept at a very low

FIGURE 7. Area Larviciding Study: CAMRS El Salvador;
January – June, 1969.



level. On one occasion when a larval source was not completely sprayed for a couple of weeks, the adult count increased. This emphasizes that, for larvicidal operations to be successful, constant vigilance must be maintained at all times to assure application of insecticide to all breeding areas.

TABLE 31

Adult Mosquitoes Beneath Bridges in Larvicide Study Area: *Anopheles albimanus* and *A. pseudopunctipennis*, both sexes

Date	(Control) Huiza	Grande	Concha-liyito	Concha-liyón	Coma-sagua	Majagual	Tunco
2-24	33	4	0	0	0	0	0
3- 3	37	5	0	0	13	1	2
3-10	22	7	0	0	22	0	4
3-17	43	10	0	0	4	1	4
3-21	116	8	1	1	6	0	0
4-10	-	22	4	0	2	0	5
4-15	-	42	9	0	2	1	3
4-21	-	59	3	1	3	0	2
4-28	-	4	0	0	4	1	0
5-23	-	3	0	0	5	2	0

TABLE 32

Adult Mosquitoes Captured at Stables Within the Larvicide Study Area During Early Evening Hours: *Anopheles albimanus* and *A. pseudopunctipennis*, both sexes

	Control	Treated Area	
Date	Establo Melara	Establo San José	Establo Sta. Emilia
Jan. 28	-	-	94
Feb. 6	-	-	4
Feb. 11	24	1	2
Feb. 20	41	2	12
Feb. 27	32	0	16
Mar. 6	13	4	6
Mar. 11	6	1	5
Mar. 18	89	0	0
Mar. 26	58	1	3
Apr. 10	-	6	2
Apr. 21	-	253	11
Apr. 28	-	67	45
May 27	-	1	46

To maintain control of anopheline larvae in the 2500-acre study area, 240 man-hours per week were required for inspection (pre- and post-treatment larval dipping), preparation and maintenance of equipment and supplies, and application of larvicide.

Each week 45,540 linear feet of river courses were sprayed, and from 7,000 to 9,000 linear feet of estuary margins were also sprayed depending on fluctuating water levels. Approximately 24 to 30 man-hours per week of the 240 man-hour total were required for inspection and treatment of all positive sources within the 150 acres of irrigated pasture included in the study.

At the rate of 0.04 lb/acre, one quart of insecticidal concentrate was consumed per week. Pre-measured dosages of Abate in 25 ml. capacity vials were carried to the field by the spraymen. The contents of a vial were added to two gallons of water to form the finished spray.

To facilitate record keeping and assist in orientation of spray crews, the rivers in the study area were marked into 500 meter sections. In determining the larval reductions within each marked area, 100 pre- and post-treatment dips were made. The reductions recorded in each section were then pooled, and the average reduction for all sprayed sections on each river were reported (Table 33).

TABLE 33

Larval Reduction (24 hr. reading) of *Anopheles albimanus* and *A. pseudopunctipennis* from Abate Applied in River Systems

Date	Chilama	Concha-liyito	Concha liyón	Majagual	Coma-sagua	Tunco	Grande
At 0.02 lb/acre:							
Jan. 20-24	99%	100%	100%	99%	100%	98%	Not treated
Jan. 27-31	100%	100%	100%	100%	100%	93%	100%
Feb. 3- 7	94%	100%	100%	100%	100%	99%	70%
Feb. 10-14	96%	100%	94%	100%	92%	99%	85%
Feb. 17-21	93%	100%	100%	98%	95%	Not counted	100%
Feb. 24-28	91%	98%	100%	99%	97%	100%	98%
Mar. 3- 7	100%	100%	100%	100%	97%	99%	97%
Mar. 10-14	97%	90%	100%	99%	92%	93%	60%
Mar. 17-21	92%	100%	100%	98%	100%	97%	98%
Mar. 24-28	92%	99%	100%	98%	97%	91%	93%
Apr. 7-11	81%	100%	100%	100%	90%	100%	89%
Apr. 14-18	92%	100%	100%	100%	98%	85%	88%
At 0.04 lb/acre:							
Apr. 21-25	99%	100%	100%	100%	100%	100%	100%
Apr. 28-30	100%	100%	100%	100%	100%	100%	100%
May 5- 9	100%	100%	100%	100%	100%	100%	100%
May 12-16	-	100%	100%	100%	99%	100%	100%
May 19-23	99%	100%	100%	100%	100%	100%	100%
May 26-30	100%	100%	100%	100%	100%	100%	100%

The increase in dosage from 0.02 lb. to 0.04 lb. of Abate per acre on April 20 was accompanied by closer supervision of the spraying during that and subsequent applications. No evaluation can be made of the relative importance of the increased dosage or the improved supervision in the achievement of the better results (Table 33) which followed these changes. However, since the results achieved during the first two weeks of the study, when the relatively inexperienced spraymen were receiving close supervision, were almost as good as those obtained after April 20, it must be concluded that the renewal of good supervision was an important factor in the improved results.

Water Management Studies

A water management engineer served as a CAMRS consultant in El Salvador from March 11 to June 6, 1969, studying local past, present, and possible future projects for reduction of potential mosquito breeding sources. Highlights of these observations and studies were as follows:

a. Drainage Project in San Miguel. This project began under Rockefeller Foundation sponsorship (1932-1935), during which time 100,000 square meters of terrain were drained by construction of 1,010 linear meters of main canals and 2,533 linear meters of underground pipe. Beginning in 1942 the project was extended under sponsorship of the Inter-American Cooperative Service for Public Health (S.C.I.S.P.). By 1950, the San Miguel system included 7,946 linear meters of lined ditches and 525 linear meters of dirt ditches. The limited maintenance required for this drainage system has been provided by three employees of the municipality of San Miguel and/or the CNAP. When visited in April 1969, this source reduction project, which is 20 to 35 years old, was still functioning effectively, representing very low cost protection per capita per year.

b. Irrigation-Drainage Project near Sonsonate. The lined ditches of this project at Nahuilingo, constructed under S.C.I.S.P. sponsorship and inaugurated in 1944, totaled 6,442 linear meters and served a double function: drainage during the rainy season and irrigation during the dry season. When inspected in April 1969, the 25-year-old system was still functioning efficiently.

c. Survey of Large-scale Irrigation Projects. Irrigation specialists of the Ministry of Agriculture and Livestock have extensive plans for land reclamation or irrigation systems in El Salvador. The only project actually under development is that of the Valley of Zapotitan, involving approximately 12,000 acres. Neither the current project nor those on the drawing boards include any considerations of possible mosquito production or abatement aspects.

d. Possible Water Management to Control Salinity in Estuaries or Lagoons. The Pacific coastal areas of Guatemala, El Salvador, and Honduras are marked by numerous estuaries and lagoons where rivers empty into the ocean. Many of the estuaries are closed during the dry season by sandbars thrown up by wave action. These normally remain closed until the next rainy season. At the time of sandbar formation, the trapped waters are highly saline and are gradually diluted by limited stream flow from the hills along the coast. Entomologic-chemical studies in the La Libertad area (see page 8) revealed that the dry season decline in salinity permits development of heavy anopheline breeding in the estuary lagoons. Larval surveys made during and immediately following the engineer's visit indicated that artificial opening of the sandbars by cutting channels or by culverts and tide gates offers excellent possibilities of eliminating or reducing anopheline breeding by reducing the areas inundated and by permanent salination of the estuary lagoons.

ANTIMALARIA DRUG STUDIES

The causal relationship of taking antimalaria drugs and the occurrence of objectionable side effects has not been definitely established. At least a part of the treated population in El Salvador is convinced that such a relationship does exist. Because of this belief, studies were undertaken to define the drug side effect relationship more precisely and to find drugs or drug combinations that, while being more acceptable to the drug taker, would be efficacious against the parasite.

Pyrimethamine + Primaquine vs. Amodiaquine + Primaquine

Pyrimethamine and primaquine are well known antimalaria drugs with proven favorable therapeutic/toxic ratios. Based on favorable results obtained by workers in Panama with this combination, a trial was started in El Salvador in January 1969 to determine the acceptability and effectiveness of this combination tablet in comparison with the amodiaquine-primaquine combination in routine operational use.

The National Anti-Malaria Campaign (CNAP) set aside for this study two districts in Zone 2 of the Collective Drug Program (PTC). These districts are located in the western coastal plain of El Salvador (Figure 8) and each area has a registered population of approximately 5,500 persons. Collective drug treatment has been used intermittently since 1966, but a continuing program has been in effect only since March 1968.

During September 1967, a complete census was conducted in both districts (Table 34), following which collective drug distribution at fortnightly intervals was initiated and continued during the months of September, October, November, and December 1967.

Following the end-of-year holidays of 1967 and until the third week of March 1968, the areas were under "observación estricta." No collective drug distribution was in progress, but active case detection--house-to-house fever case surveys--was carried out. All fever cases received presumptive medication.

From the third week of March 1968 until the present time, both areas have been under drugs. Table 34 lists the available drug program information for the two districts for the years 1967 and 1968. This background information permits a comparison with the information obtained since the CAMRS-CNAP drug study began in January of 1969. At that time, a combination of 75 mg. of pyrimethamine and 50 mg. of primaquine (adult dose) was substituted in District 8 for the regular amodiaquine (450 mg.) plus primaquine (15 mg.) combination (adult dose) being used by the CNAP in its mass drug distribution program. District 13, where the amodiaquine-primaquine combination has been continued, was selected to serve as a "control" in evaluating the side effects, efficacy, and acceptance of the two combinations.

