Reprinted from American Journal of Tropical Medicine and Hygiene
Vol. 2, No. 4, July, 1953
Printed in U.S.A.

STUDY OF A RESIDUAL POPULATION OF *ANOPHELES L. LABRANCHIAE* FALLERONI IN THE GEREMEAS VALLEY, SARDINIA

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STUDY OF A RESIDUAL POPULATION OF *ANOPHELES L. LABRANCHIAE* FALLERONI IN THE GEREMEAS VALLEY, SARDINIA[1]

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The island of Sardinia in the Mediterranean Sea has been the site of a large-scale experiment to establish the feasibility of the eradication of an indigenous malaria vector, *Anopheles l. labranchiae* Falleroni. The progress of this project has been reported by Logan (1950) and is to be the subject of a full account edited by Logan (Monographic Series of the American Journal of Hygiene). One of us (T.H.G.A.) made a survey of the anophelines of the island during the summer of 1946 and was present throughout the entire eradication period. The full-scale eradication operation, a joint effort of the Italian Government in cooperation with UNRRA and later ECA, under the technical direction of The Rockefeller Foundation, was carried on from 1947 through 1950. Since 1950 the domestic insect control service of the Sardinian Regional Government has carried on the work as funds permitted.

While throughout most of its range *labranchiae* is a coastal marsh breeder, in Sardinia this mosquito was very generally distributed in the quiet water of streams even at considerable elevations. Thus, for a time during the eradication operation, all water surfaces on the island were treated as actual or potential *labranchiae* breeding places. Later, however, only the places where *labranchiae* was found were subjected to larvicide treatment. At the conclusion of the ERLAAS[3] campaign in 1950 only very few *labranchiae* could be found anywhere on the island. The efforts of the local government since that time have been directed at the elimination of the species from these few remaining foci.

As the eradication effort proceeded, a number of fundamental and perplexing problems emerged. With eradication within a limited time as the prime objective, it was not possible to investigate these problems, which were in many respects wholly new and without precedent, there having been no prior attempt to eradicate so well-established an indigenous mosquito in so large an area of such varied terrain. Of particular interest was the question of how the minute residual population of *labranchiae* would respond to the vacuum created by the eradication effort, once control measures were suspended or reduced. The writers spent much of the active mosquito-breeding season of 1952, from late June through the end of September, in an attempt to resolve certain of these problems.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the Division of Medicine and Public Health of The Rockefeller Foundation with the cooperation of the Italian Government.

[2] On leave of absence from the Gorgas Memorial Laboratory, Panama.

[3] "Ente Regionale per la Lotta Anti-Anofelica in Sardegna" was the designation of the organization which undertook the eradication of *Anopheles l. labranchiae* in Sardinia.

While the Sardinian regional health authorities were continuing to treat all foci of *labranchiae* which could be found, within the limitations of the funds available to them, an agreement was reached to permit the writers to study one focus from which all treatment would be withheld, at least during the 1952 season. The primary objectives of this study were the following:

1. The determination of the rate of increase of a *labranchiae* population in a suitable breeding place in the absence of treatment. One of the curious phenomena which greatly hampered the later work of ERLAAS was that in such *labranchiae* larval breeding places as were found, the larvae were in most cases present in only very small numbers. Factors influencing the finding of larvae under these conditions have in part been brought out by Trapido (1951). The small number of larvae in breeding places in which they formerly abounded indicated that there must be a large element of chance in finding larvae, despite conscientious sampling of all water surfaces. It had been assumed from the general knowledge of the high reproductive potential of mosquitoes and the rapid increase of the population possible in only a few generations, that breeding areas missed on one scouting cycle would be easily found on later scouting. The validity of this assumption was to be tested by carefully following known breeding places throughout the summer, in the absence of treatment.

2. Related to this first objective was the establishment of the rate of dispersal of the species by determining the rapidity with which larvae appeared in breeding places adjacent to the original focus.

3. Adult *labranchiae* had become more difficult to find than larvae as the eradication project had progressed. While this might be due to the persisting effectiveness of residual DDT treatments of all known resting places, it was desirable to find at what larval densities significant numbers of adults again appeared in human habitations and domestic animal shelters.

4. Soon after the ERLAAS plan of attack had been shifted from the treating of all water surfaces (except the water accumulations in tree holes, the breeding place of *Anopheles plumbeus*) to the larviciding of the water surfaces in the areas where *labranchiae* could be found, it became apparent that the other anophelines underwent a shift both in distribution and abundance. The question of how these changes in the balance of the anopheline fauna might affect *labranchiae* through possible competition was also to be considered.

Essential to the investigation of problems such as these was the selection of an area from which treatment could be withheld, but which had a small residual focus of *labranchiae*. Other necessary features of the proposed area of observation were these:

1. That the area be large enough to be representative of the general situation, but small enough to be readily kept under intensive surveillance.

2. That it be enclosed by a natural barrier to limit the spread of *labranchiae* if the population of the mosquito diffused rapidly.

3. That the area be conveniently located for treatment, if the regrowth of the *labranchiae* population was rapid and a malaria transmission hazard developed.

