THE EFFECTS OF FREEZING ON THE LARVAE OF AEDES AEGYPTI[1]

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INTRODUCTION

It is well known that the yellow fever mosquito, *Aedes Aegypti* (Stegomyia fasciata), being a domestic species, having a fairly long life in the adult stage, and exhibiting the custom of hiding itself in most ingenious ways, is particularly subject to carriage for long distances on vessels, railroad trains, in baggage, etc. It is assumed that in this way it was carried from America to Africa, or *vice versa.* Through such transportation agencies this mosquito is distributed over great distances, and during warm seasons it may be carried far beyond areas where it is capable of maintaining itself permanently. It has been presumed also that when carried during the warm seasons to colder climates, it is annually exterminated by the cold, after breeding for a certain number of generations, and, consequently permanent distribution is limited in a general way, apparently, by the frost line.

Marchoux, Salimbeni, and Simond (1903) called attention to the fact that one of the most striking characteristics of *Aedes Aegypti* is sensibility to changes in temperature, an observation that has been confirmed by many investigators. Greatest energy is displayed when the temperature ranges in the vicinity of 28° C.; beyond 39° C. the heat is fatal. If the temperature goes below 15° C. or 16° C., adults become sluggish; at 12° C. to 14° C., they become torpid. Otto and Neumann (1905) placed these mosquitoes outdoors with the temperature at freezing and found that they died very quickly. When exposed to 4° C. for an hour and then placed in a warm room they revived, but longer exposure killed them. At a constant temperature of 7° to 9° C. these investigators kept *Aedes Aegypti* alive for periods ranging from three to eighty-two days. A few of the females died during the first two weeks and half of them survived thirty days; all of the males died within fifteen days, but three females lived until the fiftieth day, two until the sixty-first day and one to the eighty-second day. Carter (1901) states that "Guiteras placed

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thirty-three mosquitoes in an ice box at 8° C to 10° C. without food or water; three of them survived to the eighty-seventh day, when they were eaten by ants."

The eggs of *Aedes Aegypti* manifest remarkable resistance to external influences. Thus, Reed and Carroll (1901, p. 641) state:

> The resistance of stegomyia's eggs to external influences is worthy of note. Drying seems to be but little injurious to their subsequent fertility. We have found that eggs dried on filter paper, and kept for periods of from 10 to 90 days, will promptly hatch when again submerged in water. Dried eggs brought with us from Havana, in February, were easily hatched during the month of May, in Washington, furnishing about 60% of the usual number of larvae hatched from fresh eggs. Freezing does not destroy the fertility of the eggs. Although freezing with a mixture of salt and ice for 30 minutes has several times seemed to prevent subsequent hatching; on one occasion a batch of 155 eggs, freshly deposited, which were frozen at a temperature of —17° C. for one hour, then thawed out at room temperature and placed in the incubator at 35° C., began to hatch on the sixth day, the majority furnishing active larvae on the eighth day. In another observation, freshly deposited eggs, frozen at —17° C. for half an hour on two successive days, began to hatch on the third day as usual at incubator temperature. The resistance of stegomyia's eggs to drying for a period of three months would appear to demonstrate that this genus of mosquito could survive the winter in Havana without the presence of hibernating females.

It is apparent, therefore, that the eggs will survive low temperatures down to the freezing point, and it is probable that under natural conditions the eggs will survive out of water even longer than the longest period achieved experimentally. Further, the eggs of many of the species of *Aedes* do not hatch until the following year, and, with some species, a portion of the eggs probably remain dormant until the second year.

Under favorable conditions the larvae of *Aedes Aegypti* develop with great rapidity. However, under very unfavorable conditions larval life may be extended over relatively long periods, the larvae displaying great resistive powers. Agramonte (1902) places the minimum period of development from egg to imago at ten days; Dupree and Morgan (1902) obtained imagos in from six to eight days; while Taylor (1903) places the period at nine days.

Howard, Dyar, and Knab (1912, p. 289) state:

> The French observers determined a distinct relation between temperature and the rate of development. At Rio in the most favorable season, when the night temperatures were from 26° to 27° C. (78° — 81° F.) and the day temperatures from 28° to 31° C. (82° — 88° F.), they observed that some of the larvae of *calopus* reached the pupal stage 7 days after the hatching of the eggs, and the adult condition on the ninth day, and generally most of the larvae from the same laying of eggs produced imagos about the tenth day. They found that when this rapid breeding occurred it was necessary that the egg, as well as the larva, should have had the right temperature and the egg a rapid incubation. The temperature being lower, the evolution naturally becomes longer; and at Petropolis, with a night temperature lower than 22° C. (72° F.), they found the larvae taking from 40 to 60 days to reach the pupal state, and from 3 to 5 days longer

before the perfect insect issued. Ordinarily, according to their observations, the pupal stages last only from 30 to 50 hours. They found that the larvae do not perish at a temperature near the freezing point, but they grew very slowly and took an indeterminate time to become adults.

