THE DISPERSAL OF *AEDES TAENIORHYNCHUS*

III. Study Methods for Migratory Exodus

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Earlier studies of brood dispersal in *Aedes taeniorhynchus* (Provost 1952, 1957) had supported the thesis ascribing ultimate dispersal in migratory mosquitoes to an initial non-appetential or non-searching migration and to subsequent appetential or searching flights (Provost 1953). Subsequent investigations by members of this laboratory have confirmed the occurrence of special migratory flights initiated at the emergence site in a departure referred to as a migratory exodus (Nielsen 1958; Nielsen and Nielsen 1953; Nielsen and Haeger 1960; Haeger 1960). Methods of studying the migratory exodus, other than the important unaided observation, were especially advanced in two experiments on the east coast of Florida, one near Fort Pierce in which the mosquitoes were marked and the other near Vero Beach with unmarked mosquitoes. These two experiments are here described for the primary purpose of placing on record the techniques applied to the problem of mosquito dispersal at this laboratory.

A. The Fort Pierce Experiment

In the summer of 1952 an exodus study was undertaken in Ft. Pierce on the mid-east coast of Florida in combination with a study of marking techniques. Larvae were to be marked with radioactive phosphorus (cf. Provost 1952, 1957) and the resulting radioactive adults, in smaller groups, with aniline dyes in the dust formulation used by Clarke (1938). The experiment yielded valuable information on (1) mass egg production, (2) rearing technique, (3) marking with radioactive phosphorus, and (4) capture of adults in stationary nets at exodus.

1. *Egg Production*

The method of sod-transfer used in our second dispersal test on Sanibel Island (Provost 1957) was used and circumstances forced us to bring the egg-laden sod here from Sanibel, 180 miles away. But Sanibel Island by midsummer was all under water with no readily available egg deposit, although adult mosquitoes were phenomenally abundant. It was decided to drain an amenable area and thereby induce the adults to lay eggs there. The "Duck Pond" was selected, the same area from which sod had been taken for the June experiment (q.v.).

On July 15 an earth dam was thrown up to dike off a 250-foot length of swale and a ditch dug down the center of the narrow swale. Near the road a sump was dug 3–4 feet deep and 5 feet in diameter. A small pump was then set up which pumped the water out of the hole and delivered it across the road. It took ten days of almost continuous pumping to remove the standing water and the water seeping in through and under the dikes. During the two weeks of pumping, July 24 to August 7, rains were frequent.

On August 4, samples of the *Philoxerus vermicularis* sod averaged 2383 eggs per square foot. The sod for transfer to Ft. Pierce was cut off by shovel in approximately square-foot chunks about three inches thick. It was placed on 80 2x8-foot masonite litters. The litters were stacked on two flat-body trucks. The 1280 square feet contained an estimated three million eggs. The sod remained on the trucks in storage until September 5. Testing showed unappreciable loss of viability in these eggs over the 32 days of storage. Small portions of the sod were retained after the Ft. Pierce experiment. After five months there appeared to be little loss of eggs. By the sixth month, however, a great loss of via-

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bility had occurred, most eggs having collapsed.

2. *Larva Production*

The marking was done at a grassy clearing surrounded by salt marsh and mangrove on the eastern shore of the Indian River some five miles northeast of Ft. Pierce. This meadow on the McConville property was described in detail by Nielsen and Nielsen (1953) in their initial paper on the behavior of *Aedes taeniorhynchus* (q.v.).

A special larval nursery ditch was dug by dragline on the very edge of the Indian River so that a small pump could move water either out or into the ditch, a high dike being thrown around it to keep out any possible high water. The ditch was 15 feet wide and 80 feet long, oriented east-west, with the bottom 18 to 24 inches below the ground level at the site. A sump three feet deep was dug along the west or river side to facilitate pumping. On September 4 the pumping was begun into the river, aimed at having the ditch bottom free of water before dawn of the next day.

The plan called for producing two larval broods spaced 8 hours apart at egg-hatching. On September 5 at $05^h30'$ one truck-full of egg-laden sod was laid, like a lawn, on the eastern half of the ditch. The transfer of 640 square feet of sod from truck to ditch took one hour. The sod on all but the uppermost litters was quite moist but the vegetation (mostly *Philoxerus*) was all yellowed. There was evidence of mildew on the sod but no disagreeable or acid odors. One difficulty encountered was that the soil had glued itself to the masonite so that machete blades had to be run under the dirt to facilitate sliding the sod off.

