

PRELIMINARY REPORT ON MOSQUITO FLIGHT DISPERSAL STUDIES WITH RADIOISOTOPES IN CALIFORNIA, 1950 *



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INTRODUCTION

Knowledge of the flight dispersal of mosquitoes is of basic importance in the epidemiology of the diseases they transmit and in the planning of control measures. Aware of this fact, malariologists have investigated rather extensively the problem of the flight range of anopheline mosquitoes because of their known relationship to malaria. Eyles (5) has prepared a critical review of the literature related to the flight and dispersal habits of anopheline mosquitoes. Since his summary, numerous additional studies related to the flight and dispersal of anophelines have been conducted such as those of Eyles, Sabrosky, and Russell (7), Goodwin (8), and Correa, Lima, and Coda (4). Exhaustive literature comparable to that of the flight of anopheline mosquitoes does not exist with respect to culicine mosquitoes although a few highly significant studies have been conducted. Among these are the work of Stage, Gjullin, and Yates (14); Horsfall (10); Reeves, Brookman, and Hammon (13); Causey and Kumm (2); and Causey, Kumm, and Laemmert (3).

Investigations of the biology of California mosquitoes in irrigated pastures were begun in 1949†. It was early realized that studies of the flight range of these mosquitoes would be basic in establishing relationships that they might have to the transmission of disease and in allowing for better definition of the zones of importance to mosquito control. The three mosquitoes of major importance issuing from irrigated pastures are *Aedes nigromaculis* (Ludlow), *Culex tarsalis* Coquillett, and *Aedes dorsalis* (Meigen).

The studies of Reeves, Brookman, and Hammon (13) established basic information concerning the flight range of *C. tarsalis*. *A. dorsalis*, which in the years up to 1940 was the principal *Aedes* problem in the Central Valley, has been relegated, because of events of the past decade, to a position of much less importance than that of *A. nigromaculis*. Thus, the mosquito flight studies undertaken during 1950 were concentrated on investigating the range and dispersal patterns of *A. nigromaculis*.

For these studies the "release" emergence of mosquitoes was made in an irrigated pasture designated as the "Study Pasture" located in Township 5 S, Range 9E, Section 9 in Stanislaus County, Calif., about 8 miles west of Turlock. The pasture selected consisted of approximately 90 acres in an inverted L-shape and the release point for the radioactive mosquitoes was located at the southern border of the inside of the L. The closest human habitation was approximately 1/8 mile to the south and across a small field.

MATERIALS AND METHODS

A majority of the work done on anophelines and flight range up to the time of Eyles' summary involved capturing adult mosquitoes, marking them with a stain, releasing them, and recapturing marked specimens. The marking agents necessitated spraying with aqueous aniline dye solutions, or dusting with metallic bronzing powder. Another technique consisted of using dilute solutions of Giemsa's, Wright's, or methylene blue stains in which the larvae were reared. Zukel (16) developed methods for marking anopheline mosquitoes with fluorescent compounds; rhodamine B, among others, proved to be quite suitable. Reeves, Brookman, and Hammon (13) selected rhodamine B as their tagging material. Recently, with the work of Hassett and Jenkins (9), Bugher and Taylor (1), and Jenkins (11) the use of radioisotopes for tagging mosquitoes became a possibility. Lindquist and Yates (personal

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communication (12)) have made extensive studies of the use of radiophosphorus for tagging mosquitoes in the laboratory. After review of the work of the above authors, two methods of tagging mosquitoes were selected for use during the 1950 season. These were: (1) the use of aqueous solutions of rhodamine B applied to mosquitoes under natural conditions with a Tifa aerosol fog generator; and (2) the production of radioactive mosquitoes by allowing the larvae to develop and emerge in an aqueous solution of radiophosphorus. Results of the study using the rhodamine B dye are not available at this time.

Use of Radiophosphorus. The isotope used was secured from the Isotope Division of the Atomic Energy Commission at Oak Ridge, Tenn. Phosphorus-32 was selected as the most practical because of its extremely short half-life, approximately 14 days, and because it is an emitter of beta radiations which have a relatively short range. Both these factors contributed to personnel safety in the handling of the material. The isotope was handled through the facilities of the Western Division of Tracerlab, Inc., a commercial laboratory designed for this purpose. At this laboratory the phosphorus was converted to a neutral sodium phosphate (Na_2PO_4) solution for transportation to the field.

