REPRODUCTIVE BEHAVIOR OF *CULEX PIPIENS QUINQUEFASCIATUS* RELEASED INTO A NATURAL POPULATION[1]

R. E. LOWE, H. R. FORD, B. J. SMITTLE AND D. E. WEIDHAAS

Insects Affecting Man Research Laboratory, Agr. Res. Serv., USDA, Gainesville, Florida 32601

ABSTRACT. A study was conducted to determine the longevity and reproductive behavior of adult *Culex pipiens quinquefasciatus* Say mosquitoes. Two releases of radioactively labeled (^{32}P) mosquitoes (50,000 and 27,000 females, respectively, plus equal numbers of males) were completed 7 weeks apart on Seahorse Key, an island in the Gulf of Mexico near Cedar Key, Florida. There was a definite cycle of oviposition after the first release, with radioactively tagged rafts first collected 5 days after the release. Peak numbers of rafts were recovered first on day 8, then every 4th day, until the 29th day, when radioactivity had degraded to undetectable levels. Of the total of 9,956 egg rafts collected, 8,869 were radioactive, and 1,087 were untagged, that is, they had been oviposited by the indigenous population. Thus, the return was 17.7 rafts per 100 females released. After the second release, the first radioactive rafts were collected 4 days after release, the first peak occurred the 7th day, and subsequent peaks occurred in a 3-day cycle. A total of 2,490 rafts was collected over 21 days, 2,033 radioactive and 457 indigenous, that is, 7.5 rafts per 100 females released. The longevity of the released females was apparently shorter during the second study.

INTRODUCTION. Recent advances in methods of controlling populations of insect vectors of disease other than chemical methods have made it necessary to gain a broader knowledge of population dynamics of such mosquitoes in nature. We already do have a considerable amount of data from controlled laboratory tests concerning the rearing of many species, the survival of various stages, adult longevity, and egg laying capacity. In addition, information is available concerning flight range, mating activity, and host feeding preferences in the field. However, if we are to utilize new methods of biological or integrated control, we must also have a thorough knowledge of the dynamics of a population in the field, we must be able to understand and predict the habits and population fluctuations of a pest species over time, and we must be able to relate these data to the environmental pressures.

Lindquist *et al.* (1967) used ^{32}P-labeled *Culex pipiens fatigans* Wiedemann in a test in Burma and reported that dispersal of this mosquito was sufficient so that population control by sterile male release was feasible. Patterson *et al.* (1970) found that sterile male releases could eliminate a population of *Culex pipiens quinquefasciatus* Say if the males were required to disperse over only short distances, and Weidhaas *et al.* (1971) reported comprehensive data concerning the biotic increase of this species. Also, Hagstrum (1971) studied the survival and dynamics of larvae and adults of *Culex tarsalis* Coquillett in an isolated population.

More recently, Weidhaas *et al.* (1973) reported preliminary experiments in which small numbers of *C. p. quinquefasciatus* tagged with ^{32}P or sterilized were released daily to determine the egg-laying potential and survival of females and the migration of males. They found that the first oviposition period usually occurred about 8 days after the females emerged and that only about 10 percent of the females laid eggs. Also, a few females mated with sterile males that had been released 0.5 mile from the females. However, their studies were mainly concerned with the development of the technique of sterile male release and did not provide information concerning patterns of oviposition or survival.

In the present paper, we present the re-

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA.

sults we obtained from two releases of *C. p. quinquefasciatus* tagged with ^{32}P. The experiments were designed to determine the ovipositional patterns and adult longevity at different times of the year. Since each single release involved a large number of adults (100,000 and 54,000), the observations were made on mosquitoes of a known age. In a companion paper, Smittle *et al.* (1973) discuss techniques of rearing and labeling of the mosquitoes and the assay of the radioactive egg rafts.