On June 21, 1952, regional scouts found larvae of *labranchiae* along a branch

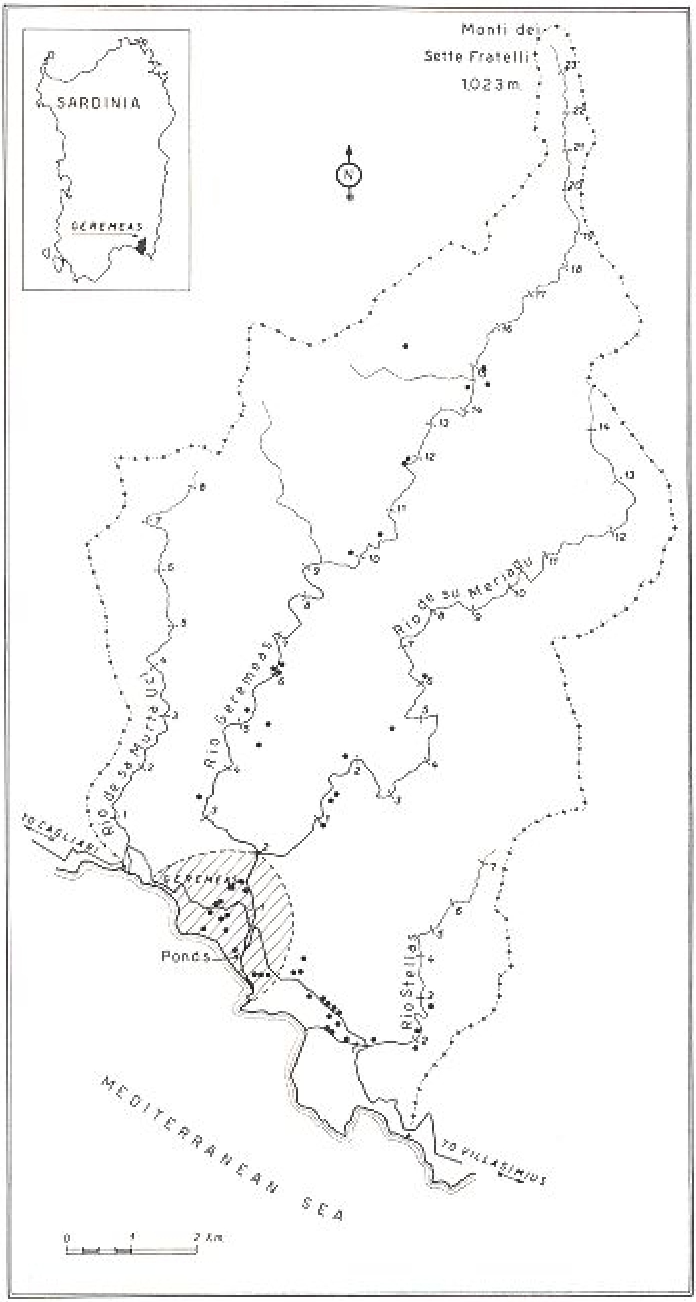

FIG. 1. Sketch map of the Geremeas area, Sardinia. The area of the concentrated studies by the writers is indicated by hatching; the black dots represent houses or shelters in which search for adult mosquitoes was made; the numerals along the rivers indicate distances in kilometers from each river mouth.

of the Rio Geremeas within 750 meters of the point where this river reaches the sea. On June 24, breeding places in the immediate vicinity received a treatment of fuel oil augmented with the spreading agent Triton X-100 (the larvicide did not contain DDT). Further treatment was suspended pending the decision of Professor G. Brotzu, "Assessore all'Igiene, Sanità e Pubblica Istruzione della Regione Autonoma Sarda," as to whether the valley of this river might be used as an observation area during the summer of 1952. Agreement to withhold treatment from the area was reached on July 12, and the active study of the *labranchiae* population was begun as soon as possible thereafter.

The experimental area is shown in the sketch map (see Figure 1). It included the valley of the Rio Geremeas and the tributary Rio Meriagu, together with the adjacent smaller valleys of the Rio sa Murta Uci and the Rio Stellas. It was limited by the ridge of the Serra Marapintau on the west, the Sette Fratelli massif to the north, the ridge of the Serra de Misa on the east, and the sea on the south. The ridges on the sides of the area rise rapidly from the sea to 700 and 800 meters, and in the north to the summit of the Monti dei Sette Fratelli with an elevation of 1,023 meters. The area encompassed was approximately 84 square kilometers.

The region, like practically all of Sardinia, has long ago been deforested. The steeper and less accessible slopes are covered with scrub vegetation, while the more gentle slopes are planted with winter wheat or set out with groves of almond trees. The upper reaches of the valley, where there is older second growth, are now being cut over and the wood burned for charcoal. The only considerable level area is within two kilometers of the sea, and is under cultivation with plantings of winter wheat, vineyards, and almond, citrus and peach trees. The peach orchards are irrigated during the dry summer months, but the irrigation is minimal and water does not stand long enough to support mosquito breeding (see Figure 2).

The bulk of the human population is concentrated in a group of farm buildings about 200 meters from the main river and 1.5 kilometers from the sea (see Figure 3). Here too are concentrated most of the domestic animals, a census of which is given in Table 1. For the rest there are only scattered small shelters, in a few cases of stone but for the most part of brush, in which shepherds and charcoal burners live. The total human population during the summer of the investigation was about 185 persons.