Mosquito larvae for tagging were collected in irrigated pastures including the one encompassing the release point. The method of collection used was that of dipping the larvae and pouring them through a funnel lined with muslin to concentrate the larvae in large quantities on the muslin. As soon as several thousand were obtained, they were transferred to two large galvanized iron rectangular tanks. These tanks were 4 by 5 ft. and were 6 in. deep. The quantity of water used in the tanks was 39.6 gal. which gave a depth of slightly under 4 in. Across the water surface of one of the tanks a seine was stretched and floated on corks to provide footing for the emerging adult mosquitoes. Floating plants were used in the

other tank for the same purpose. The majority of the larvae collected (about 99 percent) were *A. nigromaculis*, except when special collections of *C. tarsalis* were made.

The isotope was added to the tanks after the larvae were introduced in their final concentration. The radioactive solution was poured into the tank slowly (figure 1) in order to allow even dispersal of the material without creating "hot" spots. The concentration of the isotope used was based on the recommendations of Mr. Lindquist from his work in Oregon and was 0.1 microcurie per ml.

Precautions in handling the isotope were necessary to prevent possible accidents to personnel in and about the release point. A fence was erected around the tanks. Workers entering the enclosure wore rubber gloves, boots, and wrist badges and were monitored in the Turlock Laboratory for possible contamination after each visit. Contaminated equipment and material were marked as such and carefully stored in containers for that purpose. The release point was selected because of its isolated position with respect to human habitat and curiosity seekers and because this same pasture was used as a study area for the extensive ecological study of irrigated pastures being conducted during 1950.

Study of Exposure Results. The results of exposure to phosphorus-32 were studied by taking

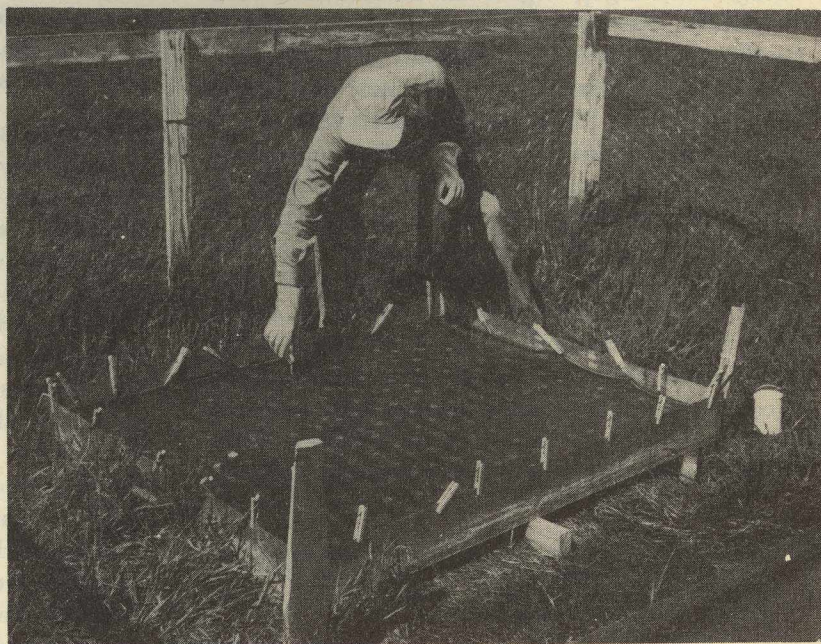


Figure 1. A radio-physicist pours the radioactive phosphorus solution into the tanks. The seine clipped to the sides of the tanks and floating on corks is for the purpose of allowing emerging adults a place to stand. (Photo courtesy Turlock Mosquito Abatement District and Lindblom Photo Service).



Figure 2. The Geiger counter was used to examine light trap collections for radioactive mosquitoes. (Photo courtesy Turlock Mosquito Abatement District and Lindblom Photo Service).

samples of larvae from the tanks, washing them several times, removing the water from their exterior surfaces, and finally monitoring them to determine how radioactive they might be.

