165

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STUDIES ON THE COLLECTION, REARING, AND BIOLOGY OF THE BLACKFLY (*Cnephia ornithophilia*)

by

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CONTENTS

FIGURES

ABSTRACT

A 5-year study (1968-72) on Cnephia ornithophilia was carried out in Maryland at the Patuxent Wildlife Research Center; data on the biology and ecology of this fly are given, along with information on collecting and rearing it. Larvae were collected at seven sites on the Center between 14 November and 29 April; pupae, between 11 January and 22 April; and adults, between 2 and 17 March. Immature stages were found in water flowing at speeds of 0.49 to 3.30 feet per second and at depths of 1 to 12 inches. The water temperatures varied from 0.5 to 15°C, the oxygen content was 10 to 13 ppm, and the pH was between 6.9 and 7.4. Most of the larvae and pupae were found on a variety of artificial and natural debris in two streams. When water temperatures were 0.5 to 10°C, larvae were found beneath the debris and vegetation in the water; and when the temperatures were 11 to 15°C, the larvae were found on top of the debris and vegetation. Immature stages were reared to adults in small modified plastic mouse cages, and in a large epoxy-coated marine plywood tank; water currents were created by use of airstones and centrifugal pumps. First to third instar larvae, placed in rearing tanks at water temperatures of 15, 19, and 22°C, pupated in 2 to 21 days, and emerged as adult flies 4 to 23 days later. Larvae with well-developed histoblasts pupated in as little time as 3 hours, with adults emerging 2½ hours later. The pattern of emergence was always more males than females the first day and more females than males on the succeeding 5 days. During the study approximately 14,242 ♂♂ and 19,152 ♀♀ emerged.

INTRODUCTION

Since its inception, the Seney National Wildlife Refuge, Seney, Michigan, has suffered major setbacks in Canada goose gosling, Branta canadensis, production due to Leucocytozoon, a protozoan parasite transmitted from bird to bird by black flies.

In 1964, I commenced studies at the Seney Refuge on the transmission of the disease and on the biology and ecology of black flies that might serve as vectors of Leucocytozoon in the goslings. Of the more than thirty ornithophilic species of black flies found on and near the Seney Refuge between 1964 and 1972, only four [Cnephia invenusta (Walker), C. taeniatifrons (Enderlein), Simulium innocens Shewell, and S. rugglesi Nicholson and Mickel] were recovered from exposed Canada geese. Of these four species, I found S. innocens to be the principal vector of Leucocytozoon in Canada geese at the Seney Refuge, causing high gosling mortalities (71 to 84 percent) every fourth year.

Though hundreds of black fly breeding sites on and surrounding the Seney Refuge were examined, only one small stream was found to harbor the immature stages of S. innocens and never in great abundance. Thus, to expediate the study it was necessary to find the immature stages of another ornithophilic species that could be collected in large numbers and reared in the laboratory to the adult stage, and that was capable of transmitting Leucocytozoon experimentally. No such fly was available in Michigan. The search continued in Maryland where on 6 February 1968, Tarshis and Stuht (1970) found larvae of Cnephia ornithophilia Davies, Peterson and Wood in the runoff stream of the Gravel Pit Pond on the grounds of the Patuxent Wildlife Research Center near Laurel; they took larvae on 15 November, pupae on 14 February, and adults on 28 February. This was the first time the species had been found in Maryland.

The very first specimen of C. ornithophilia was collected 20 May 1949 by D. M. Davies from an emergence cage placed in a stream of rapids just below Lake Sasajewun Dam in Algonquin Park, Ontario, Canada. His specimen was a female, at that time tentatively identified as C. dacotensis (Dyar and Shannon). Other females were taken by D. M. Wood in 1956 and 1957, by G. F. Bennett in 1958, and by G. F. Bennett and B. V. Peterson in 1959--all from the type locality (Davies et al. 1962). Bennett (1960) reported on the feeding habits of the species on woodland birds under the name of Cnephia "U".

Stone and Snoddy (1969) reported finding C. ornithophilia for the first time in Louisiana, Mississippi, South Carolina, and Virginia. They collected larvae in South Carolina between 11 January and 18 March, and adult females emerged from pupae on 6 February; they collected females in Louisiana and Mississippi between 6 February and 1 May; they collected larvae and pupae southern Virginia, and females emerged on 1 May; and an adult fly was collected on a flicker, Colaptes auratus, in Mississippi on 8 February.

Since finding C. ornithophilia in Maryland, I have continued studying this species in its natural environment and in the laboratory. This paper reports on studies at the Patuxent Wildlife Research Center relating to the collection, rearing, and the biology of the species. The mass rearing technique described in this paper can be useful for furthering other transmission studies utilizing laboratory-reared black flies as vectors of various human and animal diseases.

