Maxon no. 2125/912/11/111000, obtained from Trident Engineering Co. Ltd., Wokingham, Berkshire, England. All other parts of the trap, including the printed circuit board were made by the authors.

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A QUANTIFIED MASS-REARING TECHNIQUE FOR *TOXORHYNCHITED RUTILUS RUTILUS* (COQUILLETT)[1]

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ABSTRACT. Methods were devised for the mass production of the mosquito predator *Toxorhynchites rutilus rutilus* (Coquillett). These techniques permit one person to produce several thousand adults every two weeks; this represents a 5-fold increase in the number of pupae produced per standard tray and a 3-fold increase in fecundity over techniques reported previously.

Recently, it has been demonstrated that in some situations the genus *Toxorhynchites* (Theobald) has potential as a biological control agent against container-breeding mosquitoes (Gerberg and Visser 1978, Trpis 1972 Unpublished, WHO.) It is obvious that virtually all biological control strategies, with perhaps the exception of certain inoculative type programs, will involve mass production of the biological agent. It may also be true that certain deficiencies of a particular biocontrol agent may be compensated for by reducing the cost of production (Focks et al. 1978). We report here techniques that were used for the production of several thousand adult *Toxorhynchites rutilus rutilus* (Coquillett) on a biweekly basis by one person; the procedures would allow production of much larger numbers.

METHODS

Approximately three hundred and thirty 0- to 24-hr-old *Tx. r. rutilus* eggs were set (day 0) in a 50 × 40 × 10-cm plastic tray containing 7 liters of water (26–27°C), ca. 7,000 eggs or newly-hatched larvae of *Aedes aegypti* (L.), and a slurry containing 3 g of a mixture of liver

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

powder and hydrolyzed yeast (3:2). A second similar tray was set up, but with only 4 liters of water and ca. 7,000 eggs or 1st-instar *Ae. aegypti.* Tray temperatures were maintained by using a proportional power supply and resistance heating tapes (Dame et al. 1978). Both trays received another 3 g of liver and yeast on day 2. On day 3, the prey (2nd tray) which were mostly 4th instar, were strained from the rearing water with a screen of ca. 20 mesh and added to the 1st (predator) tray. The prey tray was then reset with prey and fresh water (day 3) and handled as before; the larvae were added to the predator tray on day 6. This was done again to provide more prey on day 9. On day 9, two prey trays were set so the predators could be fed on days 12 and 13.

The predator larvae were reared from egg to pupae under a long photoperiod, i.e., 15 hr light:9 hr dark, to prevent them from diapausing in the 4th instar (Bradshaw and Holzapfel 1977).

When the larvae were handled as described, the 1st pupae appeared on day 11 and ca. 96% of the pupation was completed by day 15 when the pupae were "picked" for the 1st time. After this 1st harvest, the few remaining larvae were returned to the same rearing media but in 1 or more trays of ca. 150 larvae each, given an additional tray of prey, and picked a final time on day 17 or 18. We found that it was important to return these remaining *Tx. r. rutilus* larvae to water that had been used for rearing the predators; when they were placed in clean water, the subsequent pupation tended to be prolonged and asynchronous.

Collected pupae were rinsed on a screen with a gentle stream of water to remove any surface film from the rearing trays before they were put into emergence containers; this step was important to insure low pupal mortality and successful emergence. We also found that placing more than ca. 3 pupae per cm^2 of water surface in the emergence containers resulted in a reduced harvest due to drowned adults. The pupal containers were placed in a 1 × 1 × 2-m acrylic plastic cage lined on 3 or 4 sides with 16-mesh aluminum screen designed to provide purchase for the adult predators. Each such cage was stocked with 1,000 to 1,500 pupae, water wicks to provide humidity (80–85% RH is optimum), and fresh apple slices and/or 50% honey on sponge wicks as a carbohydrate source for the adults. The length of the pupal and pre-oviposition periods varied with temperature but were ca. 5 days each at 26–27°C. Our colony stocks require no crepuscular light period but do require a regular photophase to insure adequate mating; insemination rates may be monitored by examination of the spermathecae.

Two or three 500 ml beakers painted black on the outside and half filled with water were provided as oviposition sites in each cage. The *Tx. r. rutilus* females hovered over the oviposition site while ejecting their eggs onto the surface of the water (Olinger 1957); the eggs rest upon the water without becoming wet. Because the embryonation period is ca. 50 hr at 26–27°C, we collected eggs on a daily basis using a large, coarse fritted glass funnel to separate the eggs from the oviposition water. If the eggs were to be stored for more than a few hours, they were held over a few ml of water in a sealed vial to prevent desiccation (2 hr at 50% RH produced a 60% weight reduction and variably reduced hatch).

Gerberg (1970) has described rearing techniques for *Ae. aegypti.* Dry pourable *Ae. aegypti* eggs were obtained from gravid females of the laboratory colony by allowing oviposition to occur on the moist filter paper lining of a beaker containing a few centimeters of water. After a 2-day incubation period, the eggs were removed from the paper with a stiff brush and shaken through a coarse screen to remove detritus and break up egg clusters. These eggs were held at ca. 80% RH; higher humidities (e.g., storing the eggs in sealed vials) promoted fungal growth, and lower humidities reduced egg viability. Stored properly, these eggs could be

used over several weeks (4–6) with little deterioration in hatch.