As soon as the sod was all in the ditch, a dike was built separating the ditch into two pools, and the one just filled with sod became Pool I. The pump was reversed and water brought into Pool II. When the water was a foot deep the cross-dike was broken at one end and all the water allowed to flow over the sod in Pool I. The dike was then repaired and another foot of water added to Pool II, and the procedure repeated. This was done until the water in both pools was close to the surface of the ground. In this manner there was always a strong current from Pool II to Pool I, making it impossible for any early hatching larvae to work their way into Pool II. At $09^h00'$ the cross-dike was about 8 inches above the water table. All the sod in Pool I had been flooded between $08^h07'$ and $08^h30'$.

At $14^h30'$ the pump was started and drained Pool II. Again it took about an hour to transfer the sod from the second truck to the ditch bottom. The pump was reversed and Pool II was filled with water from the Indian River. The sod in Pool II was all flooded between $16^h02'$ and $16^h30'$. The water was left at the same level as that in Pool I. It was noted at this time that the water in Pool I, having stood in the sun eight hours, was much warmer than the water just introduced into Pool II from the river.

In Pool I some eggs hatched almost immediately on flooding but it was apparently several hours before the hatch was complete. The hatch was not nearly as synchronous as was the case in the Sanibel experiment in June (Provost, 1957) although actually the flooding was much quicker in the present case. The 8-hour initial lag of Pool II behind Pool I seemed to increase from the standpoint of larval development as time went on, probably because the 8-hour advantage of Pool I was during the heat of the day.

The shallow, warm water resting on the rich organic substrate from which the eggs had hatched resulted in a food-rich situation in which the larvae grew apace.

3. *Marking Methods*

Hurricane threats forced us to postpone the experiment twice. The 80 mc of P^{32} shipped from Oak Ridge on the morning of August 25 arrived at Vero Beach, by air, Tuesday morning, August 26. The acid was diluted to one liter on the afternoon of September 8 and divided into three equal portions for field use.

At $15^h00'$ on September 8 the larvae were seined out of Pool I and placed in tanks A and B. These were wooden troughs $6' \times 3' \times 8''$. The seining was done as it had been done on Sanibel Island in June (Provost 1957). A lot of broken and loose vegetation was unavoidably transferred also and most, at least of the large pieces, were removed thereafter. By $18^h00'$ it was estimated that each tank held 250,000 larvae in about 5 inches of water. The P^{32} was introduced into tanks A and B at $18^h30'$ and $18^h40'$ respectively.

On September 9 at $05^h30'$ the first task was to set up tank C approximately 30 feet away from tanks A and B. Then 4-1/2 inches of water from Pool II were put into it and the seining of larvae from that pool to the tank was begun. The larvae were balled up in the pool; this permitted seining from the bank, so that much less vegetation and trash were transferred to tank C than had been to tanks A and B. At $07^h45'$ it was estimated that between five and six hundred thousand larvae were in tank C. More seining was done until a good estimate indicated 600,000. The P^{32} was introduced at $08^h15'$.

By the time it was used, the P^{32} had so decayed that tanks A and B received 13 mc each, and tank C, 12-1/2. With 5 inches of water in the tanks, the initial P^{32} dosage became .065 μc/ml in tanks A and B, and .063 μc/ml in tank C but .052 μc/larva in tanks A and B, and .021 μc/larva in tank C. The latter tank therefore compared favorably with two of the tanks on Sanibel Island in June where the dosage was .077 μc/ml and .022 μc/larva. However the latter produced adults yielding 500 CPM for males and 2,000 CPM for females, whereas the adults for tank C of this experiment yielded adult activities of 4,377 CPM in males and 6,371 in females. The causes for this great difference will be discussed below. In tanks A and B of the present experiment the adult activity at emergence was 6,428 CPM in males and 10,866 CPM in females. By final comparison, in the August 1951 experiment on Sanibel Island (Provost 1952), dosages of .066 to .101 μc/ml but adjusted to .019 μc/larva yielded adults which at emergence had activities averaging 715 CPM for males and 3200 CPM for females.