MATERIALS AND METHODS

Protective Sleeves

Latex obstetrical sleeves, shoulder length (No. 829 from Davol, Inc., Providence, R. I.*) were used over woolen mittens while collecting immature stages of C. ornithophilia and other simuliids in streams during the winter and early spring months when the water temperatures were 1 to 9°C and the water depths from 8 to 18 inches. Since the obstetrical sleeves aremade only for the right hand and are sold singly, two sleeves must be purchased and the second turned inside out for use on the left hand. The sleeves should be one or two sizes larger than hand size.

Collection of Immature Stages of Cnephia ornithophilia

Eggs

Bottom samples were taken from various streams at the Patuxent Center during 1970 with the intention of rearing larvae from eggs contained in the silt. The samples were scooped up with an empty 1-pound coffee can and poured into plastic bags which then were placed in other empty 1-pound coffee cans. These coffee cans were covered with plastic lids, and the samples were transferred to the laboratory. When ambient temperatures were above 21°C, samples were transported in iced styrofoam coolers. In the laboratory, the samples were stored at 4°C in refrigerators or placed directly in rearing tanks held at 21 to 25°C. All silt samples were broken up and mixed thoroughly with the water in the rearing tanks. Silt samples held in storage at 4°C for indefinite periods of time were kept wet to prevent any contained eggs from drying.

Larvae

When natural or artificial objects with attached larvae were collected, either the object with larvae was immersed in water in a transport container or the larvae were washed from the object in the container. Larvae, when disturbed during collection, emit silken threads, and if too many are placed in a container, they become entangled in the numerous threads and die. Agitating the water in the container avoids overcrowding and reduces larval mortality.

*Use of trade names does not imply endorsement of commercial products by the Federal Government.

Prior to 1971, larvae were collected in streams in empty 3-pound coffee cans, and transferred to the laboratory where they were placed in rearing tanks. In early 1971, I commenced agitating the water in transport containers utilizing a battery-operated air pump (No. 595 from Childre and Sanders, Box 535, Foley, Ala. 36535) and a 6-inch airstone (Addlife Products Co., Inc., 2482 Third Avenue, Bronx, N. Y. 10454). I then substituted three-gallon plastic buckets and 5-gallon galvanized metal buckets for the coffee cans. Prior to collecting larvae, the air pump, which was attached to each bucket with a metal clip, was turned on. Some stream vegetation also was placed in the bucket to provide attachment sites for the larvae, though they will adhere to the sides of the bucket if necessary (fig. 1). Additionally, this vegetation sometimes harbors the eggs of simuliids that would otherwise go undetected.

Another method of collecting the larvae was to place 3-foot-wide by 6-foot-long strips of gauze in stream beds. The gauze was left in the streams 24 to 72 hours, and then the attached larvae were rinsed off into transport containers. Any pupae on the gauze were placed in 70 percent alcohol to preserve them for identification.

Rearing Tanks

Small plastic tanks (fig. 2) and a large marine plywood tank, were used for rearing C. ornithophilia larvae. For stream bottom samples and larvae, 10-gallon plastic mouse cages (Maryland Plastics, 9 East 37th St., N.Y., N.Y. 10016) were used. They are 11½ inches wide, 18 inches long, and 7 inches high with a ½-inch-wide lip at the top. Streams of air bubbles were produced with airstones. An 11½-inch-long by 3/4-inch-square airstone (Addlife Products Co., Inc., 2482 Third Avenue, Bronx, N. Y. 10454) was used on one long side of the tank, and a 6-inch-long by 3/4-inch-square airstone was used on one short side. The airstones were placed on the bottom of the tank so that the airstones' air inlet tubes faced each other and could be connected. A 3-inch length (1/8-inch ID) of flexible, aquarium polyethylene tubing was attached to each inlet tube, and the two pieces of tubing were connected at the corner of the tank to a metal "T" connector. The metal "T" connector was then connected to an 18-inch piece of polyethylene tubing that was attached to an air compressor or an aquarium pump. The airstones and tubing were secured to the bottom and sides of the tank with 1- by 3-inch strips of pressure-sensitive waterproof tape (No. 471 from Minnesota Mining and Mfg., Co., St. Paul, Minn.).