Exposure periods of 12 to 18 hours proved to be sufficient to tag these larvae with easily detectable amounts. It was found that pupae could not be tagged unless they remained in the solution from 48 to 60 hours. In general the best results were obtained when early fourth stage larvae were used. Samples of mosquitoes emerging from the tanks were taken to determine the amount of radiation which they emitted. It was found there was considerable variation ranging from counts just detectable above background in some individuals to counts of more than 20,000 per minute in other individuals. In all cases in which mosquitoes were introduced into the tanks as larvae, detectable counts of radiophosphorus were found in the adults.

Tagged specimens taken from the tanks were tested with a Geiger Counter, the SU-3A Laboratory Monitor issued by Tracerlab with a T G C - 2 tube (figure 2). With this monitor single specimens with about 100 counts per minute were easily detectable even when buried among thousands of other mosquitoes. Specimens with several thousand counts per minute would cause the monitor to register even though the specimens remained in ice cream cartons in which the specimens were brought from the field. The most difficult speci-

mens to detect were those which had assimilated only a very small quantity of phosphorus (since they were exposed during the pupal stage) and the sensitivity of the monitoring equipment used was not sufficient.

Using this type monitor, a standard had to be established for deciding when radioactive specimens were located. No single specimen which did not consistently register above 50 counts per minute was included in our list of "positives" for this reason. When specimens which emitted several radiations per minute above this arbitrary background were placed in a container under the counting tube, the presence of the phosphorus could be detected frequently from

the mass of specimens when radiations from single specimens were considered questionable.

Capture of Tagged Mosquitoes. In attempting to capture tagged mosquitoes two principal methods of collection were used. The first and most important was the operation of mosquito light traps. For this purpose about 70 different light trap stations were established with a greater concentration within 3 miles of the release point and the remainder distributed radially to a distance of 10 miles. The majority of the traps were distributed on a northwest-southeast line approximately in a line with the movement of the wind. The concentration of the traps within the 3-mile zone allowed for the establishment of a dispersal pattern which indicated the concentration of trapping stations that would be necessary for future dispersal studies of this type. For the 30 traps located within the 3 miles of the release point collections were made daily with a few minor exceptions. The remainder of the traps were scheduled to be collected twice each week, usually Monday and Thursday.

The second method of collection was that of hand collecting by means of aspirators and chloroform jars. Since these pasture *Aedes* will fly from surrounding vegetation onto an operator's clothes during all hours of the day, it is possible to make hand collections at any location where these mosquitoes are present. Thus random collections were made at a large number of stations up to 1 mile

from the point of release and at a few more distant points. But because of the limitation of manpower available for this task, no regularly visited stations were established at more than a quarter of a mile from the release point.

RESULTS

Estimates of the number of mosquitoes which were tagged indicate there were about 400,000. The difficulties involved in making estimates of the number of tagged mosquitoes were as follows: (1) no good method could be established for counting the concentrated larvae when they were being moved from the field to the tanks; (2) while samples for estimating number of larvae could have been taken from the tanks, the difficulties of handling the radioactive material made this somewhat impractical; and (3) there was a high mortality of larvae at certain periods which reduced the number actually emerging. Larval counts were thus ignored in favor of pupal skin counts. It was found that the pupal skins would float in great mats on the surface of the water (figure 3). By estimating the areas covered by these skins and computing the number of skins per unit area, it was possible to arrive at a reasonable indication of the total number of emerged mosquitoes. This proved satisfactory for the first emergence since the mosquitoes from this group came out within a short period, leaving an even mat. The presence of untagged mosquitoes and poorly tagged mosquitoes

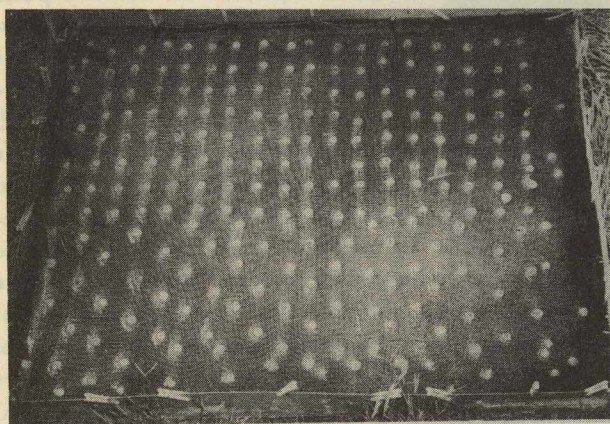


Figure 3. Photograph of tank I following the emergence of most of mosquitoes. The dark border at the edge of the tank is a floating mat containing many thousands of pupal skins.

in this emergence made the estimates more difficult. After the second and third introductions of larvae the tanks were so contaminated with algae that the pupal mats did not form properly and satisfactory estimates were impossible. Thus, the

estimate that approximately 400,000 mosquitoes were tagged is influenced by several uncontrollable factors which may have introduced errors.

