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**RESEARCH ARTICLE**

## Medical Entomology

# Effect of oviposition-site deprivation on reproductive performance and life history parameters of dengue vector *Aedes aegypti*†

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**Abstract:** Dengue and dengue haemorrhagic fever constitute one of the most significant arthropod-borne viral diseases that occur in tropical and subtropical regions in the world. Annually 390 million new dengue cases are being reported from the 128 dengue-endemic countries. *Aedes aegypti* is the primary vector that transmits the disease. Since the primary vector is a container breeder, source reduction appears to be a good vector control method. Source reduction limits the oviposition of females through oviposition-site deprivation. Therefore, the current study was conducted to determine the effect of oviposition-site deprivation on the fecundity, fertility, life-history parameters, and longevity of *Ae. aegypti*. Oviposition-site deprivation was enabled by delaying the access to the oviposition substrate. Female mosquitoes were allowed to access the oviposition substrate separately on the day of blood feeding and 2, 4, 6, and 8 days after blood feeding. The results showed that oviposition-site deprivation significantly increased fecundity with an increase in the number of egg retention days. The number of eggs laid by the female increased by 69% when the female was compelled to retain the eggs for 8 days. The highest recorded fecundity was 100 ± 5. Nevertheless, fertility, percentage larval mortality, total larval duration, pupal duration, and longevity were not affected by the number of egg retention days. Thus, it is imperative to have a clear awareness about the effect of oviposition-site deprivation on the reproductive performance of the vector mosquitoes when adopting vector control strategies.

**Keywords:** *Aedes aegypti,* oviposition-site deprivation, reproductive performance.

## INTRODUCTION

The first report, which was thought to be of a dengue epidemic, occurred in 1779 and 1780 in the three continents of Asia, Africa and North America (Hirsch, 1883). The disease was thought to be allied with an insect associated with water and the disease was called water poison by the Chinese people (McSherry, 1982). From 1780 to 1940, the dengue virus became endemic in urban tropical areas and World War II created ideal conditions for it to be converted into a global pandemic (Gubler, 1998). Dengue control measures used in many countries include source reduction through destruction of breeding places. However, an understanding of the reproductive performance and life history parameters is also essential eventually.

The virus is transmitted to humans through the bite of an infective mosquito. Vitarana *et al*. (1997) stated that *Aedes aegypti* is the primary vector while *Ae. albopictus* is the secondary vector of dengue in Sri Lanka. Early morning, 2 to 3 hours after daybreak and several hours before dusk are the peak biting hours of the vector (WHO, 2014a). During a single blood meal, they bite several people. Hence, it can transmit the virus to many people within a short period (Gubler & Rosen, 1976; Putnam & Scott, 1995; Platt *et al*., 1997). That is why *Ae. aegypti* is considered to be an efficient epidemic vector (Gubler, 1998). It is predicted that by the year 2070 the

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mean relative vectorial capacity of dengue fever transmission in Sri Lanka would increase (WHO, 2014b). Since *Ae. aegypti* is common in urban areas it prefers to breed in man-made containers which are commonly found in and around the houses (Gubler, 1998; WHO, 2014a). These include gutters, flower vases, old tires, buckets, water storage tanks, septic tanks etc. (Gubler, 1998). Kusumawathi and Fernando (2003) have stated that water storage tanks and barrels are the most productive breeding sites of *Ae. aegypti* in some areas of Sri Lanka. They attach the eggs to the walls of the containers just above the water level. *Ae. aegypti* females usually do not lay all the eggs in a single oviposition site. Instead, they deposit few eggs in several different oviposition sites (Harrington & Edman, 2001). This behaviour may increase the survival of the larvae and enable wider dispersal of the larvae. In addition, eggs of *Ae. aegypti* can remain viable for about 6 months without water in dry conditions. Usually, *Ae. aegypti* is an indoor breeder whereas *Ae. albopictus* is an outdoor breeder (Noordeen *et al*., 2018). Both the *Ae. aegypti* and *Ae. albopictus* can survive in natural and artificial systems with clean or organically rich water (Kusumawathie & Fernando, 2003).

