MOSQUITO CULTURE TECHNIQUE

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The rearing of mosquitoes in captivity is being carried on to a greater extent now than in previous years. Research on such mosquito-borne diseases as malaria, dengue, yellow fever, filariasis, and equine encephalomyelitis has involved the use of laboratory-reared insects for some years. Furthermore, mosquitoes have been used for a long time in control investigations and in the transmission of malaria for the treatment of paretics. Now, however, a more widespead interest and need is manifesting itself. Global war has stimulated investigations on repellents and insecticides, and has intensified research on certain of the mosquito-borne diseases and their treatment. In this country, studies on poliomyelitis and its possible vectors have included experiments with mosquitoes. Papers along the above lines are published in "The Proceedings of the New Jersey Mosquito Extermination Association" (1944).

Specialists in subjects other than entomology, and experts in raising insects other than Culicidae are often faced with the problem of rearing mosquitoes; and as a result there are numerous requests for practical suggestions on rearing and for references to literature pertaining to mosquito culture and to the basic principles underlying mosquito development and behavior. In addition, there is a very real interest on the part of the general public in mosquitoes — their life-history, habits, and control. In recognition of the widespread need, it is proposed to make available in this article information along the following lines:

1. SMALL-SCALE REARING. Objectives. Equipment and procedure.
2. NOTES ON DEVELOPMENT AND BEHAVIOR. Water and food requirements. Effects of temperature, humidity, and light variations.
3. LARGE-SCALE CONTINUOUS REARING. Brief discussion of contributions from literature.
4. SELECTED BIBLIOGRAPHY. Articles referred to in text. Articles recommended for additional information.

SMALL-SCALE REARING

Maintenance of small colonies of mosquitoes does not require the exacting work nor complicated equipment necessary in large-scale projects where production schedules must be met. Small-scale rearing is valuable in research problems where only a few mosquitoes are needed from time to time. It has its place, also, in making available mosquitoes in all stages for study and demonstration in classrooms and other closely supervised assemblies, such as public educational exhibits maintained by mosquito abatement commissions, local improvement committees, and museums. These demonstrations can serve to educate children and adults by furnishing exhibitions of specimens and by

showing the actual effects of control measures recommended by county commissions or similar authorities. Small-scale oiling and dusting operations can be carried out in beakers or culture dishes; and small-scale tests or demonstrations of the effectiveness of sprays can be carried out in small cages.

It should be noted here that at times continuous rearing is not desirable or possible. Some species can be raised from larvae to adults with little difficulty, and females already fertilized when captured will generally deposit viable eggs. However, adults reared from these larvae or eggs may not mate in small cages, or they may not lay viable eggs. In these cases, only one generation of captive adults may be obtained for experimental purposes or for definite association of the immature and mature forms for identification. The following specific directions may be utilized for these purposes as well as for small-scale continuous rearing. It is possible, and entirely probable, that through numerous attempts and consequent modifications, additional species will be found adaptable to continuous rearing.

Practical suggestions for maintaining small colonies of mosquitoes must be prefaced by the statement that to my knowledge there are only four species of mosquitoes in the United States for which definite steps may be recommended for continuous rearing in small cages. These are *Anopheles quadrimaculatus* Say, a common human malaria mosquito; *Aedes aegypti* (Linn.), the yellow fever mosquito; *Culex pipiens* Linn., the northern house mosquito, and *Culex quinquefasciatus* Say (= *fatigans* Wiedemann of European workers), the southern house mosquito. We have reared a colony of *Anopheles quadrimaculatus* from October until August in a cage 10½ x 10½ x 15½ inches. We are securing viable eggs from individuals of this species emerging and

