Letter to the Editor

Overlooked Oviposition on Water Surfaces: A Potential Source of Bias in *Aedes aegypti* Surveillance Programs

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(Received 22 Aug 2026; accepted 08 Sep 2026)

Dear Editor

Our recent observations of *Aedes aegypti* oviposition behavior (1), conducted both in controlled insectary settings (Fig. 1) and natural environments in Bandar Abbas (27°11′46″N, 56°17′16″E), Iran, reveal a critical nuance that may significantly affect the accuracy of current vector surveillance programs.

Traditional models often assume that *Ae. aegypti* females lay eggs exclusively on specific oviposition substrates. Gravid females preferentially deposit eggs on moist surfaces above the waterline. This evolutionary behavior, combined with the desiccation resistance of their eggs, forms the fundamental principle underlying ovitrap surveillance and larval control programs. Standard protocols for counting eggs and calculating indices, such as the Ovitrap Index, rely on the assumption that eggs are deposited on the provided substrate above the water surface. However, our observations in the insectary showed that *Ae. aegypti* lays approximately 20–30% of its eggs directly onto the water film during each oviposition event (1). We also observed this phenomenon in ovitraps and containers placed in the field.

This finding has direct and concerning implications for the effectiveness of ovitrap-based surveillance, which remains a cornerstone of *Ae. aegypti* monitoring worldwide. Current ovitraps are primarily designed to capture eggs laid on moist surfaces above the waterline. Given that standard substrates do not capture eggs deposited directly on the water surface, routine sampling methods are highly likely to overlook a significant portion of the egg population.

Field and laboratory studies in Brazil (2, 3) have reported that *Ae. aegypti*, in addition to laying eggs above the waterline, can also lay eggs directly on the water surface. These studies have confirmed that 28 to 62 percent of all eggs are laid directly on the water surface. Kairomones present in the water, particularly chemical cues from water where *Ae. aegypti* larvae previously developed, can significantly increase the proportion of eggs laid on the water surface. These cues signal to gravid females that the aquatic environment is suitable for offspring development (2–4). Our insectary study also confirmed this finding (1). However, it is important to note that oviposition behavior observed in laboratory colonies may not fully reflect field conditions, and further semi-field studies are warranted. Eggs laid on the water surface hatch faster, as they do not experience a dry period, but they are at greater risk if the water evaporates (2).

Our findings reveal a fundamental aspect of mosquito evolutionary success: a dual-path strategy that maximizes fitness under unpredictable environmental conditions. When eggs are deposited on the water surface, they often bypass the obligatory embryonic diapause, which is a period of developmental delay (2). If the aquatic habitat is stable and rich in resources, the eggs can hatch immediately, allowing the mosquito population to take advantage of the available resources before competitors arrive or before the water source evaporates. Additionally, the strategy of laying eggs above the waterline serves as an evolutionary adaptation to environmental uncertainty (2, 3).

Table 1 compares the egg fitness and survival rates for two oviposition behaviors of *Ae. aegypti*: laying eggs on the water surface versus above the waterline.

Table 1. Comparison of egg fitness and survival rates in two *Aedes aegypti* oviposition behaviors, on the water surface and above the waterline

Feature	On the water surface	Above the waterline
Type of strategy	Immediate hatching (opportunistic strategy)	Gradual drying (evolutionary adaptation strategy)
Primary goal	Exploitation of resource abundance	Resilience mechanism against environmental
Vulnerable to desiccation	High	Low (Drought resistance)
Larval survival	Generally higher if the breeding site persists	Variable; dependent on synchronous flooding
Seasonal/ecological fitness contribution	Contributes most to population growth during wet/stable seasons	Contributes most to population maintenance during dry/unstable seasons or between rainfall events

Fig. 1. Oviposition behaviors of *Aedes aegypti*. (Left) Usual oviposition behavior with eggs deposited on the substrate above the waterline in an ovitrap. (Right) Oviposition behaviors on the water surface and above the waterline in a bowl of water for placing pupae in adult mosquito cage, observed in an insectary in Iran

This finding suggests that current ovitrap-based surveillance methodologies may be subject to systematic biases. Therefore, when using ovitraps for monitoring and surveillance, it is essential to account for eggs laid on the water's surface as well as those deposited above the waterline. Not considering this may lead to underestimating infestation levels and reproductive activity in a given area.

The consequences of this surveillance gap are twofold:

1. Underestimation of population density: Current monitoring protocols may report significantly lower egg densities than those actually present in the environment, which may lead to a misleading perception of low vector abundance.

2. Delayed detection of infestation outbreaks: A false sense of low vector activity can lead to inadequate or delayed control measures, especially in regions where environmental factors, such as humidity and temperature fluctuations, may shift oviposition behavior toward the water surface. An incorrect assessment of egg density may lead to delayed or inadequate public health responses, potentially allowing *Aedes*-borne disease outbreaks going unnoticed.

Based on these observations, we propose several recommendations for future research and practice in vector control:

1. If and when current entomological surveillance protocols are revisited, we suggest that attention be given to the water surface as an additional egg-bearing compartment. Incorporating a simple collection step for floating eggs would require minimal extra effort but could substantially improve the representativeness of the data obtained.

2. Innovative trap design: The development of ovitraps incorporating floating substrates to capture oviposition patterns both at the waterline and on the water surface.

3. Further comparative research should investigate the hatchability and fitness of surface-deposited eggs compared to those laid on the substrate to determine whether this behavior confers any selective advantage.

4. Recognizing oviposition on the water surface as a characteristic feature of *Ae. aegypti* is not merely an observational interest; it highlights a shift in our understanding of this species' behavior. This is a new biological insight that has been overlooked until now. Detecting eggs in the mosquito's preferred breeding sites is challenging, as these sites often have dark surfaces. Our observations were facilitated by the white surfaces of the water bowls (Fig. 1). We recommend that monitoring and surveillance programs for this vector include checking the water surface in various containers using appropriate tools, such as a flashlight. In natural settings, when a container is nearly full of water, there is minimal space above the waterline. Behavioral plasticity allows female mosquitoes to select the most suitable strategy (above the waterline or on the water surface) for oviposition under different container conditions. This adaptability is likely a key factor in the ecological success of this species in urban environments.

Addressing this behavioral issue is essential for improving the accuracy of mosquito surveillance and the overall effectiveness of integrated vector management (IVM) programs.

We recommend updating entomological monitoring protocols to include oviposition behavior at the waterline and on the water surface. This could involve incorporating additional sampling methods or modifying ovitrap designs to effectively capture eggs deposited at the water-level interface. By integrating these behavioral insights into surveillance strategies, we can achieve a more accurate understanding of *Ae. aegypti* population dynamics, ultimately enhancing the effectiveness of vector-borne disease control programs.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Wordvice AI and Grammarly AI in order to improve readability and language of the work. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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