AN ANALYSIS OF *CULEX TARSALIS* COQUILLETT LABORATORY REARING PRODUCTIVITY [1]

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ABSTRACT. Rearing trials of larvae of *Culex tarsalis* Coquillett mosquitoes were performed with known numbers, standardized physical conditions, and selected feeding regimens. The best results were obtained with a diet calculated on the basis of 0.2 mg of a 46 percent protein commercial feed (TetraMin [2]) per larva per day for 2 weeks and 0.029 mg Terramycin per larva on 4 days. The average pupation was 70 percent, and adult production was 58 percent. Adults emerged between the 9th and 18th day with 11 of the 12 feeding regimens evaluated. Projection of the data obtained for estimation of mass rearing of colonized *C. tarsalis* indicates that it would require more time, personnel, and equipment than needed for rearing comparable numbers of *Aedes aegypti* (L.).

The mosquito, *Culex tarsalis* Coquillett, has been successfully maintained in laboratories since the 1950's (Brennan and Harwood, 1953; Brennan *et al.*, 1954; Trembley, 1955; Bellamy and Kardos, 1958). Larvae are usually reared in white enamel pans, about 20 x 30 x 5 cm, containing water at depths of 2.5 to 5 cm. A protein diet of one of the commercially available feed pellets is often used, with or without yeast or other supplemental foods. The amounts of feed are seldom accurately measured, since trial and error experience in larval rearing usually results in mosquito colony production that is satisfactory for many kinds of laboratory studies. This report describes the evaluation of several *C. tarsalis* larva rearing conditions and feeding regimens as part of a project for the development of procedures for the mass rearing of this species. The data obtained reveal that the procedures for rearing larvae of *C. tarsalis* should be improved in order to attain the efficiency and productivity required for mass rearing.

MATERIALS AND METHODS. The Greeley, Colorado, strain of *C. tarsalis* was used for the study. This colony has been maintained continually since 1959.

Rafts of eggs obtained from mosquitoes that had fed on chicks in the stock colony hatched 2 days after oviposition. The larvae were dispersed in 1 liter of water by a magnetic stirring rod, and the average number of larvae per ml was calculated from 20 2-ml samples. The proper volume of dispersed larvae was transferred to a rearing pan to stock it with the desired number of larvae, as described by Morlan, Hayes, and Schoof (1963). Selected samples showed that this method was accurate within 3 percent for obtaining aliquots of 350 larvae. White enamel rearing pans, 20 x 30 x 5 cm, were used for rearing in 1 liter of water, 1.3 cm deep; the water surface area was 522 cm^2. The rearing trials were conducted with 350 and 735 larvae per pan. The pans were placed on metal shelves 27 cm apart in an insectary at temperatures of $27 \pm 2°$ C.

Twelve feeding regimens were compared; four were 12.5 percent protein animal feed, with or without yeast supplement; eight were 46 percent protein tropical fish feed with or without yeast or antibiotic supplements. The feed provided varied according to the regimen being tested. The feeding schedules evaluated are listed in Table 1. The 12.5 percent protein animal feed was Purina Horse Chow Checkers containing ground corn, sorghum, barley, oats, wheat, soybean, alfalfa, molasses, and chemical ad-

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[2] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

TABLE 1. *Culex tarsalis* pupa and adult production in trials with 350 larvae.

Regimen	Feed	Fed mg per larva per day							Trials	Production percentages	
										Pupation	Adult
		0	1	2	3	4	5	6–12		Range (avg.)	Range (avg.)
A	12.5% protein	.2	.3	.4	.6	.6	.6	.6	8	4–66 (32)	3–51 (29)
B	12.5% protein and yeast	.2 .2	.3 .3	.4 .4	.6 .6	.6 .6	.6 .6	.6 .6	8	19–83 (45)	17–69 (33)
C	12.5% protein and yeast	.2 0	.3 0	.4 .2	.6 .3	.6 .4	.6 .6	.6 .6	8	11–57 (38)	8–43 (29)
D	12.5% protein and yeast	.2 0	.3 0	.4 0	.6 .2	.6 .3	.6 .4	.6 .6	8	22–53 (41)	16–45 (31)
E	46% protein	.2	.2	.2	.2	.2	.2	.2[a]	5	11–72 (16)	8–63 (14)
F	46% protein	.2	.2	.2	.4	.4	.4	.4[a]	3	3–23 (10)	0–5 (2)
G	46% protein	.4	.4	.4	.4	.4	.4	.4[a]	3	5–18 (10)	0–12 (4)
H	46% protein	.2	.3	.4	.6	.6	.6	.6[a]	3	1–40 (16)	0–34 (11)
I	46% protein	.2	.2	.2	.2	.2	.2	.2[b]	18	34–86 (60)	25–70 (46)
J	46% protein and yeast	.2 .1	.2 .1	0 0	.2 .1	.2 .1	.2 .1	.2[c] .1[c]	4	53–69 (61)	48–65 (57)
K	46% protein and Terramycin	.2 .029	.2 0	.2 0	.2 .029	.2 0	.2 0	.2[b] 0[d]	14	33–85 (70)	31–74 (58)
L	46% protein and neomycin	.2 .029	.2 0	.2 0	.2 .029	.2 0	.2 0	.2[b] 0	6	0 (0)	0 (0)

