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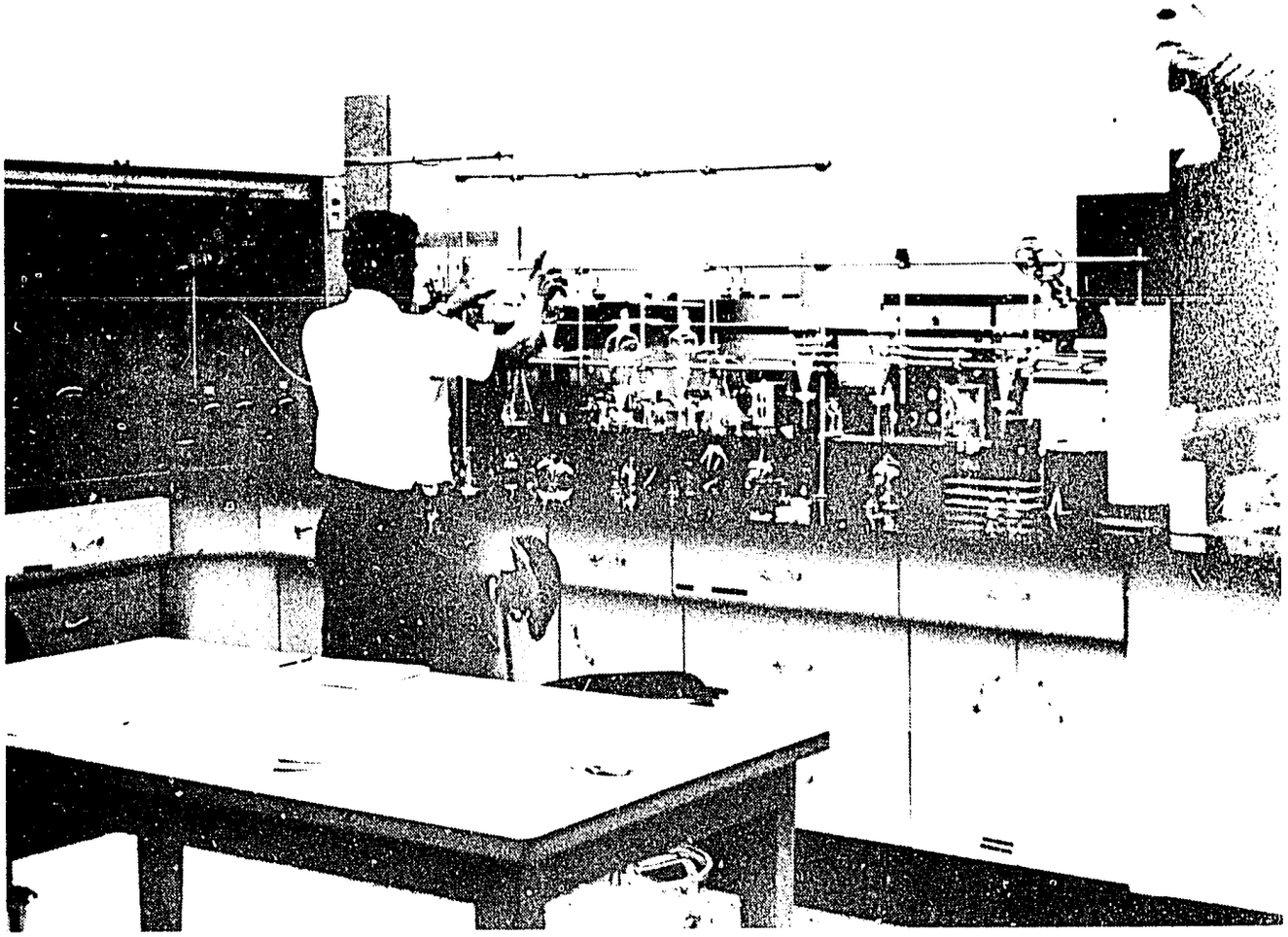

ACTIVITIES FOR YEAR ENDING JUNE 30, 1971

central america malaria research station

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE — PUBLIC HEALTH SERVICE

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central america malaria research station

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INTRODUCTION

The Central America Malaria Research Station, a joint effort of the U.S. Agency for International Development and the Center for Disease Control (CDC) of the U.S. Public Health Service, was inaugurated in February 1968, and has, as of June 30, 1971, completed a third full year of operation.

During the year the facilities of the Station were improved by the addition of a new outdoor insectary shelter and new insectary isolation rooms, and by partitioning of several areas in the main building and the entomology building to make available additional and more efficient work space. In general the buildings and equipment remain in excellent condition and are highly satisfactory for the accomplishment of the planned future programs. Two new vehicles (1 Jeep Commando and 1 Jeep Wagoneer) were received during the year, making available adequate transport for the current field investigations.

Library facilities remain excellent, with the continuing addition of texts and reprints of periodic literature in the fields of interest represented by Station programs.

Staff

During the year, the total staff of nine U.S. technicians was on duty. A full complement of 35 national employees was on hand at the end of the year. The staffing of the nine U.S. positions during FY-1971 was as follows:

Position	Staff 1971	Dates	
		Assumed	Vacated*
Chief	Geoffrey M. Jeffery	Nov. 69	-
Epidemiologist	Paul C. White, Jr.	Oct. 69	-
Parasitologist	McWilson Warren	Jan. 70	-
Research Entomologist	Samuel G. Breeland	Nov. 67	-
Research Entomologist	Joseph R. Austin	June 69	Mar. 71
	Jesse H. Hobbs	Apr. 71	-
Research Biologist	Charles W. Miller	Oct. 68	-
Public Health Educator	Anna M. Obert	Oct. 68	-
Biological Laboratory Tech.	Morris H. Price	Oct. 68	June 71
Administrative Officer	Allen J. Norton	June 70	-

* No date vacated indicates that the position was filled on June 30, 1971.

During the year, consultants at the Station included a botanical specialist for a total of three weeks (two visits), and an entomologist for two weeks. A number of staff members from the Malaria Program and other CDC components visited and worked at the Station for periods of temporary duty. These included a research entomologist (Technical Development Laboratories) for three weeks, an enteric disease epidemiologist (Epidemiology Program) for ten days, two parasitologists, and one epidemiologist (Laboratory Division) for a total of 16 days to discuss seroepidemiology, and a number of Malaria Program headquarters staff members for periods varying from a few days to two weeks.

Working Relationships with Various Groups

National Organizations

CAMRS has maintained continuing cordial and mutually beneficial relationships with the Ministry of Public Health and Social Welfare of El Salvador, with the National Antimalarial Campaign (CNAP), and with other agencies and ministries of the Salvadoran government. The Director and staff of CNAP have afforded indispensable assistance to CAMRS, providing epidemiologic and entomologic background data, making available investigational areas and situations for CAMRS activities, and providing several field personnel to work with CAMRS staff in cooperative studies.

Similar cordial and useful associations were maintained with the National Malaria Eradication Services (SNEM's) of all the countries of the Central America-Panama region, both through the medium of staff visits to and cooperative studies within these countries and through representation at the several regional meetings of the Directors of these Programs. Several members of the staff represented CAMRS at the tenth and eleventh meetings of the Working Group for Coordination of Malaria Eradication Programs in Central America and Panama, held, respectively, in San Jose in August 1970, and in Managua in May 1971. At these meetings, resumés of the programs and progress of CAMRS were presented and discussed.

Requests have been received from several countries for CAMRS to undertake studies on particular problems in these countries, and such studies have been initiated in Costa Rica, Panama and Nicaragua.

International Organizations

The staff continued to work closely with Pan American Health Organization/World Health Organization (PAHO/WHO) officials on problems and studies of mutual interest in the region. The PAHO advisors assigned to various countries hosting staff visits or projects were helpful in accomplishing the desired purposes.

Collaborative studies were continued with the AMRO-0216 (PAHO/WHO) insecticide testing group conducting studies in El Salvador to evaluate the usefulness of propoxur (OMS-33, Baygon) as a residual house spray. CAMRS personnel conducted chemical studies on the fumigant and surface activity of the insecticide to correlate with the entomologic findings in sprayed

premises, and conducted epidemiologic studies in a pilot area to determine the extent of continuing malaria transmission.

A number of visits were made to and received from personnel of the PAHO Headquarters in Washington and the Zone Headquarters in Guatemala to discuss the progress of current joint efforts, to explore future cooperative projects, and to review and discuss the CAMRS work plans.

U.S. Embassy and USAID

Attendance at the monthly staff meetings of USAID/San Salvador aided in maintaining a mutually helpful relationship with this Mission. The Joint Administrative Services, the USAID Controller's Office, and other Embassy-based offices continued to serve many administrative needs of the Station. A continuing liaison was maintained with the Mission through the office of the Public Health Advisor.

In visits to other countries where projects were underway or under discussion, necessary arrangements and contacts have been ably provided by USAID and Embassy personnel. The Chief Malaria Advisor at the Regional Office for Central America and Panama (Guatemala) has been kept informed of CAMRS activities and has provided close liaison with national and international malaria eradication activities in the region. Through the facilities of ROCAP a Spanish translation of the 1970 CAMRS annual report was provided for distribution in the Central American region.

PHS (Center for Disease Control) Personnel and Programs

The Station has continued to serve as a local contact for developing cooperative efforts between several CDC programs and national groups of El Salvador. Through these efforts a cooperative project to investigate the epidemiology of and possible immunization against Shigella dysentery was established, with the assignment of a full-time epidemiologist from CDC to the Ministry of Public Health and Social Welfare of El Salvador.

Laboratory support and field assistance were provided for a TDL Entomologist to study the applicability of hormonal larvicides under natural conditions in an endemic malaria area.

Collaborative studies with the Laboratory Division, CDC, have provided opportunities for evaluation and testing in the field of the indirect hemagglutination method (IHA) for usefulness in epidemiologic studies on malaria.

Miscellaneous Associations

Cooperative studies on biological methods of control of anophelines have been initiated with the U.S. Department of Agriculture Entomology Research groups, Gainesville, Florida, and Lake Charles, Louisiana. These include studies on mosquito pathogens and the use of sterile male release.

Active contacts have been maintained with such regional research groups as the Gorgas Memorial Laboratory (Panama), the Middle America Research Unit of the National Institutes of Health (Panama), the International Center for Medical Research and Training of Louisiana State University and the National Institutes of Health in San José, the Institute of Nutrition of Central America and Panama (Guatemala), and university groups in several of the countries.

Additional cooperative contacts outside the region have included the University of California (Riverside), the Ross Institute (London), the National Institutes of Health Laboratory of Parasitic Diseases (Bethesda, Md., and Chamblee-Atlanta, Ga.), the Tennessee Valley Authority Environmental Biology Branch (Wilson Dam, Ala.), the Environmental Protection Agency Laboratories (Perrine, Fla.), and others.

Visitors to CAMRS

During the year the Station received 82 visitors, for varying lengths of time, representing a wide range of interests and sources, including research, academic, diplomatic and commercial.

At the request of the Malaria Program at CDC, Leonard J. Bruce-Chwatt, M.D., of the London School of Hygiene & Tropical Medicine, reviewed CAMRS activities in February 1971. The research activities of CAMRS, he pointed out, are geared toward all aspects of human malaria; all the current studies are of interest, but priority should be given to research on the ecology of the vector, the parasitology of malaria, and epidemiology in its wide sense. Calling CAMRS "a much needed scientific unit that may be able to unravel some of the existing problems of malaria transmission in Central America," he recommended that the unit be used as a research training ground for national scientists from various countries of Central America. "CAMRS should be considered as an excellent investment in scientific approach to malaria eradication, and not a short-term attempt to find a new insecticide or drug that will urgently overcome all the obstacles."

Miscellaneous Staff Activities

During the year various staff members attended and participated in meetings of professional groups and societies, including the American Society of Tropical Medicine and Hygiene, the American Society of Parasitologists, the Second International Congress of Parasitology, the Annual Conference of Health Educators of Central America and Panama, the American Mosquito Control Association, the Malaria Commission of AFEB, the Entomological Society of America, the Society of Public Health Education, and the American Public Health Association.

Inter-American Malaria Research Symposium

CAMRS staff was active in the preparation of plans for the Inter-American Malaria Research Symposium to be held in November 1971. This Symposium is a joint effort of the Center for Disease Control, the Pan American Health Organization and the Government of El Salvador, and will be

held in conjunction with the Meeting of the Directors of the National Malaria Eradication Services of the Americas.

Development of Research Projects

The research program of the Station continues to pursue its major objectives of identifying and defining problems which impede the progress of eradication programs, further developing the basic information on the vector, the host, and the parasite, and exploring and demonstrating feasible tools and methods for effecting solutions. Many of the projects under study during previous years were continued or completed during the current reporting period. With the growing emphasis on changing the strategy of malaria eradication programs, diverse attack measures are being investigated and the evaluation of malaria endemicity approached in various ways. Both attack and target studies promise to improve malaria control.

Studies in vector ecology, including the relationships of *Anopheles* with *Plasmodium* and man, have been highly significant sources of information relative to the design of control programs. Such relationships, as exemplified by feeding preferences, susceptibility to parasites and activity patterns, represent the ultimate controlling factors in the transmission of malaria.

Epidemiologic evaluation needs to be improved, for the sake both of program design and determination of program effectiveness. Studies on the several available means of surveillance, including the newer serological techniques, have been intensified during the year, and have been utilized in the planning of programs which include multiple methods of control instituted on a timely basis.

The CAMRS program has considered all possible methods for vector and disease abatement. These have included the time-honored methods of source reduction by water management or larviciding, mass administration of antimalarials, newer insecticides for residual domiciliary application, and more recently the yet unproven biological control of anopheline populations. An important aim of the Station is to provide needed information on an array of eradication methods which can be used selectively and appropriately in programs of malaria eradication or control, and which would be feasible within the means and the abilities of the countries of the region and elsewhere.

Many of the studies undertaken by the Station have been located within El Salvador, where many of the problems common to Central America exist and where transportation and communication problems are minimal. However, important studies have been initiated in other countries of the region, particularly Nicaragua, Costa Rica and Panama, and others are in the planning stages. Requests for assistance have increased greatly, and CAMRS is striving to accommodate these requests within the limits of staff and budgetary considerations.

A detailed summary of the major fields of investigation of CAMRS during the year 1970-71 follows:

VECTOR ECOLOGY STUDIES

Anopheline Breeding Habitats and Seasonal Distribution

Previous sequential studies on the breeding habitats of anophelines in dry and rainy seasons in the coastal region of El Salvador, which have established patterns for such regions of Central America, were continued through this year, with particular emphasis on changes in larval habitats during periods of seasonal change. Habitat changes demonstrate anopheline potential for adaptation to drastic changes in environment. Knowledge of larval habitats is important in planning attacks with various vector control measures.

Changes in Larval Habitats from Dry to Rainy Season

In 1970, the rains began in the second week of May, and as heavy rains continued the river breeding areas were flushed out. Reflecting this seasonal change, the density of larvae in rivers of the San Diego area of La Libertad (Aquisquillo, Huiza, Jute and San Antonio), which had reached a peak in April, began to decline in May, and was negligible after mid-June. The adult population remained at a high level somewhat longer, but began to decline in June. Some adults were found under bridges as late as July 6, but not thereafter.

TABLE 1

Anopheline Densities of Rivers in Transition from Dry to Rainy Season. La Libertad, 1970.

Date (1970)		No. Dips	No. Larvae	No. Larvae per 100 dips*	No. Adults**
Apr.	1	300	625	208	-
	7	300	676	225	116
	13	-	-	-	160
	20	300	890	297	-
May	6	300	337	112	-
	7	-	-	-	109
	14	-	-	-	67
	21	400	442	110	-
	25	-	-	-	264
June	5	-	-	-	73
	16	400	23	6	-
	22	-	-	-	44
	24	400	0	0	-
July	6	-	-	-	3
	26	400	0	0	0
Sept.	17	-	-	-	0

* Totals from Rios Aquisquillo, Huiza, Jute and San Antonio.
** Totals from daytime collections under bridges of the same rivers.

Sampling in the water and on the beach of Estero San Diego revealed a similar pattern. In May and June the estuary was closed to the ocean, providing a body of fresh water ideal for anopheline breeding. Larval density reached a peak on June 20. With the exception of a small sample on July 20, no larvae were found after the estuary opened to the ocean on June 26. Adults, sampled by means of a New Jersey light trap, decreased rapidly following the depletion of larvae, indicating that the trap was measuring breeding specific to the estuary (Table 2). Figure 1 shows the dramatic effect of the opening of the estuary on both larval and adult populations associated with Estero San Diego.

TABLE 2

Anopheline Densities in Estero San Diego in Transition from Dry to Rainy Season, 1970

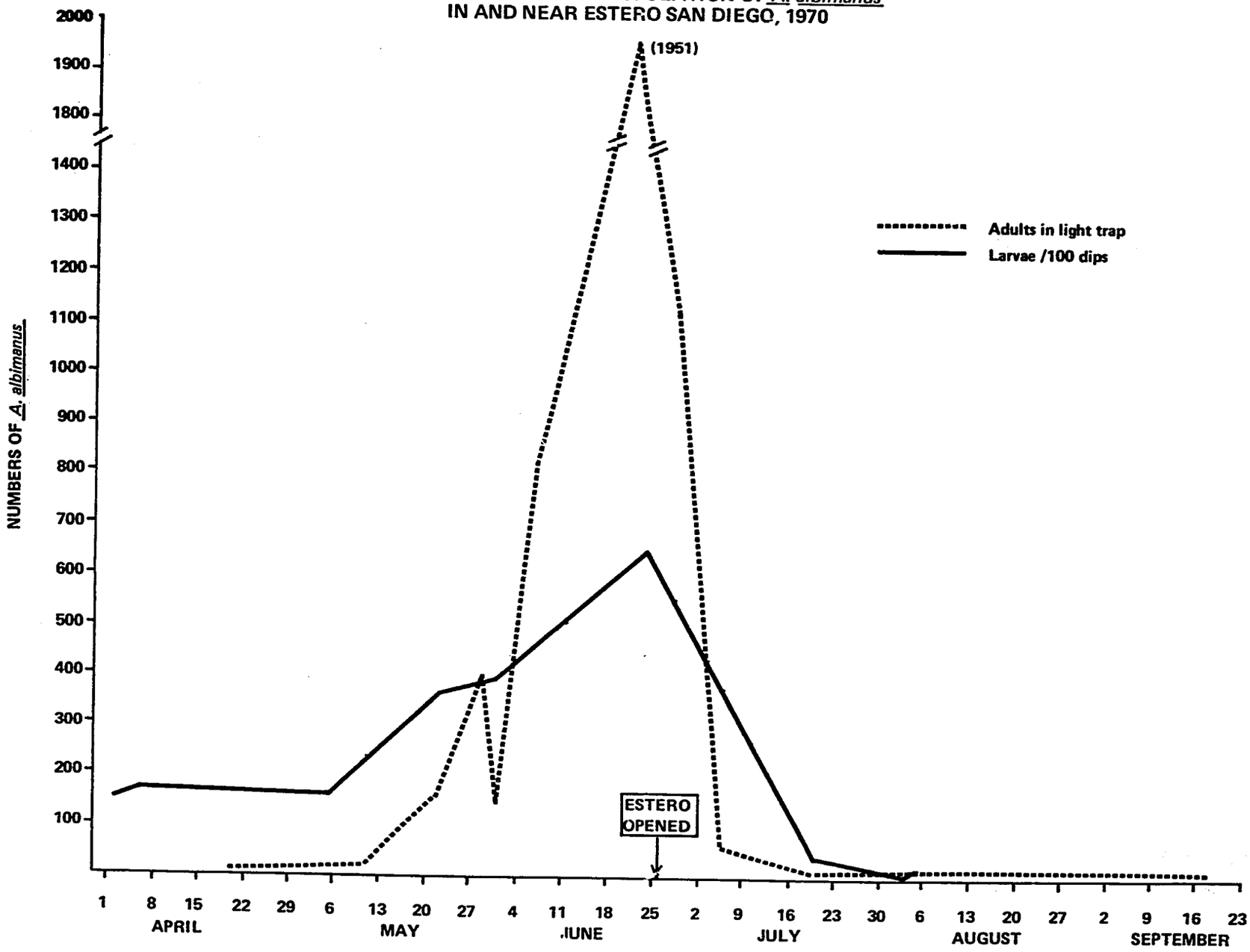
Date (1970)		Avg. No. Larvae per 100 Dips	Adults in Light Trap
Apr.	2	154	-
	6	164	-
	20	-	5
	27	-	10
May	5	160	17
	11	-	45
	22	362	164
	29	-	397
June	1	390	158
	8	-	856
	22	-	1951
	24	648	-
	*		
	29	-	1127
July	6	-	65
	20	32	11
Aug.	3	0	5
	20	0	5
Sept.	18	0	16

* Estero opened to the ocean, June 26, 1970.

Since rivers and estuaries are the main coastal region sources of *Anopheles* in the dry season, it is interesting to examine anopheline adaptation to the sudden loss of these habitats. Table 3 shows changing larval counts in coastal marshes and associated basins, exemplified by Pantano Ticuiziapa, near La Libertad. These areas were dry during the dry season; the same rains that rendered the rivers and estuaries unsuitable for anopheline breeding prepared first the pantano and later other types of depres-

Figure 1

LARVAL AND ADULT POPULATION OF *A. albimanus* IN AND NEAR ESTERO SAN DIEGO, 1970



sions as breeding habitats. Larvae were first detected in Pantano Ticuiziapa on May 22, shortly before the virtual disappearance of river breeding, thus providing the necessary overlap. It can be seen (Figure 2) that the productivity of the coastal pantano is relatively short-lived, being limited by increasing pollution of the standing water; meanwhile, other inundations (roadside ditches, irrigation overflows, puddles, etc.) overlap and compensate for the loss of the pantano habitat.

