that ovariole contractions reduce the outgrowth. Contractions of the muscle layer may dislodge the exposed ovarioles. Because explants must be attached to produce many cells, contractions detract from the quality of the culture. Unfortunately, ovariole contractions appear in earlier stages of differentiation in vitro than in vivo. These premature contractions also damage follicles and probably have an adverse effect upon the follicle cells. Thus ovaries for explant cultures should be excised before the muscular sheath is able to contract. Differentiation often continues in vitro, particularly in *G. mellonella,* and ovariole contraction may commence after several days in culture.

These criteria suggest that the ovaries of young insects produce the best cultures. However, the application of this suggestion is limited by practical consideration. In many species, the larval ovaries are so small that it is not feasible to collect quantities of tissue. Also the tissues of young larvae may not be sufficiently differentiated to yield competent cells, as Grace (1958) demonstrated with the early instars of *B. mori.* Thus, the optimum stage is the result of a compromise between several conflicting demands. The stage of choice should provide an adequate weight of ovaries containing healthy intermediate layer cells, young follicular epithelium, and preferably, undifferentiated ovariole sheath muscle.

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Collecting and Rearing Black Flies[1, 2, 3]

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ABSTRACT

This paper, based on a study carried out at the Seney National Wildlife Refuge, Seney, Michigan, and the Patuxent Wildlife Research Center, Laurel, Maryland, describes methods and techniques for collecting, storing, and rearing 8 species of Simuliidae. Included in the study were *Cnephia dacotensis* (Dyar & Shannon), *C. mutata* (Malloch), *Prosimulium fuscum* Syme & Davies, *Simulium aureum* Fries, *S. decorum* (Walker), *S. venustum* Say, *S. verecundum* Stone & Jamnback, and *S. vittatum* Zetterstedt. Simuliid eggs collected from a variety of habitats, packed with wet materials in plastic bags and held at 4°C, have remained viable, thus far, up to 424 days. Eggs held at 0° to —70°C failed to hatch when placed in rearing aquariums. Air-dried eggs held at 4°C for 339–535 days failed to hatch after being held in aquariums for 166 days. Immature stages of black flies were successfully reared in 1¼-gallon Pyrex jars and 15-gallon plexiglas aquariums in which the water was agitated by compressed air and aquarium pumps. Water velocities of 0.250–0.542 feet per second were obtained in streams of air bubbles created by use of air stones. Of 21 larval diets used, one consisting of Purina Dog Chow (60-mesh or finer), brain-heart infusion broth, and brewer's yeast powder, and a second consisting of Wayne's dog chow (60-mesh or finer) and brain-heart infusion broth without brewer's yeast powder, were most satisfactory. Larvae were fed 1 gram of diet per 1¼-gallon aquarium and 2 grams per 15-gallon aquarium biweekly. In most of the 225 cultures of *S. aureum, S. decorum, S. verecundum, S. venustum,* and *S. vittatum* it took from 1 to 5 days for the development of eggs to larvae (165 cultures, 73%) and the same number of days for the development of pupae to adults (172 cultures, 76%). Development time for larvae to pupae was fairly closely divided between 11–15 days (63 cultures, 28%) and 16–20 days (53 cultures, 23.6%). The entire period for development from egg to adult after placement of eggs in rearing aquariums was from 21 to 25 days for 26.7% of the cultures.

In studies relating to the biology and ecology of black flies as vectors of *Leucocytozoon* infection in Canada geese, *Branta canadensis,* at the Seney National Wildlife Refuge, Seney, Mich., I undertook a survey to determine the species of black flies that occurred on the Refuge. The only black flies of importance in the epizootiology of *Leucocytozoon* are those that are ornithophilic, but I studied various mammalophilic and anthropophilic species as background for carrying out research with ornithophilic species.

Although several species of black flies were collected during each spring and early summer over a 4-year period (1963–66) it is probable that those collected represent only a part of the total present. The species collected included *Cnephia dacotensis* (Dyar & Shannon); *C. mutata* (Malloch); *C. taeniatifrons* (Enderlein); *Prosimulium fuscum* Syme & Davies; *Simulium aureum* Fries; *S. decorum* (Walker); *S. emarginatum* Davies, Peterson, & Wood; *S. rugglesi* Nicholson & Mickel; *S. tuberosum* (Lund-

[1] Diptera: Simuliidae.
[2] Accepted for publication August 31, 1967.
[3] Mention of proprietary products does not necessarily imply their endorsement by the Department of the Interior.

ström); *S. venustum* Say; *S. verecundum* Stone & Jamnback; and *S. vittatum* Zetterstedt.

The 95,535-acre Refuge is situated in the east-central portion of Michigan's Upper Peninsula. The Refuge is composed of 4 broad habitat types: cropland 416 acres; upland (brush and timber) 26,911 acres; marshland 60,065 acres; and open water 7243 acres. Most of the open water is contained in 21 pools in which water levels are controlled. These pools, which range from 27 to more than 1000 acres, receive a constant water supply from the Driggs River, Walsh Ditch, and Marsh Creek through a system of diversion ditches.

In the latter part of 1964 a rearing program was undertaken to determine if immature stages of black flies could be reared in the laboratory and if they could be collected in sufficient numbers to carry on a large-scale rearing program. I believed that by rearing flies in this way I might find some species that would not be captured as adults, data might be obtained that would be valuable later in developing controls for these flies, and valuable knowledge would be obtained for rearing quantities of flies for use in experimental transmission studies on *Leucocytozoon*.

Although various methods of rearing Simuliidae have been developed by other investigators (Jobbins-Pomeroy 1916; Puri 1925; Wu 1930; Bradt 1932; Smart 1934; Bequaert 1934; Mackerras and Mackerras 1948; Dalmat 1955; Fredeen 1959a; Ussova 1961; Hall and Harrod 1963; Wenk 1965; Wood and Davies 1965, 1966) these methods were, for the most part, unsuitable for my specific needs. Since I was primarily attempting to rear large numbers of black flies, I had to develop and utilize inexpensive equipment and techniques that would be easy to set up and handle in the field and in the laboratory, and yet would work well for the wide variety of species taken from many breeding sites.

MATERIALS AND METHODS

Collection and Storage of Immature Stages.—Eggs.—Eggs of Simuliidae were collected along the shores of pools, streams, rivers, and diversion ditches; above and below spillways and waterfalls; from trailing and standing vegetation; and from various objects found floating or lodged in the water. Along pond shores near spillways, black fly eggs were taken on masses of washed-up water-starworts, *Callitriche verne,* and slender spike rush, *Eleocharis acicularis.* In the hope that simuliid eggs might be recovered from the silt in stream bottoms, samples were taken also with an Ekman dredge.

Whenever feasible, pieces of vegetation, small stones, tree limbs, twigs, and other objects harboring egg masses were collected in toto. Eggs on pilings, spillway boards, and spillway-board supports were carefully collected with a spatula or tongue depressor.

