EFFICACY AND AUTODISSEMINATION OF AEDESTECH MOSQUITO HOME SYSTEM (AMHS) ON *Aedes* MOSQUITOES IN LABORATORY AND SMALL-SCALE FIELD STUDY

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by

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LIST OF SYMBOLS

&	And
ß	Beta coefficient
m^3	Cubic meter
°C	Degree Celsius
$	Dollar
=	Equals
F	F-stastistic
>	Greater than
Hz	Hertz
H	Kruskal-Wallis's test statistic
<	Less than
L	Liter
m	Meter
µl	Microliter
ml	Milliliter
mm	Millimeter
-	Minus
×	Multiplication
ln	Natural logarithm
%	Percent
±	Plus-minus
p	p-value
R^2	R- squared value
®	Registered trademark
V	Volt

z	Z-score

LIST OF ABBREVIATIONS

12L:12D	12 hours of light :12 hours of darkness
ADE	Antibody-dependent enhancement
Ae. aegypti	*Aedes aegypti*
Ae. albopictus	*Aedes albopictus*
AI	*Aedes* Index
am	*ante meridiem* (Latin); Means before noon
AMHS	AedesTech Mosquito Home System
ANOVA	Analysis of Variance
ATA	Aplikasi Mudah Alih AedesTech / AedesTech Mobile App
BI	Breteau Index
Bs	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis israelensis*
CDC	Centers for Disease Control and Prevention
CO_2	Carbon dioxide
COMBI	Communications for Behaviourial Changes
COVID-19	Coronavirus disease of 2019
DENV	Dengue virus
DENV-1	Dengue Virus Type 1
DENV-2	Dengue Virus Type 2
DENV-3	Dengue Virus Type 3
DENV-4	Dengue Virus Type 4
df	Degrees of freedom
ER	Kadar Kemunculan / Emergence Rate
EI	Emergence Inhibition
et al.	*et alia* (Latin); Means and others
GLM	General Linear Model
HI	Indeks Penetasan / Hatching Index
IBMP	Instituto Biologia Molecular do Paraná
IGR	Insect growth regulators
iOS	iPhone Operating System
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services

L: D	Light:Dark
L3	Larval stage 3
L4	Larval stage 4
LC50	Lethal Concentration 50
LC90	Lethal Concentration 90
mg	milligram
MHAQ	Larutan Mosquito Home Aqua / Mosquito Home Aqua solution
mins	Minutes
MIR	Minimum Infection Rate
MOH	Ministry of Health
NMRR	National Medical Research Register
OI	Indeks Ovitrap / Ovitrap Index
OviTo	Oviposition Towel
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PPF	Pyriproxyfen
ppm	parts per million
QR Code	Quick response code
RCNN	Regional Convolutional Neural Network
RNA	Ribonucleic acid
Rpm	Revolutions per minute
RT-PCR	Reverse transcription polymerase chain reaction
Sdn. Bhd.	Sendirian Berhad
Sig.	Significance
sp.	Species
spp.	Species pluralis
SPSS	Statistical Package for the Social Sciences
T. urticae	Tetranychus urticae
TBE	Tris/Borate/EDTA
Tukey’s HSD	Tukey's Honestly Significant Difference
UPM	Universiti Putra Malaysia
USM IACUC	Universiti Sains Malaysia Institutional Animal Care and Use Committee
VCRU	Vector Control Research Unit
VOC	Volatile organic compounds

WHO	World Health Organization

KEBERKESANAN DAN SEBARAN AUTO BAGI AEDESTECH MOSQUITO HOME SYSTEM (AMHS) TERHADAP NYAMUK *Aedes* DALAM MAKMAL DAN KAJIAN LAPANGAN SKALA KECIL