In connection with this study about 10,000 *C. tarsalis* were introduced into the tanks and a number of these were recovered. Special attempts were made to obtain tagged *C. tarsalis*; therefore, 5,084 specimens were taken from resting stations mostly at distances between 1 and 2 miles from the release point. No radioactive *C. tarsalis* were taken in these resting stations although approximately 170 collections were made. All radioactive *C. tarsalis* were taken in the light traps, the most distant occurring at 1 mile downwind.

Emergence Peaks. Under natural conditions, pasture *Aedes* usually emerge in very definite broods, all of the adults coming from the water in 24- to 48-hour periods. When these mosquitoes were placed in rearing tanks in the third and fourth stages, they had a tendency to emerge over a longer period, usually 4 to 5 days, with occasional stragglers remaining in the aquatic stage as long as 10 days. The radiophosphorus was introduced into the tanks on August 9. At that time about 350,000 mosquitoes were in the tanks. Of these about 200,000 are believed to have become tagged and emerged. Many of them, however, emitted only a few radiations so that the easily detected group amounted to an estimated 100,000. The peak of emergence of this group occurred on August 12 and the first recovery of a specimen in the light traps was on the morning of August 13 when a male *A. nigromaculis* was taken $\frac{1}{4}$ mile to the northwest. The second period of emergence came about following the introduction of several hundred thousand additional larvae during the period of August 19 and 20. The peak of emergence occurred about August 22. The third period of emergence reached a peak about September 2 and 3 as a result of the introduction of the third concentration of larvae several days earlier. Usually the collections of tagged mosquitoes were made about 2 to 3 days after the peak of emergence and there was no evidence to indicate that the radioactive mosquitoes existed for more than 7 or 8 days following emergence.

Recoveries. There was a total of 673 radioactive mosquitoes passed under the monitor at the field station laboratory. This did not include a number of low count specimens which, having only a few radiations per minute above the arbitrary background, were not easily detected unless several were present in the same box. This occur-

red since for the first release a large number of pupae were introduced into the tanks along with the larvae, and these pupae were either not tagged or were tagged with small quantities of P^{32} . Of the 673 radioactive mosquitoes taken, 198 were from the release point itself and collected either on the pans and tanks or on the grass surrounding the tanks.

The radioactive mosquitoes collected with aspirators amounted to 226 specimens with the most distant recovery at about $\frac{1}{2}$ mile from the point of release. Results of this collecting method are shown in table 1. A total of 27,000 specimens was taken in 422 collections in which these 226 mosquitoes were included. Mosquito light traps took 1,366 collections and included an estimated 2,500,000 mosquitoes. Of these, 249 were radioactive with the most distant recovery at $1\frac{7}{8}$ miles from the release point. Results of this method of collecting are shown in table 2.

The recovery rate for radioactive mosquitoes (excluding 198 specimens collected at the

release point) was slightly more than 0.1 percent of those released.

Pattern of Dispersal. As indicated in figure 4 it was found that there was a tendency for mos-

