minimum air temperatures are maintained above freezing.

Hibernating *C. apicalis* females were also collected on several occasions along with *C. tarsalis*.

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SOME SPECIES OF MOSQUITOES REARED FROM DRY MATERIALS[1]

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INTRODUCTION. It is well known that some mosquitoes deposit their eggs on the water surface, while others lay them in moist or dry depressions which will later be subjected to flooding. It has also been established that the eggs of those species that are not deposited directly in water are capable of withstanding considerable drying. In fact, in some cases they may remain viable after having dried for a year or more (Bates, 1949). When the depressions containing the eggs are flooded, some of the eggs present will not hatch at this time, but require subsequent drying. In most cases, the eggs which will hatch do so within a few minutes to a few hours after water is added. As a consequence of these characteristics, larvae of many species of *Aedes* and *Psorophora* can be collected in quantity only a few days after a rain. During periods of drouth they are seldom found in permanent pools.

The fact that larvae of many species of *Aedes* and *Psorophora* can be collected in large numbers only for a short time after a rain creates a problem for anyone interested in obtaining larvae during periods of drouth. Theoretically, one should be able to gather debris and soil from depressions, flood the material in the laboratory and obtain larvae of *Aedes* and *Psorophora*. So far as could be determined, however, such work has never been done on a large scale except for special situations. Dunn (1926), while investigating the breeding habits of the yellow fever mosquito in west Africa, collected dry material from tree holes and made an extensive study of the species that hatched when flooded in the laboratory. He found several species of *Aedes* common to that locality in addition to *Aedes aegypti* (L.). Wilkins and Breland (1949) recovered larvae from approximately 50% of the samples of dry material taken from tree holes in Texas. *Aedes triseriatus* (Say) and *A. zoosophus* D. & K. (= *Aedes alleni* Turner) were represented in this latter work as well as *Culiseta inornata* (Will.). Stone and Reynolds (1939) obtained specimens of *Culex, Anopheles* and *Psorophora* in Panama by flooding slightly moist material from depressions.

THE PRESENT STUDY. This study had

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two principal objectives. First, to discover what species of mosquitoes could be recovered from dry materials; and second, to determine whether or not this type of collecting would be a practical method of obtaining species of *Aedes* and *Psorophora* during periods of drouth.

This work was done between September, 1949, and July, 1950. Much of the material was collected within a radius of some sixty miles of Austin, Texas, but substantial numbers of samples were taken from areas considerably farther east, west and south. Since collecting mosquitoes in this way was comparatively new, the methods of collecting and treating the materials were changed from time to time as seemed necessary.

About a quart of soil and other debris per sample was collected at first, but this proved an insufficient amount and was increased to approximately a gallon, and soon to half a bushel. Small quantities were flooded in shallow procelain pans, while number three galvanized tubs were used for flooding the larger samples. The samples consisted of soil scraped from the bottom of dried depressions, tracks of animals, cracked earth and under leaves, as well as other debris in low areas subject to flooding. Leaves, grass, small twigs and partially rotted materials were also collected. All samples were dry at the time of flooding, that is, free of noticeable moisture. During the winter and early spring much of the material was damp at the time of collection. These samples were dried in the laboratory several days before flooding.

It has been established that the eggs of certain species of *Aedes* and *Psorophora* need some stimulus before hatching. This stimulus varies with the species and may take the form of certain chemicals, a change in oxygen concentration or other factors (Horsfall 1949; Bates 1949). There is still much to be learned regarding the nature and action of these stimuli, but in natural habitats they apparently occur. In the laboratory, eggs submerged in tap water often do not hatch, indicating an absence of proper stimuli or the presence of inhibiting substances. In the present work, therefore, fresh pond or river water was used. All such water was strained through cloth to remove possible eggs or larvae that might be present. Pans and tubs were kept covered to avoid contamination by female mosquitoes in the laboratory.

As has been previously stated, all eggs in a single batch of material often do not hatch at the same time; therefore, some samples were flooded several times with a long period of drying between each flooding. This practice was usually not carried out with the large samples because the bulk would have required more space for drying than was available. However, several small samples of soil taken from edges of ponds and other places subject to flooding, such as tree holes and rock holes, were treated in this way.

