DETERMINING THE FLIGHT OF MOSQUITOS.

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INTRODUCTION.

This report presents a method for determining the flight factors of mosquitos. The scheme was developed and tried out on the canal zone and has given results which warrant its publication. Apart from its purely scientific standpoint, the knowledge of the flight of Culices enables us to direct better our efforts toward the eradication of these insects from our habitations, and thus greatly reduce the possibilities for transmission of such diseases as yellow-fever, malaria, dengue, etc., and to a large measure do away with the insect as a pest.

DESCRIPTION.

Briefly stated, adult mosquitos are bred, colored with an anilin dye and then liberated at stations about the town selected for study. Systematic collections of adults are made in the buildings of this town, and these adults are tested for the presence of color.

A. GENERAL CONSIDERATION.

Dispersal includes *everything* involved in the movements of animals from one place to another. It is a more or less eccentric movement because the paths taken are usually those of least resistence and economy. In mosquitos, dispersal is limited to four general means: (1) flight of the adult, (2) the adults may be carried by the wind, (3) they may be carried in trains, other vehicles, on the clothing of man or on other animals, and (4) the eggs, larvæ, pupæ and to some extent the adults, may be carried down stream or across a pond by current or wind action.

Such mosquitos as transmit diseases to man, especially when they serve as intermediary hosts in such transmission, are usually limited in their breeding area to the vicinity of human habitations. This is well illustrated by *Aedes calopus* Meigen which transmits yellow fever, and *Anopheles albimanus* Wiedemann, responsible for E. A. malaria. Such mosquitos (verified by us in the two cited species) are not distant travellers, and if they do come from distant places, it is through gradual infiltration. Some species of *Culex* are powerful fliers; others apparantly remain only near their breeding place.

To merely liberate colored adults is almost futile. The study is an ecological one and requires a knowledge of all the physical, biotic and historic factors that in any way enter into the environment of the species studied. The statements given under the three subheads following are not intended to be exhaustive, and they must be amplified according to the species selected.

1. Physical Factors.

A good map of the region selected for experimentation is necessary. It must indicate with fair accuracy the topography, commercial projects, habitations, streets, roads, and inlets of oils or poisonous refuse into streams or ponds and the extent of this pollution.

A recording anemometer should be in operation at the central station, and in addition to the velocity per hour intervals, should give the eight main directions. If more than one such instrument is available, the others may be distributed at stations where decided wind deviations take place. Small portable anemometers will greatly augment the data. A self-recording rain guage is a valuable addition.

The necessity of a well-kept, tabulated record for the data should not have to be mentioned. The following reproduction of an arrangement found satisfactory in our work may be of help to other investigators. This method gives the investigator