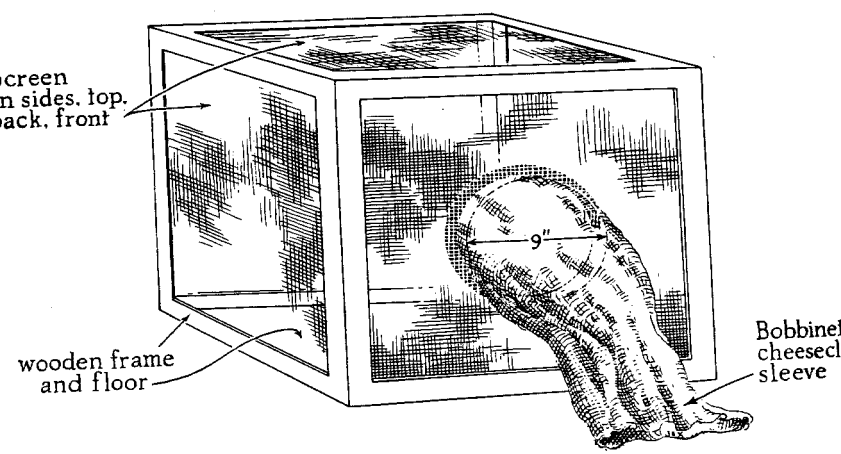

Figure 1. Mosquito Cage—2 x 2 x 2 feet.

mating in a cage only 10 inches square, and we have continued this lot since the middle of July of this year. We have reared *Aedes aegypti* continually for over two years in lantern globe cages (as described by Woke in 1937); the cages are approximately 6½ inches high and 3½ inches in diameter at the base. Huff (1937) also reported maintenance of *Culex pipiens* and *C. quinquefasciatus* in lantern globe cages.

EQUIPMENT FOR ADULTS

Cage.

With the above facts before us, it would seem practicable to use cages approximately 2 x 2 x 2 feet for all three genera, Anopheles, Aedes, and Culex. As we have noted, small cages can be used, but larger ones are more convenient, because they will provide room for the emergence pan for pupae, food for emerging adults, pan for deposition of eggs, and space for a host introduced to furnish a blood meal. The cage (Fig. I) should have a wooden floor and a sturdy wooden frame covered with wire screening or bobbinet. The screening should be copper or galvanized wire to prevent rusting, and the wooden frame should be painted to prevent warping. The screen should have at least 18 meshes to the inch to retain the smallest adult mosquitoes. The sleeve providing entry into the cage may be made of bobbinet or heavy cheesecloth. The cage may be varied in several ways; for example, a sliding panel or hinged door may be added, or the sleeve may be set in a hinged door. It is advisable to raise the cage an inch or two from the surface of the table so that water cannot collect under it. It is also advisable to cover the cage floor with paper or cloth which can be removed when soiled. Four towels, preferably Turkish, should be provided to cover each cage and these kept moist in order to provide necessary humidity.

Suction Tube.

Although adults may be caught in the cage and transferred by means of a vial about 1 inch in diameter and 4 or 5 inches long, a suction tube (Fig. II) is much more practicable. This consists of a rubber tube ¼ in. in diameter and about 18 in. long, slipped over one end of a glass tube ¼ in. in diameter, and about 6½ in. long. A plug in the end of the glass tube next to the rubber tubing prevents inhalation of the mosquito when suction is applied. The plug is made and inserted as follows:

A piece of index card, or preferably celluloid or old photographic film 1¼ x ¾ in. is rolled into a hollow cylinder ¾ in. long, with its diameter slightly less than that of the glass tubing. One end is placed in the center of a 1¼ in. square of cheesecloth (double thickness may be advisable) and both are pushed into the glass tube. This results in a thin cloth barrier stretched across the tube about ¾ in. from the end over which the rubber tubing is now slipped.

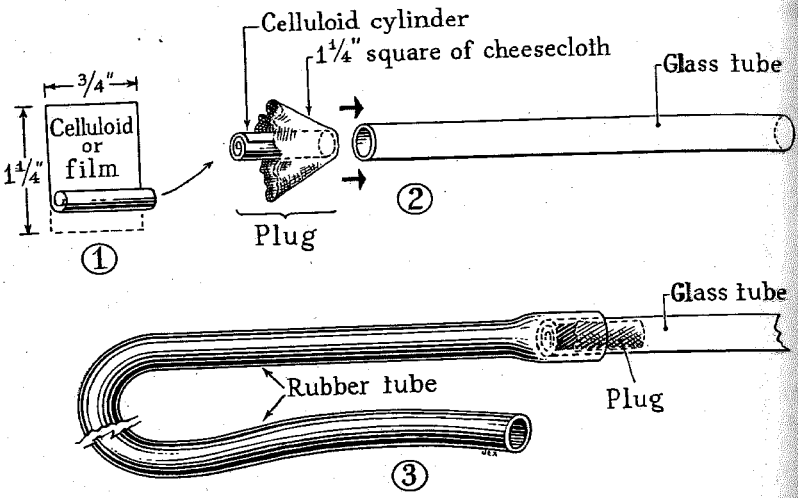

Figure II. Suction Tube
1. *Celluloid before rolling into shape.*
2. *Insertion of plug—semi-diagrammatic.*
3. *Completed suction tube.*

Feeding Apparatus.