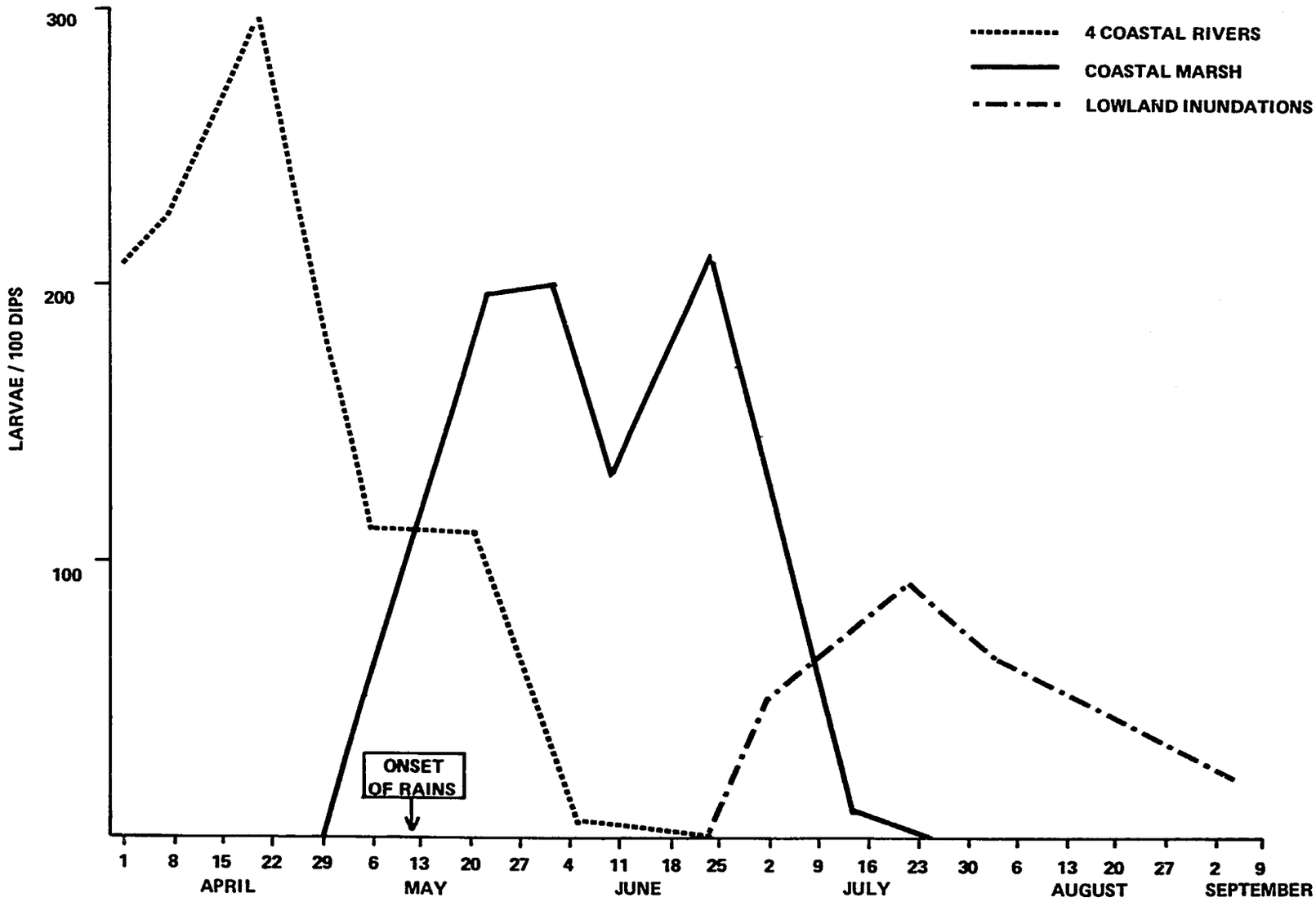
TABLE 3

Larval Densities in Coastal Situations During Transition from Dry to Rainy Season, La Libertad, 1970

Date	Anopheline Larvae/100 Dips		Comments
	Pantano Ticuiziapa	Miscellaneous Inundations	
Apr.	0	0	Dry conditions.
May 22	196	0	Pantano recently flooded, no miscellaneous inundations.
June 2	200	0	Pantano recently flooded, no miscellaneous inundations.
10	131	0	Pantano recently flooded, no miscellaneous inundations.
24	210	0	Pantano recently flooded, no miscellaneous inundations.
July 2	130	50	Roadside ditch, first miscellaneous breeding detected
14	10	-	Pantano polluted, and with broken surface film, habitat becoming unsuitable.
22	0	92	Pantano with solid surface film, unsuitable; breeding in flooded puddles.
Aug. 3	0	65	Pantano unsuitable; breeding in pools and puddles.
Sept. 4	0	23	Pantano unsuitable; breeding in drainage ditch.

Figure 2

SEASONAL CHANGES IN LARVAL DENSITIES IN
FOUR COASTAL RIVERS, A COASTAL MARSH, AND MISCELLANEOUS INUNDATIONS, 1970



Changes in Larval Habitats from Rainy to Dry Season

By early October 1970, rainy season breeding was at a peak in permanent bodies of water, inland marshes, and collectively in miscellaneous habitats. The late rainy season habitats of November gave rise to some rather phenomenal larval densities in certain situations, such as marshes with reduced water areas or habitats freshly re-exposed by intermittent rains, and lakes with gradually receding shorelines exposing new areas of marginal vegetation. As rains decreased in late November and in December, typical dry season habitats began to be productive; by the end of December dry-season breeding conditions prevailed.

After Estero San Diego opened to the ocean in early July, breeding in the estuary stopped. By late February, when the dry season was established, the estuary closed; larvae were detected for the first time on March 5 (288 larvae in 135 dips, or an average of 2.1 per dip). Thereafter, larval counts rose rapidly, averaging as high as 12.3 larvae per dip on March 16, 1971. At the end of June the estuary was still intact, and populations remained at a high dry-season level.

Sampling of larvae in 11 coastal rivers immediately east and west of La Libertad revealed no breeding in October, and only an occasional positive find in November. In December breeding accelerated and by the end of the month all 11 rivers had reached expected dry-season breeding levels (2,935 larvae in 135 dips, or an average of 171 per 100 dips). As expected, larval densities varied considerably from river to river, from 7 larvae per 100 dips in Aquisquillo to 848 per 100 dips in Conchaliyito. *Anopheles albimanus* made up 37.9% of the larval population and *A. pseudopunctipennis* 62.1% (Table 4). Both the average larval density and the species ratio found in these rivers were about the same as in previous years.

Several noteworthy features appear in Table 4. First, the two most productive rivers during this early stage of dry season breeding were the Conchalyito with 848 larvae per 100 dips and the Majagual with 771 larvae per 100 dips. No other rivers approached these densities. Further, the Conchaliyito supported *A. albimanus* to the exclusion of *A. pseudopunctipennis* and the Majagual supported *A. pseudopunctipennis* to the exclusion of *A. albimanus*. The influence of the early season on the biotic potential of the two rivers and the conditions favoring one species to the exclusion of the other are unknown. A physicochemical and biological study of these two rivers as well as the San Antonio on a sequential basis throughout the season would be useful. Observations during previous seasons have shown the San Antonio to be an "albimanus" river.

Some lakes in El Salvador support anopheline breeding on a year-round basis, but densities fluctuate from season to season, influenced by the amount of habitat provided by floating or emergent vegetation, water levels, and agitation of surface water by rains and wave action.

Lake Ilopango, a permanent crater lake, supports anopheline breeding year-round. During the rainy season its margin extends to inlets and overflows shallow areas. Moderate to dense breeding occurs in marginal emergent vegetation and some breeding occurs in floating vegetation in the lake

TABLE 4

Larval Densities of Rivers in Transition from Rainy to Dry Season, La Libertad, December, 1970.

River	No. Dips	No. Larvae	Larvae per 100 Dips	Percent alb.	Percent pp.
Aquisquillo	200	14	7	66.7	33.3
Chilama	200	78	39	14.8	85.2
Comasagua	225	258	115	20.0	80.0
Conchaliyito	25	212	848	100.0	0
Conchaliyon	75	182	243	40.0	60.0
Grande	100	84	84	39.7	60.3
Huiza	200	40	20	88.8	11.2
Jute	190	56	30	25.0	75.0
Majagual	200	1542	771	0	100.0
San Antonio	200	236	118	96.4	3.6
Tunco	100	233	233	26.3	73.7
Total	1715	2935	171	37.9	62.1

alb. - *Anopheles albimanus*

pp. - *Anopheles pseudopunctipennis*

proper. Thus, during October 3,398 larvae were taken in 540 dips in combined habitats near San Agustin for an average of 6.3 larvae per dip. In December, when marginal habitats had become dry, breeding became more concentrated in floating vegetation in the lake proper; on December 8, the density in this floating vegetation had reached 12.2 larvae per dip. Such adaptation compensates for the loss of marginal breeding sites.

Lakes San Diego and Güija in the Metapán area support significant breeding only during the rainy season. During October, the density in San Diego reached 35.9 larvae per dip, and in Güija, 10.9 per dip. When water levels decrease in these lakes, breeding moves to nearby rivers and irrigation waters.

Lake Apastepeque, near San Vicente, supports year-round breeding with moderate densities. During transition periods larval densities increase; new inundations appear in early rainy season and re-exposed vegetation breaks the water surface as the lake recedes in early dry season. Thus, densities which averaged around 1.5 larvae per dip during the rainy season rose to 5.0 on November 12 when new vegetation was exposed, and dropped to 3.0 per dip on December 22 as waters receded below the maximum vegetation line toward dry season stability.

In El Salvador, both coastal and inland marshes support rainy season breeding. Usually these marshes are near large bodies of water (estuaries on the coast and lakes or river bottoms in the exterior), and may be heavily used as pasture when not inundated. These habitats play an important role in the continuity of anopheline breeding in given areas and behave somewhat like lakes, since, in effect, they could be considered temporary lakes. Typically, coastal marshes such as Ticuiziapa, associated with Estero Ticuiziapa, show early rainy season breeding with fresh inundations, followed by a void period due to stagnation, then late rainy season breeding due to the fresh inundations from intermittent rains after periods of drying. Thus, from July to mid-October, Pantano Ticuiziapa supported little or no anopheline breeding. However, with later fresh inundations densities reached 0.8 larvae per dip on October 23, 1.5 per dip on November 27, and 1.0 per dip as late as December 10. Because of the high water tables in the coastal region, a minimum rainfall will produce these late season habitats. Irrigation in the pantano and nearby pastures provides the dry season continuity of breeding habitats.

In addition to Pantano Ticuiziapa, a large pantano associated with Lake Apastepeque supported breeding at densities of about 2.0 larvae per dip throughout the rainy season until it was without water by December 1. Thereafter, the nearby lake could compensate for this lost habitat.

Larval density reached a peak of 16.3 larvae per dip in a pantano separated from the margin of Lake Ilopango at San Agustin on November 10. By November 18, the pantano was dry, but the floating vegetation of the lake remained available as an alternate habitat.

The other rainy season habitats such as roadside ditches, irrigation channels, and temporary pools supported heaviest breeding in October, and continue as long as water is available. Between October 23 and December 3,

1970, densities in irrigation channels averaged 10.8 larvae per dip in the La Libertad area and 9.2 larvae per dip in the Metapán area. Roadside ditches averaged 1.5 larvae per dip in the Metapán areas, and miscellaneous pools in the La Libertad area averaged 1.0 larvae per dip. Collectively, these sites play important roles as temporary breeding sites during a period when rainy season habitats are being lost and dry season habitats develop.

Cumulative Results of Larval Habitat Studies, 1968-1971

The 1970 CAMRS Annual Report (Fig. 1, p. 6) included a graphic presentation of the seasonal changes in larval habitats. Data accumulated over four years have traced the same habitats as they developed, stabilized, declined, disappeared, and reappeared along with the dry and rainy seasons. Impressions and hypotheses formed early in the study were tested and validated during the later phases.

Seasonal changes exert a profound effect on larval habitats. No major breeding habitat in El Salvador supports production of a given species of *Anopheles* evenly throughout a year. Survival of anopheline populations in the dry season is not necessarily explained by physiological adaptations such as aestivation or postponement of eclosion in the mosquito itself. Rather, the organism depends on shifting sites, since the same seasonal changes that cause one breeding place to become unsuitable for breeding will at the same time bring another aquatic environment into production.

Table 5 presents cumulative monthly results of larval sampling by habitat from 1968 to 1971. River and estuary breeding begins modestly in December, building to a rather steady plateau of heavy breeding from January through May, and declines in June to a negligible level in July.

Irrigated pasture breeding continues year-round, but depends upon a different set of conditions from season to season. During the dry season, pasture breeding results from the overflow of irrigation water, and during the rainy season, from general inundations. The data show a rather steady breeding pattern ranging from 0.84 to 2.51 larvae per dip, the low point coming in August and September, probably associated with stagnating pasture waters.

Even though permanent bodies of water may be available year-round as breeding sites, their larval yield fluctuates more than that of other habitats, with monthly averages ranging from 0.18 larvae per dip in April to 16.8 larvae per dip in November. Water level and vegetation changes interact to affect breeding habitat availability. The miscellaneous habitats are most available during the rainy season months when practically every natural basin contains water.

Omitting the transitional months of June and December, dry season habitats are sufficiently abundant to account for dry-season survival of both *A. albimanus* and *A. pseudopunctipennis* (Table 6). There was an average of 1.80 larvae per dip (45.4% *A. albimanus*) in 49,858 dips for dry season months and very nearly the same average, 1.76 per dip (88.4% *A. albimanus*), in 15,303 dips for rainy season months.

TABLE 5

Cumulative Monthly Larval Densities by Habitat. El Salvador, 1968 to 1971.

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Sample Identification A. alb.	Sample Identification A. pp.
Rivers[1]	No. Larvae	5,117	13,578	18,173	10,213	6,646	418	12	-	-	-	-	1,314	55,471	2,106	4,566
	No. Dips	3,295	7,934	8,338	7,490	4,010	1,370	600					1,970	35,007		
	Avg. No. Larvae/Dip	1.55	1.71	2.18	1.36	1.66	0.31	0.02	-	-	-	-	0.67	1.58	31.6%	68.4%
Estuaries[2]	No. Larvae	4,478	4,425	4,264	5,341	4,023	1,294	55	0	0	-	82	73	24,035	1,644	45
	No. Dips	2,187	3,480	2,905	2,930	1,310	895	250	330	200		705	350	15,542		
	Avg. No. Larvae/Dip	2.05	1.27	1.46	1.82	3.07	1.45	0.22	0	0	-	0.12	0.21	1.55	97.3%	2.7%
Pastures[3] (Pantanos)	No. Larvae	2,141	1,125	1,252	987	196	1,315	1,533	1,073	1,649	1,593	5,221	912	18,997	3,554	879
	No. Dips	1,050	655	529	400	100	798	1,035	1,210	1,970	905	2,084	450	11,186		
	Avg. No. Larvae/Dip	2.04	1.72	2.37	2.47	1.96	1.65	1.48	0.89	0.84	1.76	2.51	2.03	1.69	80.2%	19.8%
Lakes & Ponds	No. Larvae	4,323	439	402	80	1,739	314	92	624	472	4,677	2,015	2,443	17,620	1,631	1,079
	No. Dips	905	520	435	450	260	290	350	600	300	757	120	265	5,252		
	Avg. No. Larvae/Dip	4.78	0.84	0.92	0.18	6.69	1.08	0.26	1.04	1.57	6.18	16.80	9.22	3.35	60.4%	39.6%
Other[4]	No. Larvae	422	4	271	2	-	3,517	757	1,323	1,890	1,862	2,038	533	12,619	1,643	19
	No. Dips	150	150	300	75		1,793	665	1,240	539	773	670	285	6,640		
	Avg. No. Larvae/Dip	2.81	0.03	0.90	0.03	-	1.96	1.14	1.07	3.51	2.41	3.04	1.87	1.90	98.8%	1.2%

1 - Sum of 8 rivers (Aquisquillo, Chilama, Comasagua, Grande, Huiza, Jute, San Antonio and Tunco.

2 - Sum of 3 estuaries (Majagual, San Diego, Ticuiziapa).

3 - Irrigation waters during dry season, flooded marshes or pastures during rainy season.

4 - Miscellaneous inundations (Roadside ditches, wheelruts, transient water).

TABLE 6

Larval Densities in Dry and Rainy Seasons.* El Salvador, 1968-1971.

Source	Dry Season (January-May)					Rainy Season (July-November)				
	No. Dips	No. Larvae	Avg. No. Larvae/Dip	Species** alb.	Species** pp.	No. Dips	No. Larvae	Avg. No. Larvae/Dip	Species** alb.	Species** pp.
Rivers	31,067	53,727	1.73	1,885 31.0%	4,194 69.0%	600	12	0.02	-	-
Estuaries	12,812	22,531	1.76	1,624 97.2%	45 2.8%	1,485	137	0.09	20 100.0%	0
Marshes and Pastures: Dry Season Irrigation	2,734	5,701	2.09	529 78.0%	149 22.0%	-	-	-	-	-
Rainy Season Inundations	-	-	-	-	-	7,204	11,069	1.54	1,775 88.0%	242 12.0%
Lakes and Ponds	2,570	6,983	2.72	273 24.4%	847 75.6%	2,127	7,880	3.70	1,075 81.2%	249 18.8%
Miscellaneous Habitats	675	699	1.04	42 82.4%	9 17.6%	3,887	7,870	2.02	943 99.2%	8 0.8%
Total	49,858	89,641	1.80	4,353 45.4%	5,244 54.6%	15,303	26,968	1.76	3,813 88.4%	499 11.6%

* Transition months of June and December excluded to prevent overlap between seasons.

** alb. - *A. albimanus*, pp. - *A. pseudopunctipennis*.

In addition, the data show that rivers and estuaries are dry season habitats; that pastures are fed by both dry season irrigation and rainy season inundation; and that permanent waters and miscellaneous habitats produce year-round breeding, but favor the rainy season. The relative prevalence of *A. albimanus* varies by habitat and season, ranging from 24.4% in dry season lake and pond habitats to 100% in estuary collections.

Relationships Between Plants and Anopheline Production

Dr. T. F. Hall, a Botanist-Consultant, visited CAMRS during late August and early September 1970 and again in March 1971, with the objective of studying relationships between plants and anopheline production in El Salvador during peak anopheline density periods of each season. A summary of results, adapted from Dr. Hall's report, follows:

Anopheline larval populations were observed in pools containing various types of aquatic plants and in open-water areas, using adjacent paired dipping sites. One of the paired sampling sites had plants and/or plant structures intersecting the water surface, and the other site presented a clean water surface. Other than the presence or absence of vegetation intersecting the air-water interface, the sites were assumed to be similar for parameters such as temperature, light, and hydrogen ion concentration. Samples were taken with standard dippers and in a comparative series with the Hess square-foot sampler. The densities of anopheline larvae in given sites with and without certain vegetation types intersecting the water surface were compared only for areas of high productivity at the sampling time. Study areas included volcanic and other lakes, ditches, pastures, pantanos, and esteros.

Aquatic Vegetation as Larval Habitat

In a comparison between watery sites that contained vegetation and those that did not, 1500 samples were dipped from various locations in August-September 1970 (rainy season) and March 1971 (dry season). Of 1385 dips in water where plants intersected the surface, 534 contained larvae. In contrast, of 245 dips taken where no plants rose above the surface of the water, only two were positive. Larvae were virtually absent even in clean water areas that were adjacent to breeding areas. These results show that breeding in both the rainy season and the dry season occurs in an environment including a plant-air-water interface, which we term the "intersection line" (Table 7).

Comparison of Plant Types as Larval Habitats

Larvae were sampled in selected plant communities and adjacent clean water areas during periods of high productivity in both rainy and dry seasons (Table 8). Use of a square foot sampler in the rainy season habitats yielded significant amounts of anopheline larvae in marginal grasses and water lettuce, but almost none in adjacent clear water areas. Likewise, when dips were taken among surface-breaking filamentous algae of river pools and along the plant-air-water interface of detached floating leaves in understory pools of mangrove swamps, larvae were plentiful; no larvae were found in adjacent clear water. These observations lead to the conclusion that the local anophelines breed in association with all types of plants studied and with detached plant parts. In addition to the above cited examples, anopheline breeding appears to occur in most other local aquatic vegetational types.

TABLE 7

Anopheline Larvae in Plant Intersection Lines and in Clear Water. El Salvador, 1970-71.

Season	Inspection Dates	Water Surface	Dips Total	Dips Pos.	Dips % Pos.
Rainy	8-27-70 to 9-3-70	Plants Intersecting	660	199	30
		Clean	100	1	1
Dry	3-15-71 to 3-18-71	Plants Intersecting	725	335	46
		Clean	145	1	1

TABLE 8

Larval Frequency and Density associated with Different Plant Types. El Salvador, 1970-71.

Location	Larvae in plants breaking water surface: Dips No.	Dips % pos.	Avg. No. per dip	Larvae in clean water surface: Dips No.	Dips % pos.	Avg. No. per dip
			Rainy Season (Square Foot Sampler)			
Lago de Apastepeque (marginal grasses)	30	100	24	30	1	0
Cantora Area San Miguel (water lettuce)	30	60	1	10	0	0
			Dry Season (Regular Dipper)			
Rio Chilama (filamentous algae)	55	76	8*	10	0	0
San Diego Estero Mangrove Swamp (detached leaves)	50	100	12**	25	0	0

* All *A. pseudopunctipennis*

** All *A. albimanus*

Comparative Anopheline Densities in Plant Communities at a Single Location.

At Laguna de Apastepeque, a segment of shoreline with plant zonation and heavy anopheline breeding was dipped for larvae during the rainy season using the Hess square-foot sampler. The vegetational zones consisted of (1) marginal grasses (Bermuda and others) with algae, (2) a middle zone of aquatic primrose (*Jussiaea*), and (3) an outer or lakeward zone of tall tule (*Eleocharis*). "Water holes" or openings existed in the vegetated zone and these presented areas of clean water surface for sampling. The results (Table 9) showed significantly different numbers of anopheline larvae per square-foot, these being: 24.3 for the grass zone, 3.5 for the primrose zone, 0.13 for the tule zone, and negligible numbers in areas with a clean-water surface. These observations indicate that the exact type of plant community existing in a given area can have a significant influence upon the magnitude of the overall anopheline population added to man's environment. It should be recognized that the importance of anopheline breeding areas is determined not by density alone but by density in combination with the size of breeding grounds. In addition, naked-stem tule, which has a low intersection value, showed minor production compared to the grass and primrose zones of much higher intersection values.