In the 1951 experiment (*q.v.*, Provost 1952) water and larvae from each of the four tanks were placed into five beakers per tank immediately after introduction of the P^{32} into the tanks, and both water and larvae were monitored every six hours from all four tanks and twenty beakers. This study revealed that ingestion of isotope was proportional to dosage per water unit and not to dosage per larva, which is logical. Monitoring of pupae, pupal skins, and adults showed that the skin's activity was only 8.3 percent of the adult in the female, compared to 15.6 percent in the male—evidence that the usual higher activity of the adult female is the result of her greater feeding activity in the larval state. It was also demonstrated then that larval increments of radioactivity per time unit were proportional to larval size. In this 1951 experiment the larvae became hungry and two of the four tanks were fed dog biscuit. The maximum larval radioactivity was 7,500 CPM before feeding and 9,500 CPM after feeding. In the unfed tanks the same maximum, 9,500, was reached, but two days later. One unfed tank was retained for observation after the big emergence and all larvae eventually died, with no further pupation. A further finding of the 1951 experiment was that 16 percent of total adult radioactivity was in the legs, while the percent activity in the head-thorax-abdomen was 12–61–11 for males and 6–31–46 for females. While the total ratio of radioactivity in females over males was 5 to 1, it was 2 to 1 in the head and thorax and 22 to 1 in the abdomen.

Returning to the Ft. Pierce 1952 experiment, by $09^h00'$ of September 9 the larvae which had been overnight in tanks A and B showed pronounced signs of hunger, and by $11^h00'$ so did those in tank C after only three hours in that tank. All were fed ground dog biscuit. By late September 11, 50 ounces of dog biscuit had been fed these larvae or 1.3 mg per larva. This heavy feeding was the result of transferring the larvae from pools to tanks in the late third instar instead of in the early or middle fourth instar. It also accounted for the high activity of adults compared to the June experiment on Sanibel Island when the transfer was when they were 16 to 24 hours old as fourth instars (Provost 1957). The record of feeding in the three tanks is shown in Fig. 1, showing the accompanying rise in larval radioactivity.

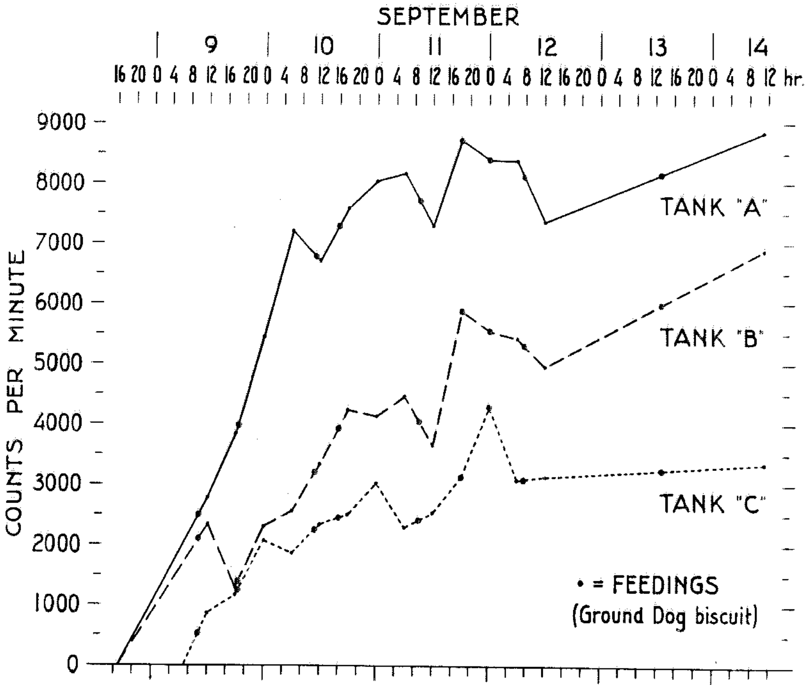

Fig. 1.—Uptake of radioactivity by *A. taeniorhynchus* larvae in three tanks. Ft. Pierce, 1952.

The experience of three radioactive-marking experiments leads us to the following conclusions. Transferral from pools and natural food to tanks for radioactive-marking may be done either in the late third or fourth instar. The earlier it is done the more artificial feeding will be necessary and the greater will therefore be the threat of mortality in the tanks. If the exigencies of the case have resulted unavoidably in a necessarily low P^{32} dosage, transferral in the third instar may be necessary for good marking. If sufficient P^{32} is on hand, however, good marking with little or no feeding and much reduced mortality can be better achieved by delaying transferral to the tanks until the larvae are well into the fourth instar and compensating by an increased P^{32} dosage.

4. *Exodus Study Methods*

By noon of September 10 pupation had advanced to 50 percent in the warm waters of Pool I but only to 10–25 percent in the shaded tanks A and B. No pupae were found in Pool II and in tank C. Because of feeding complications, heavy rains, and the failure of dyeing methods it proved impossible properly to follow the course of pupation in the tanks. Adult emergence began on September 12, with a minor exodus that evening at twilight. There was a larger exodus at twilight on September 13 and a slightly lesser one at twilight on September 14. Observations of behavior during exodus, including the first observation of mass mating, are reported elsewhere (Haeger 1960).

