STUDIES IN LABORATORY REARING OF ANOPHELES QUADRIMACULATUS SAY[1]

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Since a constant supply of healthy, vigorous, adult mosquitoes was needed for use in various studies being made on insect repellents, laboratory rearing of *Aedes aegypti* and *Anopheles quadrimaculatus* was initiated. No difficulties were encountered in rearing *Aedes* but the same cannot be said for *Anopheles*, consequently this paper deals only with the latter species.

A generous supply of *Anopheles quadrimaculatus* eggs was obtained from the U. S. Department of Agriculture, Bureau of Entomology Laboratory at Orlando, Florida, in November, 1943. The method of rearing followed at that time was obtained from notes made by Dr. C. E. Venard at Orlando and from the publication by Crowell (1940). In following these methods certain difficulties were encountered in rearing the larvae through the four instars. It was learned that other laboratories were having similar rearing troubles. This paper is written to report several modifications of the above mentioned techniques with the hope that the suggestions offered will be of value to those now engaged in similar work and helpful to those who may later become interested in such activity.

The rearing room was 11 x 6½ feet with a ceiling 10 feet high and lighted by a fluorescent lamp containing two 40-watt white tubes, which was on only during the day. The temperature was maintained at approximately 80° F. and the relative humidity varied from 40 to 70 per cent, but most of the time it was very near 50 per cent.

ADULTS.—The adults for egg-laying were maintained in a 16 mesh screened thirty-inch cubical sleeve cage. Water was available to the mosquitoes in the oviposition pan. As a source of food each cage was supplied daily with a piece of absorbent cotton approximately one inch square saturated with a 5 per cent honey solution in water and in addition a blood meal was furnished by allowing them to feed from the clipped belly of an immobilized rabbit introduced into the cage for one-half hour. The rack for holding the rabbit was similar to that used by Campbell, Barnhart and Hutzel (1941) with the exception that the bottom, sides and ends were completely enclosed. This eliminated all hiding places and very few mosquitoes escaped into the room when the apparatus was removed from the cage. Another precaution taken to prevent escape was the use of a rubber tube from a compressed air line to blow the mosquitoes off the rack and away from the sleeve as the rabbit was being withdrawn. It was found that the rabbit lay more quietly if its head was not exposed to the mosquitoes in the cage, consequently the sleeve of the cage was pinned around the neck and the head remained outside.

The population was maintained by placing jars containing pupae in the stock cage. A screen wire cone with 1½ inch opening at the top was placed over each jar to allow the emerging adults to escape into the cage, but prevent egg-laying adults from ovipositing in them. On alternate days a 5″ x 5″ cylindrical screen wire cage with a 1″ x 4″ opening and a sliding celluloid lid was placed over a jar where emergence was underway and the adults thus obtained were placed on a shelf to give a series of cages of "dated" mosquitoes for use in laboratory tests.

[1]The work described in this paper was done under a contract recommended by The Committee on Medical Research, between The Office of Scientific Research and Development and the Ohio State University Research Foundation.

EGGS.—For oviposition a white enameled pan 10 inches in diameter and 2¾ inches deep containing water to a depth of 1 to 2 inches was placed in the stock cage daily. On the following day it was removed and all adults picked from the surface with a pair of forceps. The water was siphoned off by means of a device made from a block of wood ¾″ in thickness, and a glass tube with dimensions as shown in Figure 1. The pan was set on a gently sloping surface so that the water