All egg samples, except those taken with a spatula or dredge, were placed in water-filled, plastic-lidded 1-lb coffee cans (metal containers 5½ in. high and 4 in. diam). The cans were placed in Freezesafe® styrofoam coolers[4] containing blocks of ice.

Egg samples taken with a spatula were held in disposable plastic petri dishes in the coolers. Dredge samples were placed in plastic bags, the openings secured with rubber bands, and the bags placed in plastic-lidded coffee cans which were then taped closed.

In the field, the egg samples were held in the coolers up to 24 hr. All samples, when removed from the coolers, were placed in refrigerators and held at 4°C. The egg samples on vegetation and rocks can be shipped in the cans with water or removed from the water and wrapped in wet cotton or cheesecloth. The choice would depend on storage space, transport space, and the length of time involved. One to 2 weeks would be the maximum time recommended for storage in the cans, unless one first lined them with plastic bags, as the cans do rust. The egg samples in the petri dishes were shipped in the dishes and the dredge samples were shipped in the cans in which they had been collected.

Eight×12-in. pieces of wet absorbent cotton, Dacron batting, cheesecloth, or gauze (3 layers) were used to wrap egg samples. Before being wrapped, each sample of vegetation was cut to a 4-in. length. The cut pieces were laid out crosswide down the center of the wrapping material, about ½-in. apart. The 2 sides of the material were folded over the ends of the vegetation and then the material and eggs were rolled up and placed in a plastic bag, which in turn was rolled up and fastened with a rubber band. Rocks and twigs were wrapped in the same type of wet material and placed in plastic bags. The petri dishes were placed in plastic bags and secured with rubber bands. All packages were labeled with pertinent data. The packaged samples were held at 4°C in a refrigerator in the field laboratory prior to shipment and at the home laboratory in Maryland. Styrofoam® coolers were used for shipment of the packaged eggs and cans of dredge samples from Michigan to Maryland. Blocks of ice were made by freezing water in 1-lb coffee cans. Six plastic-lidded cans of ice were used in each cooler. This amount of ice was sufficient for storage for 60 hr or longer even in the heat of August. Dry ice cannot be used as it freezes the eggs. All my experiments to date have been with mixed cultures, because the eggs I have collected in the field have always been of mixed species. I have not had any known pure cultures of eggs to work with, since none of my laboratory-reared flies have mated or oviposited in the laboratory.

Larvae.—Larvae were collected in the same water habitats and from the same kinds of vegetation and objects as eggs. Pieces of vegetation, small stones, small tree limbs, twigs, and other small objects harboring larvae were collected in toto. A pair of forceps was used to removed larvae attached to large objects, such as spillway boards and boulders. Additionally, larvae were collected on pieces of gauze (Tarshis 1968).

[4] Manufactured by Polyfoam Packers Division, Glo-Brite Foam Plastics, Chicago, Ill.

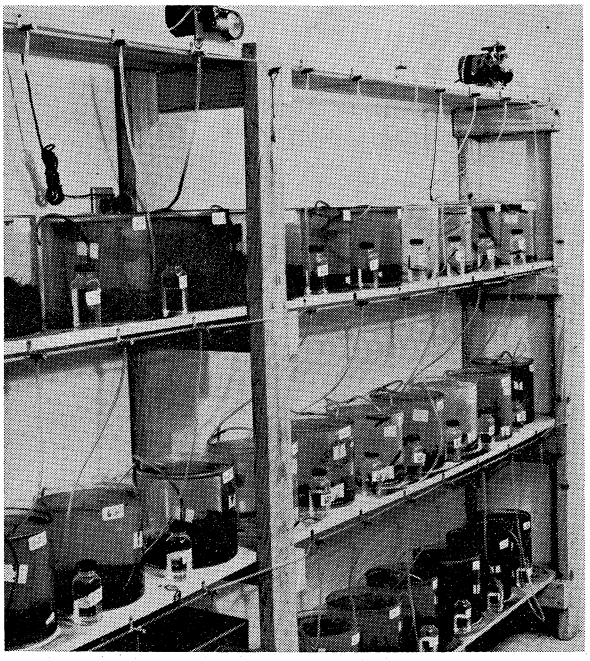

FIG. 1.—Equipment for rearing simuliids: 1¼-gal Pyrex aquarium and the 2 types of pumps used to agitate water in the aquariums. The pump top left is the Supreme 100 and the pump top right is the Titan III.

Collected larvae were handled in 1 of 2 ways: if field collections were to take less than 8 hr, the larvae were placed in cans of water and held in styrofoam coolers for transport to the field laboratory; if collections were to take longer, the larvae were placed in lidded coffee cans and held in a larval transport box containing equipment for agitating the water within the containers (Tarshis 1966b). Larvae have a maximum survival time of only 8 hr in nonagitated water. The transport box was used also for transporting larvae by truck from Michigan to Maryland. Upon arrival at the field laboratory in Michigan or at the home laboratory in Maryland, the larvae were placed in aquariums.

Pupae.—Methods for collecting and transporting black fly pupae are identical to those for collecting and transporting larvae. (Since writing the paper on collection of larvae (1968), I have found that gauze left in streams several days will harbor pupae as well as larvae.) Pupae were taken for identification purposes only, and they were preserved in 70% alcohol.

During the cold spring months a waterproof neoprene-coated nylon coverall garment was worn over warm clothing for fording extremely cold streams (Tarshis 1966a).

Rearing.—Aquariums.—Two sizes of aquariums were used for rearing Simuliidae: 1¼-gal Pyrex® animal jars (Corning Glass, Stock no. 6941), 8¼-in. diam and 8 in. high (Fig. 1); and 15-gal aquariums fabricated from ¼-in.-thick plexiglass, 20¼-in. long, 14 in. wide, and 12½-in. high (Fig. 2).

Agitation of Water.—All aquariums were provided with equipment for agitating the water. Compressed air was used when available. Since most compressed air lines have some water and oil flowing through them, a side-arm Pyrex flask, half-filled with absorbent cotton and placed between the compressed air outlets and the aquarium inlets, was used as a trap for water and oil.

Where compressed air was not available, aquarium pumps were substituted. The Supreme Airmaster® Model 100[5] and the Titan III®[6] aquarium pumps were very satisfactory for this work. The Airmaster delivered enough air for 15 small or 3 large aquariums. The Titan III is an electric pump with 3 compressors, each independent of the others. Each compressor has 2 nozzles allowing 2 hoses to be used, the air source being common to both nozzles. This pump delivered enough air for 50 small and 10 large aquariums.

Streams of air bubbles were obtained by using airstones. One of the better airstones was found to be Halvin's Bluestone®, Stock no. 66B.[7] At the beginning of the experimental studies, 1 airstone was used in each small aquarium. Subsequently, it was found that the greatest number of larvae were in the vicinity of the air bubbles, so 3–6 airstones were used in each small aquarium. Each airstone was connected to a 12-in. piece of ⅛-in. ID flexible aquarium polyethylene tubing and the 3 pieces of tubing were connected to the aquarium pump air outlet by means of a metal aquarium "cross."[7] When 6 airstones were used, 3

[5] Manufactured by Eugene G. Danner Mfg., Inc., 1660 Summerfield Street, Brooklyn, N. Y.