TABLE 9

Larval Densities (Hess Square-foot Sampler) in Different Plant Types Along One Segment of Shoreline of Laguna de Apastepeque. San Vicente, El Salvador, September 2, 1970.

Plants Sampled	Dips		Larvae		
	Total	Pos.	Total	per sq. ft.	per shoreline mile (Est.)
Marginal grasses and algae	30	30	730	24.3	1 million
Water primrose	30	27	106	3.5	1/4 million
Tule	30	3	4	0.13	5,000
Open water areas within marginal band of vegetation	30	1	1	0.03	500

An Example of Reciprocal Relations in Anopheline Production

Seasonal changes in the vegetation intersection line of Lago de Ilopango, a large lake near San Agustin produced a reciprocal effect on the biotic potential of the two species, *A. albimanus* and *A. pseudopunctipennis*. During the dry season, when the water level is low, normally submersed plants such as elodea associated with filamentous algae were noted breaking the water surface. Larvae were common (averaging 7 per dip) in areas where the plants were not completely submersed. But in adjacent clear water areas, just as at times when the plants were under water, larval counts were negative.

The dry-season, low water level anopheline population was all *A. pseudopunctipennis*. In the rainy season, when all vegetation in the lake was submersed, the fields bordering the lake were inundated, and larvae could be found in the flooded fields. This rainy-season larval population was all *A. albimanus*. It seems reasonable that at some intermediate water level position there is a water surface system simultaneously unfavorable to larvae of both of these species. The shifts in species composition at San Agustin in Lago de Ilopango appear to be but a small unit example of succession in breeding areas in the topographic space of this country spanning the time interval from rainy-to-dry-to-rainy season in the annual cycle.

Classification of Plant Types

There follows a suggested classification of plant types which may be helpful in (1) following seasonal shifts in breeding sites, (2) developing a capability to predict from plant communities the potentially important anopheline breeding areas in time and in space, and (3) evaluating potential preventive and/or corrective measures.

Classification of Plant Types*

A. Woody Species

1. Mangrove Swamps

Mangle	- *Rhizophora mangle*
Arbol de Sal	- *Avicennia nitida*

2. Willow Stands

Sauce	- *Salix humboldtiana*

B. Herbaceous Species

1. Free Floating

Ninfa or Lechuga	- *Eichhornia crassipes*
Lechuga de Sapo	- *Pistia stratiotes*
	- *Spirodela polyrhiza*
	- *Salvinia auriculata*
Doradilla	- *Azolla caroliniana*

2. Anchored Submersed

Lechuga de Rana	- *Najas guadalupensis*
Barbona	- *Ceratophyllum demersum*
Elodea	- *Hydrilla* (?)
Chichicaste	- *Najas marina*
Zacatillo	- *Potamogeton* sp.

3. Anchored Floating Leaf

Ninfa	- *Castalia ampla*
Ninfa	- *Nymphoides humboldtianum*

4. Anchored Floating Mat

- *Jussiaea sedoides*

5. Anchored, erect, naked

Tule	- *Eleocharis* sp.
Tule	- *Typha angustifolia*

6. Anchored, erect, leafy

Carrizo	- *Phragmites communis*
Plantanillo	- *Thalia geniculata*

7. Anchored with flexuous leaves or stems

-*Echinochloa colonum*

8. Algae

Filamentous	-*Spirogyra*
Microscopic	- Desmids

* Most scientific and local names after Standley and Calderon.

Anopheline Densities

Comparison of Methods

The measurement of anophelism, at least in Central America, has been largely limited to methods for measuring dwelling-oriented activities of adult *A. albimanus* as required by the residual wall spraying regimen of malaria eradication programs in the region. Thus, such methodology has been restricted essentially to those methods that would measure segments of the vector population considered to be important in evaluating the effectiveness of residual spray deposits. The research objectives of CAMRS and the needs of diversified malaria eradication programs require a wider variety of anopheline density measurement techniques than are commonly used in Central America, and it has been necessary to evaluate additional methodology.

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

Entomological studies conducted by CAMRS since January 1968 in a coastal malarious area extending 10 kilometers east of the port city of La Libertad have afforded the opportunity to appraise comparatively various methods used to measure anophelism in the area. In terms of numbers collected, the New Jersey light trap was superior to other collecting methods with 93.9 *A. albimanus* per collection, 96.9 percent of which were females. It was particularly useful in measuring seasonal fluctuations, in determining the nocturnal activity peak, and in detecting the presence of the species in a given locality. This method was inadequate for measuring *A. pseudopunctipennis* populations. Collections from cattle corrals in the area yielded an average of 84.4 specimens of *A. albimanus* per collection, 99.9 percent of which were females. This method compares favorably with the light trap, but requires the collectors to be present during nighttime hours. Likewise, it yields few specimens of *A. pseudopunctipennis* (average of 1.1 per collection). Diurnal collections from natural resting sites yielded an average of 20.2 *A. albimanus* per collection and 5.8 *A. pseudopunctipennis* per collection. The percentage of males, 71.1% and 56.3%, respectively, for the two species, was the highest of any method used. Collections from beneath bridges accurately measured breeding in the associated rivers and yielded an average of 7.2 *A. albimanus* (74.4% females) and 9.7 *A. pseudopunctipennis* (63.3% females) per collection. This collection system has been the most useful of the six for obtaining host preference and longevity study specimens.

Collecting with human bait near houses yielded an average of 29.5 *A. albimanus* per collection and no *A. pseudopunctipennis*. This method, along with that of inside house captures, which accounted for an average of 9.4 *A. albimanus* (65.1% females), and only a single *A. pseudopunctipennis* female, measures only a segment of the anopheline population, i.e., that

portion attracted to a specific human bait source or disposed to enter particular houses at a given time. Such collections are erratic, but highly useful if properly understood and related to specific measurement purposes. A summary of anopheline density measurement data is given in Table 10.

A seasonal consideration of these anopheline measurements shows a consistently higher dry-season population density. While this may be due, in part, to the effect of cotton spraying on adults in the area during much of the rainy season, it seems more likely that the general prevalence of adults during the dry season reflects the predominance of beneficial dry-season breeding habitats (estuaries, rivers, irrigated pastures, and permanent bodies of fresh water). Dominating this area are two rather large estuaries, several rivers, and a large irrigated pasture. There are no permanent bodies of fresh water and the only typical rainy season habitat is the Ticuiziapa marsh which serves as a favorable habitat only during early rainy season in June and July, shortly after inundation (See Figure 2). Later in the season, the water in this marsh becomes polluted from cattle droppings accumulated during the dry season, and unsuitable for anopheline breeding, leaving only the miscellaneous rainy season water collections to fill the gap.

Comparison of Light Trap Models

Preliminary studies were done on the comparative efficiency of three light trap models--the standard New Jersey light trap, the CDC miniature light trap, and a new portable miniature light trap received from WHO for evaluation. The latter model is similar to the CDC trap, but utilizes an ultra-violet light source operating on a 12-volt battery. The comparative evaluations have been done on an *ad hoc* basis and the results to date are detailed in Table 11. Generally the WHO trap appears to attract *A. albimanus* somewhat better than the CDC trap, but is much less attractive than the New Jersey trap, using a 25-watt incandescent bulb. None of the three appeared to be very useful for attracting *A. pseudopunctipennis*. Preliminary conclusions suggest that relative portability of the CDC or the WHO-UV trap, both of which require heavy batteries, does not compensate for the superior efficiency of the New Jersey trap. Even with the added expense of generators or current inverters and batteries, the New Jersey model offers advantages for use in the measurement of *A. albimanus* populations. There remains a real need for a trap attractive to *A. pseudopunctipennis*.

TABLE 10

Summary of Anopheline Captures by Collecting Method. La Libertad Area of El Salvador, January 1968-June 1971.

		A. albimanus					*A. pseudopunctipennis*				
	No. Coll.	No. Pos.	% Pos.	No. Adults	Avg. No. Per Coll.	% Females	No. Pos.	% Pos.	No. Adults	Avg. No. Per Coll.	% Females
Lt. Trap, San Diego	167	151	90.4	24,765	148.3	97.1%	9	5.4	13	0.08	100.0%
Lt. Trap, Ticuiziapa	191	170	89.0	8,837	46.3	96.3%	4	2.1	4	0.02	100.0%
TOTAL, 2 Traps	358	321	89.7	33,602	93.9	96.9%	13	3.6	17	0.05	100.0%
Natural Diurnal Resting	78	62	79.5	1,577	20.2	18.9%	9	11.5	453	5.8	43.7%
Cattle Corrals											
El Coco	60	50	83.3	4,545	75.7	100.0%	13	21.7	48	0.8	100.0%
Melara	47	45	95.7	4,486	95.4	99.9%	19	40.4	73	1.6	100.0%
TOTAL, 2 Corrals	107	95	88.8	9,031	84.4	99.9%	32	30.0	121	1.1	100.0%
Rio Aquisquillo	34	22	64.7	391	11.5	89.8%	8	23.5	26	0.8	80.8%
Rio Huiza	21	15	71.4	162	7.7	67.3%	18	85.7	932	44.4	71.0%
Rio Jute	50	41	82.0	305	6.1	65.9%	45	90.0	508	10.2	48.4%
Rio San Antonio	47	38	80.9	230	4.9	64.3%	5	10.6	14	0.3	57.1%
TOTAL, 4 Rivers	152	116	76.3	1,088	7.2	74.4%	76	50.0	1,480	9.7	63.3%
Human Bait	23	18	78.3	677	29.4	100.0%	0	0	0	0	0
House Captures	72	29	40.3	676	9.4	65.1%	1	1.4	1	-	-

TABLE 11

Effectiveness of Three Light Traps by Location and Time.
El Salvador, October-December 1970.

Time	Trap Placement	New Jersey			CDC			WHO-UV		
		No. of Traps	Anophelines alb.	Anophelines pp.	No. of Traps	Anophelines alb.	Anophelines pp.	No. of Traps	Anophelines alb.	Anophelines pp.
6PM-6AM	Stable	1	46	1	3	7	2			
		1	68	3				1	22	0
		1	22	1	1	1	0	1	0	2
	Village				1	11	0	1	9	0
	House Patio	1	71	0	3	0	0	1	12	1
6PM-9PM	Inside House	1	9	0	1	0	0	1	1	0
Total			216	5		19	2		44	3

All specimens female; no males trapped.

Host Preference Studies

The results of microprecipitin tests to determine vertebrate host preferences show that *A. albimanus* feeds almost exclusively on domestic animals and man. Out of 1,008 specimens collected inside houses, 46% had fed on humans and 30.6% had fed on cattle. In contrast, collections from outdoor diurnal resting sites included only 2.1% human feedings and 87% cattle. The remainder of the specimens are divided among other animals; double feedings do occur (Table 12). Five kinds of animals (humans, cattle, dogs, pigs, and horses) had furnished feedings for 95.7% of the specimens collected indoors, and 96.7% of specimens collected out of doors.

TABLE 12

Host Preferences of *Anopheles albimanus*.
El Salvador. 1970-1971.

Host	Intradomiciliary Collections		Collections from Natural Resting Sites		All Collections	
	No.	%	No.	%	No.	%
Man	444	44.0	18	2.0	462	24.3
Cow	289	28.7	772	86.6	1061	55.9
Dog	158	15.7	9	1.0	167	8.8
Pig	47	4.7	43	4.8	90	4.7
Horse	3	.3	16	1.8	19	1.0
Unknown	33	3.3	29	3.3	62	3.3
Other	10	1.0			10	.5
Man/Cow	15	1.5	1	.1	16	.8
Man/Dog	3	.3			3	.2
Man/Pig	1	.1			1	.1
Man/Racoon	1	.1			1	.1
Cow/Horse	2	.2	1	.1	3	.2
Cow/Dog	2	.2			2	.1
Cow/Pig			2	.2	2	.1
Total	1008		891		1899	

Figure 3
LOCALITIES OF CAMRS STUDIES ON VECTOR ECOLOGY

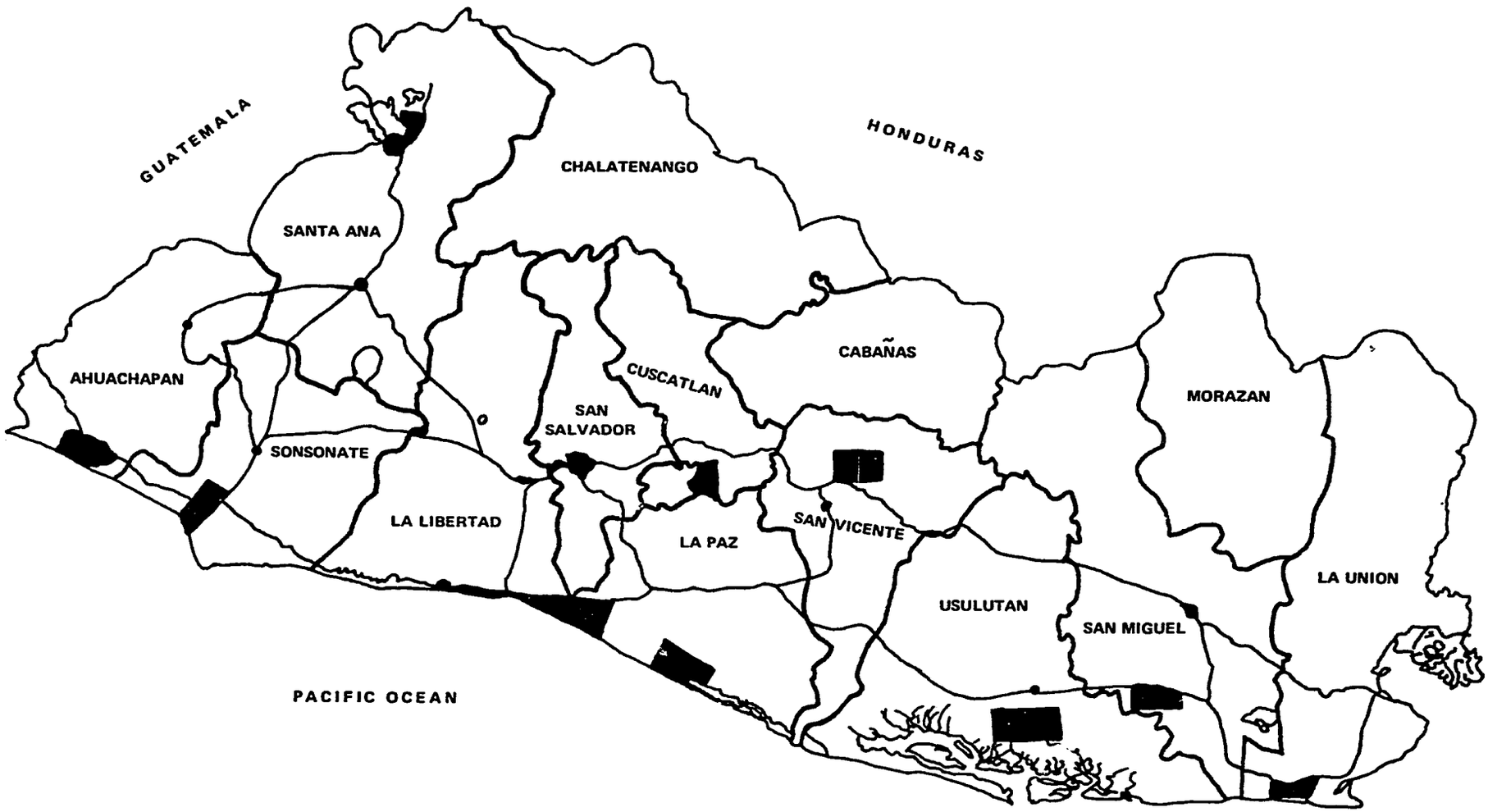


TABLE 13

Percent of *A. albimanus* containing Human Blood.
Intradomiciliary Collections. El Salvador, 1970-1971.

Department and Localities	1970										1971		
	Dry Season			Rainy Season							Dry Season		
	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
La Libertad (San Diego, Melara)	-	-	-	83	-	50	-	-	-	-	-	-	-
La Paz (Zunganera)	-	-	97	-	85	81	60	-	-	-	-	-	87
San Miguel (Jocotal/ Curruncha)	24	63	50	72	83	82	60	-	-	-	-	-	25
La Unión (Condadillo)	-	50	33	62	60	-	43	-	-	-	-	-	-
San Vicente (Chamoco, Anonas, San Carlos, Las Mesas)	-	-	-	-	90	-	40	60	-	38	-	33	15
Usulután (Puerto Avalos, Salinas)	-	-	-	-	11	12	-	18	-	-	-	95	-
Sonsonate (Atalaya)	-	-	-	-	-	-	-	-	-	-	-	-	16

Seasonal and local changes in the percentages of *A. albimanus* containing human blood (according to precipitin tests) are shown in Table 13. For example, the anthropophilic index is high in the Zunganera area of La Paz in both rainy and dry season months, while human biting rates are high in the Jocotal area of San Miguel, the Condadillo area of La Unión, and the San Vicente localities in the rainy season, and in the Usulután localities in the dry season.

Table 14 presents the comparative feeding pattern of *A. albimanus* and *A. pseudopunctipennis* from natural resting sites. In both species, the same five animals (cattle, horse, pig, dog, and man) accounted for essentially 97% of all feedings for both species, with cattle being the major host and man a very minor one in both cases, an expected finding in outdoor collection sites. Likewise, when specimens of both *A. albimanus* and *A. pseudopunctipennis* were taken in common collections, feeding patterns again involved only cattle, horse, dog, pig and human sources (Table 15). These collections were dominated by cattle, 93.7% for *A. albimanus* and 80.7% for *A. pseudopunctipennis*. *A. pseudopunctipennis* fed almost exclusively on cow and horse, with no human samples found in these collections from outdoor sites.

TABLE 14

Host Preferences of *A. albimanus* and *A. pseudopunctipennis* in Natural Resting Sites. El Salvador, 1970-71.

Host	*A. albimanus* No.	*A. albimanus* %	*A. pseudopunctipennis* No.	*A. pseudopunctipennis* %
Cow	772	86.6	316	81.7
Pig	43	4.8	3	.8
Man	18	2.0	4	1.0
Horse	16	1.8	45	11.6
Dog	9	1.0	6	1.6
Unknown Mammal	29	3.3	10	2.6
Man/Cow	1	.1	1	.3
Cow/Pig	2	.2	1	.3
Cow/Horse	1	.1	1	.3
Total	891		387	

TABLE 15

Host Preferences of *A. albimanus* and *A. pseudopunctipennis* from Common Collections. El Salvador 1970-71.

Department	Locality and Habitat	*A. albimanus*						*A. pseudopunctipennis*					
		Cattle	Horse	Dog	Pig	Man	Unknown Mammal	Cattle	Horse	Dog	Pig	Man	Unknown Mammal
La Libertad	Comasagua River, Beneath Bridge	6	1	-	-	-	-	3	5	-	-	-	-
La Libertad	"	3	2	-	-	-	-	16	6	-	-	-	-
La Paz	Apanta River, Beneath Bridge	2	-	-	1	-	1	4	-	1	2	-	1
Sonsonate	Atalaya, natural daytime resting near stable #3	30	-	-	-	-	-	20	-	-	-	-	-
Sonsonate	"	30	-	-	-	-	-	11	1	-	-	-	2
Sonsonate	"	29	-	-	-	1	-	9	1	1	-	-	1
San Miguel	Lake Jocotal, natural, daytime resting	23	-	-	-	1	2	21	-	-	-	-	2
San Miguel	"	10	-	-	-	-	-	12	-	-	-	-	-
Total No.		133	3	0	1	2	3	96	13	2	2	0	6
Percentages		93.7	2.1		0.7	1.4	2.1	80.7	10.9	1.7	1.7		5.0

Natural Plasmodium Infections in Anophelines

Since March 1970, 3,657 *A. albimanus* females from 426 collections in nine Departments of El Salvador have been dissected for malaria parasites. Through June 1971, the dissections showed all of 2,881 salivary glands negative for sporozoites and all of 776 mid-guts negative for oocysts. Additionally, a total of 80 *A. pseudopunctipennis* yielded 74 negative glands and 6 negative mid-guts. Although no mosquitoes positive for malaria parasites were detected by dissections during the reporting year, two CAMRS entomologists developed malaria during the third quarter of the year (January-March), one *P. vivax* and one *P. falciparum*, following evening adult captures in the same general area of the Pacific coast. Careful consideration of the activities of these subjects assures that these were the only opportunities for exposure, and seems to confirm the presence of infected *A. albimanus* at the collection sites.

In July 1971, shortly after the end of the reporting year, positive salivary glands were found in the dissection of four *A. albimanus*, two each from San Diego Beach and Zunganera Beach in the neighboring coastal Departments of La Libertad and La Paz, respectively. Both San Diego specimens were from intradomiciliary collections, while one of the Zunganera specimens was intradomiciliary and the other was from a natural resting site. It is interesting that both cases of malaria reported above are traceable to these localities. Also of note are the data of Table 13 which show high anthropophilic indices for both of these localities. These positive glands were the first seen in about 4,000 dissections examined at CAMRS.