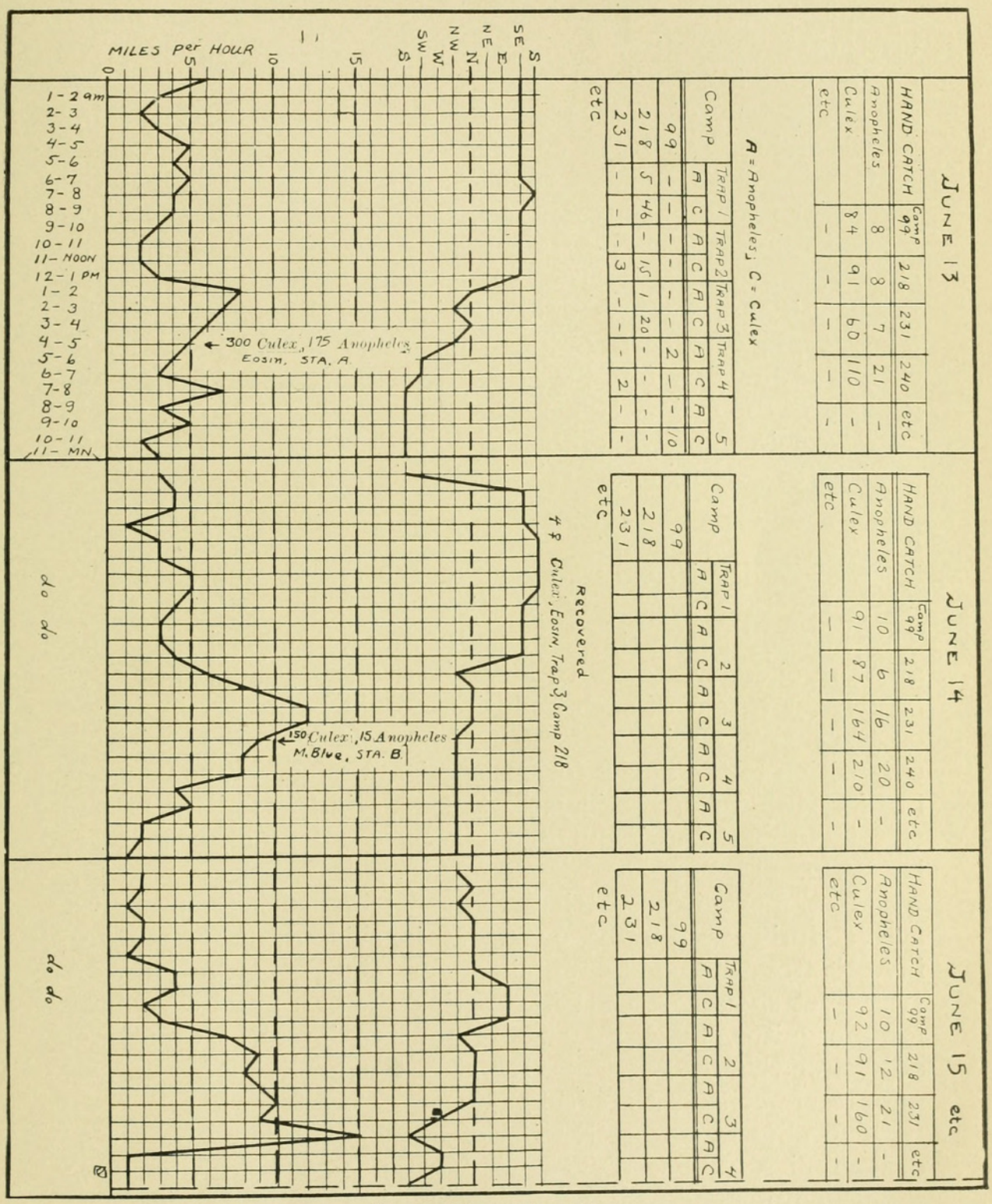

Fig. 1. *Correlation Chart.*

at a glance four distinct data, graphically placed in proper relation to each other, viz: (1) the velocity and direction of the wind per hour intervals, for six or more days, according to the size of paper used; (2) the quantity, species, and sex of mosquitos liberated, time and place of liberation and the color used; (3) the quantity, species, sex, source and color of recovered adults, and (4) the total mosquito catch in all buildings. A simple system of cross-reference to data sheets containing details will save time and energy.

Tracing cloth, so ruled that the ordinates correspond to the above form, can have recorded thereon the quantity and duration of rainfall, cloudbursts, fogs, barometric pressure, frosts, etc. By superposition on the above tabulated form, the relation, if any, of these factors to flight, will be seen. A similar tracing, made to correspond to the map, should indicate the extent of prairies, forests, forest fires, drainage, marshes, the geology of the region, etc.

Porcelain cup evaporimeters should be installed at many stations to determine the relative humidity.

2. Biotic Factors.

Weekly or biweekly surveys of the entire area should be made for the purpose of locating mosquito breeding areas. These should be charted on smaller maps. If portions of this area are oiled, treated with larvacides, or subjected to noxious fumes, the extent of such pollution should be clearly indicated on the maps. It is necessary to know the time interval from oviposition to the adults for the species studied. When searching for *Anopheles*, particularly the malaria-transmitter, a safe rule is to go to unfrequented places, small puddles in grass land, etc.

Most mosquitos, particularly the blood-suckers, are most active during and after dusk. It is evident, then, that an investigator should be detailed for night observations. A sweep-net should be used for beating the grass and shrubbery for mosquitos, traps may be set out to intercept or attract adults, or the observer may remain quiet, expose his arm, and note the ferocity of the biting.

The abundance or scarcity of such predacious animals as dragon flies, robber flies, ants, toads, frogs, fish, bats, etc., should be noted. Marked oscillations in the numbers of mos-

quitos will occur through the ravages of these animals. Collections of these forms must be made and the stomachs examined. The best time for such collection is at or just after dusk when they feed upon these dainty morsels. Unless the stomachs are examined that evening, they should be preserved in 95% alcohol, containing about one percent of thymol, the latter to arrest enzymic action.

