

Long-term storage and viability of *Ochlerotatus albifasciatus* eggs (Diptera: Culicidae)

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The viability of Ochlerotatus albifasciatus (Macquart) eggs stored at room temperature and at 5°C was studied over 31 months. After 12, 18 and 31 months of storage, eggs were acclimatized at 22°C for ten days, and then inundated twice every seven days. The effect of the storage period on the percentage of hatching was analyzed by one way ANOVA. Differences on the hatching response between the first and second flooding were analyzed by paired t-test. Differences on the hatching response between the two storage conditions were analyzed by Mann-Whitney rank test. Results showed that (1) Oc. albifasciatus eggs were able to survive and hatch over 31 months; (2) the percent hatching of eggs stored at 5°C was higher than that of eggs stored at room temperature; and (3) low temperatures and long periods without water favor installment hatching.

Key words: Culicidae - floodwater mosquitoes - egg dormancy - diapause - egg survival - hatching

Ochlerotatus albifasciatus (Macquart) is a floodwater mosquito of medical importance (Mitchell et al. 1987) distributed from tropical Brazil (Forattini et al. 1988) to cold areas in Argentina (Rossi et al. 2006). Larval sites are temporary, semi-permanent and ephemeral pools, with a high variation in the water level (Maciá et al. 1995). As a consequence of the heterogeneous larval sites, *Oc. albifasciatus* eggs have adopted strategies that allow them to survive under unfavorable environmental conditions. When eggs are either immersed in cold water or remain dry they enter a quiescence or diapause (Campos & Sy 2006, 2007). When environmental conditions turn favorable a proportion of the eggs hatch while others remain in dormancy until the next flooding (Campos & Sy 2006). This behavior, characterized by the occurrence of partial hatchings, is called installment hatching, and has been interpreted as a reproductive strategy that permits multivoltine species that breed in ephemeral pools to survive for long periods (Andreadis 1990). Thus, installment hatching and resistance to low temperature and lack of water, permit *Oc. albifasciatus* survive in regions where rainfall and temperature are highly variable (Campos & Sy 2007).

Several studies on Aedine mosquitoes, mainly from tree hole and container habitats, have been carried out to estimate egg survival (e. g., Christophers 1960). For example, Gubler (1970) estimated that *Aedes albopictus* (Skuse) eggs are able to survive for 243 days. On the other hand, studies on floodwater mosquitoes showed that *Aedes*

and *Psorophora* eggs resist in dormancy for approximately four years (Breeland & Pickard 1967), while *Aedes vexans* (Meigen) and *Aedes sticticus* (Meigen) eggs are able to survive for two years if the substrate remains moist (Gjullin et al. 1950). Survivorship of floodwater mosquito eggs has been studied from different regions all over the world, but is unknown from neotropical species.

The objective of this study was to determine the resistance of *Oc. albifasciatus* eggs to long dry periods, testing the hypothesis that *Oc. albifasciatus* eggs survive after a long dry period independently of storage temperature.

The experiment was carried out with eggs obtained from wild-caught females of *Oc. albifasciatus* captured with a manual aspirator at Pereyra Iraola Provincial Park (34° 51' S; 58° 08' W), province of Buenos Aires, Argentina, from May to June 2004. Mosquitoes were blood fed on a guinea pig and stored individually in tubes (6 cm in height x 3 cm in diameter) with a moist piece of towel paper resting on a cotton pad at the bottom of the tube. After oviposition, females were withdrawn and the tubes sealed with plastic film to maintain humidity. Eggs remained at room temperature for over nine days to ensure complete maturity of embryos (Fava et al. 2001).

Batches (batch = group of eggs laid by a single female during one gonotrophic cycle) were pooled and randomly assigned to one of two treatments. Eggs from one treatment were kept at room temperature, and the other was stored in the dark at 5°C. All batches with fungal or bacterial contamination during storage were excluded from the experiment.

After 12, 18, and 31 months of storage, 20 batches from each group were flooded except for the group of 18 months at 5°C where 19 batches were used. Batches were acclimatized for 10 days at 22°C and 12:12 h light-dark photoperiod after flooding. To stimulate hatching, eggs were transferred to Petri dishes (10 cm in diameter, 1.5 cm in height) containing 40 ml of tap water and 10 mg of yeast. After 24 h, larvae were counted and unhatched

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eggs were returned to the original tube and stored at 25°C until the next flooding, which was set up seven days later. Chorions of eggs that remained unhatched after the second flooding were bleached with 50% commercial sodium hypochlorite solution to check viability. Embryos that were creamy white with eyespots, hatching spine, and distinct abdominal segmentation, were assumed to be viable. Yellow-brown or red-brown embryos were assumed to be nonviable (McHaffey & Harwood 1970).