[6] Manufactured by Ray-Wayland Corp., Box 919, Oak Park, Ill.

[7] Manufactured by Halvin Products Co., 1916 McDonald Ave., Brooklyn, N. Y.

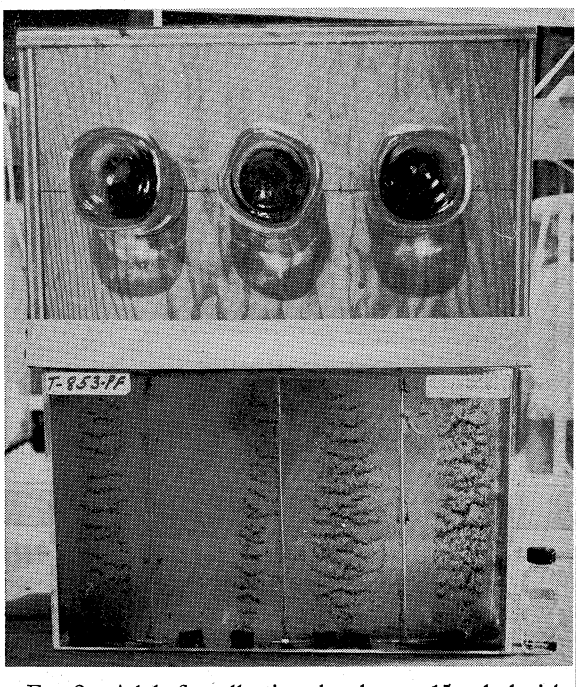

FIG. 2.—Adult-fly collection chamber on 15-gal plexiglas aquarium. Note concentration of simuliid larvae in air streams above airstones in aquarium.

sets of 2 airstones each were connected by a metal aquarium "T."

Ten pairs of airstones (20) were used in each of the large plexiglas tanks, 6 on each side and 4 on each end. The 2 airstones of each pair were connected with a metal aquarium "T" and the "T" was connected to the main air supply outlet by a 12-in. piece of ⅛-in. ID rigid polyethylene tubing.

To ensure that the streams of air bubbles were flowing against the inside walls of the aquariums, the air lines leading to the airstones were secured to the aquarium walls with pressure-sensitive tape. Polyester film tapes no. 471 and no. 853[8] were satisfactory. When securing these tapes it is essential to have the inside walls of the aquariums free of grease and moisture and to press the tapes tight so that no air spaces are left under them.

Artesian well water taken directly from the tap was used for rearing the immature stages. It was found unnecessary to age the water, the normal procedure when using tap water containing chlorine.

Collection of Reared Adults.—A fly-collection chamber (Fig. 3) was fitted over the top of each aquarium to collect adults emerging from pupae. The chamber was a box constructed of ¼-in.-thick exterior plywood, or ¼-in. tempered masonite, 9 in. wide, 8 in. high, and 8½-in. deep. The box was put together with ¾-in. 18-gauge nails and waterproof glue. The bottom was inset 2 in. to keep the chamber from falling off the aquarium and had a hole cut out that was slightly smaller than the inside diameter of the aquarium. The opening has to be as close as possible in diameter to the aquarium opening, so that emerging flies will not be blocked as they crawl up the walls of the aquarium to the collection chamber above. A ⅛-in. hole was drilled in the back of each adult fly collection chamber for the air line. A 3½-in.-diam hole was cut in the front of each chamber and a wide-mouth Ball® mason jar lid was secured in the opening with epoxy glue. A 1-qt jar was screwed into the lid. Since Simuliidae are positively phototropic, emerging flies went directly into the glass jar. Once a day the jar was removed and the flies were collected from it with an aspirator tube. To prevent fly escapes the jar was covered during the collecting. To make the cover, a 1½-in. hole was cut in a jar lid. Two 2-in. squares of dental dam, each with a 1-in. slit cut in it, were taped to the lid over the hole. The 2 pieces of dental dam were placed so that the 2 slits formed a cross and made escape of the flies impossible, while making it very easy to insert and remove the aspirator from the jar.

The fly-collection chamber for the large Plexiglas aquarium was made of ½-in.-thick exterior plywood, 20¼ in. long, 14 in. wide, and 12 in. high. Its design was essentially the same as that for the smaller aquarium, except that 3 collection jars were attached to the front of this larger chamber.

[8] Manufactured by Minnesota Mining and Manufacturing Co., St. Paul, Minn.

RESULTS

Prolonged Storage of Eggs.—Eggs of *S. aureum, S. decorum, S. venustum, S. verecundum,* and *S. vittatum* held at 4°C have remained viable, thus far, as long as 424 days. Although the wet cotton used to wrap the eggs decomposed and became slimy, this feature had no apparent effect on the eggs, as all those placed in an aquarium hatched within 1–5 days, the normal time for egg hatch.

No attempt was made to obtain separate data on each species. Various species will oviposit in the same place, often resulting in the placement of several layers of eggs on the same object. It was not possible to physically separate species without destroying the eggs.

Black fly eggs are held together with a protective gelatinous mass. Sterilization of eggs with 5% NaOH (to destroy surface contaminants) as suggested by Fredeen (1959b) tends to dissolve the mass, but also destroys most of the eggs. So far I haven't felt it necessary to sterilize the eggs, since they have remained viable without sterilization.

Brine flotation (Fredeen 1959b) for the extraction of eggs from sand and other dredged material from streams was felt to be impractical, since Fredeen recovered so few eggs for the many man-hours he expended in obtaining them. Therefore, I stored the eggs I collected in dredge samples just as I took them in the field, without separation. The temperature during storage was 4°C. When I wished to ascertain whether there were viable eggs in the dredge samples I emptied the stored samples, in toto, into aquariums and waited for the larvae to separate themselves from the mud and silt after they hatched from the eggs. The aquariums were filled with water, and the water was agitated with compressed air and held at 22°C from the time the samples were placed in them.

An accidental temperature change from 4° to 33°C for 18 hr had no detrimental effect on stored black fly eggs. Samples of the heat-exposed eggs hatched within 4–5 days after placement in the aquariums. Either the eggs were sufficiently protected from the heat by being wrapped in wet cotton and held in a plastic bag or this high temperature doesn't have the effect on eggs that one might expect.

These studies demonstrate the ease with which the eggs of some species of Simuliidae can be stored in the laboratory for production of adult flies whenever needed for experimental studies.