Generally, dengue outbreaks occur in Sri Lanka twice a year, which are seasonal followed by the monsoonal rains. They usually occur during June/July or October to December and are correlated with the increase of vector density (Sirisena & Noordeen, 2014). Since high rainfall causes flooding leading to increased breeding, the vector population density increases (Noordeen *et al*., 2018).

Investigators have stated that female *Ae. albopictus* can retain mature eggs for a certain period until oviposition (Hitchcock, 1968). The egg retention and oviposition are affected by chemical and physical factors including visual, tactile, and olfactory responses (Bentley & Day, 1989; Dhileepan, 1997). Oviposition will be interrupted due to the lack of a suitable aquatic medium or due to oviposition deterrents (Xue *et al*., 2005). This forced egg retention influences the oviposition patterns (Chadee, 1997) and vitellogenesis (Else & Judson, 1972) of *Ae. aegypti*. Once the female takes a blood meal, oogenesis begins, and it is followed by vitellogenesis (Else & Judson, 1972). These processes are governed by the hormones secreted from the neurosecretory cells of the brain and corpora allata (Lea, 1970). The duration of egg retention plays an important role in the reproductive performance and life history parameters of the mosquito. Judson (1967) has found that *Ae. aegypti* females can complete two gonotrophic cycles when the oviposition is prevented. Moreover, the amount of blood taken for the initiation of the second gonotrophic cycle was reported to be higher than the usual amount of blood required (Judson, 1967). Later, Else and Judson (1972) found that oviposition-site deprivation has prevented the initiation of the second gonotrophic cycle in many females and has significantly delayed the initiation time of the second gonotrophic cycle in other female *Ae. aegypti*. However, the study conducted by Meola and Lea (1972) has concluded that with the retention of a batch of mature eggs, a second gonotrophic cycle would not take place in *Ae. aegypti* because of the complete inhibition of vitellogenesis.

During dry seasons and dry spells during wet seasons, the oviposition sites may not be available for several days and weeks. Therefore, once female mosquitoes become gravid during these dry periods, there will not be oviposition sites to lay eggs on time. As a result, the female *Aedes* may retain eggs for more extended periods than usual.

Since there is no effective vaccine or drug against dengue fever, alternative strategies against *Aedes* vectors have been widely implemented. As *Ae. aegypti* is a container breeder, controlling dengue vectors through larval source reduction has become the most common and widely practised method. Such anthropogenic practice will create a similar situation to dry weather, where gravid mosquitoes will not find oviposition sites in the natural environment. This may lead to retention of eggs and may influence the reproductive performance of female vectors.

Thus, it is hypothesized that oviposition site deprivation created by natural and human influenced factors may affect the reproductive performance and life-history parameters of *Ae. aegypti.* In the absence of such knowledge on *Ae. aegypti* from Sri Lanka, a laboratory study was designed. The objective of the study was to determine the effect of oviposition site deprivation up to 8 days after blood feeding on the fecundity, fertility, larval mortality, total larval duration, pupal duration, and longevity of *Ae. aegypti.*

## MATERIALS AND METHODS

### Test insects and place of study

The eggs of *Ae. aegypti* used for the study were donated from the parent colony which has been maintained for 2 years at the Faculty of Medicine, University of Colombo. A pure colony of adult *Ae. aegypti* was maintained throughout the study period, in the insectary at the Department of Zoology and Environmental Management of the University of Kelaniya (6º58'20.91" N; 79º54'52.83" E) under the same temperature (27 ± 2 ºC), relative humidity (75–80%), and photoperiod (12L : 12D) as provided for the parent colony at the University of Colombo.