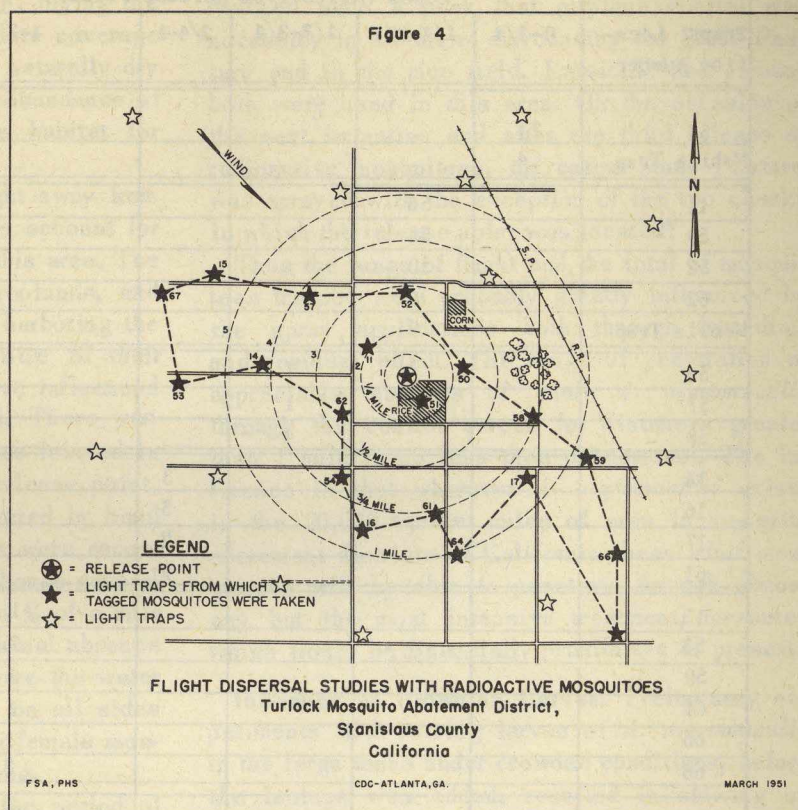


Table 1

RESULTS OF HAND COLLECTING AS A MEANS OF CAPTURING RADIOACTIVE MOSQUITOES,
TURLOCK, CALIF., 1950

Distance from Release Point, in Feet.	First Emergence-Peak Aug. 12				Second Emergence Peak-Aug. 22	Third Emergence Peak-Sept. 2
	Before Aug. 14	Aug. 14	Aug. 15	After Aug. 15	After Aug. 22	After Sept. 2
50	16	16		6	120	1
50 - 251		27				
251 - 500		13			1	
501 - 750		4			2	2
751 - 1,000		7		1	3	
1,001 - 1,500		1	1			
1,501 - 2,000			1			
2,001 - 2,500			2			
2,501 - 3,000			2			
Total	16	68	6	7	126	3
Grand Total	226					

Table 2

**RESULTS OF THE OPERATION OF LIGHT TRAPS IN CAPTURING RADIOACTIVE MOSQUITOES NEAR
TURLOCK, CALIF., 1950**

Traps: Location Number	Radioactive Mosquitoes Captured for Each Distance Zone Zones (Miles from Release Point)					Number of Collections with Radioactive Mosquitoes
	0-1/4	1/4-1/2	1/2-3/4	3/4-1	1-2	
51	71					13
Mobile Trap	4					1
11		56				15
50		58				12
52		10				6
62		2				2
Mobile Trap		9				2
54			7			7
57			1			1
58			4			3
14				3		3
16				3		3
17				2		2
61				9		6
13					1	1
53					2	2
59					2	2
64					1	1
65					1	1
66					3	3
Totals	75	135	12	17	10	86
Av. per Trap (Stationary traps only)	71	31	4	4.5	1.6	
Total radioactive specimens caught249						

quitoes to disperse downwind and somewhat into the wind after they had extended themselves beyond the $\frac{1}{2}$ -mile zone. The collections made by hand were neither sufficiently regular nor large to establish a proper pattern, but in general they supported the evidence shown by the light trap collections.

FACTORS INFLUENCING RESULTS

The Pasture Habitat. The pasture area surrounding the release point on three sides provided conditions believed to be extremely favorable to the maintenance of the *A. nigromaculis* population. Portions of the area were covered with knee high

clover, grass, and weeds which sheltered the adults during the daylight hours. Moisture on the surface of the ground was always present in some of the areas of deeper grass. Animals, mostly dairy cows but including dogs and squirrels, as well as birds, were present in the pasture. The farmer and the field workers of the study unit spent many hours in the pasture. Blood meals were thus readily available. Good oviposition sites for the females were present in every check as evidenced by the fact that larvae would hatch almost anywhere in the pasture after flooding. The wind was gentle both day and night throughout almost all the period of the study, seldom reaching more than

10 miles per hour and frequently only 1 or 2 miles per hour. This gentle wind came almost constantly from the northwest and infrequently swung to blow directly from the north. The temperature reached high peaks during the day, sometimes above 100° F., but dropped more than 30° F. during the night. Tempered by the almost complete coverage of the area with irrigation water, the naturally dry air is humid. Thus the area with its abundance of low vegetation creates a favorable habitat for the existence of pasture mosquitoes.