The water in the valley is all contained in the streams and their feeding springs, and several small ponds at the mouth of the main river near the sea. At flood times these ponds become part of the lower course of the stream, but throughout the summer months of the study they remained discrete beside the river bed. With the lack of forest cover on the valley slopes, the winter rains turn the water courses into torrents but during the dry summer months these are reduced to intermittency. The two kilometers of the stream nearest the sea were particularly affected by drying during the summer of the study, but stretches of water remained near the originally-found *labranchiae* foci, and the larger of

FIG. 2. View of the lower portion of the Geremeas valley from a point about four kilometers from the sea, showing the barren open nature of the country and the area near the river mouth under cultivation.

FIG. 3, View of the main group of farm buildings at Geremeas center, including the quarters of farm laborers, a sheep pen, cattle yard, horse stable and pigsty. The one adult *Anopheles l. labranchiae* taken during the study was in the pigsty here.

the ponds, in some cases as much as two meters deep, contained water throughout the summer.

The principal rainfall is during the fall, winter, and early spring months. The summers are hot and dry, with little or no rain. Meteorological data are not available from the Geremeas valley itself, but in Table 2 are given the data on rainfall, temperature and humidity from the Elmas airport, about 35 kilometers northwest of Geremeas. During the period of the study at Geremeas

TABLE 1

Domestic animal census, Geremeas center, Sardinia, August 1952

Cattle	13	Dogs	23
Sheep	370	Cats	22
Donkeys	2	Rabbits	7
Horses	1	Chickens & Chicks	116
Pigs	7	Ducks	26

TABLE 2

Meteorological data from Elmas airport, Sardinia, 1952

	MEAN TEMPERATURE (DEGREES CENTIGRADE)	MEAN RELATIVE HUMIDITY (%)	PRECIPITATION (MILLIMETERS)	DAYS OF RAIN
January	8.1	77	41.2	15
February	8.1	74	23.6	9
March	12.5	75	9.6	4
April	13.2	71	27.6	5
May	17.4	71	9.4	5
June	23.3	68	0.0	0
July	25.6	61	12.4	2
August	25.6	69	0.6	2
September	21.9	69	50.2	8
October	18.3	75	17.4	9
November	13.1	73	41.2	14
December	10.8	82	54.8	13
Total precipitation and rainy days			288.0	86

there was no significant precipitation except a heavy rainfall from September 14 to 17.

While the knowledge of the biology and habitats of the anophelines of Sardinia has been reviewed by Aitken (in Logan *et al.*, 1953) it will be necessary here briefly to describe the main features of the biology of the anophelines present in the valley.

At Geremeas we found *Anopheles hispaniola* to be the anopheline most intimately associated with *labranchiae*. The larvae are characteristic of clear sun-lit water with some freshening by surface movement or by seepage, and are most often associated with growths of the alga, *Spirogyra*. Larvae were not found in water rich in organic material from the decay of aquatic vegetation. The ERLAAS experience was that the species built up slowly during the summer, becoming most abundant in the last half of the summer and early fall. Unlike *labranchiae*, the adults are not found in houses or domestic animal shelters even in the presence of heavy larval concentrations in nearby breeding places. The adults are thus not subject to the attrition produced on anopheline species that rest in structures treated with DDT or other residual toxicants. The species has been taken in Sardinia from sea level to at least 800 meters, but was not commonly found before 1949.

Anopheles claviger is also a species found in clear fresh water, but is usually in shaded situations. It is also encountered under sun-lit conditions, but usually where the water is cool (ordinarily less than 20°C). Most collections of larvae in Sardinia were made in springs and the rivulets from springs. The species is most abundant in spring and fall, and exhibits a decline in numbers during the hot summer months. Adults are occasionally taken in man-made structures, but the bulk of those found by ERLAAS were resting in natural grottoes or in vegetation. It has been taken as high as 1,300 meters in Sardinia.

Anopheles marteri is a species of the cool shaded waters of mountain streams. It was most commonly associated with *claviger* and only very rarely with *labranchiae*. The few adults taken by ERLAAS were in grottoes or similar natural shelters along water courses.

Quite rare in the Gerenieas experimental area during 1952 was *Anopheles algeriensis*. Larvae are usually in still water which is partially shaded. It has been collected in brackish water. While not many adults were taken by ERLAAS, a few were found in man-made shelters.

At the beginning of the eradication effort in 1947, *Anopheles l. labranchiae* was the dominant, most widespread and most generalized anopheline in Sardinia. While it was only rarely found in brackish water, it was widely distributed in fresh-water situations up to 1,000 meters. In this the species differed in its larval habitats from the situations described on the Italian mainland where it is considered a coastal breeder, often in brackish water. Hackett (1949) has remarked on the fresh-water distribution of this mosquito in Corsica, Sardinia, Sicily and the Barbary Coast of North Africa and attributed it to the lack of fresh-water competitors. In Sardinia *labranchiae* was most often taken in sun-lit water, usually with some surface vegetation. The water might be clear, or could be rich in organic material, quiet as in ponds or swamps, or slowly moving as at the margins of streams. At first adults were common in pigsties, stables and houses, but these were all subject to residual treatments with DDT and later Octa-chlor, and adults became exceedingly rare in such situations. In addition to man-made structures they were taken in much lesser numbers in grottoes.