### Rearing of mosquitoes

A plastic tray (23.0 × 18.0 × 5.0 cm) was taken, and the egg sheet was placed in it. Two-third of the tray was filled with water boiled up to 100 ºC and cooled to room temperature (Zheng *et al*., 2015). The tray was covered with a 0.5 mm mesh net to prevent oviposition by other mosquitoes in the surroundings. After 24 h, the first instar larvae that hatched from the eggs were counted carefully under a light source. Then, 100 larvae were transferred to a 2 L plastic tray. A total of 6 trays were prepared with 100 larvae per each. The first, second, third, and fourth instar larvae were fed with 5, 6, 7 and 8 mL of the liquid diet of krill respectively, twice daily. The water quality of the larval rearing medium was maintained by carefully siphoning the water from the trays and replacing with new aged tap water daily. Once the larvae pupate, they were transferred into 500 mL beakers which contained aged tap water. The beakers were kept inside mosquito rearing cages until the adults emerged. A 10% sugar solution was used to feed the adults regularly (Helinski & Harrington, 2011; Zheng *et al*., 2015). Human blood which was collected from the Central Blood Bank, Narahenpita was used to feed the female mosquitoes and they were starved for 24 hours before blood feeding. Blood-feeding was achieved using the membrane feeding technique (Owens, 1981).

### Effect of oviposition site deprivation on fecundity, fertility, life-history parameters and adult longevity of *Aedes aegypti*

Four experiments were carried out to assess the effect of oviposition site deprivation on below parameters.

(i) Fecundity
The number of eggs laid within 120 h (5 ds) after the females were allowed to oviposit, was counted under a low-power stereo microscope at 15X magnification.

(ii) Fertility
After oviposition, a piece of egg sheet with 100 eggs was taken and was placed on a 2 L tray filled with water boiled up to 100 ºC and cooled to room temperature. The number of first instar larvae that hatched out from the eggs after 24 h, were carefully counted under a light source and recorded. Another 24 h were spent in counting the number of 1st instar larvae.

(iii) Life-history parameters
After oviposition, the egg sheet was taken and was placed on a 2 L tray filled with water boiled up to 100 ºC and cooled to room temperature. Twenty-four hours later, 50 first instar larvae were counted and transferred to another 2 L tray. Larval mortality, total larval duration, and pupal duration were recorded.

(iv) Longevity
After oviposition, the 10 females were transferred to a separate mosquito rearing cage. They were regularly fed with a 10% sugar solution (Helinski & Harrington, 2011; Zheng *et al*., 2015). The average number of days that all the 10 females survived was recorded (Artis *et al*., 2014).

Each experiment was repeated 3 times.

For these experiments, two hundred female mosquitoes were allowed to mate and were blood-fed for 30 min. Fully engorged 50 females were collected and 10 mosquitoes each were separately allowed for oviposition on the day of blood-feeding, and 2, 4, 6, and 8 ds after blood feeding. Black colour cylindrical plastic cups (200 mL) with a diameter of 7.0 cm and depth of 6.0 cm were used to prepare the oviposition substrates. The cups were soaked in a water bath for 1 wk to avoid the plastic odour. A filter paper stripe of length 22.0 cm and width 5.0 cm was placed along the interior circumference of the plastic cup which acted as the substrate for egg-laying. One-third of the cup was filled with aged tap water (Figure1).

**Figure 1.** Oviposition substrate with a filter paper stripe (22×5 cm)

### Statistical analysis

Data obtained during the experiments were analysed using Minitab 18 software. All the data were tested for the Anderson-Darling normality test. As the data followed a normal distribution, one-way analysis of variance (ANOVA) was carried out to check whether there was a significant difference among the data obtained for the effect of oviposition site deprivation on the fecundity, fertility, and life history parameters of *Ae. aegypti* with the number of egg retention days. Tukey's test was used to test the differences among sample means for significance. Pearson correlation and regression analysis were applied to determine the functional relationship between the fecundity of *Ae. aegypti* mosquitoes and the number of egg retention days.

