SURVIVAL OF *AEDES* (*NEOMELANICONION*) EGGS IN THE LABORATORY

THOMAS M. LOGAN AND KENNETH J. LINTHICUM

U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011

ABSTRACT. Adult *Aedes mcintoshi* and *Ae. circumluteolus* were produced from eggs stored up to 9 months in the laboratory. Under standard hatching conditions, eggs stored at 24° and 26°C consistently produced adults for up to 9 months after collection. In contrast, eggs stored at 15°C were significantly different, producing <1% adults from 6–12 months after collection. *Aedes* (*Neomelaniconion*) eggs can be stored and serve as a reliable source of specimens to be used in vector competence and other studies.

In Kenya, *Aedes* (*Neomelaniconion*) species may complete up to 2 generations per year, typically coinciding with unusually heavy rainfall during the rainy seasons. However, during periods of average rainfall, habitats may not become flooded and eggs must persist for varying periods of times. Little is known about the effect of environmental temperature on the survival of *Aedes* (*Neomelaniconion*) eggs in nature or in the laboratory. In nature, the longevity of these eggs is an important factor in the epidemiology of Rift Valley fever (Linthicum et al. 1985). For laboratory studies of vector competence, storage of field-collected eggs is important because these species have not been successfully colonized. This study was conducted to determine the effect of storage temperatures on the rate of hatching and the survival of larvae to adults over an extended period of time.

Aedes species eggs were extracted by the methods described by Horsfall (1956) from multiple soil samples (ca. 0.3 m diam × 5 cm deep) taken on June 21, 1990, from a dambo located along the Kiu River in Central Province, Kenya, in an area described previously (Linthicum et al. 1988). This dambo and adjoining dambos had flooded in April and May 1990 and produced a population of *Aedes* species. Because more than 90% of *Aedes* (*Neomelaniconion*) eggs in a dambo hatch during the first flooding (Logan et al. 1991), we assumed that most of the eggs used in this study were laid during May and June 1990.

In the final step of the egg-extraction procedure, eggs with a diamond shape, characteristic of the subgenus *Neomelaniconion* of *Aedes*, were pooled. This step eliminated all eggs belonging to species of the subgenus *Aedimorphus*, the other commonly occurring subgenus of floodwater *Aedes*. Unfortunately, eggs could not be identified to species; identification was inferred by rearing to adult stage. Although it was not possible to distinguish the eggs of *Ae. mcintoshi* Huang from *Ae. circumluteolus* (Theobald), we believe that the ratio of adult specimens may be representative of the relative abundance of their eggs because the adult species ratio was similar to that observed in the area after flooding of natural habitats (Linthicum et al. 1988, 1989). Approximately 10,000 extracted eggs were placed on moist filter paper, sealed in a petri dish and shipped to our laboratory in Maryland. Upon receipt, a sample of noncollapsed eggs was removed, counted and exposed to environmental conditions known to stimulate egg hatching (Gerberg 1970). Larvae were counted and reared under standard conditions at 26°C (Gargan et al. 1983). Adults were counted and identified after emergence. The remainder of the extracted eggs were divided into 3 groups and placed on moist filter papers in a sealed containers. These were placed in incubators at 15° or 26°C or at a variable room temperature (23–25°C), referred to below as 24°C. Eggs were checked to maintain the moisture of the filter papers and prevent mold growth. Periodically, samples (n = 100–649) of eggs from each temperature regimen were tested, as described above, to determine the percentage of eggs hatching and reaching the adult stage. Eggs that failed to hatch during the initial flooding were reflooded up to 3 times.

Over 12 months, 7,464 eggs were subjected to hatching stimuli and 2,056 (28%) hatched, yielding 761 (10%) adults. Immature mortality occurred almost exclusively in the larval stages. Nearly all pupae (98%) survived to produce viable adults. *Aedes* (*Neomelaniconion*) *mcintoshi* comprised the majority of the adults that emerged, representing 89% of the specimens, followed by *Ae.* (*Neo.*) *circumluteolus* (11%). The number of males and females of both species that survived to adults was 375 males and 386 females.