During the anopheline survey of Sardinia in 1946 *labranchiae* was found to be common in the Gerenieas valley. Collections of larvae were obtained at a number of places sampled along the course of the stream, and while the detailed records are not available, we know it occurred up to an elevation of several hundred meters and a dozen or more kilometers from the sea. The last *labranchiae* in the valley, prior to 1952, were found in 1949. None were found in the intensive

ERLAAS search during 1950, or the less concentrated scouting conducted by the Regional Government in 1951. The nearest positives during 1950 and 1951 were along the east coast in the vicinity of, and to the south of, San Priamo, an area separated from the Geremeas valley by several high, barren and dry mountain ridges. The year 1952 was thus the third one during which no larviciding was done in the valley. The last DDT residual spraying of houses in the valley by ERLAAS was in September 1949. Although no *labranchiae* had been found here, as part of a general precautionary program the provincial medical authorities had also sprayed houses with DDT on June 5, 1951.

In 1952 the Geremeas valley was under routine observation by scouts of the Regional Government during the months prior to its designation as an experimental area for our use in July. The search was primarily directed at the finding of larvae. Scouting started during the week beginning April 28. Only *claviger* was found consistently during this and subsequent weeks, but in addition there were a few collections of *marteri* in the Rio Bacu Mandara, a tributary of the Geremeas, and in mid-June some *algeriensis* were found in the ponds at the river mouth and in a few water holes in the river bed upstream. As a result of the discovery of *labranchiae* in the lower Rio Geremeas on June 21, the adjacent Rio sa Murta Uci was scouted the following week, but only *marteri* and *hispaniola* were found, and these in the lower part of the stream. At the time *labranchiae* was found on June 21, there was, interestingly enough, little else in the Rio Geremeas, possibly because the river was still running too fast as a result of the spring rains, and there had not yet been enough development of *Spirogyra* to provide stream margin shelter for larvae. It is noteworthy that up to this time in the Rio Geremeas and the tributary Rio Meriagu, *hispaniola*, which was later the dominant species, had not been taken at all. During the critical week when *labranchiae* was first found, the collections consisted of 15 larvae of *labranchiae*, 19 of *algeriensis* and 69 of *claviger*. No adult anophelines were taken during this period.

LARVAL STUDIES

Methods

In order to locate anopheline findings as closely as possible, the streams were marked off in the field into tracts 250 meters long and were given serial numbers. The number of tracts in each river was as follows:

Rio Geremeas	96 (24.0 km.)
Rio Meriagu	56 (14.0 km.)
Rio sa Murta Uci	33 (8.25 km.)
Rio Stellas	30 (7.5 km.)

The first scouts were employed on July 17, preliminary scouting began on July 18, and by the week of July 21 ten scouts were employed. It was subsequently found that this number of men was not sufficient for intensive coverage of the entire area and two additional scouts were added on July 31. The men, working in pairs, were assigned one kilometer of stream to scout each day.

These men had all had previous experience in hunting anopheline larvae and quickly became familiar with the terrain. The crews were rotated so that each pair of men had a different stretch of stream to cover each week. The attempt was made to search the most important parts of the main streams (the Rio Geremeas and Rio Meriagu) each week, and the streams with less suitable *labranchiae* habitats (the Rio sa Murta Uci and Rio Stellas) on alternate weeks. The field work was completed on September 26.

To follow the picture of *labranchiae* distribution and abundance in even greater detail in the lower part of the Geremeas valley where the species was first found, the portion of the Rio Geremeas within 1.5 kilometers of the sea was further subdivided into 50-meter tracts, and all small ponds and pools outside the stream course itself were numbered. Two scouts were assigned exclusively to this area and spent a full week covering it.

TABLE 3

Numbers of anopheline larvae collected by scouts at Geremeas, Sardinia (July–September, 1952)

Anopheles	RIO GEREMEAS	RIO MERIAGU	RIO SA MURTA UCI	RIO STELLAS	TOTAL
labranchiae	242	1	0	0	243
hispaniola	3,917	1,224	281	3	5,425
claviger	376	292	8	71	747
marteri	29	1	25	535	590
algeriensis	10	0	0	0	10
Total	4,574	1,518	314	609	7,015

Apart from the searching necessarily delegated to the scouts, the writers, together with their associate Dr. Sergio Bettini of the Istituto Superiore di Sanità in Rome, undertook to follow certain of the water surfaces in the immediate vicinity of the original positives.

Results of routine scouting

The story of what happened to *labranchiae* is a very simple one, and rather different from what might have been expected in this area in which we knew it had formerly been the dominant species. There was no rapid build-up of the population in the original breeding places, and only a negligible and transitory appearance of larvae in adjacent appropriate breeding places.