The data in Table 34 and Figures 9 and 10 indicate a great increase in the population registered during the cotton harvesting season, with many nomadic groups apparently moving along the coast as the work proceeded. Even with this influx of a large floating population, the acceptance levels have remained roughly the same.

As indicated in Table 34, study District 8 (pyrimethamine and primaquine) had acceptance varying from 48 percent to 66 percent during the January-June 1969 period. During the same twelve cycles, control area District 13 (amodiaquine and primaquine) had acceptance ranging from 56 percent to 64 percent.

The percent of the total population sampled and the actual number of positive slides are shown in Table 35. This table also furnishes background information for the previous years.

FIGURE 8.

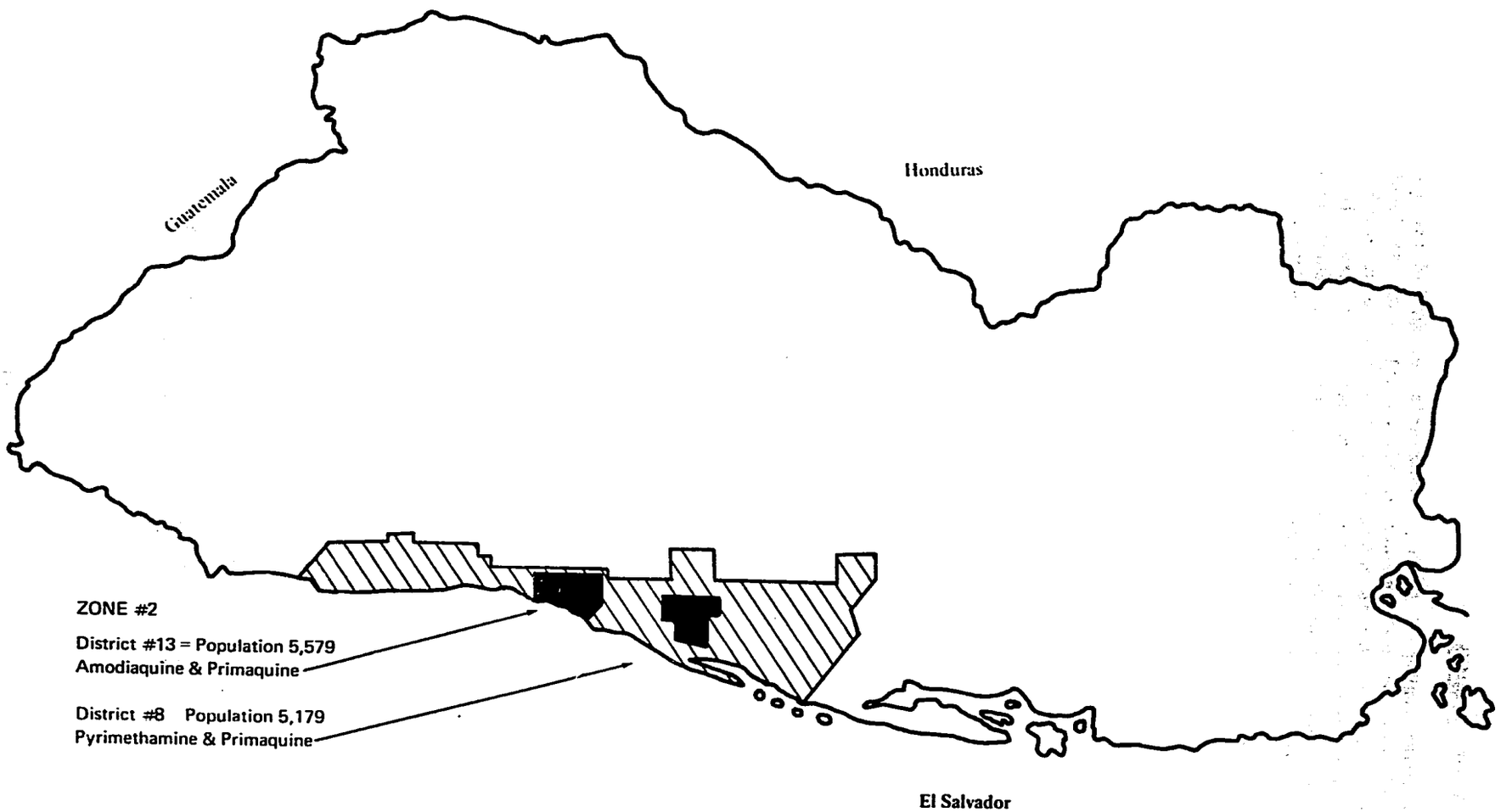

Guatemala
Honduras
ZONE #2
District #13 = Population 5,579
Amodiaquine & Primaquine
District #8 Population 5,179
Pyrimethamine & Primaquine
El Salvador

TABLE 34

Persons Registered and Level of Drug Acceptance (Treatment) in Districts 8 and 13, CNAP PTC Zone 2, El Salvador during 1967, 1968, and 1969

	Persons Registered						Acceptance Level (%)					
	1967		1968		1969		1967		1968		1969	
District:	8	13	8	13	8	13	8	13	8	13	8	13
					**6530	6536					**66	62
Jan.			*(5062	5262)	6403	6966			*(5	4)	57	64
			*(5212	5175)	6385	6492			*(4	4)	58	61
Feb.			*(4888	5260)	5386	6977			*(4	5)	48	56
			*(5147	5206)	5086	5470			*(5	3)	51	59
Mar.			5026	5286	4995	5386			62	66	57	63
			5080	5240	5026	5366			54	64	57	61
Apr.			4894	5310	5093	5408			58	61	55	61
			4944	5410	5100	5477			60	64	56	60
May			5137	5434	5072	5653			58	60	60	63
			5119	5477	4996	5594			59	63	61	61
June			5247	5527	5179	5592			59	63	64	62
			5363	5623					56	58		
July			5254	5780					56	62		
			5370	5822					56	58		
Aug.			5441	5870					54	56		
			5290	5769					53	52		
Sept.	5263/	5365/	5295	5821			5/	6/	51	53		
	5436	5249	5157	5714			77	72	51	55		
Oct.	5506	5267	5228	5684			68	70	52	54		
	5779	5383	5296	5697			62	65	52	54		
Nov.	6492	5460	6051	5928			58	61	56	53		
Dec.	6528	5677	6819	6228			51	63	60	53		

__/ Data from census made in September 1967 prior to initiation of collective drug distribution (amodiaquine-Cl + primaquine) by CNAP.

*( ) Collective drug distribution temporarily suspended (1st four cycles of 1968). Active case detection, with slides taken from and medication given to fever cases only.

** Pyrimethamine + primaquine replaced amodiaquine-Cl + primaquine in District 8 beginning with first cycle in 1969.

FIGURE 9. Number of Persons Registered in Districts Eight (8) and Thirteen (13) for the Months Indicated in the Years of 1968 and 1969.

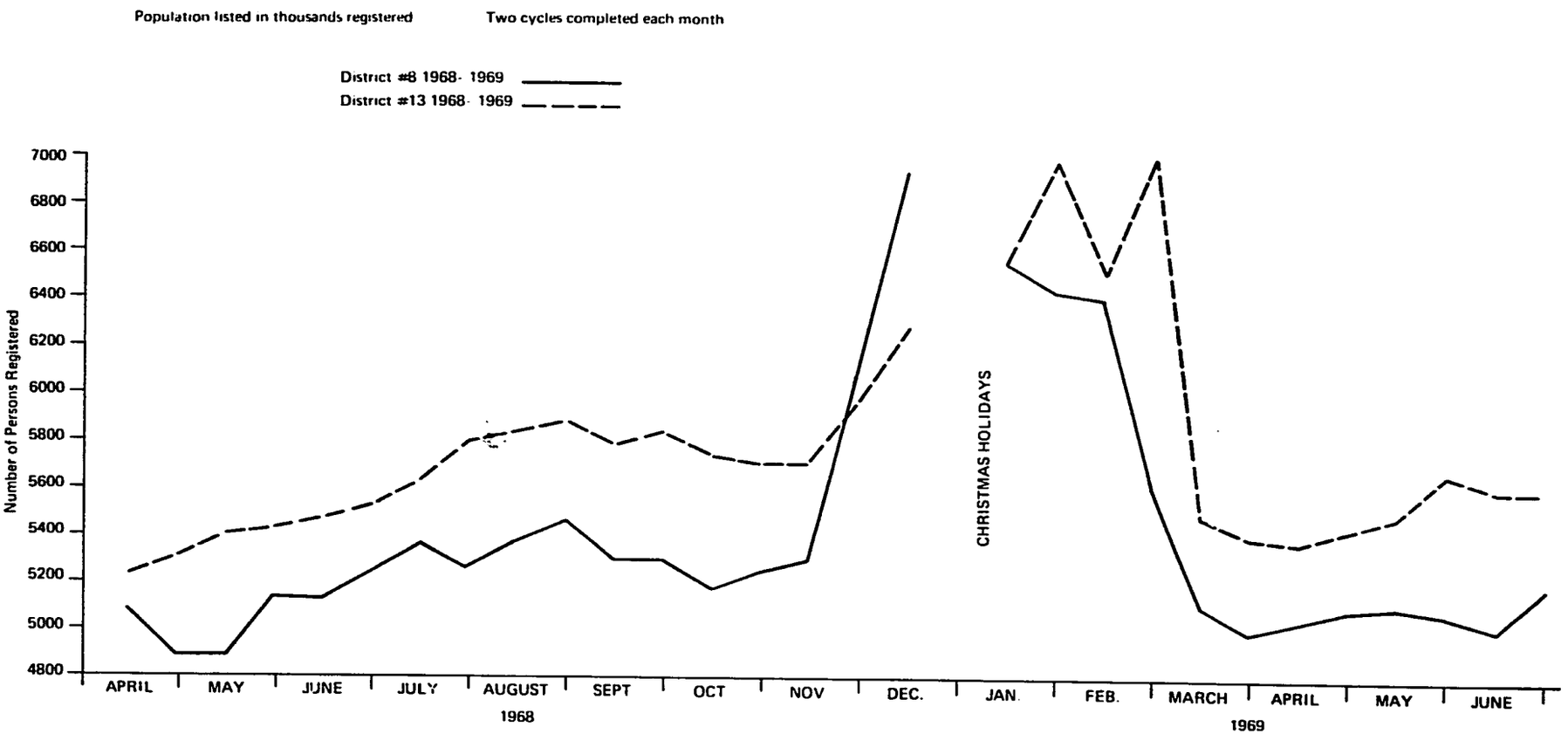


FIGURE 10. Drug Acceptance Levels (Percentages) for Districts Eight (8) and Thirteen (13) for the Months Indicated in the Years of 1968 and 1969.