Desiccated Eggs.—In preliminary studies the eggs of *S. aureum, S. decorum, S. venustum, S. verecundum,* and *S. vittatum* collected in streams on cattail and sedge were removed from the water, air dried, placed in quart-sized cardboard containers, and held at 4°C for varying periods. After 22 days, only a few of 1 group of eggs hatched when they were placed in an aquarium. Development occurred in the normal length of time and resulted in the production of 3 adult flies. Eggs in 6 other groups failed to hatch when they were placed in aquariums after 24–40 days' storage. Later, 25 additional groups of eggs were

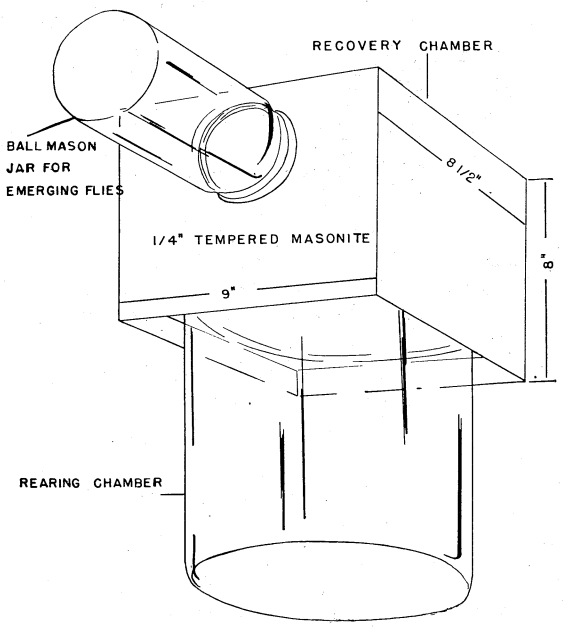

FIG. 3.—Schematic drawing of 1¼-gal Pyrex black fly rearing aquarium and adult-collection chamber.

collected on cattail and sedge, air dried, and held at 4°C for 339–535 days. All of these failed to hatch after being in aquariums for 166 days. Eggs of these species either will not develop after being desiccated for prolonged periods, or take much longer for hatching than do eggs held in a wet or damp condition.

Horsfall (1962) stated that the eggs of some species of Simuliidae, like flood-water mosquitoes, can withstand desiccation. Horsfall, in personal communication, noted that this statement was based on the works of Ussing (1925), Grenier (1949), and Fredeen (1959b). Although Ussing (1925) reported collecting the eggs of *S. latipes* (Meigen) from stream beds that had little to no water he stated: "I have always seen a little dampness under the stones in the bed of the stream just where I have found eggs of *S. latipes* at midsummer tide." Grenier (1949) quoted O'Kane (1926) who said, in regard to *S. hirtipes* (Fries):

> "Since the spring collections show larvae present in countless numbers in streams that dry up in midsummer, and since the species does not appear again in the larval stage until late in the fall, when it may be found in streams that had been dry for many weeks previously, it is evident that at least some representatives of this species must spend the midsummer period either in the adult or in the egg stage. Of the two assumptions the latter appears the more probable. The adults are the first black flies to appear in the spring and would not be likely to survive in numbers throughout the summer and fall. If they lay their eggs at this time, as is likely, and place them close to the surface of running water, the eggs would probably soon be uncovered because streams are falling rapidly at this time of year. The eggs might, presumably, remain unhatched through the summer season. With the coming of fall rains and the rise of water in the streams in October and November, the eggs would again be covered and presumably might then hatch."

Thus, O'Kane only presumed that the eggs of *S. hirtipes* remained unhatched in a desiccated state throughout the summer months. In actuality they may have been kept moist in seemingly dry river beds by damp stones, as Ussing suggested, or in some other way. There always is the possibility that underground streams and springs are keeping stones, mud, and sand moist enough to preserve eggs laid on or in them, even though one may not readily observe this.

I was never able to get development of immature stages of black flies from stream-bed dredge samples that had dried out, although wet dredge samples yielded several species of black flies (*Cnephia mutata, S. venustum, S. verecundum,* and *S. vittatum*). Fredeen et al. (1951) and Fredeen (1959a) reported successes in recovering the eggs of several species of black flies from wet dredge samples. Fredeen (1959b) reported keeping viable the eggs of *S. venustum* from dredge samples for a year or more by placing eggs collected on vegetation on damp filter paper in sealed jars.

For a long time it was thought that hibernation of Simuliidae took place only in the larval stage. Edwards (1920) was the first to suggest that some Simuliidae may hibernate in the egg stage. He suggested that the presence of a hard shell and absence of an enclosing jelly on *S. aureum* eggs may be an adaptation to life in streams that are completely dry for long periods, and that such species might survive long periods of desiccation in the egg stage. It would seem that the choice of the word "desiccation" is misleading as the description indicates the opposite: the hard shell on the eggs protected them from just such a danger. Sommerman et al. (1955) in their studies of Alaskan Simuliidae found that most Alaskan species they studied overwintered in the egg stage.

Wu (1930) subjected *S. vittatum* eggs to various periods of desiccation in the laboratory and then submerged them in water. A small number hatched from eggs that had been exposed to air for 10–14 hr, but none from eggs that had been out of water from 18 hr to 5 days. She, as I, found that when simuliid eggs were exposed on objects above water, only those that were kept moist hatched. Others beyond the reach of water shrank, died, and failed to hatch even when water was again available.

Ussova (1961) found that small creeks do not always freeze completely through in the winter. Often there will be water flowing along the bottoms of these creeks under the ice. She found both eggs and larvae in such creeks, showing once again that apparently dry waterways are not always as dry as one might presume.

Thus, from my own studies and those of others, it does not appear that there is any real evidence that simuliid eggs can survive true desiccation as certain

flood-water mosquitoes can. However, the need for more work is certainly indicated.

Effect of Temperature on Egg Survival.—In studies with the eggs of *S. aureum, S. decorum, S. venustum, S. verecundum,* and *S. vittatum* I found that freezing the eggs at 0° to −70°C destroyed the embryos. No eggs hatched when placed in aquariums after 5, 10, 15, 30, or 60 days at these low temperatures. However, at temperatures of +2°–+9°C eggs of these species wrapped in wet material remained viable as long as 424 days.

Fredeen (1959b) was unable to store the eggs of *S. venustum* and *S. vittatum* at −79°C; however, he found that the eggs of *C. dacotensis, S. arcticum* Malloch, *S. aureum, S. decorum, S. luggeri* Nicholson & Mickel, and *S. meridionale* Riley remained viable when stored at +0.5°–+1.5°C for 2–9 months. He found also (1959a) that eggs of *C. dactotensis* oviposited in the laboratory had to be kept for 5–7 months at natural stream temperatures before hatching commenced, and that 1–2 months of refrigeration at 0°C, to simulate overwintering conditions failed to accelerate or increase the rate of the hatch.

