

MASS REARING OF *CULEX PIPIENS* L.¹

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Mass rearing of mosquitoes may be defined as the synchronous production, on a regular schedule of a standardized or specified mosquito in large quantities. Arbitrarily, "large quantities" are considered as over 10,000 individuals per rearing unit or tray, or weekly production of at least 100,000 individuals. "Mass production" implies the application of mechanization, standardization, quality control, and the other techniques of large scale industrial production to a biological problem.

Exciting developments in the field of biological control, as exemplified by the release of sterile male screw worm flies, by the release of cytoplasmic-incompatible mosquitoes and other genetic methods, point to the importance of the development of efficient economic means of mass production of insects.

Fay, Baker and Jensen (1959), working on radioactive tagging of *Culex*, describe their techniques for rearing large quantities of mosquitoes. De Meillon and Thomas (1966) briefly describe a mass rearing technique for *Culex*. Our system for mass rearing of *Culex pipiens* is as follows:

EGGS. Egg rafts are collected in a plastic container,² 12.7 x 17.78 x 6.35 cm (5 x 7 x 2½") in size, to which water has been added to a depth of approximately 2.5 cm (1"). Oviposition containers are placed in the mosquito cages 72 hours after the females have been provided with a blood meal. The container is normally placed in

the mosquito cage in the afternoon and left overnight. The number of eggs per raft averages approximately 175 in our laboratory with an 86 percent hatch rate per raft. Hatching occurs in approximately 30 hours at 26.7° C. (80° F.). Maximum egg raft production occurs between the third and the fifth day after the blood meal.

LARVAE. Larval rearing trays can be seeded with first instar larvae by placing one egg raft per 150 larvae desired in each tray, or they can be estimated by the use of an aliquot apparatus as described by Gerberg *et al.* (1968).

Larval rearing trays are 137.6 x 76.2 x 5.08 cm (54 x 30 x 2"). They are constructed of galvanized iron. Each tray has a stoppered drain hole at the front corner. The tray is filled 24 hours prior to the introduction of larvae with 26.5 liters of water to a depth of 2½ cm. This allows stabilization of the water temperature at 26.7° C. (80° F.).

Each larval tray is seeded with approximately 10,000 first instar larvae, or 1 larva for each square centimeter of surface area, or 2.65 cc of water.

Larval food consists of Purina dog chow³ ground to 40 mesh and fed on the following schedule:

Day 0*	1.5 gms/tray
Day 1	2.5 gms
Day 2	2.5 gms
Day 3	3.0 gms (plus 10 ml of 11% dried brewer's yeast well mixed with water)
Day 4	3.0 gms
Day 5	4.0 gms
Day 6	5.0 gms
Day 7	7.0 gms
Day 8	7.0 gms
Day 9	7.0 gms

* Day 0 is day of introduction or hatching of larvae.

¹This study was supported by the U. S. Army Medical Research and Development Command, Dept. of the Army, under Contract DA-49-193-MD-2771, under the auspices of Walter Reed Army Institute of Research. This paper is contribution number 576 from the Army Research Program in Malaria.

²Tri State Plastic Molding Co., 400 North Wells Street, Chicago 10, Illinois.

³Purina dog chow, Ralston Purina Co.

PUPAE. First pupation occurs on day 6 and is usually completed on the eleventh day with maximum pupation occurring on the eighth and ninth day. Pupae are separated initially on day 7 with a yield of approximately 85 percent males and 15 percent females. On day 8 the ratio of males to females is about 50-50. On day 9 male pupae approximate only 23 percent of the total, the females reaching their peak yield on this day. On day 10, 90 percent of the pupae are females (Fig. 1).

sluiceway. The gaps between the bottom of the sluiceway and the plates range from openings of $25/1000''$ to the largest gap of $54/1000''$.