DISCUSSION. For this study 195 samples of dry materials have been collected and flooded. A total of 5 genera of mosquitoes, including approximately 22 species, were recovered. Some species could not be determined with certainty, because only one or a few specimens were obtained, and these either died before attaining the fourth larval instar, or were in poor condition for determination. The results of this work are summarized below. A few additional observations relative to specific species may be of interest.

It has been noted previously that some samples were flooded several times with periods of drying between floodings. Samples taken from tree holes usually yielded more larvae when reflooded than other samples. From one sample of tree hole debris to which water was added thirteen times, *Aedes triseriatus* (Say) was recovered twelve times and *Aedes zoosophus* Dyar and Knab five times.

In an effort to obtain egg deposition, fifty adult *Aedes sollicitans* (Walker), reared from dry material were placed in a cage measuring approximately 18 x 18 x 18 inches. The cage was covered with wet burlap bags to increase the humidity, and dishes of grass and mud dampened with sea water were supplied. Pieces of

oranges and apples were placed in the cage for the males, and through sock openings females fed freely on one of the writer's arms. Mortality was much higher among males than females, and all of the males were dead within 15 days. Several females survived for more than 40 days, but all except one were dead on the forty-fifth day. This lone female attained an age of fifty-six days before she died. Egg deposition apparently did not occur, since no larvae were recovered, although the debris in the dishes were flooded and dried several times.

Perhaps the most significant fact connected with this work was the recovery of several larvae of *Culex, Anopheles* and *Culiseta,* as described below. During this time egg rafts of *Culiseta inornata* (Williston) were easily collected in quantity in the field and experiments were designed to test the reaction of the eggs to drouth and freezing temperatures.

SYNOPSIS OF DATA ON MOSQUITOES RECOVERED FROM 195 SAMPLES OF DRY MATERIALS (September, 1949 to July, 1950). 1. *Aedes aegypti* (Linn.): Austin, Travis County; from soil in an old bucket, 1 sample yielded larvae, 5 recovered. 2. *A. sollicitans* (Walker): Corpus Christi, Nueces County, Copano Bay, Aransas County; from salt marsh grass, 5 samples yielded larvae, 550 recovered. 3. *A. atropalpus* Coq.: Junction, Kimble County, Del Rio, Val Verde County; from soil and sand in rock holes, 2 samples yielded larvae, 150 recovered. 4. *A. vexans* Meig.: Palmetto State Park, Gonzales County; from leaves, soil and rotted debris, 6 samples yielded larvae, 200 recovered. 5. *A. infirmatus* D. & K.: Palmetto State Park, Gonzales County; from leaves and soil, 1 sample yielded larvae, 4 recovered. 6. *A. triseriatus* (Say): Junction, Kimble County, Huntsville, Walker County, Livingston, Polk County, Woodville, Tyler County; from rotted wood from tree holes, 8 samples yielded larvae, 100 recovered. 7. *A. zoosophus* D. & K.: Junction, Kimble County; from rotted wood from tree holes, 1 sample yielded larvae, 40 recovered. 8. *A. trivittatus* (Coq.): Junction, Kimble County; from drift material, 1 sample yielded larvae, 13 recovered. 9. *A. thelcter* Dyar: Palmetto State Park, Gonzales County; from leaves and soil, 2 samples yielded larvae, 8 recovered.

10. *Psorophora ferox* (Humboldt): Austin, Travis County, Palmetto State Park, Gonzales County; from leaves, drift and soil, 6 samples yielded larvae, 25 recovered. 11. *P. longipalpus* Roth: Palmetto State Park, Gonzales County; from leaves, drift and soil, 3 samples yielded larvae, 25 recovered. 12. *P. horrida* (D. & K.):* Palmetto State Park, Gonzales County; from leaves and surface soil, 1 sample yielded larvae, 6 recovered. 13. *P. cyanescens* (Coq.): Copano Bay, Aransas County; from *Andropogon* and marsh grass, 1 sample yielded larvae, 10 recovered. 14. *P. signipennis* (Coq.): Junction, Kimble County, Palmetto State Park, Gonzales County; from leaves, grass and trash, 2 samples yielded larvae, 55 recovered. 15. *P. confinnis* (Lynch Arribalzaga): Raymondville, Willacy County, Palmetto State Park, Gonzales County; from grass, leaves and soil, 3 samples yielded larvae, 12 recovered. 16. *P. ciliata* (Fab.): Williams Creek, Travis County, Palmetto State Park, Gonzales County; from leaves and surface soil, 6 samples yielded larvae, 50 recovered. 17. *P. howardii* Coq.: Williams Creek, Travis County, Palmetto State Park, Gonzales County; from leaves and surface soil, 6 samples yielded larvae, 56 recovered. 18. *P. varipes* (Coq).:* Palmetto State Park, Gonzales County; from leaves and surface soil, 1 sample yielded larvae, 2 recovered.

