**JOURNAL OF SUSTAINABLE NATURAL RESOURCES**
e ISSN: 2716 7143

**J-SuNR**

Vol. 5 No. 2 (2024) 24-30
https://publisher.uthm.edu.my/ojs/index.php/jsunr

# An In-depth Analysis of the Contemporary Biological Characteristics of *Aedes* Mosquitoes in Laboratory Conditions

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DOI: https://doi.org/10.30880/jsunr.2024.05.02.004

**Article Info**

Received: 21 August 2024
Accepted: 27 November 2024
Available online: 17 December 2024

**Keywords**

*Aedes aegypti*, *Aedes albopictus*, arbovírus, biological dynamics, laboratory conditions

**Abstract**

*Aedes aegypti* and *Aedes albopictus* are recognized vectors of various arboviruses, including dengue, Zika, and chikungunya, contributing significantly to global public health challenges. The investigation aimed to clarify essential aspects of the biological dynamics of both *Aedes* species under standardized laboratory conditions. This included developmental duration, adult lifespan, gonotrophic cycle, and morphometric parameters across various life stages. Findings revealed that the lifecycle completion of *Ae. aegypti* and *Ae. albopictus* in laboratory settings averaged approximately 8.75 days and 10.35 days, respectively, with no statistically significant difference observed between the two species. Intriguingly, a notable difference in longevity was observed between male and female mosquitoes, with females showing significantly longer lifespans ($P<0.05$). Specifically, male *Ae. aegypti* demonstrated a mean survival time of 24 days, contrasting with females' longevity of 33.80 days. Similarly, male *Ae. albopictus* displayed a mean lifespan of 16.60 days, while females persisted for approximately 21.40 days. Morphometric analyses revealed subtle yet noteworthy differences, with *Ae. aegypti* exhibiting slightly larger egg dimensions (0.567 mm) compared to *Ae. albopictus* (0.526 mm). Similarly, pupal cephalothorax measurements revealed a slight difference, with *Ae. aegypti* measuring 2.021 mm and *Ae. albopictus* measuring 1.928 mm. In conclusion, these findings provide valuable insights into the intricate biology of *Aedes* mosquitoes, thereby facilitating the development of targeted strategies for disease control and mitigation. Such endeavors are crucial in the ongoing battle against arboviral diseases and underscore the significance of rigorous scientific inquiry in public health initiatives.

## 1. Introduction

*Aedes,* a genus encompassing more than 950 mosquito species within the Diptera order, comprises significant disease vectors and nuisance biters capable of transmitting deadly pathogens to humans and animals. These mosquitoes are native to various temperate and tropical regions worldwide but have expanded their range due to human-mediated introductions and environmental changes [1]. It is known that *Aedes aegypti* and *Aedes albopictus*, originally from Africa and Asia, respectively, have facilitated the spread of debilitating infectious diseases such as chikungunya, dengue, and Zika fevers. The mosquito *Ae. aegypti,* commonly known as the primary urban vector for dengue viruses worldwide, plays a significant role in the transmission of these diseases.

Meanwhile, *Ae. albopictus,* an ecologically versatile species, has recently been reported as more abundant in human-dominated urban and suburban areas [2] compared to previous years. It prefers highly vegetated urban environments over rural areas [3]. Despite being associated with tropical climates, historical and contemporary records indicate its persistence beyond these regions. *Aedes* spp. exhibits a strong affinity for human habitats, often entering buildings to feed and rest [4]. Unlike many other mosquito species, *Aedes* primarily feeds on humans and exhibits day biting behaviour, often seeking multiple hosts during a single reproductive cycle.

Dengue, primarily considered an urban malady, is showing an annual increase in prevalence [5]. In Malaysia, both *Ae. aegypti* and *Ae. albopictus* serve as dengue vectors, thriving near human settlements [6]. These mosquitoes breed in both natural and artificial water containers, with *Ae. aegypti* favouring indoor receptacles and *Ae. albopictus* favouring outdoor habitats. The larvae of *Aedes* mosquitoes require stagnant water for their development, making any standing body of water a potential breeding ground [7]. The productivity of breeding sites is influenced by water quality factors. Higher mosquito yields are observed in poorly circulated, warmer water bodies with higher organic content [8]. However, current reports on the shifting behaviour of *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus* pose a significant concern for the potential outbreak of dengue fever [9]. The current scenario involving *Aedes* spp. raises alarms, as it suggests a heightened risk of dengue transmission in densely populated regions where human mosquito interactions are frequent. Additionally, urbanization and climate change further exacerbate the situation by creating favourable habitats for these mosquitoes. This can potentially intensify the transmission dynamics of dengue fever, necessitating proactive surveillance and control measures to mitigate the risk of outbreaks [9].