Various devices are used to hold small animals quiet while female mosquitoes take a blood meal. We have used the apparatus illustrated in Fig. III; but there are others, and more can be improvised. A guinea pig or rabbit can be restrained in a tight-fitting hardware-cloth cylinder, closed at either end by wires, or by a fold of the material (Fig. III, 1). There should be no latitude for movement on the part of the animal, otherwise the mosquitoes will be rubbed off. A convenient chick-holder is made from a muslin square or "blanket," a cardboard or wooden stand, a heavy rubber-band, and several straight pins (Fig. III, 2-4). The rubber-band is slipped under the flaps and stretched across the top of the stand and the muslin square is slipped under the band, so that the hole in the "blanket" and the opening in the stand coincide. The chick is then placed so that its clipped back rests over the openings; then the sides of the "blanket" are brought up and pinned securely with straight pins.

EQUIPMENT FOR LARVAE

White enamel pans are preferable for raising larvae, because the small immature forms show up better against a white background. Many workers use glass culture dishes, or even medium-sized glass or china bowls. In fact

I raised larvae for three years in culture dishes about 8 inches in diameter by 3 inches deep because they were readily available. A white background, such as a sheet of paper or a towel, is helpful when transparent containers are used. Oblong trays, such as developing trays 16 x 9½ x 2 inches are excellent rearing pans. They are used in this laboratory for culturing 300-400 larvae of either Anopheles or Aedes. They hold at least 3 quarts of water. Round pans of similar capacity are likewise widely used. Briefly, containers for rearing larvae should have a capacity of at least 3 quarts, be 2 or 3 inches deep, and preferably, but not necessarily, white and opaque.

A convenient, but not essential, piece of equipment is a 50 ml. volumetric transfer pipette, shortened to 8 or 10 inches, and fitted with a rubber bulb. (Fig. IV). This is useful when one wishes to transfer several hundred larvae or a quantity of water to another container.

Salt-shakers are a convenient means of storing and dispensing powdered food to the larvae.

EQUIPMENT FOR PUPAE

Medicine droppers with the sharp end filed off to make a wide mouth are used for removing pupae. These droppers can be made by cutting 4 inch sections of ½ inch or ⅜ inch glass tubing and fitting each with a rubber bulb. Small white enamel pans or china bowls about 3 or 4 inches deep are excel-

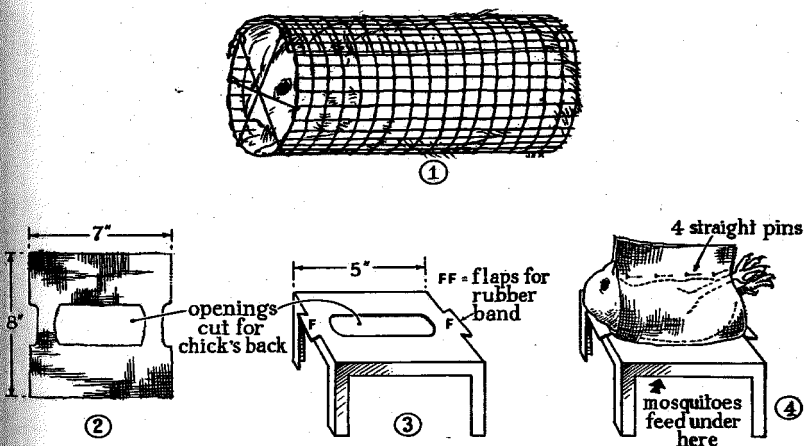

Figure III. Devices for restraining guinea pig and chick
1. *Guinea pig in hardware-cloth cylinder.*
2. *Muslin square for wrapping chick.*
3. *Small stand for holding chick.*
4. *Chick in place, ready to be set in cage.*