The species remained confined to the first five tracts of the Rio Geremeas (within 1.25 kilometers of the sea) throughout the summer, with only two exceptions. On July 10, a scout of the Regional Government, later employed as our chief scout, found four 4th instar larvae in a portion of the Rio Geremeas, subsequently numbered by us as tract 24–25, six kilometers from the mouth of the river at an elevation of 75 meters. An intensive search of this tract by us the following week failed to produce additional larvae, and routine searches by scouts during ten subsequent weeks were also negative for *labranchiae*. The only other appearance of *labranchiae* outside the small concentration near the river mouth, was a single 3rd instar larva taken by the scouts on September 8 in tract 33–34 of the Rio Meriagu, 10.5 kilometers from the sea at an elevation of 475 meters. The heavy rains of September 14 to 17 flushed out this river so that few anophelines of any species were taken during the following two weeks before the project was terminated, and *labranchiae* was not again encountered away from the mouth of the river.

Of particular interest is the general distribution and large numbers of larvae of *Anopheles hispaniola* taken in the Rio Geremeas and the Rio Meriagu. This species was not found in the anopheline survey of Sardinia by Aitken in 1946, and it was not until late 1949 or 1950 that it was first found in the Geremeas area.

The fundamentally altered relationship among the anophelines is nicely demonstrated by the fact that during the week of the first discovery of *labranchiae* in June, when 15 larvae of this species were taken, no larvae of *hispaniola* were found; yet in the subsequent collections made from July through September, only 243 additional larvae of *labranchiae* were found, while 5,425 larvae of *hispaniola* were taken (see Table 3). Thus during a period sufficient to permit the development of the *hispaniola* population from a level so low that no larvae were found by routine search, up to the point where it was the dominant species, *labranchiae* was barely able to maintain itself.

Results of intensive pond studies

Near the mouth of the Rio Geremeas, within a few hundred meters of the sea, are several depressions, either at the edge of the stream bed which was dry during the summer, or within a few meters of it. The larger of these depressions contained water throughout the summer and a rich growth of aquatic vegetation (*Typha angustifolia*, *Juncus litoralis*, *Scirpus nigrescens*, *Phragmites communis*, *Iris pseudocornus*, *Potomogeton natans*, *Carex* sp., and *Lemna* sp.). The organic richness of the water in these ponds, produced by the decay of the associated vegetation, would we supposed favor the breeding of *labranchiae* over *hispaniola* since the latter species is usually found in clear water with some freshening by circulation. These ponds were all in the immediate vicinity of the originally discovered *labranchiae* foci or were themselves positive at the beginning of the study. We thought that they would be ideal breeding places for *labranchiae* and arranged to follow the growth of the population in them by personal examination each week throughout the summer. Three of the ponds were selected for this study (Nos. 2, 7 and 12). In these ponds the entire water surface intersected by aquatic vegetation was swept by using a large oval net (65 x 30 cm.), and the concentration of surface debris then minutely examined in white enamel basins or tubs (see Figure 4). All anopheline larvae found were microscopically identified in the field and returned alive to the ponds from which they had been taken. The numbers of anopheline larvae taken each week are recorded in Table 4. The interesting result of this study is that only *labranchiae* was found, these breeding places not being invaded by either *hispaniola* or *algeriensis* (the latter

FIG. 4. Concentrated search for anopheline larvae at one of the ponds near the mouth of the Rio Geremeas. The entire intersection surface of the pond was swept with the large net and the accumulated debris transferred to pans where larvae were sought by visual inspection.

FIG. 5. The portion of the Rio Geremeas where studies of the relations between *Anopheles l. labranchiae* and *A. hispaniola* were carried on, both along the stream margin and in the artificial pool in the foreground.

was present in very small numbers close by, near the mouth of the Rio Gereneas). But, despite the absence of any other anopheline larvae which might have created a situation of competition, the *labranchiae* population did not build up, and in fact no larvae at all were found the last two weeks of September, following the rains of mid-September. The hot, dry weather of August may have resulted in a short life span of adults which would have been unfavorable to the rapid growth of larval populations, but there was also no build-up during the cooler weather of September.

TABLE 4

Numbers of Anopheles l. labranchiae *larvae in natural ponds at Gereneas, 1952*

	POND NO. 2	POND NO. 7	POND NO. 12	TOTAL
24 July	10	—	—	10
8 August	0	1	22	23
16 August	12	9	20	41
22 August	4	5	13	22
30 August	0	2	10	12
4 September	0	10	45	55
10 September	1	5	7	13
19 September	0	0	0	0
26 September	0	0	0	0
Total	27	32	117	176

TABLE 5

Numbers of Anopheles l. labranchiae *and* A. hispaniola *larvae in artificial pool No. 1, Gereneas, 1952 (dug July 30, 1952)*

	NO. DIPS	*labranchiae*	*hispaniola*	TOTAL	PER CENT *labranchiae*
7 August	20	0	4	4	0
14 August	15	17	7	24	71
20 August	6	27	73	100	27
27 August	5	9	41	50	18
4 September	10	0	14	14	0
10 September	10	0	1	1	0
19 September	20	0	17	17	0
26 September	20	0	66	66	0
Total	106	53	223	276	

Results of intensive artificial pool studies

To provide conditions intermediate between the rich organic waters of the natural ponds and the fresh flowing water of the stream, we dug two pools, two meters in diameter and 20 centimeters deep, about a meter's distance from the river edge, where there would be some subsurface freshening of the water