Regular monitoring of *Aedes* mosquito biology is crucial for effective disease surveillance and control strategies. By understanding the population dynamics, distribution patterns, and behavioural adaptations of *Aedes* mosquitoes, authorities can anticipate and respond to potential outbreaks of diseases like dengue fever more effectively. Continuous surveillance enables the early detection of changes in mosquito abundance and behaviour, facilitating the timely implementation of targeted interventions such as insecticide spraying or community-based mosquito control measures. Moreover, monitoring *Aedes* biology provides valuable data for research on vector ecology, helping scientists develop more efficient control methods and strategies to mitigate the transmission of mosquito-borne diseases in the long term. Regular monitoring of *Aedes* mosquito biology is essential for proactive public health initiatives aimed at reducing the impact of dengue and other mosquito-borne diseases.

## 2. Methodology

### 2.1 Immature Development Times and Adult Emergence

A total of 800 laboratory strain mosquito eggs, freshly laid and less than one week old, were sourced from the Vector Control Research Unit (VCRU) at the School of Biological Sciences, Universiti Sains Malaysia, for this study. The eggs were divided into four batches of 200 each and immersed in dechlorinated water for 24 h to promote hatching. The complete development time of the immature stages (instars 1-4 and pupa) was observed and recorded at 8 h intervals (3 P.M., 11 P.M., and 7 A.M.). At each interval, the temperature was measured, and the average was calculated for analysis. During this period, larvae were fed a powdered food mixture composed of dried cow liver, dog biscuits, powdered milk, vitamin B complex, and yeast. Upon reaching the pupal stage, the insects were transferred to small laboratory glass bottles, which were then placed in a cage. The pupation rate was determined by counting the number of emerged adult mosquitoes and the remaining pupal cases in the glass bottles.

### 2.2 Lifespan of Adult *Aedes aegypti* and *Aedes albopictus*

The emerged adult mosquitoes were transferred using an aspirator into plastic cups covered with nets, with each cup containing 10 individuals of the same species and sex. For each sex, five replicates were prepared. The mosquitoes were continuously provided with a 10% sucrose solution delivered through a cotton ball, which was replenished daily. Observations and mortality recordings were conducted every 24 h to measure longevity or lifespan.

### 2.3 Egg, Larvae, Pupae, and Adult *Aedes* Mosquito Sizes

For size measurements, the specimens were immobilized by chilling at 5°C for 30 min. They were then observed under a light microscope at 4x and 10x magnifications to ensure accurate morphological measurements. For the eggs, their lengths were measured. Larvae were measured from the head to the last segment of the body, while for pupae, the length of the cephalothorax was recorded. Adult measurements involved determining the body length from the head to the last segment of the abdomen. Images of the specimens were captured and recorded using an image documentation system, OLYMPUS cellSens Standard Software, and under OLYMPUS SZX16-CCD microscope.

## 3. Result and Discussion

### 3.1 Immature Development Time and Life Span of *Aedes*

Table 1 presents a comparison of the developmental times for the immature stages of *Ae. aegypti* and *Ae. albopictus* under laboratory conditions, covering larvae (L1-L4), pupae, and adults. The larvae of *Ae. aegypti* took approximately 2.98±0.41 days to progress from L1 to L4, whereas *Ae. albopictus* required a longer developmental period of about 3.73±0.21 days. This trend was consistent across all stages, with *Ae. albopictus* taking an overall development time of 10.35±0.79 days from L1 to adulthood, compared to 8.75±0.44 days for *Ae. aegypti*. The comparison indicates that *Ae. albopictus* generally requires more time to develop from the L1 stage to adulthood than *Ae. aegypti*. An independent samples t-test was conducted to compare the mean development times between the two species. The analysis yielded a t-value of 1.68 ($P>0.05$), indicating no statistically significant difference between the species.