Data collected included: (1) percent of hatching; (2) eggs unhatched with dead embryos after two successive flooding; (3) spontaneous hatch after the previous flooding; (4) eggs collapsed after the previous flooding; and (5) eggs without embryos. The percentage of eggs hatching from each batch was calculated as the number of larvae after flooding/number of total eggs (hatched and un-hatched) \times 100, and each batch was treated as a replicate (Parker 1988). These percentages were arcsine-transformed before ANOVA using the Freeman-Tukey's transformation. Differences in the hatching response between treatments stored for 20 months were analyzed by Mann-Witney rank test. The percentage of hatched eggs from the first and second flooding were analyzed by a paired t test. Differences in the hatching response among storage times in treatments stored at 5°C were analyzed by one-way ANOVA. When significant differences were detected by ANOVA, multiple comparisons of means were made using Tukey's honestly significant differences test for unequal data (Zar 1996).

Results of the present study show that spontaneous hatchings occurred in eggs that remained stored at both conditions, and increased when the storage time was long (Table I). These spontaneous hatchings could be attributed to water condensation during storage which stimulated the pharate larva to hatch. A similar pattern was observed for the percentage of collapsed eggs in relation to the storage time (Table I). On the other hand, the mortality of pharate larva after the second flooding, in both groups varied between 1.7 and 33% and showed no relation with the storage time (Table I).

Viability (eggs hatched + eggs unhatched with embryo alive) of *Oc. albifasciatus* varied between 12 or 31 months if eggs were stored at room temperature or 5°C

respectively. In the group of eggs stored at room temperature 13.8% of hatch was recorded but only in the treatment of 12 months storage. In contrast the group of eggs stored at 5°C hatchings was observed after the three storage times (Table I), and the percentage of hatched eggs decreased significantly ($F = 12.37$, $df = 2$, $p < 0.001$) as time increased. Comparison between treatments stored during 12 months showed that the percentage of hatching eggs stored at 5°C, was significantly higher than that of eggs stored at room temperature ($T_{(20,20)} = 299$, $p = 0.003$) (Table I). Ludueña-Almeida and Gorla (1995) observed that *Oc. albifasciatus* eggs stored at drought condition survive for six months, decreasing their viability as time increase. Our result show that mortality increases when the storage time is long, independent of storage conditions.

In this study the treatments that required two floodings to hatch were the eggs stored for 12 and 18 months at 5°C. The number of hatched eggs during the second flood ($n_{(Treat,12)} = 3$, $n_{(Treat,18)} = 109$), was significantly lower (Paired: $t_{(Treat,12)} = 5.18$, $df = 15$, $p < 0.001$; $t_{(Treat,18)} = 2.99$, $df = 11$, $p = 0.012$) than hatched eggs from the first flood ($n_{(Treat,12)} = 934$, $n_{(Treat,18)} = 586$) (Table II). All eggs stored at room temperature hatched during the first flooding while a few eggs stored at 5°C hatched after the second flooding (Table II). This installment hatching behavior observed in the treatment at 5°C was reported previously in a study where some eggs after being stored and flooded during winter required up to three consecutive flooding to hatch, while eggs flooded in spring hatched during the first flooding (Campos & Sy 2006).

I concluded then that (1) *Oc. albifasciatus* eggs are able to survive and hatch over 31 months; (2) the percent hatching of eggs stored at 5°C is higher than that of eggs stored at room temperature; (3) low temperatures and long dry periods favor installment hatching.

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TABLE I

Percentage of *Ochlerotatus albifasciatus* eggs (from 20 batches by treatments), that hatched after being stored at two temperature conditions for three storages periods

Eggs	Storage condition					
	Room temperature			5°C		
	Months			Months		
	12	18	31	12	18	31
Hatched after two successive flooding	13.8	0	0	41.5	33.4	0.1
Unhatched with embryo (dead)	33	29.5	14.7	18.2	1.7	11.6
Spontaneous hatch previous flooding	8.1	12.8	14.4	3.7	9.9	12.6
Collapsed (with and without embryo)	38.5	51.9	54.9	31.5	29.8	46.7
Without embryo	6.6	5.8	16	5.1	25.2	29

TABLE II

Number of *Ochlerotatus albifasciatus* eggs (from 20 batches per treatment) that hatched in two successive flooding, after being stored at two temperature conditions for three dry periods

Flooding	Storage condition					
	Room temperature			5°C		
	Months			Months		
	12	18	31	12	18	31
1st	278	0	0	934	586	3
2nd	0	0	0	3	109	0

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