To collect the emerging adult flies, a screened housing was placed on the top of each plastic tank. The housing frame of 1- by 1-inch fir stock was 11½ inches wide, 18 inches long, and 18 inches high. All sides of the housing except the front and bottom were covered with 20-mesh aluminum screen cloth. The front was covered with a sheet of clear butyrate plastic, 0.05 inch thick (Read Plastics, 317 Cedar St., N.W., Washington, D.C. 20012). The bottom of the housing was left open because it is placed over the open top of the tank. The screen cloth and plastic sheet were nailed to the housing frame with 3/4-inch, 16-gauge nails. A 10-inch-diameter hole was cut in the center of the plastic front, and a piece of surgical stocking material (9½ inches by 36 inches) was attached over the opening. Acetone was

used to melt the plastic and form a bond with the stocking, which overlapped the periphery of the opening by 2 inches when stretched and sealed. The long end of the surgical stocking was tied in a knot after the larvae were introduced into the tank or after the adult flies were removed. The screened housing was secured all around to the lip of the tank with 2-inch-wide strips of pressure-sensitive waterproof tape.

The second rearing tank (fig. 3) was constructed of 3/4-inch-thick marine plywood. This tank was 8 inches deep, 18 inches wide, and 58 inches long; it was first put together with waterproof glue and then screwed together with 3-inch-long wood screws placed 4 inches apart. The tank was waterproofed with fiberglass cloth and epoxy resin applied according to directions contained in booklet No. 13547 on fiberglassing, repairing or rebuilding boats (Sears, Roebuck and Co., Philadelphia, Pa. 19132). One caution must be added to the directions: wear rubber gloves when working with epoxy resins because they are very toxic (Anonymous, 1970). A wooden support stand for the tank was constructed of 2- by 4-inch fir stock with 4- by 4-inch fir stock for legs (fig. 3C).

To produce the best flow of water, a baffle board (7¼ inches high, 42 inches long, 3/4-inch thick) was placed down the center of the tank, and a piece of clear butyrate plastic (0.05 inch thick, 5¼ inches wide, 24 inches long) was attached at each end of the tank to form a curved wall (fig. 3B). The plastic was secured to the inside walls of the tank with 2-inch-wide strips of pressure-sensitive waterfroof tape. For creating water currents, two polyethylene centrifugal pumps were used (No. 7001 from Cole-Parmer Instrument Co., 7425 N. Park Ave., Chicago, Ill. 60648).

In one end wall of the plywood tank, four holes (7/8-inch-diameter) were drilled 1 inch down from the top of the tank; the two outside holes were 2½ inches from the sides of the tank, and the two center holes were 6½ inches apart (fig. 3B). Four, 3-foot lengths of 7/8-inch-diameter plastic garden hose were put through the holes for letting water in and out of the tank. The two outlet hoses extended 6 inches into the tank, and the hose ends were just above the water line; the two inlet hoses extended 6 inches into the tank, and the hose ends curved down into the water. A "J"-shaped piece of ½-inch hardware cloth, 7 by 12 inches, was secured across the inlet side of the tank. After attaching the hoses to two pumps (fig. 3C), 1-inch expandable clamps were placed over the hose ends and tightened. The base of each pump was attached to the stand shelf with wooden screws.

To collect the emerging adult flies, ascreened housing (fig. 3A). was placed on top of the rearing tank; the frame was 1- by 2-inch fir stock. The housing was covered on all but the front and bottom with 20-mesh aluminum screen cloth. The front of the housing had one hinged door and two stationary panels, all covered with clear butyrate plastic. The screen cloth and butyrate plastic were nailed to the housing frame with 3/4-inch 16-gauge nails. A 10-inch-diameter hole was cut in each plastic panel, and surgical stocking material was attached over each hole as previously described. The inside junctures between the top of the plywood tank and the bottom of the screened housing were sealed with a waterproof silicone caulking compound.

The plywood tank was kept in an environmental room where humidity, temperature, and light were thermostatically controlled. One 6-foot-long fluorescent daylight fixture was attached to the ceiling above the screened housing, and another was attached to the ceiling on the opposite side of the room. The lights were controlled with an interval timer to stay on 12 hours and off 12 hours.

A 1- to 2-inch layer of sand and fine gravel was placed on the bottom of the plywood tank, and medium-sized rocks were placed on the sand-gravel layer. Finally, a moderate quantity of dead leaves and twigs was placed on the rocks; this material was used by the larvae for pupation.

Water Supply

Artesian well water treated with a neutralizing agent was used for rearing all immature stages; the pH of the water was 7.5 and the oxygen content 9.5 ppm. The stream flow was 2.00 feet per second.

Larval Diet

The diet used to feed the larvae was comprised of Purina dog chow, brain-heart infusion broth, and brewers yeast (Tarshis, 1968). Larvae in the 10-gallon plastic tanks were fed 10 grams of larval medium weekly, those in the plywood tank 25 grams bimonthly. I found that it was best not to overfeed larvae with the prepared medium because large quantities of natural food always were brought in on the vegetation that was taken from streams when larvae were captured.

