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SHORT REPORT

PRE-OVIPOSITION BEHAVIOR OF *AEDES AEGYPTI* IN THE FIELD: FIRST REPORT OF EGGS LAYING ON WATER SURFACE IN AN ARTIFICIAL BREEDING SITE IN THE CITY OF RIO DE JANEIRO

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ABSTRACT

This study presents the first report of *Aedes aegypti* laying eggs on the water surface of an artificial breeding site in the field. This report occurred in the city of Rio de Janeiro inside a white bucket containing 11 liters of rainwater. Conspecific larvae had previously been found in this bucket. From the 219 eggs obtained, 135 (61.6%) were laid on the water and 84 (38.3%) on the border of the bucket. Larvae (4th instar) and adults obtained from the eggs were identified as *A. aegypti*. This behavior may influence the population dynamics of *A. aegypti* and expands the knowledge about the species adaptation. This report confirms the species' ability to lay eggs on the water surface of artificial breeding sites in the field and suggests the hypothesis that pheromones in the water may influence the females to lay eggs on water.

KEY WORDS: Ethology; *Aedes aegypti*; adaptation; disease vectors; mosquito vectors.

The control of *Aedes aegypti* still represents an important challenge in the prophylaxis of arboviruses, with emphasis on dengue, Zika and Chikungunya viruses. Common control strategies focused on the use of insecticides have not provided the necessary effectiveness. Despite the proposal and the use of alternative methods, no satisfactory prophylactic results have been achieved so far, particularly in the extensive and complex endemic areas from Brazilian tropical cities.

Establishing feasible and effective control strategies in regions with major social and environmental obstacles requires a good understanding on the behavior of adult species in the field, in particular the behavior of females before oviposition when choosing a breeding site. Despite the significant literature on *A. aegypti* oviposition and breeding sites, the strategies of females´ egg

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laying behavior in the field is yet to be further understood. In mosquitoes the pre-oviposition behavior is mediated by semiochemicals, in addition to tactile, physical, chemical stimulus and availability of breeding sites (Bentley & Day, 1989; Navarro-Silva et al., 2009; Day, 2016; Miwingira et al., 2020). In this context, laying eggs on the water surface is a lesser-known behavior in the field. Eggs laying on the water surface have been largely reported in the laboratory (ASPH, 1907; Young, 1922; O'Gower, 1957; Wood, 1963; O'Gower, 1963; Goma, 1964; Tirapatsakun et al., 1981; Madeira et al., 2002; Gomes et al., 2006; Swan et al., 2018), but very poorly observed in the field; in addition, those studies have used traditional oviposition traps, with small black pots and wooden paddles (Chadee et al., 1995; Soares et al., 2015; Wermelinger et al., 2015). This study presents the first report of *A. aegypti* oviposition on the water surface of an artificial breeding site in the city of Rio de Janeiro.

In a white bucket covered with a translucent plastic plate outside the house, which originally was used as a chlorine container commercialized for swimming pool cleaning, some *A. aegypti* larvae were found. No eggs were noticed in the water. The bucket had the following characteristics: 29 cm in height and 26 cm in diameter, approximately 11 liters of rainwater (Figure 1). After removing the larvae, the bucket was intentionally left open, without a lid, at the same place and with the same water. Following a weekly inspection, over a month later eggs floating on water surface have been found (Figure 2). Eggs in the water were carefully sieved, counted, and isolated for the larvae to hatch. Eggs founded on the border of the bucket were gently removed with a small brush and then counted and isolated. All eggs were transported to laboratory in white plastic trays containing water from the breeding site. The larvae were bred until they reach the adult stage. All adults and the remaining larvae were identified under a stereoscopic microscope.

This oviposition was found in the fall, 05/20/2021, in a residential housing neighborhood in the city of Rio de Janeiro. Two hundred nineteen eggs were counted, 135 on the water surface (61.6%) and 84 on the edge of the bucket (38.4%). Twenty-nine adults and 15 4^{th} instar larvae hatched from the eggs on the surface, while two larvae emerged from the eggs on the edge. All individuals were identified as *A. aegypti*.

This first report of *A. aegypti* oviposition at the water surface in an artificial breeding site in the field (rather than in oviposition traps) expands knowledge about the adaptive capacity of the species.

Probably this oviposition behavior in an artificial breeding has not been previously observed because of its difficulty to detect the eggs in the mosquito preferred breeding places, usually with dark surfaces. The current account was favored by the white surface of the bucket. The knowledge that female mosquitos lay their eggs on wet surfaces can also draw attention to eggs deposited on water of the breeding sites.

Figure 1. Bucket with *Aedes aegypti* laying of eggs in water.

Figure 2. Aedes aegypti eggs deposited on the water surface in an artificial breeding in the field.

This report improves the knowledge on the species' adaptive capacity. Its eggs are highly capable to resist desiccation for long periods and its larvae are active foragers, an appropriate behavior to explore transitory breeding sites without aquatic predators and where food resources and time are ecological constraints. These characteristics suggest that the species successfully explored transitory breeding sites throughout its evolutionary process, with the great advantage to maybe avoid the presence of aquatic predators and competitors. However, the species is known to explore perennial breeding sites where it is possible to lay eggs on the water surface. Many reports of eggs laid on the water in the laboratory suggest that this behavior could be an innate characteristic and most eggs may be laid on the water depending on the type of the container (Christophers, 1960). However, in the field, this behavior may be significantly influenced by the presence of semiochemicals, possibly signing favorable conditions for females to deposit directly on the surface. In this report, *A.aegypti* larvae have been found in the breeding water. Several studies have demonstrated that females are stimulated to ovipose in places with a previous presence of conspecific larvae or eggs (Soman & Reuber, 1970; Ravindrakumar & Reuben 1970, Benzon & Apperson, 1988; Allan & Kline, 1998; Tilak et al., 2005; Serpa et al., 2008; Wong et al., 2011; Mwingira et al., 2020), suggesting an effect of attractive compounds or bacteria (Benzon & Apperson, 1988; Ponnusamy et al., 2008). This attractiveness may have occurred in this case. The preference for laying eggs directly in the water (61.6%) may have been induced by this attractiveness, or by the smooth surface of the bucket (inappropriate), or even by the presence of kairomones left by natural enemies (e.g. ants) on the edge. It is possible to suppose that the development cycle from egg to adult may decrease with the eggs deposited directly in the water and which influence the population dynamics of *A. aegypti* and, above all, the productivity of perennial breeding sites.

This report confirms the species' ability to lay eggs on the water surface and suggests the hypothesis that the presence of pheromones in the water can stimulate the female mosquitos to lay eggs on the surface of water. If this hypothesis is confirmed, it is possible to assume that females may prioritize known breeding sites, in particular, where they emerged and, consequently, not dispersing.

CONFLICTS OF INTEREST

All authors declare that there is no conflict of interest to disclose.

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