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FACTORS INFLUENCING THE SEARCH FOR ANOPHELINE LARVAE SARDINIA

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The progress of the ERLAAS project for the eradication of *Anopheles l. labranchiae* in Sardinia has been reviewed by Logan, 1950. Once the great bulk of the mosquitoes had been destroyed, the problem arose of finding those that remained so that efforts could be concentrated on their destruction. I was invited to participate in the work of finding these last mosquitoes on the island during the 1950 breeding season.

The fact that *Anopheles l. labranchiae* is a species indigenous to Sardinia presented a situation very different from that encountered in the successful eradication campaigns which had previously been waged against the invading *Anopheles gambiae* in Brazil (Soper and Wilson, 1943) and in Egypt (Shousha, 1948). Despite the most painstaking scouting of all known water surfaces for anopheline larvae, as well as searches of possible adult resting places, *A. l. labranchiae* continued to be found occasionally in areas that had been thought to be clean for as much as, or in some cases more than, two years. The present account deals with studies of anopheline larval behavior in Sardinia, and how this behavior may affect the ability of the entomologist to detect larvae when they are present in only small numbers.

The conventional method of searching for anopheline larvae is that of using a dipper or net to skim up portions of the water surface and examining this surface water against the convenient white background of an enamel pan or dish. A multitude of variations of this method have been reported. In Sardinia during the 1950 season dippers of the sort described and figured by Russell, West and Manwell, 1946, were used. These dippers have a diameter of 15 cm. and a capacity of about 400 cc. To measure the total larval population of limited areas other methods have been described, such as that of fencing off sections of water surface and thoroughly dipping the enclosed water from which the larvae cannot escape by lateral movements (Bates, 1941). When the first method is used, the surface of a large area may be sampled; when the second method is used, the total population may be determined, but only for a small fraction of the entire area of the presumed breeding place. The experience in Sardinia indicated the need to stress that both these methods only sample the population of a breeding area; neither method is adequate for determining the absolute presence or absence of larvae in an extensive breeding place when the populations are very low. This is a point generally overlooked by writers who report a "100 per cent kill" of larvae when they have dipped in an area and failed to find larvae following field tests of larvicides.

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The difficulty of finding larvae, and an expression of how great the element of chance in such finds is, when population levels are low, was well illustrated by an area in the valley of the Riu Mulargia, Sardinia, found positive for *A. l. labranchiae* during the first week of August 1950. Scouting of this isolated river valley had been negative during 1949 and through July 1950. During the first week of August scouts found larvae of *labranchiae* in two small pools in the partially dried up stream-bed. A subsequent intensive search of these pools produced nine fourth-instar and one third-instar larvae. The scouts concentrated their search in the area but found no additional larvae. At a point about 100 meters from one of the positive pools was a gully beside the main stream course with a strip of water 40 meters long, two meters wide, and a half meter deep, richly provided with submerged and surface vegetation and a moderate amount of emergent vegetation (figures 1 and 2). The water was still and fully exposed to the sun, an ideal *labranchiae* habitat. The scouts working the area had found this pool to be negative for *labranchiae*, although it supported a heavy culicine population.

I started dipping at one end of this pool, and on the fourth dip a third-instar larva of *labranchiae* was found along with numerous culicine larvae. Additional dipping, until 50 dips had been made within one meter of where the first larva had been taken, failed to produce any more larvae of *labranchiae*. At this point 15 scouts were placed around this end of the pool shoulder to shoulder and they proceeded to cover the restricted area of about four by two meters. Observation of these men showed that each one made an average of 12 dips per five minutes. It was 10 minutes later, and after approximately another 360 dips, that the first *labranchiae* larva was taken by these scouts. Continued dipping for one hour in the same restricted area of eight square meters produced an additional 19 second- and third-instar larvae. No larvae were found in subsequent intensive dipping of the remaining 36-meter length of the strip of water (figure 2).

Thus in a restricted area, where there were not less than 21 larvae in eight square meters of water, it took about one-thirtieth of a man-hour to find the first larva, but it took two and a half man-hours to find the second; that is, it took 75 times as much effort to find the second larva as it did the first. The impossibility of finding these larvae, using this method, by anything but chance is clearly demonstrated.