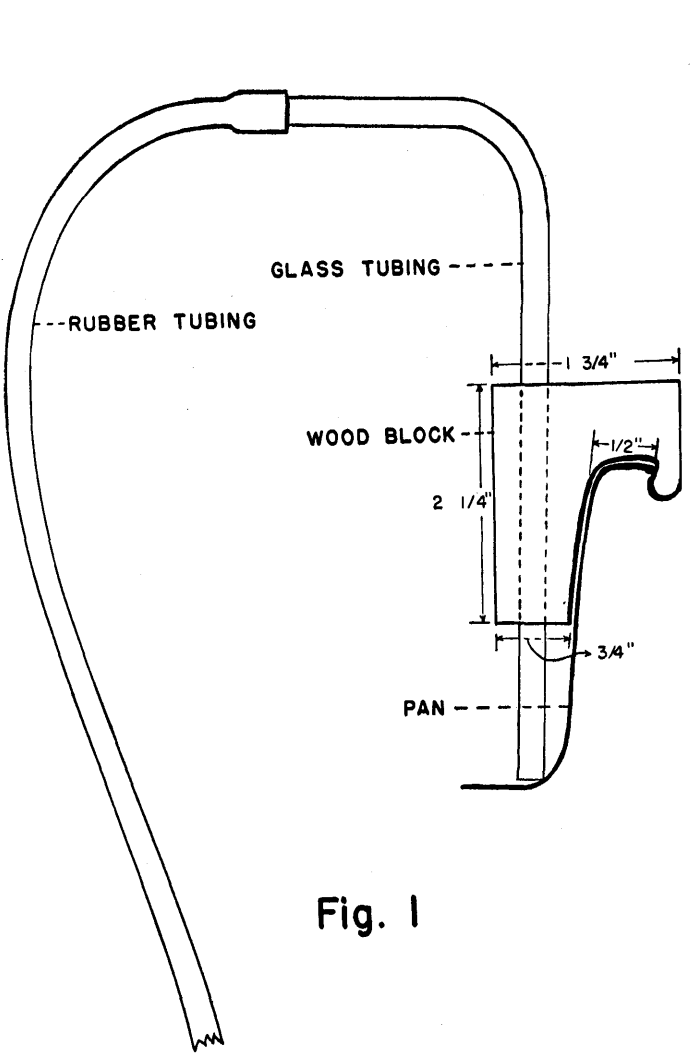

Fig. 1

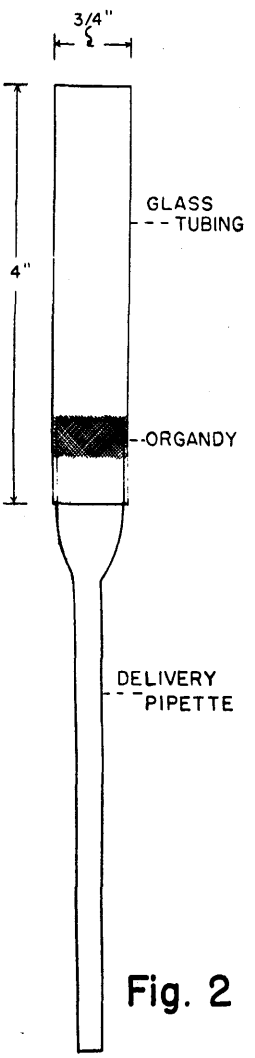

Fig. 2

Figs. 1 and 2. Devices for concentrating Anopheline eggs.

would run to the lower side. The flow from the siphon was stopped just as the floating eggs near the outlet were about to be carried off in the stream of water. The eggs were left on the sides and bottom of the pan. They were then washed into a 100 ml. beaker by means of a wash bottle, and the contents poured slowly into a funnel sieve. Care should be exercised especially when large batches of eggs

have been collected as they may partially block the passage of water and rapid pouring may result in an overflow. The funnel sieve shown in Figure 2 may be held in position by a burette clamp on a ring stand or held in the hand while the eggs are being washed from the beaker. It consisted of a glass tube four inches in length, open at both ends, the outside diameter of which was three-fourths inch and large enough to allow the top half of a 10 ml. delivery pipette with the end covered with two thicknesses of a fine mesh organdy to fit snugly inside. After the eggs had been washed into the funnel, the delivery pipette was pushed upward through the open tube and the organdy removed.