Life history studies should be made at the laboratory and all possible data bearing on the ecological problem collected.

3. Historic Factors.

This includes the geology of the region, the plant and animal association and their past history and present trend, past human disturbances still exerting an influence on the biota, and the past history of the mosquitos studied.

B. DETAILED DESCRIPTION.

Mosquitos are delicate organisms, the majority of the species unable to endure intense dry heat, absence of water or shelter, high winds, heavy rains, etc. They are dainty morsels to hosts of alert forms. So far as our experiments are concerned, additional factors enter to lessen the number of released adults which may be recovered. First, the female almost exclusively is able to suck blood. Second, mosquitos are not dependant upon human blood alone. We have noted mosquitos sucking the blood of horses, mules, dogs, cats, monkeys and fowls. The need, then, for releasing large numbers of colored adults is evident. Better results will follow if thousands of mosquitos are liberated.

1. Collection and Care of Larvæ and Pupæ.

A. Collection: Mature or nearly mature larvæ and all pupæ, of the species selected for study, should be collected. Young larvæ thrive poorly in the field laboratory. All predacious larvæ must be excluded from the receptacles containing larvæ and pupæ.

A white enameled or porcelain saucer is very satisfactory in "dishing-up" water and algæ to note whether mosquito larvæ are present. The larvæ, if there, stand out in bold relief against the white back-ground. If the larvæ and pupæ are abundant, a large white enameled dressing bucket (such as is used by hospitals) should be used to dip up quantities of the

water and algæ. This prevents frequent disturbance of the water, and allows the frightened mosquitos to regain their equilibrium. When dishing-up the water, a shadow should not pass over the surface of the pond, as this causes the larvæ to wriggle away. The algæ in the bucket should be removed after the larvæ clinging to them have been dislodged. The contents of the bucket may then be strained through a clean piece of surgical gauze. In this manner the larvæ and pupæ are not lost, while the very young larvæ, small debris, etc., are allowed to wash into the pond or stream. The gauze should be inverted over a wide-mouthed jar, and water applied very carefully with a pipette to the larvæ. These are thereby released from the gauze and placed in the jars. Small pails are as servicable as jars.

The receptacles containing the larvæ and pupæ must be kept in a cool, shaded spot, otherwise the water will quickly foul. Not more than one and a half inches of water should be allowed in these jars. The larvæ should not be left in these containers for more than one half a day. Overcrowding must be avoided and at least once each half day the water should be aerated. A Paquelin Cautery bulb, with a capillary tube attached, serves well this object, and one or two bulbfuls will be found ample.

B. Transportation: The larvæ in these wide-mouthed jars should be taken each half day to the field laboratory, and here emptied into plates or larger receptacles. Prior to transportation, the jars should be placed into a basket and separated from each other with excelsior or cotton wadding, and while carried, shaking must be reduced to a minimum. Constant shaking prevents the larvæ from reaching the surface of the water to breathe and hence repeated unsuccessful attempts to reach the surface bring fatigue and a large percent if not all of the larvæ succumb as a result. Protection from heat and direct light must be considered. The jars themselves must be covered with a close-mesh gauze to prevent the escape of adults emerged en route. If larvæ or pupæ are transported in trains or vehicles, extra precaution should be taken regarding shaking, and additional precaution to prevent inquisitive people from handling these jars and shaking them "to see the wrigglers wriggle,"

Mosquito larvæ and pupæ must be considered as delicate organisms and rough treatment en route makes nil the whole day's work and gives but little encouragement for further work.

C. The Field Laboratory: The field laboratory is a necessity, but it need not consist of more than a small screened house, about eight feet square, protected from direct sunlight and heat. If located convenient to the breeding places, the collected larvæ will suffer but little from jarring en route. Several such houses may be erected at convenient places, however, for all purposes one such house will suffice. It means a concentration of the collected larvæ at one place and one attendant can give these his undivided attention. No staining of adults should be made at the laboratory as this would involve transferring of colored adults and the possible escape en route of some of these.

D. Breeding-out Methods: As soon as the larvæ and pupæ in the wide-mouthed carrying jars reach the field laboratory, they should be transferred into a large pan. The pupæ should be picked out by means of a pipette and confined in jars, these covered with gauze.