Recovery of marked mosquitoes for the first few days of exodus was attempted by stationary nets, at the meadow discussed below, and by the usual truck traps, rotor traps, and light traps within five miles. Three rotor traps, three truck traps, and 12 light traps were in operation. In addition a large funnel mounted on a fast boat was operated up and down the Indian River for two miles north and south of the release point. It caught no marked mosquitoes. An attempt was made to sample the air 50 to 100 feet up by a Piper "Cub" plane equipped with funnel nets. This also proved abortive.

The June experiment on Sanibel Island had convincingly shown that the insects in the process of migrating could not be adequately sampled by any of the common collecting devices: light traps, truck traps, or bait traps. The rotor trap 40 feet from the tanks did catch departing migrants, however, and taught us that at least the migratory exodus could be better studied with collecting devices.

For the Ft. Pierce experiment, we employed the stationary nets described by Nielsen (1960). The large funnels are specially designed to trap wind-borne or migrant insects while not retaining many insects in ordinary searching flights. Four pairs of these two-meter square nets (Fig. 2) were mounted, one pair as near as terrain permitted in each of the four cardinal points, 75 feet from the tanks and facing them. The low net was centered 6 feet above the ground and the upper net, directly above it, 15 feet above the ground.

Collections made at various hours of the night with these traps are summarized in Table 1. There was a very high density of wild, unmarked mosquitoes on the meadow, making all work there very painful unless repellents were used. Not having operated a rotor trap there (no electricity), however, we had no measure of the ratio of newly emerged, marked mosquitoes on the exodus nights to the wild, unmarked mosquitoes. For the three successive nights of operation the percentage of mosquitoes marked, in the stationary nets, was (♂ — ♀) : 89–1, 47–14, and 49–31 for a total of 56 percent of the males and 18 percent of the females. The unmarked could have represented some escapes from the rearing pool residual which we had unwisely not destroyed. The lower nets yielded a higher percentage of marking in the males than the upper

TABLE 1.—Captures of *Aedes taeniorhynchus* in stationary nets, September 12–15, 1952. Ft. Pierce experiments

		Directional									
		N		E		S		W		Total	
		Lo	High	Lo	High	Lo	High	Lo	High	Lo	High
♂	wild	5	3	5	2	11	49	1	3	22	57
	marked	6	1	14	2	21	42	9	6	50	51
♀	wild	53	12	73	21	35	55	31	35	192	123
	marked	1	0	4	0	12	50	0	2	17	52
percent marked	♂	55	25	74	50	66	46	90	67	70	47
	♀	2	0	5	0	26	48	0	0	8	30
♂	wild	8		7		60		4		79	
	marked	7		16		64		14		101	
♀	wild	65		94		90		66		315	
	marked	1		4		62		2		69	
percent marked	♂	47		69		51		79		56	
	♀	2		4		41		3		18	