Another instructive instance of how difficult it may be to find larvae, when populations are low, was the case of a positive finding reported from a swamp, the Palude Salone in the northeastern corner of Sardinia, which I had the opportunity to investigate with and through the kindness of Dr. Thomas H. G. Aitken. This swamp was several acres in extent, in part overgrown with the tall sedge *Juncus* and emergent grasses and in part open water with heavy mats of surface vegetation. It had been repeatedly found positive for *labranchiae* and treated with larvicide during the years prior to 1950. Scouts reported the finding of larvae there in mid-May 1950. A morning search by Dr. Aitken and myself several days after the report of the positive finding failed to produce any larvae. The following day Dr. Aitken had a group of 30 scouts investigate the area thoroughly under his supervision. The men were placed in a line extending across the swamp about two meters from each other and started to dip at 10:00 A.M. following a systematic pattern across the swamp (figure

FIG. 1. Valley of the Riu Mulargia, north of Donigala, Sardinia. The gully in the foreground forms part of the partially dried up course of the stream. The group of men are clustered about a pool in which larvae of *Anopheles l. labranchiae* were found on 2 August 1950.

FIG. 2. Closeup of a pool positive for larvae of *Anopheles l. labranchiae* in a gully beside the main course of the Riu Mulargia. The scouts are engaged in intensive dipping of the strip of water two meters wide and 40 meters long.

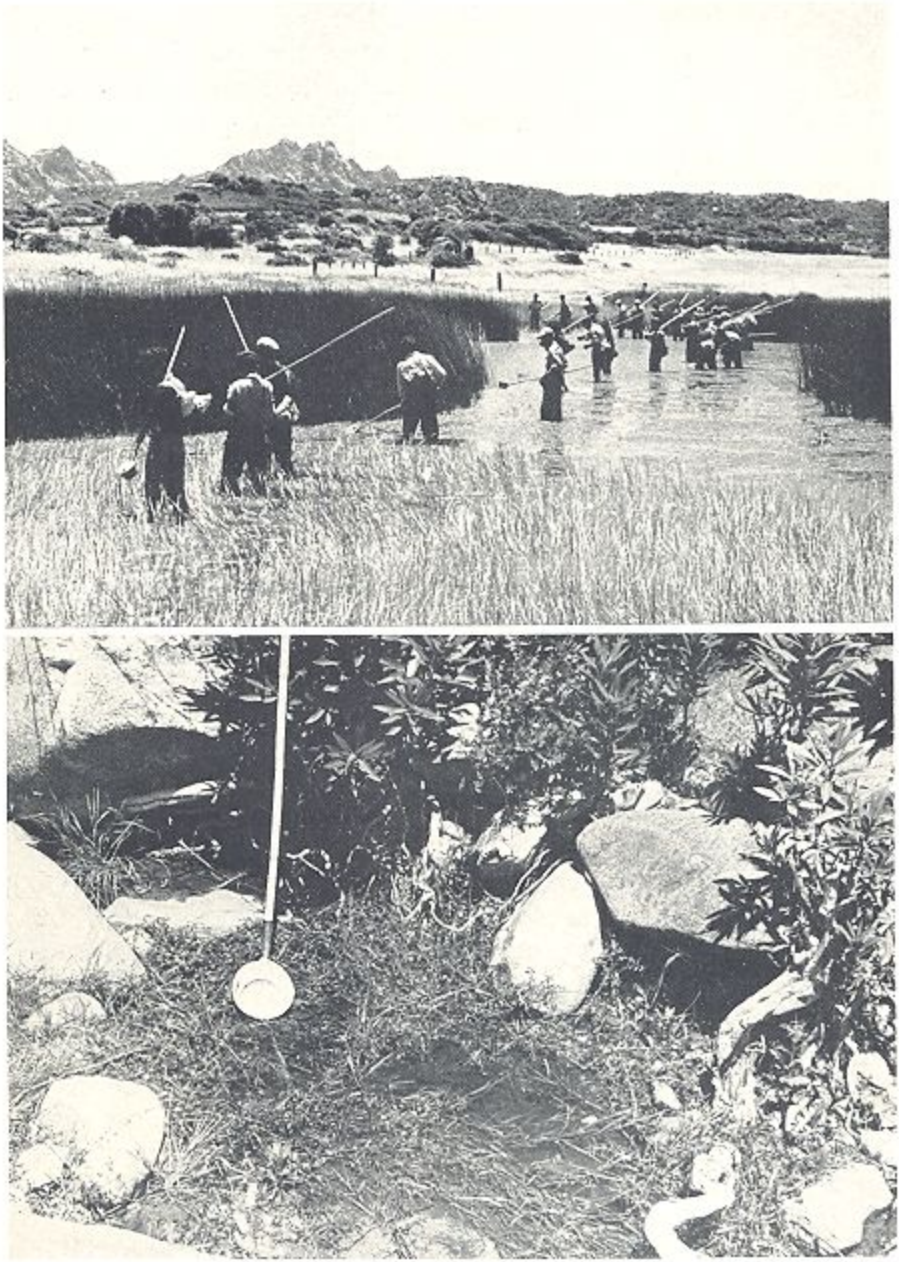

FIG. 3. The Palude Salone, east of Arzachena in northeastern Sardinia. Approximately 17,000 dips were made by 30 scouts before the first larva of *Anopheles l. labranchiae* was found in this swamp.