Experimental Location and Methods. The area selected for the study was an island, Seahorse Key, off the Gulf Coast of Florida near Cedar Key. Several factors make this site excellent for a biological study: (1) it is large, a mile long and as much as ¼ mile wide; (2) it is isolated, since it is separated from the nearest land by 2 miles of salt water; (3) it is a wildlife refuge, so birds and other animals are available for the blood meal required by the mosquitoes; (4) *C. p. quinquefasciatus* occur naturally; and (5) the mosquito breeding potential of the island has been well-defined by previous sterile male releases. Permission to use Seahorse Key for these studies was granted by the USDI, Fish and Wildlife Service, and the University of Florida, which maintains a marine laboratory at the location.

The island has a high central ridge running lengthwise and is densely foliated except for a T-shaped clearing that extends from the marine laboratory near the dock to an old lighthouse at the top of the hill. No important natural fresh water breeding areas are present except a septic tank and a fresh water cistern, and the earlier studies appeared to establish that the populations of *Culex* sp. were confined to the areas near the cluster of buildings.

In the earlier tests, 10-gallon plastic wash tubs containing 2½ gallons of water infused with a mixture of liver powder, brewer's yeast, and hog supplement (1: 1: 1) had proved to provide attractive oviposition sites. Therefore, for the present test, 14 such tubs were placed on the island—7 near the laboratory and adjoining work sheds at the base of the hill, 3 midway up the hill near a generator shed and salt water cistern, and 4 on the highest ridge of the island near the lighthouse. Some tubs were in sheltered areas near the buildings, and others were placed in or at the interface of wooded areas. In addition, one tub was placed on a second island, Atsena Otie Key, 2 miles from Seahorse Key and thus closer to the mainland city of Cedar Key. Most other breeding sites were removed, that is, the septic tank and the fresh water cistern were sealed and any containers, debris in the woods, and large seashells capable of holding rainwater were emptied or destroyed. All tubs were emptied and reset on a schedule, every third or fourth day, to overcome the problem of surface scum and predator breeding and to assure an active infusion that would have the most oviposition attractancy.

The oviposition tubs were set up the first week of April, which was a month earlier than the test species had been collected in recent years. Thereafter, collections of egg rafts were made 3 times a week. The first release was made May 28–30; the second was made July 15–17. Four *Culex* species were collected: *C. p. quinquefasciatus, C. restuans* Theob., *C. salinarius* Coq., and *C. nigripalpus* Theob. All egg rafts were held for hatch, and the larvae were reared to the fourth instar for identification. (None of the larvae were returned to the island.)

Rearing of mosquitoes for the releases was accomplished by using a series of large plastic trays each containing 2000 larvae hatched from approximately 13 egg rafts. For the first release of 100,000 radioactive males and females, 50 trays were maintained on a normal rearing regimen until the larvae reached the third instar. Then they were removed from the infused water, and 2 trays were consolidated into a clean tray containing 3500 ml of clear water with 8 to 10 μ Ci of radioactive phosphorous (^{32}P). This approximated one larva/0.002 μ Ci ^{32}P/

ml in each of the 25 trays. The larvae remained in this solution for 6 to 7 hours, at which time fresh infusion was added as a food source.

As pupation occurred, pupae were separated from larvae and sexed with a pupal separator. Once the relative numbers of each sex had been ascertained, the pupae were placed in enamel pans lined with damp filter paper. (An aliquot of each group was allowed to emerge in the laboratory so we could determine actual sex ratios and numbers.) Then each pan was covered with a plexiglass top, and the pans were transported to the island in a large styrofoam cooler containing ice (to retard activity). On the island, clean water was added to the pupae in each pan, and the pans were placed under the marine laboratory to allow normal adult emergence. The methods of rearing and release for the second study were identical, except that the larvae were treated with ^{32}P for 18 hours (11 to 12 hours longer) to increase radioactivity.