Longevity Studies (Physiological Age Grading)

As pointed out by a WHO drafting Committee in "Terminology of Malaria and of Malaria Eradication" (Geneva, 1963), the determination of the age of female anophelines is of importance for full understanding of the epidemiology of malaria and for assessment of the efficacy of anti-anopheline measures, most of which aim at shortening the average lifespan of the population of the malaria vector. The determination of physiological age according to the number of gonotrophic cycles undergone by a female mosquito is basic. By means of age grading it is possible to determine the age composition of the mosquito population and to find out the proportion of females which are in the epidemiologically dangerous age. At present two methods are increasingly used: The first is a simple technique for distinguishing between parous and nulliparous female anophelines by examination of the tracheoles of the ovaries, permitting assessment of the nulliparous-to-parous ratio, from which the probability of survival of mosquitoes, and therefore their average longevity, can be calculated. A second, more elaborate technique is based on the dissection of the ovaries to count the number of dilatations left in the ovarioles subsequent to each ovulation and oviposition. The number of dilatations found usually corresponds to the number of gonotrophic cycles, and gives more direct evidence of the physiological age of the females.

CAMRS has used the second of these two methods. Between April and September 1970, involving parts of both rainy and dry seasons, the *A. albimanus* population samples were very young (Table 16). Of 423 females dissected from nine coastal departments, only 49 (11.6%) were parous as determined by examination of the ovarioles. The 49 parous specimens included only one showing evidence of more than one gonotrophic cycle.

These studies were temporarily discontinued because of laboratory evidence that *A. albimanus* exhibits gonotrophic dissociation whereby blood ingestion by the female may not always result in gonadal development. In the laboratory numerous specimens have taken several blood meals without developing eggs. Since interpretation of age-grading studies of this type depends upon gonotrophic concordance in the species, laboratory studies are underway to determine the level of confidence that may be placed in the technique. If this method proves unreliable, an alternative, such as the tracheole method, will be used.

TABLE 16

Results of Dissections to Determine the Physiological Age of Anopheles albimanus. El Salvador, April-September 1970.

Department	No. Dissected	Parous		Gonotrophic Cycles	
		No.	%	1	2
Ahuachapán	13	0	-	-	-
La Libertad	111	20	18.0	20	0
La Paz	31	6	19.4	6	0
La Unión	74	4	5.4	3	1
San Miguel	86	9	10.5	9	0
San Vicente	48	4	8.3	4	0
Santa Ana	15	0	-	-	-
Sonsonate	22	2	9.1	2	0
Usulutan	23	4	17.4	4	0
Total	423	49	11.6	48	1

Identification of Physiologic Variants in *Anopheles albimanus*

When one considers the usual criteria for an efficient malaria vector it is sometimes difficult to understand how the human malaria parasite can continue to survive in Central America. There seems to be little doubt that *Anopheles albimanus* is the primary vector of human malaria in this area and yet this mosquito species fails to meet most of these criteria. Host preference studies indicate that this species, as a whole, prefers other animals than man as a source of blood, and there is a frequent tendency for the mosquitoes to rest and feed outside of houses. Dissection records have revealed very few natural malaria infections in these mosquitoes. These observations combined with others, such as anophelism without malaria in areas above 300 meters, apparently efficient malaria transmission during the dry season when the anopheline population is reduced, and rapid disappearance of malaria from the Atlantic coast of Central America while the same methods of attack were much less effective with the same vector species on the Pacific coast, strongly suggest the presence of physiologic variants within what may become a species complex. If some of these variants have a distinct preference for human blood or are more susceptible to the human malaria parasite, then a partial explanation for some of the epidemiologic peculiarities of human malaria in this area would have been found. This is of course, not a new concept. The *A. gambiae* story in Africa and the *A. leucosphyrus* complex in southeast Asia are two examples of persistent investigation developing what was at one time a single morphological species into a series of species, frequently with markedly different habits of breeding and feeding.

At the present time no proposal is being made to divide *A. albimanus* into several species. However, a species with such a wide geographic distribution as *A. albimanus* has a real potential for physiologic variations which would be reflected in differences in ecology and habits, and such differences might materially influence the vectorial capacity of the mosquito. CAMRS is interested in this concept and is anxious to identify any behavioristic or morphologic groupings in *A. albimanus*. Initially, efforts have been concentrated on geographic isolates, from both in and outside of El Salvador. Each isolate, once it is colonized, is being studied for specific physiologic and morphologic characteristics which might separate it from other isolates. Susceptibility to human malaria parasites will be determined for all isolates by feeding on malaria-infected monkeys, and all isolates will be tested for sensitivity to the commonly used insecticides.

Certain biochemical techniques will also be applied to each group of mosquitoes. One such technique, investigated as a possible means of establishing the existence of strain variants, was the determination of the presence and quantity of various pteridine compounds present in extracts from fourth instar larvae. These compounds fluoresce, and can be isolated by paper chromatography and quantitated by scanning the chromatogram in a fluorometer. Preliminary studies on the heterogeneous insectary colony were very promising. Two additional mosquito colonies, representing distinct geographical separations within El Salvador, were established in the laboratory and used to further evaluate the technique with material of known habitat variation and, hopefully of a homogeneous nature. Initial analysis comparing single extracts from these two colonies yielded significant qualitative and quantitative results. Repeat analyses, however, were not consistent, but

varied widely both within a colony and between colonies. These variations were too great to be attributed solely to chance, so replicate tests were made and analyzed using larvae from only one colony. Again the results were widely divergent. Because this technique appears unsuitable for the purpose of delineating specific characteristics with any degree of consistency, further attempts using it have terminated. Hopefully, work will continue on the problem of strain occurrence utilizing the more exact methods of electrophoresis and gas chromatography.

Any morphological character which might identify one of these isolates will also be exploited. Interest is now being directed toward pupal and egg characteristics. In cooperation with Dr. John Belkin of UCLA a thorough morphological analysis will be made on any isolate that for whatever reason seems to hold promise for separation. To supplement these investigations, chromosomal studies will also be carried out on any isolate that in terms of either behavior or morphology seems to be distinct from the species as a whole.

Colonies of *A. albimanus* have been established from the following areas in El Salvador: (1) Laguna San Diego in the mountainous interior of the country; this is a permanent breeding site and maintains mosquito populations even during the dry season; (2) Melara on the coast of El Salvador in an area that is highly malarious; this colony will be used as a base for future physiologic and susceptibility studies; and (3) Guayabo near the hydroelectric project on the border between the departments of Cabañas and Chalatenango; this is an area where extensive longitudinal sero-epidemiologic studies are being carried out. Work is currently underway to establish colonies of *A. albimanus* from Cinquera and Guarjilla in El Salvador, both of which are above 300 meters and apparently without malaria transmission.

There is one relatively well known morphologic character in the pupae which may aid in identifying certain groups. All naturally isolated groups of *A. albimanus* from El Salvador produce a certain proportion of pupae with a prominent dorsal white stripe. The percentage of striped vs. nonstriped pupae varies with area of isolation. Striped and nonstriped pupae have been isolated from the San Diego colony and are being maintained separately. The striped colony is now producing more than 80 percent striped pupae, an increase from the initial 20 percent. Physiologic analysis and malaria susceptibility studies will be carried out on these isolates in the near future.

Through the cooperation of Dr. Georghiou of the University of California (Riverside), a striped backed colony from Haiti is now being established in the laboratory. Dr. Arnaldo Gabaldon of Venezuela has also agreed to send *A. albimanus* eggs from that country, which approaches the southern-most extension of the distribution range of the species. The same system of analysis will be applied to all of these isolates and at the present time plans and efforts are underway to acquire materials for colonizing dry season survival populations and isolates from the Atlantic coast of Central America.

VECTOR CONTROL STUDIES

Residual Insecticides

During the year, studies on residual insecticides were narrowed down to two compounds, both carbamates: propoxur (OMS-33 or Baygon) and Landrin (OMS-597). The fumigant qualities of propoxur were investigated and evaluated by chemical methods, and small-scale exterior house-spraying was expanded into a large-scale trial. Laboratory studies early in the year on Landrin led to a village-wide field trial of this new compound.

Exterior Application of Propoxur

In the previous year, propoxur had been sprayed on the exterior surface of dwellings in a small village trial. The results suggested that such an exterior application effectively decreased inside biting for at least five to six weeks, suggesting larger-scale use. Thus, when in the course of studying multiple malaria control methods the opportunity arose in June 1971 for the application of residual insecticides on a large scale, an exterior spray of propoxur was selected. A total of 1,260 houses were sprayed, with coverage of all exterior walls and any overhanging surfaces protected from rainfall. The insecticide formulation was propoxur 70% w.d.p. (water dispersible powder), at a dosage of 2 gm/m^2. This operation proceeded more rapidly than conventional inside spraying, since the time required to prepare a house for outside spraying is minimal. In this cycle, an average of 9.5 houses were completed per sprayman-day, compared with 5.9 houses for conventional inside spraying in this area. Entomological evaluation of this trial is proceeding, based on weekly searches in the same 40 selected houses for resting anophelines, as well as simultaneous inside and outside human bait captures from 6 to 8 PM in eight houses. The houses for human bait captures were chosen to represent the different construction types and degrees of openness found in the houses of coastal El Salvador.

Chemical evaluations of fumigant activity of the insecticide when used in this manner are discussed below.

Studies on Landrin (OMS-597)

Laboratory studies: Landrin has been reported to be similar to propoxur (OMS-33) for the purpose of residual indoor application, and holds some advantages in cost and toxicity. Because of the possibility of future field testing in Central America, preliminary laboratory testing of the effectiveness of the compound was initiated. Table 17 presents mortality rates of anophelines exposed to specially treated test papers, using standard WHO susceptibility equipment. Further tests will be needed to determine a reliable LD-50.

TABLE 17

Percent Mortality of *A. albimanus** 48 Hours After Exposure to Landrin.

Dose in gm/m^2	Length of Exposure 30 min.	15 min.
.016	56.5**	0**
.032	100.0	86**
.063	100.0	100.0
.125		
0 (Control)	8	12

* 25 blood-fed female *A. albimanus* per trial.
** corrected mortality.

Using a plywood panel treated with one gram Landrin per m^2, contact, non-contact, and humidity tests were performed; results indicate that in fumigant activity and effects of humidity on effectiveness, Landrin resembles propoxur. On retesting three weeks after treatment, the panels retained their effectiveness. In summary, these preliminary and incomplete tests indicate that Landrin is a very active insecticide against adult *A. albimanus*, even at low dosages and short periods of exposure, with a fumigant activity which might make it useful in the open-type houses of this region.

Village-scale trial with Landrin: In June 1971 an area was selected for the village scale trial of Landrin in the locality of La Zunganera, District San Juan Talpa, in the coastal area of El Salvador. Preliminary entomological evaluations had shown the high anopheline density of this area. Furthermore, there was evidence of resistance to the chlorinated hydrocarbon and organophosphorus insecticides in the mosquito populations. After entomological evaluation, geographical reconnaissance, and mapping, houses which represent most of the construction types found in coastal El Salvador were selected for spraying.

The spraying took place during early July. Single pump charges of Landrin (760 grams) were weighed and sealed in plastic bags for transport to the field. The five spraymen, trained by the National Malaria Eradication Campaign (CNAP), used Hudson X-pert pumps of 8 liter capacity fitted with T-Jet HSS 8002 nozzles. A 5% suspension of Landrin was prepared by individual spraymen and applied at a target dose of 2 gm/m^2. All inside wall surfaces of 197 houses were sprayed to a height of 3 meters from the floor. No difficulties were experienced in preparing the suspensions, nor did the material clog the nozzles or settle unduly in the pump tanks during the operations. No toxic symptoms were noted among the spraymen or householders during the course of the spraying. Spraymen were instructed to wash hands and face after completing a house, to bathe at the end of the day, and to change to a clean uniform daily. Householders were instructed to remove all food-

stuffs and cooking utensils from the house, and to remain outside for one hour after the spraying.

The entomological evaluations will include weekly bio-assay testing of sprayed surfaces, following the techniques described in the Seventeenth Report of the WHO Expert Committee on Insecticides (1970). The test insects will be wild-caught *A. albimanus* females. Mosquitoes will be exposed, under plastic cones, to wall surfaces of palm thatch, brick, wood, and mud. Approximately 180 mosquitoes will be exposed to each of the surface types weekly, and mortalities recorded 24 hours after exposure.

In addition to entomological evaluations being conducted with this insecticide, various chemical studies are in progress. Although no symptoms of pesticide intoxication were noted among spraymen, air samples were collected during spraying operations which correspond to the amount of insecticide inhaled by the individuals during the course of one day's operation. Urine samples were also collected before, during and after exposure over a three day operational period.

Using samples of treated surfaces collected at various time intervals from date of application, the persistence of the insecticide is also being studied. Air samples similar to those taken in the propoxur studies have been collected at the same time as the surface samples to determine the possible presence of a fumigant effect and to what extent volatilization might reduce the residual activity of the insecticide. Analysis of all samples is presently in progress and the results will be forthcoming in the near future.

Studies on the Fumigant Activity of Propoxur (OMS-33)

CAMRS and PAHO personnel in El Salvador and PAHO-SNEM in Nicaragua are continuing to evaluate the effectiveness of propoxur (o-isopropoxyphenyl-N-methylcarbamate), for malaria eradication and control. In three studies, propoxur was used in a wettable powder formulation and applied at a target dose of 2 grams per square meter by conventional house spraying techniques. Each study involved a different target surface and/or area, affording CAMRS an opportunity to test the non-contact toxicity (fumigant activity) of propoxur under a variety of conditions. In the Nicaragua study, spraying covered all interior wall surfaces as well as any outside surfaces that were protected from rainfall. The PAHO (AMRO-0216) tests in El Salvador involved application of the insecticide in two non-overlapping one-meter wide swaths to the interior roof-wall junction. In the CAMRS tests, insecticide is applied to all exterior walls and the under-surface of projecting roof areas.

CAMRS measured the fumigant activity of propoxur by sampling the air around treated surfaces. Air-sampling equipment was positioned near a treated surface and air drawn through a glass Greenberg-Smith impinger containing a solution of 0.05N sodium hydroxide which traps the insecticide contents of the air sample. Air was sampled for one-hour periods, at rates varying from 0.1 to 0.3 cubic feet (2.6 to 8.4 liters) per minute, depending on the type of sampling unit. Samples were returned to the laboratory for preparation of a chlorinated derivative of the insecticide. Using an electron capture gas chromatograph, the concentration of propoxur was determined.

During the previous year it had been found that relatively small amounts of the insecticide were present in air sampled after spraying in periods of low relative humidity. With the onset of the rainy season, the increase in relative humidity was accompanied by an increase in the insecticide concentration in the air. The influence of relative humidity on the fumigant activity of propoxur was more clearly demonstrated this year.

In the AMRO-0216 program, where the insecticide was applied every 35 days, samples were collected the day following application and every 7 days thereafter through 9 spray cycles, 4 during the dry season and 5 during the rainy season. As graphically presented in Figure 4, the insecticide concentration in the air was consistently higher during the rainy season than during the dry season. That relative humidity was the principal factor involved in these higher concentrations can be seen in Table 18. During the sixth, seventh and eighth spray cycles, the temperature remained constant while the relative humidity decreased. Accompanying this decrease was the reduction in the air concentration of the insecticide.

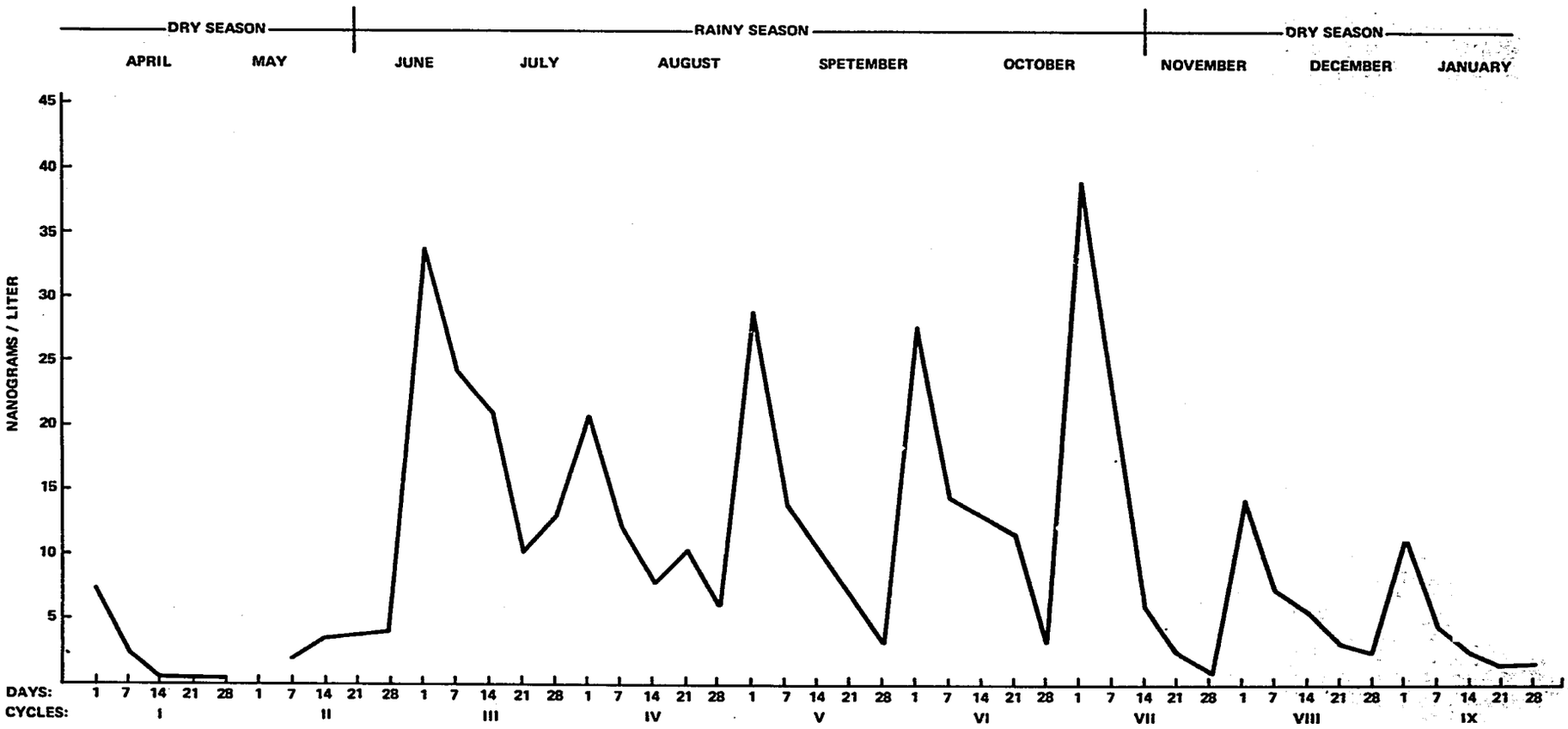
TABLE 18

Average Air Concentration of Propoxur in Three Thatch-roofed, Pole-walled Houses Relative to Temperature and Humidity. El Salvador.

Spray Cycle No.	Days after Application	Temperature (°C)	Relative Humidity %	Concentration Nanograms per liter
	1	29.5	87	27.6
	7	28.5	88	14.5
6	14	29.5	81	12.0
	21	29.0	82	11.7
	28	29.5	73	3.0
	1	31.0	83	39.2
	7	29.0	80	25.0
7	14	29.5	77	6.0
	21	28.5	57	2.1
	28	28.5	62	0.6
	1	30.5	35	14.0
	7	29.5	47	7.3
8	14	31.0	49	5.7
	21	28.5	50	3.8
	28	28.5	45	3.3

It has also been theorized that the more applications made, the greater will be the concentration and duration of insecticide in the air. In the Nicaragua trials, the insecticide was applied as a total interior spray once every three months. The predominant type of house construction in the trial area is rough, unpainted wood planking. Air samples were collected in this

Figure 4
AVERAGE AIR CONCENTRATION OF PROPOXUR IN PARTIALLY SPRAYED HOUSES (AMRO-0216)



area after both the first and second cycles. The concentration of insecticide in the air after the second cycle was approximately twice that found after the first cycle (Table 19). Since there was very little difference in relative humidity between the two sampling periods, the increase in concentration cannot be attributed solely to environmental conditions. It is believed that, at the time of the first application, a large proportion of the insecticide is irreversibly sorbed by the treated surface and is unable to change from the solid to the vapor state. During the period between applications some of the chemical would be released from these sorption sites, probably a concentration below our level of detectability, and would also undergo degradation processes so that numerous sorption sites would again be available. Nevertheless, with the second application, many of the sites would still be occupied and thus more chemical would be available to undergo a change from the solid to the vapor state.

TABLE 19

Average Air Concentration of Propoxur in Wood Houses Receiving Full Interior Spray. Zelaya, Nicaragua.

Spray Cycle No.	Days after Application	Temperature (°C)	Relative Humidity %	Concentration Nanograms per liter
	1			-
	2			-
	3			5.5
	4			-
1	5	29.0	76	7.1
	6			7.8
	7			5.7
	8			6.4
	9			4.1
	1			21.4
	2			17.5
	3			12.7
	4			11.3
2	5	27.0	80	17.8
	6			11.7
	7			10.8
	8			9.8
	9			9.5

Recent samples of propoxur in a large-scale CAMRS test of exterior wall spraying in El Salvador may aid in substantiating the relationship between multiple spraying, humidity, and fumigant activity. The insecticide was applied to all exterior walls of the houses and to any overhanging areas protected from rainfall. Air samples were collected from areas closely adjacent

to the sprayed houses at intervals for 14 days after spraying (Table 20). In these samples the chemical had virtually disappeared from the air by the 14th day, even though the relative humidity at the time ranged from 57 to 70 percent. Further studies will attempt to link humidity and multiple application and also will seek to determine the residual amounts of propoxur which may persist on various types of surfaces during variable conditions of temperature and humidity and after variable numbers and spacing of applications.

TABLE 20

Air Concentration of Propoxur in Exterior Applications, El Salvador.