FIG. 4. A spring-fed pool habitat of *Anopheles claviger* in the bed of the Riu Sa Murta Uei in southeastern Sardinia. This was one of the habitats in which intensive searching of 50 dips per square meter was carried out.

3). At 3:00 P.M. the first larva was found after four hours of dipping (one hour was taken out for lunch). The place where the larva was taken was about one-third of the way across the swamp in water about half meter deep and covered with surface vegetation. This place was in no apparent way different from the remainder of the open area of the swamp. Concentrating half the scouts in the area where the first larva had been taken produced about 40 larvae from first- to fourth-instar during the next hour. The larvae were taken in two groups about 15 meters apart. No larvae were taken elsewhere.

Allowing the usual rate of 12 dips per five minutes per man, some 17,280 dips would have been made before the first larva was found in this swamp, which was known to be positive. Here again was a striking demonstration of the capricious distribution of very small larval populations which may be located only by chance using present scouting methods.

Field experience of the sort outlined above indicated the desirability of more detailed studies of the effectiveness of dipping as a means of finding anopheline larvae. As a consequence of the ERLAAS campaign, *Anopheles l. labranchiae* was so rare that it was not feasible to work with this species. Habitats of *Anopheles claviger*, a species present in modest numbers, were therefore chosen for further study. What appeared to be favorable breeding places of this species, clear, cool, gently running water with some surface and emergent vegetation, were selected, and an area of one square meter was marked off. A dipper of the sort described above was used. Figure 4 illustrates one such habitat. Fifty dips were made in each sample plot, chosen as a possible breeding place on the basis of its favorable ecological features, and after consulting the ERLAAS records to establish that the area had not received larvicidal treatment for a year or more. The protocols of five such field trials, which proved positive for *claviger* larvae, are given in table 1. In these trials an average of two dips were made per minute and about 25 minutes devoted to making the 50 dips arbitrarily chosen as the number to cover each one square meter plot. This obviously represents the most intensive sort of search, yet it is evident from the scattered appearance of the larvae that had the number of dips been extended to a hundred or more, and even an hour devoted to each square meter, it might still have been possible to find additional larvae. These field trials raised the question of how it was possible to cover so restricted an area so thoroughly and still continue to find larvae.

In dipping for anopheline larvae at or near the water surface it is generally presumed that the larvae are at or near that surface. This is known to vary with different species, however. It was observed that larvae of *Anopheles claviger* would often remain at the bottom of the dipper sometimes wholly or partially concealed in the debris scooped up with them. Bates (1949) has described behavior of this sort as "alarm reactions" of larvae and points out that larvae may remain at the bottom in a rigid "death-feigning" position to which psychologists have applied the term "letisimulation."

As a result of this field observation it seemed desirable to establish the pattern of how long larvae avoided the surface when disturbed by the mechanical agitation of the water surface by dipping. At various times it was possible to obtain larvae of four species of *Anopheles* as follows: *A. claviger*, *A. hispaniola*, *A. algeriensis* and *A. l.*

TABLE 1

The occurrence of larvae of Anopheles claviger *in one square meter plots in the course of 50 dips*