The eggs were placed in an enameled pan 10 inches in diameter to hatch. The pan was filled with water to a depth of 1½ to 2 inches. A paraffined cardboard ring, 3½″ o.d. and 2¼″ i.d. was floated in the water. This was used to prevent the eggs from floating to the edge of the pan and adhering to the sides following evaporation of the water. The organdy covered with eggs was then placed on the

TABLE I

A Comparison of Rate of Larval Development in Shallow versus Deep Water

Number Days After Hatching	Number Pupae Collected	
	Shallow Water	Deep Water
10	2	0
11	14	0
12	38	0
13	34	5
14	51	24
15	50	53
16	32	45
17	18	52
18	5	51
19	0	11
20	0	6
21	0	2
Total Pupae	250	250
Larval Mortality	7	22
Percentage Mortality	2.7	8.5

surface of the water inside the ring. The eggs floated off and the cloth sank to the bottom and was carefully removed with a pair of forceps. Two days after the eggs had been "set," hatching occurred. Several counts of the total number of larvae hatching from one day's supply revealed than from 3,000 to 10,000 eggs were deposited.

Larvae.—Following hatching 250 larvae were counted into each of two oblong Pyrex dishes (No. 232) containing approximately 150 ml. of water. The number of larvae could be determined quickly and accurately by the use of an ordinary medicine dropper and a Veeder hand tally or counter. Groups of recently hatched larvae were sucked up in the medicine dropper and as they were slowly forced out into the water in the dish the number was recorded on the counter in the other hand. A white background was necessary in order to see the larvae easily. No further manipulation was required until pupation occurred.

The Pyrex dishes were selected because they were a standard product generally available, they had a flat bottom and were uniformly rectangular in shape which made possible efficient use of shelf space. They were 12 inches long, 8 inches wide and 2 inches high.

Depth of water was determined by a study of larval development in shallow and deep water. Two Pyrex dishes (No. 232) were used in the test, each containing approximately 250 larvae. Water depth in one was maintained at one-eighth inch which required 150 ml. of water, and in the other at one-half inch, requiring 600 ml. of water. The quantity of powdered food supplied each dish was the same. Repeated tests gave results similar to those shown in Table I.

These data show that the larvae in the shallow water developed more rapidly than those in deep water and with less mortality. This may be due to the fact that larvae in the shallow water swept the bottom with their mouth brushes and obtained the food that settled. They can do this with their respiratory plates remaining at the surface. The water was clear with no scum, and very few organisms such as bacteria or protozoa could be found indicating that they were unnecessary for proper larval development. The pupae from the deep water dish were smaller in size indicating inadequate food supply. Additional food added to the deep water dish to compensate for that which the larvae could not reach

TABLE II

A Comparison of Rates of Development of Larvae Using Dog Food and Yeast in Different Proportions

Number Days After Hatching	Number Pupae Collected Daily		
	Dog Food and Yeast, Equal Parts	Dog Food—1 part Yeast —2 parts	Dog Food—2 parts Yeast —1 part
9	9	1	1
10	59	21	15
11	93	91	81
12	67	88	82
13	14	28	53
14	1	8	11
15	0	1	1
Total Pupae	243	238	244
Mortality	7	5	9
Percentage Mortality	2.6%	2.05%	3.56%

because of settling, resulted in scum formation, a considerable amount of gelatinous, flaky, suspended material and murky water. Microscopic examination of the water from such dishes revealed a very high population of bacteria, principally *Escherichia coli* and protozoa of the genera *Oikomonas, Hartmanella,* and *Colpoda* as determined by Professor W. J. Kostir and Dr. C. E. Venard. All these protozoa are air borne forms and primarily bacteria feeders. Once a dish became clouded or murky, death of most of the larvae was certain and if held for further development, a few larvae usually reached the pupal stage but the rate of development was greatly retarded. Results indicated that when this occurred it was best to dispose of the contents of the entire dish. An over-abundance of food, especially in the earlier instars increased the number of cloudy dishes.

A logical explanation for the greater mortality in the deep water dishes seemed to be that the excess food resulted in an abundance of bacteria, which appeared to be an ideal medium for protozoa because they increased greatly in numbers. The bacteria, being anaerobic, deoxygenated the water and along with the excess of food, a scum was produced on the surface that interfered with the dissolving of oxygen of the air into the water. The scum also clogged the respiratory plate

of the larvae causing them to struggle to free the plugged area, resulting in many of them being trapped in the flaky, gelatinous suspended material.