Most of the better reasons for flight away from the pasture, if reasons are needed to account for mosquito movement, were lacking in this area. The surrounding terrain, pasture land, croplands, and open areas were no better suited to harboring the adult *A. nigromaculis* than the place of their origin. Two significant facts may have influenced the flight pattern shown in figure 4. There was an extensive grove of eucalyptus trees located in a northeasterly direction from the release point. Only one tagged mosquito was recovered by hand collecting within this area and none were recovered in the traps beyond the grove although several were in operation only about ½ to ¾ of a mile away. The other fact noted was the virtual absence of adult *Aedes* on the borders or above the water of the rice field although the fields on all sides were uninhabitable because of biting female mosquitoes present during the early evening.

Spraying Operations. Throughout the period of the flight studies the active work of the Turlock Mosquito Abatement District was being conducted. This program was chiefly one of larviciding with jeeps on which spray booms are mounted. The larvicide which was usually applied at the rate of 0.2 to 0.4 lb. per acre on pasture land was a DDT emulsion. During August it is frequently necessary to apply about twice as much as during the spring months because of the lessened effectiveness of the DDT due to higher temperatures. All sources within the Mosquito Abatement District are treated as often as mosquito larvae are found; in irrigated pastures, this generally occurs with each irrigation at 10- to 20-day intervals. The Study Pasture was surrounded by the Turlock and East Side Mosquito Abatement Districts on the east for a distance of about 20 miles and to the north for at least 15 miles. On the west the active control program extended to the western foothills about 8 or 9 miles distant. The San Joaquin River bisects this western area on a north-south line at about 3½ miles from the release point.

Before the first emergence peak, August 12, the spraying approached only to the outer border of the study area and did not include the rice fields. Just before the second release, August 22, the adult mosquito population in surrounding areas reached such a peak that airplane control was necessary in all areas surrounding the Study Pasture and in the rice field. Larvicide and aerosol both were used in this area. On the occasion of the next irrigation and after the third release of radioactive mosquitoes, the entire Study Pasture was sprayed with the exception of the two checks in which the release point was located*.

Thus the range of flight and the total of mosquitoes trapped were probably greatly influenced by the spray applications both through immediate and residual effect. The range of penetration of appreciable numbers of adult *A. nigromaculis* through the control screen for distances greater than 1 mile is no less than phenomenal. The inference is that wherever *A. nigromaculis* exists in the 20,000 square miles of area in mosquito abatement districts of California these adult mosquitoes will be able to penetrate through almost any but the most intensive treatment, measures which would be financially prohibitive at present.

Influence of Crowding Larvae. Preliminary experiments with rearing larvae of *A. nigromaculis* in the large tanks under crowded conditions, before the isotope was added, resulted in slowing up development of the larvae and a more extended emergence period than occurs in natural water. A large proportion of the adults which emerged was very small. Naturally the question arises as to the probability that differences in the dispersal pattern and distance of flight may exist between these tank-reared and naturally developing pasture-reared *A. nigromaculis*.

Influence of the Isotope. To determine the influence of this factor, larvae and pupae in small enamel pans were reared separately from the large tanks but within the release point enclosure (figure 5). Into four of these pans a concentration of 0.1 microcurie of P³² per milliliter of solution was introduced; into the next group of four, 0.01 microcurie per ml.; third group, 0.001 microcurie

*Through the courtesy of Mr. G. E. Washburn, Manager, Turlock Mosquito Abatement District, the mosquito control operations were modified and delayed whenever possible to allow for emergence and dispersion of the radioactive mosquitoes. Control work was necessitated through public pressure on the District, however.