Collection and Storage of Adult Flies

Emerged adults of C. ornithophilia were collected daily with a suction apparatus. Emerged males and females were separated utilizing the CO_2 anesthetization technique described by Tarshis (1957). The females were stored in quart-sized, cylindrical, cardboard containers made from oyster or ice cream cartons 3 3/8 inches in diameter and 7 inches high. The ends of the containers were removed, and the openings were covered with 30-mesh plastic netting taped fast with 1-inch-wide masking tape. One end was placed in a petri dish containing a slice of apple to provide nutrient, and the other end had a pad of non-sterile gauze saturated with water. Flies were introduced or removed from the container through a 1-inch-diameter hole in the wall covered with two, 2-inch squares of slitted dental dam (The Hygienic Dental Mfg., Co., Akron, Ohio 44310). Each piece of dam was separately taped to the container over the hole, one on top of the other with the slits at right angles to each other. The females were held at 21 to 25°C or in a refrigerator at 4°C. Males were preserved in 70 percent alcohol in shell vials.

RESULTS AND DISCUSSION

Collection of Developing Stages of Black Flies from Streams

Eggs

Stream bottom samples were collected from seven sites on 26 October 1970 at the Patuxent Wildlife Research Center, placed in several 10-gallon plastic tanks, and held at 23°C. No larvae of any simuliids were found in the streams at the time the silt samples were taken. Three days later, 1st-instar larvae of C. ornithophilia Davies, Peterson and Wood, Prosimulium gibsoni (Twinn), Simulium decorum Walker, S. venustum Say, and S. vittatum Zetterstedt were observed in the airstreams (just above the airstones) in some of the rearing tanks. These larvae developed only from the silt samples taken from the Gravel Pit Pond runoff stream.

Larvae

C. ornithophilia larvae were collected from seven sites on the Patuxent Center (fig. 4): (1) above and below the culvert before Gravel Pit Pond, (2) Gravel Pit Pond runoff stream, 20 feet below the spillway, (3) runoff stream from culvert at juncture of Service and River Roads, (4) third temporary stream on River Road, (5) runoff stream from Snowden Pond, (6) east spillway of Cash Lake, and (7) Cash Creek runoff stream. The runoff stream of the Gravel Pit Pond (fig. 5) was the best site for collecting larvae and pupae of C. ornithophilia until the spring of 1969 when large populations of immature stages were found in Cash Creek, 2 miles east of the main gate of the Patuxent Center at State Highway 197, and 1.9 miles west of Bowie, Md. Cash Creek is 75 feet above sea level and varies in depth (under natural conditions) from 1 to 12 inches. Cash Creek is formed by the runoff from Cash Lake, a large, open, medium-shallow pond, several hundred feet north of the overpass on State Highway 197. From the overpass south, the Creek takes a tortuous route for 1.23 miles and empties into the Patuxent River. Cash Creek varies in width from 4 to 12 feet, and its sloping banks are 2 to 8 feet in height. Its banks support a variety of medium- to large-sized trees, dense brush, briars, and grass (fig. 6); its bottom is composed partly of fine- to medium-sized gravel and rocks, and partly of clay and silt. Ten to 20 feet north and south of the overpass, the Creek is littered with a variety of artificial debris (e.g., old automobile tires, petticoat insulators, newspapers, concrete slabs, bricks, pieces of electrical wiring, glass and metal beverage containers, stockings, pieces of construction lumber). The presence of this debris did not seem to hinder development of larvae, for large populations were found on these objects. The remaining portion of the Creek, south of the overpass to the Patuxent River, was laden with tree limbs and rootlets, moss, a variety of aquatic plant segments, trailing grasses and vines, and fallen fence posts and railings. Large populations of larvae also were found on these objects.

From all seven sites, larvae were found in water flowing at speeds of 0.49 to 3.30 feet per second. Water temperatures varied from 0.5 to 15°C during the collection periods from November to April. Oxygen content was 10 to 13 ppm, and the pH was between 6.9 and 7.4. When water temperatures

0.5 to 10°C, larvae were found beneath the debris and vegetation in the water; and when the temperatures were between 11 and 15°C, the larvae were found on top of the debris and vegetation. In late November and early December, when the ambient temperatures were 12 to 14°C, larvae of S. venustum and S. vittatum were taken along with larvae of C. ornithophilia; however, as water temperatures dropped, C. ornithophilia larvae were the only ones found in the streams. By the end of December, C. mutata (Malloch) appeared; C. ornithophilia and C. mutata were found together until mid-March, when S. venustum and S. vittatum generally appeared again. In March 1971, which was colder than in the preceding 3 years, C. ornithophilia and C. mutata were the only species found. In 1972, small populations of S. venustum and S. vittatum were taken along with large populations of C. ornithophilia between 8 February and 14 April; and large populations of S. decorum and S. vittatum were taken between 15 April and 15 May.