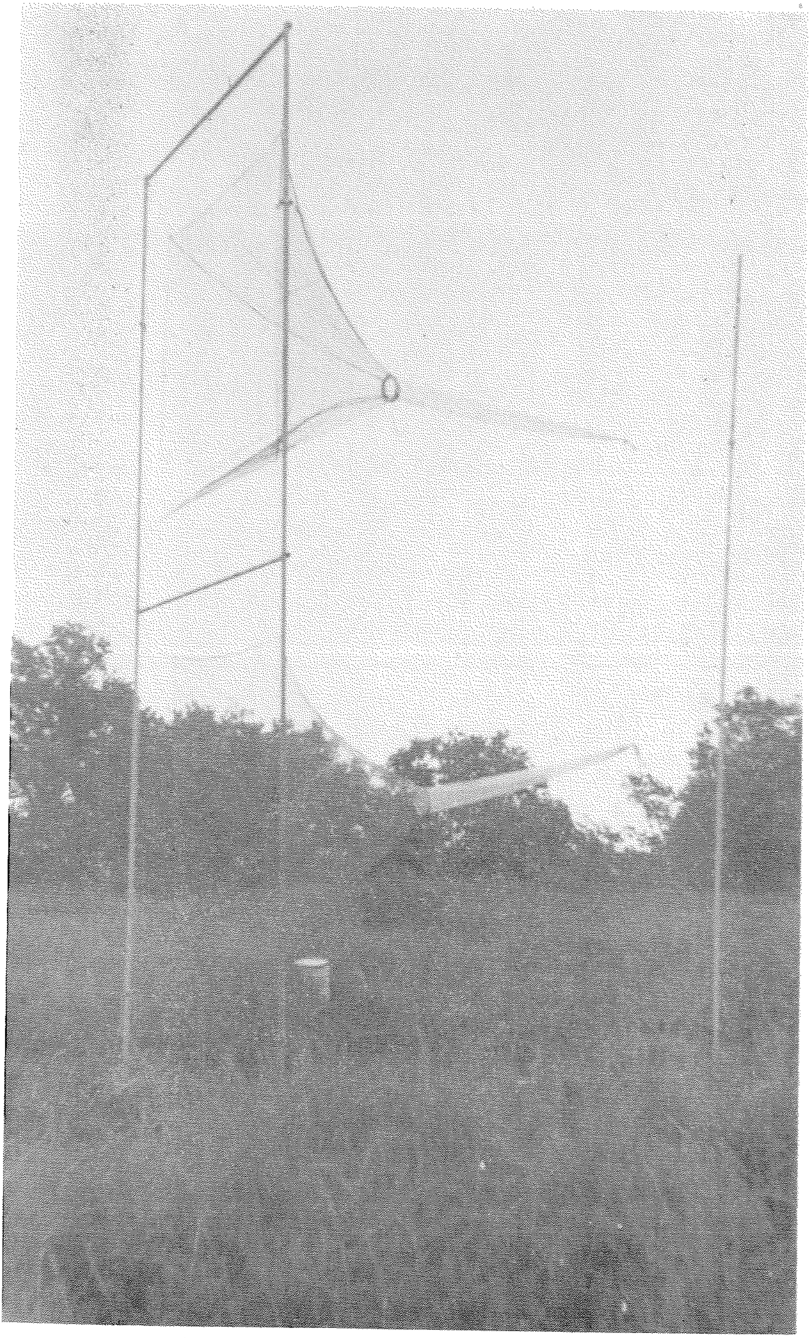

FIG. 2.—Stationary nets as set up for interception of mosquito exodus.

nets, whereas this difference was pronouncedly reversed in the females. The sex ratio in the upper nets, 39 percent males, compared to the lower nets, 26 percent males, seemed to indicate a higher flight for the males, but this conclusion was thrown in doubt by the much higher percentage of males in the lower nets among the marked mosquitoes, 75 percent *vs.* 50 percent. With respect, therefore, to height of migratory flight, the stationary nets yielded inconclusive data, but the possibilities of the method were shown to be excellent.

With respect to direction of exodus, the stationary nets showed themselves most useful. The nets were slightly clock-wise deviant from the cardinal points, so that the south net was actually SSW. The wind during the exodus nights was NE. Disregarding heights, the percentage of total marked catches in the south net was (64/101) 64 percent for males and (62/69) 90 percent for females, indicating a strong downwind displacement. The unmarked, as percentages, were 10 percent N, 9 percent E, 76 percent S, and 5 percent W for males and 20 percent N, 30 percent E, 29 percent S, and 21 percent W for females. The unmarked males followed the same southerly trend as the marked mosquitoes, but the omnidirectional trend of the unmarked females suggested that searching flights of older, non-migrating females were the source, this being borne out by their preponderance in lower nets, 192 out of 315 or 61 percent so caught. The probable reconstruction is therefore that migrants of both sexes went downwind and were mainly intercepted by the upper nets, whereas the older non-migrant females were about their searching flights in all directions and were mainly intercepted by the lower nets. The unmarked males, most likely newly emerged from the breeding pools, also went downwind and were caught mostly (72 percent) in the upper nets. These conclusions are highly speculative and are given only to emphasize the potential usefulness of these stationary nets.

By contrast with the results obtained at the meadow, distant collecting methods were quite unsuccessful. Truck traps (cf. Provost 1957) operating throughout the exodus nights over three routes within five miles of the meadow yielded only three marked specimens, a female on the beach road within 2 miles of the meadow to the southeast (Sept. 15 between $06^h00'$ and $06^h10'$), a male on the river road 2 miles southwest (Sept. 15 between $05^h50'$ and $06^h02'$), and a second male on the ridge road 3 miles west (Sept. 14 between $05^h29'$ and $05^h37'$). Three rotor traps, 2 miles north, 2 miles southwest, and 2-1/2 miles southeast, operating all night yielded no marked mosquitoes. The light traps yielded marked females only, and these only starting the third night after the first exodus when 13 were recovered. On the fourth night 31 were caught, on the fifth only 2. The traps were not run beyond this as we were not studying the ultimate dispersal. Directionally, 34 out of 46 marked females recovered, or 74 percent, were north; 8 were south, 2 southeast, and 2 west. There is no indication that this distribution reflects the final distribution, as the twilight exodus three nights in a row indicated a very likely migration far beyond the light traps operated, especially to the southwest.