**Table 1** *Development time of Aedes mosquitoes from larvae to adult*

| Stages | Development time in days (mean±SE) | |
|---|---|---|
| | *Aedes aegypti* | *Aedes albopictus* |
| L1-L2 | 1.55±0.17 | 1.75±0.09 |
| L3 | 1.10±0.19 | 1.20±0.22 |
| L4 | 2.98±0.41 | 3.73±0.21 |
| Pupa | 3.00±0.17 | 4.43±0.56 |
| L1- adult | 8.75±0.44 | 10.35±0.79 |

In a study conducted by Rozilawati in 2016 [10], it was found that *Aedes* mosquitoes from two different locations, Kuala Lumpur and Selangor, took a longer time 11 days to complete their lifecycle from the L1 larval stage to adult emergence. This variation could be attributed to several factors, including temperature, food availability, and larval density [11][12]. In the current study, the average recorded temperature was 29°C, which is considered ideal for the development of *Aedes* larvae. Whilst, at 39°C, embryonic development is inhibited, leading to larval death just hours after hatching [13]. Ezeakacha's study in 2019 [14] demonstrated that temperature influences the life-history traits of developing larvae, particularly under competitive interactions, and that discrepancies between larval rearing and adult habitat temperatures can impact adult fitness. This discovery is essential for understanding how different life-history stages of *Ae. albopictus* and other disease vectors might adjust to changing climate conditions with optimal temperatures accelerating insect development. This faster development at favorable temperatures may enhance their potential as arbovirus vectors [15].

Table 2 compares the fecundity and survival rates of *Ae. aegypti* and *Ae. albopictus* during their immature stages under laboratory conditions. A total of 800 eggs were hatched for both species, however *Ae. albopictus* exhibited a higher initial survival rate, with 92.1% of its eggs progressing to the larval stage, compared to 84.9% for *Ae. aegypti*. At the larval stage (L1-L4), *Ae. albopictus* maintained a stronger survival rate, producing 737 larvae, while *Ae. aegypti* had 679. This trend continued into the pupal stage, where *Ae. albopictus* achieved a 98.4% survival rate, resulting in 725 pupae, whereas *Ae. aegypti* had a slightly lower survival rate of 97.5%, yielding 662 pupae. In the final stage, from pupa to adult, *Ae. albopictus* once again outperformed *Ae. aegypti*, with 89.8% of its pupae emerging as adults, totaling 718 individuals, compared to 81.8% for *Ae. aegypti*, which resulted in 654 adults. In summary, *Ae. albopictus* consistently showed higher survival rates across all developmental stages, suggesting a more robust development process under the same laboratory conditions. Studies indicate that factors such as temperature, diet, and density significantly influence the development rate and survival of mosquito species [16].

Table 3 compares the average lifespan of male and female mosquitoes from two species, *Ae. aegypti* and *Ae. albopictus*. Overall, males from both species exhibited shorter lifespans compared to their female counterparts. Specifically, the male *Ae. aegypti* has an average lifespan of 24.00±2.10 days, while the male *Ae. albopictus* lives for 16.60±0.98 days. On the other hand, the female *Ae. aegypti* has a longer lifespan of 33.80±0.86 days, compared to 21.40±0.40 days for the female *Ae. albopictus*. The data reveals significant differences in lifespan between the sexes within each species, as well as between the two species overall. Female mosquitoes of both species outlive their male counterparts. The results of the t-test analysis confirm that these differences in lifespan between males and females within each species, as well as between the two species, are statistically significant ($P<0.05$). Notably, female *Ae. aegypti* live nearly 10 days longer than males, and female *Ae. albopictus* outlive males by approximately

5 days. These differences could be that male mosquitoes tend to expend more energy during mating activities, which may reduce their lifespan. In contrast, females may utilize energy more efficiently due to their blood-feeding behavior, which sustains them for a longer period [17].

**Table 2** *Fecundity and survival rate of Aedes immature stages*

| Mosquito | Stage | Total | Survival Rate (%) |
|---|---|---|---|
| *Aedes aegypti* | Eggs | 800 | 84.9 |
| | Larvae (L1-L4) | 679 | 97.5 |
| | Pupae | 662 | 81.8 |
| | Adults | 654 | |
| *Aedes albopictus* | Eggs | 800 | 92.1 |
| | Larvae (L1-L4) | 737 | 98.4 |
| | Pupae | 725 | 89.8 |
| | Adults | 718 | |

Additionally, environmental factors such as temperature and humidity significantly impact mosquito lifespan, which may explain the observed differences between *Ae. aegypti* and *Ae. albopictus*. A study conducted by Delatte et al. in 2009 [17] found that the average lifespan at 25°C was 18 days for males and 30 days for females. At all observed temperatures, females consistently outlived males, a finding supported by other studies using constant temperatures [18]. The longer lifespan of female mosquitoes is likely attributed to biological and reproductive factors. Females require a longer lifespan to secure a blood meal, lay eggs, and ensure the survival of the species. Since males do not require as much time to mate, their lifespan is generally shorter. This observation aligns with a study by Cui et al. in 2021 [19], which also found that female mosquitoes generally live longer than males across various species, primarily due to their reproductive needs.

