CHAPTER 18

EFFECT OF MULTIPLE IMMERSIONS ON EGGS AND DEVELOPMENT OF IMMATURE FORMS OF *HAEMAGOGUS JANTHINOMYS* FROM SOUTH-EASTERN BRAZIL (DIPTERA: CULICIDAE)

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ABSTRACT: The effect of multiple immersions on *Haemagogus janthinomys* Dyar, 1921 eggs and the development of its immature forms were studied. Eggs of *Hg. janthinomys* from Linhares (state of Espírito Santo) were submitted to multiple immersions, counting the number of hatched eggs and observing the periods for development until adult. The egg-hatching proportion attained 42-88% (mean=68.2%), after 16 immersions, in three trials. After 8-9 immersions, the effect of additional immersions was negligible. The periods for development of immature forms were 3.67±0.89, 1.2±0.66, 1.12±0.63, 4.86±1.77, 2.25±0.67 and 12.40±0.82 days, respectively for 1st-4th instar larvae, pupae and the total. The postponement of the egg-hatching can be important for the production of mosquitoes, from one egg batch, during several months. The total period of larval development is very similar to previous indirect field calculations in the Brazilian state of Para and to mosquitoes from Panama studied in the laboratory.

KEYWORDS: Egg-hatching; immersion, development, diapause; drought; *Haemagogus janthino- mys*; yellow fever

Haemagogus janthinomys Dyar, 1921 has been shown to be the most important vector of yellow fever in Brazil (Antunes and Whitman, 1937; Vasconcelos, 2003) and other countries (Trapido and Galindo, 1957), and has also been found infected by Mayaro (Hoch et al., 1981) and Ilhéus virus (De Rodaniche and Johnson, 1961). *Haemagogus janthinomys* feeds mostly in primates (Vasconcelos, 2003), but also on several other animals (Alencar et al., 2004).

This species lives mostly in primary forests, as is usual in other species of *Haemagogus*, and its immature forms develop in tree holes and in bamboo oviposition traps (Arnell, 1973). *Haemagogus* mosquitoes have a preference for ovipositing in bamboo traps with large horizontal holes, instead of those with small lateral holes, preferred by *Sabethes* mosquitoes (Galindo et al., 1951, 1955). The effect of immersions on the eggs of some species of *Haemagogus* from Panama was studied, but the resistance of eggs of *Hg. janthinomys* and the effect of immersions is poorly known (Galindo et al., 1955). Mattingly (1973, 1974) and Linley and Chadee (1991) described the morphology of the egg of this species; its dorsal surface adheres to surfaces by a glue, probably indicating a tendency to be fixed to surfaces, more accessible in cut bamboos than in holed ones.

A study on the effect of multiple immersions of eggs of *Hg. janthinomys* on their hatching was developed. The development of larvae was observed until the emergence of adults.

METHODS

Egg collection in the field and processing

Haemagogus janthinomys eggs were obtained from ovitraps baited with leaves, utilized in a primary forest at Linhares Municipality (19°18'S 40°04'W, 25m a.s.l.), in the Brazilian south-eastern State of Espírito Santo; the locality was described by Borgonovi (1983). Ovitraps and their utilization were previously described (Alencar et al., 2005); briefly, 1-litre black flowerpots, containing four wood plates (2.5 x 14 cm), were exposed at 2.5-6 m above ground. Plates were exposed for 3-4 days, examined for the presence of eggs, and their number was recorded. The positive plates were transported dry to the laboratory in plastic bags, in polystirene boxes. The plates were then dipped in white trays, with tap water, at 29±1°C. The plates were maintained three days in the water, and were then removed, counting the hatched larvae. The egg-hatching was observed in three trials, respectively for 206, 156, and 495 eggs. The dipping was repeated 16 times, with intervals of three days.

Development of immature forms

Twenty-one first instar larvae were observed until the emergence of adults. The larvae received fish food (Tropi-Fish), and the excess of food and sediment was cleaned daily. The temperature was maintained at 28±1°C, and the photoperiod was 10:14 (L:D). The difference between the total periods for development for females and males was analysed by ANOVA. The genera were abbreviated as proposed by Reinert (2001).

RESULTS

Egg-hatching proportion varied, after 16 immersions, from 42 to 88%; the evolution of the proportion of hatching after the immersions can be seen in Fig. 1. After 8-9 immersions, the effect of additional immersions was usually negligible. As previously observed (Alencar et al., 2004), all eggs on the plates belonged to *Hg. janthinomys*. Williams' mean, a modified logarithmic mean (Bidlingmayer, 1969) was utilized.