B. The Vero Beach Experiment

In October 1958 an experimental study of a natural *Aedes taeniorhynchus* emergence near the laboratory was undertaken as a test of various exodus study techniques to be employed in future dispersal experiments. The emergence was in a half-acre slough grown mostly to Sea Oxeye (*Borrichia frutescens*) and surrounded by cabbage palms, oaks, and other medium-sized trees. The hatch resulted from the windblown spring tides of October 7 and 8. Water temperatures averaged 24° C. during the larval period. Pupation started on the morning of October 13. The weather was very uniform during the main pupal interval, October 13–16, with water temperatures varying little from 25° C. Using information now available on pupal duration (Nielsen and Evans, MS) and age at

Fig. 3.—Kytoon net for upper-air sampling of mosquito exodus.

exodus (Nielsen, 1958; Haeger, 1960) it was predicted that the first emergence peak would be about 20^h on October 15, or a good 2 hours after sunset and over 10 hours before sunrise, presenting thus the likelihood of a middle-of-the-night exodus.

On October 15 emergence started at $18^h30'$ and increased quickly to a high level lasting from 20^h to $22^h30'$. By the latter time the grass and palmettos were covered with resting adults. Assuming that an exodus of a gradual type would occur, peaking at 03^h, word was sent out for observers to gather and a Kytoon net to be in readiness. This net (Fig. 3) for sampling upper-air strata was as described in Nielsen and Greve (1950) but instead of a sounding balloon a steadier "Kytoon" was used. This device was to be manned by two men and operated downwind from the point of exodus.

No exodus occurred. A few individuals trickled away between 01^h and 04^h which could have been disturbed by the observers. No major activity developed during the morning twilight either. For watching both resting and flying mosquitoes through the night 12-volt spotlights with yellow filters proved very satisfactory. The Kytoon collecting did not, of course, materialize. Older mosquitoes, from a previous brood, were biting badly enough that most observers used repellents. A sample of the new mosquitoes, taken at 01^h, was taken alive and retained several days to establish egg maturation without a blood-meal; 38 percent were autogenous. Sweep net samples of the resting mosquitoes (Table 2) between $01^h35'$ and $05^h30'$ yielded 17 percent to 27 percent females, and these females were from 30 percent to 60 percent mated (by spermathecal examination). Since midday collections the next day at the same site yielded only half as large a female ratio and all of them unmated, it is evident that about half the females collected at night were old females not belonging to the new emergence. The males in these same sweep net collections revealed sexual potencies (hypopygial rotation $\geqq 135^\circ$) increasing from 3 percent at $01^h35'$ to 15 percent at $05^h30'$, indicating that by sunrise ($06^h23'$) less than one male out of five was potent.

Not having left during the night, for unaccountable reasons, the new mosquitoes spent the daytime, October 16, at the emergence site, assuring a twilight exodus which did, of course, occur. Daytime collections gave a truer sex-ratio for the first day's emergence (Table 2): 7–8 per cent females. By $15^h45'$ the mosquitoes were averaging 20 hours of age; the females were still 100 percent virgin, even though by then two-thirds of the males were potent. Late in the afternoon, females coming to probe were collected and examined chromatographically for age;[2] at $16^h30'$ 36 percent were under 24 hours of age but at $18^h00'$ only 3 percent were of this age. This seemed to indicate that even though many of the new females came to a blood source, the great majority of biting females, especially near sunset, were old ones not belonging to the emergence under study. Both young and old coming to bite probably did so only after disturbance had set them flying, however.

In anticipation of the exodus at twilight, J. S. Haeger set out two "sticky nets" of the type he had recently been experimenting with. These were 22-inch round nylon nets covered with adhesive, 20 feet above the ground, one south and the other west of the emergence site. The nylon net averaged 11 hexagonal cells to the linear inch, and the adhesive formula was:

1-1/2 lb. amber gear grease
1 qt. #20 motor oil
1 pt. mineral spirits

By $17^h50'$ all was in readiness for observation of the twilight exodus: sticky nets, Kytoon standing by, spotlights, nets, pill boxes, forceps, etc., etc. Daytime temperatures had averaged 27° C., the relative humidity 60 percent, and the wind out of the west at 5 to 7 miles per hour.