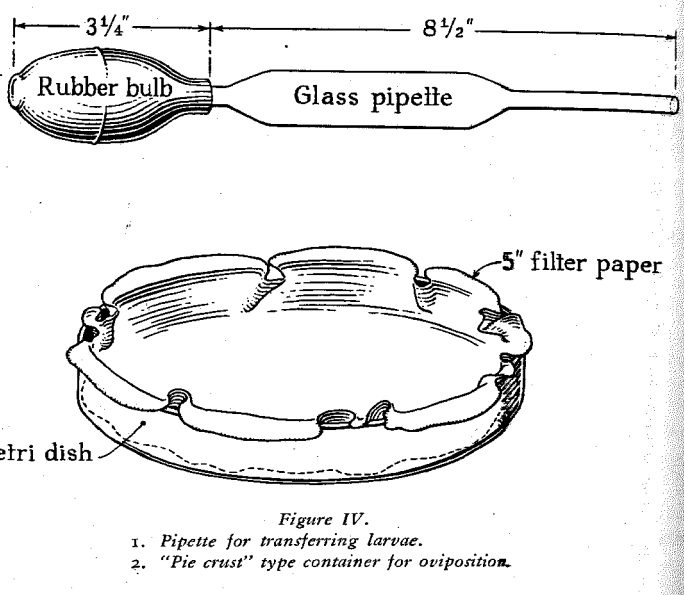

Figure IV.
1. *Pipette for transferring larvae.*
2. *"Pie crust" type container for oviposition.*

lent receptacles for water containing pupae awaiting emergence. The top and bottom halves of small petri dishes 3½ in. (9 cm.) in diameter and ½ in. (12 mm.) deep, may be used for this purpose also, but they hold only a small amount of water which can evaporate quickly.

EQUIPMENT FOR EGGS

Any of the containers used for pupae may be used for holding the water on which caged females may oviposit. If transparent glass dishes are used, a white background should be provided, such as a sheet of paper or a towel placed underneath the dishes, so that the eggs may be observed more easily. For mosquitoes that will not oviposit on open water, cellucotton pads are cut to fit into one-half of a 9 cm. petri dish, saturated with water, and covered with a circle No. 3 Whatman 9 cm. filter paper. Another useful egg-collector is a thin circle of paraffin about 9 cm. in diameter, with small holes punched in it to allow it to float just below the surface of the water in a small bowl.

Our most efficient egg-collector is one developed in our insectary and very aptly called a "pie-crust dish" (Fig. IV.). It consists of one-half of a 3½ inch (9 cm.) petri dish into which a 5 inch (12.5 cm.) filter paper has been fitted so that the fluted margins extend slightly over the edge like the lower pie-crust in a pan. The filter paper may be fitted easily without tearing if it is moistened before handling. The advantages of such a device are (1) that

the entire container remains moist as long as there is any water in the dish, and eggs laid on the sides and rim will not dry out as the water level recedes; (2) eggs are more easily washed from wet filter paper than from moist glass. In fact they frequently cannot be removed from the latter without injury.

FOOD SUPPLY AND PREPARATION

The following foods are suggested for the maintenance of a winter mosquito colony:

Dog biscuits (Purina dog chow is preferred)
Dehydrated skimmed milk
Whole oat grains (to be cut in half)
Dextrose
Ripe apples

Substitutions may be made to meet the wishes or demands of the worker. Adult mosquitoes will feed on fruit such as apples, grapes, moistened dried prunes, raisins, peaches, etc. in addition to sweetened water solutions, such as honey-water, and syrup-water. A 10% dextrose solution is prepared by pouring 50 grams (5 level tablespoonfuls) of dextrose into 450 cc. (approximately 1 pint) of distilled water. The dextrose dissolves more easily if it is added to the water, rather than the water poured over it. We have fed *Aedes aegypti* for several years on just granulated sugar dissolved in water.

Dog biscuits crushed to a coarse powder with a hammer or rolling-pin are ready for *A. aegypti* larvae. *Anopheles quadrimaculatus* require the same food as a very fine dust. This is prepared by grinding the coarse powder with a mortar and pestle, and then rubbing it through a double-thickness of fine cheesecloth. If necessary, this sieving is repeated until the powder is fine enough to float on the surface of the water in which the Anopheles are living.

WATER SUPPLY AND PREPARATION

Natural waters vary, and the choice of tap, pond, well, filtered, etc. will have to be left to individual workers. Distilled water, however, is almost always safe, and we use it exclusively. Bates "Medium S," which will be discussed later, consists of the following:

1 liter of distilled water
1 gram magnesium sulfate ($MgSO_4 \cdot 7H_2O$)
0.5 gram calcium sulfate ($CaSO_4 \cdot 2H_2O$)
0.5 gram sodium chloride (NaCl)

Rather than buy comparatively expensive chemicals, it is suggested that ordinary table salt and epsom salts be substituted for sodium chloride and magnesium sulfate, respectively.