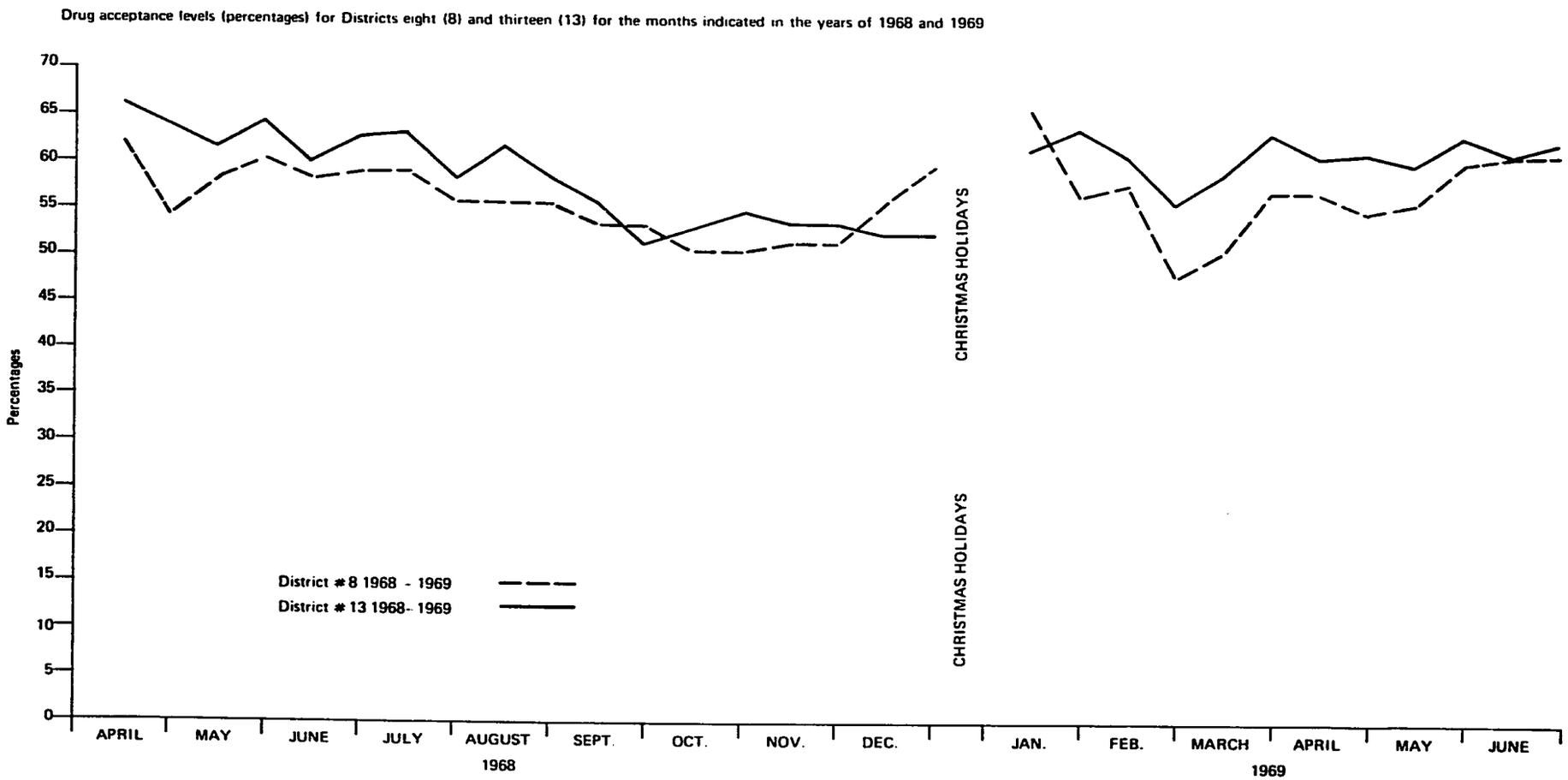


TABLE 35

Percentage of Registered Population Sampled and Number of Positive Slides in Districts 8 and 13, CNAP PTC Zone 2, El Salvador during 1967, 1968, and 1969

	Percentage of Population Sampled						Number of Positive Slides					
	1967		1968		1969		1967		1968		1969	
District:	8	13	8	13	8	13	8	13	8	13	8	13
					4.0	8.0					4	3
Jan.			*(5.6	5.8)	4.0	4.0			*(19	24)	2	3
			*(5.0	5.4)	4.0	4.0			*(20	30)	0	2
Feb.			*(5.4	6.2)	42.0	6.0			*(34	24)	2	3
			*(5.4	6.0)	4.0	6.0			*(40	41)	1	3
Mar.			2.0	3.0	5.0	54.0			22	27	3	11
			1.0	2.0	5.0	9.0			9	11	0	6
Apr.			4.0	6.0	56.0	4.0			7	27	2	2
			3.0	4.0	2.0	6.0			4	25	2	7
May			3.0	6.0	4.0	64.0			11	13		14
			3.0	5.0	3.0	3.0			10	25		16
June			3.0	4.0	51.0	7.0			15	15	2	35
			2.0	4.0					13	22		
July			3.0	5.0					11	12		
			4.0	4.0					10	28		
Aug.			4.0	4.0					5	7		
			4.0	4.0					3	15		
Sept.	5.0/	7.0/	4.0	8.0			119/	146/	0	10		
	2.0	4.0	5.0	6.0			34	67	2	0		
Oct.	2.0	2.0	5.0	5.0			3	18	0	3		
	10.0	4.0	4.0	6.0			11	16	1	6		
Nov.	2.0	3.0	4.0	6.0			3	6	2	2		
Dec.	1.0	2.0	3.0	6.0			8	6	2	4		

__/ Data from census made in September 1967 prior to initiation of collective drug distribution (amodiaquine-Cl + primaquine) by CNAP.

*( ) Collective drug distribution temporarily suspended (1st four cycles of 1968). Active case detection, with slides taken from and medication given to fever cases only.

___ Survey cycles by CAMRS personnel.

CAMRS surveys are conducted every fourth cycle, alternating between the two districts, and are designed to provide the following information on each person interviewed: (1) vital statistics, (2) history of malaria, (3) education, (4) occupation, (5) spontaneous complaints, and (6) complaints solicited from refusers. These surveys are carried out by CAMRS personnel. During the three routine cycles between survey cycles, the local malaria program personnel record complaints on the house cards (CNAP TC-1) which are picked up and tallied upon completion of the study.

The most common and specific complaints received in the two districts (8 and 13) during the two CAMRS surveys already completed are listed in Table 36.

Table 37 presents the numbers of "complainers" in the two districts who expressed single or multiple complaints. These complaints are tabulated as 1, 2, 3, or 4 per person. The percentage of complainers (complainers/persons contacted X 100) registered in the second CAMRS cycle in each district declined significantly from the figures for the first cycle. It is possible that the higher complaint figures in the first survey cycle in each district were due to the presence of new personnel (both national and foreign) in the PTC program.

An attempt was made to classify the specific complaints (Table 36) into four broad groups. These were listed as: gastrointestinal, neurological, psychological, and allergic (Table 38).

At the close of the reporting period it was difficult to evaluate the effectiveness of the pyrimethamine + primaquine combination tablets. The acceptance levels of this combination, as shown in Table 34, were slightly lower than those of the country tablets (amodiaquine-Cl + primaquine), but the amount of information available was not sufficient to justify conclusions.

Table 39 shows the average registered population, actual numbers of slides taken, and slide positivity rates in Districts 8 and 13 during the January-June periods of 1968 and of 1969. The sharp reduction in the slide positivity rate (SPR) from 1968 to 1969 in the pyrimethamine + primaquine area (from 9.1% to 0.26%) and the lesser reduction in the control area (9.2% to 1.1%) may be attributable in part to the inclusion of greater numbers of non-febriles in the 1969 sampling. However, the significant reductions in absolute numbers of positives in both areas, but especially notable in the pyrimethamine + primaquine area, indicate the effectiveness of the medication and/or a possible reduction in malaria potential.