And earlier (p. 120, 1912) the same authors state:

In temperate North America a few species pass the winter in the larval state. Such is well known to be the case with *Wyeomyia smithii.* Larvae of all sizes are overtaken by cold weather in their habitat, the water in the leaves of the pitcher plant. They become enclosed in the solid ice and when liberated in the spring continue development in the normal manner. This appears to be the only way in which this species passes the winter and the hardiness of these larvae can be understood when one considers that the species occurs as far north as Minnesota and Ontario. *Culex melanurus* hibernates in the larval state. The larvae live in spring-holes in marshes and remain at the bottom during cold weather. The larva of *Megarhinus septentrionalis,* which normally lives in hollow trees, hibernates, although, apparently, it will not survive freezing. It goes to the bottom in cold weather and remains submerged. The larva of *Bancroftia signifer,* which usually is found associated with *Megarhinus,* also hibernates.

In England and in the more southerly part of Europe, where the winters are much less severe than in North America, one species of Anopheles (*A. bifurcatus*) regularly hibernates as larva. The larvae in various stages of development have been found in the winter, living beneath the ice, by a number of observers, and these larvae later produced imagos. In Switzerland, Galli-Vallerio and his collaborators have made observations through about 10 consecutive winters that show that *Anopheles bifurcatus* and *Culiseta annulatus* regularly hibernate there as larvae.

The object of this study was to determine whether the larvae of *Aedes Aegypti* would revive after a period encrusted in ice, and, if so, for how long. The larvae were collected from an old wooden barrel which contained many stegomyia in all stages of development. This investigation was carried on during the months of October and November, 1932.

Method

A series of small glass tumblers (about 1½ inch in diameter) were half-filled with rain water, and several lively larvae of *Aedes Aegypti* were placed in each tumbler. Some of the tumblers were placed in a "cold room," the temperature of which was gradually lowered by thermostatic control from 20.5° C. until the contents of the tumblers froze. The drop in temperature was regulated so that exactly seven hours elapsed before freezing started. Freezing was completed in forty-five minutes more, and the temperature of the room was then — 2° C. This temperature was maintained during the periods of exposure. Individual tumblers were removed after freezing was complete at the intervals shown in the tables, placed in a compartment kept at 20° C., and allowed to slowly thaw out. Controls were kept at laboratory temperature in similar containers and in the same water.

Observations

The results obtained are recorded in Tables 1 and 2. The first series of larvae were kept frozen for from 2 to 11 hours (Table 1).

TABLE 1

Larvae Frozen from Two to Eleven Hours

Tumbler No.	Hours Frozen	Time Required for Recovery	Time to Maturity
		Hours	
1	2	2.00	Same 24 hours as controls
2	3	2.50	"
3	4	2.00	"
4	5	2.00	"
5	6	2.50	"
6	7	2.75	"
7	8	2.50	"
8	9	3.00	"
9	10	2.75	"
10	11	Did not revive	....

TABLE 2

Larvae Frozen from Ten to Sixteen Hours

Tumbler No.	Hours Frozen	Time Required for Recovery	Time to Maturity
		Hours	
11	10	3.25	Same 24 hours as controls
12	11	Did not revive	....
13	12	"	....
14	13	"	....
15	14	"	....
16	15	"	....
17	16	"	....

A second series of larvae were then exposed to freezing for a period of from 10 to 16 hours (Table 2).

Discussion

The larvae of *Aedes Aegypti* will revive after having been encrusted in ice for periods of not longer than 10 hours, and will proceed to maturity about as rapidly as controls. If encrusted in ice for 11 or more hours the larvae of this species are killed. Consequently, it is probable that larvae of *Aedes Aegypti* which are overtaken by freezing temperatures and frozen solid for 11 or more hours do not survive the winter months, although larvae which submerge may possibly hibernate. The eggs of this species are seemingly more resistant to freezing than are the larvae (1901). In cold climates if the larvae of *Aedes Aegypti* are frozen solid during the winter months, survival of the species is dependent upon the eggs, or the hibernating females, or both.

The fact that the larvae of *Aedes Aegypti* will revive after having been encrusted in ice for 10 hours or less increases somewhat the problems of health authorities and stresses the importance of careful inspection of shipments and carriers, as well as passengers, from countries where yellow fever exists, particularly in cases of transportation by air. Lloyd and Russell (1932) have emphasized the potential danger of reinfection with yellow fever of centers of population which once had the disease but have now been freed of it, because of the rapid development of air transportation. Yellow fever is still a menace to life and to commerce in the United States, and the threat of reinfection demands the continuous and persistent efforts of all public health agencies.

A recent Associated Press dispatch (1933) informs us that Dr. Henrique Aragao, a yellow fever expert of Rio de Janeiro, has discovered that the Brazilian tick can transmit yellow fever. If this is true, the menace is more acute.

Bibliography

Agramonte, A. 1902. *Mosquitoes and Yellow Fever,* Chap. 9, 91-106.

Aragao, H. 1933. Ticks Transmit Fever. *The Commercial Appeal.* Vol. 124, No. 107, p. 6 (April 17).

Carter, H. R. 1901. A Note on the Spread of Yellow Fever in Houses. *Med. Record, N. Y.*, 59: 933-937.

Dupree, J. W., and H. A. Morgan. 1902. Mosquito Development and Hibernation. *Science, n. s.* 16: 1036-1038.

Howard, L. O., H. G. Dyar, and F. Knab. 1912. *Mosquitoes of North and Central America and the West Indies,* Carnegie Institution of Washington, D. C., 1: 289.