Figure 5. For the purpose of determining the effect of radioisotopes on the mosquito larvae, a number of small pan tests were run containing various concentrations of the isotope. Results of the emergence data from these pans were compared with emergence data from pans in which no isotope was present. (Photo courtesy Turlock Mosquito Abatement District and Lindblom Photo Service.)

per ml.; fourth group, 0.0001 microcurie per ml.; and several pans were left as checks. The larvae used were almost all late fourth stage. No differences in development and emergence between the pans without the P^{32} and those containing even the greatest concentration were observed under the conditions of the test.

Comparison with Other Studies. Eyles (5) gives a tabular summary of distances of flight for *Anopheles* showing the greatest flight distance experimentally demonstrated to be 8.7 miles (Swellengrebel and Nykamp, (15)) for *Anopheles maculipennis atroparvus*. Correa, Lima, and Coda (4) found *Anopheles albitarsis* flew 12-13 km. (7.45 to 8.07 miles) under conditions existing near Iguape, Brazil. For the North American species *Anopheles quadrimaculatus* Eyles and Bishop (6) had found tagged mosquitoes as far as 2.5 miles from the point from which they were released. Eyles, Sabrosky, and Russell (7) later found that this mosquito ranged 3.63 miles under certain conditions.

In Arkansas, Horsfall (10) found *Psorophora confinnis* could fly as far as 9 miles. Stage, Gjullin, and Yates (14) recovered an *Aedes vexans* female at 3 miles and several *Aedes sticticus* females 5 miles from where they were stained.

In more recent studies by Causey and Kumm (2), tagged *Aedes scapularis* were recovered after flights of 4 km. (2.48 miles) while species recovered at distances over 1 km. (0.62 mile) were *Aedes serratus*, *Psorophora ferox*, *Aedes crinifer*, and *Chagasia fajardoi*. Later Causey, Kumm, and Laemmert (3) found, among others, *Haemogogus spegazzini* to fly 11.5 km. (7.13 miles), *Aedes leucocelaneus* 5.7 km. (3.534 miles), *A. serratus* 11.5 km. (7.13 miles), *P. ferox* 10.8 km. (6.696 miles), and *Aedes terreus* 5.6 km. (3.472 miles).

Reeves, Brookman, and Hammon (13) found the following maximum distances of flight in their studies for the following California species: *Culex quinquefasciatus*, 2.5 miles; *Culiseta incidens*, *Anopheles psuedopunctipennis franciscanus*, 0.6 miles; *C. tarsalis*, 2.5 miles; and *Culex stigmatorhina*, 1.0 mile.

In the present study of pasture mosquitoes in California, radioactive *A. nigromaculis* were recovered at 1 7/8 miles downwind and 1 1/2 miles up but slightly crosswind indicating that these mosquitoes were, under the conditions of the experiment, either limited in capabilities or in stimulus to make greater flights. The factors which may have influenced the flights of these individuals are given above.

PREDATORS

Insects and other predators on both the larval and adult mosquitoes devoured large numbers of the experimental specimens. Water beetles and water bugs of several species inadvertently introduced with the larvae or entering the tanks later from nearby sources consumed larvae throughout the study. Several of these emitted radiations of more than 20,000 counts per minute.

Emerging adults were attacked by myriads of ferocious spiders that took a heavy toll. Spiders taken in the vicinity of the tank were highly radioactive. Small frogs also participated in eating the mosquitoes as they crawled from the tanks. Some frogs even jumped in the tanks.

Several dragonflies, captured in the vicinity, failed to cause any reaction under the Geiger tube.

CONCLUSIONS

1. Phosphorus-32 is a valuable isotope for biological tracer work and well suited to use in

mosquito dispersal studies.

2. Methods of tagging large numbers of mosquito larvae using P^{32} under field conditions proved both feasible and practical for biological investigations.

3. The flight range of radioactive *A. nigromaculis* allowed to emerge in mosquito abatement district using larvicides and aerosols for mosquito control, during midsummer in California, may be as much as 1 7/8 miles, while significant numbers of flights of 1 mile were noted.

4. A large number, 475 specimens, of radioactive tagged mosquitoes were recovered. This is believed to be only a very small portion, about 0.1 percent, of the estimated number of specimens allowed to emerge.

5. The flight range of *A. nigromaculis*, unhampered by insecticide applications, was not established, nor can the potential effects of large untreated sources (contiguous sections of irrigated pasture lands) on neighboring abatement districts be determined without additional study.

ACKNOWLEDGMENTS

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