White enameled or porcelain soup plates gave the best results as breeding receptacles for the larvæ. If the plates are tilted slightly, both shallow and deep water is afforded to the larvæ. Debris and filamentous algæ should be reduced to a minimum. The food of the larvæ should be known; if diatomaceous, a few pebbles covered with diatoms will suffice. Predacious larvæ of all sorts must be eliminated. Some species of mosquitos prefer sunlight, others do not, or there is preference for foul water, etc. These peculiarities must be known. *Successful breeding depends upon a careful attendance to the peculiar environmental factors of each species.*

Unless the water in the dishes is changed weekly, fouling will ensue and cause heavy mortality. This is obviated by pouring the contents of the dishes over a piece of clean surgical guaze and then inverting over a clean plate containing fresh water. The larvæ when in contact with the water will free themselves from the meshes of the cloth. Sudden additions of fresh water were found to be detrimental; best results were obtained with water which had been standing in the room for twenty-four hours. Careful observance to these environmental requirements has reduced mortality among our own larvæ from ten percent to less than one percent.

The water in the breeding dishes must be aerated twice daily. The Paquelin Cautery bulb method referred to on page 10 does well when the number of places is few. When these plates are numerous, it is better to construct an aerating device such as shown in figure 2, using old tins, tubing, etc. There

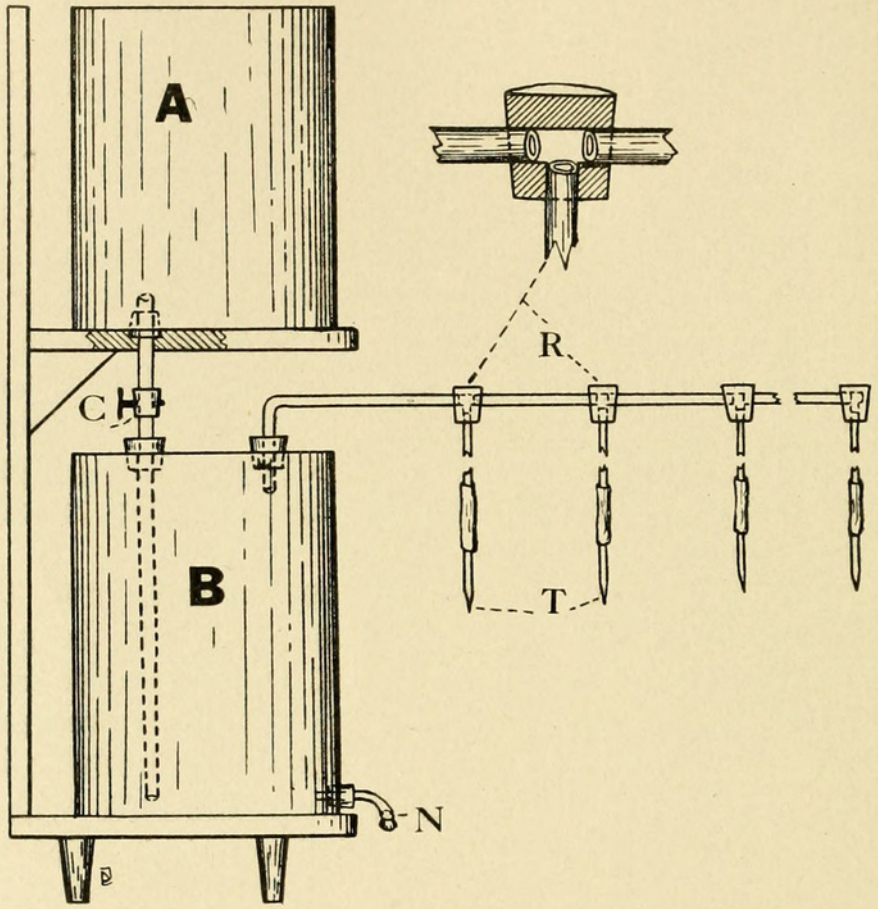

Fig. 2. *Aerating Device.* A, water reservoir, open at top; B, air chamber; C, stop-cock regulating flow of water into B; R, tee unions made of cork; T, terminal capillary tubes.

should be as many feeders as there are dishes to aerate. To set the apparatus into operation, adjust feeders to plates, fill chamber "A" with water and open stop-cock leading to the air chamber.

