INVESTIGATION OF THE SKIP-OVIPOSITION BEHAVIOUR OF *Aedes aegypti* AND *Ae. albopictus* IN A LABORATORY SETTING

A. P. S. Perera[1,2*], K. O. Bandaranayaka[1], M.D.S. Janaki[2] and H. T. R. Jayasooriya[1]

[1]*Department of Zoology, The Open University of Sri Lanka, Sri Lanka*
[2]*National Dengue Control Unit, Sri Lanka*

In Sri Lanka, dengue fever is currently considered as the main vector-borne illness. The lack of a vaccine or other effective treatment has made it important to control the vectors. Research on behaviour of *Aedes aegypti* and *Ae. albopictus*, the dengue vectors, is crucial for monitoring and controlling the disease outbreaks. Skip-oviposition behavior is characterized by the dispersal of eggs among multiple containers within a single gonotrophic cycle exhibited by a number of container breeding *Aedes* species. It is known as a behavioural adaptation among females to increase the survivability of the eggs from adverse conditions in breeding sites. This study examines the skip-oviposition patterns of *Ae. albopictus* and *Ae. aegypti* in a controlled laboratory environment. Laboratory reared three males and a fully engorged female *Ae. aegypti* and *Ae. albopictus* were placed in two separate, rearing cages measuring 47.5 cm x 47.5 cm x 47.5 cm in size. Each cage had five black ovitraps lined by white fabric ovitrap strips, filled up to two-thirds with tap water, along with 10% sucrose solution for adults. After six days, ovitrap strips were collected and were observed for the number of eggs laid by each species for each ovitrap separately. For each species, five replicates were carried out in order to compare the behaviour among the two species. Both *Ae. aegypti and Ae. albopictus* exhibited skip-oviposition behavior. The mean number of eggs laid in ovitraps were not significantly different between the two species (Kruskal Wallis test; H=0.02, P=0.882). However, the distribution of *Ae. aegypti* and *Ae. albopictus* eggs was not uniform across all oviposition traps. Both *Ae. aegypti* and *Ae. albopictus* oviposited more than 50% of their eggs in a single oviposition cup. Therefore, they are more likely to choose one ovitrap to lay more eggs than other ovitrap. Semi-field studies with more replicates are recommended to fully understand the skip-oviposition behaviour to further define effective monitoring of vector density.

Keywords: *Aedes aegypti*, *Aedes albopictus*, Skip-oviposition Behaviour, Dengue Vector

[*]*Corresponding Author:* paramikaperera90@gmail.com

INVESTIGATION OF THE SKIP-OVIPOSITION BEHAVIOUR OF *Aedes aegypti* AND *Ae. albopictus* IN A LABORATORY SETTING

A. P. S. Perera[1,2*], K. O. Bandaranayaka[1], M.D.S. Janaki[2] and H. T. R. Jayasooriya[1]

[1]Department of Zoology, The Open University of Sri Lanka, Sri Lanka
[2]National Dengue Control Unit, Sri Lanka

INTRODUCTION

Aedes aegypti is recognized as the primary vector of dengue in the Asian region and *Ae. albopictus* is identified as the secondary vector of dengue, playing a crucial role in the persistence of the virus (Dalpadado *et al.*, 2022). Dengue is currently considered a primary vector-borne disease in Sri Lanka. Moreover, dengue and dengue haemorrhagic fever have become essential health issues, mainly due to the absence of a vaccine or any other promising drug (National Dengue Control Unit, 2019). As a result, research on behaviours of *Ae.aegypti* and *Ae.albopictus*, the dengue vectors, is crucial for monitoring and controlling of the disease.

A female mosquito has the ability to distribute her eggs to multiple oviposition sites within a single gonotrophic cycle. This phenomenon is generally known as skip-oviposition (Day, 2016).

Skip-oviposition is a strategy employed by mosquitoes to mitigate resource competition among larvae. By dispersing their eggs, *Aedes* mosquitoes decrease the likelihood of all larvae hatching in a single location, potentially resulting in insufficient food availability or increased vulnerability to predators (Reiter, 2007). Consequently, this phenomenon has an impact on the population density of mosquitoes (Reinbold *et al.*, 2021). However, the phenomenon of oviposit skipping still needs to be investigated, despite its significant role in the biology of *Aedes* vectors (Reinbold *et al.*, 2021). Therefore, this study was done to investigate the skip-oviposition behaviour of *Ae. aegypti* and *Ae. albopictus* and compare their skip-oviposition patterns under controlled laboratory circumstances.