During the six-month period covered by this report, there were no reported positive slides from persons reported to be taking the pyrimethamine-primaquine combination. During this same period, there were five positive slides from persons claiming to have taken the amodiaquine-primaquine combination; three of these were *Plasmodium falciparum* and two were *P. vivax*. The five cases were investigated by the CNAP and reported as doubtful if the drugs had actually been taken.

TABLE 36

Physiological Classification of Complaints from Pyrimethamine-Primaquine Study Area (District 8) and from Amodiaquine-Primaquine Control Area (District 13)

CNAP Collective Drug Distribution Program, Zone 2, El Salvador

1969

Category of Complaint	First CAMRS Survey				Second CAMRS Survey			
	District 8 February 17 - March 2		District 13 March 17 - 30		District 8 April 21 - May 2		District 13 May 19 - 31	
	Number Complaints	% of Total Complaints	Number Complaints	% of Total Complaints	Number Complaints	% of Total Complaints	Number Complaints	% of Total Complaints
Vomiting	251	21	368	20	163	18	123	16
Dizziness	247	20	337	19	147	16	129	17
Headache	179	15	272	15	109	12	64	9
Abdom. Pain	125	10	121	7	103	11	52	7
Anorexia	83	7	79	4	50	5	31	4
Diarrhea	82	7	93	5	62	7	79	11
Nausea	66	5	68	4	39	4	23	3
Other Complaints*	177	15	472	26	243	27	247	33
Total Complaints	1,210	100%	1,810	100%	916	100%	748	100%
Total Number of Persons Complaining	986		1,323		792		679	
Total Number of Persons Contacted	3,201		3,555		3,509		3,987	

* Great varieties of "other complaint" categories were represented in the spontaneous (unsolicited) complaints. The number of these categories for the surveys were as follows: First Survey; District 8: 51 categories; District 13: 92 categories; Second Survey; District 8: 77 categories; District 13: 64 categories.

TABLE 37

Numbers of Complainers Presenting Single or Multiple Complaints in Pyrimethamine-Primaquine Study Area (District 8) and in Amodiaquine-Primaquine Control Area (District 13)

CNAP Collective Drug Distribution Program, Zone 2, El Salvador

1969

Persons Presenting	First CAMRS Survey				Second CAMRS Survey			
	District 8 February 17 - March 2		District 13 March 17 - 30		District 8 April 21 - May 2		District 13 May 19 - 31	
	Number Persons	Total Complaints	Number Persons	Total Complaints	Number Persons	Total Complaints	Number Persons	Total Complaints
1 Complaint	775	775	923	923	690	690	615	615
2 Complaints	199	398	320	640	82	164	59	118
3 Complaints	11	33	73	219	18	54	5	15
4 Complaints	1	4	7	28	2	8	-	-
Totals	986	1,210	1,323	1,810	792	916	679	748
Persons Contacted	3,201		3,555		3,509		3,987	
$\frac{\text{complainers}}{\text{persons contacted}} \times 100 =$	30.8%		37.2%		22.6%		17.0%	

TABLE 38

Proportion of Physiological and Non-physiological Complaints from Pyrimethamine-Primaquine Study Area (District 8) and from Amodiaquine-Primaquine Control Area (District 13)

CNAP Collective Drug Distribution Program, Zone 2, El Salvador

1969

Comprehensive Complaint Category*	First CAMRS Survey		Second CAMRS Survey	
	District 8 Feb. 17-March 2	District 13 March 17-30	District 8 April 21-May 2	District 13 May 19-31
Gastrointestinal	52.4%	42.6%	50.3%	46.1%
Neurological	37.8%	40.0%	30.4%	28.7%
Psychological	6.0%	12.2%	15.5%	19.1%
Allergic	1.8%	3.5%	2.7%	3.7%
Other	2.0%	1.7%	1.1%	2.4%

*Categories and classifications made by Ernest Feigenbaum, M.D.

TABLE 39

Comparison of Slides Taken and Slide Positivity Rates in Pyrimethamine + Primaquine and Amodiaquine-Cl + Primaquine Areas during January - June; 1968 and 1969

	1968	1969
DISTRICT 8 (pyrimethamine + primaquine)		
Average Population	5100	5455
Number Slides Taken	2103	6984*
Number Slides Positive	191	18
Slide Positivity Rate	9.1%	0.26%
DISTRICT 13 (amodiaquine-Cl + primaquine)		
Average Population	5488	5850
Number Slides Taken	2846	9459*
Number Slides Positive	262	105
Slide Positivity Rate	9.2%	1.1%

* Includes two CAMRS special surveys.

CHARACTERIZATION OF CENTRAL AMERICAN MALARIA PARASITES

No information is available on the relapse pattern, drug susceptibility, or virulence of the malaria parasites prevalent in Central America. Because of this lack of information, it was decided that CAMRS would collaborate with other U.S. research groups in such a study, using *Aotus trivirgatus* (owl monkeys) as much as possible to transport the infections to the collaborating laboratories.

During April 1969, a CAMRS team accompanied a researcher from Stanford University, California, on a field trip to the Marcovia area of Honduras for a collection of infected bloods. The researcher brought four owl monkeys with him and CAMRS furnished three. The four animals from Stanford University were inoculated with three strains of *P. falciparum* while the three animals from CAMRS were inoculated with two strains of *P. vivax* and one of *P. falciparum*. The three animals from CAMRS were delivered to the Primate Section of the Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH/NIAID), U.S. Public Health Service, Chamblee, Georgia, for use in connection with human volunteer studies being carried out at the U.S. Penitentiary in Atlanta, Georgia. At the close of the reporting period, patent parasitemia had appeared in one of the monkeys returned to Stanford University and one of those sent to the NIH/NIAID, both being *P. falciparum* infections.

SEROLOGIC STUDIES

Indirect Hemagglutination Inhibition Tests

The CAMRS is collaborating with the Parasitology Section of the NCDC Laboratory Program in evaluating the Indirect Hemagglutination Inhibition Test as an epidemiologic tool in malaria eradication activities. CAMRS personnel are collecting blood specimens on filter paper strips from field populations in El Salvador for the laboratory tests being performed by the Laboratory Program in Atlanta, Georgia. Several hundred specimens were submitted to the Parasitology Section during the report period. This collaborative study will continue over at least another year before adequate data will become available for conclusive evaluation.

EPIDEMIOLOGIC STUDIES

Determination of G-6-P.D. Levels in Erythrocytes of Various Population Groups in El Salvador

Although no difficulties have been encountered in the CNAP's mass drug distribution program involving the fortnightly administration of primaquine, little information was available on the G-6-P.D. levels of the general population receiving the antimalaria drugs. To provide information on this question, CAMRS began a survey of G-6-P.D. levels in El Salvador in collaboration with the Biochemistry Department of the Medical School of the National University of El Salvador. The blood samples are to be taken and the reagents supplied by CAMRS, with the Medical School conducting the laboratory tests.

During the period February to June 1969, 500 samples were taken in the western coastal plains area around Sonsonate, El Salvador. Since G-6-P.D. deficiency is sex-linked, only the male population was sampled. An additional 500 samples will be taken in the eastern coastal plains and Highlands areas of El Salvador.

Of the 500 samples taken by the end of the reporting period, one was lost in handling. Of the remaining 499 examined, 21 were found with deficiencies, representing 4.2 percent of the male population tested.

HEALTH EDUCATION ACTIVITIES

As was evident in the developments which ultimately led to the establishment of the Central America Malaria Research Station, refusal by mounting numbers of people to take antimalarial drugs in community wide treatment programs has resulted in a serious dilution of such programs as an eradication measure. In terms of human behavior, this was seen as the most serious single problem confronting eradication efforts in Central America and the one most urgently calling for improved educational action. Consequently CAMRS health education resources have been centered primarily on a better definition of this problem and ways to attack it.

Because El Salvador, in effect, serves as the pilot country for CAMRS in this field of activity, exploration of health education research possibilities and initial steps to develop studies have been centered largely with personnel of the National Malaria Eradication Service (CNAP) of this country.

During the past year, the following three major types of activity emerged as holding greatest possibility for feasible health education study effort within the limits of available resources:

1. Field surveys, manageable operational studies on behavioral influences, and health education methods and materials.

2. Studies of relevant data from national programs and CAMRS activities.

3. Supportive services to other CAMRS activities.

The process of developing health education studies in the operational setting has been a slow one. A large share of the responsibility for such studies must be shouldered by personnel in the national program. The Salvadoran staff has been interested and cooperative but, as is more often than not the case in operational malaria eradication programs, day-to-day pressures are numerous and urgent.

Understandably, the demands of current operational eradication efforts, coupled with the normal reservations present when experience in research and studies as a part of field operation has been limited, have been conducive to a cautious approach. By the same token, CAMRS, too, has exercised caution in favor of careful assessment of the value and feasibility of possible studies. The most practical and acceptable approach has been to build on activities which were initiated by the National Malaria Eradication Campaign.

Field Study of Factors Which May Influence Acceptance in Collective Drug Treatment Programs

Early in 1969, CNAP made a decision to place La Libertad, a coastal town of approximately 6,000 inhabitants, under communitywide antimalaria drug treatment ("Collective drug treatment"). The Zone II (La Libertad) Medical Chief and health education auxiliary, with concurrence from the central office, decided to survey health attitudes and knowledge shortly before the drug distribution was to start. Influenced by a prior survey in another coastal community, the Zone II staff approached personnel in the secondary school about having students conduct the survey through household interviews. The response was affirmative and enthusiastic.