The writer noted on four occasions a large roach drinking water from breeding pans, at the same time devouring larvæ. On seven occasions ants were seen reaching after such larvæ as were near the edge of the plates and while under observation, two larvæ were successfully withdrawn from the water. These observations suggest strongly the need of protection against these inroads. Keep lookout for mice.

Frequently through chemical or physical changes in the water, produced by excess of heat or food, improper food or foreign substances, waste, etc., the larvæ become sluggish and pupation is greatly retarded. If the cause is not due to fouling of the water, then table salt added not in excess of three percent, will make the larvæ active and accelerate pupation. The dead or sick larvæ in such pans should be eliminated.

Pupæ should be segregated from the breeding dishes daily, preferably morning and evening. They should be confined in wide-mouthed jars, the depth of water not exceeding one and one half inches, and the number of pupæ not more than two hundred and fifty. The mouths of these jars should be provided with paper cones, the tip truncated, and both cone and neck of the jar inserted into a screen cage as shown in figure 3.

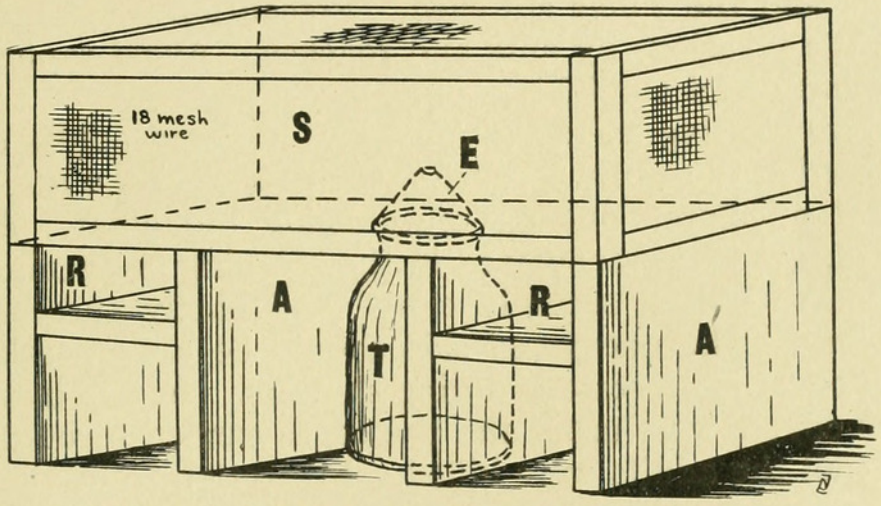

Fig. 3. *Breeding-out Cage.*S, screen cage, hinged back; A, wooden "H" supports; R, lofts for wetted waste; T, jar containing pupae; E, truncated paper cone trap.

The cone acts as a trap, thus preventing the adults which enter the cage from returning into the jar and being drowned. All crevices about the jars and in the cages must be stopped with cotton waste.

It is advisable to place moist cotton on the floor of the screen cage, also to fill cavities "A" of the "H" supports with wet waste. The top ought to be covered with a wet cloth. These simple measures keep the inside of the cage cool and sweet, and adults can be kept in good condition for at least six days. The cages must be protected from direct sunlight, heat and rain. If ants are present, isolation by water barriers is necessary.

Several times the writer noted roaches in the cages, and the crops of the dissected roaches, as well as the appearance of the adults in the cages, showed the "why" of their presence.

2. The Care of Adults.

A. At the Field Laboratory: It is necessary each morning to remove the jars containing pupæ from the screen cages. It will be found that no few adults remain on the sides of the glass jars. These are readily transferred into the screen cage by holding the cage in direct sunlight and tapping the jar briskly with the hand. When the jars are removed, the holes in the cage which served to receive them, must be plugged snugly with cotton waste to prevent the escape of any adults. The cage must now be placed in a sheltered corner and left for several hours, or until the chitinous portions of the exoskeleton have hardened and the wings stiffened. Precaution must be taken against the invasions by ants and roaches. Avoid rapid evaporation and direct sunlight.

B. Transporting Adults to Stations: Colored adults should not be carried to several localities, the danger of the accidental escape of a colored one en route being too great. Adults, unstained, are best transported in the morning or evening, and each cage should be securely closed and partly encased in a damp cloth. The uncovered side should be underneath. An oil cloth cover is neccessary during showers. In two instances, when no covers were used, and the cages carried through light showers, all the adults were killed. Protection from wind was found necessary. Air currents cause rapid evaporation which the mosquitos cannot withstand.