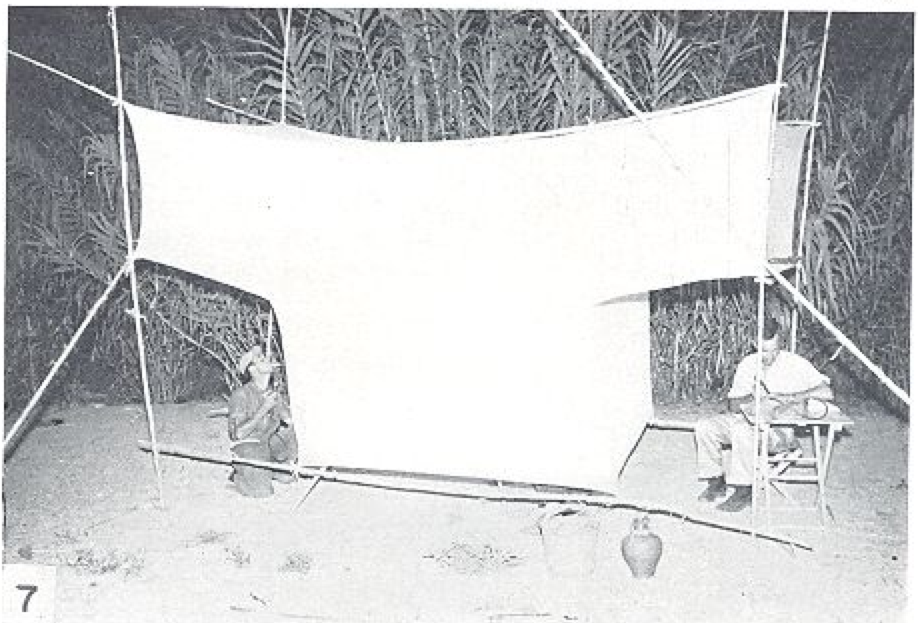

FIG. 6. The white stakes along the margin of the stream mark the four-meter stretches where the balance between larvae of *Anopheles l. labranchiae* and *A. hispaniola* was studied each week. As much as three hours was necessary for the examination of each four-meter stretch. In this breeding place, formerly the habitat of *Anopheles l. labranchiae*, *A. hispaniola* predominated throughout the summer.

FIG. 7. The Shannon light and net trap set up beside a breeding place in the stream at night. Mosquitoes attracted by the light inside the central "box" of the trap were collected by hand. The only anophelines taken by this means were *A. hispaniola*.

through the sandy soil. One of these, which had been dug on July 30, was examined by us each week from August 7 to September 26 (see Figure 5). The pool was seeded with algae and a few aquatic plants from the adjacent stream. Collections were made with conventional dippers and larvae returned alive after identification. The results of the weekly sampling of this pool are given in Table 5. *Anopheles hispaniola* invaded the pool at once, but on the second examination, on August 14, *labranchiae* was dominant, 71 per cent of the larvae being of this species. After this, however, the balance shifted in favor of *hispaniola*, with *labranchiae* disappearing altogether at the last, just as it did in the natural ponds.

TABLE 6

Numbers of Anopheles l. labranchiae *and* A. hispaniola *larvae along stream margin, Geremeas, 1952*

	NO. DIPS	*labranchiae*	*hispaniola*	TOTAL	PER CENT *labranchiae*
25 July	353	16	42	58	28
26 July	239	1	82	83	1
2 August	194	2	52	54	4
7 August	164	3	125	128	2
14 August	131	1	34	35	3
Total	1,081	23	335	358	

Results of intensive stream margin studies

We also attempted a concentrated study of the progress of the balance between *labranchiae* and *hispaniola* in the stream habitat formerly occupied by *labranchiae*. Several tracts of four meters of river margin were examined by us each week until the drying of the lower portion of the Rio Geremeas made the tracts unsuited for anopheline breeding in the latter part of August (see Figure 6). The results of this study are given in Table 6. It will be seen that at all times *hispaniola* was the dominant species.

ADULT STUDIES

In addition to the men employed as larval scouts, two men were engaged to search for adult mosquitoes in human habitations and domestic animal shelters. On the sketch map (see Figure 1) are indicated by black circles practically all man-made shelters in the study area. The types of shelters and the number of times they were examined are shown in Table 7. The net result of this intensive search for adult anophelines was the finding of one adult female *claviger* in a stable at Casa Oghittu, Sa Corti, on August 21, and a single *labranchiae* female in the pigsty at Geremeas center on September 3. Following the finding of this one *labranchiae*, the pigsty was searched daily from Monday through Friday of each week, but no additional specimens were taken.

Samples of wall scrapings were taken at Geremeas center on August 25 for determination of the amount of DDT residue from the spraying of 1951. Through the courtesy of Professor M. Alessandrini and Dr. V. Amormino, DDT determinations were made at the Istituto Superiore di Sanità, Rome; the results are given

TABLE 7

Inspections for adult anophelines in the Geremeas valley during the 10-week period July 20–September 27, 1952

SHELTER TYPE	NUMBER OF ROOMS		ROOM INSPECTIONS		AVERAGE NUMBER OF INSPECTIONS	
	Geremeas center	Other areas	Geremeas center	Other areas	Geremeas center	Other areas
House	42	61	233	286	5.5	4.7
Stable	8	38	67	199	8.4	5.2
Pigsty	14	11	128	83	9.1	7.5
Hen house	8	6	64	27	8.0	4.5
Rabbit hutch	3	2	22	8	7.3	4.0
Shed	5	16	31	100	6.2	6.2
Shepherd's hut	6	35	33	182	5.5	5.2
Grotto	0	1	0	6	0	6.0
Bridge	0	10	0	37	0	3.7
Total	86	180	578	928	6.7	5.2
Grand Total	266		1,526		5.7	

Note: One *labranchiae* in pigsty on September 3; one *claviger* in stable on August 21.