## RESULTS AND DISCUSSION

Although several studies have been conducted to determine the effect of oviposition site deprivation on some lepidopterans and dipterans, limited studies have been conducted to determine the effect of oviposition site deprivation on the reproductive performances, life history parameters, and longevity of adult *Ae. aegypti*. According to the current study, the mean number of eggs laid by the females which were subjected to oviposition site deprivation had varied significantly (one-way ANOVA, $F = 12.19$, $DF = 4$, $p < 0.05$). Tukey's test showed that the fecundity of the females which were subjected to 6-day and 8-day egg retention periods were significantly different from the rest. The highest fecundity ($100 \pm 5$) was observed once the females were subjected to an 8-day egg retention period and the lowest fecundity ($59 \pm 4$) was observed when the females were subjected to a 0 day egg retention period (Figure 2a). The mean number of eggs laid was positively correlated with the number of egg retention days within the female (Pearson's correlation, $p < 0.05$, $R^2 = 0.986$; Figure 2b).

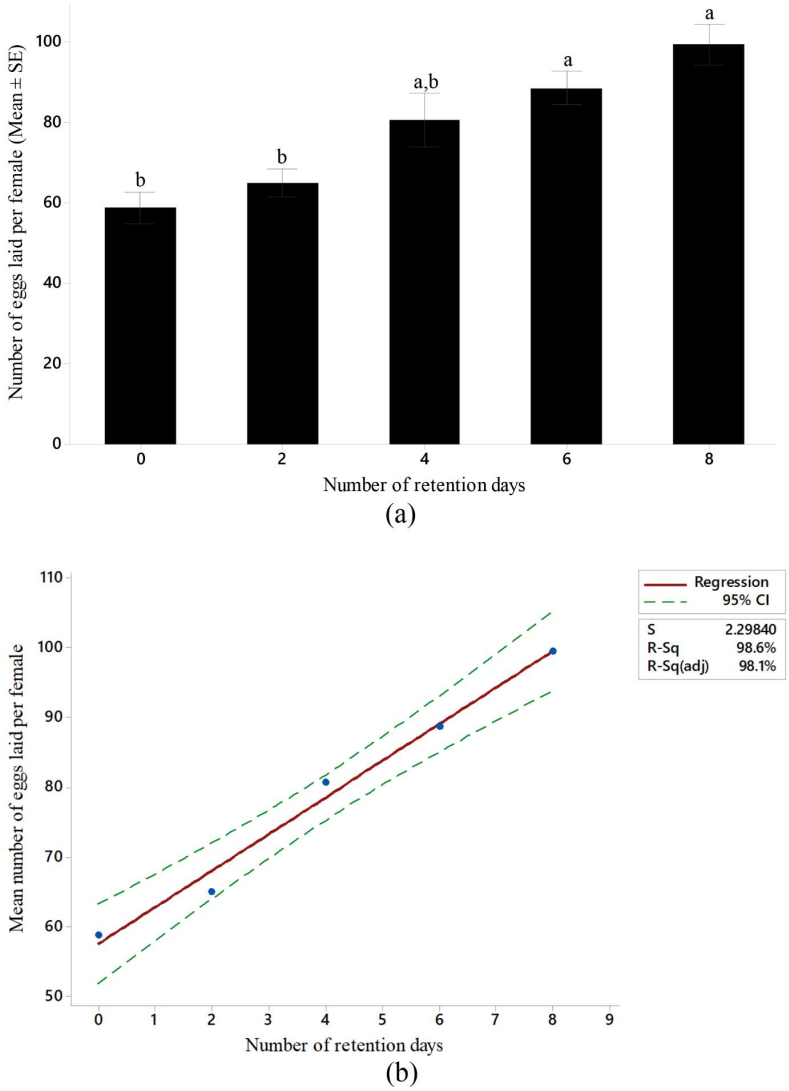


**Figure 2**: (a) Fecundity of female *Ae. aegypti* with number of egg retention days within the female. Bars with different letters are significantly different from each other ($p < 0.05$). (ANOVA, Tukey's test, $p < 0.05$). (b) Relationship between the fecundity of female *Ae. aegypti* with number of egg retention days within the female. (Pearson's correlation, $p < 0.05$)