3. Coloring of the Adults.

A. Dyes Used: Aqueous solution of eosin, fuchsin, gentian-violet, bismarck-braun, methylene-blue and orange-g, were used with good success, the proportions of dry stain to water being about one gram to fifty cc. It is best to make small quantities at a time as stock solutions may deteriorate. All stains should be kept locked up. Likewise staining operations should be known only to a few. Curiosity too frequently gives birth to trouble.

It may not be amiss to state under this section two other "markers" which may be used effectively with larger diptera. In our work they were not as serviceable as the dyes. The

first is a 1 : 20 aqueous solution of phenolphthalein. It was found satisfactory on typhoid flies and is detected readily when a drop or two of one percent solution of ammonium or sodium hydroxide is added to the suspected specimen. A deep red color indicates presence of the drug. The second agent is corn starch and it is detected by applying tincture of iodin, a purple color ensuing in its presence. We had no opportunity to give this latter method a fair trial.

B. Staining the Adults: The mosquitos in the rectangular screen cages should be stained preferably toward evening, about two hours before they are to be released, and always at the station where they will be liberated. A shelter must be provided for these cages. The stains must be applied lightly and must be dry on the insects before they may be allowed freedom. Small globules of water on the wings weight these down to such an extent that the mosquito cannot fly, and it is then easily captured by ants, roaches or more alert forms.

The aqueous solution of the anilin dyes is converted unto a very fine spray through a vaseline-nebulizer, or a fine atomizer, and this spray is allowed to *fall* upon the mosquitos. Direct and forceful projection of the stain against the sides of the mosquito is productive only of death to the insects. Too concentrated solutions must be avoided. The idea is not to encrust the mosquito with the stain, but to place a minute speck only upon the body. Hundreds of tests, using mere specks of the stain gave perfect results when tested for color. The danger of too-protracted a staining is that spiracles become sealed with the stain, mouth parts glued together, sensory areas covered, wings folded, etc.; in other words the mosquitos are no good.

After the mosquitos have been liberated, the cage should be washed in clear water to dissolve all superfluous stain adhering to the screening or sides of the cage. Such crusts, if allowed to remain, create a foulness about the cage which is detrimental to the mosquitos confined therein. It is best, though the statement seems hardly necessary, to keep separate cages for each color used.

To remove stains from fingers and hands, received during the coloring operations, wash hands in acid alcohol. The best way is to use rubber post-mortum gloves when staining mosquitos.

4. Liberating Colored Adults.

The experiments conducted on the canal zone suggest the advisability of liberating adults at or about dusk, or from then on till midnight. The stations selected may be few or many, depending upon the complexity of the physical and biotic factors presented at the time. All that is necessary for liberation is for someone to open the lid of the cage containing the colored mosquitos. This observer should note the time when he liberated the mosquitos, the climatic conditions at the time, and the direction taken by the mosquitos. If people move about near the place of liberation, particularly after dusk, and go to the town from there, this should be carefully noted. The person delegated for this duty should be a keen observer, and honest, too.

The habits of the mosquitos vary with the species—not all cry for the warm blood of man. Then there are some that can't be without it. The writer liberated in the bush, about one quarter miles from Corozal, Canal Zone, at eleven a. m., about fifty stained *Anopheles albimanus* Wiede., and noted three of these soon clinging to his dark colored trousers, and by walking slowly—just as the natives do—he brought these with him into the town of Corozal. This illustrates one of the avenues of dispersal, practically independant of wind, and we must reckon with it, especially since this species is responsible for most of the malaria on the canal zone.

A precaution, based on the above observation, was found necessary. *Brush your clothing* carefully after liberating colored mosquitos, and if possible, *wear a light colored suit.* The latter suggestion proved very helpful. Note also if people passing along the highways, walk toward or from the townsite, and whether they saunter or walk fast, or are quiet or boisterous.

5. Collection and Examination of Adults in Buildings.

The recovery of liberated mosquitos in the buildings will demand thorough search and great precaution. Unless this is done, much fruit cannot be expected for the labors and patience expended.