Ussova (1961) noted that during winter there is a layer of air between the water and the ice cover of streams. She found the eggs and larvae of several species of Simuliidae in the water under the ice, but felt that the majority of Simuliidae overwinter as eggs. The eggs she took from the bottoms of streams were viable. She evidently did not try to rear eggs found in ice, because she said she did not know whether the embryo dies at low ice temperatures. However, earlier (1956) she reported that she had found that eggs would survive 21 days at −10° to −18°C and concluded by saying that it could be assumed eggs frozen in ice by chance might remain alive. She based this conclusion on the fact that Zernov (1928) found the middle layers of ice to be between −0.9° and +0.1°C and the water temperature to be an almost constant +0.6°C. Ussova further reported that the organisms were usually found in the middle and lower layers of ice. Ussova (1961) found that, in winter, the larvae of Simuliidae migrated into deeper parts of the water where the current was slower. They attached themselves to the roots of aquatic plants, to grasses, to branches, and, very rarely, to stones. The development of larvae at low temperatures (+0.5° to +2.0°C) was very slow. Many larvae died during the winter and the number of resultant pupae was very small compared with the number from larvae which hatched in the autumn. Thus, she said, hibernation in the egg stage seems an important adaptation of most species of Simuliidae, ensuring their existence under the climatic conditions of the north.

Emergence of Larvae and Larval Growth.—Effect of Water Temperatures.—During my studies, where the room and water temperatures were constantly 10°C, small numbers of larvae of *S. venustum, S. verecundum,* and *S. vittatum* hatched from eggs after 7–38 days in aquariums. After 18–50 days larvae commenced to pupate. It took 21 days after pupation for the adults to emerge.

In another test, with the room and water temperatures at 15°C, egg hatch occurred in 1–8 days, larval development took 7–62 days, and pupal development took 1–26 days. When water temperatures were between 20° and 25°C egg hatches occurred in 1–5 days, larval development took 11–29 days, and pupal development took 1–5 days.

At the Seney Refuge black fly eggs do not hatch in appreciable numbers until the water temperature reaches 15°C, usually in early May.

Effect of Water Agitation.—Eggs of *S. decorum, S. venustum,* and *S. vittatum* were collected at 6 sites at the Seney Refuge. Half of these eggs were placed in aquariums in which the water was agitated and the other half were placed in aquariums in which the water was not agitated. All were held at room temperature (20°–21°C), and although eggs hatched within 2 days in each of the 12 aquariums, far fewer larvae were found in those in which the water was not agitated.

It is possible that the same number of larvae emerged from the eggs in both groups of aquariums. I have found that larvae of Simuliidae cannot live more than 8 hr in still water; therefore I believe that some larvae may have died in the aquariums in which the water was not agitated. To ensure against larval losses in routine rearing, one should always place the eggs in aquariums in which the water is being agitated.

Rearing.—Larval Diets.—Black fly larvae feed principally on food particles suspended in flowing water. Food requirements do not seem to be particularly exacting. According to Miall (1895), Kellogg (1901), and Johannsen (1903) black fly larvae feed on microscopic algae, such as desmids and diatoms, and parts of phanerogamous plants. Jobbins-Pomeroy (1916) found that the color of the larvae varies according to the available food, and that they seem to thrive best in streams containing a large proportion of such organisms as *Euglena viridis* and *Spirogyra.* Cameron (1922) and Anderson and Dicke (1960) found also that black fly larvae feed primarily on diatoms and other algae, some of which are scraped from the substratum with their mouth parts. The latter authors noted also that water containing large amounts of eroded soil particles and other detritus were unfavorable for larvae. Puri (1925) and Peterson (1956) found also that black fly larvae scrape food from the substratum with their mouth parts. In laboratory studies Puri (1925), Bradt (1932), and Smart (1934) were able to keep black fly larvae alive in water rich in algae. Bradt (1932) found skim milk powder and powdered yeast, and Vargas (1945) found different types of ground Purina® to be effective larval diets. Rubtzov (1956) reported that black fly larvae were difficult to maintain in the laboratory, but that they could be kept alive when the water in which they were kept was rich in "microorganisms." Fredeen (1959a) found that black fly larvae would feed on suspensions of live yeast cells, Pablum®, and

laboratory-cultured unicellular algae. He found also (1964) that 3 common species of Simuliidae (*Simulium venustum, S. verecundum,* and *S. vittatum*) developed from 1st-instar larvae to adults when offered washed suspensions of *Bacillus subtilis, Aerobacter aerogenes,* or *Escherichia coli* as food. Wood and Davies (1966) reared adults of *S. aureum, S. vittatum, S. verecundum, P. fuscum, P. gibsoni* (Twinn), *P. magnum* Dyar & Shannon, *C. mutata, S. pugetense* (Dyar & Shannon), and *S. pictipes* Hagen from eggs and larvae on suspensions of yeast cells.

During my studies, of 21 larval diets used, one consisting of 25 parts of Purina Dog Chow (60-mesh or finer), 10 parts of brain-heart infusion broth, and 1 part brewer's yeast powder, and a second consisting of 25 parts of Wayne's Dog Chow (60-mesh or finer) and 10 parts of brain-heart infusion broth without brewer's yeast powder yielded the best results (Table 1). Once the eggs hatched the larvae were placed on a diet of 1 g of the selected food/1¼-gal aquarium and 2 g/15-gal aquarium bi-weekly.

It is fairly easy to count pupae and adult flies, but I never found a satisfactory method for exactly counting masses of blackfly eggs without destroying them. All the eggs I worked with were encased in a gelatinous mass; the eggs had to be separated out from the mass for counting, and removal of this gelatin from around the eggs killed them. Therefore, for these larval diet studies I used the following method to gauge the number of eggs used for each experiment. I removed the eggs from a 1-in. piece of vegetation (cattail or sedge) and placed them in a counting chamber. Three counts were made of the eggs and the average was noted. I did this with several pieces of vegetation and from then on used this overall average to gauge how many eggs were on each piece of vegetation I used in the experimental work. The counting of masses of larvae is almost as difficult as the counting of eggs. Therefore, as exact a count as possible was made of the larvae that had emerged from approximately 16,000 eggs in each of 5 aquariums and, then, the overall average of these numbers was used as the gauge for this study.

All solid laboratory foods and wheat germ were ground to 60-mesh or finer with a Model 4 E Disc Mill.[9] Because ground laboratory-animal foods in combination with brain-heart infusion broth and/or heart-infusion broth absorb moisture and cake at room temperature, these media were stored in a refrigerator. The caked media are difficult to break up or dissolve in water and they do not regrind well. When caking occurred it was found best to discard the medium and make up a fresh batch. The ground laboratory-animal foods without the broths do not cake and can be kept at room temperature.

From these and subsequent studies I found that I could obtain excellent development of larvae up to and including the 3rd instar with any of several of the diets. However, after that, production decreased rapidly. Whether this decrease was caused by the diet or was a matter of overcrowding is difficult to ascertain at this time. Loss of larvae after reaching the 4th instar occurred in both sizes of aquariums.