Sunset was at $17^h52'$. During the twilight interval the wind remained from the

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[2] Aging by chromatography is being developed as a technique by Dr. P. T. M. Lum. It has not yet been published.

TABLE 2.—Summary of collections during October 1958 *Aedes taeniorhynchus* exodus study at Borrichia Slough. Collection symbols: Sw = sweep net, Asp = aspirator, St = sticky net

		October 16											17		
		Sw 0100	Sw 0135	Sw 0230	Sw 0330	Sw 0430	Sw 0530	Sw 1200	Sw 1545	Asp 1630	Asp 1800	St 1810	Sw 1035	Asp 1630	St 1810
Total	♂		201	239	74	153	114	121	117			282	870		45
	♀		41	49	27	39	24	9	10			73	1023		131
	% ♂		83	83	73	80	83	93	92			79	46		26
Males	Rotation of Hypopygia														
	0°		50	47	12	24	7	4	0			0	32		0
	45°		63	89	16	42	14	5	0			11	172		0
	90°		33	34	13	30	14	81	39			36	358		11
	135°		4	4	1	7	5	25	56			112	40		18
	180°		1	0	0	3	1	1	18			73	4		14
	% mature		3	2	2	9	15	22	65			80	7		75
	Damaged		50	65	32	47	73	5	4			50	264		2
	% damaged		25	27	43	31	64	4	3			18	30		4
Females	Virgin		5	4	7	5	7	9	10			15	80		75
	Mated		5	6	3	5	3	0	0			5	0		51
	% "		50	60	30	50	30	0	0			25	0		41
	> 24 " old									19	29			8	
	< 24 " old									11	1			52	
	% "									36	3			86	
	Ov. advanced	14												4	
	Ov. not "	23												7	
	% autogenous	38												36	

east but increased slightly in velocity, 8–9 miles per hour with gusts up to 18 miles per hour. The temperature fell to 24° C. and leveled off. The relative humidity rose to 90 percent. Barometric pressure was quite stable at 1016 millibars.

Although a few specimens left between $18^{h}00'$ and $18^{h}05'$ the mass exodus was at $18^{h}06'$, or 14 minutes after sunset. The mosquitoes appeared to rise almost straight up, 15 to 20 feet, before being carried away by the northeast wind. Exactly simultaneously, thousands of males began swarming above the tops of every tree, utilizing every branch tip and palm frond. No one observed what could be surely identified as mating in these numerous and large swarms—even with the combination of amber spotlights and binoculars which made examination of the more distant swarms most satisfactory. The virtual eruption of swarms at the precise moment of mass exodus, the ubiquity and large size of the swarms, and the absence of mating—all these were most impressive.

The strong gusts of wind made the Kytoon net collecting impossible, but the sticky nets caught 73 female and 282 male *A. taeniorhynchus* during the short interval ending at $18^{h}30'$. Since the resting population at midday was 93 percent and 92 percent male (Table 2) and the sticky-net catch 79 percent, one wonders whether the missing 13 percent may not represent those males which went immediately to swarming, especially since the same happened the next evening (see below). Of the 232 males with determinable hypopygial rotation, 80 percent were complete ($\geqq$ 135°), the estimated age (by temperature correlation) of all males at exodus time averaging 21 hours. The females caught in these sticky nets were 25 percent mated. Since the resting females were 100 percent virgin at $15^{h}45'$ there appears to have been some mating on the exodus—if we assume the nets to have caught only the new females. Of the two nets, the south one picked up 77 percent of the collection and the west one 23 percent. The wind data were inadequate for us to relate these catches to wind direction.

The salient points of these twilight observations were, then: (1) the males were 92 percent of the resting population, by sweep net, and 79 percent of the departing population, by sticky net; (2) the specimens averaged 22 hours of age at sunset and 80 percent of the males were judged able to copulate, i.e. had hypopygial rotations of over 135°; (3) the resting females were 100 percent virgin while those departing, as represented in sticky nets, were 25 percent mated; (4) simultaneously with the mass exodus, 14 minutes after sunset, there was a mass and most impressive formation of male swarms; (5) no mating was observed in these swarms.