All pupae sized between $.034''$ and $.0415''$ were found to be males. All pupae tested on days 7, 8 and 9 that were $.043''$ or larger were found to be females. On day 10, male pupae were found mixed with females at $.042''$ and $.044''$. We believe this is due to the reduction in tray population due to removal of pupae on

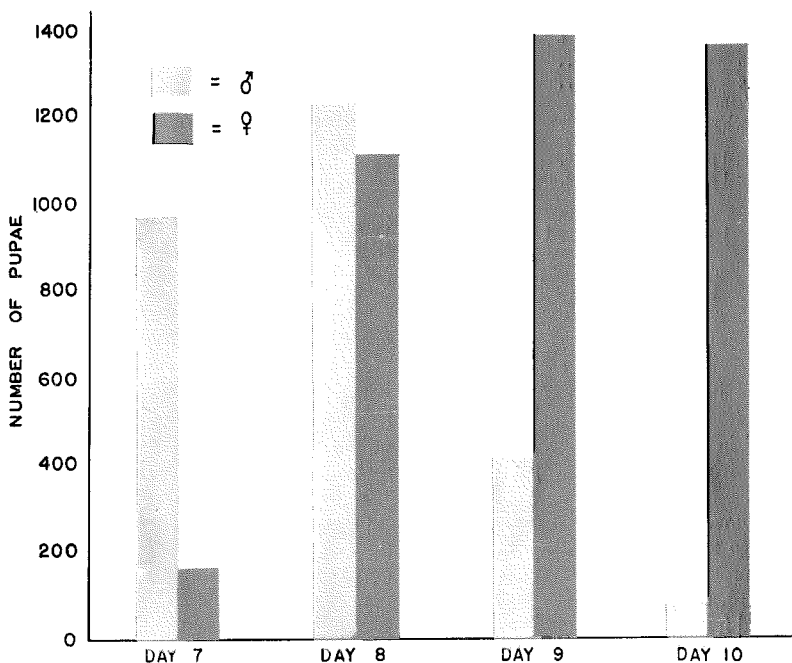


FIG. 1.—Daily yield of male and female *Culex pipiens* seeded at 10,000 per tray.

Pupae are sized to determine the point at which pure males or pure females could be separated. The pupae are segregated according to the variations in the diameter across the cephalothorax. The sizer used for this purpose is made up of a series of plates suspended above a sluiceway. The plates are machined in such a way that a graded series of openings are present between the bottom of each plate and the

the previous days, and to the increased amount of food each remaining larva has available.

Mixed males and females were found between $0.42''$ and $.0425''$ on days 7, 8 and 9. On day 10 the range increased, spanning $.042''$ through $.044''$, therefore to obtain only females it would be necessary to set the sizer at $.045$ or larger (Table 1).

TABLE I.—Sexual distribution of *Culex pipiens* pupae relative to the diameter of the cephalothorax and day of separation.

Diameter inches	Day 7		Day 8		Day 9		Day 10	
	♂	♀	♂	♀	♂	♀	♂	♀
.0340	4	..	13
.0350	25	2
.0370	6	..	1	..	1
.0380	5	..	1
.0385	11	..	13	..	2
.0390	35	..	25	..	9	..	3	..
.0415	6	..	1	..	5	..	2	..
.0420	5	6	3	7	15	..	7	2
.0425	2	2	1	2
.0430	..	5	..	1	..	2	2	3
.0440	..	3	..	10	..	11	4	8
.0460	4	6
.0480	..	6	..	1	..	27	..	29
.0500	13
.0515	6
Pupae/sample	121		80		77		85	
% of each sex	81.8	18.2	71.3	28.7	45.5	54.5	21.2	78.8

Total percent of ♂♂ 57.6.

Total percent of ♀♀ 42.4.

The larvae are returned to trays for 24 hours, after which time they are sent through the sizer for an additional yield of pupae. Pupae are volumetrically measured into lots of 1,000, placed into emergence dishes and are allowed to emerge into adult cages. Approximately 2,000 female and 1,000 male pupae are placed in a cubic foot cage. Adult emergence from pupae is completed in 48 hours.