**Table 3** *Comparison lifespan between male and female of Aedes mosquitoes*

| | Male (M) | Female (F) | T-test between (M) and (F) | T-test between sex-species |
|---|---|---|---|---|
| *Aedes aegypti* | 24.00±2.10 | 33.80±0.86 | F = 3.38; t=-4.32; df=8; *P*= 0.003 | F = 2.61; t=-3.20; df=8; *P*= 0.013 |
| *Aedes albopictus* | 16.60±0.98 | 21.40±0.40 | F=3.24; t=-4.54; df=8 *P*=0.002 | F = 2.06; t=-13.07; df=8; *P*= 0.000 |

## 3.2 Egg, Larvae, Pupae, and Adult *Aedes* Mosquito Sizes

The *Aedes* mosquito undergoes distinct developmental stages, each with unique characteristics. The eggs are dark, oval-shaped, and boat-like in appearance. Larvae have elongated, segmented bodies with a prominent head, while pupae are comma-shaped with a well-defined cephalothorax. Adults are slender, featuring long legs and scaled wings (Fig 1).

Figure 2 shows that the mean egg lengths of both species are similar, with *Ae. aegypti* eggs measuring 0.567 mm and *Ae. albopictus* eggs measuring 0.526 mm. Upon hatching, the larvae of both species, initially transparent at the L1 stage, gradually darken as they grow. The larval size increases with each successive molt, progressing through four instars. In *Ae. aegypti*, the larval length increases from 1.325± 0.02 mm at L1 to 7.558±0.07 mm by L4. Similarly, in *Ae. albopictus*, the larval length ranges from 1.338±0.03 mm at L1 to 6.985±0.08 mm by L4. The cephalothorax of *Ae. aegypti* pupa measures 2.021±0.030 mm, slightly larger than that of *Ae. albopictus* which measures 1.928± 0.024 mm. Additionally, adult females of both species were observed to be significantly larger than their male counterparts ($P$ <0.05).

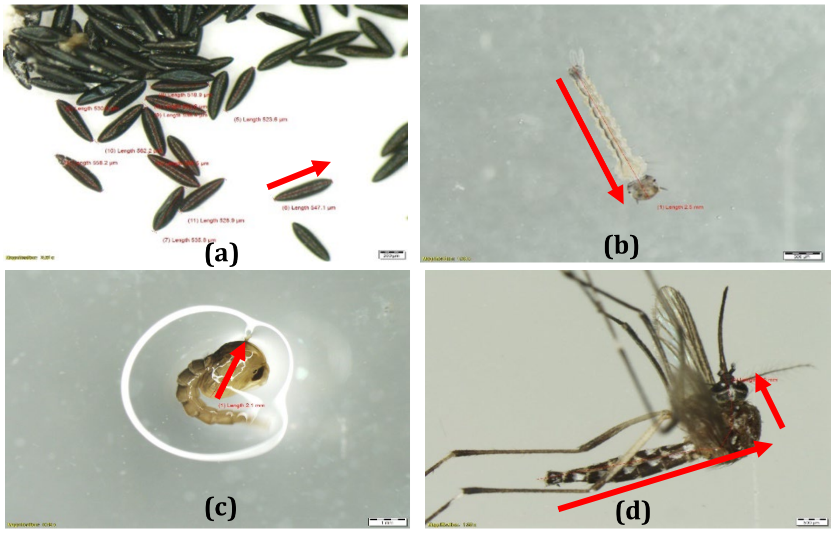
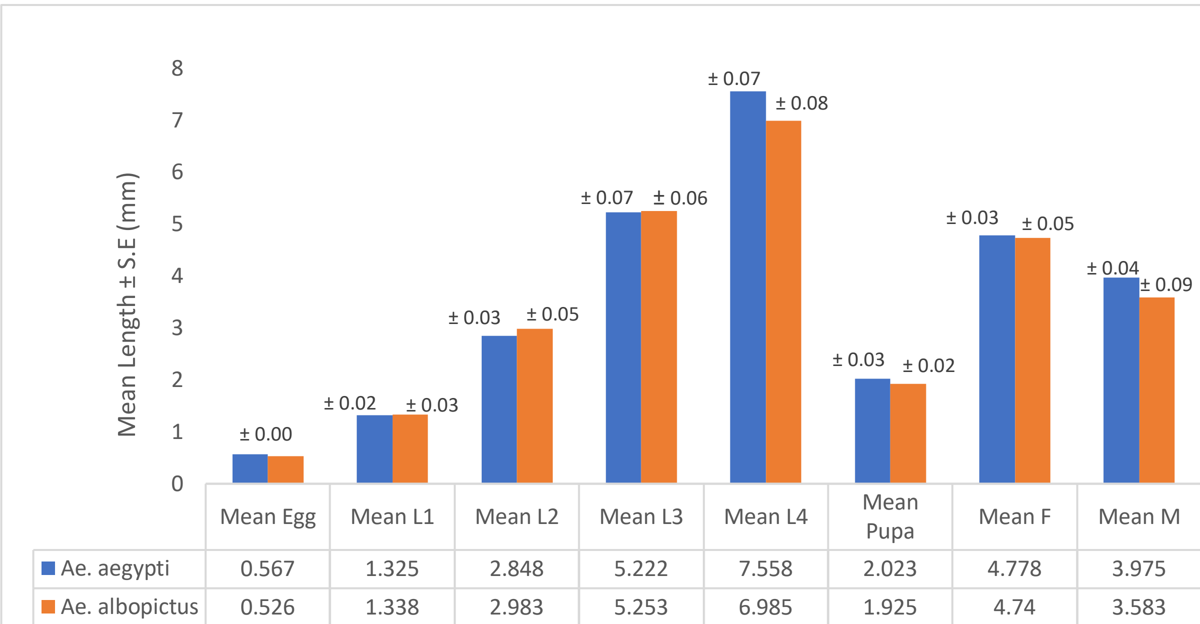