Days after Application	Concentration in Nanograms per liter by Type of House Construction			Temperature	Relative Humidity
	Pole	Palm	Mud	(°C)	%
1	40.7	12.5	5.7	31	72
2	13.2	5.5	4.8	31	60
7	4.8	3.7	1.2	32	57
14	0.0	0.6	0.0	32	58

To demonstrate the influence of relative humidity on the release of propoxur from sprayed surfaces, the insecticide was applied to two 36" x 36" sheets of paper at a rate equivalent to 1 gram per square meter. The papers were air-dried and then placed inside sealed plywood boxes 4' x 4'. Air samples from the box were collected through a glass tube inserted through a rubber-stoppered hole centered in the top of each box, and extended to the midpoint of the box. These boxes were maintained in the laboratory at a temperature of 25° C. Twenty-four hours after positioning the papers in the boxes, air samples were collected. Each sample contained approximately the same concentration of insecticide (34 nanograms per liter). The papers were then removed, placed outdoors in a sheltered area and allowed to weather for two weeks. The papers were then repositioned in the boxes. One box contained a drying agent which maintained the relative humidity at 40%, whereas the other box contained pans of water which raised the relative humidity to 74%. Air samples were again collected after 24 hours. In the 40% RH environment, the insecticide concentration was 3.3 nanograms per liter, as compared to 11.9 nanograms per liter in the 74% RH environment. The relative humidity conditions in each box were then reversed, the papers remaining in place. Again the more humid air contained greater amounts of the insecticide (13.8 nanograms per liter) than the drier atmosphere (2.0 nanograms per liter). The experiment was repeated using 12" x 12" sheets of paper and, again, the insecticide concentration in the more humid environment exceeded that of the drier. Thus it has been concluded that relative humidity exerts a great influence on the release of the insecticide from treated surfaces.

Larviciding

Petroleum Larvicides

Limited larvicidal tests were conducted during January 1971 using Flit MLO in a variety of habitats including estuary, river, lake and pasture situations. Although efforts were made to obtain good surface coverage at acreage rates in the 1-3 gallon range, more often higher rates (5.0 - 6.5 gal/acre) were required to produce a visible film over the entire surface of the plots. The material was applied with a two-gallon Hudson hand sprayer equipped with a Teejet 8002 fan-type nozzle under a gauge-measured pressure of 40 psi. This spraying resulted in larval reduction varying from 97.7% to 83.0% reduction (Table 21). The results in the lake, with 83.0% and 87.7% reduction at dosage rates of 5.8 and 2.9 gallons per acre, respectively, suggest that smaller dosages might be as effective as larger ones. Obtaining good coverage with the smaller amounts of liquids presents difficulties, so that future tests will compare the effectiveness of varying nozzle types and pressures.

Flit MLO does seem to offer promise in selected situations which can be determined on the basis of accessibility, toxicity, cost requirements and other factors. Further testing to determine the optimum application techniques and dosage rates in various situations are planned for this as well as other promising larvicidal materials.

Abate

Extensive trials using Abate as a larvicide applied by aircraft to dry season river breeding habitats were conducted in association with a program of integrated control in a coastal area of El Salvador. The results of these trials are described elsewhere in this report.

TABLE 21

Effect of Dry-Season Flit MLO Application on *Anopheles* populations, El Salvador, 1971.

Locality & Habitat	Dosage (Gal/Acre)	Plot Size	Before Application			After Application			Percent Reduction After 24 hrs.
			No. Dips	No. Larvae	No. Larvae per Dip	No. Dips	No. Larvae	No. Larvae per Dip	
Estero Ticuiziapa, Mangrove Swamp	5.0	5,000 ft^2	145	2,137	14.7	150	50	0.3	97.7
Hda. Santa Emilia, Irrigated Pasture	6.5	4,400 ft^2	50	229	4.6	50	25	0.5	89.1
Tunco River, Pooleo Water	5.3	4,340 ft^2	150	501	3.3	150	110	0.7	78.1
Lake Ilopango, Floating Vegetation	5.8	4,000 ft^2	100	1,030	10.3	100	176	1.8	83.0
Lake Ilopango, Floating Vegetation	2.9	4,000 ft^2	55	1,031	18.7	55	127	2.3	87.7

Adulticiding

ULV Ground Application

A single test of the newly-acquired Leco ULV Cold Aerosol Fogger was conducted during the dry season in the early evening (around 6:00 p.m.). With the fogger mounted on a pick-up truck, its nozzle directed backwards and 45° above horizontal, technical malathion was sprayed on a target area south and downwind of the intersection of the Coastal Highway and the San Diego Beach road. The fogger was calibrated to deliver 3 fluid ounces per minute by use of the flow meter and vehicle speed, with the calibrated application rates estimated to be 0.3 ounces per acre, based on a 300-foot swath target. Expected effective coverage would be somewhat less.

As measures of exposure, three cages of wild-caught *albimanus* females from the Malathion-susceptible area of Acajutla had been placed 100, 300, and 600 feet from the discharge point. At each station, the three cages were placed on either side of the road, separated from each other by no more than 30 feet. The cages were collected 45 minutes after exposure and the adults were transferred to clean holding cups for mortality counts 24 hours later. An overall mortality of 43.3% was obtained at the 300-foot distance with lesser mortalities of 15.7% and 28.1% at the 100-foot and 600-foot distances, respectively (See Table 22). Even though the mortalities were low, there is evidence that good downwind distribution was achieved.

Developmental trials of this fogger are indicated, using various combinations of vehicle speeds, flow rates, nozzle directions, etc., for each of a number of candidate insecticides.

ULV Air Application

During late October 1970, the U. S. Military and the U. S. Department of Agriculture (Gainesville, Florida Laboratory) collaborated in an aerial application of ultra-low-volume (ULV) fenthion over a 20 square-mile area of the Panama Canal Zone. Because of its prior experience and continuing interest in the potential of this method for *A. albimanus* control, CAMRS was invited to observe and participate in the tests.

The tests differed from those conducted by CAMRS in 1968-69 in two important respects, i.e., (1) in the use of fenthion, an organophosphorus compound, effective against both larvae and adults and (2) in early evening (5:30-6:10 p.m.) applications when mosquitoes are expected to be active. A U. S. Air Force C-123 equipped with 14 nozzles (Spraying Systems 800 4) was flown at a speed and altitude to deliver 1.0 fluid ounces (.075 lbs. per acre) of technical fenthion per acre in 2,000 foot swaths. The target area was covered in 40 minutes flying time.

Effects of fenthion were measured using 10 bioassay stations, consisting of a cage of adults and a container of larvae in both open and canopied situations. Supporting pre- and post-treatment evaluations were made from horse trap and human bait capture stations. Preliminary findings indicated good results from the evening application of this insecticide. CAMRS will continue to be interested in the development and field testing of ULV methodology.

TABLE 22

Percent Mortality of *A. albimanus* Females 24 hours after Application of Technical Malathion by ULV Cold Aerosol Fogger. El Salvador, 1970.

Station No.	Distance from Discharge	Trap	Cover	Caged Females* Alive	Dead	Total	Percent Mortality
1	100'	A	Open	18	5	23	21.7
		B	Hedge row	23	1	24	4.2
		C	Heavy brush	18	5	23	21.7
Total, Station 1				59	11	70	15.7
2	300'	A	Open	12	11	23	48.0
		B	Low brush	13	10	23	43.5
		C	Tall tree	14	8	22	36.4
Total, Station 2				39	29	68	43.3
3	600'	A	Open	13	6	19	31.6
		B	Hedge row	18	5	23	21.7
		C	Heavy brush	15	7	22	31.8
Total, Station 3				46	18	64	28.1
All Stations				144	58	202	23.2
Control - 1				17	0	17	0
Control - 2				19	0	19	0
Control - 3				20	0	20	0

Insecticide Resistance in *A. albimanus*

CAMRS continued to collaborate with the University of California at Riverside in a study of *A. albimanus* resistance to organophosphorus and carbamate insecticides. During the year, three shipments of *A. albimanus* eggs from field sources were made to Riverside for studies on the genetics of developing resistance.

A colony of *A. albimanus* from adults collected at Hacienda Melara, La Libertad, was used for tests conducted at Riverside. The LC_{50} of propoxur, carbaryl, malathion, parathion, and methyl parathion for this colony had increased by 1.1 to 3.4 times in comparison to a mixed susceptible strain from Haiti and Panama. When the El Salvador strain was subjected to larval selection pressure by propoxur at the 90-95% level of mortality, the larval LC_{50} rose only gradually at first, i.e., from the parental .53 ppm to .68 ppm in the F_1 generation and to 1.5 ppm in the F_2. A change in slope of the dosage-mortality line of the F_2 generation from 3.3 in F_1 to 1.4 was more indicative of impending resistance. Selection of a third generation resulted in a remarkable increase in resistance to propoxur. Concentrations of 10 ppm to 500 ppm produced 24-hour mortalities ranging from 3 to 15%; and 1000 ppm gave only 81% mortality.

The studies from which the foregoing data were taken (Ariaratnam and Georghiou, WHO/VBC/71.277) are being continued and it is expected that these studies will provide needed qualitative and quantitative information on the nature of the resistance factors involved. The information so far obtained, in the opinion of the above authors, is of major importance in mosquito control and malaria eradication strategies.

CAMRS has also maintained a colony of malathion-resistant *A. albimanus* from material collected at Hacienda Melara in coastal El Salvador. Material from this colony has been provided to several institutions for additional studies on insecticide resistance.

Determinations of DDT on Tarsal Segments of Mosquitoes

A method of determining the amount of DDT sorbed by the tarsi of mosquitoes has been developed. In the laboratory, a direct correlation can be made between the amount of chemical on the tarsi and both the concentration to which the individual mosquito was exposed and the length of exposure. The objective of the method is to establish the degree of contact exposure in mosquitoes caught biting indoors and/or exiting from sprayed houses. The method might also be combined with precipitin tests for specimens taken from natural harborage catches in order to indicate the likelihood of outdoor transmission. In areas of sporadic or non-DDT coverage, it may be valuable in determinating the amount of exposure to the chemical as used in agricultural practices. The method may also serve as an indicator of the presence and amount of DDT-resistance in mosquito populations.

A cooperative project which employs this technique is presently underway with the MEP, Philippines. For two separate tests, sets of coded samples were received with various histories of DDT exposure. On the basis of residue determinations, the first set was matched exactly with the corresponding history data but it was not possible to repeat this success with the second set. To date, 50 such samples have been received and analysed. Confirmation of the chemical interpretations with the actual history of the samples is still pending.

Source Reduction by Water Management

Coastal estuaries in El Salvador and elsewhere in Central America provide anopheline breeding sites which may be significant under certain seasonal conditions. These estuaries are formed by numerous rivers flowing across the coastal plain and emptying into the Pacific Ocean through areas which closely approach sea level, allowing for a certain amount of water collection at the terminal points. During the rainy season sufficient water is carried in these rivers to maintain a constant flow into the ocean and to keep the estuary openings patent. During this period the estuaries are flushed by the tides every twelve hours, precluding the possibility of anopheline breeding. As the river flow decreases during the dry season, many of these estuaries are closed by the formation of sand bars created by wave action, and large bodies of impounded water may form. While the water is at first of high saline content, the continued entry of fresh water from the rivers, albeit at a reduced rate in the dry season, results in a gradual decrease in salinity, and an accompanying expansion of water surface into heavily vegetated areas. The situation is then ideal for *Anopheles albimanus* breeding. Several months after the beginning of the rainy season, the river flow becomes sufficient to cut a channel through the sand bar and the area opens naturally to the ocean again.

Studies during previous years have delineated the seasonal breeding potential of estuaries relative to salinity and other factors. One such estuary, Estero San Diego, has been studied intensively for the past two years and has been shown to be a primary breeding habitat during the dry season. Investigations have been made during the current year on the use of several methods for eliminating this source of anopheline breeding. This estuary generally closes to the ocean during February, and prior studies have shown that approximately one month later the ecology of the estuary permits anopheline breeding. The elimination of this location as a breeding habitat would require either that the opening to the ocean be maintained continuously throughout the dry season, or at least be opened periodically with no more than a month elapsing between reclosing and reopening. A number of techniques have been considered for opening the estuary to the ocean, including explosives, hand labor, and use of heavy machinery. In any such undertaking, feasibility and economy are important factors.

Because of its known effectiveness, low cost, and availability in many ditching procedures, dynamite was selected for initial trials. With the assistance of an engineer and demolition expert from the U.S. Southern Command (Panama) test charges of one, four, and twelve sticks of dynamite were placed at a depth of three feet in the sand bar and detonated. The results of this test were poor because of the high velocity of the explosive used, coupled with the absence of rigid compactness of the sand. The largest charge tested (12 sticks) produced a conical hole 4' wide by 4' deep. Since the charge had been placed at a depth of 3', it was obvious that no great advantage had been gained. Considering the time involved in planting and setting the charge, the amount of sand moved was little or no greater than that which one man with a shovel could move in an equal amount of time. Other explosives of lower velocity, such as ammonium nitrate, were considered, but the cost made these alternatives impractical.

The second method tested for opening the estuary passage was hand labor. About one month after the natural closing of the estuary, larval development had been observed in the estuary at salinity values as high as 63 percent sea water. At this time assistance was requested of the Civil Action Brigade of the Salvadoran Army, which provided men and hand equipment to dig a channel between the ocean and the estuary. Nine men in three shifts, working one hour per shift, were able to dig a channel 6 feet wide between the two bodies of water in approximately 6 hours. However, the incoming tide prevented the construction of a suitable grade down which the estuary water could flow, and, as the sea water entered the ocean end of the channel it undermined the sides and sealed the channel for one-third of its length.

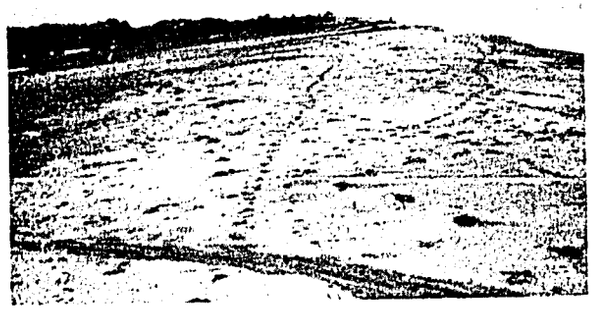
On the following day the channel was dug again, but at a steeper grade starting from the estuary. In order to prevent the rapid inflow of water and the resultant hindering of the digging effort, no initial direct opening was made into the estuary. When the water table was reached, underground seepage proved troublesome, and although the men worked very hard and succeeded in obtaining a somewhat steeper grade the incoming tide prevented drainage of estuary water. During the high tide, some sea water entered the estuary before the channel was again sealed by the sliding of the sand walls due to the passage of this sea water. The volume of water entering was insufficient to change the salinity of the estuary water to the extent necessary for interruption of larval development. The effort involved and changes effected are partially conveyed by the series of photographs in Figure 5.

In considering the results of this operation, it is evident that at least three criteria must be met before a suitable opening can be achieved. The channel must be constructed with a suitable grade down which the impounded water may flow, and at the same time must be wide enough so that sliding sand from the sides will not impede the flow of water. Further, the opening should be timed to coincide with a low tide so that tidal action will not prevent an out-flow of water, at least for the first tidal cycle. Because of the difficulty of working below the water table and the size requirements for the channel it seems unlikely that the use of hand labor, such as that demonstrated, will be adequate. Consideration is being given to the use of heavy equipment which may be economically available in the area, such as a "clamshell" excavator. It is anticipated that this equipment will be available for trial at the appropriate time, when the estuary again closes from the ocean after two or three months of dry season conditions.

The potential of such estuaries as sources of malaria transmission is being evaluated epidemiologically. The area around Estero San Diego was surveyed and showed a parasite rate of 3.2 percent early in the anopheline breeding season. In the same survey, a positivity rate of 28 percent by the fluorescent antibody serological method indicated the presence of malaria in the population during the past 3 years. Similar evaluations will be made before and at intervals after the removal of the anopheline breeding site to determine the effectiveness of this method under this special set of circumstances.

Figure 5

SOURCE REDUCTION BY WATER MANAGEMENT



1. View of sand bar, Estero San Diego. The object was to dig a channel connecting the estuary on the left with the ocean on the right.

2. Civil Action Brigade, Salvadoran Army digging the channel.

3. Completed channel, with ocean water entering.

4. Wave action caused erosion of sides of channel, closing the area.

5. Condition of channel after the closing.

6. The channel was made wider and deeper the next day, however the same sequence of events occurred and the channel was again closed, as this photo illustrates.

Biological Control

During the year, biological and non-conventional chemical methods of controlling anopheline populations, including mosquito pathogens, synthetic hormones as larvicides, and sterile-male release measures, were studied.

Mosquito Pathogens

WHO and various governmental and academic investigative groups have a continuing interest in developing collaborative projects in the area of biological control of mosquitoes. A protocol has been developed which will permit staff members from various institutions to join in a single screening program to determine the identity and extent of naturally occurring diseases and enemies of anopheline vectors of malaria in Central America. Initial collaborators include CAMRS, the CDC Technical Development Laboratory, the TVA Environmental Research and Development Laboratory, and the USDA Laboratories of Gainesville, Florida, and Lake Charles, Louisiana. A coordinator has been agreed on and some preliminary work has begun.

Personnel from the CDC Technical Development Laboratory and the USDA Lake Charles Laboratory have made on-site investigations in El Salvador in cooperation with CAMRS. From this work, it is already evident that a number of pathogens do occur naturally among anophelines in El Salvador, and that they are receptive to infection by parasites under laboratory conditions. Two microsporida have been so far identified: *Thelohania* sp. (near *legeri*, but probably a new species) from *A. pseudopunctipennis* larvae from three breeding sites, and *Thelohania* sp. (probably *obesa*) from *A. pseudopunctipennis* larvae, from two breeding sites. Dissection of 90 adults of *A. albimanus* were negative for pathogens. Other studies have shown that both *A. albimanus* and *A. pseudopunctipennis* were quite susceptible to the nematode, *Reesimermis nielsoni*, in the laboratory. The level of induced infections in surviving larvae ranged from 76 percent (2.5:1 ratio of preparasitic nematodes to exposed larvae) to 100 percent (20:1 ratio of preparasitic nematodes to exposed larvae) for *A. albimanus*, and from 80 to 100 percent at the same ratios for *A. pseudopunctipennis*.

Hormonal Larvicides

The CDC Technical Development Laboratory has been studying the effects of a number of synthetic juvenile hormone compounds on mosquito larvae, including anophelines. Certain of these compounds show definite promise against anopheline larvae with 95 percent mortalities at levels of approximately 0.1 ppm. These materials exert their effect on developmental processes of the mosquito usually in the larval or pupal stages, but even apparently normal adults which survive treatment in the aquatic stages may be damaged in terms of longevity, blood-feeding, infectivity, or fecundity. Laboratory testing of some of the compounds advanced to the point that field testing was conducted in El Salvador during April 1970. A number of field plots containing larvae of *A. albimanus* and/or *A. pseudopunctipennis* were treated by CDC-TDL personnel from Savannah. CAMRS collaborated by making appropriate surveys and density studies and in evaluating results. Final results were not available during the reporting period, but early impressions are that the

compounds offer much promise for further testing under field conditions.

Sterile Male Release

In certain malarious areas anopheline control with the standard residual spray technique has been ineffective because of vector resistance, because the biting or resting habits of the vector do not bring it into contact with the insecticide-treated surfaces, or for other reasons. Thus it has become necessary to review and evaluate other methodology. Recent developments in the sterile male technique demonstrated by the Gainesville, Florida, USDA Laboratory indicate that this method offers promise against anophelines, especially among isolated populations.

A. albimanus has certain important biological characteristics that make it adaptable to the sterile male technique. For example, it is readily colonized, indicating compatibility in the mating behavior of laboratory-reared males and wild females. Also, the species can be reared in large numbers. CAMRS has already developed adequate information on the bionomics of *A. albimanus* in an area amenable to the requirements of a sterile male release project, and the USDA group at Gainesville has the necessary expertise in the technique. Therefore, it has been possible to initiate a trial to determine the feasibility of sterile male release in controlling or eradicating *A. albimanus* for the purpose of interrupting transmission of malaria. The Lake Apastepeque area near San Vicente, El Salvador, has been selected as the location for this study.

Pre-release surveys were begun on a systematic basis in May 1971. Prior to this trial, a sufficient amount of data was accumulated to predict seasonal population buildup on which the release schedules will be based. Larval populations are being sampled by a special device to determine the average number of larvae per square foot of breeding surface. These sample figures can be projected to give an estimate of total production and emergence of mosquitoes, which in turn will be the basis for estimating the numbers of chemosterilized males needed for release at a given time to result in proper sterile-male to normal-male ratios.

Methodology for estimating adult densities is also being developed with the idea of determining the best method for evaluating results of population control measures. It appears that a combination of New Jersey light traps, cattle corral counts, and natural resting counts will be utilized. All of these methods have detected adult populations reflecting larval population increases. Meanwhile investigations at Gainesville will be directed toward the development of specific techniques for the mass rearing, exposure and release of mosquitoes, and the acquisition of equipment for shipment to El Salvador.

A colony of *A. albimanus* from the Apastepeque area has been established at CAMRS and mass rearing in the CAMRS laboratory is targeted to reach a peak of 50,000 male pupae per day by March 1, 1972, with sterile male releases scheduled to begin about April 1, 1972, and to continue for 10 generations.

PARASITE BIOLOGY

Characterization of Malaria Parasites of Central America.

Life Patterns and Normal Drug Sensitivity

Efforts to characterize the malaria parasites of Central America by their life patterns (including relapse patterns) and their sensitivity to commonly used antimalaria compounds have continued through the year. Parasites from active infections in various parts of the region have been sought for isolation in the "owl" or "night" monkey, *Aotus trivirgatus*, which is usually susceptible to the human parasites. A colony of these monkeys is maintained at CAMRS. Cooperative arrangements with the NIH/NIAID (National Institute of Allergy & Infectious Diseases) Laboratory at Chamblee, Georgia, have made possible the study of Central American Malaria parasites both in additional monkeys and in human volunteers.