Each time immature stages were found in a stream, several collections were made, at 15-foot intervals, to be certain all species present would be collected. Collecting after heavy rainstorms was difficult because many larvae were washed downstream from their usual attachment sites and were less easily located.

About 1,000 larvae of C. ornithophilia were introduced at a time into each of the 10-gallon rearing tanks, and 2,000 to 5,000 larvae into the marine plywood tank.

Pupae

C. ornithophilia pupae were taken as early as 28 December and as late as 22 April. They were found only in the Gravel Pit Pond runoff stream and in Cash Creek, where they occurred sparsely on rocks, twigs, tree limbs, and leaves. However, when the fabric technique was employed to recover larvae in the Gravel Pit Pond runoff stream, as many as 1,896 pupae of C. ornithophilia were recovered from four, 3-foot-wide and 8-foot-long pieces of gauze placed over rocks and gravel, and left in the stream for 7 days. For the most part, the pupal cases were loosely woven of silken threads and particles of silica; a few pupae were naked (i.e., without cases). Pupae of C. mutata, P. gibsoni, P. vernale Shewell, S. venustum, S. vittatum, and S. decorum also were collected on gauze strips. Pupae from the gauze strips were preserved in 70 percent alcohol because they could not be removed from the gauze without causing injuries. However, pupae brought in on leaves, twigs, various vegetative segments, and rocks continued developing in rearing tanks until adult flies emerged. Pupae removed from their substrata, of whatever nature, did not develop into adults.

Adults

C. ornithophilia adults were taken in the field between 2 and 17 March; however, because larvae of this species have been taken as late as 29 April, adults probably emerge until the middle of May. Several hundred adults were collected from emergence cages placed over several larval breeding sites in the Gravel Pit Pond runoff stream, and a few adults were collected on strips of gauze harboring pupae. No adults were collected from mallard and Pekin

white ducklings exposed along the banks of the Gravel Pit Pond runoff stream; this may be due to the absence of C. ornithophilia at ground levels or perhaps the birds exposed were the wrong hosts for the flies. No attempts were made to expose various woodland birds. In collections made at Algonquin Park, Ontario, Canada, Bennett (1960) and Khan and Fallis (1970) found C. ornithophilia in abundance in the canopy. No attempts were made to collect this species in the canopy along the banks of either the Gravel Pit Pond runoff stream or Cash Creek.

Rearing Adults from Larvae in the Laboratory

Until the large populations of C. ornithophilia larvae were found in the Gravel Pit Pond runoff stream and in Cash Creek, it was difficult to obtain large numbers of adults from the small samples of eggs and pupae that were collected. Once the large concentrations of larvae were discovered in the streams on the Patuxent Center, however, collections could be made continuously from mid-November to mid-April; larvae then were collected weekly or bimonthly.

All collected larvae were placed in the rearing tanks, where water temperatures of 15, 19 and 22°C were maintained. The 1st-, 2nd-, and 3rd-instar larvae collected between 14 November and 15 December and placed in the rearing tanks pupated in 2 to 21 days; adults emerged 4 to 23 days later. After 15 December, larvae were found in all stages of development in streams. Larvae with well-developed histoblasts pupated in as little time as 3 hours after being placed in the rearing tanks; adults commenced emerging 2½ hours later. Many adults emerged 12 to 24 hours after their introduction as larvae, and the remainder emerged 1 to 23 days later.

Of 2,925 C. ornithophilia adults that emerged in 1970 between 26 February and 6 March, 495 were ♂♂ and 246 ♀♀ on the first day of emergence; 523 were ♂♂ and 877 ♀♀ on the 2nd day; 144 were ♂♂ and 546 ♀♀ on the 3rd and 4th days; and 14 were ♂♂ and 80 ♀♀ on the 5th day. This same emergence pattern of more males on the first day and more females every day thereafter was typical for each group of adults that emerged each year from 1968 through 1972. In 1968, 1,262 ♂♂ and 1,107 ♀♀ emerged; in 1969, 2,774 ♂♂ and 4,492 ♀♀; in 1970, 2,789 ♂♂ and 4,248 ♀♀; in 1971, 2,376 ♂♂ and 3,298 ♀♀; and in 1972, 5,041 ♂♂ and 6,001 ♀♀.

Storage of Emerged Females

When many female C. ornithophilia emerged, some were utilized immediately while others were placed in storage containers for future use. Usually about 100 females were placed in each storage container.

In 1969, I did 15 longevity experiments with newly-emerged females. Groups of 50 to 100 females were placed into storage containers and held at 21 to 25°C on apple slices and water. In one experiment, all the flies died after 9 days of storage; in seven experiments, they died after 12 to 20 days; in six experiments, death occurred in 21 to 28 days; and in the last experiment, all were dead at 35 days.