DIP NUMBER	1ST TRIAL NUMBER OF LARVAE	2ND TRIAL NUMBER OF LARVAE	3RD TRIAL NUMBER OF LARVAE	4TH TRIAL NUMBER OF LARVAE	5TH TRIAL NUMBER OF LARVAE
1st	1 (3rd)*	0	0	1 (4th)	0
2nd	0	0	0	0	0
3rd	0	1 (1st)	1 (2nd)	0	0
4th	0	0	0	0	0
5th	0	0	0	0	0
6th	0	0	0	0	1 (3rd)
7th	0	0	0	0	0
8th	0	0	0	1 (4th)	0
9th	1 (3rd)	0	0	0	0
10th	0	0	0	1 (3rd)	0
11th	0	0	0	0	0
12th	1 (3rd)	14 (1st)	0	0	0
13th	1 (4th)	4 (1st)	0	0	0
14th	0	0	0	0	0
15th	0	0	0	0	0
16th	0	1 (1st)	0	1 (3rd)	0
17th	0	0	0	0	0
18th	0	0	0	0	0
19th	0	0	0	0	0
20th	0	0	0	0	0
21st	0	0	0	0	0
22nd	0	0	0	0	0
23rd	1 (3rd)	0	0	0	0
24th	0	0	0	0	0
25th	0	1 (1st)	1 (3rd)	0	0
26th	0	1 (1st)	0	0	0
27th	0	0	0	0	0
28th	0	0	0	0	0
29th	0	0	0	0	0
30th	0	0	0	0	1 (3rd)
31st	0	0	0	0	0
32nd	0	0	0	0	0
33rd	0	0	0	0	0
34th	0	0	0	0	0
35th	1 (2nd)	0	0	0	0
36th	0	0	0	0	0
37th	0	0	0	0	0
38th	0	0	0	0	0
39th	0	0	0	0	0
40th	0	0	0	0	0
41st	0	0	0	0	0
42nd	0	0	0	0	0
43rd	0	1 (1st)	0	0	0
44th	1 (3rd)	0	0	0	0
45th	0	0	0	0	0
46th	0	0	0	0	0
47th	0	0	0	0	0
48th	0	0	0	0	0
49th	0	0	0	0	0
50th	0	0	0	0	0
Total no. positive.....	7	23	2	4	2

* Figures in parentheses indicate larval stage.

labranchiae. When larvae were obtained, one larva was placed in a dipper of the sort described above and mechanically disturbed by poking at it. The time period during which the larva avoided the surface was then read from a stop-watch and recorded. In most cases 10 trials were made with each larva, after which it was discarded. The results of these trials are shown grouped by convenient frequencies in table 2. These

TABLE 2

Water surface avoidance by Sardinian Anopheline larvae following mechanical disturbance

LARVAE SUBMERGED IN EXCESS OF THE FOLLOWING TIME INTERVAL* MIN.: SEC.	*Anopheles l. labranchiae*		*Anopheles claviger*		*Anopheles hispaniola*		*Anopheles algeriensis*	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
:01	328	100	300	100	258	100	31	100
:02	175	53	281	94	255	99	29	94
:03	86	26	257	86	245	95	28	91
:04	44	13	243	81	243	94	28	91
:05	23	7	235	78	241	93	27	87
:10	16	5	209	70	225	87	26	84
:20	12	4	198	66	185	72	24	73
:30	9	3	184	61	147	58	21	68
:40	9	3	157	52	125	48	20	65
:50	6	2	134	45	104	40	16	52
1:00	6	2	116	39	93	36	14	45
2:00	5	2	52	17	54	21	6	19
3:00	2	1	25	8	36	14	3	10
4:00			17	6	25	10	1	3
5:00			12	4	19	7		
6:00			8	3	14	5		
7:00			7	2	11	4		
8:00			6	2	10	4		
9:00			5	2	9	3		
10:00			5	2	7	3		
11:00			5	2	3	1		
12:00			4	1	1	0.4		
13:00			2	0.7	1	0.4		
14:00			2	0.7	1	0.4		
35:00					1	0.4		

* Larvae which returned to the surface immediately are arbitrarily recorded under 1 second.

data show a marked difference between the behavior of *A. l. labranchiae* and the other three species studied. Thus after a lapse of five seconds all but seven per cent of the *A. l. labranchiae* had returned to the surface, while 78 to 93 per cent of the other species were still submerged, at 30 seconds the comparable figures are three per cent, contrasted with 58 to 68 per cent; at one minute two per cent contrasted with 36 to 45 per cent. At four minutes, in 328 trials, all the *labranchiae* larvae had returned to the surface but six per cent of the *claviger* and 10 per cent of the *A. hispaniola* were

still submerged. *Anopheles claviger*, in the course of 300 trials, was observed to remain below the surface for as long as 14 minutes, while in one of 258 trials with *hispaniola*, one larva remained submerged for as long as 35 minutes.

The ability of *Anopheles claviger* to remain submerged for long periods may be related to the fact that this species is apparently capable of cuticular respiration (Bates, 1949), passing the winter in the larval state, with the larvae capable of survival in water under ice. In Sardinia *claviger* and *hispaniola* larvae, which remained submerged the longest times in these tests, occur along streams in water which probably has a high dissolved oxygen content. This would aid the larvae in maintaining themselves by cuticular respiration under the water surface.

These data are significant in interpreting the adequacy of scouting for larvae by dipping at the surface, and possibly also in explaining the survival of larvae following treatment of water surfaces with oil-base larvicides which affect only the water surface.