In subsequent studies with 87 cultures using a mixture of Purina Dog Chow, brain-heart infusion broth, and brewer's yeast powder, approximately 1,392,000 eggs produced 12,545 pupae and 9,442 adults. In 92 cultures, using Wayne's Dog Chow and brain-heart infusion broth without brewer's yeast powder, approximately 1,472,000 eggs produced 11,836 pupae and 9,983 adults. The species emerging were *S. aureum, S. decorum, S. venustum, S. verecundum,* and *S. vittatum.*

In my field laboratory at the Seney Refuge, approximately 76,000 larvae in varying stages of development, collected from 4 breeding areas, produced 21,608 pupae and 18,019 adult flies. The species included *C. dacotensis, S. aureum, S. decorum, S. verecundum, S. venustum, S. vittatum,* and *P. fuscum.* The larvae were reared in two 15-gal plexiglas aquariums and were fed on a mixture of Purina Dog Chow, brain-heart infusion broth, and brewer's yeast powder.

When Fredeen (1959a) fed *S. arcticum* on dehydrated baker's yeast, he obtained 23 adults from 17,000 1st-instar larvae. In his most successful trial with the same species and diet he obtained 20 adults from 750 1st-instar larvae. In rearing experiments with *S. venustum,* using a diet of Pablum, Fredeen (1959a) obtained a 16% emergence of adult flies from 38,000 eggs and 1st-instar larvae. In their rearing experiments with *S. ornatum* Meigen, Hall and Harrod (1963), using a mixed culture of diatoms and desmids, obtained 140 adult flies (14%) from 1001 1st-instar larvae hatched from 3000 eggs. Wood and Davies (1966) reported obtaining a 50–80% yield of adults of *S. aureum* reared in the laboratory and fed on baker's yeast. They used 150 batches of eggs with 300–800 eggs in each batch. They did not mention how many larvae or pupae they obtained from these eggs.

Developmental Periods.—Wu (1930) found the incubation period for eggs of *S. vittatum* to be 4–5 days during the summer months when the water temperature was 20°–22°C. She found also that in standing water at an unstated temperature the incubation period lasted from 5–55 days. In my experiments, I found that eggs of *S. decorum, S. venustum,* and *S. vittatum* will hatch in 2 days in standing water at room temperature (20°–21°C).

Opinions differ regarding the duration of the larval period in Simuliidae. Emery (1913) reported the larval period of *S. vittatum* to be about 11 weeks and Puri (1925) reported it for *S. aureum* and *S. erythrocephalum* (DeGeer) to be 4–5 weeks during the summer months. Wu (1930) reported that under favorable conditions *S. vittatum* passed through all its larval instars within 13–17 days. Fredeen (1959a) found *S. aureum* to have about a 14-day larval period.

The duration of the pupal period seems to vary with investigators, perhaps because of the diverse rearing factors and species involved. Durations of pupal peri-

[9] Quaker City Mill, Philadelphia, Penn.

Table 1.—Diets used for rearing *Simulium aureum, S. decorum, S. verecundum, S. aureum,* and *S. vittatum.*[a]

Diets utilized	Proportions of ingredients	Pupae produced/aquarium			Adults produced/aquarium			
		No.	% from eggs	% from larvae	No.	% from eggs	% from larvae	% from pupae
Purina Dog Chow Brain-heart infusion broth Brewer's yeast powder	25 10 1	589	3.7	5.9	366	2.3	3.7	62.1
Wayne's Dog Chow Brain-heart infusion broth	25 10	385	2.4	3.9	296	1.9	3.0	76.9
Purina Dog Chow Heart infusion broth	25 10	250	1.6	2.5	206	1.3	2.1	82.4
Wayne's Dog Chow Brain-heart infusion broth	25 5	217	1.4	2.2	204	1.3	2.0	92.2
Wheat germ	100	215	1.3	2.2	126	0.8	1.3	58.6
Krackes blood media	100	177	1.1	1.8	91	.6	0.9	51.4
Non-fat whole dry milk	100	145	0.9	1.5	80	.5	.8	55.2
Wayne's Dog Chow Brain-heart infusion broth Brewer's yeast powder	25 10 1	124	.8	1.2	94	.6	.9	75.8
Liver media	100	86	.5	0.9	47	.3	.3	54.7
Brewer's yeast powder	100	80	.5	.8	22	.1	.2	27.5
Purina Dog Chow Brain-heart infusion broth	25 10	50	.3	.5	25	.2	.3	50.0
Cooked meat media	100	45	.3	.5	25	.2	.3	55.6
Brain-heart infusion broth	100	43	.3	.4	24	.2	.2	55.8
Wayne's Dog Chow Heart infusion broth	25 5	40	.3	.4	36	.2	.4	90.0
Purina Dog Chow	100	35	.2	.4	14	.1	.1	40.0
Guinea Pig Chow Brain-heart infusion broth Brewer's yeast powder	25 10 1	30	.2	.3	17	.1	.2	56.7
Mouse Chow Brain-heart infusion broth Brewer's yeast powder	25 10 1	25	.2	.3	13	.1	.1	52.0
Washed suspensions of *Bacillus subtilis*	100	10	.1	.1	7	.1	.1	70.0
Yeast extract	100	0			0			
Gelatin	100	0			0			
Suspension of microscopic algae	100	0			0			

[a] Avg of 5 aquariums/diet. About 16,000 eggs were used for each 1¼-gal aquarium, yielding about 10,000 larvae/aquarium. Water temperature 15.6°–19.4°C.

ods have been reported as follows: Newstead (1907), 2–6 days for *S. ornatum;* Jobbins-Pomeroy (1916), 3½–9 days for *S. venustum;* Cameron (1922), 5–7 days for *S. simile* Malloch; Puri (1925), 5 days for *S. nolleri* Fries; Wu (1930), 3¼–5¼ days for *S. vittatum;* Fredeen (1959a), 20 days for *C. dacotensis,* 16 days for *S. decorum,* 21 days for *S. luggeri,* 8 days for *S. venustum,* and 11 days for *S. vittatum.*

The days required for adult emergences from eggs of several species were reported by Fredeen (1959a) as follows: 30 days for *C. dacotensis,* 17 days for *S. aureum,* 20 days for *S. decorum,* 25 days for *S. luggeri,* 13 days for *S. venustum,* and 14 days for *S. vittatum.*

I followed developmental periods in 225 mixed cultures of *S. aureum, S. decorum, S. verecundum, S. venustum,* and *S. vittatum* (Table 2). In most of the cultures it took 1–5 days for the development of eggs to larvae (165 cultures, 73.3%) and the same number of days for development of pupae to adults (172 cultures, 76.4%). However, the developmental time for larvae to pupae was fairly closely divided between 11–15 days (63 cultures, 28%) and 16–20 days (53 cultures, 23.6%). The entire time for eggs to develop to adults after placement in aquariums was from 21 to 25 days for 26.7% of the cultures.

Water Current Rates.—Under natural conditions simuliid larvae have been found in streams varying from those with no currents to those with exceedingly rapid currents. Sommerman et al. (1955), in their

Table 2.—Days for development of 225 mixed cultures of *Simulium aureum, S. decorum, S. venustum, S. verecundum,* and *S. vittatum* at water temperatures of 15.6°–19.4°C.