[a] Fed through day 8.
[b] Fed through day 14.
[c] Fed also on days 7 and 9.
[d] Fed also on days 8 and 12.

ditives; crude protein comprised not less than 12.5 percent, crude fat not less than 1.5 percent, and crude fiber not more than 25.0 percent. The 46 percent protein was TetraMin containing fish meal, fish liver, cod liver oil, shrimp, milt, brine shrimp, roe, aquatic plants, egg yolk, oat flour, and wheat germ oil; crude protein comprised not less than 46 percent, crude fat 5 percent, and crude fiber 8 percent. The use of TetraMin for rearing *Aedes stimulans* and *A. vexans* has been reported by Kardatzke and Liem (1972) and for *Culiseta inornata* by Pappas (1973). The TetraMin in this study was heated for 10 minutes in a dry oven at 190° C. Both types of feed were ground to pass through a 40-mesh screen, and measured amounts of food were used to provide the amount designed for each feed regimen. The yeast used was a blend of brewer's and primary dried yeast that is commercially available from E. R. Squibb and Sons, Inc.; it also was ground and passed through a 40-mesh screen. The Terramycin (oxytetracycline HCl) was a soluble powder produced by Charles Pfizer and Co., and the neomycin (neomycin sulfate) was a soluble salt produced as Mycifradin Sulfate by the Upjohn Company.

The efficacy of each rearing procedure was based upon the number of pupae produced and the number of adults emerging within 18 days. The pupae production and the adult production were both evaluated to determine if excessive mortality occurred between the time of pupation and adult emergence. Each feeding regimen was compared in from 3 to 18 trials, with 350 larvae per pan, and only a few additional trials were made with 735 larvae per pan. Deionized water was used, and the water level in the pans was kept constant throughout the trials by adding water. The surface of each pan fed the horse chow was skimmed daily with a strip of paper towel to remove any surface scum (pellicle); whereas, the pans fed the TetraMin were skimmed infrequently and only when a pellicle was observed.

RESULTS. The *C. tarsalis* pupa and adult production obtained with 11 feeding regimens using 3 to 18 replicates of 350 larvae per tray is summarized in Table 1. The best pupa and adult production was obtained with TetraMin (regimen K) fed at the rate of 0.2 mg per larva per day for 2 weeks and supplemented with 10 mg Terramycin (0.029 mg per larva) on the day of hatch (day 0) and on days 4, 8, and 12. Regimen K produced an average of 70 percent pupation and 58 percent adult emergence of the total larvae in 14 trials; the percentage of pupation ranged from 33 percent to 85 percent, and the percentage of adult emergence from 31 percent to 74 percent. Sixty percent pupal production was obtained in 18 trials with TetraMin alone, fed at the 0.2 mg per larva rate (regimen I). In a series of only four trials, 61 percent pupation and 57 percent adult emergence was obtained with the 0.2 mg TetraMin rate fed on days 0, 1, 3 through 7, and 9, supplemented with yeast fed at the rate of 0.1 mg per larva on the same days (regimen J). The other regimens tested produced lower pupation and emergence rates (Table 1). No pupation or emergence was obtained from six trials of regimen L with TetraMin fed daily for 2 weeks at 0.2 mg per larva supplemented with neomycin added on days 0 and 3 at the rate of 10 mg per pan (0.029 mg per larva). The larvae in the six trials generally died within 6 days, and all such trials were terminated within a week.

Adult mosquitoes emerged between days 9 and 18. In the trials with TetraMin regimens they began to emerge on days 9 through 12; whereas, in the trials with horse chow they began to emerge on days 11 through 15.

Another noticeable characteristic of the rearing trials with TetraMin alone was the absence of a heavy scum on the water surface. Some scum was noted in the TetraMin trials with the Terramycin supplement, and this generally occurred on the days after the antibiotic was added.

The production obtained in trials with the 12.5 percent protein horse chow was surprisingly low; the average pupation ranged from 32 percent to 45 percent. Adult emergence also was low, with averages ranging from 29 percent to 33 percent. The pans usually developed a heavy scum between days 5 and 9, and the water surface had to be skimmed daily to remove the pellicle.

Single trials with 735 larvae per pan of the 12.5 percent horse chow regimen, with and without yeast supplement, resulted in an average pupation of only 12 percent and emergence of 11 percent. Low production also resulted from duplicate trials of 735 larvae per pan, with 46 percent protein TetraMin diets fed through day 8 at the rates listed in Table 1 as E through H. These resulted in less than 10 percent pupation and no adult emergence, so trials with 735 larvae per pan were discontinued.