The activity was billed as a general health survey, and CNAP staff purposely kept in the background in order to introduce as little bias as possible with respect to interviewers and respondents. For the Zone II staff, the stated purpose of the survey was to gather information to help devise the content of promotional materials and activities in the collective drug treatment program.

The health education auxiliary drafted an interview guide and, at this point, asked CAMRS for help with revision. Through combined effort, a questionnaire was developed that could be handled by the relatively inexperienced youthful interviewers and from which responses could easily be tabulated.

With 120 students participating, the survey was completed in one day. All homes were visited; and, in 731, at least one adult (15 years of age or above) was at home. There were no refusals. The CAMRS health educator, present as an observer, noted that the students did remarkably well and that the people were obviously proud of their enterprising youth.

Zone personnel tabulated responses immediately following the survey and used the findings as a basis for public information as intended. They were more than willing, however, for CAMRS to utilize the completed questionnaires for further study of data. Subsequently, information which might have relevance for health education programming or serve as an indicator for further investigation was extracted from the data. Study of the data was on the basis of answers for themselves or for the household by the 731 members of individual households who served as respondents.

On the basis of the data studied, the profile of respondents which emerged was:

- Based on a total population of 6,000, respondents comprised 12.2% of La Libertad residents.
- Based on 900 households (number cited by CNAP) respondents represented 81% of the households.
- 66% were women (Table 39).
- 68% were between the ages of 20 and 49 (Table 39).
- 72% were probably not functionally literate, i.e., had not completed a minimum of five years of schooling (Table 40).
- 16% had had malaria more frequently than any other disease.
- 31% reported that they or someone else in the household had had malaria.
- 93% of respondents wanted to be protected against malaria.
- Respondents to the interview question, "How do you get rid of malaria in the community?" gave the following answers (Table 41):

  41% - Take medicine
  40% - Kill mosquitoes
  5% - Kill mosquitoes and take medicine
  8% - Gave other responses
  6% - Had no opinion

- 19% (142 respondents) believed malaria to be the most dangerous disease (Table 42). Among these 142 respondents there were the following findings:

  70% were between 20 and 49 years of age (Table 43).
  77% were functionally illiterate (Table 44).
  44% reported that they or someone else in the household had had malaria.
  80% responded that taking medicine or killing mosquitoes was the best way to eradicate malaria. The responses were equally divided between the two answers. In no instance did a respondent reply with both measures.

TABLE 39

Distribution of Respondents by Age Group and Sex

(Years)	Total in Age Group Number	%	Distribution by Sex M	%	F	%
15 - 19	72	9.8	19	2.6	53	7.2
20 - 34	287	39.3	73	10.0	214	29.3
35 - 49	212	29.0	79	10.8	133	18.2
50 - 59	91	12.4	39	5.3	52	7.1
≥60	69	9.4	37	5.1	32	4.4
TOTAL	731	100	247	33.8	484	66.2

TABLE 40

Distribution of Respondents by Age Group and Educational Level

Age Group (Years)	No.	Distribution by Education Level: No Formal Education No.	%	Less than Elementary No.	%	Elementary (6 Yrs.) No.	%	Jr. High (3 Yrs.) No.	%	Sr. High or Vocational No.	%	University No.	%	No Answer No.	%
15 - 19	72	14	19.4	21	29.2	28	38.9	5	6.9	4	5.6	0	-	0	-
20 - 34	287	90	31.4	99	34.5	71	24.7	15	5.2	8	2.8	1	0.3	3	1.0
35 - 49	212	108	50.9	67	31.6	27	12.7	3	1.4	6	2.8	1	0.5	0	-
50 - 59	91	52	57.1	26	28.6	10	11.0	1	1.1	1	1.1	0	-	1	1.1
≥60	69	39	56.5	15	21.7	12	17.4	0	-	1	1.4	0	-	2	2.9
TOTAL	731	303	41.5	228	31.2	148	20.2	24	3.3	20	2.7	2	0.3	6	0.8

TABLE 41

Responses on Most Important Malaria Eradication Measure by Age and Sex

Age	Sex	Number	Kill Mosq. and Take Medicine		Kill Mosq.		Take Medicine		Other		No Opinion	
			No.	%	No.	%	No.	%	No.	%	No.	%
15 - 19	M	19	0	-	4	21.1	12	63.2	2	10.5	1	5.3
	F	53	0	-	24	45.3	19	35.8	4	7.5	6	11.3
20 - 34	M	73	1	1.4	36	49.3	26	35.6	6	8.2	4	5.5
	F	214	17	7.9	77	36.0	92	43.0	15	7.0	13	6.1
35 - 49	M	79	4	5.1	38	48.1	24	30.4	8	10.1	5	6.3
	F	133	8	6.0	50	37.6	61	45.9	9	6.8	5	3.8
50 - 59	M	39	1	2.6	17	43.6	15	38.5	5	12.8	1	2.6
	F	52	1	1.9	20	38.5	25	48.1	3	5.8	3	5.8
≥60	M	37	1	2.7	15	40.5	11	29.7	8	21.6	2	5.4
	F	32	0	-	9	28.1	17	53.1	2	6.3	4	12.5
All	M	247	7	2.8	110	44.5	88	35.6	29	11.7	13	5.3
	F	484	26	5.4	180	37.2	214	44.2	33	6.8	31	6.4
TOTAL		731	33	4.5	290	39.7	302	41.3	62	8.5	44	6.0

TABLE 42

Disease Believed To Be Most Dangerous

Responses	Number of Respondents	% of Total
Cancer	283	38.7
Malaria	142	19.4
Tuberculosis	109	14.9
Other*	88	12.0
Gave more than one answer	37	5.1
"Don't know"	66	9.0
No answer	6	0.8
TOTAL	731	100

* In the general categories of gastrointestinal or acute respiratory.

TABLE 43

Distribution by Age Group of Those Who Believed Malaria To Be the Most Dangerous Disease

Age Group (Years)	Distribution by Age and Sex					
	Total in Group		M		F	
	No.	%	No.	%	No.	%
15 - 19	14	9.9	3	21.4	11	78.6
20 - 34	56	39.4	19	33.9	37	66.1
35 - 49	44	31.0	18	40.9	26	59.1
50 - 59	14	9.9	6	42.9	8	57.1
≥60	14	9.9	4	28.6	10	71.4
All	142	100	50	35.2	92	64.8

TABLE 44

Distribution by Sex and Educational Level of 142 Respondents in the La Libertad Survey Who Gave Malaria As "The Most Dangerous Disease"

Formal Education	Total in Group		M		F	
	No.	%	No.	%	No.	%
None	63	44.4	17	27.0	46	73.0
Less than Elementary	47	33.1	20	42.6	27	57.4
Elementary (6 Yrs.)	28	19.7	11	39.3	17	60.7
Jr. High (3 Yrs.)	2	1.4	2	100.0	0	-
Sr. High or Vocational	2	1.4	0	-	2	100.0
TOTAL	142	100.0	50	35.0	92	65.0

A comparison between respondents who believed malaria to be the most dangerous disease and total number of respondents in the survey is as follows:

	Subgroup (142)	Total Group (731)
20 - 49 yr. age group	70%	68%
Functionally illiterate	77%	72%
Reported having had malaria more frequently than any other disease	27%	16%
Reported that one or more household members had had malaria	44%	31%
Wanted protection against malaria	93%	93%

The analysis of the La Libertad survey data did not provide sufficient information to develop a sound educational program. It emphasized the need for additional information and the desirability of follow-up on the survey findings. One question that it raised was the degree to which data are representative of the La Libertad community. For example, two thirds of the respondents were women. Therefore, to what extent did this represent men at work and to what extent did it reflect households without men? The interview guide did not include a question about head of household or composition of household.

La Libertad is, for El Salvador, a town of fair size, a principal port, and generally considered to be a better educated and more sophisticated community than average. The degree to which respondents were representative of the educational level is not known. The survey did not include a question on the education level of other members of the household; follow-up surveys should include this question.

Another aspect of the study, and one on which follow-up has been initiated, is to determine the relationship between some of the answers given by respondents and acceptance of medication. Tentatively, the follow-up will include a re-survey of households by students, study of statistical data which has accumulated on acceptance of medication, and visits by CAMRS and/or CNAP staff to a random sample of households.

Some of the questions which may be answered are:

1. Has a respondent who answered "take medicine" to eradicate malaria a better acceptance record than one who gave some other answer? What is the household record in cases where the respondent said "take medicine" versus the record of those in which the respondent gave some other answer?

2. What relationship, if any, is there between acceptance and the answer that some one in the household has had malaria?

3. In practice, what is the relationship between educational level and acceptance?

4. How does acceptance of respondents who believed malaria to be the most dangerous disease compare with that of those who named some other?

5. What has been the acceptance level of students who participated in the survey and of other members in their households? What will be the effect of a second student survey on community acceptance of antimalaria medication?

Study of Factors Affecting the Acceptance of Collective Drug Treatments

The study of relevant data available from CNAP and CAMRS activities was seen as a permanent, ongoing aspect of health education investigation. Analysis of this kind of data may validate or disqualify subjective impressions about factors which affect acceptance of antimalaria drug medication as well as bring to light factors which have not heretofore been identified as behavioral influences with respect to acceptance or refusal of medication in collective treatment programs. This type of analysis was begun during the report year.