*Ae. aegypti* is a container breeder. As such, the majority of the dengue elimination programmes are targeted at removing and destroying the breeding sites of the mosquitoes. Source reduction prevents or delays the oviposition of the females because of the limited number of available breeding sites. When the mosquitoes lack their preferred oviposition sites, they will be forced to retain eggs and lay them with a delay, in whatever the site available. However, the current study showed that there is an effect of oviposition site deprivation on fecundity. Fecundity increased by 69% compared to the 0 days, once the period of egg retention was increased by 8 days. Nevertheless, the exact physiology behind this phenomenon is unknown. It is assumed that the females utilize the food reserves that are available for general metabolism or reabsorb some egg rudiments and trigger a second gonotrophic cycle before the previous batch of eggs is laid. Some species such as *Anopheles pharoensis* (El-Akad & Humphreys, 1988) and *An. maculatus* (McDonald & Lu, 1972) respond to short-term oviposition site deprivation (2-5 days) while some other species like *Ae. albopictus* (Xue *et al*., 2005) and *Culex quinquefasciatus* (Yang, 2008) respond to long-term oviposition site deprivation (10–70 days). Dieter *et al*. (2012) stated that the effect of oviposition site deprivation is modified by the nutritional condition of the diet taken before the egg retention period. When the females of *An. gambiae* were given a supplemental blood meal after long-term oviposition site deprivation, the fecundity increased compared to the fecundity of the females

which were not subjected to oviposition site deprivation (Dieter *et al*., 2012). Therefore, in the wild condition mosquitoes may take another blood meal to increase their fecundity if they are subjected to oviposition site deprivation. This will lead to an increase in vector population. Ultimately the disease transmission intensity will also be increased due to the increased vector population.

Hatching rate of eggs represents the fertility of adult mosquitoes. The percentage of eggs hatched did not show any significant difference among the females which were subjected to different egg retention periods (one-way ANOVA, $F = 0.12$, $DF = 4$, $p > 0.05$; Table 1). The mean percentage of eggs hatched was 84.93 ± 0.47. In contrast, the study conducted by Govoetchan *et al*. (2013) has shown a significant decline in egg hatch rate with the increase of the duration of oviposition site deprivation for a maximum of 15 days in both the KISUMU strain and wild strain of *An. gambiae.* The hatching rate was not increased when the oviposition site deprived females were treated with supplemental insemination. In addition to the waning in the hatching rate, some of the eggs of *An. gambiae* failed to tan (Dieter *et al*., 2012). As all eggs tanned in the current study, there may not have been any effect of oviposition site deprivation on the tanning of *Ae. aegypti* eggs. Xue *et al*. (2005) stated that the number of eggs that failed to tan increased with the increase of duration of oviposition site deprivation. This may happen when the accumulation of L-tyrosine and other compounds necessary for the tanning of the chorion is prevented due to egg retention. When the eggs fail to fully tan within 6 to 8 hours after oviposition, the eggs may be exposed to dry conditions and they may collapse leading to a reduction in fertility (Clements, 1992). Yang (2008) has shown that both fecundity and fertility were high in wild-caught *Culex quinquefasciatus* females after 4 weeks of oviposition site deprivation.

Limited number of studies have been carried out to evaluate the effect of oviposition site deprivation or forced egg retention on larval mortality, total larval duration and pupal duration. The current study revealed that oviposition site deprivation has no impact on the life-history parameters of *Ae. aegypti*. The percentage larval mortality did not show a significant difference with the number of egg retention days within the female (one-way ANOVA, $F = 0.90$, $DF = 4$, $p > 0.05$; Table 1). The mean percentage larval mortality was 14.33 ± 1.16. The total larval duration was measured by the sum of the larval duration of first, second, third, and the fourth instar larvae. The total larval duration did not show a significant difference with the number of egg retention days within the female (one-way ANOVA, $F = 0.60$, $DF = 4$, $p > 0.05$; Table 1). The mean total larval duration was 105.6 ± 2.4 hours. The pupal duration did not display a significant difference with number of egg retention days within the female (one-way ANOVA, $F = 0.30$, $DF = 4$, $p > 0.05$; Table 1). The mean pupal duration was 41.60 ± 0.98 hours. Similarly, adult female mosquitoes which were subjected to the different number of egg retention days did not show any significant difference in longevity (one-way ANOVA, $F = 0.40$, $DF = 4$, $p > 0.05$; Table 1). The mean adult longevity was 19.40 ± 0.29 days.