Feeding Starved, Laboratory-Reared Females on Ducklings

Newly-emerged female C. ornithophilia were held in storage containers for 48 hours on apple slices and water. The flies were deprived of nutrient for 24 hours and water for 3 hours before being exposed on Pekin white and Khaki Campbell ducklings. In 86 feeding experiments done between 24 February and 24 May 1972, 1,645 of the 2,479 flies fed. The flies became engorged in 5 to 8 minutes. For complete details on feeding C. ornithophilia on waterfowl see Tarshis (1972).

ACKNOWLEDGMENTS

My appreciation and thanks go to John Stuht, who aided in the construction of the marine plywood rearing tank, and in laboratory and field studies in 1968; to Terrell Goodman, who aided in the collection of immature stages in 1970; and to Julian O. Knisley, Jr., who aided in the collection of immature stages in 1972. I also thank my wife, Bernice, for drawing figure 3.

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Figure 1.--Larval transport buckets with air pumps attached. (Photo I. B. Tarshis)

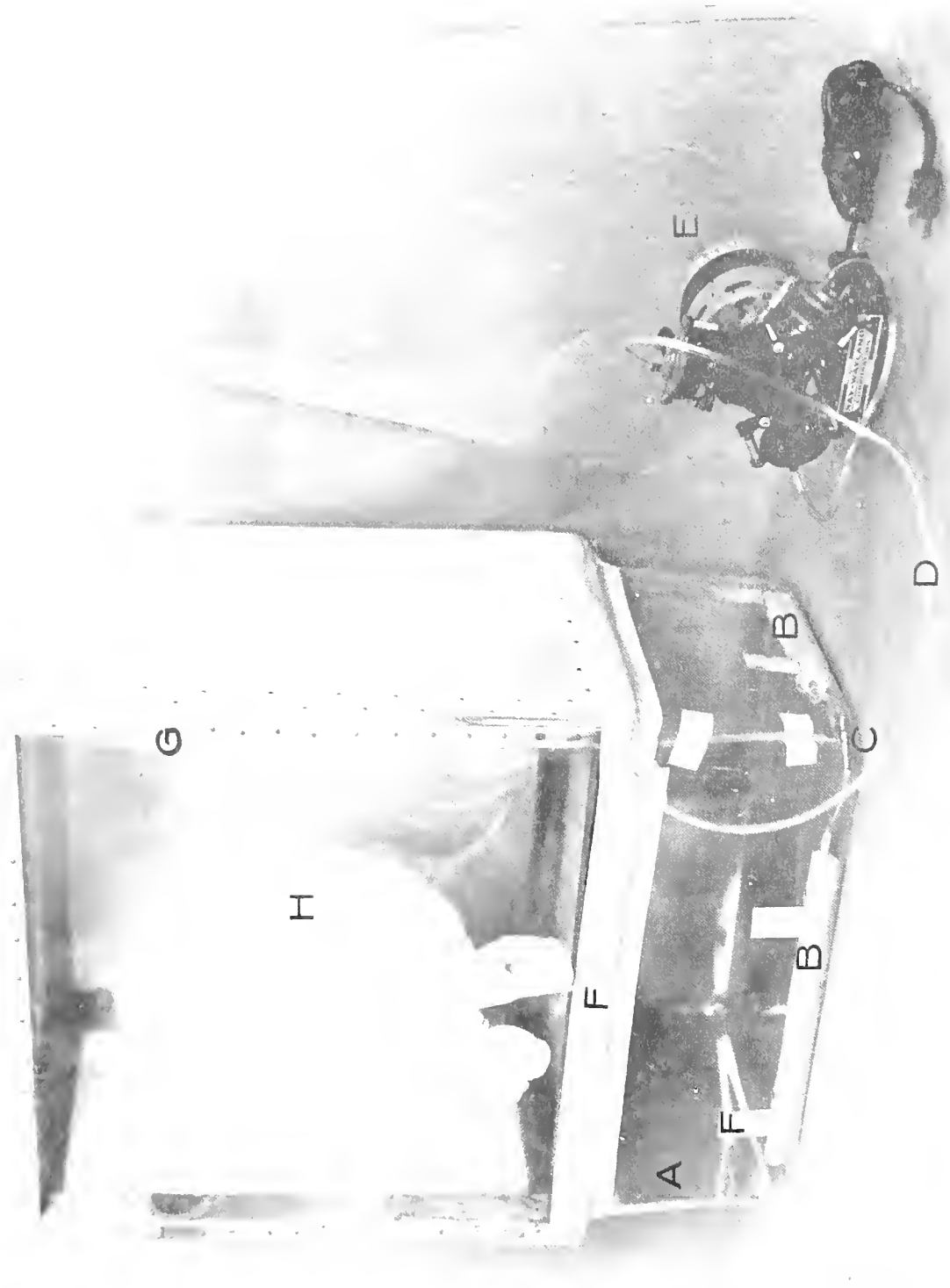

Figure 2.--Construction details of 10-gallon rearing tank: A-plastic mouse cage, B-airstone, C-"T" connector, D-polyethylene tubing, E-air pump, F-waterproof pressure sensitive tape, G-screened housing for emerging flies, H-surgical stocking material. (Photo I. B. Tarshis)

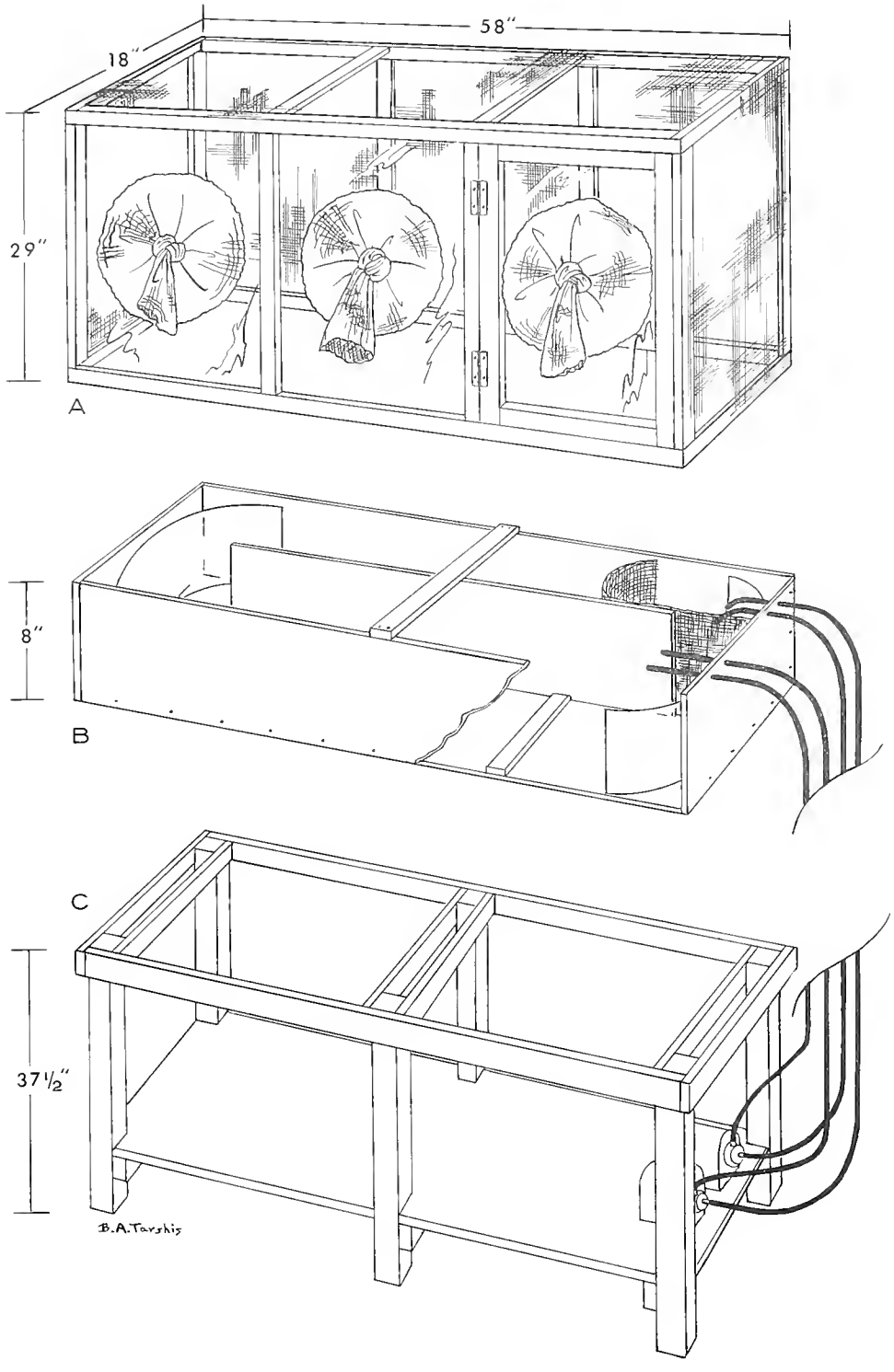

Figure 3.--Construction details of marine plywood rearing tank: A-screened housing for emerging adult flies, B-rearing tank for larvae and pupae, C-support stand for rearing tank and screened housing.