EQUIPMENT FOR TEMPERATURE AND HUMIDITY

Maintenance of a colony of several hundred mosquitoes does not require constant temperature and humidity control devices. A temperature of 80° F.-85° F. is excellent for larvae, while 75° F. favors the longevity of adults. For general rearing work in one room, it is best to keep the temperature between

75° F. and 80° F. day and night. A maximum-miminum thermometer is useful for keeping a check on room temperatures in one's absence. A relative humidity of 80% is a good all-around humidity for this type of rearing, and it can be provided by means of 4 wet towels to each cage of adults. The towels are wet, wrung out to prevent excess dripping, and placed over 4 sides of the cage; one side is better left open. These towels should not be allowed to dry out. The moisture in a cage may be supplemented, if necessary, by a slight modification of Rozeboom's method (1936). An ordinary red clay flower pot is plugged at the bottom with cloth and filled with sand. This sand is kept wet, so that the pot will provide additional moisture and also a damp place for resting mosquitoes. The top is covered to prevent the females' ovipositing on the moist sand.

PROCEDURE FOR ANOPHELES QUADRIMACULATUS

We shall assume that you have *A. quadrimaculatus* eggs on damp filter paper. Prepare a larval rearing tray for each 300 expected larvae. Fill each tray with at least 3 quarts of water — preferably Bates' "Medium S." Add about ½ pint (250 cc.) of water from an old pan just prior to putting in the eggs; this furnishes food to the young larvae which should hatch within 48 hours. When the minute larvae are seen wriggling, dust a very small amount of powdered dog food on the surface of the water. Sprinkle small quantities of food into the trays every morning and evening unless the larvae are not consuming the food previously furnished. Excess food or food which has dropped to the bottom of the tray should be removed with a pipette, but care should be taken not to remove the larvae. Add water to replace that lost by evaporation. A fungus growth usually occurs on the sides and bottom of healthy trays of our *Anopheles* larvae. (This fungus was isolated by Dr. C. W. Emmons, Principal Mycologist, National Institute of Health, and has been identified by Dr. J. N. Couch, University of North Carolina, as a species of *Pythium*.) This is allowed to remain unless it ensnares the larvae; in this case, it may be removed in strips by means of forceps.

Developing larvae (especially Anopheline) should be protected from currents of air which disturb the water surface and often keep them in almost imperceptible but continuous motion. We discovered that the reason we could rear larvae on one shelf of a rack and not on the others was because of an air current from the air-conditioner. Such situations can be remedied by hanging up towels or arranging some other kind of barrier. If trays of larvae are placed in a glass enclosed cabinet, such as a bookcase, this situation is automatically taken care of.

At the end of about two weeks, the larvae will have attained full growth and pupae will begin to appear. Transfer these carefully by means of a wide-mouthed medicine dropper to a small container partly filled with water. Only about 30 pupae should be placed in a pan 4 inches in diameter and 2 inches deep, because crowding prevents complete emergence of adults. After all of the pupae have been picked, place the pans in a cage and fill each with water.

Small flat pieces of cork, about ½ inch square often assist newly-emerged adults in leaving the water.

A slice of ripe apple or a cellucotton pad saturated with dextrose solution may be placed in a small dish inside the cage. The 4 wet towels should be hung over the cage immediately, because the adults will begin to emerge within 24 hours. Keep the towels moist; and be sure that food is present at all times.

In about a week, the female mosquitoes will be ready for their first blood meal, which is necessary for oviposition. Feeding is encouraged by withholding fruit or dextrose for the 24 hours preceding the blood meal. Blood may be offered by placing your arm (the knuckles and fingers protected by a heavy glove) in the cage and allowing the mosquitoes to feed, or it may be furnished by a small animal such as a guinea pig, rabbit, or chick. The hair or down on the animal's back and sides is cut with clippers or small scissors, the animal partially immobilized by one of several methods (see instructions under *Equipment for Adults*), and placed inside the cage for 15 minutes. Removal of towels from the cage at this time will disturb the females and thus encourage feeding. Several hundred mosquitoes may bite, and it may be advantageous to quiet the guinea pig or rabbit by letting it nibble on a small piece of apple or carrot. We have seen guinea pigs accustomed to handling sit quietly without restraint in a cage with several thousand mosquitoes. After feeding, the mosquitoes should be allowed to settle down in the cage before you attempt to remove your arm or the animal; otherwise you will run the risk of some escaping when the sleeve is opened. Blood-meals should be furnished at least once a week.