The objectives of the first release were to determine the longevity and behavior of this species. The objectives of the second release were to determine (a) whether seasonal changes influenced behavior, (b) whether the recovery of labeled rafts as much as 30 days after release was correlated to the original number of females released, and (c) whether the radioactive labels were intensified by longer treatment.

All egg rafts in the oviposition tubs were collected and identified before each release, and daily collections were made for assay for 30 days after each release. In addition, three people stayed in residence on the island through the first week after both releases to observe resting locations, flight activity, swarming and oviposition behavior. All egg rafts collected from each oviposition tub after a release were returned to the laboratory in lots of as much as 20 rafts per vial. The assay procedure is reported by Smittle *et al.* (1973).

RESULTS. On April 12, 5 *C. p. quinquefasciatus* egg rafts were collected from Atsena Otie Key, but the species was not found on Seahorse Key until April 20 and did not become prevalent until May 6. By May 20, all rafts collected were *C. p. quinquefasciatus.* Daily collections from this date until the first week of July produced an average of 36 egg rafts each day from the indigenous population of the island.

As noted, the first release of 50,000 tagged females and an equal number of males occurred from May 28 to 30. Adult emergence of these insects was complete by June 1. (Males were released to insure that all females were inseminated.) Prolonged searches in late afternoon and early evening after the releases revealed only a few resting adults, but, by 2015 hours the evenings of May 29 to June 7 numerous adults were evident, and the flight activity increased as darkness progressed with mating swarms occurring until soon after 2100 hours. By this time the swarms became less organized and the adults dispersed into the woods. Most activity occurred the first 4 evenings. This pattern was the same for the second release, including the times at which swarming occurred.

Collection of a sample of adults from the swarms on June 3 showed that the sex ratio was approximately 1: 1. Some of the females appeared gravid and ready to oviposit. The swarms were isolated in sheltered areas between the sheds and under the cistern and always at the lower or middle elevations. Oviposition began about 2130 hours and continued until about midnight, but additional rafts were deposited during the early morning hours from just before dawn until about 0700 hours; also, on cloudy or overcast days, newly laid rafts were collected as late as 1100 hours. Checks made throughout the evening and early morning hours on 2 successive days during peak oviposition showed that approximately 60 percent of the rafts were laid in the evening hours on each of these 2 days.

The first radioactive rafts were collected the morning of June 2, 5 days after the initial release. Subsequently, on days 6, 7, and 8, a rapid increase occurred in the total number of rafts, almost all of which were radioactive. The numbers decreased on days 9 and 10 and increased slightly on day 11, and a second peak occurred on day 12 (Figure 1). Another decrease was registered on days 13, 14, and 15 followed by a small increase on day 16. The numbers of rafts collected were large enough so that a fourth peak was apparent on the 19th day after release, but thereafter the number decreased gradually to day 29 (Table 1). After day 25, more indigenous (untagged) egg rafts than radioactive rafts were collected, and by day 29, the mean counts/minute of radioactivity in tagged rafts was so close to background that it could not be accurately identified.

As Figure 1 shows, a definite cycle of oviposition occurred with peak numbers of rafts present every 4th day. Also, the number collected was greater than expected, perhaps because of the optimum environmental conditions that prevailed. A total of 9,956 egg rafts was collected on the island during the 29 days after release. Of these, 8,869 were radioactive and 1,087 were oviposited by the indigenous population; however, 177 of the indigenous rafts were collected the first 5 days after release before any labeled rafts were recovered. Thus, the average oviposition was 17.7 rafts for each 100 females released. (Rafts collected after day 29 could have been from the released females, but they were unidentifiable because of the degradation of radioactivity.) The fact that the collection of unlabelled rafts (average of 36 rafts per day for 30 days) remained stable indicates that the removal of eggs from the ovitraps did not decrease the indigenous population.