Time in days	Cultures Completing Each Stage							
	Eggs to larvae		Larvae to pupae		Pupae to adults		Complete cycle	
	No.	%	No.	%	No.	%	No.	%
1–5	165	73.3	0		172	76.4	0	
6–10	36	16.0	29	12.9	38	16.9	0	
11–15	9	4.0	63	28.0	9	4.0	2	0.9
16–20	9	4.0	53	23.6	3	1.3	40	17.8
21–25	4	1.8	29	12.9	1	0.4	60	26.7
26–30	1	0.4	11	4.9	2	.9	36	16.0
31–35	0		8	3.6	0		29	12.9
36–40	1	0.4	14	6.2	0		11	4.9
41–45	0		6	2.7	0		16	7.1
46–50	0		7	3.1	0		17	7.6
51–55	0		4	1.8	0		4	1.8
56–60	0		0		0		4	1.8
61–65	0		1	.4	0		2	0.9
66–70	0		0		0		0	
71–75	0		0		0		1	.4
76–80	0		0		0		3	1.3

studies of Alaskan Simuliidae, recorded current flows for 5 species of *Prosimulium* to be between 1 and 7 ft/sec, for 4 species of *Cnephia* to be between 2 and 10 ft/sec, and for 13 species of *Simulium* to be between stagnant and 7 ft/sec. Peterson and Wolfe (1958) and Wolfe and Peterson (1959), working on Canadian Simuliidae, found larvae of 1 species of *Prosimulium,* 3 species of *Cnephia,* and 8 species of *Simulium* in those parts of streams where the current velocity ranged from 0.3 to 1.0 ft/sec and a few species (*P. hirtipes, S. decorum,* and *S. pictipes* Hagen) in water where the current velocity was greater than 1 ft/sec. Fredeen and Shemanchuck (1960) found black fly larvae in irrigation canals where the water flow ranged from 1–2 ft/sec, but found a few also in canals where the flow was less, and others in drops and flumes where the velocity went as high as 5 ft/sec. Where the water was rich in plankton, as in some secondary reservoirs, they found dense colonies of *S. vittatum* in flows as slow as 0.6 ft/sec and smaller numbers in water that was scarcely flowing at 0.1 ft/sec.

When I took 200 reading with a Pigmy Price Flow Meter at the Seney Refuge between 1964 and 1966 I found stream velocities to be 0.29–4.93 ft/sec at 25 breeding sites for the following species of Simuliidae: *C. dacotensis, C. mutata, C. taeniatifrons, P. fuscum, S. aureum, S. emarginatum, S. decorum, S. rugglesi, S. tuberosum, S. venustum, S. verecundum,* and *S. vittatum.*

Wu (1930) found that larvae of *S. venustum* and *S. vittatum* were still well established where the water velocity was 6 ft/sec. Bequaert (1934) pointed out that Lutz (1910) was the first to demonstrate that if black fly larvae are placed in a glass jar through which water is kept moving, the larvae will migrate to spots where the flow is the swiftest. Fredeen (1959a) tried to rear 1 species of *Cnephia* and 6 species of *Simulium* in tap and river water circulated at 0.3–1.4 ft/sec by platform shaker or compressed air. However, while larvae of *S. arcticum* developed to the 3rd instar in water flowing at this rate, it was only in water flowing at 4.5 ft/sec that even a few pupae and adults of this species were produced.

In my rearing experiments using larvae of *S. aureum, S. decorum, S. venustum, S. verecundum,* and *S. vittatum,* newly hatched larvae always migrated immediately to air streams produced by airstones on

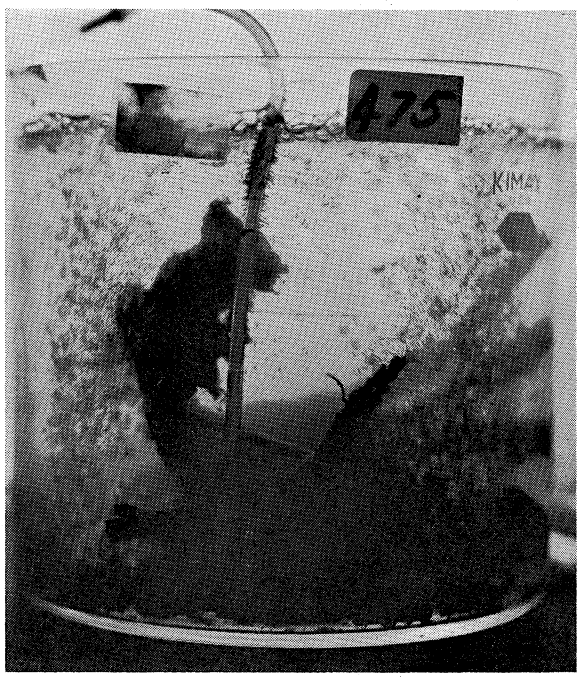

FIG. 4.—Concentration of newly emerged black fly larvae in air streams above airstones in 1¼-gal Pyrex aquarium.

Table 3.—Velocities of air streams in rearing aquariums.

Location of meter	Flow rate (ft/sec)[a]	
	15-gal tank	1¼-gal tank
1 in. below surface of water	0.479	0.542
Middle of aquarium	.250	.348
1 in. above airstone	.342	.250

[a] Avg of 10 readings/location of meter.

the bottoms of the aquariums (Fig. 4). Readings of stream flow were taken in the air streams of the aquariums with the aid of the Pigmy Price Flow Meter (Table 3).

Table 4 summarizes details of the rearing experiments. It should be noted that all data collected from the inception of this program are included here. Therefore, the results reflect all the usual initial problems with such things as perfecting equipment; formulating techniques; air, heat, power, and water failures; and inadequate larval diets. Such problems account for all the incompleted cultures of eggs listed in the 1st section of the table as well as some of the other results. The negative results obtained with 60 Ekman Dredge samples were because of drying of the samples before they were placed in the aquariums.

DISCUSSION

Though several species of Simuliidae were obtained each spring and early summer over a 4-year period at the Seney Refuge, the 12 identified species collected are believed to represent only a small number of the total species present on or in close proximity to the Refuge. This belief is based on the fact that there are such diversified breeding sites involved. It was presumed from the start of the investigative studies that a greater number of species of Simuliidae might be found if immature stages of black flies could be collected and reared to adults. With this premise in mind (as well as for other reasons), an extensive rearing program was initiated. Eggs and larvae have been collected from more than 75 widely separated breeding sites on the Refuge so as to determine not

Table 4.—Rearing Simuliidae in the laboratory.