METHODOLOGY

Samples of *Aedes* eggs collected from five oviposition traps (05) which were kept in Battaramulla and CMC D4 MOH areas were used for this study. The collected ovitrap strips were kept in larval trays with tap water for hatching under controlled laboratory conditions (25±2 °C, 65±5% RH). Larval food was added to the tray facilitating the larval feeding. L3 stages of *Ae. aegypti* and *Ae. albopictus* were identified using morphological identification key (Rueda, 2004). Identified *Ae. Aegypti* and *Ae. albopictus* larvae were reared separately and the emerging adults from pupae were placed in separate mosquito-rearing cages (30 cm x 30 cm x 30 cm). A sucrose solution (10%) was used to provide the necessary energy to the adult mosquitoes. After two days female mosquitoes in each cage were fed with human blood supplied from the National Blood Bank, by using a Hemotek membrane feeder. The blood fed female mosquitoes of both species were collected separately with a mouth aspirator. They were placed inside a plastic cup covered in a small mesh net. A fully engorged female mosquito from each species were transferred into a large mosquito-rearing cage (47.5 cm x 47.5cm x 47.5 cm) with three males from the same species. Then, five black plastic ovitrap cups (diameter of 11 cm and height of 9 cm) with the ovitrap substrate (ovitrap strip) made from white fabric were placed inside the mosquito rearing cage. After that, the ovitrap cups were filled with two-thirds tap water. A sucrose solution (10%) was provided for the mosquitos for their nourishment. Two mosquito-rearing cages were kept under controlled laboratory conditions (25±2°C, 65±5% RH) and the oviposition was observed. The ovitrap strips were collected separately after six days, and the number of eggs were counted. Five trials were conducted during the study period.

RESULTS AND DISCUSSION

Mean Number of eggs laid by the two *Aedes* species in ovitraps were not significantly different (Kruskal Wallis test; H = 0.02, P = 0.882) (Table 1). Replicates indicated that *Ae. aegypti* and *Ae. albopictus* females were involved in skip-oviposition behaviour. Characteristically each female mosquito had laid 50% or more of their eggs in one ovitrap out of five ovitraps in the cages (Table 2).

Table 1. Comparison of skip-oviposition behaviour of *Ae. aegypti* and *Ae. albopictus*

	Ae. Aegypti		*Ae. albopictus*	
Replicate	No of cups positive/ no of cups placed	Mean no of eggs per ovitrap ± SE	No of cups positive/ no of cups placed	Mean no of eggs per ovitrap ± SE
1	4/5	14.6±6.6	4/5	13.6±6.6
2	3/5	9.8±6.7	3/5	9.4±6.0
3	3/5	12.4±7.8	3/5	12.2±8.6
4	3/5	11.8±8.1	4/5	13.4±5.8
5	3/5	12.4±8.0	3/5	11.0±6.8

SE – Standard Error of mean

Table 2. Skip- oviposition data and favourite cup percentage of *Ae. aegypti* and *Ae. albopictus*

Ae. aegypti	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Ovitrap cup 1	21	0	6	12	0
Ovitrap cup 2	37	0	0	4	6
Ovitrap cup 3	0	35	14	43	0
Ovitrap cup 4	8	12	42	0	43
Ovitrap cup 5	7	2	0	0	13
Ovitraps with *Ae. aegypti* eggs	**4**	**3**	**3**	**3**	**3**
Number of ovitrap used	5	5	5	5	5
Favorite ovitrap percentage %	50.6	71.4	67.7	72.9	69.4

Ae. albopictus	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Ovitrap cup 1	6	0	46	11	7
Ovitrap cup 2	9	0	7	35	37
Ovitrap cup 3	0	11	8	8	0
Ovitrap cup 4	15	32	0	0	11
Ovitrap cup 5	38	4	0	13	0
Ovitraps with *Ae. albopictus* eggs	**4**	**3**	**3**	**4**	**3**
Number of ovitrap used	5	5	5	5	5
Favorite ovitrap percentage %	55.9	68.1	75.4	52.2	67.2