The percentage of eggs hatching and of those reaching the adult stage after being held at various temperatures for different times is shown in Table 1. Hatching percentage of newly received eggs (approximately 1 month after eggs were recovered from soil samples held at 24°C) was high (74%; 74/100) and 95% of these survived to adults. Two months after egg recovery, the percentage of eggs hatching at 24°C and

Table 1. Percentage of *Aedes* (*Neomelaniconion*) eggs hatching and adults yielded from eggs held at differing temperatures.

Months after collection	15°C		24°C		26°C	
	Egg hatch	Adult	Egg hatch	Adult	Egg hatch	Adult
1	—*	—	74	95	—	—
2	7**	0***	52	44	66	64
4	60	76	57	8	51	14
6	24	0	16	90	20	44
7	44	0	22	42	63	61
8	22	11	15	67	6	15
9	9	0	5	71	16	83
10	10	0	9	0	31	1
12	27	0	0	0	0	0

* Eggs were placed at 15° and 26°C 1 month after collection and were tested starting 2 months after collection.
** Percentage of eggs hatching after subjection to hatching stimuli.
*** Percentage of those eggs that hatched that survived to adult.

26°C was 52% (64/124) and 66% (61/93), respectively; however, only 7% (7/100) of the eggs held at 15°C hatched. From 4–9 months, egg hatch was generally similar among various temperatures and varied from a low of 5% (14/285) (month 9; 24°C) to a high of 63% (281/447) (month 7; 26°C). When compared with all other storage conditions, eggs held for 4 months at 15°C produced the highest percentage of adults (46%). By 10 months, no adults were produced from eggs held under any of the storage regimens, even though some egg hatching occurred. Twelve months after recovery, no hatching occurred in eggs held at 24° (0/441) and 26°C (0/344), and none of the larvae (0/98) from the 15°C group survived to adult. The proportions of the number of eggs hatched/total eggs flooded and the number of adults emerged/number of hatched eggs were calculated and transformed using the arcsine transformation for proportions (Snedecor and Cochran 1989). The transformed variables were analyzed by analysis of covariance with temperature as a classification variable and month after egg recovery as a covariate, using the computer program PROC GLM (SAS Institute Inc. 1988). The slopes of the temperatures over time were different for the proportion of eggs hatched/total eggs flooded with the 15°C slope differing from the higher temperatures ($P = 0.03$). Although some eggs held at 15°C hatched on all 8 floodings, these produced adults only 2 of the flooding periods (Table 1). The slope of temperature over time for the proportion of adults emerged/number of hatched eggs was not significant ($P = 0.14$). The effect of temperature was significant after removing the effect of time ($F[2,19]$, $P = 0.03$). The difference between temperatures least square means showed 15° significantly different from 24° and 26°C (LSD, $P < 0.05$) but, 24° and 26°C were not significantly different. Adult mosquitoes were consistently produced from eggs stored at 24° or 26°C for up to 9 months after collection.

Although Logan et al. (1991) found in a field study that a small percentage of *Aedes* (*Neomelaniconion*) eggs would hatch after each of 4 successive floodings, virtually all eggs hatched in the laboratory after the first flooding. This suggests that laboratory conditions were optimal for egg hatching (Novak and Shroyer 1978).

Erratic egg hatching has been documented in several subgenera of *Aedes* including (*Neomelaniconion*) [as (*Banksinella*)] by Gillett (1955). Frequent variation in egg hatching and larval survival has been observed frequently in eggs extracted from soil samples and flooded without holding in the laboratory (T. M. Logan, unpublished data). In spite of fluctuations in egg hatch and larval survival, 16% of the eggs held at 24° and 26°C yielded viable adult *Ae. mcintoshi* and *Ae. circumluteolus* for up to 9 months after collection. Eggs held at 15°C produced less than 1% adults (9/2353) from 6–12 months after collection (Table 1). *Aedes* (*Neomelaniconion*) eggs can be stored and may serve as a reliable source of specimens to be used in vector competence and other studies, even though the factors affecting the variability observed are not understood.

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