After the females have had their first blood-meal, a small container filled with water should be kept in the cage continually for oviposition. As the water evaporates, it must be replenished in order to prevent eggs from becoming stranded on the sides of the container. Every third day they should be washed into trays for the next larval generation.

PROCEDURE FOR AEDES AEGYPTI

Each larval tray is filled with about 3 quarts of distilled water and prepared for eggs by adding food 12 to 24 hours in advance, or by adding about ½ pint (250 cc.) of water from a previously used pan just prior to putting in the eggs. This procedure is followed in order to stimulate hatching of the older eggs, as explained by Gjullin and co-workers (1941), and at the same time to furnish food for the young larvae. If eggs are received dried on filter papers, immerse the papers in the water and allow them to remain until at least several hundred larvae have hatched in each pan. This may require several minutes or several hours. All of the eggs may not hatch at the first submergence, and it is possible to remove the filter papers, dry, and store them for subsequent re-submergence and additional hatching. When the young wrigglers appear, add some coarsely powdered dog food and some oat grains cut in half. The larvae should be divided so that only about 300

are in each tray. Watch the pans carefully so that you do not overfeed. An excess of food will cause a film or pellicle to form on the surface of the water and kill the larvae. Normally, the water will discolor slightly with time, but this is not the cloudy, scummy appearance forecasting mortality. A small amount of dog food and about 20 oat grains cut in half are a daily quota for one pan of healthy larvae.

In about a week, pupae will begin to appear. These should be transferred to the cage in the same way as given for the *Anopheles quadrimaculatus*. The male pupae of *Aedes aegypti* are smaller than the females of the same lot, as described by Cantrell (1939). By saving only the females and a few males for mating, the maintaining of excess males may be eliminated. Unwanted pupae can be easily killed in hot water. Wet towels and food are provided as for the *Anopheles quadrimaculatus*. A blood meal is furnished as with the above species, but eggs are collected in a slightly different manner. We suggest that one-half of a small petri dish be fitted with a pad of cellucotton, saturated with water, and covered with a circle of No. 3 Whatman filter paper for oviposition. Fifty females will deposit on an average of 2,000 to 3,000 eggs a week on such a paper. When the paper is well-covered with eggs, remove the entire pad and allow it to dry at room temperature for about 3 or 4 days. In this way, the developing eggs are "conditioned" as explained by Shannon and Putnam (1934), and a greater per cent of hatch can be expected. These eggpapers once "conditioned" may be dried for several days or a year. However, the per cent of hatch decreases with time, and it is better if eggs are used within two months. A paraffin circle floated just below the surface of the water in a small bowl, as described under *Equipment,* is a less expensive, but not as efficient a method of egg collection.

Procedure for Culex pipiens and C. quinquefasciatus

Directions for hatching eggs and rearing larvae continuously are given by Huff (1937); and we suggest that the eggs be washed into the trays of water and the same method be followed as with the rearing of immature *Aedes aegypti*. Dehydrated skimmed milk is the food Huff recommends, and this should be given carefully with attention being paid to the amount the larvae are consuming. A combination of ground dog food and brewers' yeast is an excellent larval food, also. Pupae are removed to the rearing cage, which is furnished with wet towels, and fruit or dextrose for the emerging adults, and blood-meals provided — as with the preceding species. Eggs are laid on open water in dishes as are those of *Anopheles quadrimaculatus,* or they are deposited on saturated filter papers over cellucotton pads as are those of *Aedes aegypti*.

Precautions to be Observed

Everyone makes mistakes. For your convenience and from our own experience and observations, we have listed some pitfalls to be avoided.