**Fig. 1** *Morphological characteristic and measurements of Aedes mosquito across developmental stages (a) Eggs; (b) Larva; (c) Pupa; (d) Adult*




| | Mean Egg | Mean L1 | Mean L2 | Mean L3 | Mean L4 | Mean Pupa | Mean F | Mean M |
|---|---|---|---|---|---|---|---|---|
| Ae. aegypti | 0.567 | 1.325 | 2.848 | 5.222 | 7.558 | 2.023 | 4.778 | 3.975 |
| Ae. albopictus | 0.526 | 1.338 | 2.983 | 5.253 | 6.985 | 1.925 | 4.74 | 3.583 |

**Fig. 2** *Comparative measurements of Aedes mosquitoes across developmental stages*

The slight size difference between *Ae. albopictus* and *Ae. aegypti* can be attributed to several interrelated factors. A significant factor is genetic variation. *Aedes albopictus* possesses a slightly larger genome than *Ae. aegypti*, which may influence physical characteristics, including size [20]. Over time, this genetic variation can lead to noticeable differences in their morphology. Additionally, *Ae. albopictus* is renowned for its ecological plasticity, allowing it to adapt to a wide range of environmental conditions. This adaptability likely provides a competitive advantage in terms of growth and development, contributing to its larger size. Furthermore, *Ae. albopictus* exhibits strong competitive abilities, enabling it to thrive in diverse environments and access resources more effectively, which supports its more substantial growth. Nevertheless, the temperature of the environment, food availability, and habitat conditions also play crucial roles in determining the size of these species [21][22]. These factors interact in complex ways, resulting in the observed size differences between the two mosquito species.

## 4. Conclusion

Understanding the developmental timelines of *Ae. aegypti* and *Ae. albopictus* is crucial for understanding their roles as disease vectors. Although statistical analysis revealed no significant differences in developmental stages between the species, the extended developmental period of *Ae. albopictus* may enable it to synchronize population growth and seasonal abundance with *Ae. aegypti*, potentially supporting continuous virus transmission within the ecosystem. This complementary timing could be key to maintaining viral loads. Future research should include long-term monitoring of mosquito populations, environmental impact assessments, and genetic studies to explore underlying mechanisms. Integrating these insights into vector management strategies and public health programs could significantly enhance disease prevention efforts.

## Acknowledgement

Findings of this research is made possible through the facilitations by VCRU, School of Biological Sciences, Universiti Sains Malaysia.

## Conflict of Interest

There is no conflict of interest regarding the publication of the paper.

## Author Contribution

*The authors confirm contribution to the paper as follows:* ***study conception and design:*** *Azlinda Abu Bakar ;* ***data collection:*** *Muhammad Nur Hilmi Zainudin;* ***analysis and interpretation of results:*** *Azlinda Abu Bakar and Muhammad Nur Hilmi Zainudin;* ***draft manuscript preparation:*** *Azlinda Abu Bakar. All authors reviewed the results and approved the final version of the manuscript.*

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