A review was made of CY 1967 and CY 1968 operational data from the CNAP collective drug treatment program in Zone III (Usulutan, El Salvador). In this study, factors which were identified for analysis in relation to acceptance of antimalaria drug medication were:

1. Sequence of treatment cycles.

2. Time of year in which a cycle occurs.

3. Malaria case rate at the time that collective drug treatment was introduced.

4. Size of population in a locality.

The examination of each of these factors in relation to acceptance of medication was limited by the extent to which comparable data for the various localities could be extracted from statistics based on program activities as presented in the report used for the study.

In Zone III, 49 localities were placed under collective drug treatment in 1967. Dates on which treatment was introduced and the number of treatment cycles conducted thereafter during 1967 varied. In 25 localities, collective drug treatment was introduced between May 8 and June 19. In 24 localities, the first cycle began August 21 and continued for nine cycles without interruption until December 22, 1967.

The program was resumed in 1968 in 44 of the 49 localities under collective drug treatment in 1967. However, there were variations among localities with regard to the beginning date of the initial 1968 cycle, the number of cycles conducted, and the date on which the last cycle for the year ended. Variations stemmed from both epidemiologic and administrative circumstances.

The populations in the 49 localities ranged from 300 to 4,500. The malaria case rate at the time that collective drug treatment was introduced varied from 3 to 100 per 1000 population.

Study of the data was as follows:

1. Sequence of Treatment Cycles

To examine the effect of sequence of cycle <u>alone</u> on drug acceptance, two groups of 16 localities each, matched for population size by localities, could be compared for the first nine cycles following the introduction of collective treatment. In one group (Group A), the beginning date for the first cycle in the respective localities fell sometime between May 8 and June 19, 1967. In the other group (Group B), the beginning date for all localities was August 21, 1967.

Percentage levels of acceptance by cycle were added for each group. The potential total per cycle for each group was therefore 1600.

Figure 11 illustrates the fluctuation in acceptance of medication for each of the two groups during their first nine cycles. It will be noted that, in both Groups A and B, the highest level of acceptance was during the first cycle and that both had a sharp drop in the second cycle. For the remaining cycles, the trend for Group A was primarily downward. Group B had a more erratic pattern and ended with an upward trend for cycles 8 and 9.

A second examination of the effect of cycle sequence was made on the basis of eighteen localities that had a total of nine consecutive cycles in 1967 following the introduction of collective treatment. The first cycle began August 21 and the ninth ended December 22. In these localities, collective treatment was not resumed until June 3, 1968, a lapse of five months.

The nine cycles in 1967 were compared with the first nine cycles in these same communities in 1968. As was the case when two different groups of communities were compared in the same year, a sharp drop in acceptance occurred in the second cycle (Figure 12). Fluctuations and downward trend were less pronounced in 1968. However, in general, the acceptance level was lower throughout the nine cycles in 1968.

There was somewhat more similarity in the pattern of fluctuation in the comparison between cycles for the same communities in two different years than for the two different groups of communities in the same year.

An examination of fluctuation in levels of acceptance for a large number of sequential cycles could be made for only eight of the 49 localities. Collective drug treatment was introduced in these eight on August 21, 1967, and terminated for that year on December 22. In 1968, the program was resumed on April 15 and continued without interruption for 15 cycles. Figure 13 shows the variations that occurred. It would appear that drug acceptance maintained a slightly steadier level for the last 12 cycles.

2. Time of Year in Which a Cycle Occurs

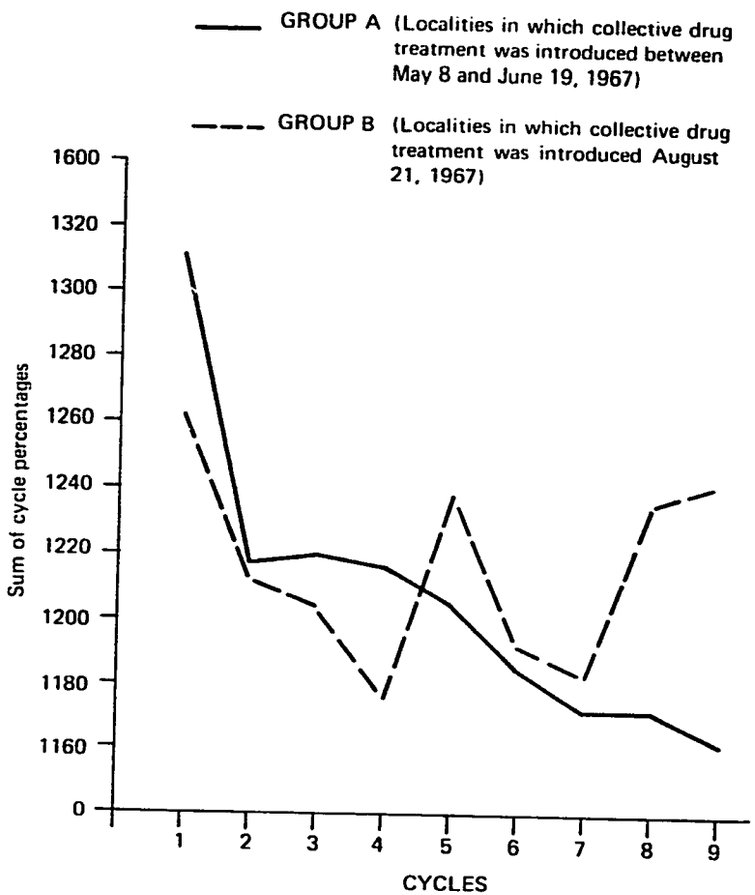
The data were examined to determine the relationship between drug acceptance and the month in which the cycle occurred without regard for any other factor which may have influenced acceptance. Twenty-six localities, ranging in size from the smallest population to the largest, were included and were representative of the various population sizes in the 49 localities in the study. Table 45 shows the sum of cycle percentages of drug acceptance for 23 cycles; the highest possible total per cycle would be 2600.

TABLE 45

Sum of Cycle Percentages by Dates of Cycle

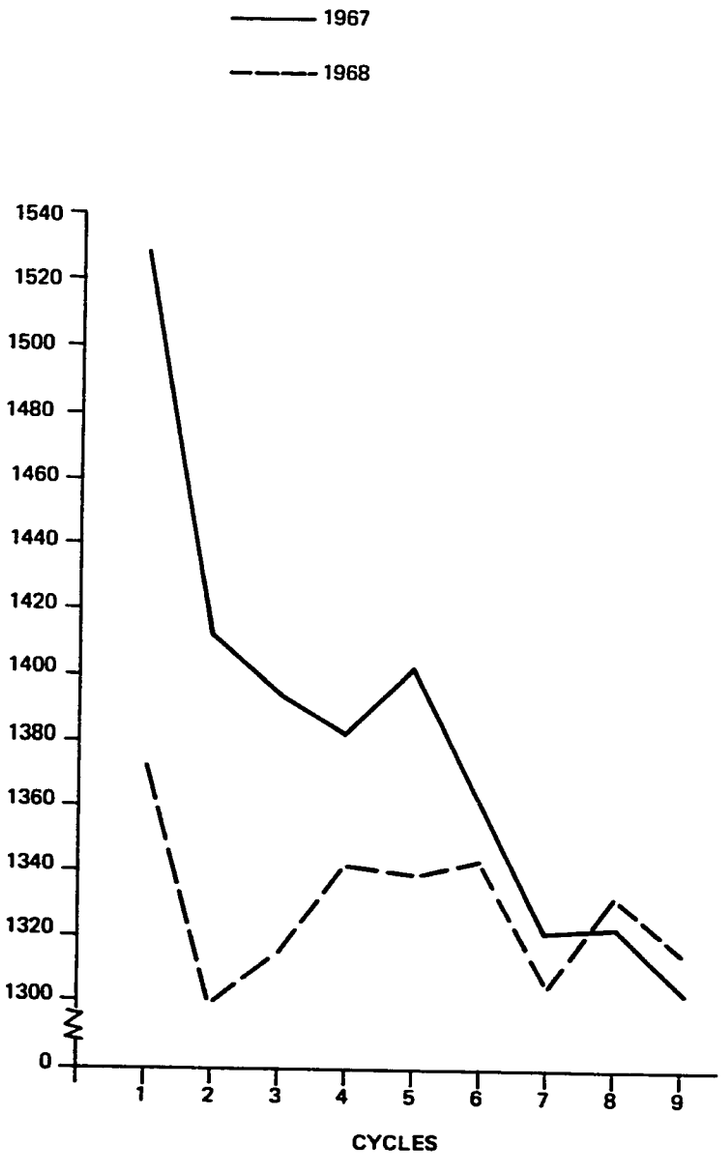
Dates - 1967	Sum	Dates - 1968	Sum
Aug. 21 - Sept. 2	1970	Apr. 15 - May 4	1938
Sept. 4 - Sept. 16	1913	May 6 - May 18	1862
Sept. 18 - Sept. 30	1928	May 20 - June 1	1820
Oct. 2 - Oct. 14	1810	June 3 - June 15	1867
Oct. 16 - Oct. 28	1874	June 17 - June 29	1922
Oct. 30 - Nov. 11	1863	July 1 - July 13	1867
Nov. 13 - Nov. 25	1950	July 15 - July 27	2006
Nov. 27 - Dec. 9	2030	Aug. 5 - Aug. 17	1897
Dec. 11 - Dec. 22	2075	Aug. 19 - Aug. 31	1887
		Sept. 2 - Sept. 14	1880
		Sept. 16 - Sept. 28	1916
		Sept. 30 - Oct. 12	1887
		Oct. 14 - Oct. 26	1967
		Oct. 28 - Nov. 9	1905

FIGURE 11. Relationship Between Drug Acceptance and Cycle Sequence in Two Groups of 16 Localities Each for Their First Nine Cycles Following the Introduction of Collective Treatment.*



*Based on data supplied by the collective treatment program in Zone III USULUTAN, EL SALVADOR.