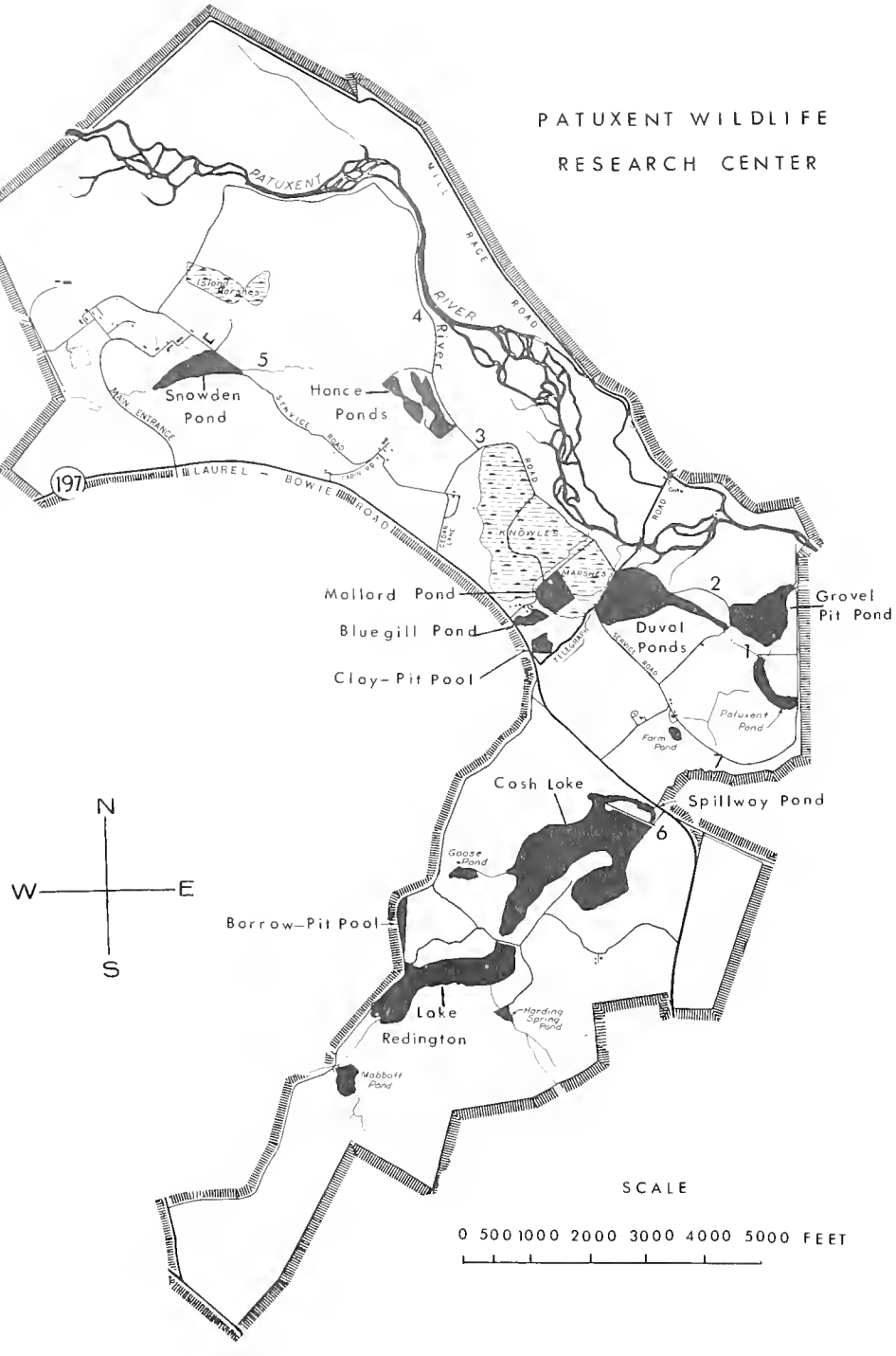

Figure 4.--Collection sites for C. ornithophilia: 1-above and below culvert before Gravel Pit Pond, 2-Gravel Pit Pond runoff stream, 3-runoff stream at juncture of Service and River Roads, 4-third temporary stream on River Road, 5-runoff stream from Snowden Pond, 6-east spillway of Cash Lake, and 7-Cash Creek runoff stream.

Figure 5.--Breeding site for C. ornithophilia in the Gravel Pit Pond runoff stream. (Photo I. B. Tarshis)

Figure 6.--Breeding site for C. ornithophilia in Cash Creek. (Photo I. B. Tarshis)

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