**Table 1:** Effect of oviposition site deprivation on fertility (number of eggs hatched), percentage larval mortality, total larval duration, pupal duration and adult longevity of female *Aedes aegypti* with different number of egg retention days within the female: 0, 2, 4, 6 and 8 days.

| Parameter | Number of egg retention days within the female | | | | |
|---|---|---|---|---|---|
| | 0 days | 2 days | 4 days | 6 days | 8 days |
| Percentage of eggs hatched (Mean ± SE) | 86.0 ± 1.5 | 84.7 ± 2.7 | 85.7 ± 3.9 | 83.3 ± 2.9 | 85.0 ± 3.5 |
| Percentage larval mortality (Mean ± SE) | 10.3 ± 1.5 | 15.0 ± 3.2 | 16.7 ± 3.5 | 16.3 ± 3.0 | 13.3 ± 2.0 |
| Total larval duration in hours (Mean ± SE) | 100.0 ± 4.0 | 108.0 ± 6.9 | 100.0 ± 4.0 | 112.0 ± 4.0 | 108.0 ± 12.0 |
| Pupal duration in hours (Mean ± SE) | 40.0 ± 4.0 | 44.0 ± 4.0 | 44.0 ± 4.0 | 40.0 ± 4.0 | 40.0 ± 4.0 |
| Adult longevity in days (Mean ± SE) | 20.0 ± 1.2 | 18.3 ± 0.3 | 19.7 ± 0.7 | 19.7 ± 1.2 | 19.3 ± 1.3 |

Although Govoetchan *et al*. (2013) has shown that the longevity of the gravid females of *An. gambiae* was higher than that of non-gravid females, oviposition site deprivation has not increased the survival of *An. gambiae*. The study conducted by Artis *et al*. (2014) showed that longevity and blood-feeding rate of *An. gambiae* have declined leading to a reduction in vectorial capacity. However, when the oviposition site deprived females receive access to a supplemental blood meal their survival is increased (Artis *et al*., 2014).

Vector density is an important component of vectorial capacity (Dye, 1992). Climate, longevity, and fecundity of the females are the factors that determine the vector density (De Jesus & Reiskind, 2016). According to Ponlawat and Harrington (2009) vector control is the most successful way to control dengue and dengue haemorrhagic fever. Thus, it is important to know the reproductive biology and life history parameters of the vector mosquito.

The current study provides valuable information useful for planning vector control programmes. Dieter *et al*. (2012) has stated that vector control through source reduction would interrupt the oviposition of females and suppress the vector population. Consequently, source reduction leads to oviposition site deprivation due to the removal of breeding sites. In addition to source reduction, dry spells during wet seasons leads to the same. Therefore, gravid mosquitoes will retain the eggs due to the lack of breeding sites to lay them. The incidence of egg retention will significantly increase the fecundity of the females. Ultimately, increase in fecundity may lead to an increase in larval population that may contribute to an increase in the adult vector population. Thus, source reduction alone may not be an effective way to control vector population of *Ae. aegypti*. Hence, a focus on the early control of adult mosquitoes in control programmes is important to prevent large numbers of eggs being released to the environment.

Also, the current study revealed that the highest fecundity of the mosquitoes could occur when the mosquitoes were forced to retain eggs for 8 days. This knowledge can be applied in mass-rearing of mosquitoes to be used in the sterile insect technique. This is because the production of mosquitoes can be increased when the adults are allowed to oviposit at the most suitable age which will increase their fitness.

## CONCLUSION

The present study indicates that delaying the oviposition of *Ae. aegypti* increases the fecundity of the female. However, parameters including fertility, percentage larval mortality, total larval duration, pupal duration, and longevity are not significantly affected. Early control of adult mosquitoes is important in the control programmes in view of the findings of the study. Furthermore, the study provides information on reproductive performance that is useful in mass rearing of mosquitos, which is an essential procedure in sterile insect release and other methods currently experimented in Sri Lanka.

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