On the average, scouts made 12 dips per man, per five minutes. Allowing five seconds for dumping the water from the dipper and making a fresh dip, the dipping interval would be about 20 seconds. According to the data given in table 2, 66 per cent of the *claviger*, 72 per cent of the *hispaniola*, and 73 per cent of the *algeriensis* would not have returned to the surface during this time period following the disturbance of the water surface. On the other hand, all but four per cent of the *labranchiae* would be at the surface after 20 seconds. When the larval densities are very low and the possibility of detecting them slight, even under the best conditions, a larger percentage of such larvae as might be present, of the species *claviger, hispaniola,* and *algeriensis*, would not be detected since they would not be at the surface.

The behavior of larvae in avoiding the water surface is also of importance in evaluating the degree of success that can be expected from the use of oil-base larvicides which are presumed to produce kills of larvae at the water surface. Concerned particularly are the stream-breeding species *claviger*, and *hispaniola*, which, as shown above, demonstrate significant surface avoidance following disturbance of the water surface. Oil-base larvicides fortified with toxicants such as DDT, and even with added spreading agents, may not be expected to persist on moving water for prolonged periods. Following the disturbance of the water by the movements of the larvicider, surface avoidance exhibited by *claviger* and *hispaniola* might well be expected to result in their failure to return to the water surface before the oil surface film was dissipated. This may in some measure explain why the ERLAAS experience showed a closer approach to eradication of *labranchiae* than *claviger* or *hispaniola* at the time when all ground water surfaces were being treated with larvicides although other factors may have been involved as well.

SUMMARY

Field experience in Sardinia illustrates how uncertain the method of dipping for anopheline larvae is in establishing their presence or absence, when populations are very low.

The premise that anopheline larvae are at or near the water surface is not always

valid. Experiments are reported demonstrating the extent of surface avoidance following mechanical disturbance for the species *Anopheles claviger*, *A. hispaniola*, *A. algeriensis*, and *A. l. labranchiae*. *Anopheles l. labranchiae* is shown to return to the surface more rapidly than the other three species. One minute after being disturbed, two per cent of the *labranchiae*, 39 per cent of the *claviger*, 36 per cent of the *hispaniola*, and 45 per cent of the *algeriensis* were still submerged. In 328 trials with *labranchiae* larvae the maximum submergence time was three minutes. The comparable figures for the other species were: *claviger*, 14 minutes in 300 trials; *hispaniola*, 35 minutes in 258 trials; *algeriensis*, 4 minutes in 31 trials.

REFERENCES

Bates, Marston 1941. Field studies of the anopheline mosquitoes of Albania. Proc. Ent. Soc. Wash., **43**: 37–58.

Bates, Marston 1949. The Natural History of Mosquitoes. MacMillan Co., New York, N. Y., 379 pp.

Logan, John 1950 *Anopheles labranchiae* eradication in Sardinia: An interim report. Amer. Jour. Trop. Med., **30**: 313–323.

Russell, Paul F., West, Luther S., and Manwell, Reginald D. 1946. Practical Malariology. W. B. Saunders Co., Philadelphia, Pa. 684 pp.

Soper, Fred L. and Wilson, D. Bruce 1943. *Anopheles gambiae* in Brazil, 1930 to 1940. The Rockefeller Foundation, New York, N. Y., 262 pp.

Shousha, Aly Tewfik 1948. Species eradication. The eradication of *Anopheles gambiae* from Upper Egypt, 1942–1945. Bull. World Health Org., **1**: 309–352.

RESUMEN

La experiencia adquirida en el campo en Sardinia demuestra cuán incierto el método de inmersión es para establecer la presencia o ausencia de larvas anofelinas cuando sus poblaciones son muy bajas.

La premisa que las larvas anofelinas se encuentran en o cerca de la superficie no es siempre válida. Se haa reportado experimentos que demuestran lo extensamente que las especies *Anopheles claviger*, *A. hispaniola*, *A. algeriensis* y *A. l. labranchiae* pueden evadir la superficie después de alterarse ésta mecánicamente. *A. l. labranchiae* parece retornar a la superficie más rápidamente que las otras tres especies. Un minuto después de la alteración dos por ciento de las *labranchiae*, 39 por ciento de *claviger*, 36 por ciento de *hispaniola* y 45 por ciento de *algeriensis* continuaron sumergidas. En 328 pruebas con larvas *labranchiae* el tiempo máximo de sumersión fué tres minutos. Las figuras comparables para las demás especies son: *claviger*, 14 minutos en 300 pruebas; *hispaniola*, 35 minutos en 258 pruebas; *algeriensis*, 4 minutos en 31 pruebas.