Monkeys inoculated with parasites from six human infections had been provided the NIH group for study previously, and one additional parasite was acquired during the current year. During late August, 1970, a trip was made to Nicaragua to seek isolates of *P. vivax* in the Rama area, i.e., on the Atlantic side of the country. Until this time, all of the Central American isolates had been either from the Pacific coastal regions or from Panama. A search in the Rama area located two cases, and parasites were inoculated into two *Aotus* monkeys. The monkeys were returned to CAMRS and subsequently shipped to the NIH Laboratory in Chamblee, Georgia, for study. In one of the monkeys, parasites were observed about 15 days after inoculation. The strain has been preserved and inoculated into additional animals. Mosquitoes have been infected and further studies are underway. Currently four *P. vivax* strains are under intensive study in the NIH laboratory: one from Panama, two from El Salvador, and the one isolated in Nicaragua.

Interesting results from studies on the earlier isolates from Panama and El Salvador are becoming available. While much of the work has dealt with the course of infection in the non-human primates and in the infectivity of the parasites to mosquitoes, significant information has also been acquired on the normal relapse patterns in man. To date all of these strains have shown moderately long latent periods after treatment of primary attacks, with much shorter periods of latency after treatment of relapses. For example, ten volunteers inoculated with the Panama strain developed a primary attack in a median of 14 days. Two of this ten received curative therapy (chloroquine-primaquine); of the other eight, treated with various non-curative antimalarials, all experienced a first relapse, with a median latent period of 165 days after inoculation. One of these was given curative treatment, but six of the remaining seven had experienced a second relapse in a median of 219 days after original inoculation, or a median of about 53 days after the previous parasitemia. To date, three of these had experienced a third relapse at a median of 269 days, with about 54 days between the second and third relapse. The El Salvador strains, though based on a smaller number of cases, showed similar patterns. Of five primary attacks of the Salvador I strain not receiving a curative drug, three have relapsed, with a median latency of 207 days. The two relapses seen with the Salvador II strain

appeared after 172 and 262 days. The first of these has relapsed a second and third time with latent periods of 31 and 28 days respectively. The absence of relapses in some cases may indicate only that enough time had not yet elapsed, since the volunteers were not all inoculated at the same time.

Judging from the literature, this is the first firm information available on the life patterns of Central or South American strains of *P. vivax*. In the past, strains from other areas possessing the same pattern have been classified as "temperate zone" strains, since the long latency period could allow the parasite to over-winter in its vertebrate host, the relapsing infection providing a new reservoir of infection for the warm season return of the anopheline populations. One could equally attribute the parasites' ability to survive a prolonged dry season period of low anophelism, such as exists in many areas of Central America, to the same delayed relapse of parasites acquired toward the end of the previous transmission season.

Field studies during the past several years seem now to have shown the effect of this long period of latency, and some of the findings can be interpreted more reasonably. As an example, in several studies the case rates of *P. vivax* have risen during the months of February and March, a puzzling occurrence in the absence of any significant vector populations at that time. There followed an equally unexplainable decrease in positivity during the next two months. With the knowledge of parasite relapse patterns now available, it seems apparent that the rise in positivity may have been due solely to relapses of infections acquired during the previous high transmission period in September and October. Clearly this information will be crucial in the planning and evaluation of antimalaria programs.

Additional strains of *P. vivax* are being sought from Costa Rica (Atlantic and Pacific sides), from Honduras, and from other areas where epidemiologic or geographic factors suggest possible differences in life patterns. Based on information now available or being developed, field studies on the effects of these known patterns on transmission cycles are underway and planned. Predicting relapses after a known outbreak of vivax malaria would be important in the selection and timing of antimalaria measures. These studies will also facilitate differentiation of relapses from primary transmissions during periods of increasing case rates, one of the difficult problems in assessing levels of endemicity and evaluating the effectiveness of antimalaria measures.

At the same time, drug studies are being done on all strains studied by the NIH group to determine normal sensitivity to the commonly used antimalarials. Preliminary results suggest normal sensitivity to chloroquine, chloroguanide, pyrimethamine, and quinine, but further confirmation is needed. In all cases tried, the routine 14-day primaquine treatment has effected radical cures of the infections, i.e., prevented relapses. Information is being sought on the minimum effective primaquine regimens, in view of the current wide usage of a 5-day course of treatment, as well as weekly or biweekly primaquine, in the Central American region.

Malaria strains are being studied at CAMRS, utilizing the colonies of *Aotus* (owl) monkeys and *Samiri sciurious* (squirrel) monkeys now available.

Parasite Infectivity to Mosquitoes

Preliminary studies are underway to determine the susceptibility of the anophelines of the region to the malaria parasites of the region. Parasite infections have been established in monkeys, providing a continuing reservoir for such studies. Initial studies have been done, utilizing several isolates of *A. albimanus* available in colonies at CAMRS, but results are as yet insufficient for reporting.

Studies done by the NIH Laboratory at Chamblee, Georgia, on strains of *P. vivax* provided by CAMRS have produced some interesting and provocative results. The vectors (*A. freeborni* and *A. maculatus*) used in these studies are not coindigenous with the parasites but preliminary evidence suggests, for example, significant differences between two *P. vivax* isolates from El Salvador in their infectivity to mosquitoes. The parasite infectivity relationships have been quantitated for two strains from El Salvador, one from Panama, and one from Nicaragua, using this vector-parasite system. These results suggest significance in the parasite-vector relationships in Central America, and repeat studies at CAMRS using coindigenous vectors and parasites have been planned.

MALARIA CONTROL DEMONSTRATION PROJECTS

Drug Studies

A previous study of two drug combinations as antimalarials was summarized in the 1970 Annual Report. Regimens of pyrimethamine-primaquine and amodiaquine-primaquine were instituted in comparable coastal El Salvador endemic areas (Districts 8 & 13, Zone 2, Fig. 6). The objectives of the study were to compare the acceptance and effectiveness of the two drug combinations, and also to explore the reasons why drug programs have, in the past, failed to serve as effective eradication methods. The drugs utilized in malaria eradication have encountered resistance from the people: complaints of side effects have been high, and general drug acceptance has been low. This 1969 study sought to determine patterns of acceptance and refusal, to identify and catalog the specific complaints of drug recipients, and to find measures which might increase population acceptance. In comparing the two drug combinations, the 1970 annual report revealed little or no difference in acceptance of the two combinations, little difference in complaints of side effects, and a slight anti-malarial differential in favor of the pyrimethamine-primaquine combination (probably related to the lower level of endemicity in the area in which the combination was used). The bi-weekly dosage schedules are presented as follows.

Pyrimethamine-Primaquine Dosages in Mg. Base.

Weight of Recipient (Pounds):	<20*	20-49	50-99	≥100
Pyrimethamine	9.375	18.75	37.5	75.0
Primaquine	6.25	12.5	25.0	50.0

* Children under 6 months not treated.

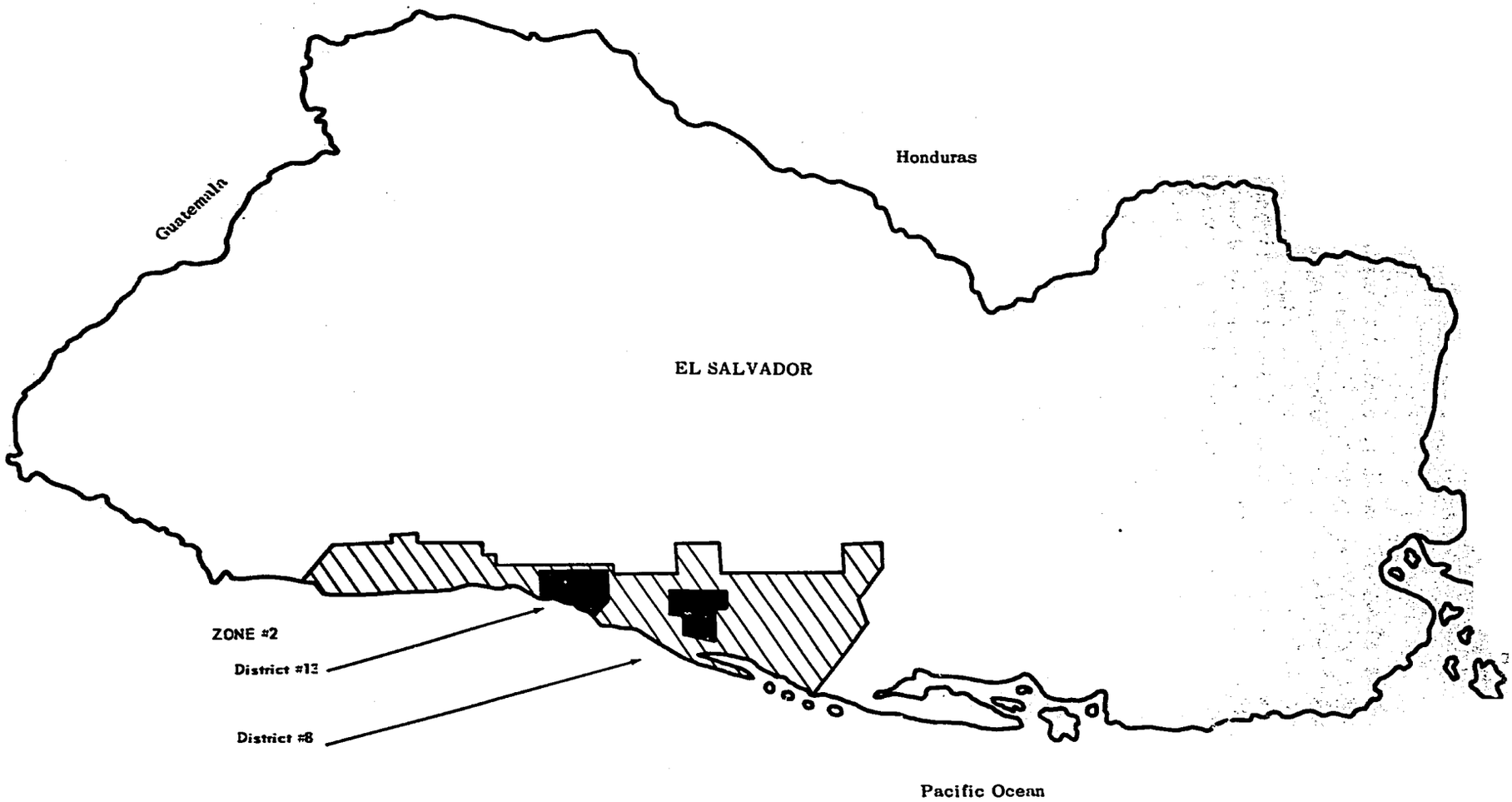
Amodiaquine-Primaquine Dosages in Mg. Base.

Age of Recipient (Years):	<1*	1-2	3-6	7-12	≥13
Amodiaquine	75**	150	225	300	450
Primaquine	7.5	15	22.5	30	45

* Children under 6 months not treated
** Chloroquine.

Figure 6

Drug Study Areas, El Salvador



In order to determine the influence of differences in district conditions on drug effects, a study in which the combinations were reversed in the two districts was planned. Unfortunately, District 8 had been included in a large-scale PAHO insecticide test and was unsuitable for the drug study. Nevertheless, the pyrimethamine-primaquine combination was introduced into District 13, on a biweekly schedule from March through December, 1970. Comparative data are presented in Table 23 and Figure 7; all three of the drug programs followed essentially the same pattern, with an insignificant rise in acceptance of pyrimethamine-primaquine in 1970. Likewise, no significant differences in slide positivity rates occurred. District 8, which had been sprayed with propoxur in 1970 instead of receiving drugs, had nearly the same slide positivity rate as in the previous year. If general survey results are used rather than the relatively flexible active and passive case detection mechanisms, positivity rates are: District 13, 1969, 0.8% and 1970, 1.3%; District 8, 1969, 0.8% and 1970, 0.5%. In both districts the proportion of infections due to *P. falciparum* was much lower than in 1969 (5.8% in District 13 and 5.4% in District 8). Thus, at least as far as *P. falciparum* is concerned, pyrimethamine-primaquine seems to compare well with a propoxur spraying program as a malaria control method.

The complaint patterns did not appear to vary significantly between the two combinations or between the two years of pyrimethamine-primaquine use (Table 24). The slightly lower number of complaints with pyrimethamine-primaquine did not permit a conclusion on comparative side effects, particularly in the face of variable observations on acceptance (Table 23). There did seem to be smaller numbers of multiple complaints with this drug combination. A compilation of complaints by category (Table 25) and of the most frequently occurring specific complaints (Table 26) yielded little of significance in comparing the two combinations. If the 2 regimens are compared in the same population (District 13) but in different years, the pyrimethamine-primaquine combination appears to have some advantage in complaint patterns. The specific complaints were fewer with this regimen, and there appeared to be an increase in those classified as "psychological", suggesting an increased amount of subjectivity in the reasons for not accepting the drugs. This, along with a somewhat lower rate of refusal, could be interpreted as evidence of fewer side effects. However, the complaint patterns of the two regimens used in the same year, but in different populations, were very similar. On the whole it is not possible to conclude that either combination has any notable advantage.

Whatever the cause, the acceptance rate, which averaged 60 to 66 percent through the entire study, varied between 48 and 74 percent in individual cycles. It is obvious that malaria transmission, although not eliminated, was reduced considerably, confirming impressions derived from previous use of mass chemotherapy in the region. It was further obvious that the pyrimethamine-primaquine combination has no advantage that would justify recommending any expensive changeover for country programs with large stocks of the standard drugs on hand or available.

TABLE 23

A Comparison of Amodiaquine + Primaquine with Pyrimethamine + Primaquine, District 13 and District 8, El Salvador, 1969-1970.

	District #13		District #8	
	1969 Amodiaquine + Primaquine	1970 Pyrimethamine + Primaquine	1969 Pyrimethamine + Primaquine	1970 Intradomiciliary Propoxur
Average Population	5,952	5,842	5,643	5,244*
Accepting (%)	63.6	66.3	60.1	-
Refusing (%)	34.6	31.9	35.9	-
Absences (%)	2.8	1.8	4.0	-
Total Slides Taken	22,975	16,636	16,203	20,314
Total Positives	528	551	123	203
Slide Positivity Rate	2.3	3.3	0.8	1.0
Percent *P. falciparum*	43.6	5.8	29.5	5.4

* Average number contacted in surveys.

Figure 7

Acceptance Levels Pyrimethamine–Primaquine (District 8, 1969; District 13, 1970) and Amodiaquine–Primaquine (District 13, 1969)

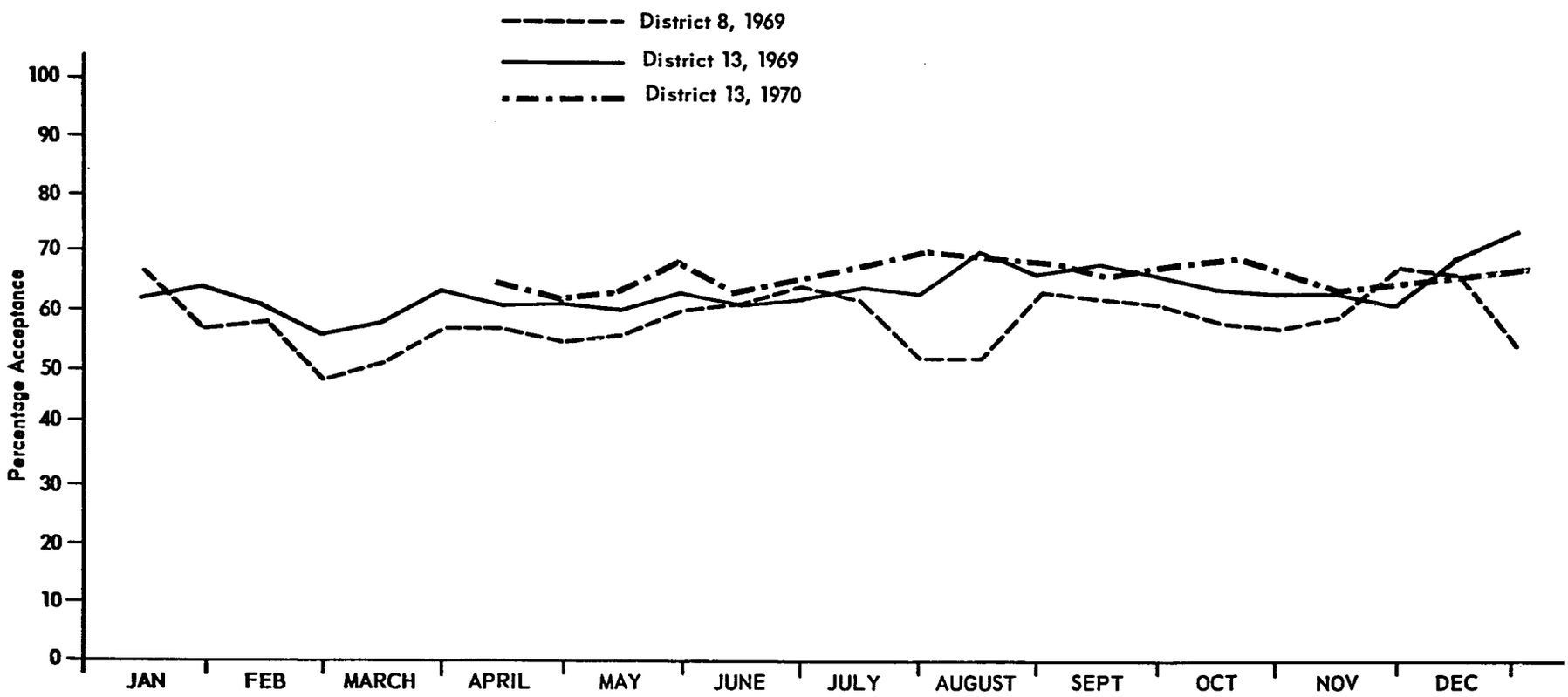


TABLE 24

Complaints Associated with Amodiaquine + Primaquine and Pyrimethamine + Primaquine. District 13, 1969-1970 and District 8, 1969, El Salvador.

	District #13		District #8
	1969 Amodiaquine + Primaquine	1970 Pyrimethamine + Primaquine	1969 Pyrimethamine + Primaquine
Number of Complaints	Number of Complainers		
One	3,440	2,371	3,015
Two	704	625	465
Three	131	50	44
Four	7	1	3
Total	4,282	3,047	3,527
Total Contacts	18,564	16,220	17,224
No. of Contacts Complaining	4,282	3,047	3,527
% Contacts Complaining	23.1	18.8	20.5

TABLE 25

Types of Complaints with Amodiaquine + Primaquine and with Pyrimethamine + Primaquine, District 13, 1969-1970 and District 8, 1969, El Salvador.

		District #13		District #8
		1969 Amodiaquine + Primaquine	1970 Pyrimethamine + Primaquine	1969 Pyrimethamine + Primaquine
Complaints*				
Gastrointestinal:	No.	2,157	1,296	1,864
	%	11.6	8.0	10.8
Neurological:	No.	1,587	683	1,272
	%	8.6	4.2	7.4
Psychological:	No.	1,210	1,613	803
	%	6.5	9.9	4.7
Allergic:	No.	166	41	72
	%	0.9	0.3	0.4
Others:	No.	149	142	68
	%	0.8	0.9	0.4
Total Complaints		5,269	3,775	4,079
Total Contacts		18,564	16,220	17,224

* Complaint percents based on the total contacts.

TABLE 26

Specific Complaints with Amodiaquine + Primaquine and with Pyrimethamine + Primaquine, District 13, 1969-1970 and District 8, 1969, El Salvador.

		District #13		District #8
		1969 Amodiaquine + Primaquine	1970 Pyrimethamine + Primaquine	1969 Pyrimethamine + Primaquine
Complaints*				
Vomiting	No.	902	563	775
	%	4.9	3.5	4.5
Dizziness	No.	839	265	676
	%	4.5	1.6	3.9
Headache	No.	577	361	475
	%	3.1	2.2	2.8
Abdominal Pain	No.	339	147	324
	%	1.8	0.9	1.9
Anorexia	No.	324	261	261
	%	1.7	1.6	1.5
Diarrhea	No.	334	126	245
	%	1.8	0.8	1.4
Nausea	No.	161	124	143
	%	0.9	0.8	0.8
Total Contacts		18,564	16,220	17,224

* Complaint percents based on the total contacts.

Multiphasic Control in an Endemic Area

Organization

Based on information on District 13, Zone 2, El Salvador (Figure 6), obtained during the previous two years when the area was used both in a drug study and for continuing epidemiologic and entomologic surveillance, a study of a multiphasic approach to malaria control was begun in January 1971. Located on the Pacific Coast, the 100 square kilometer area contains a population of 5,900 in 1,350 dwellings. The area's six localities are characterized by large haciendas where cotton and cattle are raised. A variety of habitats are available for anopheline breeding, including rivers, estuaries, ditches, irrigated pastures, and areas flooded during the rainy season. The dry and wet seasons are clearly differentiated, with over 95 percent of the annual rainfall (average: 1,745 mm) falling between April and October.

The incidence of malaria is monitored in three ways: (1) blood smear surveys of the entire population at 8 week intervals, (2) Active Case Detection (ACD), or contacting the entire population every 2 weeks and taking blood smears from persons reporting current or recent fever, and (3) Passive Case Detection (PCD) through the medium of voluntary collaborator posts, where persons with malaria symptoms may go for diagnosis and medication. Because migrant workers move through the area during certain periods of the year, records are maintained on all persons contributing a blood film so that cases may be classified imported or indigenous. In order to make them more accessible to the population, additional voluntary collaborator posts were established in the area, bringing the total to 14. These posts are canvassed once weekly to collect blood slides and to replenish their supplies.

Persons visiting a voluntary collaborator post are treated with a single dose of amodiaquine (600 mg. for adults, and a corresponding dose in children, based on age and weight). All persons found to be infected, whether by ACD, PCD, or survey, are recontacted as soon as the diagnosis is made and given a course of amodiaquine (600 mg. single dose) and primaquine (15 mg. per day for 5 days). A five day course of primaquine is provided on the first day, along with instructions for daily dosing, but only the first dose is taken under medical supervision. Because of the time required for blood smear diagnosis, treatment usually begins one or two weeks after acquisition of the blood smear.

Entomologic surveillance proceeds by weekly larval collections made in 93 selected sites throughout the area and by evening human bait captures at 12 selected stations in the area.