DISCUSSION

The skip-oviposition behaviour of *Aedes* mosquitoes is a crucial determinant that might influence the dispersal (Edman *et al.*, 1998) and, consequently, the survival, longevity, and flight range of this mosquito. Understanding oviposition behaviour can enhance monitoring and management of this significant disease carrier (Nazni *et al.*, 2016). According to the previous studies both *Ae. aegypti* and *Ae. albopictus* displayed skip-oviposition behaviour (Reinbold-Wasson *et al.*, 2021). Even though the current study reports a similar behaviour of skip-oviposition there was no difference in egg-spreading behaviour (skip-oviposition) between the two species considered *Ae. aegypti* and *Ae. albopictus* (H= 0.02, P=0.882). Moreover, it was observed that both the species used a similar quantity of oviposition cups, as reported by Reinbold-Wasson *et al.*, (2021). The studies indicated that the distribution of *Ae. aegypti* and *Ae. albopictus* eggs was not uniform across all oviposition traps. They are likely to choose one cup to lay more eggs than other cups. During the study, both *Ae. aegypti* and *Ae. albopictus* oviposited more than 50% of their eggs in a single oviposition cup. A similar observation was made by de Abreu *et al.*, (2015) where the *Aedes* female oviposited at least 40% of their eggs in one preferred oviposition cup when they were in an environment with multiple identical oviposition cups. This may be due to the fact that both *Aedes* vector mosquitoes prefer to oviposit one cup when there are more cups in the system. Aberu *et al.*, (2015) identified this as the preferred cup factor of *Aedes* mosquitoes.

Recommendations and Conclusion

Both *Aedes* species exhibit skip-oviposition behaviour and the number of eggs laid by the two *Aedes* species in ovitraps did not reveal a significant difference. However, they do not oviposit in the same manner in all ovitraps. They have a strong preference for a specific ovitrap and lay 50% or more of their eggs in that particular container. Nevertheless, the study's final conclusions would have been more precise if it had been carried out in semi-field circumstances with a greater number of containers. Hence, further research in semi-field situations with additional *Aedes* vector containers is recommended to be carried out in future.

References

Abreu, F. V. S. D., Morais, M. M., Ribeiro, S. P., & Eiras, Á. E. (2015). Influence of breeding site availability on the oviposition behaviour of *Aedes aegypti*. *Memórias do Instituto Oswaldo Cruz*, *110*, 669-676.

Dalpadado, R., Amarasinghe, D., Gunathilaka, N., & Ariyarathna, N. (2022). Bionomic aspects of dengue vectors *Aedes aegypti* and *Aedes albopictus* at domestic settings in urban, suburban and rural areas in Gampaha District, Western Province of Sri Lanka. *Parasites & Vectors*, *15*(1), 148.

Day, J. F. (2016). Mosquito oviposition behavior and vector control. Insects, 7(4), 65.

Edman, J. D., Scott, T. W., Costero, A., Morrison, A. C., Harrington, L. C., & Clark, G. G. (1998). *Aedes aegypti* (Diptera: Culicidae) movement influenced by availability of oviposition sites. *Journal of Medical Entomology*, *35*(4), 578-583.

National Dengue Control Unit, Ministry of Health. (2019). *Sri Lankan situation.* https://www.dengue.health.gov.lk/web/index.php/en/information/sri-lanka-situation (Accessed on 29/03/2024).

Nazni, W. A., Bandara, M. R. S. S., Azahari, A. H., Craig, R. W., & Lee, H. L. (2016). Skip oviposition behavior of laboratory, field and transgenic strain of *Aedes Aegypti* (L.). *Southeast Asian J. Trop. Med. Public Health*, *47*, 680-690.

Reinbold-Wasson, D. D., & Reiskind, M. H. (2021). Comparative skip-oviposition behavior among container breeding *Aedes* spp. mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, *58*(6), 2091-2100.

Reiter, P. (2007). Oviposition, dispersal, and survival in *Aedes aegypti*: implications for the efficacy of control strategies. *Vector-Borne and Zoonotic Diseases*, *7*(2), 261-273.

Rueda, L. M. (2004). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. *Zootaxa*, 589(1), 1-60.