The second release of 27,000 tagged adult females and an equal number of tagged males was accomplished July 15 to 17, by which time radioactivity from the first release could not be detected in any collected rafts (no overlap from females that might have survived from the first release). Swarming was observed

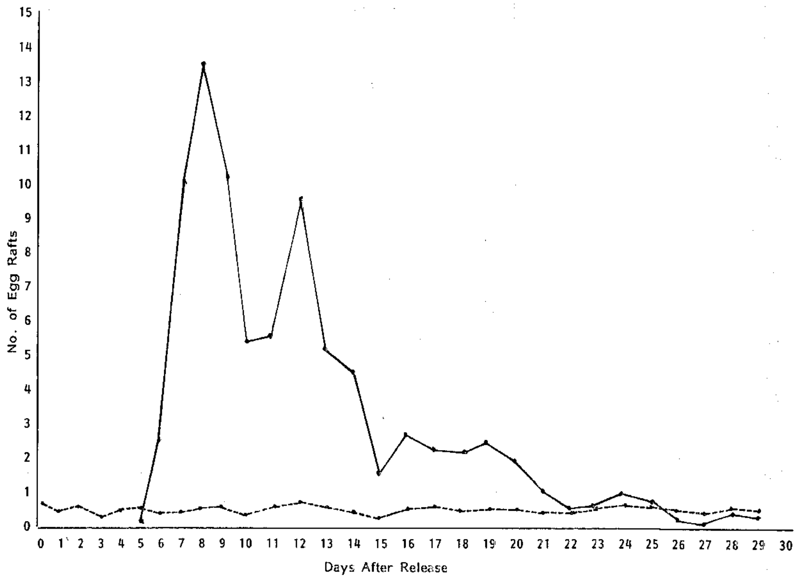

Fig. 1.—Number of *C. p. quinquefasciatus* egg rafts collected daily from Seahorse Key after the first release May 28–June 26, 1971. (Solid line = radioactive rafts; dotted line = indigenous rafts.) (Number of egg rafts x 100.)

Table 1.—Oviposition of *C. p. quinquefasciatus* following the first release of females (50,000) labeled with ^{32}P.

Days after release	Number indigenous egg rafts	Number radioactive egg rafts
0	55	..
1	38	..
2	42	..
3	10	..
4	32	..
5	39	6
6	29	255
7	30	1,039
8	43	1,367
9	49	1,087
10	25	555
11	41	588
12	57	997
13	44	533
14	32	455
15	13	194
16	37	291
17	39	235
18	24	234
19	46	246
20	49	205
21	36	149
22	32	81
23	41	99
24	45	109
25	42	56
26	35	27
27	19	13
28	36	29
29	27	19
Total	1,087	8,869

from July 18, or 2 days after release, until day 6 after release, and the greatest activity occurred on day 4. The swarms were in the same areas as before and again occurred between the hours of 2030 and 2115. Only a few resting adults could be found after swarming activity ceased and during the day. As in the first release, oviposition again was divided between the evening and dawn hours, and some rafts laid as late as 1000 hours.

The first radioactive rafts were collected on July 20, 4 days after the initial release, the first peak of oviposition occurred on day 7. The numbers dropped on days 8 and 9, and a second peak occurred on day 10 (Table 2). (This second peak actually was greater than the first, undoubtedly because day 10 was the first warm, clear day after the release.) A drastic drop then occurred in the daily numbers of rafts, a slight increase occurred on day 13, and by day 21, there were only 2 radioactive rafts. However, by day 18, the indigenous rafts outnumbered the labeled rafts (Figure 2), and by day 21, the radioactive counts were almost too low for accurate detection.

Figure 1 shows that the trends of the collection after the second release were similar to those after the first release. However, the peaks occurred on a 3-day cycle rather than on a 4-day cycle, longevity was reduced, and numbers were lower, naturally enough since fewer adults were released. A total of 2,490 egg rafts was collected (Table 2); of these 2,033 were radioactive and 457 were indigenous. Thus we collected 7.5 rafts per 100 released females in the second release.