A. Collection by Hand: Hand collections in the buildings are best made at dawn and just at dusk, the mosquitos at these two periods trying to get out and into, respectively, of the

buildings. The men selected for this work should be provided with a killing-tube made of a heavy walled test tube, 6 inches by 1¼ inches, containing a four inch cotton plug saturated with chloroform, over which are a few circular pieces of blotter paper. The collector merely superimposes the mouth of his tube over the mosquito he sees on the wall or clothing, the chloroform vapor, readily generated by the aid of the heat of his palm, quickly kills the insect. A few days' work will render the novice an expert. All the mosquitos caught in one day in one building should be placed by the collector into a circular pill box, of which he should have a good supply. This box should be labeled, giving the date, house, and the initials of collector. At the close of day, these boxes should be turned over to the person in charge, who should check them and rectify any existing errors. The next step is to examine these captured mosquitos for color. (See pp. 19-20).

B. Collection by Traps: If the buildings are *well-screened* and holes and crevices blocked, mosquito traps may be used to excellent advantage. This is being done on the canal zone,

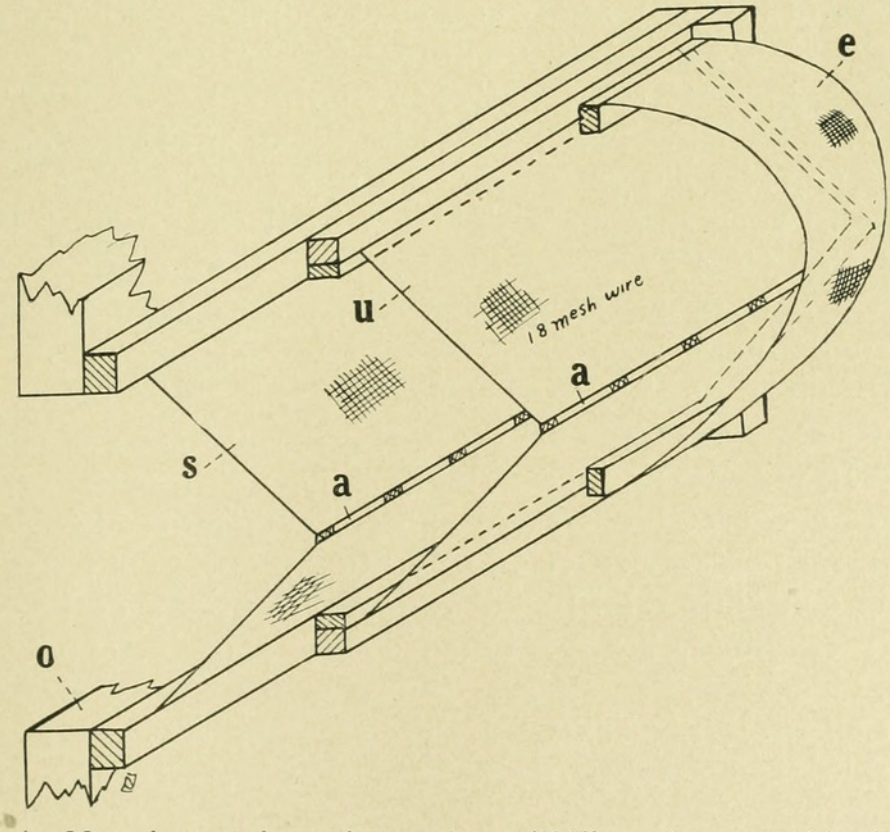

Fig. 4. *Mosquito trap*, in section. **s**, inner "V" section; **u**, middle "V" section; **a**, slits in the "V" sections; **e**, semicircular outer envelope; **o**, sill of building. The "V" sections are detachable.

and the trap illustrated was developed by Mr. Chas. H. Bath, sanitary inspector. Such or similar traps greatly add to the data, and if placed to buildings that harbor a large number of people asleep, will attract many mosquitos, save them, and in regions of malaria, greatly reduce the number of such cases. The traps should be numbered and recorded on charts where their location with respect to the wind is seen at a glance.

Traps should be taken down each morning, at about nine o'clock was found best, and the adults in these killed and placed into pill-boxes, one box for each trap, and each box properly labeled. There is no apparent need for blocking up the opening in the wall when the traps are removed. During five months with these traps, the writer never found a single mosquito that entered during the daytime. The method used was to place a new trap in the place of the one taken out.