1. Check your screening. Lay a ruler on the material and count the number of meshes yourself. Are there at least 18 meshes to one inch?
2. Verify the night and week-end temperatures of your rearing-room. Sudden changes in temperature play havoc with insect colonies.
3. See that pans for eggs and immatures are rinsed thoroughly. Unexplained larval mortality has been traced to a residue of soap powder.
4. Avoid undue disturbance of cages and pans containing mosquitoes. Emerging adults and ovipositing females should be absolutely quiet.
5. Do not pour water containing living larvae or pupae into the sink. Strain the water through cheesecloth or muslin and destroy the insects in boiling water. This will prevent development of mosquitoes in the sink-trap.
6. Avoid the use of insecticidal sprays in close proximitity to rearing room. Fly or roach sprays used five rooms away have adversely affected adult mosquitoes. Aeresols and DDT are even more dangerous.

NOTES ON DEVELOPMENT AND BEHAVIOR

The basic requirements of mosquitoes and the conditions affecting their development have led to much research on the water and foods favorable to mosquito rearing and on the responses of mosquitoes to varying degrees of temperature, humidity, and light.

WATER AND FOOD

A variety of waters — pond, rain, filtered, tap, and distilled — are among those recommended as best suited for larval culture. Natural waters vary, and the treatment of tap water in cities differs. In this laboratory, we find that more certain results follow the use of distilled water than with that drawn from the tap. Larvae will not develop and pupate consistently in tap water in the vicinity of Washington, D. C. Mosquito-workers from other parts of the country relate similar experiences. Granett and Haynes (1944) report the use of filtered water at New Brunswick, N. J.

Alkalinity or acidity of water (hydrogen-ion content or pH) has been the subject of much investigation. MacGregor (1929) concluded in part that "the influence of pH is of unquestionable importance and that its significance lies in the fact that, under natural conditions, it indicates the favorable or unfavorable association of chemical and biological factors in the breeding-places upon which the successful or unsuccessful development of the larvae depends." We used Squibb's Nitrazine papers to ascertain the approximate pH of cultures in our laboratory. New, old, thriving, average, and sickly lots were tested. *Anopheles quadrimaculatus, A. punctipennis, Aedes aegypti,* and *A. vexans* were tested according to directions, and the resulting color comparison showed no variations which could be correlated, except a tendency for the older cultures toward neutral. Water when placed in the pans is usually about pH 5.5.

Effects of various chemicals on larval breeding have been of interest to many workers. Bates (1941) found that the addition of certain salts to distilled water was favorable to the development of larvae of *Anopheles atroparvus, A. labranchiae, A. maculipennis,* and *A. superpictus.* We use his "Medium S" for much of our *Anopheles* rearing.

Larval nutritional studies have resulted in abundant literature. It is of interest to note that our experiences in raising Anopheline larvae seem to verify Hinman's assumption (1933) that a fungus may produce a growth-stimulating factor. Our thriving cultures of *Anopheles quadrimaculatus* exhibit a fungus growing along the sides and bottom of the pans, which if not thick enough to entangle larvae, is allowed to remain.

Adults have been fed a variety of fruits and sweetened waters. Research has shown that females and males will thrive on such diets, but that with a few exceptions, and for all practical purposes, blood meals are a pre-requisite for deposition of viable eggs.

EFFECTS OF TEMPERATURE, HUMIDITY, AND LIGHT VARIATIONS

Temperature and humidity conditions have been reported on by many workers, including Shannon and Putnam (1934). Huffaker (1944) reared *Anopheles quadrimaculatus* in abundance for temperature studies, and discovered that the optimum temperature for larval development was around 31° C. (87° F.). Hurlburt (1943) also studied the rate of growth of this species in relation to temperature, and found that a constant temperature of 35° C. (95° F.) prevented rearing of this species while constant temperatures of 82° ± 1°, 74° ± 2°, and 64° ± 1° F. permitted continuance of the life-cycle. He concluded that there is an increased rate of growth with a rise in temperature, although he did not state that it was directly proportional. Both Huffaker and Hurlburt reared the larvae in accordance with the recommendations of Crowell (1940). Our general experience over a period of 8 years has been that adult mosquitoes will accommodate themselves to a rise in temperature more rapidly than to a decrease in humidity.

Jobling (1937) experimented on the development of *Aedes aegypti, Culex pipiens,* and *C. quinquefasciatus* in complete darkness. This is an informative paper, and should be read by those planning to maintain colonies of mosquitoes. Tate and Vincent (1932) found that *Culex pipiens* fed readily in the dark only after prolonged exposure to light. Marshall and Staley (1932) reported that *Anopheles* spp. *Culiseta* spp. (=*Theobaldia*) and *Aedes* spp. fed in daylight on human blood, while *Culex pipiens* was reluctant to engorge under the same conditions. Seaton and Lumsden (1941) have compared the effect of light and darkness on *Aedes aegypti's* biting. They state that light reduced by one-half the number of mosquitoes biting. We find that, for our purposes, *Anopheles quadrimaculatus, A. punctipennis, Aedes aegypti,* and *A. vexans* feed more readily in a subdued light, although when hungry enough, they feed equally well under a bright light or in almost total darkness.