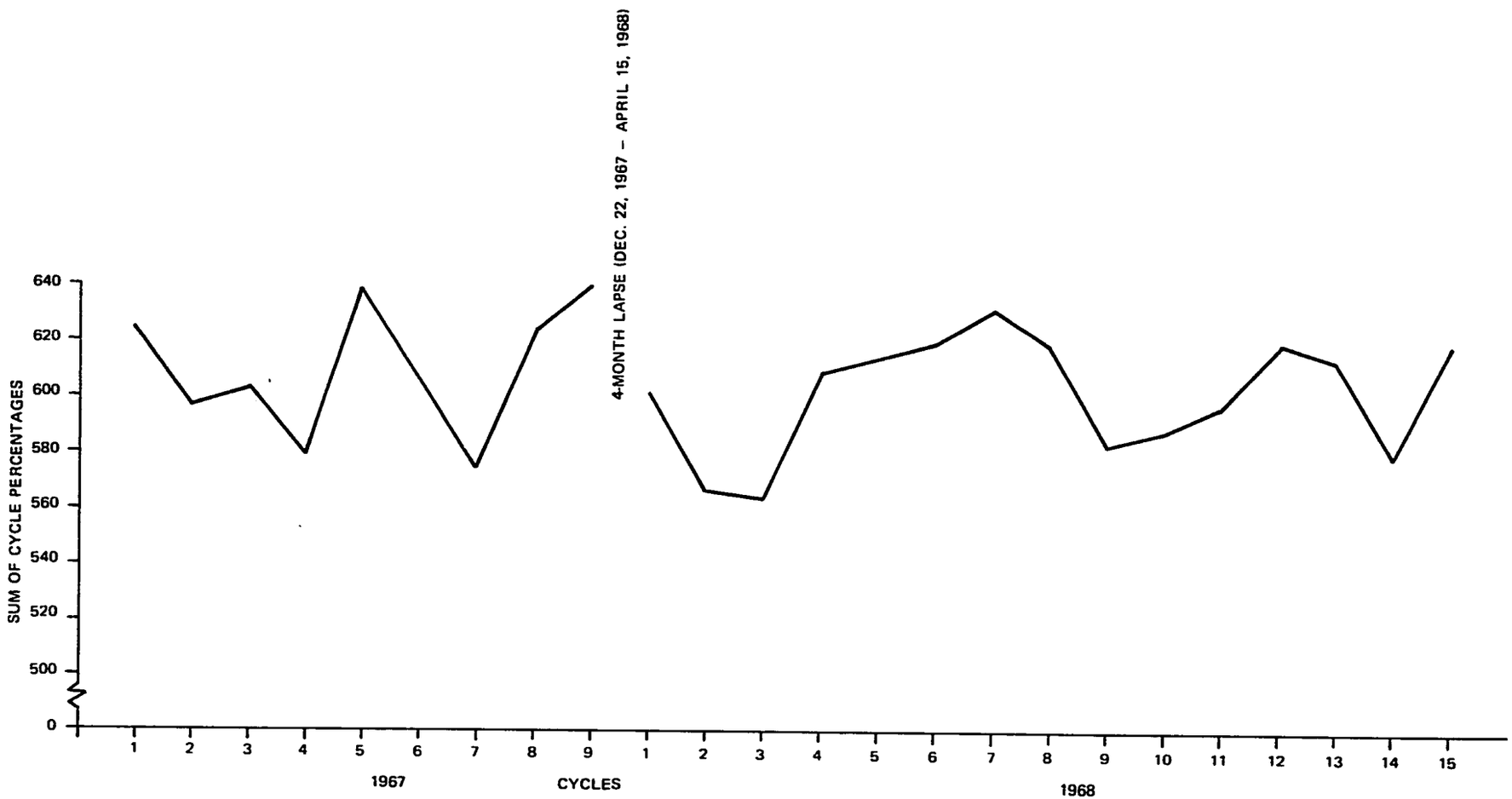
FIGURE 12. Relationship Between Drug Acceptance and Cycle Sequence in 18 Localities With a Five-Month Lapse Between 1967 and 1968 Cycles.*



*Based on data supplied by the collective treatment program in Zone III USULUTAN, EL SALVADOR.

FIGURE 13. Drug Acceptance by Cycle Sequence for 8 Localities with a 4-Month Lapse Between 1967 and 1968 Cycles.*

*Based on data supplied by the collective treatment program in Zone III USULUTAN, EL SALVADOR



The data show that the range of sums of cycle percentages is from 2075 (80% of 2600) to 1810 (70%). However, within this overall total there was one locality with a low of 41 percent on one cycle and a high of 69 percent. The highest that any locality reached on one cycle was 92 percent.

The drug acceptance level tended to be higher in 1967 but appeared to be on the increase at the end of 1968. Overall, one is not able to identify a specific relationship between drug acceptance and time of year from these data.

3. Malaria Case Rate at the Time That Collective Treatment Was Introduced

The percent of the population that accepted antimalaria drugs during the introductory cycle in each of the 49 localities was examined in relation to the malaria case rate when the cycle was initiated. Table 46 below summarizes the results of this study.

TABLE 46

Relationship Between Percentage of Drug Acceptance for the Introductory Cycle and Malaria Case Rate When the Cycle Was Initiated

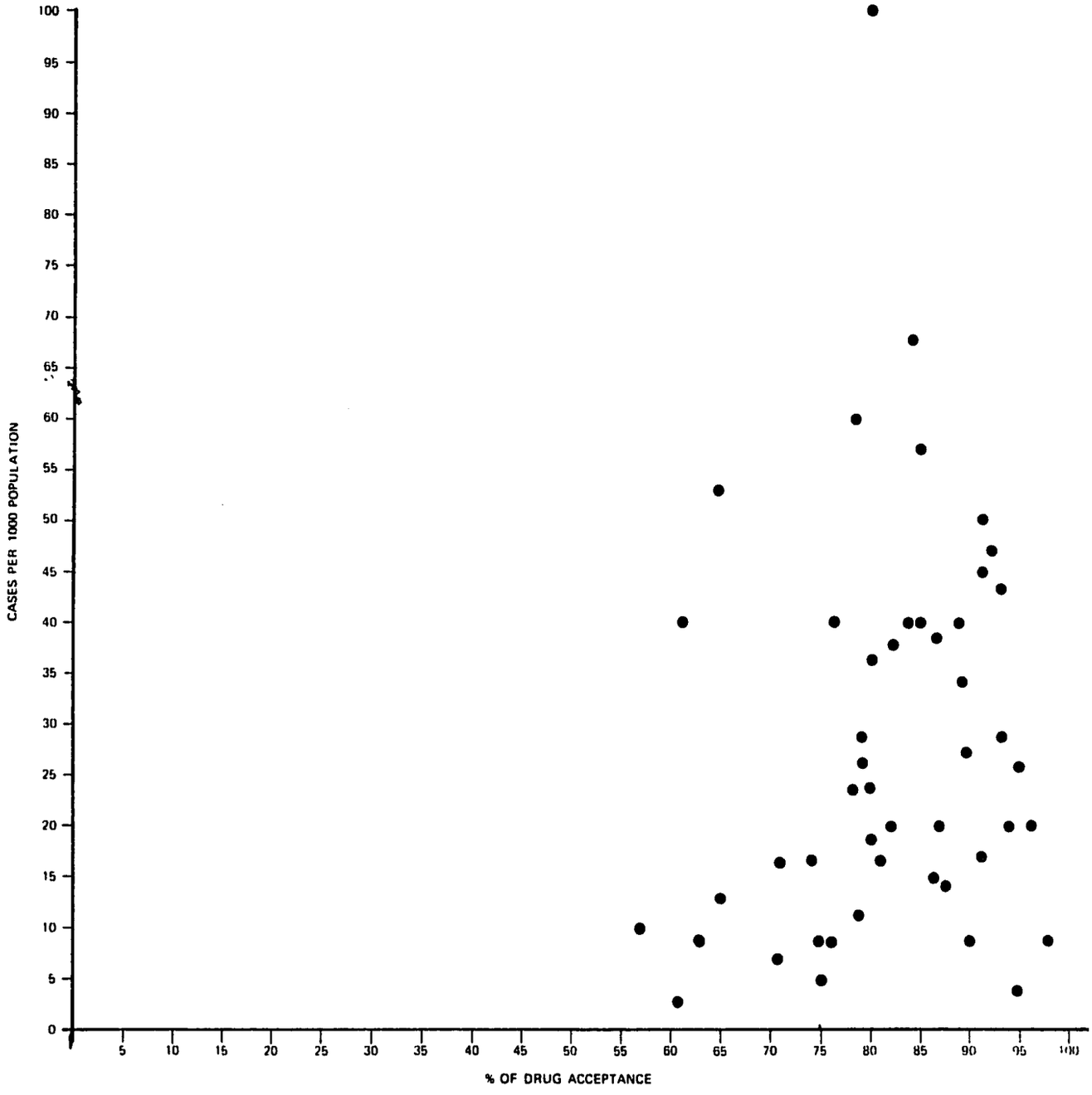
Malaria Cases per 1000 population	No. of Localities	Percentage of Acceptance				
		65 or lower	65-74	75-84	85-94	>94
3 - 10	10	3	1	3	1	2
11 - 20	13	0	3	4	5	1
21 - 30	8	0	0	4	3	1
31 - 50	13	1	0	4	7	1
50 - 100	5	0	1	3	1	0
TOTAL	49	4	5	18	17	5

The data show that when the case rate exceeded 20 per 1000 population there tended to be a rise in the acceptance level. In the review of individual localities, the highest percentage of acceptance obtained was 99 percent in one locality with a case rate of 34 per 1000 population. Total population of this locality was 821. This same locality had a total of 29 cycles in 1967 and 1968 with an average of nearly 90 percent acceptance for the two years. Following the initial cycle, only three malaria cases were confirmed. One of these occurred in the 12th cycle in 1967, one in the 4th cycle in 1968, and one in the 6th cycle in 1968.