A very satisfactory and quick way to kill the mosquitos in the traps is to place the trap into a closed chamber and fumigate with sulphur dioxide. The question arises whether or not this gas combines with the moisture in the mosquito to form sulphurous acid (H_2SO_3), and whether or not this will bleach what color is on the mosquitos. The data following, of a series of tests made, indicate the negative is true:

50 Culex sp. Stained lightly with eosin, left in SO_2 chamber for 3 hrs.; no bleaching.

100 Culex sp. Stained lightly with eosin; 100 Culex sp. with gentian-violet, exposed 13 hrs.; no bleaching.

30 Culex, 70 *Anopheles albimanus* et *malefactor* stained lightly with methylene-blue, exposed to burning sulphur and generated steam for 3½ hrs.; O. K.

10 Culex sp. each slightly stained with all stains cited, exposed 15 minutes; no bleaching.

Paper and blotters, wetted and colored, exposed for 6 hrs.; no bleaching; no acid reaction to litmus.

Vials containing 1 : 10000 aqueous solutions of bismarck-braun, methylene-blue, gentian-violet and eosin, exposed 3½ hrs.; no bleaching; no acid reaction.

C. Collection in Tents. If patient and honest men are procurable, army tents may be pitched at suitable places radiating from the releasing point, and these men placed, one to a tent, with a lantern, killing tube and boxes, to catch all mosquitos that enter the tent. The lamp should burn dimly,

and the men cautioned to be as quiet as possible, and if they must move about, to so do with little commotion. Contrary behavior shews mosquitos away. It seems these gnats wait at the door till the occupant is quiet. The mosquitos caught in a given tent during each hour interval, should be placed in a pill-box, and this one properly labeled, containing in addition to what had already been indicated, the particular hour's catch represented.

D. Collections with a Beating Net: Important clues bearing directly upon the movements of adult mosquitos will be obtained by systematic sweeping in the grass and shrubbery, using for this purpose a large entomological beating net. The adults thus captured should be placed into pill-boxes, these labeled to show the place where caught, character of the vegetation, and hour when captured. The note book should contain data concerning the temperature, wind direction, velocity, humidity, cloudiness, smoke, etc. The writer noted from a series of sweepings that *Anopheles albimanus* Wiede. and certain Culices (*C. quinquefasciatus* Say et *Mansonia titillans* Walker) were more abundant in the grass when the winds were above four miles per hour, than when these winds were less. Its bearing upon the problem can only be determined after a series of careful tests.

E. Examination of Adults: The mosquitos in a single pill-box should be emptied upon a piece of glass plate under which is a white blotter or paper. With a camel's hair brush these are spread over the plate and each specimen is wetted with a testing solution containing three parts of glycerine, three of alcohol and one of chloroform. If any color is present upon any mosquito, it will be revealed as soon as the testing solution reaches it, diffusing outward. Thus each colored specimen becomes a distinct nucleus of diffusion—hence non-colored adults cannot receive through accident some of the diffusing color and thus confuse the observer. The number, species, sex, date and where captured, of all recovered mosquitos, should appear on the data sheets and charts. In addition, a record should be kept of the total mosquito catch, properly tabulated.

It is advisable that only one person be detailed for this examination, and care must be exercised to select a man free from either amnesic or general color blindness. His working table must be kept clean. He should make preliminary tests

to note the action and peculiarity of each color when tested. Accidental rupture of the abdomen of a mosquito, thus extruding the contained blood, should cause no confusion as this blood does not diffuse as does a stain, and furthermore, after a few minutes in the solution, it turns brown. If a spectroscope is available, all colors recovered should be confirmed. As a precaution, all tested mosquitos should be destroyed daily. It is advisable that each day's catch be examined as soon as possible, and whenever delay is necessary, afford protection from ants.

SUMMARY.

1. It is essential, first of all, to have a good map of the territory, to keep a record of climatic conditions, to know the topography and plant associations, the species of mosquitos studied, etc.

2. Larvæ and pupæ must be collected in large numbers, cared for at a field laboratory and the adults that emerge kept in first class condition until ready to be colored and released.

3. These adults must be stained lightly and carefully, without injury to the insect, and the stain allowed to dry on the mosquitos before they are released. Color at liberating station.

4. Release the adults, noting conditions under which this is done. Brush your clothing.

5. Collect daily as with a fine comb, the mosquitos that entered the buildings, tents, and traps. Test these for any color present.

6. *Lastly, interpret rightly your results.*

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November 25, 1912.

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