LARGE-SCALE REARING

Large-scale rearing of mosquitoes has been carried on in laboratories all over the world. In the United States, material has been published on the rearing of some of our native species, *Anopheles quadrimaculatus* Say, *A. punctipennis* (Say), *Aedes aegypti* (Linn.), *Culex pipiens* Linn., *Culex quinquefasciatus* Say (=*fatigans* Wiedemann of European workers) and *Culiseta inornata* (=*Theobaldia inornata* Williston).

Anopheles spp.

Anopheles quadrimaculatus and *A. punctipennis* were maintained in large colonies by Boyd and co-workers who published accounts over a period of years (1926, 1930, 1932, and 1935). The 1932 and 1935 papers were arranged and published in a compilation by Galtsoff, Lutz, Welch, and Needham (1937). Equipment and procedures are carefully explained in all of these articles. Larvae were fed on hay or wheat infusions supplemented by yeast. Adults were kept in cages 8 x 13 x 10 feet.

Crowell (1940) rearing *A. quadrimaculatus,* found that he could substitute powdered dog biscuit as a larval food, and that one pound was sufficient for rearing 29,000 larvae. Larvae were reared in tap water in enamel pans 20 x 12 x 2 inches. Adults were fed fruit or 10% dextrose solution and were kept in cages with sides 40 inches square.

Burgess and Young (1944), in a well illustrated paper, summarize the method employed for *A. quadrimaculatus* rearing in the Columbia, S. C., laboratory.

Anopheles albimanus Wied. was maintained in a laboratory in Panama by Rozeboom (1936). He succeeded in keeping adults of this species in a cage 2 x 2 x 2 feet, at a temperature of 80° F. to 86° F. with relative humidity at about 80%. His method of supplementing moisture in the cage is noteworthy. He plugged one end of an unglazed drain tile, 12 inches long by 5 inches in diameter, with paraffin and cloth, then added sand, which he moistened daily. This provided the necessary extra moisture as well as a damp place for the resting adults.

Aedes aegypti (LINN.)

Large colonies of *Aedes aegypti* are maintained in laboratories throughout the world. This species was raised in abundance in a laboratory in Brazil, and excellent papers by Shannon and Putnam (1934) and Putnam and Shannon (1934) discuss methods and equipment thoroughly.

Granett and Powers (1937) describe their method of maintaining supplies of *A. aegypti* and *Culex* mosquitoes during the dormant season. They recommend a mixture of powdered skim milk, Harris brewers' yeast or Fleischman's yeast, and powdered dry bread.

Granett and Haynes (1944) report on the rearing of approximately 12,000 *A. aegypti* weekly for repellent tests. They use water filtered as explained in

their paper given at the Thirty-first Annual Meeting of the New Jersey Extermination Association.

At the same meetings, a method for providing at least 1200 female *A. aegypti* weekly, was offered by Trembley (1944). Larvae were reared in distilled water and fed oat grains cut in half, or a combination of oats and crushed dog biscuits.

Culex spp.

Culex pipiens and *Culex quinquefasciatus* are common rain-barrel or house mosquitoes. They are widespread, and much has been published on rearing them in captivity.

Huff (1937) in concluding a paper on rearing methods for *C. pipiens* and *C. quinquefasciatus* states: "When attention is directed to the essential requirements of these species it will be found that they may be grown with ease." He found a temperature of 80° F. preferable. He reared larvae in white enameled pans or refrigerator dishes and recommends dehydrated skimmed milk as food. The adults were kept in lantern globe cages and fed preferably raisins which had been cooked in a small amount of water. Further recommendations for the raising of these two species of *Culex* may be found in the paper by Granett and Powers (1937) already referred to, and other papers in the bibliography.

Culiseta inornata (=*Theobaldia inornata* WILLISTON).

Culiseta inornata was successfully established and maintained in a captive colony by Owen (1937 and 1942). A semi-balanced aquarium provided food and habitat for the larvae.

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