Murphy (Snow *et al.*, 1957); Lake Junaluska (Jamnback and Wirth, 1963). Since our concepts of this species were altered in 1963 (Jamnback and Wirth), the first three records above may actually refer to *C. sanguisuga* (Coquillett). *C. paraensis* (Goeldi): South Mills (NMNH). *C. sanguisuga* (Coquillett): Jackson, Macon, and Rowan Cos. (Jamnback and Wirth, 1963). *C. venustus* Hoffman: Camp Davis (Foote and Pratt, 1954); Murphy (Snow *et al.*, 1957). There are now 15 *Culicoides* spp. known from North Carolina.

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# AXENIC REARING OF *CULEX SALINARIUS* [1]

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INTRODUCTION. The experimental rearing of mosquito larvae in sterile media was initiated by Barber (1927, 1928) in his examination of the role of microorganisms in larval nutrition. He reported no growth of *Culex quinquefasciatus* and *Aedes aegypti* larvae in sterile media, or in water containing only dead organic water.

Later, Hinman (1930, 1932) was able to rear a few *A. aegypti* to the adult stage, but obtained no growth of *Culex* or *Anopheles* larvae. Rozeboom (1935), in his study of the relationship of bacteria and bacterial filtrates to the development of larvae, found that sterilization of the medium rendered it unsuitable for larval development since bacteria were utilized as food. However, Trager (1935a, 1935b, 1936 and 1937) in study of growth requirements of larval *A. aegypti* found that growth occurred in media free of microorganisms when essential nutritional components were incorporated in it. Since then, investigators have axenically reared this species for various purposes (Trager and Sabbarow, 1938; Sabbarow and Trager, 1940; Trager, 1935a, 1935b, 1936, 1937, 1948; Buddington, 1941; Goldberg and DeMeillon, 1948a, 1948b; Lea *et al.* 1956; Lea, 1957; Singh and Brown, 1957; Grace, 1966; Akov, 1962; and Peleg and Trager, 1963). However, only a few species other than *A. aegypti* have been

[1] This study initially supported by USPHS Research Grant from the National Institutes of Health, No. 5 RO1-GM 12362-05.

[2] A portion of this work is included in the junior author's study that was conducted in partial fulfillment of the essay requirement for the degree of Master of Public Health, Yale University School of Medicine.

grown in sterile media: *Culiseta incidens* (Frost *et al.*, 1936), *Culex pipiens pipiens* (Buddington, 1941), *Culex pipiens molestus* (Lichtenstein, 1948) and *Aedes taeniorhynchus* (Nayar, 1966).

The present work on axenic rearing of additional species of mosquitoes was initiated in association with mosquito cell culturing attempts requiring sterile tissue of larvae and pupae, and the purpose of this paper is to report the first successful axenic rearing of *Culex salinarius.*

MATERIALS AND METHODS. The mosquitoes were obtained from the colony of *C. salinarius* established in the laboratory (Wallis and Whitman, 1968). Egg rafts were deposited on distilled water placed in the colony cage. They were allowed to develop for 24 hours and examined under a dissecting microscope for signs of growing embryos. Those appearing viable were transferred with a small sterile spatula to the surface of White's solution (White, 1931), for surface sterilization of the egg caps. In the initial techniques the egg rafts were submerged in the solution and individual eggs broken away from the raft for washing. When larvae failed to hatch from eggs treated in this way, a modified procedure which gave less exposure to the disinfectant was followed. In this undertaking the rafts were not immersed in the solution; instead, they were carefully placed in an upright position and floated on the surface of the disinfectant for 20 to 30 minutes. They were then floated on the surface of 2 ml of nutrient medium (the mosquito tissue culture medium described by Singh (1967), supplemented with 16 percent of commercial medium 199, with glutamine and 2X Earle's base (Cat. No. 115EP, Grand Island Biological Co., New York)) in 5 ml sterile test tubes until hatching occurred during the next 24-hour period. Any tubes showing signs of contamination during this time were discarded.

Newly hatched larvae remaining uncontaminated were transferred in sterile pipettes to 8 ml of fresh medium in sterile 30 ml plastic (Falcon) flasks (15 larvae per flask) which were then sealed. These were incubated at 28° C. until completion of larval, pupal or adult development, without reopening the flasks.

Usually ten flasks providing a total of 150 larvae, were set up at one time. This procedure was repeated 28 times during a period of 16 months to provide live sterile *C. salinarius* larvae, pupae, and adults for experimental tissue culture.

RESULTS AND DISCUSSION. Initially very poor results were obtained in the process of surface sterilization of the eggs. When egg rafts were broken up to allow complete immersion of eggs in White's solution, no hatching occurred. However, when the procedure was modified so that only the portion of the rafts that came in contact with the nutrient medium was surface-sterilized by flotation on White's solution for 20 to 30 minutes, over 90 percent of the eggs remained viable. Of larvae hatching into tubes of nutrient medium, only an average of 10 percent were lost due to initial contamination. When this occurred, bacterial growth in the nutrient medium appeared during the first 24 hours after hatching.

The larvae remaining uncontaminated during the initial hatching period, when transferred to fresh medium and sealed in the plastic flasks, grew normally through the four larval instars. The only exception was a somewhat longer than normal larval developmental period. They pupated in a 2- to 3-week period, usually without larval mortality. Pupal development occurred and both male and female adults emerged in the flasks after a 2- to 4-day pupal period. Adults were left in the rearing flasks in only two of the replications of the axenic rearing procedure, but good viability was observed. Most adults survived the first few days, and an average of 80 percent remained alive for 1 week if left undisturbed. However, as increased flight activity was attempted in the narrow confines of the flask, most of the adult mosquitoes became trapped in the surface film of the medium and died during the second week of life.

Since the purpose of this work was to provide live sterile mosquitoes in various stages of development for tissue culture starts, in most of the replications of the rearing procedure, larvae and pupae were sacrificed during various developmental periods, and no attempt is made to give cumulative totals of numbers surviving the different growth stages under axenic rearing conditions. It can be concluded, however, that this culture method is suitable for repeated production of all growth stages of *C. salinarius* in sufficiently large numbers to make it a practical procedure for providing sterile material for tissue culture work with this species.

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