TABLE 8

DDT determination of wall samples from Geremeas center

Samples collected August 25, 1952[1]

NO. OF SAMPLE	DESCRIPTION OF SAMPLE	WEIGHT OF MATERIAL (GRAMS)	UNALTERED DDT PER SQUARE METER (GRAMS)
1	Whitewashed wall, surface material	0.05	0.10
2	The same, but deeper scraping	0.6	0.32
3	The same, another place	0.08	0.12
4	The same, but deeper scraping	1.00	0.80
5	Wooden beam of pigsty, extracted with acetone on cotton	—	0.40
6	The same, another location	—	0.6
7	Stone wall of pigsty, extraction with acetone on cotton	—	trace
8	The same, another location	—	trace

[1] Analyses performed at the Istituto Superiore di Sanità, Rome.

in Table 8. It is difficult to say how effective these residual deposits ranging from a trace to 0.6 gram per square meter would be in killing adult *labranchiae* under field conditions. But if these mosquitoes were visiting the main group of farm buildings at Geremeas in even modest numbers, more than the one specimen should have been taken in the repeated daytime and nighttime searches.

We also attempted to find adult *labranchiae* in places other than conventional shelters. Adults were sought in tall grass and sedges beside the ponds in which larvae were present, by placing a mosquito net over an area of about 2.5 square meters and blowing smoke into the vegetation with a bee smoker. This procedure was performed 79 times between the end of July and mid-September. While a number of culicine mosquitoes were found by this means, no anophelines were taken.

Anophelines were also sought in rabbit burrows by covering the entrances with netting and spraying in acetone to stir up any mosquitoes inside. About 75 burrows were thus treated during August and early September, but no mosquitoes were found.

In an attempt to find *labranchiae* at night when the adults would be active, we set up a Shannon net and light trap beside a portion of the Rio Geremeas where larval breeding was going on (see Figure 7). The results were as follows:

July 30	8:20 to 10:20 P.M.	2 *hispaniola*
August 18	8:00 to 10:00 P.M.	6 *hispaniola*
August 25	8:00 to 10:00 P.M.	negative
August 27	7:30 to 9:30 P.M.	negative

Two inspections were made at night in the principal pigsty at Geremeas center, but only culicine mosquitoes were found.

MALARIA SURVEY

Two blood surveys of the population of the valley were completed during the period of the study. While 133 thick smears were examined on the first survey and 91 on the second, the actual number of persons from whom blood was obtained on the two surveys was 185. Their age distribution was as follows: (classification after Russell, West and Manwell, 1946):

0–1 year, infants	5
2–4 years, pre-school children	9
5–9 years, school children	21
10–14 years, adolescents	23
15 or more, adults	127
Total	185

One positive slide with a very few parasites of *Plasmodium vivax* was obtained on the first survey from a 56-year-old male without clinical symptoms. The man concerned, a shepherd, lived in the upper valley of the Rio Stellas, an area where no *labranchiae* were found. It was concluded that this was an old infection.

DISCUSSION

It is apparent from the study of this one summer at Geremeas that in the absence of any antimosquito measures, residual populations of *labranchiae* do not necessarily build up rapidly, nor do they necessarily diffuse widely.

This observation might be qualified by noting that Geremeas is a confined valley and the sequence of events might have been different in an open-plain situation. The failure of the *labranchiae* population to grow or diffuse significantly during the three summer months after its discovery in an area to which it was well suited by the evidence of its prior endemicity remains, however, a most surprising result. If we assume that this residual population of *labranchiae* has the same genetic composition and thereby the same behavior pattern and biotic potential as the population endemic here before the eradication effort, there are three factors which might have mitigated against a normal build-up of the population.

1. The *labranchiae* adult females would have sought their blood meals primarily in human and domestic animal shelters, and the still-persisting residue of DDT on the surfaces of these structures would have produced a mortality great enough so that barely enough females survived to maintain the population. While this possibility cannot be entirely discounted we have no real evidence that this happened, for if *labranchiae* visited the main group of farm buildings in even modest numbers, more than one specimen should have been taken in the repeated daytime and nighttime searches.

2. The *labranchiae* positive was not found at Geremeas until June 21, and if we assume it was the result of a recent introduction, the species may not have become well established until the onset of the hot, dry summer weather, which would have limited the adult life span, thus reducing the number of egg depositions.

3. A third possibility is that there is a critical population density below which there are so few adults in an area that swarming of males and consequent fertilization of females is only partially successful. While the larval population of *hispaniola* was at least as low as that of *labranchiae* at the beginning of the summer, the overwintering adult population of *hispaniola*, which was not detected since the species is not domestic, was probably substantial. The breeding stock of adult *labranchiae*, on the other hand, must have been very small since the species had not been found in the area in the preceding two years.