The larvae were fed *twice daily* with a mixture of equal parts by weight of finely ground[2] Purina dog food and powdered dried brewer's yeast. This mixture was tried in other proportions for effects on development and the results are shown in Table II. Peak pupation was reached more quickly when equal parts of dog food and yeast were used and mortality was greatest when dog food predominated and lowest when yeast predominated.

Studies were made on the amount of food required for each instar and the results are presented in Table III. Because all the larvae did not molt at the same time a safe range on quantity of food was given for the third and fourth instars which were the periods of greatest growth and food consumption. With practice an approximation of these amounts could be administered by means of tapping a salt shaker containing the food mixture a given number of times. Just as the food landed on the clear and ambered colored water, the small particles scattered rapidly over the entire surface. It was noted that this surface tension reaction did not take place in the dishes where the water was clouded.

TABLE III

QUANTITIES OF FOOD FOR EACH INSTAR REQUIRED TWICE DAILY FOR 250 LARVAE

	MILLIGRAMS OF FOOD PER DISH OF 250 LARVAE, FED TWICE DAILY			
	INSTARS			
	1	2	3	4
1. Slow Growth Low Mortality Water Clear	10	20	50	75
2. Normal Growth Low Mortality Water Clear	20	30	60–80	80–90
3. Normal Growth High Mortality Water murky plus scum and suspended flaky food material	30	40	90	100

Before the shallow water technique was proven to be superior to that of using deep water, a study was made of the possibility of changing the physical properties of the food so that it would float on the surface for several hours. After numerous trials this was accomplished by thoroughly mixing 160–175 ml. of water with a mixture of 50 grams of powdered dried brewer's yeast and 50 grams of finely ground dog food using an electric kitchen mixing machine. A teaspoonful of the resulting paste was then placed in the center of one-half of a sheet of waxed paper 6 x 12 inches. The other half of the paper was folded over the mixture and pressed between two panes of glass. This quantity of food mixture made a very thin layer six inches in diameter when treated in this way. When all of the food mixture had been pressed, the halves of the papers were separated and allowed to dry, after which they were crumpled to remove the flakes of food. These were broken up

[2]Laboratory model "Micropulverizer" with Herring bone Screen No. 035H B. S1.

so as to pass through 30 mesh screen, but in breaking them up some fine particles were produced and these were removed by sifting through an 80 mesh screen.

A check was then made on the relative merits of flaked versus powdered food in deep and shallow water. Two Pyrex trays were filled with 150 ml. of water and two with 600 ml. of water. Into each tray was then counted 255 larvae. The trays were fed once daily the same quantity of food. The results are given in Table IV. Repeated tests gave similar results.

TABLE IV

A Comparison of Rate of Development and Mortality in Shallow and Deep Water Using Flaked and Powdered Food

Number Days After Hatching	Number Pupae Collected			
	Powdered		Flaked	
	Shallow	Deep	Shallow	Deep
10	.....	.....	.....	.....
11	42	7	30	12
12	125	56	89	48
13	65	82	84	75
14	1	18	42	59
15	.....	13	8	36
16	.....	4	1	15
17	.....	1	.....	3
Total pupae	233	181	254	248
Percentage Mortality	8.6%	29.0%	0.39%	2.7%

The results indicate that when powdered food was used the shallow water technique was superior to that of using deep water. Where flaked food was used very little difference in mortality resulted. The pupae from the deep water trays receiving powdered food were noticeably smaller. Since making flaked food is an additional chore its use is not recommended even though in this test it gave slightly lower mortality than powdered food in the shallow water trays.

Pupae.—The pupae were removed daily by means of a wide mouth medicine dropper and placed in four to six inches of fresh tap water in jars six inches in diameter. Jars this size will accommodate 600–800 pupae. Following removal of pupae the larvae from the last two or three dishes were combined into one dish to conserve space. The above described technique produced a daily average for the 7½ month period from December 1, 1944, to July 22, 1945, of 490 pupae with 94.8 per cent emergence and peak pupation in from 10 to 13 days after hatching.

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