DISCUSSION. The weather during the

Table 2.—Oviposition of *C. p. quinquefasciatus* following the second release of females (27,000) labeled with ^{32}P.

Days after release	Number indigenous egg rafts	Number radioactive egg rafts
0	32	..
1	38	..
2	29	..
3	28	..
4	21	7
5	21	55
6	14	191
7	18	400
8	16	269
9	6	141
10	19	460
11	15	137
12	25	82
13	18	116
14	18	60
15	9	43
16	8	8
17	22	26
18	44	17
19	34	16
20	10	3
21	12	2
Total	457	2,033

first release was typical for the season—warm days, slight breezes, and little rain or thunderstorm activity. The bird population was high, and the nesting activity on the island provided an excellent opportunity for the mosquitoes to obtain a blood meal. In addition, a minimum of human activity took place in and around the buildings during the evening hours and night. By the second release, conditions had changed. Daily thunderstorms and heavy rainfall accompanied by continual winds prevailed. The numbers of nesting birds and their young had decreased, and most had left the island within 2 weeks after the second release. In addition, an average of four to six people were in residence at the lighthouse engaged in marine research at the laboratory. This activity included operation of generators during the evening, water usage with the ensuing runoff, extensive use of lights, production of noise, routine ingress and egress of boats and equipment, and the use of some repellents around the working and sleeping areas at night.

The increased activity, unfavorable weather, and fewer hosts for blood meals may partially explain the apparently decreased longevity of females after the second release. Also, these conditions may be the reason fewer rafts were collected per 100 females released. When the oviposition cycles of the two releases are compared (Figures 1 and 2) one can see that the relative numbers collected each day would have been similiar if conditions had permitted earlier oviposition of the first egg rafts after the second release. Obviously, environmental conditions in the field influenced oviposition. Also, the average numbers of indigenous rafts collected per day after releases 1 and 2 were 36 and 21, respectively, a reduction of 42 percent. If the results of the second release are adjusted by this percentage, then the total number of rafts collected after the second release compares favorably with the numbers collected after the first release. Specifically, the second release was only one-half of the first release, so if recapture had been 17.7 rafts per 100 females, the collection would have numbered 4780 rafts. However, the 42 percent reduction in population that prevailed at the time of the second release would have produced only 2770 rafts. Thus, the actual collection of 2033 rafts is not exceptionally low.

The collection of 17.7 rafts per 100 released females attained in the first study (with a 4-day ovipositional cycle) indicates that the estimated average daily mortality rate of females was 24 percent. This rate would have increased to 34 per-

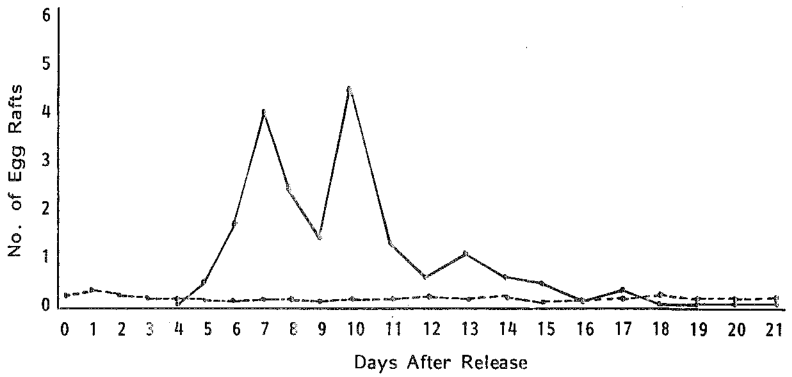

Fig. 2.—Number of *C. p. quinquefasciatus* egg rafts collected daily from Seahorse Key after the second release July 16–August 6, 1971. (Solid line = radioactive rafts; dotted line = indigenous rafts.) (Number of egg rafts x 100.)

cent to account for the reduction to 7.5 collected rafts per 100 released females observed in the second release (a 3-day cycle of oviposition). The pressures that reduced the indigenous population at the time of the second release also acted to reduce the number of released mosquitoes.