Cultured from	No. cultures[a] Total	Negative	Incomplete (larvae and/or pupae only)	Yielding adults	No. pupae produced	No. adults emerged from pupae	Duration of cultures[b] (days)	Species reared
Eggs on rocks	46	4(9%)	7(15%)	35(76%)	2,544	1,740(68%)	1–49(21)	*C. dacotensis, S. aureum, S. decorum, S. venustum, S. verecundum, S. vittatum*
Eggs on tree limbs	8	5(62%)	0(0%)	3(38%)	285	187(66%)	23–45(34)	
Eggs scraped off abutments and spillway board supports	41	13(32%)	5(12%)	23(56%)	2,652	2,045(77%)	1–64(19)	
Eggs on vegetation[c]	393	150(38%)	45(12%)	198(50%)	24,434	19,545(80%)	1–77(22)	
Subtotal	488	172(12%)	57(12%)	259(53%)	29,915	23,517(79%)		
Eggs from Ekman dredge samples	150	109(73%)	22(14%)	19(13%)	325	285(88%)	1–28(10)	*C. mutata, S. verecundum, S. venustum, S. vittatum*
Desiccated eggs on vegetation[d]	36	34(94%)	1(3%)	1(3%)	20	2(10%)	1	*S. vittatum*
Larvae on rocks, leaves, and twigs	28	1(4%)	0	27(96%)	6,492	5,322(82%)	4–54(16)	*C. dacotensis, S. aureum, S. decorum, S. verecundum, S. venustum, S. vittatum, P. fuscum*
Larvae on spillway boards	18	1(6%)	0	17(94%)	5,000	3,821(76%)	6–37(20)	
Larvae on gauze	12	0	0	12(100%)	3,703	2,834(77%)	10–84(48)	
Larvae on vegetation[e]	31	1(3%)	0	30(97%)	6,353	6,042(95%)	7–24(16)	
Subtotal	89	3(3%)	0	86(97%)	21,608	18,019(83%)		
Grand total	763	318(42%)	80(10%)	365(48%)	51,868	41,823(81%)		

[a] Total numbers and number of species in each culture were unknown; water temperatures 16.1°–21.1°C; water velocities 0.250–0.542 ft/sec.
[b] Avg duration in parenthesis.
[c] Cattail, *Typha latifolia;* sedges, *Carex lasiocarpa* and *C. lacustris;* slender spikerush, *Eleocharis acicularis;* wildcelery, *Vallisneria americana;* soft rush, *Juncus effusus;* bluejoint, *Calamagrostis canadensis* common elodea, *Elodea canadensis;* slender pondweed, *Potamogeton pusillus;* variable pondweed, *P. gamineus;* northern naiad, *Najas flexilis;* water-starwart, *Callitriche verne.*
[d] Cattail, *T. latifolia;* sedges, *Carex lasiocarpa* and *C. lacustris.*
[e] Cattail, *T. latifolia;* sedges, *C. lasiocarpa* and *C. lacustris;* bluejoint, *Calamagrostis canadensis;* wildcelery, *V. americana;* common elodea, *E. canadensis;* slender pondweed, *P. pusillus;* variable pondweed, *P. gamineus.*

only the species present, but also the distribution of the species on the Refuge.

Contrary to expectation, no new species have been identified among the laboratory-reared flies. Five species, *C. taeniatifrons, S. rugglesi, S. emarginatum, S. excisum,* and *P. fuscum,* collected as adults, have not been identified as yet among the reared flies. However, because of the extensiveness of the program, it has not been possible to identify all the specimens reared thus far.

The cultures that were negative in all probability contained nonviable eggs. It was not possible to check the viability of eggs collected. When eggs have been taken from new-growth vegetation, indicating comparatively recent oviposition, hatching percentages have been fairly high. Eggs from older vegetation, rocks, twigs, and concrete abutments sometimes have had high percentages of hatch and sometimes have not. All unhatched egg samples were left in the aquariums several months before being discarded to be certain they were not undergoing diapause or dormancy.

It is generally possible to judge whether eggs are viable by examining them with a high-magnification hand lens. Viable eggs are plump, and the embryonic masses are visible in them. Nonviable eggs are depressed and oftentimes openings can be seen through which previously developed larvae have emerged. However, it is not always possible or feasible to examine every egg sample before placing it in an aquarium. Oftentimes fresh and old eggs are intermingled on vegetation or rocks and it is necessary to include nonviable eggs in cultures. It makes little difference from a practical standpoint, but should be borne in mind when reading results.

In the rearing experiments reported here no attempts were made to establish any self-sustaining colonies of Simuliidae. We were primarily interested in determining what species of flies could be reared from immature stages and whether massive numbers of black fly cultures could be maintained at one time in the laboratory.

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The Genus *Phytoseius*[1] in Egypt and the Sudan[2]

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ABSTRACT

Mites of the genus *Phytoseius* are generally found inhabiting low-growing vegetation in Egypt and in the Sudan. Two species have been collected from Egypt; *P. plumifer* (Canestrini & Fanzago) and *P. **solanus,*** n. sp. So far in the Sudan *P. **perforatus,*** n. sp., is the only species representing this genus. Both new species described and illustrated are found associated with tetranychid mites and with the nymphs of white flies. Although food habits of these mites are unknown, preliminary observations indicate that they are at least partly predaceous. Investigations of such mites and their relationships with other phytophagous hosts are urged.

The biology and systematics of the predatory mites of the family Phytoseiidae have received increasing attention in recent years since these mites have been found very important in the natural control of phytophagous mites and other arthropod pests of crops.

Until recently the genus *Phytoseius* has not been known from the Sudan, though some phytoseiids of other genera have been described from there (El Badry 1967a). One species, *P. plumifer* (Canestrini & Fanzago), has been reported from Egypt (El Badry 1967b). Of the 2 new species described in this paper, *P. solanus* is from Egypt, *P. perforatus* from the Sudan. The species treated here are typical members of the genus *Phytoseius* as recently defined by Chant (1965). In the following descriptions, the terminology for the dorsal and ventral chaetotaxy is that followed by El Badry (1967b), which was slightly modified from that of Schuster and Pritchard (1963). The setal nomenclature for the legs is that of Evans (1963).

The type-material of the new species is deposited in the Acarina collection of the Entomological Society of Egypt.

Phytoseius perforatus, n. sp.

(Fig. 1–7)

Diagnosis.—Distinctive in that the scutum has 4 pairs of strong pores, 2 strongly invaginated pairs on the proscutum and 2 moderately invaginated pairs on the postscutum. Setae L_2 and L_4 short and smooth; L_5 twice as long as L_1. Spermatheca highly sclerotized. Leg IV with 3 capitate macrosetae.

FEMALE.—Length 284μ, width 155μ. Dorsal shield poorly sclerotized, not covering dorsum, with 4 characteristic pairs of strong pores dorsocentrally; with 15 pairs of setae—7 in the lateral, 2 in the mediolateral, and 5 in the dorsocentral series (Fig. 1). Tritosternum (Fig. 2) with long, tapering, moderately ciliate branches. Proscutum with 6 pairs of prolateral setae, all serrate except second and fourth laterals; prolaterals I 59μ, II 7μ, III 46μ, IV 11μ, V 115μ, VI 100μ in length; mediolateral I minute, 10μ long; sub-

[1] Acarina: Phytoseiidae.
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