For mass rearing purposes, adequate accuracy and efficiency were obtained by volumetric measurement of both predator and prey eggs by using a small funnel and a pipette closed at one end. There proved to be ca. 6,670 and 66,700 *Tx. r. rutilus* and *Ae. aegypti* eggs per ml, respectively. Predator eggs could be poured from the pipette directly onto the water surface of the rearing tray; *Ae. aegypti* eggs had to be wetted before they were immersed because dry eggs tend to rest above the meniscus and do not hatch. Wetting the prey eggs was accomplished by shaking a measured portion of eggs vigorously in a small vial half filled with water.

We monitored predator egg viability by placing eggs individually in vials half filled with water and examining for hatch 3 days after oviposition. The same information was obtained for the prey species by microscopic examination of *Ae. aegypti* egg shells removed from the bottom of the rearing tray 4 or more hr after they were added to the tray.

DISCUSSION

With the techniques described, we recovered an average 237±27($\overline{X}$±SD) predator pupae and 11±4 fourth-stage larvae from each tray at the 1st harvest. The overall yield from egg to pupa by day 18 was 73% (245 pupae/330 eggs set). Cannibalism was the major source of loss during larval development, but this occurred almost exclusively during the first 6 days of development; there was little or no pupal loss due to cannibalism between days 11 and 15 when the 4th-stage larvae and the supposedly vulnerable pupae were together. From pupation through emergence we typically experienced 3.6% pupal mortality and 0.4% loss due to drowned adults. The daily survival of the caged adults averaged 0.997; this resulted in a 1.5% adult loss by day 5 when oviposition began. Thus, the overall yield from egg to ovipositing adult was 70.2%.

Focks et al. (1977) reported that the fecundity of *Tx. r. rutilus* was 1.0 eggs/female per day when 110 predator larvae/tray were fed *Ae. aegypti* reared on TetraMin® staple food, a commercially-available tropical fish food. By using the diet and techniques described in the present paper, we obtained an average fecundity of 3.2 eggs/female per day. However, predator pupal weights were similar, 50.0±2.0 and 48.2±2.8 mg ($\overline{X}$±SD) for the TetraMin-prey diet and liver-yeast-prey diet, respectively, and adult survival of the 2 groups did not differ significantly. A 2nd difference between the 2 methods was the number of prey larvae required per pupa produced. The earlier technique (Focks et al. 1977) required ca. 290 *Ae. aegypti* larvae per pupa; the scheme reported here requires only 145 larvae. In contrast, Gerberg (1974 unpublished) reported that 200–250 prey larvae were required per pupal *Toxorhynchites brevipalpis*. None of the methods mentioned differed significantly in the time required for development.

An additional and significant benefit provided by rearing the predators according to the new method, i.e., at a higher density, is the savings in space; techniques presented here allow about a 5-fold increase in production with the same physical plant. Our attempts to further reduce rearing costs will focus on the development of accurate information concerning the various components and interactions of the production system. These relationships are nonlinear and complex. For example, decreasing the predator-prey ratio reduces cannibalism among *Tx. r. rutilus*, but larger numbers of *Ae. aegypti* eggs are required because prey reared at higher densities are smaller. Conversely, rearing prey in lower densities produces a larger, robust, and more nutritious larva, but additional prey trays must be set which is expensive labor-wise: also, prey reared at lower densities tend to pupate and emerge before they are consumed. The point is that there are optimums for the various inputs

of labor and materials when the effort is to minimize cost. Determining these optimums is difficult, especially when the criterion used to evaluate the rearing scheme is the quality of insect produced. At this time, we do not know the relationship between such criteria as pupal weight, protein content, larval diet, and development time, temperature regimens and important factors such as the female's ability to locate oviposition sites, field survival, and fecundity. Until such data are available, we must use rearing synchrony, pupal weight, protein content, cage survival, and fecundity as indicators of production quality.

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AN IMPROVED METHOD FOR INDUCING EGG-LAYING IN CERTAIN AFRICAN *ANOPHELES*

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ABSTRACT. A short description is given of a reliable method used to obtain eggs from *Anopheles* mosquitoes in the laboratory.

INTRODUCTION

Morphological studies of *Anopheles* mosquitoes involve rearing the progeny of single females to adulthood. A study of several adults with their associated larval and pupal pelts is then possible, such as that recently carried out by de Meillon, et al. (1977) to separate *An. funestus* from a sibling species. In the case of rare species, or where material is not easily obtainable, it is essential that the methods of rearing families are as effective as possible. Time wastage and needless frustrations can be avoided if one is certain of obtaining eggs from most, if not all, specimens collected.

The method commonly used for obtaining eggs was to introduce wild-caught females into individual glass or plastic tubes of diameter 2.5cm and height 7.5cm. These contained damp cottonwool covered with a circular piece of filter-paper; the open end was covered with fine gauze. The females were offered blood meals daily until they died. In our experience it was unusual for as many as 30% of the females to lay eggs under these conditions. Quite frequently fully gravid females died before oviposition