The ovitraps proved to be an effective method of surveying for *C. p. quinquefasciatus*. The 14 tubs placed in various locations indicated that the insects released under the marine laboratory had dispersed as adults throughout the entire test area. In addition, the collections showed that the ratio of tagged to normal rafts was fairly constant for each location though a few tubs near both swarming sites and the interface of the wooded areas consistently attracted the most oviposition. The one tub maintained on Atsena Otie Key produced few *Culex* sp. rafts during the time of the 2 releases; however, 2 of these rafts were radioactive. Therefore, these rafts may have been deposited by tagged females that migrated the 2 miles over saltwater; however, they may also have been transported accidentally by boat even though they were collected the 13th and 16th days after release on Seahorse Key.

The techniques used for ^{32}P labeling of the females were apparently not detrimental to *C. p. quinquefasciatus* since the collections (17.7 and 7.5 radioactive rafts per 100 released females) were exceptionally high compared with those in other release-recapture experiments. Provost (1952) was able to collect only 324 ^{32}P-tagged adult *Aedes taeniorhynchus* (Wied.) from approximately one million released by using various sampling methods to determine omnidirectional dispersion. Fussel (1964) collected only 0.23 percent of 275,000 ^{32}P tagged and released adult *C. p. quinquefasciatus*, and Lindquist *et al.* (1967) recaptured 0.011 percent and 0.042 percent of 583,000 and 280,000 adult *C. p. fatigans*, respectively, in two release studies. In addition, in each of these tests, collections beyond 0.5 mile from the release sites were very low, and more than 90 percent of the recaptures were within this range.

All the data from our study therefore indicate that Seahorse Key remained isolated for the duration of the experiments and that the characteristics of the released populations were similar to those of the indigenous mosquitoes. The accumulated knowledge from this study has been used in making subsequent releases in which dual tagging has been used—radioactivity and sterility.

ACKNOWLEDGMENT. The authors extend their appreciation to Mrs. A. L. Cameron for her excellent technical assistance in both the field and laboratory aspects of this study.

Literature Cited

Fussell, E. M. 1964. Dispersal studies on radioactive-tagged *Culex quinquefasciatus* Say. Mosq. News 24(4):422–426.

Hagstrum, David W. 1971. A model of the relationship between densities of larvae and adults in an isolated population of *Culex tarsalis* (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 64(5):1074–1077.

Lindquist, A. W., Ikeshoji, T., Grab, B., Demeillon, B. and Kahn, Z. H. 1967. Dispersion studies of *Culex pipiens fatigans* tagged with ^{32}P in the Kemmedine area of Rangoon, Burma. Bull. World Health Org. 36:21–37.

Patterson, R. S., Weidhaas, D. E., Ford, H. R. and Lofgren, C. S. 1970. Suppression and elimination of an island population of *Culex pipiens quinquefasciatus* with sterile males. Science 168:1368–1370.

Provost, M. W. 1952. The dispersal of *Aedes taeniorhynchus*. 1. Preliminary studies. Mosq. News 12(3):174–190.

Smittle, B. J., Lowe, R. E., Ford, H. R. and Weidhaas, D. E. 1973. Techniques for ^{32}P labeling and assay of egg rafts from field-collected *Culex pipiens quinquefasciatus* Say. Mosq. News 33(2):215–220.

Weidhaas, D. E., Patterson, R. S., Lofgren, C. S. and Ford, H. R. 1971. Bionomics of a population of *Culex pipiens quinquefasciatus* Say Mosq. News 31(2):178–182.

Weidhaas, D. E., Smittle, B. J., Patterson, R. S., Ford, H. R. and Lofgren, C. S. 1973. Survival, reproductive capacity and migration of adult *Culex pipens quinquefasciatus Say*. Mosq. News 33(1):83–87.