19. *Culex restuans* Theob.: Williams Creek, Travis County; from leaves and surface soil, 1 sample yielded larvae, 3 recovered. 20. *C. salinarius* Coq.: Palmetto State Park, Gonzales County; from leaves and surface soil, 1 sample yielded larvae, 2 recovered.

21. *Culiseta inornata* (Williston): Williams Creek, Travis County, Palmetto State Park, Gonzales County; 6 samples yielded larvae, 14 recovered.

* Of doubtful identity.

22. *Anopheles pseudopunctipennis* Theob.:* Gaines Ranch, Travis County, Williams Creek, Travis County; from leaves and surface soil, 2 samples yielded larvae, 4 recovered.

Culiseta inornata normally lays its eggs in the form of rafts on the water surface. In the southern part of the United States, *C. inornata* tends to be a winter mosquito, and in this area is usually quite common from October or November until March or April. In the north, the species breeds in the spring and summer. It is probable that this mosquito passes the winter by hibernating in the adult state in the North (Matheson 1946), but it is not known how the species survives the summers in the South (King, Bradley and McNeel 1944).

In the vicinity of Austin, Texas, Wilkins and Breland (1949) reported having collected material from a dry tree hole, which was known to have been free of water for at least two months. From this sample they obtained more than fifty larvae of *C. inornata.* They suggested that at least part of the population in this area passes the summer in the egg stage.

The writer performed two experiments with egg rafts of *Culiseta inornata* in an effort to elucidate this problem further. Twenty rafts were used in the first and 200 rafts in the second experiment. The 20 rafts were divided into 4 groups of 5 rafts each, and the 200 rafts were divided into 10 groups of 20 rafts each. Moist, well rotted leaves were placed in each of a number of small dishes. Seven of these egg groups were placed beneath the surface of the leaves, and the remainder, except for one group of 20 rafts flooded at once as a control, were placed on the surface of the leaves in dishes.

The control group of 20 rafts hatched within a short time after being placed in water. The percentage of hatch, insofar as could be determined, was close to 100 per cent. The experimental rafts, both those exposed to the air and those covered by leaves, were subjected to the same types of treatment. These included exposure to laboratory temperatures, freezing temperatures and temperatures from five to fifteen degrees centigrade. None of the rafts hatched that were on the surface of the leaves. However, many of those that were covered by leaves hatched, in some cases yielding a high percentage of larvae. The specific treatment given each group of eggs, and the approximate percentage of eggs yielding larvae, are noted below.

The approximate percentage hatch of *Culiseta inornata* eggs under moist leaves exposed to different conditions was as follows: (1) 20 rafts exposed to outside temperature for 36 hours, lowest temperature 5° C.: 75 per cent hatch. (2) 5 rafts exposed to room temperature for 72 hours, average temperature about 27° C.: 50 per cent hatch; 20 rafts under same conditions, 75 per cent hatch. (3) 20 rafts exposed to room temperature (average 27° C.) for 72 hours; then 5° C. for 24 hours, 98 per cent hatch. (4) 5 rafts exposed to room temperature (average 27° C.) for 72 hours, then −8° C. for 24 hours, 90 per cent hatch; 20 rafts under same conditions, 98 per cent hatch. (5) 20 rafts exposed to room temperature (average 27° C.) for 72 hours; then −8° C. for 48 hours, 98 per cent hatch.

These results indicate that the eggs of *C. inornata* are capable of surviving for a time in the absence of free water if the rafts are covered by leaves. It is also obvious that under the same conditions the rafts will withstand freezing temperatures for a time. The percentage of eggs that hatch, however, is actually no accurate criterion of the number of larvae that are likely to complete their development. Larvae that hatched from all groups of eggs that were exposed to freezing, began to die shortly after emerging. Approximately 50 per cent of the larvae from eggs exposed to minus 8 degrees centigrade for 24 hours survived. The larvae from eggs kept at this temperature for 48 hours all died within a few hours, an indication that the ability of the eggs to withstand freezing is a limited one.