From the start of the study to the commencement of the rainy season, the major breeding areas were larvicided by air. The larvicide Abate was sprayed at a target rate of 0.01 lbs. per acre of water surface. All known potential breeding areas were treated weekly, with application beginning as early in the morning as possible. Commercial crop-spraying aircraft were used; as they are not needed for agriculture during the dry season, they are available economically. Occasionally, small water collections not efficiently reached by the aerial method were larvicided by hand. Larvicide was first applied on January 27 and re-applied at weekly intervals through June 11. Deposition

of insecticide in target areas was routinely checked using dye-cards for recovery and enumeration of sprayed droplets.

Entomologic Evaluation

In general there appeared to be a well-defined larvicidal effect following the spraying. The adult populations of *A. albimanus* in District 13, as measured by diurnal bridge counts, were smaller during the period of aerial larviciding than in the previous two years (Table 27). Weekly human bait captures made at 12 points near major breeding places also indicated a marked reduction of adult *A. albimanus* during the months of aerial larviciding (Table 28).

TABLE 27

Adult *A. albimanus* by Diurnal Bridge Counts, Rio Huiza. El Salvador, 1969-1971.

Year	# Collections	Male	Female	Total	Average per Collection
1969	21	172	246	418	19.9
1970	18	87	164	251	13.9
1971	10	18	42	60	6.0

Epidemiologic Evaluation

In the first six months of 1971, 969 positive blood smears were taken in this area (pop. 5,900). Seventy-eight of the positives were duplicated in the multiple surveillance, yielding a corrected total of 891 cases for the 6-month period. This number is considerably higher than that seen in the first six months of the previous two years; in fact it is higher than total annual cases for each of the prior years, when the area was under mass drug distribution (Table 29). The well known efficiency of mass drug programs in reducing numbers of cases is borne out by these comparative data. What portion of the increase might be explained by the increased number of voluntary collaborator posts and the active case detection system cannot be estimated. The additional six posts established for the study yielded 44% of the 398 positives detected through the voluntary collaborator system. A comparison of the yield of the three surveillance systems in comparable periods of 1970 and 1971 is shown in Table 30. The increased yield of the active and passive case detection systems over general surveys is obvious. Taking three general surveys in 1971, as compared to two in 1970, obscured to some degree this increased yield. It seems apparent that a number of factors--enhancement of

TABLE 28

Average Adult *A. albimanus* Females
by Weekly Human Bait Capture, El Salvador, 1971.

Capture Point	January	February	March	April	May
1	2	8	0	2	8
2	0	0.3	1	0	16
3	9	5	3	2	3
4	13	4	1	0.7	4
5	0	0	0	0	0
6	20	3	1	0	0
9	4	1	0.3	0.3	0.5
11	9	5	3	0.5	1
12	6	4	0.3	0	0
13	0	0.3	0	0	0
14	12	4	4	0	0.2
15	20	1	1	0	0.3
Monthly Average All Points	7.9	3.0	1.2	0.46	2.8

TABLE 29

Malaria Positives by Month, District 13,
January 1969-June 1971.

Month	1969*	1970**	1971
January	5	12	24
February	8	36	102
March	18	60	158
April	15	33	225
May	23	18	120
June	50	17	340
July	111	45	
August	120	91	
September	144	187	
October	14	27	
November	17	18	
December	3	7	
Total Positives	528	551	969++
Attack Rate/1,000+	89.3	93.2	163.9
Total Slides	22,975	16,636	10,787
Slide Positivity Rate	2.3	3.3	9.0

* Mass biweekly distribution of amodiaquine + primaquine entire year.
** Mass biweekly distribution of pyrimethamine + primaquine March through December.

\+ Based on population estimated at 5,912.
++ 78 positives known to be repeats, due to multiple surveillance methods in operation.

TABLE 30

Production of Three Surveillance Systems: Active Case Detection (ACD), Passive Case Detection (PCD), and General Surveys. El Salvador, 1970 & 1971.

		Slides		Positives		SPR.
		Total	%	Total	%	
Jan-June 1970	PCD*	184	2.6	51	29.0	27.7
	ACD**	748	10.6	87	49.4	11.6
	Survey***	6,112	86.7	38	21.6	0.6
	Total	7,044	100.0	176	100.0	2.5
Jan-June 1971	PCD*	885	8.2	440	45.4	49.7
	ACD**	1,019	9.4	202	20.8	19.8
	Survey***	8,883	82.3	327	33.7	3.7
	Total	10,787	100.0	969	100.0	9.0

* Eight passive detection posts (voluntary collaborators) in 1970 and 14 in 1971.

** Active case search conducted in conjunction with fortnightly mass drug distribution in 1970, and without drug distribution in 1971.

*** Two general surveys in 1970; three in 1971.

the number of voluntary collaborator posts, enhancement of the efficiency of the active case search, the inclusion of an extra survey, and the inclusion of duplicate positive samples -- all may have served to increase the yield and percent of positive samples. However, in spite of these factors, the increase in the attack rate is real and of significance. Including all sampling, the attack rate per 1,000 per month, for the 6 months of January through June, averaged about 5.0 in 1970 and 27.3 in 1971. If the known duplication of positives (78) for 1971 is eliminated, the average monthly attack rate for 1971 is 25.1.

Of the total number of positive samples (969) only 8 were *Plasmodium falciparum;* all of the remainder were *P. vivax.* There was no significant difference in the proportion of positives in males or females, although, because of the difficulties of sampling the working male population, beginning at age 12 there is a significantly higher number of females in the sampled population. The greatest number of positives (54.3%) occurred in the 0 to 5 and 6 to 11 year age groups, each of these groups contributing about half of this number. Of the six localities in the area, two produced only 4.3 percent of the positive samples, while one other produced 42.7. The remainder were divided fairly evenly among the other three. Continuing transmission in the face of reduced anopheline populations is an interesting and important finding. Hopefully, entomologic surveillance in assessing the transmission potential in an area can be useful, but there are obviously obscure factors which may negate the quantitative relationships between vector densities and levels of transmission. These quantitative relationships will be closely assessed during the subsequent rainy season and during the use of other antimalaria measures in this area.

Operations

Cost is an important factor in the selection of an antimalaria measure. For this reason, very close attention has been given to the operational costs of the project, e.g., the use of aerial larviciding. Weekly aerial applications of larvicide were begun on January 27 and terminated on June 11, 1971. The spray mixture was composed of Abate applied at a target rate of 0.01 lbs/acre mixed with diesel oil. The finished product was applied at a target rate of 150-220 cc/acre. Twenty applications were made, using 30.3 gallons of Abate at a cost of $942.33 and 397.2 gallons of diesel oil at a cost of $87.39 (Table 31). A total of 44 hours were flown at a cost of $2,720, with an estimated 10,981 acres sprayed. The total cost of materials and aircraft was $3,749.72. For the 10,981 acres sprayed, this represents a cost per acre of $0.34. Based on an average population of 5,813 for 1970, the average cost per person for the five month period is $0.64.

In addition to the aerial spraying, small breeding sites were treated by hand application using Abate and AMSCO solvent. These were small isolated sites that would not have warranted the use of the aircraft, and such sites were treated during the time the field personnel were checking for larval densities. A total of slightly more than one gallon of the finished product was expended.

TABLE 31

Aerial Larviciding: District 13, El Salvador, January-June 1971.

Operation of Aircraft

No. of Weekly Applications	Est. Acres Treated		Hours Flying		Cost of Aircraft	
	Avg/Week	Total	Avg/Week	Total	Avg/Week	Total
20	549.1	10,981	2.2	44	$136.00	$2,720.00

Materials Used

ABATE				DIESEL OIL			
Gal. Used		Cost*		Gallons Used		Cost*	
Avg/Week	Total Used	Avg Cost/ Week	Total Cost	Avg/Week	Total Used	Avg Cost/ Week	Total Cost
1.5	30.3	$47.17	$942.33	19.9	397.2	$4.37	$87.39

Total Cost $3,749.72
Cost per acre 0.34
Cost per person 0.64

Average Cost/Week - $187.47

* at $31.10/gal.
** at $ 0.22/gal.

Rainy Season Control Methods

After the onset of the rainy season near the middle of June, aerial larviciding was no longer feasible because of expanding water collections. At this time, because of the moderately large number of malaria cases, it was decided to proceed with a single cycle of drug distribution through the entire district. This was accomplished during the last week in June, using the amodiaquine-OH preparation (Basoquin) at a single dose of 600 mg. for adults. Although no accurate records were kept on the number of absentees or refusers failing to receive the drug (no return visits were made after the initial household contact) the acceptance rate was estimated to be around 60 percent.

One of the factors involved in the ecology of malaria in this district is the heavy use of agricultural insecticides for the cotton crop, beginning in mid-July and reaching significant proportions in early August. At this time vector densities and malaria transmission have in the past declined to very low levels. During the present study guarded reliance will be placed on this factor for the exertion of desired levels of control during the August to September period. However, the necessity to bridge the gap between June and August dictated the use of additional measures. Therefore, in addition to the lowering of the parasite reservoir with the cycle of drug distribution, a single cycle of house spraying with propoxur (OMS-33) was planned. This house spraying cycle, applying the insecticide to the exterior walls of the houses, was done during the first week in July and is described elsewhere in this report (Vector Control Studies: Residual Insecticides). The routine entomologic and epidemiologic evaluations are continuing and will provide an assessment of the efficacy of these methods. Based on the information available at the time, additional methods including additional drug cycles, further insecticide use, etc., will be available for maintaining an acceptable level of control.

Relationship of Population Attitudes and Behavior

An additional aspect of this study is the assessment of public response to the variable methods used. The sequential introduction of multiple methods, e.g., larviciding, mass drug distribution, and house spraying, may have advantages and disadvantages over the repetitive single method programs to which the population has become accustomed in the past. For each method there are human responses which must be considered, to determine the extent of population acceptance and participation. For example, in the area in question, dry season accumulated water available for various needs of humans and domestic animals is at a minimum. Any threat to the usefulness of the water supplies through the use of an objectionable or dangerous larvicide is unacceptable. On the other hand, previous experience has shown that early in the course of a mass drug distribution program, i.e., the first few cycles, the population acceptance is relatively high, giving promise that a single cycle would be well accepted. It is important to know whether this does in fact hold true or if there are unforeseen factors in a single cycle program which would negate this acceptance, and if the introduction of promotional materials might even enhance the acceptability of a single cycle.

Prior to the initiation of aerial spraying no effort was made to conceal the nature and purpose of the procedure; neither was any made to inform the population about it. Precautionary instructions were not needed, since the larvicide, at the dosage used, was non-toxic for humans, domestic and wild animals including fish, and for aquatic insects other than Anopheles larvae. Since residents of the area were accustomed to intensive aerial spraying of cotton fields, it was not anticipated that there would be an adverse reaction to the comparatively limited spraying required to cover *A. albimanus* breeding places. Nevertheless, the spraying was being done along rivers heavily used by the population for multiple purposes, and it could not be taken for granted that the benignity of the insecticide and the familiarity of the populace with crop spraying would guarantee acceptance. After three weeks of spraying a small initial survey was conducted in the area to sound out the population on the use of the method. The survey was purposely made informally and conversationally, but was preplanned to gain information on several specific points. In essence the interviews showed that about 2/3 of the sample were aware that the spraying was being done and almost all of these connected the spraying to mosquito control, having gotten this information from malaria program personnel working in the area or from neighbors who had been so informed. There was no evidence of adverse reaction to the spraying program, with the exception of one respondent who complained that the aircraft had not passed over his house to alleviate his personal mosquito problem. Additional questioning concerning population knowledge of the malaria problem and of the measures being exerted to combat it provided background information which will be useful in future studies in the area.

A second population study in the area concerned the utilization of the voluntary collaborator posts, one of the essential elements in malaria surveillance in this program. During active case detection visits, and visits made during the general survey, all persons reporting recent or current febrile illness were instructed to go to the voluntary collaborator post for presumptive treatment. A check of the records showed that only 7 of 86 persons later diagnosed as positive for malaria actually did go to the post for treatment. Of this group 63 were interviewed, 57 who had not gone to the V.C. post and 6 who had. From these interviews it was determined that failure to seek treatment was not because of absence of symptoms: 55 of the respondants reported such symptoms, usually at the same time or relatively close to the day the slide was made and instructions received. Reasons for not visiting the post varied considerably (Table 32). Further information was also elicited from this group on their knowledge of the cause of malaria, which indicated that about 50 percent connected it with the mosquitoes, but less than 20 percent of this number were aware that the mosquito had to derive its infection from another human. Further studies of means to enhance public use of the voluntary collaborator posts are planned, including an evaluation of the use of "referral slips."

TABLE 32

Reasons Given by 57 Respondents For Not Going to a Voluntary Collaborator Post. El Salvador, 1970.

Reason	No Responses
Location of V.C. post not known	14
Fear of side effects from medication	12
V.C. post too far	7
Did not think symptoms caused by malaria	5
Because somebody came by to take blood	5
Purchased medicine	4
Was given slip of paper to take to V.C. did not want to go#	2
Never had symptoms	2
Didn't remember about V.C. post	1
Did not find V.C. at post	1
V.C. post out of medicine	1
Lack of time	1
Didn't like V.C.	1
Too sick to go	1
Away from locality	1
Never uses V.C.	1
Sick for only a short time	1
Took child but V.C. said to bring him back when he had fever	1
Total	61*

* Reasons exceed number of persons because 4 respondents gave two answers.

\# Some of those with symptoms were given a hand written reminder by program personnel.

STUDIES OF POPULATION ATTITUDES AND BEHAVIOR

Human behavior, an essential factor in the epidemiology of malaria, has suffered considerable neglect in the recent past. Always important influences, population attitudes and behavior have sometimes been decisive in the success or failure of disease prevention measures. Malaria is no exception in this regard; human behavior has a profound effect on patterns of malaria transmission and can be a controlling factor in the success or failure of antimalaria measures. There is scarcely a single area of malaria eradication methodology, either in common use or under study, where success does not depend to a degree on the attitudes and behavior of the individuals or groups directly affected. In Central America there have been specific causes of program failure identifiably related to the people's lack of compliance with antimalaria measures.

For these reasons, in every CAMRS program involving technical antimalaria methodology there has been an effort to identify and evaluate factors which may influence behavioral response to the measures, and to identify and test appropriate educational methodology for combatting attitudes and behavior which may adversely affect acceptance or compliance. Examples in which these studies have been highly important include: (1) the acceptance of periodic mass distribution of chemotherapeutic agents; (2) the acceptability of water management programs in relation to population use of available water; (3) acceptance of larviciding in water collections used intensively for human needs; (4) relative acceptability of exterior vs. interior house spraying; (5) public utilization of epidemiologic surveillance resources, particularly the voluntary collaborator network; (6) acceptance of area treatment with aerial or ground-based ULV; and (7) response to the introduction of integrated control measures. Supportive studies, generally brought out in discussions of the individual projects, have been done in many of the CAMRS control activities and demonstrations during the year, including aerial larviciding, limited cycles of antimalaria drugs, the exterior use of residual insecticides, source reduction by water management, and utilization of surveillance systems as a control method. These studies will provide an important source of guidelines for consideration of the behavioral factors important to malaria programs.

As an example of the utilization of accumulated experience, during the year CAMRS was offered the opportunity to assist in preplanning and evaluation of an educational program essential to the success of a proposed Department-wide program of house spraying with the new insecticide, propoxur (OMS-33), in El Salvador. Of great concern to the CNAP and PAHO staff involved in this planning was the possibility that there would be an adverse public reaction to the spraying, particularly if people did in fact have transitory discomfort because of failure to follow instructions about removing themselves and animals from the premises during spraying and for a short time thereafter. Wildly exaggerated rumors on side effects had previously come to the attention of program staff in another large area where strip spraying of interior walls with the same insecticide was being tested.

Through conferences with a Health Education auxiliary of the El Salvador program (CNAP), the CAMRS Health Education Specialist provided information

and suggestions, based on her previous studies, as to the probable population characteristics involved, the appropriate target for educational effort (head of household), and the level of knowledge already possessed on malaria and malaria control procedures. Person-to-person communication, as opposed to a mass media approach, was strongly recommended as the most effective means of bringing about changes in attitudes, and of developing a clear understanding of concepts and procedures associated with a given health activity. It was considered essential to explore ways to convey educational messages through interpersonal communications and to build in safeguards against inaccuracies in message content. Since spraymen are the only program personnel who normally have routine contacts with individual households in spray programs, the possibility of their serving as "educators" was discussed. Subsequently CAMRS staff worked with the CNAP Director and other key personnel to develop and implement a plan for training spraymen for an educational role.

After the beginning of the spray program, CAMRS staff conducted limited field interviews with householders to attempt to evaluate population acceptance. At that time it appeared that the householders had been adequately briefed on the purposes of the spray program and the necessary precautions, and seemed exceptionally well-informed. Operational personnel reported no cases of refusal to allow house spraying, in contrast to the usual course of events. Studies will be done in similar programs in an effort to further refine practicable methods, available within the structure of operational groups, for conveying essential information to populations.

EPIDEMIOLOGY OF MALARIA

As in the past most of the CAMRS epidemiologic investigations have been associated with other studies, particularly those involving the mass distribution of chemotherapeutic agents and the demonstration of a multiphasic control program. Such studies are described elsewhere in this report. As part of these studies it has been possible to evaluate multiple operational surveillance methods, including passive case detection (voluntary collaborators), periodic active case search, and general population surveys. Investigation of cases has been done where practicable to determine origin of cases and possible sources of transmission. An effort has been made to evaluate the role of population migration, a constant problem in the Central American countries, in the definition of malaria problems as well as in the evaluation of eradication programs and methods.

Studies on Surveillance Methodology

With the changing strategy of malaria eradication programs and the probable return to more limited control programs in many malarious areas, it becomes more important for epidemiologic surveillance methods to be useful and efficient. In the mass spraying and drug programs of the past, full coverage of populations was assumed and rapid detection of trouble areas less important. Where full coverage with any method is no longer practicable or effective it is more urgent to have mechanisms which will provide accurate and timely information on which to base the application of appropriate antimalaria measures. Through all of the CAMRS projects where the several surveillance methods are being utilized there has been an effort to catalog their advantages and deficiencies and to evaluate their comparative usefulness. The newer serological methods have been introduced and are being utilized, both for their intrinsic value in providing otherwise unobtainable epidemiologic information and also for evaluation of the traditional parasitologic methods for determination of levels of endemicity and transmission. During the year, a study of passive detection posts manned by lay voluntary collaborators was initiated to define the limitations and potential of the voluntary collaborator networks. After determining capabilities and factors which contribute to or militate against maximum effectiveness, efforts will be made to design measures for improvement of effectiveness. As a first step toward the definition of the networks a visit to Costa Rica resulted in valuable information for that country on the origin of the voluntary collaborator network, on steps in its subsequent development, on its role in the surveillance of malaria, and factors which may have a positive or negative influence on its efficacy.

A few preliminary points of interest can be reported from the Costa Rica study. Interviews with the malaria program (SNEM) staff were most productive, and identified as major problems:

1. Maintaining the interest and competence of the voluntary collaborators when they have few or no visits from persons with symptoms for long periods.

2. Maintaining public alertness to the continued threat of malaria in spite of the decreased incidence of cases.

3. Need to reorient supervision of the volunteers to correct the first two problems.

Sero-epidemiologic Investigations

Serology as an epidemiologic tool in field malaria evaluations has received increasing attention during the past five years. As an indicator of the incidence of malaria, a reliable and rapid serologic test for malaria antibody would have many advantages over the conventional examining of thick blood films. More information can be acquired in one serologic survey than in many repeated routine parasitologic examinations, particularly in areas of unstable or sporadic malaria or where transmission is markedly seasonal.

CAMRS activities in this field involve investigations designed to expand the application of sero-epidemiology into current malaria problem areas, and the interpretation of results in correlation with other epidemiologic data. In addition, a limited but growing amount of activity is in response to specific requests from national malaria programs in the region for assistance in problem areas.

The indirect fluorescent antibody technique (IFA) has now been proven in field studies in Asia and Africa as well as the Western Hemisphere. CAMRS, in cooperation with the Laboratory of Parasitic Diseases (NIH) in Chamblee, Georgia, is using this technique in all of its field sero-epidemiology studies. Specimens for serologic analysis are taken from a finger prick and one capillary tube of blood is placed onto a pre-marked area on a strip of filter paper. These specimens are allowed to dry and then stored at -20°C until they can be sent to the Georgia Laboratory for analysis. A thick blood film from each subject is prepared at the same time as the serologic specimen, both from a single finger prick, making the field collection of specimens a relatively simple matter.

Longitudinal Study - Guayabo, El Salvador

The Guayabo area of El Salvador is located in the interior of the country near the artificial lake formed by the dam of a major hydroelectric project. This is an area with consistent but low-level malaria transmission. The antimalaria measures consist of the use of residual DDT two times each year in a total house coverage program. There is also a system of voluntary collaborators in the area.

Nine localities were selected for an investigation designed to test, on a longitudinal basis, the effectiveness of the IFA technique as an epidemiologic tool. The Guayabo area was considered to be appropriate for such a study since low incidence provided a greater challenge in epidemiologic evaluations and the investigators could remain constantly aware of the actual malaria situation through regular and frequent visits to the voluntary collaborators. In addition, its malaria incidence is probably representative of a large part of Central America and information gained here would thus be applicable in other parts of the region. Surveys were designed to be conducted every six months.

The initial surveys in seven of the nine localities yielded a parasite prevalence which was quite low--less than one percent (Table 33). At the same time, the number of positive IFA responses was higher, 3.6%, but not markedly so. These results are considered to be representative of areas where transmission is at a consistent but low level. The number of active cases per year is small and the number of people who demonstrate, serologically, a recent experience with malaria remains essentially unchanged; additions to this group are more or less balanced by people whose antibody has dropped to an undetectable level due to an absence of challenge. In areas of higher endemicity, the difference between parasitologic positives and IFA positives is greater (compare the parasitologic and IFA results for San Diego in Table 37). The age-accumulated experience with malaria as demonstrated by the number of positive serologic responses can be seen in this group but not to the same degree as that seen in areas with more intense transmission. Only 10 percent of the people 30 years of age or over had measurable IFA responses to malaria in the Guayabo area. In contrast 69 percent of the same age group near Estero San Diego on the coast of El Salvador, where the amount of malaria is greater had IFA titers of 1:20 or higher. The lower degree of serologically demonstrated age-accumulated experience is another part of the epidemiologic picture of low level malaria transmission.