The locality with the lowest acceptance, 56 percent, for the introductory cycle had a case rate of 40 per 1000 population. Population of the locality was 1130. The locality had nine treatment cycles in 1967 and none in 1968. Its average acceptance level was in the low 60's. Its highest cycle percentage was 74. Following the initial cycle four cases were reported in the 3rd cycle, one in the 5th, and one in the 9th.

There were other inconsistencies between case rate and acceptance which lend interest and purpose to further analysis of such cases as well as to more extensive analysis of the positive relationship between case rate and acceptance. A scatter diagram was made to illustrate the relationship between drug acceptance and case rate (Figure 14). This diagram suggests that a correlation does not necessarily exist between drug acceptance and case rate. This distortion occurs primarily with a high drug acceptance and a low case rate.

FIGURE 14. Scatter Diagram: Relationship Between Malaria Case Rate and Percentage of Drug Acceptance for Introductory Cycle in 49 Localities.



4. Size of Population in a Locality and Drug Acceptance

Percentages of drug acceptance for the initial nine cycles (1967) in 49 localities were summed for each locality. These sums of cycle percentages were charted by size of population with the lowest population being 321 and the highest 6500 (Figure 15). The groupings are in ascending order by population. Similar distribution of percentage of drug acceptance was observed in each group. There are more low levels of drug acceptance in the 1101-1500 population group. However, one of the seven localities had the second highest acceptance level among the 49.

Summary:

An examination of the four factors thought to be possibly associated with drug acceptance levels did not, on the basis of these limited data, demonstrate any specific patterns of relationships. However, before eliminating these factors as possible influences on drug acceptance, a more extensive study should be made. Such a study should include data from all zones over a longer period of time.

It seems apparent that additional kinds of data are needed. From the data examined, it was not possible to determine whether persons refusing the drug are the same from cycle to cycle or represent different individuals each time. Is there an age, sex, occupational, and/or malaria history difference between acceptors and refusers? It appears that there may be a hard core of participation around 50 percent to 60 percent.

In order to institute a health education program specifically directed toward the non-acceptors, it is necessary to have definitive information about the behavior of these individuals. To obtain these kind of data, it may be necessary to conduct case studies within localities that have a low drug acceptance level as well as in those with a high acceptance level. Information gained from these two types of communities should be compared to determine what differences can be identified in terms of behavioral influences. This would provide the basis for designing operational studies focused on developing methods to improve drug acceptance.

Survey of San Diego Beach Households in Conjunction with a ULV Project

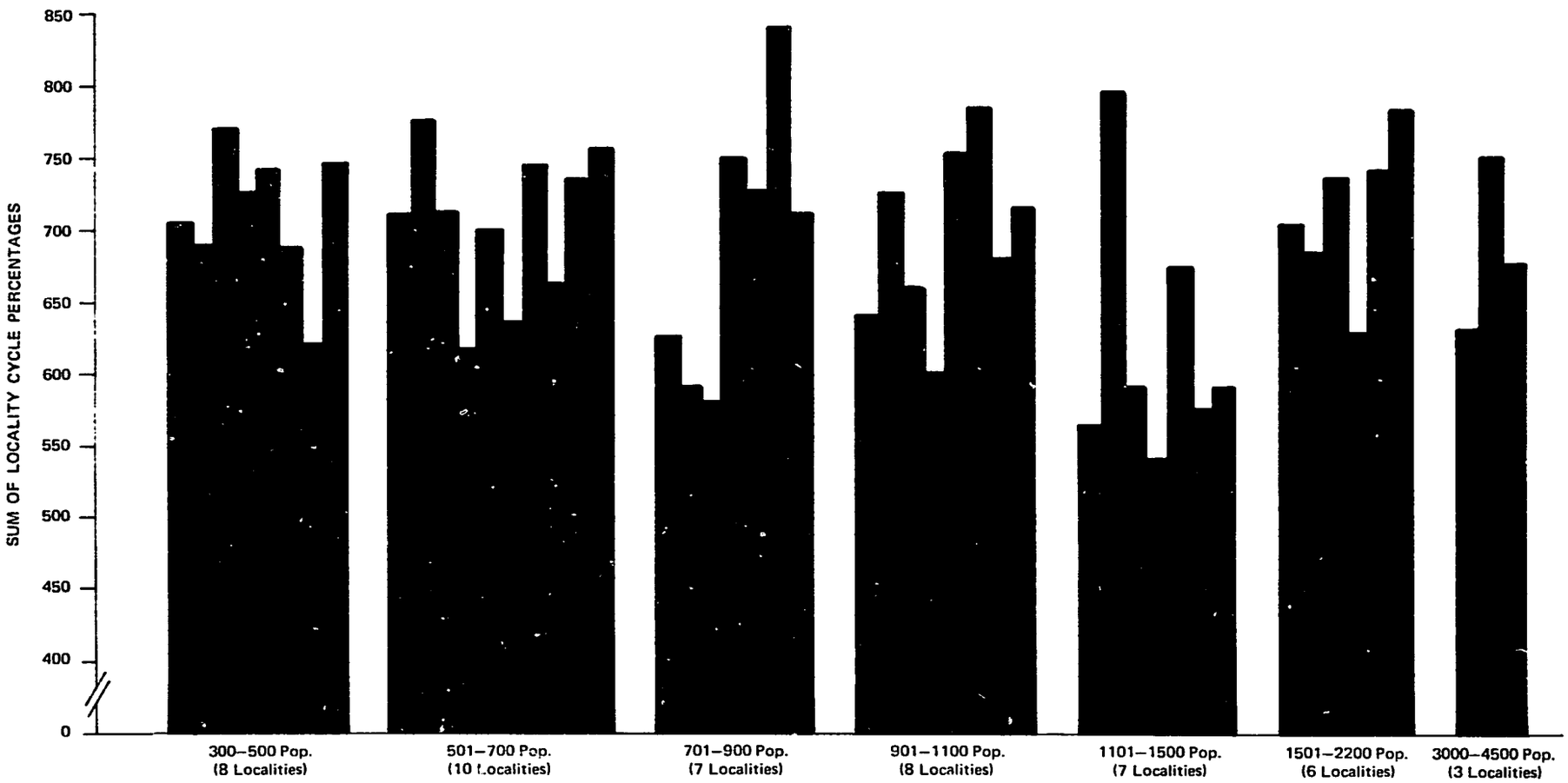
The survey was conducted among 20 percent of the households of permanent residents in the San Diego Beach area in conjunction with the ultra low volume application of malathion (see Page 30), to add another dimension to other data collected through entomologic studies. Permanent residents at San Diego Beach are families employed as caretakers of beach houses.

Respondents were interviewed before, during, and after the series of aerial applications in order to gather their impressions on current mosquito density, fluctuations in density throughout the year, characteristics of mosquitoes, including biting habits and breeding places, and effect of the malathion application. Respondents were also asked about measures they use to combat mosquitoes.

Sixty-two households were included in the survey. The first series of interviews were conducted February 13-17, 1969, the second series a month later, and the third series in May following termination of the ULV project. In the second round of interviews, it was possible to contact all 62 households. However, on the third round it was possible to locate adults in only 34 of the households.

Respondents gave detailed and graphic descriptions of the kinds of mosquitoes that bite them. Twenty-seven of them described the "small mosquito with white feet". This one, they said, did not "sing" before biting. Although the majority of respondents had had no formal schooling, they left little doubt of their power of observation with respect to mosquitoes.

FIGURE 15. Relationships Between Drug Acceptance and Population of Locality (First 9 Cycles 1967 for 49 Localities, Zone III, Usulutan, El Salvador).



Other responses included:

58 respondents said mosquitoes bite most in the evening beginning around 6:00 p.m.

35 said there was no difference between indoor and outdoor biting.

19 said there was more biting outside (only two houses were screened).

49 said mosquitoes are worst during the rainy season.

43 said the two estuaries which partially encircle the beach area are responsible for so many mosquitoes.

The kinds of control measures volunteered were as follows:

Smoke	- 26
Nets for all	- 16
Nets for children	- 7
Insecticide	- 9
Miscellaneous responses	- 10
Do nothing	- 6
Total responses	74 (Some gave more than one response.)

Fifty-two of the group had lived in the locality for more than a year.

On the second visit after the ULV program had begun, only 33 houses would offer an opinion as to whether or not the mosquito population had been reduced. Of these, 25 said it had been. The "no opinion" respondents frequently said they hoped the plane would spray again.

On the third visit, all respondents said they thought the aerial spraying was a good thing and if it were continued it probably would help. They volunteered suggestions about time of day to spray, frequency, and strength of the insecticide.