One of the principal questions that

* Of doubtful identity.

arises is whether the *Culex, Culiseta* and *Anopheles* survived in the egg or larval stage. The writers are unable to answer this question at the present time. No larvae of these genera were observed in the first instar but all were discovered in the tubs in second, third and fourth stadia. This fact is not especially significant, however. When dry material is flooded, so much debris often floats on the water surface that first instar larvae are easily overlooked if only a few specimens are present.

Stone and Reynolds (1939) recovered first instar larvae of *Culex* and *Anopheles* by flooding material that was only slightly moist. They suggested that the *Anopheles* at least had survived in the egg stage. This same suggestion has also been made in the case of *Culiseta* by Wilkins and Breland (1949). Survival in the egg stage is supported by the fact that in the present work, eggs remained viable for a time in the absence of free water.

Under the conditions outlined, the writers do not feel that routine collecting of dry material is a practical method of obtaining mosquito larvae. In many cases, few or no specimens were recovered, even though half a bushel or more material was collected. Percentage of recoveries was much higher, however, from debris collected from tree holes. This fact has been previously confirmed (Wilkins and Breland 1949). Also, a relatively small amount of tree hole debris may yield a large number of larvae. These facts indicate the practicability of obtaining species of *Aedes* from tree holes by collecting dry samples.

It appears probable that other species with a limited breeding habitat, such as salt marsh and rock hole breeders, could be collected profitably by this method. It is also suggested that a higher percentage of recoveries could be obtained by flooding damp material immediately rather than by allowing it to dry several days before flooding.

OTHER INVERTEBRATES. In addition to the species of mosquito larvae obtained in this work, a number of other invertebrates were also recovered. Inasmuch as it was not the objective of this study to investigate invertebrates other than mosquitoes, only the most prominent forms were noted. No attempt was made to identify the species, but an effort was made to place the most numerous ones in the proper order, family or genus.

Numerous Protozoa were observed in nearly every flooding of material, *Paramecium* being the most common. Many Oligochaetes were obtained from about seventy-five per cent of the samples. Those noted particularly were *Lumbricus* and *Aulophorus*. Several Crustaceans were recovered including *Daphnia* (Cladocera), *Cyclops* (Copepoda), fairy-shrimp, probably *Streptocephalus texanus* (Anostraca), *Apus* (Notostraca), and one or more species of Conchostraca.

Members of six families of insects were noted including Chironomidae; Heleidae, probably *Culicoides;* Syrphidae, possibly of the genus *Eristalis;* Psychodidae, possibly of the genus *Psychoda* (Diptera); Belostomatidae (Hemiptera), and Dytiscidae, possibly *Dytiscus* (Coleoptera). The Belostomatidae and Dytiscidae were a half centimeter or more in length when first observed, therefore, it is suspected that these insects survived drouth in the debris as nymphs and larvae instead of hatching from eggs.

SUMMARY. 1. A study has been made of the species of mosquitoes reared from 195 samples of dry material flooded with water in the laboratory. Larvae were obtained from 58 samples.

2. Some observations were also made upon the laboratory reactions of *A. sollicitans* reared from dry material, and upon the ability of egg rafts of *Culiseta inornata* to withstand drying and freezing.

3. Five genera of mosquitoes were recovered, including nineteen species positively identified and three tentatively identified. These included species of *Aedes, Psorophora, Culex, Culiseta,* and *Anopheles.* Many other invertebrates were also obtained.

4. It is not known in what stage the few specimens recovered of *Culex, Culi-*

seta, and *Anopheles* survived. The fact that egg rafts of *Culiseta inornata,* if covered by leaves, will withstand a certain amount of drying suggests that it was the egg stage.

5. Less than one-third of the samples collected yielded larvae, and in many cases only a few specimens were obtained. Under the conditions outlined, it is thus not believed that routine collecting of dry material is a practical method of obtaining mosquito larvae.

6. A relatively high percentage of dry debris obtained from tree holes yielded larvae when flooded in the laboratory. These results have also been confirmed by the writer's associates. It is thus believed that collecting dry debris is a practical method of obtaining certain tree hole breeding species. It is also suggested that other mosquitoes with a limited breeding habitat might be recovered in quantity by the collection and flooding of dry material.

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