ADULTS. Adults are maintained in 30 x 30 x 30cm (12 x 12 x 12") cages.⁴ This presents a vertical resting surface of 3,600 sq. cm. The cage is constructed of pressed form aluminum framing $\frac{3}{8}$ " x $\frac{3}{4}$ " wide and is screened with 18 x 22 mesh aluminum screening. The floor of the cage is of aluminum sheeting. A hammock constructed of fine mesh nylon is an integral part of the top of the cage. Cotton pads⁵ soaked in 10 percent sucrose are placed in the hammock to provide carbo-

hydrates for the adults. A restrained domestic chick is placed either in the hammock or in the cage. This first blood meal is provided 4 days after adult emergence. Further blood meals are provided every fifth day, for 3 weeks. The insectary is maintained at a 14-hour daylight cycle, although lighting schedules are not required for this species. The adults are maintained at 28° C. (82° F.) and 80 ± 5 percent RH.

Cages are maintained for 3 weeks. By the end of the third week, approximately 75 percent of the productive capacity of the colony has been attained and the adults are killed off. The males die very rapidly, 80-90 percent mortality occurring in the first 10 days from emergence. The majority of the dead males are found in the oviposition dishes during the first 3 days.

One thousand eight hundred twenty-four adult females were held for longevity studies in 40 pint cardboard cartons (8.5 cm in diameter and 9.0 cm in depth) containing an average of 45 females each. These were fed with 10 percent sucrose on cotton pads. Mortality at the end of 30 days was 41.2 percent. Fifty percent

⁴ Gerberg Mosquito Cage GH-1. Cornell Chemical and Equipment Co., Inc., Baltimore, Maryland 21228.

⁵ Jumbo Preptic Cotton Balls, Johnson & Johnson.

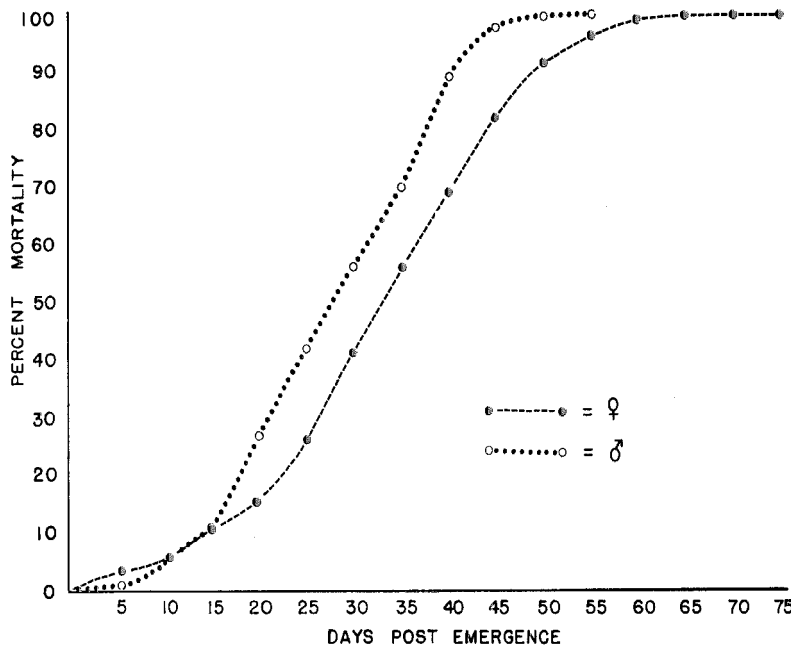


FIG. 2.—Longevity of adult *Culex pipiens* mass reared from lots of 10,000 larvae, sized for sex and mechanically separated in the pupal stage.

mortality occurred between the 33rd and 34th day. The maximum longevity achieved by one female was 74 days.

Longevity studies of 448 males were conducted in a similar manner. A 30-day mortality of 56 percent resulted from a population of 50 adults per pint carton. Fifty percent mortality occurred on the 29th day. The maximum longevity of the oldest male was 56 days (Fig. 2).

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