There remains yet another kind of explanation of what may have happened at Geremeas and in Sardinia, namely that there has been some fundamental alteration in the normal biology and behavior of *labranchiae*, or some change in the balance between it and the other anophelines. The sequence of events at Geremeas points very strongly to the conclusion that if one species of anopheline is eradicated, or nearly eradicated, the vacuum created by its absence may not be permanent, and the lacuna may be filled by another species.

At Geremeas there can be no doubt that *hispaniola* has successfully invaded and occupied what was formerly a *labranchiae* habitat. Whether or not this change in the balance between the two species is a permanent or a temporary phenomenon could only be established by leaving this valley untreated for a period of years, until any DDT residues on structures were reduced to certain insignificance, and *labranchiae* had had several full seasons to express, if it could, the vitality and aggressiveness which formerly characterized it in this area. This will not be possible since the public health authorities considered the malaria hazard too great, and the valley was again put under treatment following the completion of the 1952 observations.

That changes in the hydrology, through drainage and diking which affect salinity and other elements in the larval environment, may modify the balance between members of the *Anopheles maculipennis* group, and in consequence the malaria picture, has long been appreciated and eloquently expressed by Hackett (1937) and others. With the rapid development of a differently oriented attack on anophelines and malaria through the use of residual insecticides, the time has now come to consider whether this chemical attack may not also be so selective that it too would result in an altered balance. In the present case, the residual population of what we call *labranchiae* at Geremeas and in Sardinia may now have as its normal biology and mean behavior pattern a way of life formerly followed by only an insignificant, and thereby overlooked, fraction of the entire population. Specifically, the present *labranchiae* population may not regularly invade human habitations, and the adult habit of selection of oviposition site and source of blood meals, the larval requirements for development and the ability to evade predators may all be altered. If the control of these key functions in the life of the mosquito have a heritable basis, as is likely, then the present residual population of *labranchiae* may lack the genetic materials necessary to express the diversity of habits which resulted in its formerly being the dominant anopheline.

The selective action of residual spraying of human and domestic animal shelters in killing that part of the adult anopheline population which habitually rests in these structures is quite apparent. This phase of the eradication program would have affected the great bulk of the *labranchiae* population, only a small fraction of the *claviger* population, and *hispaniola* not at all. There is a less general appreciation of the fact that even uniform larviciding of all water surfaces would have produced a selective effect on the different anophelines, because both of extrinsic differences in the optimum environment of each species, and intrinsic differences in the habits of the various larvae in the same breeding place. The larvae of *claviger* and *hispaniola* are in situations where there is water movement, or at least some freshening of the water, while those of *labranchiae* are more likely to be in still water. Larvicides would have been more effective, as a result of a longer contact period, in the latter situation than in the former. Even within the confines of a particular breeding place, the differences in reaction of the larvae to disturbance of the water demonstrated by Trapido (1951) would result in a differential exposure, with *labranchiae* more affected than *claviger* or *hispaniola*. Thus it was shown by Trapido (1951) that one minute after disturbance of the water surface, only two per cent of *labranchiae* larvae remained submerged, while 36 per cent of the *hispaniola* were still down. In a test conducted during the present study one *hispaniola* larva survived after being submerged for 75 minutes. Thus even in the situation where larvae of both species were in the same breeding place, *labranchiae* might well be more drastically affected by larvicide treatment.

We must recognize that the behavior of a particular mosquito species or race is not rigid, but capable of a "spectrum" of variation, and that this variation is genetically controlled. The selective action of toxicants on both adults and larvae would result in a residual population which differed in its mean behavior pattern not necessarily through the addition of new elements by mutation, but with the original genetic material present in different proportions. If the selection were sufficiently rigorous and long enough sustained there would result the total elimination of the portion of the behavior pattern most susceptible to chemical attack. In this situation the lifting of the attack would result in the mosquito no longer being able to re-establish itself in its former way of life, since it would lack the genetic potential to do so. Were the chemical attack only incompletely selective and not sustained for long enough, there would be a gradual reversion to the original situation. In this second case, the factor of the increase and competition from other species would retard the rate at which the reversion would take place. The one-season study at Geremeas was carried on long enough to appreciate the possibility that one or another of these situations may exist, but not long enough to obtain certain proof.

The important implication to be derived from this interpretation of the results is that *labranchiae* at Geremeas, and perhaps elsewhere in Sardinia, has become so modified in its behavior that it may no longer be an effective malaria vector.

SUMMARY

In the absence of any treatment in the Geremeas valley of Sardinia there was no build-up of the population of *Anopheles l. labranchiae* either as larvae in the vicinity of the original focus, or as adults in human or domestic animal shelters. There was no significant diffusion of *labranchiae* from the immediate area of the original positive to other suitable breeding places in the valley. The invasion of former *labranchiae* breeding places by *Anopheles hispaniola* and the replacement of the former by the latter was demonstrated.

The factors which may have contributed to the replacement of *labranchiae* by *hispaniola* are discussed, and it is suggested that the present residual population of *labranchiae* may differ in essential behavior characteristics which would limit its effectiveness as a malaria vector, but the study was not conducted over a long enough period to establish this with certainty.

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