Observations the next day, October 17, showed the expected rise in female ratio among the second day's emergence; at $10^{h}30'$, sweep-net collections gave a 54 percent female figure (compared to 8 percent the first day). Of the males, 7 percent only had completely rotated hypopygia, indicating clearly that virtually none of the first day's males remained among these. At $16^{h}30'$ probing females were collected and 86 percent (52/60) were under 24 hours of age (by chromatography). That so many neonate females should be seeking blood was surprising. A subsample of this collection revealed 36 percent autogeny, a figure close enough to the previous day's to make it quite certain that roughly a third of the brood under observation was autogenous.

The sticky nets had worked so well it was decided to test the technique in a more elaborate manner at the anticipated second twilight exodus. Sticky nets were made 1 × 2 feet and suspended in pairs arranged in a 90° V so that when made sticky on both sides an exposure to each of the four cardinal points resulted for each V (Fig. 4). The nets, 34 of them, were fastened to 1 × 2 foot aluminum wire frames and attached at 10-, 20-, and 30-foot levels on three 30-foot poles and four 20-foot poles. These were set out in the slough in as strategic a manner as possible to intercept the exodus.

The weather during civil twilight was like the day before except for a considerable lessening of the wind; it was still northeast but only 2–3 miles per hour. The exodus took place between $18^h05'$ and $18^h15'$. It did not appear to be as rushed as the night previous, and opportunity was afforded to see some mating while the departure went on. The exodus appeared to be mainly toward south and west.

All sticky nets together gathered 176 mosquitoes, the males representing 26 percent compared to the previous night's 79 percent. Again the proportion of males in the exodus was less than in the resting population (26 percent : 46 percent), supporting the probability that many males did not depart. By the hypopygial rotation criterion, 75 percent of departing males were potent, compared with 80 percent the previous night. By the spermathecal criterion, 40 percent of the females were mated, compared to the previous night's 25 percent. On the whole then, this exodus generally resembled that of the first night, the major difference being that the proportion of females was greater and more of them were mated.

The Kytoon net worked well this evening. Sampling from 30 to 200 feet above the ground downwind from the exodus yielded nothing. Between 20 and 50 feet some mosquitoes were caught but were found escaping through the netting as fast as caught. The same could have happened, undetected, at the 30- to 200-foot collecting. Nothing was learned except that the method had definite possibilities for establishing the vertical distribution at exodus. Certain modifications of the net were obviously indicated.

The sticky nets were taken down after the exodus, the operation lasting from $18^h45'$ to $21^h00'$. The nets were labeled and laid between sheets of paper, then rolled into a large bundle and taken to the laboratory. Experimentation showed alcohol best for preserving the mosquitoes after removal from the sticky nets. Although the technique was experimental and the rolled-up catches remained in the bundled condition 2-1/2 days before the mosquitoes were removed from the nets, 96 percent (43/45) of the males were satisfactorily examined for degree of hypopygial rotation while 95 percent (126/132) of the females were satisfactorily examined for presence or absence of sperm in the spermathecae.

The potential of this sticky net method of studying migratory departures is evident from the detailed study made in this instance but not here recorded, involving such things as height and direction of flight as differing with sexes, mated state, age, etc., etc. This information is summarized here as follows: (1) the upper nets and downwind traps caught more mosquitoes than the lower and the upwind, this pattern being more pronounced in males than in females. (2) There was some circumstantial evidence that the lower and the upwind nets caught a considerable percentage of older females flying appetentially. (3) There was some evidence that swarming males got caught in the uppermost nets.

This experiment at the Borrichia Slough was therefore successful in demonstrating what can be learned about a migratory exodus of mosquitoes without marking. A three-pronged attack appears indicated: (1) *Observation*, to be concerned especially with the time sequence of the brood's development, i.e., the rhythm of pupation and emergence, the twilight flight behavior, etc., and with the simultaneous gathering of weather data. (2) *Collecting*, to be done on a strategic rather than routine schedule, and utilizing especially the sticky nets, flying nets (*cf.* Ft. Pierce experiment, above), Kytoon nets, rotor nets, sweep nets, and others as indicated. (3) *Laboratory determinations*, as exhaustively as possible to establish sex, age and physiological state of all recovered mosquitoes, with resort to spermathecal and ovarian examination of females, including testing for autogeny, to hypopygial examination of males for age determination, and to chromatography or any other aging technique regardless of sex.

C. SUMMARY

Field techniques for the study of a mosquito migratory exodus are described.

FIG. 4.—The directional sticky nets as used in the Vero Beach 1958 mosquito exodus study.

The production and marking of mosquitoes in large numbers is described. The possibilities of various collecting devices for use at exodus time are discussed and exemplified. The use of methods to determine age and physiological state of field-caught mosquitoes is discussed and exemplified also. Several migratory exodi are described.

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