TABLE 33

Parasitologic and Serologic Results by Age and Sex from Seven Localities, Guayabo Area of El Salvador, 1971.

Age in Yrs	Parasitology				Serology (IFA)			
	Examined		Positive		Examined		Positive	
	M	F	M	F	M	F	M	F
≤ 4	28	36	0	1	12	16	0	1
5 - 14	121	140	1	1	96	111	1	2
15 - 29	87	71	0	0	75	65	3	1
30 - 49	36	38	1	0	30	33	2	3
≥ 50	11	25	0	0	7	22	1	3
All	283	310	2 (0.7%)	2 (0.6%)	220	247	7 (3.1%)	10 (4.0%)

Generally, there was little variation from locality to locality in the study area. The parasitologic and serologic responses of the nine localities are summarized in Table 34. In some areas where populations are small, the percentage of people with positive IFA responses appears high. However, when the sample size is considered, these differences are probably not significant.

The village of Santa Rosa la Maraña (and, though the sample is very small, El Dique) does seem to have more malaria than its neighbors. In Santa Rosa la Maraña both the parasitologic and the IFA responses are higher than those seen in the summary for contiguous areas (Table 33): 11.2% of the population had IFA responses greater than 1:20 while 2.4% had demonstrable parasitemias at the time of the initial survey. It is interesting that the only active case of *Plasmodium falciparum* encountered in this area was in Santa Rosa la Maraña. There are no readily apparent ecologic differences between El Dique and Santa Rosa la Maraña, and other localities in the study area. Potential breeding sites are equally present in all of the localities studied.

The explanation for the difference in malaria incidence may lie in the availability of health services. The voluntary collaborator post in Santa Rosa la Maraña was, until recently, located some distance from the main population area; the people denied that there was a V.C. post in the village. In El Dique, a settlement consisting of a small group of houses belonging primarily to one family, there is no voluntary collaborator; fever cases must walk a distance of several kilometers to reach an area where they can be treated and examined. The ineffectiveness of the V.C. system in these two areas may be one of the factors responsible for their higher malaria incidence.

TABLE 34

Parasitologic and Serologic Results from Nine Localities, Guayabo Area of El Salvador, 1971.

Locality	Parasitology		Serology (IFA)	
	Examined	Positive	Examined	Positive
San Benito	59	1	41	0
San Antonio la Cruz	56	0	13	1(7.7%)
La Trinidad	180	1	155	4(2.6%)
Nombre de Jesus	88	0	86	0
El Dique	37	2	18	2(11.1%)
San Nicolás	73	0	57	4(7.0%)
Cuartel de la Guardia	97	1	97	6(6.2%)
San José Potrerillos	108	0	108	1(0.9%)
Santa Rosa la Maraña	125	3	116	13(11.2%)

Comparison of Areas Above and Below 300 Meters Elevation

It has been thought for many years that malaria transmission in El Salvador was greatly reduced in areas above 300 meters elevation, but the reasons were and are obscure. The situation was considered to be well suited to sero-epidemiologic investigations. The Guayabo study area is below 300 meters. Three nearby villages above 300 meters were selected for comparative studies. The results of these investigations are presented in Table 35. No active parasitemias were found in the localities above 300 meters and 1.9% of this population had positive IFA responses. Both of these figures are lower than those seen in the Guayabo area, which is below 300 meters. However, other aspects of this study are probably more significant. It is apparent that the positive serologic responses occur more frequently in males than females in the high altitude areas. None of these responses were found in people below 20 years of age. All but one of the positive responses had a maximum titer against *P. falciparum*. (The remaining maximum IFA titer was against *P. malariae)*. We cannot explain the preponderance of *P. falciparum* responses since *P. vivax* is the dominant malaria parasite in all parts of the country. The results presented in Table 35 also establish that there is little or no transmission in this area, since no serologic responses were seen in people under 20 years of age. *Anopheles albimanus* is found in the area but in low numbers. At the present time, it is not possible to say whether the absence of transmission in these areas above 300 meters is due to a reduced mosquito population or to the presence of a strain of mosquitoes with one or more physiologic features not compatible with the transmission of malaria.

TABLE 35

Serologic (IFA) Results by Age and Sex in Three Localities above 300 Meters Guayabo Area of El Salvador, 1971.*

Age In Yrs	IFA Examinations: Examined M	Examined F	Positive M	Positive F
≤ 4	8	11	0	0
5 - 14	81	118	0	0
15 - 29	19	40	2	0
30 - 49	25	35	0	1
≥ 50	15	23	3	1
All	148	227	5 (3.4%)	2 (0.9%)

* Parasitologic examinations on all persons examined serologically were negative.

Study of Local Epidemic of *P. falciparum*

In January 1971 a small localized outbreak of *P. falciparum* malaria occurred in Jocomontique (elevation, 180m; population, 75) in the hilly interior of El Salvador in the northernmost part of the Department of Usulután. While initially there was concern that the parasites from this area were not normally sensitive to chloroquine and other 4-amino-quinolines, it was eventually determined that they were. The parasitologic and serologic investigations carried out in the locality primarily involved in the outbreak are presented in Table 36. While at this time the PCD data did not reflect a rise in cases, the level of parasite-positive specimens was considerably higher than for other interior parts of El Salvador, with 11 percent of the sample having demonstrable parasitemias. All infections encountered were identified as *P. falciparum*, though *P. vivax* is usually the dominant malaria parasite in this country. Serologic studies, which measure malaria experience, resulted in a still higher estimate of the portion of the population who had experienced malaria. Thirty-seven percent of the sample had positive IFA's, showing evidence of recent experience with malaria, and most of these (75 percent of serologic positives and 27.7 percent of the sample) reacted to *P. falciparum*. This study suggests that serology may be an effective tool for defining, in terms of geography and impact on the population, local outbreaks of malaria.

TABLE 36

Parasitologic and Serologic (IFA) Results by Age and Sex.
Local Outbreak of *P. falciparum*, Jocomontique, El Salvador, 1971.

Age In yrs	No. Examined		Parasite Positive		IFA Positive	
	M	F	M	F	M	F
≤ 4	3	5	0	1	0	2
5 - 14	12	8	1	1	4	3
15 - 29	3	11	0	1	0	5
30 - 49	0	8	-	2	-	4
≥ 50	1	3	0	0	1	1
All	19	35	1	5	5 (26.3%)	15 (42.9%)

Malariometric Studies - Estero San Diego

Estero breeding of *Anopheles albimanus* during the dry season apparently contributes significantly to the malaria problem in coastal sections of El Salvador. A program has been proposed for dry season drainage of this estero with concomitant introduction of sea water in order to reduce the malaria vector breeding area, providing opportunity to apply sero-epidemiologic techniques to the evaluation of a specific antimalarial measure. Surveys will be carried out at approximately six-month intervals in this area and we will be particularly interested in evaluating the impact of opening the estero on the malariometric picture in the area.

The results of the initial parasitologic and serologic investigations in this area are presented in Table 37. The malaria incidence is higher here than in the Guayabo area, with IFA responses typical of areas with moderately low but consistent malaria transmission. As can be seen in Table 37, the accumulation of malaria experience with age as measured by IFA is marked, indicating that challenges remain frequent enough to maintain antibody titers in the older segments of the population. More opportunities to apply sero-epidemiology in the evaluation of antimalaria measures will be sought in the future.

TABLE 37

Parasitologic and Serologic Results by Age,
Estero San Diego Area of El Salvador, 1971.

Age in Yrs	Parasitologic Examinations			Serologic Examinations (IFA)		
	Examined	Positive		Examined	Positive	
		No.	%		No.	%
≤ 4	10	0	-	10	1	10.0
5 - 14	153	9	5.9	151	21	13.9
15 - 29	53	0	-	53	13	24.5
30 - 49	37	1	2.7	37	23	62.2
≥ 50	22	0	-	22	18	81.8
All	275	10	3.6	273	76	27.8

Sero-epidemiologic Profile in Tipitapa, Nicaragua

Malariometric studies were carried out in Tipitapa, Nicaragua, where entomological studies, including host preference investigations are planned. Tipitapa is a small village located on the shores of Lake Managua in an area where larviciding may be an effective antimalaria measure. A school survey was carried out and the parasitologic and serologic results of this investigation are presented in Table 38. More than 6 percent of the people between 5 and 15 years of age had peripheral parasitemias at the time of the survey.

Most of these parasitemias were quite intense and probably represent initial infections. It is immediately apparent that there is little difference between the percentage of this population that is parasite positive and the percent with positive serologic responses. This difference between parasitologic and serologic responses is even less when the two positive IFA titers in the teachers are not considered. It would seem that malaria transmission in this area is sporadic. Both *P. falciparum* and *P. vivax* are present but there has been a recent period of *P. vivax* transmission, since most of the school children who had positive serologic responses also had *P. vivax* parasitemias. It is difficult to predict what a total population study would reveal; however, it is probable that the percentage of the population with positive parasitemias would remain about the same while the percent with significant IFA titers would be expected to increase. Further studies in this area are planned in order to confirm these impressions. One of the goals of these varied studies is to find target populations that will serve as representatives of an entire area. Therefore, it is essential to gain experience with the sero-epidemiologic tool in a variety of endemic situations.

TABLE 38

Parasitologic and Serologic (IFA) Results by Age and Sex.
School Survey, Tipitapa, Nicaragua, 1971.

Age In Yrs	No. Examined		Parasite Positive		IFA Positive	
	M	F	M	F	M	F
5 - 9	18	20	2	0	1	0
10 - 14	13	23	0	3	2	4
15 - 19	0	3	-	0	-	0
≥ 20*	0	2	-	0	-	2
All	31	48	2 (6.5%)	3 (6.3%)	3 (9.7%)	6 (12.5%)

* Two teachers aged approximately 40 and 50.

Serologic Responses to Malaria and Chagas Disease

The influence of related antigens on any serologic technique is always of concern in field-collected specimens. The only other haemaprotozoans found in man in the Central American area are the leishmanias and the trypanosomes. Dr. Draper of the London School of Hygiene and Tropical Medicine is currently working with the IFA technique in trypanosomiasis. In order to begin to obtain information on possible antigenic relationships between the two parasites, a series of dual specimens were collected and IFA titers for the protozoan parasites determined. The results of these investigations are presented in Table 39. Cinquera, Jutiapa and Tejutepeque are mountain villages (above 300 meters) in the Department of Cabañas in El

Salvador. Chanmico is located outside San Salvador near a small lake. As can be seen, the malaria incidence is low in all of these areas. So little information is currently available on the actual distribution of Chagas disease in El Salvador that it is impossible to say whether the number of positive responses to *Trypanosoma cruzi* is high or low. These results are preliminary and as the opportunity presents itself other areas with different types of malaria endemicity will be studied in the same fashion.

TABLE 39

Serologic Results (IFA) for Malaria and Chagas Disease from Four Localities, El Salvador, 1971.*

	Malaria Pos./Examined		Chagas Disease Pos.**/Examined	
	Male	Female	Male	Female
Cinquera	3/64	2/87	7(7)/64	11(12)/88
Jutiapa	0/21	1/42	1(1)/21	3(2)/44
Tejutepeque	0/48	0/73	5(2)/48	4(5)/73
Chanmico School	0/80	0/85	1(13)/80	6(9)/85
Total	3/213	3/287	14(23)/213	24(28)/290

* Parasitologic examinations on all persons examined serologically were negative.

** Numbers in parentheses indicate doubtful positive responses for Chagas antibody.

Sero-epidemiology of Malaria in the Mato Grosso Area of Brazil

The sero-epidemiologic study in the Cuiabá Sector of Mato Grosso, Brazil, has been continued with second and third parasitologic-serologic surveys in October 1970 and February 1971. The Annual Report of CAMRS for 1970 contained full geographic and demographic descriptions of the Cuiabá Sector, and reviewed the malaria history of that area. The initial survey included in that Report revealed a very low incidence of malaria in the larger Campo Grande Sector in the southern part of Mato Grosso, dictated the cessation of planning for the proposed insecticide tests, and also resulted in the elimination of Campo Grande from further serologic studies. Surveillance data collected in both Sectors during the calendar year 1970 has been collected and tabulated.

The continuing parasitologic and serologic* (IFA) surveys in Cuiabá have sampled the same localities included in the first survey, and the results

* The IFA testing of blood samples is being done by Dr. William E. Collins, LPD/NIAID/NIH, Chamblee, Ga.

now begin to clarify a number of questions on the epidemiology of malaria in this region. A summary of the three surveys conducted to date in Cuiabá is provided in Table 40. Several points of interest can be noted from this summary. There was a slight increase in parasite positivity during the period studied, from 0.8% in April 1970, to 1.2% in September 1970, to 2.3% in February 1971. Whether this can be considered a significant increase, representing an upsurge of malaria in the area is in doubt, and will have to await further surveys to determine if the increase is real or merely seasonal. Of great interest is the shift of parasite species in the three surveys, with *P. vivax* predominating in April, a shift to *P. falciparum* in October, and a return to lesser predominance of *P. vivax* in February. This parasite shift may well reflect seasonal differences in transmission patterns, with recently acquired *P. falciparum* predominating in the high transmission season, and the *P. vivax*, with its patterns of chronicity and relapse, predominating at other times. That *P. falciparum* is the dominant parasite in the area is confirmed by the continuing higher number of maximum IFA titers for this species. In Table 41 the parasitologic and IFA serologic results of the February 1971 survey are presented for the 17 localities included. It can be seen that 137 of the 156 (88 percent) serologic positives (IFA) had maximum titers to *P. falciparum* (9 of these had equal maximums to *P. vivax*). This compares with a finding of 81 percent with maximum *P. falciparum* titers in the April 1970 survey, and 91 percent in the October survey. Localized concentrations of parasite positives (Table 41) continue to support the impressions gained through the longitudinal study that in this area malaria is characterized by sporadic, localized seasonal outbreaks. In general the IFA serological results support this conclusion.

One spurious figure appears in the IFA results for the October 1970 survey: the increase in the total positive GMRT (geometric mean of reciprocal titers) as indicated in Table 40. The rise of this figure to 125.72 during this survey, when considered at face value, would suggest a significant rise in antibody levels and malaria experience in the area. This would be puzzling in view of the reduced proportion of IFA positives and the only very slight rise in parasite positives. The explanation for this appears to be a simple one, although it diminishes to some extent the usefulness of the second survey in the longitudinal study. The technique for collection of serologic samples contained in the study protocol specifies the use of standard amounts of blood after finger puncture in a microhematocrit tube, the full amount of the tube to be expressed onto filter paper. Elution of the entire blood spot for testing would then result in a standard and consistent concentration in the eluate. During the second survey (October, 1970) there was a deviation from this technique, hematocrit tubes were not used, and blood was expressed directly onto a filter paper within a previously delineated circle. Careful estimates made after the fact, supported by actual trials, indicate that an average of 0.14 ml of blood is collected by the "soaking" method, while only 0.07 ml is collected through use of the microhematocrit tubes. Obviously, the IFA test was being run using about twice the usual volume of blood, which understandably resulted in a significant increase in the titer endpoints. However, it is considered that the higher GMRT is an anomaly and does not represent a significant increase in the antibody levels of the population.

TABLE 40

Parasitologic and Serologic Results Cuiabá Sector
Mato Grosso, Brazil, April 1970-Feb. 1971.

Survey No.	Date	Parasitologic Examinations					Serologic Examinations (IFA)			
			Positive					Positive (≥1:20)		
		Exam.	No.	%	*P.v.*	*P.f*	Exam.	No.	%	G.M.R.T.* of Pos.
I	Apr. 1970	957	8	0.8	7**	2**	920	110	12.0	69.64
II	Oct. 1970	767	9	1.2	1	8	741	69	9.3	125.72
III	Feb. 1971	1,134	26	2.3	15**	12**	1,124	156	13.8	69.52

* Geometric mean reciprocal titer
** 1 mixed infection

TABLE 41

Parasitologic and Serologic Results of a Survey of Seventeen Localities in
Cuiabá Sector, Mato Grosso, Brazil, February 1971.

	Parasitologic Examinations					Serologic Examinations (IFA)					
		Positive					Positive		Maximum Titers		
	Exam.	No.	%	*P.f*	*P.v*	Exam.	No.	%	*P. falc.*	*P. vivax*	*P.f* = *P.v*
Tres Pedras	68	2	2.9	2	-	67	17	25.4	10	8	1
Fasendinha	27	0	-	-	-	27	0	-	-	-	-
Coxipo do Ouro	38	1	2.6	-	1	38	1	2.6	-	1	-
Sao Concalo	81	0	-	-	-	81	1	1.2	-	1	-
N.S. de Guia	132	3	2.3	-	3	131	7	5.3	2	5	-
Guarita	99	0	-	-	-	99	0	-	-	-	-
Barro do Bugres	250	12	4.8	7	5	246	36	14.6	35	5	4
Porto Estrela	82	1	1.2	-	1	82	11	13.4	11	1	1
Camargo Correro	100	4	4.0	-	4	98	16	16.3	10	8	2
Bau	11	2	18.2	2	1*	10	2	20.0	2	-	-
Jao Paulo	29	1	3.4	1	-	29	9	31.0	9	-	-
Pai Luis	43	0	-	-	-	42	17	40.5	17	0	0
Passagem Sociadade	38	0	-	-	-	38	3	7.9	2	1	-
Quintanda	22	0	-	-	-	22	0	-	-	-	-
Posto Indiyina	79	0	-	-	-	79	24	30.4	18	7	1
Fazenda Santana	14	0	-	-	-	14	2	14.3	2	-	-
Ouro Fino	21	0	-	-	-	21	10	47.6	10	-	-
Totals	1,134	26	2.3	12	15*	1,124	156	13.8	128	37	9

* 1 mixed infection

TABLE 42

Passive and Active Surveillance
Campo Grande and Cuiabá Sectors, Mato Grosso, Brazil, 1970.

	Cuiabá (8 Municipios)				Campo Grande (8 Municipios)			
		Positives				Positives		
	Slides	No.	%	*P. falc.*	Slides	No.	%	*P. falc.*
Passive	5262	1759	33.4	66.3	1180	54	4.6	27.8
Active**	4211	330	7.8	66.7	5488	43	0.8	48.8
Total	9473	2089	22.1	66.4	6668	97	1.5	37.1

** 22,614 houses were visited in Cuiabá and 12,513 in Campo Grande.

The surveillance information collected for both the Campo Grande and Cuiabá Sectors during 1970 is summarized in Table 42. Although the collection of samples through the active case detection mechanisms had been sporadic and somewhat disorganized during the year the results are of interest. There seems to be little doubt that malaria is not a severe problem in the Campo Grande Sector, at least in the areas covered by this surveillance. This observation confirms the conclusions reached after the first parasitological-serological survey in April 1970, where low parasite prevalence and serological positivity prevailed. On the other hand, the total of more than 2,000 parasite positives seen in the Cuiabá Sector (22 percent of the specimens collected) suggests an incidence at least as high as that which can be derived from our survey figures. In both Sectors, the passive case detection system produced the majority of slide positives, and confirms the usefulness of this method in surveillance when properly employed and carefully interpreted.

A comparison of the surveillance data for the five years, 1966-1970, is presented in Table 43. It should be noted that even though the sampling in 1970 was less than perfect, the number of specimens obtained was considerably enhanced, principally through the installation of an active case detection system for that year. This had not been done in previous years and may have served to depress the positivity rate to some extent. A total of 4,211 slides were collected through active search, with only 7.8 percent positive for malaria parasites (Table 42). Had the source of sampling included only the passive detection posts, as had been the case in the previous four years, the positivity rate would have been 33.4 percent, suggesting a slight increase over 1969 (31.4 percent), continuing a gradual progressive increase during the previous years.

TABLE 43

Surveillance Data in Study Areas,
Cuiaba and Campo Grande Sectors, Mato Grosso, Brazil, 1966-1970.

	Cuiabá			Campo Grande		
Year	Slides	% Pos.	% *P. fale.*	Slides	% Pos.	% *P. fale.*
1966	7,109	15.0	74.9	2,708	4.7	20.3
1967	7,603	15.5	80.3	3,995	5.3	6.2
1968	6,590	24.0	78.2	4,324	9.9	71.2
1969	4,289	31.4	67.8	2,367	8.7	58.7
1970	9,473	22.1	66.0	6,668	1.5	37.1

The positivity rate remained low in the Campo Grande Sector in 1970, but here again the inclusion of the active case detection system depressed the rate to some extent. Had passive case detection alone been used the rate would have been approximately 4.6 percent (Table 43), indicating a somewhat lower rate than seen the previous year, but similar to that seen

several years previously.

As had been seen in the past the proportion of *P. falciparum* positives remained high in the Cuiabá Sector, with 66.4 percent of the positives exhibiting this species. In contrast, the positives in Campo Grande were 37 percent *P. falciparum*. In Cuiabá equal proportions of positives obtained by passive and active methods were *P. falciparum*, while in Campo Grande the active case detection system revealed more *P. falciparum* positives than the passive (49 percent vs. 28 percent).

Results of the three surveys conducted in the Mato Grosso have been assembled and programmed for computer analysis. The results to date of this programming have provided tabulations which should simplify the analysis of the results and aid in estimates of epidemiologic patterns in this area and others with similar characteristics. The usefulness of continuing this longitudinal study for an additional period and of the extrapolation of the results for interpretations of single surveys of this type in other areas seems assured. Planning for the completion of the study includes two more surveys at 6-month intervals in FY-1972, and the inclusion, at the request of the Director of the Malaria Program in Brazil, of small, targeted single surveys in marginal areas of transmission within Brazil, utilizing the experience and information attained in the major study for the definition of epidemiologic parameters in the small areas.

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