DISCUSSION. Brennan and Harwood (1953), in describing the first colonization of *C. tarsalis,* reported that their numbers of larvae per pan varied and that approximated amounts of a 32 percent protein with brewer's yeast and dried milk were fed to the larvae. Less than 2 years later, Hubert, Rush and Brennan (1954) reported they had noted behavioral changes in the colony *C. tarsalis,* and that they were able to rear the larvae on a protein diet without the yeast and dried milk supplements. Bellamy and Kardos (1958) also reported a selection effect among a *C. tarsalis* colony in California. They found that feeding large amounts of a high-protein diet to larvae was conducive to the expression of autogeny. Unpublished studies of colonized *C. tarsalis* in our laboratory have provided evidence that selection also may occur with respect to adult longevity and arbovirus infection susceptibility. Therefore, reasonable restraint should be used in equating laboratory test results among colony *C. tarsalis* strains reared under artificial conditions to biologic characteristics found among natural populations of *C. tarsalis.*

Published *C. tarsalis* rearing techniques are summarized in the American Mosquito Control Association's rearing manual (Gerberg, 1970), and the manual contains the recommendation that one high-protein animal food pellet be fed each 3–4 days, until pupation occurs, to 300 to 500 larvae per pan. In our study the rearing of 350 larvae per liter of water, a density of 0.35 larva per ml and 0.6 larva per cm^2 of surface area, was found to be markedly superior to rearing 735 larvae per liter, 0.6 larva per ml and 1.4 per cm^2. The trials with 735 larvae per pan resulted in greatly reduced production, probably due to overcrowding.

Hubert, Rush and Brennan (1954) reported finding scum on the water surface in an occasional pan among a presumably identical series of pans that were fed only "high protein pellets." The formation of scum occurred with most of the horse chow regimens that we tested, but it was not a problem with the TetraMin regimens.

The percentage of pupation obtained with the horse chow regimens was unexpectedly low, and none yielded an average pupation of more than 45 percent. The poor pupation rates may be associated with the conditions causing the scum to form. The initial series of TetraMin trials generally failed to produce adults within the 18-day test period. This was believed to be due to the discontinuation of feeding on day 8, and the subsequent TetraMin regimens, in which the daily feedings were continued through days 12 or 14 (Table 1, regimens I through K), were the most productive tested. The pupation rate obtained with regimen K (TetraMin and Terramycin) was estimated to be 70 percent with 95 percent confidence limits calculated as 62 percent and 79 percent. The rate estimated for regimen I (daily feeding of 0.2 mg TetraMin per larva) was 60 percent and for regimen J (same as I but with a yeast supplement) was 61 percent. The pupation rate under regimen K was not significantly greater than the rates under regimen I or J at the .05 level of probability but was at the .10 level, using a 2-tailed t test.

In the study of *C. tarsalis* by de St. Jeor and Nielsen (1964), several antibiotics were used to control surface scum in larva rearing pans. They reported that 0.1 gm of mouse chow per 500 larvae (equivalent to 0.2 mg per larva) was an adequate amount of food when fed every third day to 1st- and 2nd-instar larvae and every second day to 3rd- and 4th-instar larvae. Their best results were obtained with three types of tetracyclines (Terramycin, Achromycin, and Aureomycin). The antibiotic was added to give a concentration of 10 μg per ml, and the percentages of pupation ranged between 85 percent and 91 percent. We were unable to achieve such high pupation rates in the present study. Only Terramycin and neomycin were tested in this study, and neither the beneficial mode of action of Terramycin (regimen K) nor the deleterious effect of neomycin (regimen L) is completely understood. Further study is needed to determine these causal relationships, since such information could provide the basis for improving rearing productivity.

The results of this study may be used to project what would be needed to mass produce colonized *C. tarsalis*. Multiple stock colonies for egg production, or development of a method for storing accumulated *C. tarsalis* eggs, will be required. If 7-liter capacity trays are used, similar to those reported for mass rearing *Aedes aegypti* (Morlan, Hayes and Schoof, 1963), about 2,500 larvae could be reared per tray, with an expected production of 1,750 pupae and 1,450 adults per tray. Therefore, about 690 trays would be required to rear 1 million *C. tarsalis*. The period required for adult emergence would be between 10 and 18 days. Compared with the reported *A. aegypti* mass rearing procedure, the hypothetical *C. tarsalis* mass rearing is much less efficient. Whereas two men produced from 500,000 to 800,000 adult *A. aegypti* in 192 trays within 11 days, our results indicate that two men could produce no more than 280,000 adult *C. tarsalis* in the same number of trays within 18 days. Even if adult production could be increased to 80 percent (2,000 per tray), less than 400,000 adults could be produced during the 18-day period. Thus, projects requiring the mass rearing of *C. tarsalis* should include plans for adequate equipment and personnel to meet production goals. Of course, projects, such as sterile male releases, that utilize only one sex, will need to incorporate that condition into production plans.

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