One larva died in the second instar, three in the third and one in the fourth. The other 16 immature mosquitoes evolved to adults. The periods for the development of immature forms, with the ranges between brackets, were 3.67±0.89 (0-4), 1.2±0.66 (1-3), 1.12±0.63 (1-2), 4.86±1.77 (4-11), 2.25±0.67 (2-3) and 12.40±0.82 (11-13) days, respectively for 1st-4th instar larvae, pupae and the total. The mean duration for the cycle was 13.42±0.13 (13-14) and 12.75±2.75 (9-13), respectively for females and males; the difference was not significant.

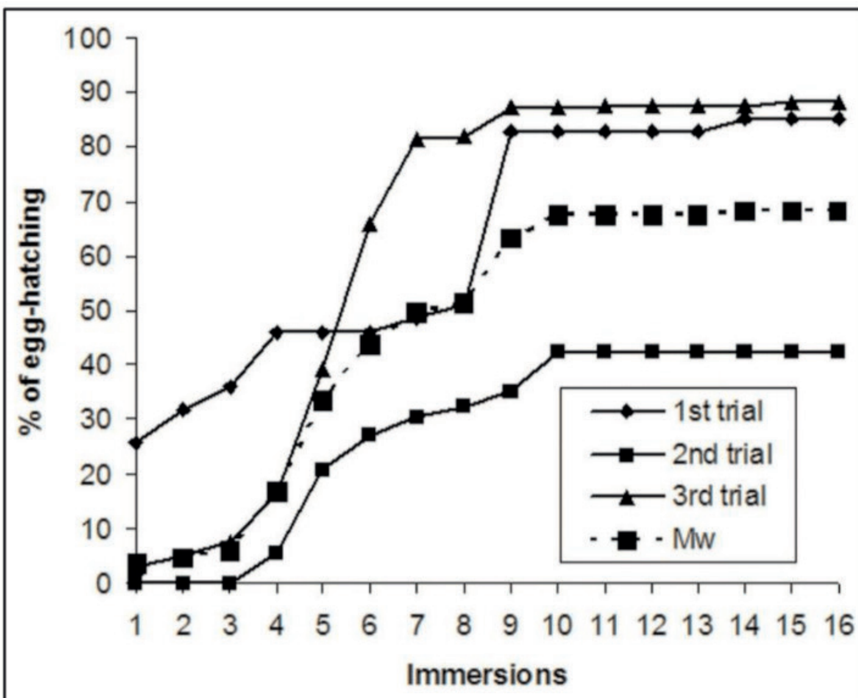

Figure 1. Effect of multiple immersions on the egg-hatching in *Hg. janthinomys*. M_w – Williams' Mean.

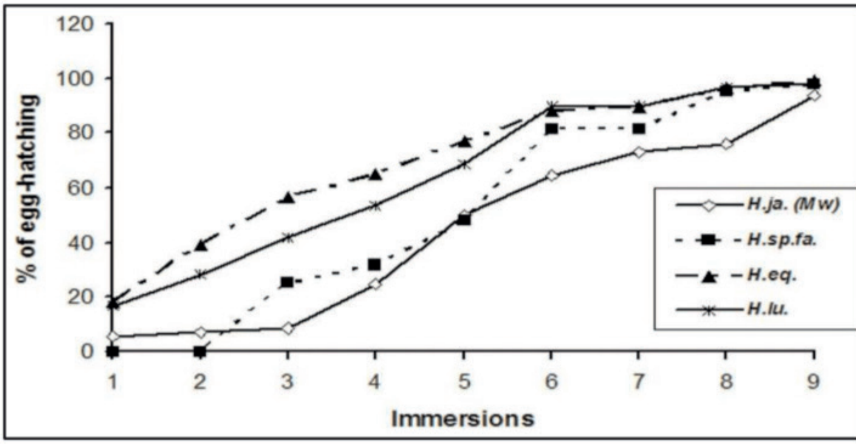

Figure 2. Effect of multiple immersions on the egg-hatching in *Hg. janthinomys* and three species of *Haemagogus* studied by Galindo et al. (1955 - Tables III and IV). H.ja. (Mw) – *Hg. janthinomys*; H.sp.fa. – *Hg. spegazzinii falco* (= *Hg. janthinomys*); H.eq. – *Hg. equinus*; H.lu. – *Hg. lucifer*.

DISCUSSION

Our results show variable effects of immersions; they are most evident when one compares the first to the third trial (Fig. 1). There was a cumulative effect of the immersions, until the 8th-10th immersions. Hovanitz (1946) observed hatching of eggs of *Hg. janthinomys* (as *Hg. spegazzinii falco*, see Arnell, *loc. cit.*) after one immersion. Galindo et al. (1955) submitted eggs from bamboo traps to 6 to 11 immersions, in two experiments, and respectively 8 and 44 eggs hatched, mostly after the 3rd and 4th immersions.

The observed proportions attained 85-90% after 9-11 immersions, in two trials, and the mean was almost 70% (Fig. 1). Although the effect of multiple immersions in the egg-hatching of *Hg. janthinomys* had been reported (Galindo et al., 1955), no complete information on the proportion of the eggs hatching was available for comparisons. The need of several immersions for the hatching of most eggs seems to occur in several populations of the species, even so distant as those from Panama and south-eastern Brazil.

If the egg-hatching index continues to rise after so many immersions, as observed for *Hg. janthinomys*, eggs deposited by the females, possibly in the same batch, can quickly produce adults after each rain strong enough to submerge the eggs. This high number of immersions and desiccations would probably be equivalent to several months, indicating that the production of adults can occur through out the year, unless meteorological conditions are unfavourable for the egg-hatching. The observed period for the larval and pupal development of *Hg. janthinomys* (12.40±0.82 days) is very similar to that supposed for mosquitoes in the nature in Para State (13 days) (Dégallier et al., 2006).

This postponing of hatching in *Hg. janthinomys* was probably correlated to the late appearance of adults, compared to *Haemagogus equinus* Theobald, 1903 and *Haemagogus* *lucifer* (Howard, Dyar and Knab, 1912) in Panama (late June or July vs. May and early June) (Galindo et al., 1951). Supposing the sums of proportions of the hatched eggs in the tables of Galindo et al. (1955 - Tables III and IV) as 68.2% of the totals, as in the present study, all these species would have similar curves, with some postponing for *Hg. janthinomys* (Fig. 2). So, this small post- poning of the egg-hatching could explain the differences among the three species. Since oviposition of females of *Hg. janthinomys* is significantly greater in wet season, compared to dry season, in Trinidad (Chadee et al., 1992), the gradual hatching of eggs may be a way to survive through the dry season. A study of seasonal variation of *Hg. janthinomys* in Rio de Janeiro is being developed (Alencar et al. – unpublished results).

Tap water was used in the present study. Maybe the presence of bacteria and nutrients (Gjulin et al., 1941; Barbosa and Peters, 1969) of a falling oxygen concentration (Judson, 1960) could influence the results. Since the hatching of *Ochlerotatus sierrensis* (Ludlow, 1905) (=*Aedes sierrensis*) (Reinert, 2000) occurred only when the oxygen concentration was very low (0.25 ppm or less) (Judson et al., 1966), the concentration for the hatching of eggs of *Hg. janthinomys* should be determined.

The effect of immersions can also be very important for survival, competition and seasonal fluctuation of some African mosquitoes in tree holes (Lounibos, 1981).

The influence of genetic programming and of physiological factors on the reaction of eggs of *Hg. janthinomys* to multiple immersions should be investigated. The eggs of these mosquitoes from Panama did not hatch before they were submitted to a period of at least two weeks under moist conditions (Galindo *et al.*, 1951). In the present study, this period was only the 3-4 days enough for the transportation of the eggs for the laboratory. This difference can also be related to biological differences due to the origin (south-eastern Brazil).

The dormancy of Aedine eggs usually ends in the first immersion, as in *Aedes aegypti* (Linnaeus, 1762), but some eggs can need more than one immersion for the hatching (Clements, 1963). This is known as *instalment hatching* (Gillett, 1955a), and is probably a strategy for the survival of mosquitoes in temporary pools submitted to several inundations (Andreadis, 1990). The regulation of this occurrence, variable between populations of the same species and eggs of the same female (Gillett, 1955b), is still not understood, and has not been studied for *Haemagogus* mosquitoes. The effect of multiple immersions on *Oc. albifasciatus* (Macquart, 1838) eggs is related to day-length and temperature (Campos and Sy, 2006).

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