ABSTRAK

Pemasangan ovitrap merupakan strategi yang berdaya maju untuk kawalan nyamuk *Aedes*. Kajian ini telah menilai keberkesanan ovitrap sebaran auto yang dipanggil AedesTech Mosquito Home System (AMHS), yang mengandungi pyriproxyfen. Kajian ini merangkumi ujian makmal dan kajian lapangan berskala kecil. Objektif kajian lapangan adalah untuk menilai secara menyeluruh kesan AMHS terhadap *Aedes*, serangga bukan sasaran, dan kadar jangkitan denggi dalam nyamuk. Dalam ujian makmal, penyelidikan ini dijalankan ke atas dua spesies nyamuk: *Aedes albopictus* dan *Aedes aegypti*. Tiga aspek berbeza telah diterokai dalam ujian makmal: pengaruh penarik terhadap oviposisi, kesan kedudukan perangkap terhadap oviposisi, dan pemilihan tapak oviposisi. Kajian lapangan yang dijalankan di Asoka Apartment, Pulau Pinang, berlangsung dalam tiga tempoh berturut-turut: kajian garis dasar, kajian keberkesanan, dan kajian sebaran auto. Pada masa yang sama, ketepatan Aplikasi Mudah Alih AedesTech (ATA) telah diteliti. Keputusan-keputusan makmal menunjukkan larutan Mosquito Home Aqua (MHAQ) dengan penarik secara konsisten menarik *Ae. aegypti* dengan berkesan (ANOVA Welch, $F(2,68.66)=5.22, p=0.01$). Walau bagaimanapun, keberkesanannya terhadap *Ae. albopictus* adalah kurang optimum berbanding rawatan lain (ANOVA Dua Hala, $F=0.16, df=2, p>0.05$), menunjukkan keperluan untuk mempertimbangkan penarik tambahan. Yang ketara, kedudukan AMHS tidak menunjukkan kesan yang jelas terhadap daya tarikannya untuk kedua-dua spesies nyamuk (Ujian-T, $p>0.05$), menggariskan fleksibiliti dalam

penempatan perangkap. Kejadian pilihan oviposisi serentak dalam replikat yang sama menunjukkan kemungkinan bahawa penarik sedia ada dalam MHAQ tidak mempengaruhi oviposisi secara signifikan ($p>0.05$). Oleh itu, penghapusan penarik adalah dicadangkan untuk mengurangkan kos pengeluaran AMHS. Dalam kajian lapangan, perangkap AMHS mengurangkan Indeks Ovitrap (OI) secara signifikan berbanding perangkap garis dasar ($p>0.05$). Perangkap yang dirawat menunjukkan pengurangan Indeks Penetasan (HI) dan Kadar Kemunculan (ER) tanpa menyebabkan kemudaratan kepada serangga bukan sasaran. Yang ketara, sebaran auto pyriproxyfen telah diperhatikan membawa kepada pengurangan populasi *Aedes* ($p>0.05$). Walaupun kadar jangkitan denggi tidak dapat diukur, kajian-kajian ini membuktikan keberkesanan AMHS dalam kawalan *Aedes*, yang difasilitasi melalui sentuhan langsung dan sebaran auto, sambil memberi kesan minima ke atas serangga bukan sasaran. Ketepatan ATA dalam pengiraan telur secara konsisten menunjukkan kadar ketepatan yang rendah sebanyak $3.53\pm0.00\%$ berbanding pengiraan manual. Tiga kajian berturut-turut tidak menunjukkan peningkatan yang ketara dalam ketepatan pengiraan ATA. Penambahbaikan berterusan adalah penting, terutamanya dalam meningkatkan ketepatan ATA dan mengurangkan kebergantungannya kepada internet. Secara keseluruhannya, penyelidikan ini menggariskan potensi besar AMHS dalam mengawal nyamuk *Aedes*, disokong oleh bukti statistik yang kukuh yang diperoleh daripada kajian makmal terkawal dan kajian lapangan.

EFFICACY AND AUTODISSEMINATION OF AEDESTECH MOSQUITO HOME SYSTEM (AMHS) ON *Aedes* MOSQUITOES IN LABORATORY AND SMALL-SCALE FIELD STUDY

ABSTRACT

Ovitrap deployment stands as a viable strategy for *Aedes* mosquito control. This study evaluated the efficacy of an autodissemination ovitrap called AedesTech Mosquito Home System (AMHS), which incorporates pyriproxyfen. This study encompassed laboratory trials and a small-scale field study. The objective of the field study was to comprehensively assess the impact of AMHS on *Aedes*, non-target insects, and dengue infection rates in mosquitoes. Within the laboratory trials, these investigations unfolded across two species of mosquitoes: *Aedes albopictus* and *Aedes aegypti*. Three distinct facets were explored in the laboratory trials: the influence of an attractant on the oviposition, the effect of trap positioning on oviposition, and the selection of oviposition sites. The field study conducted in Asoka Apartment, Pulau Pinang, unfolded in three successive periods: baseline study, effectiveness study, and autodissemination study. Concurrently, the accuracy of the AedesTech Mobile App (ATA) was scrutinized. The laboratory results indicated the Mosquito Home Aqua (MHAQ) solution with attractant consistently attracted *Ae. aegypti* effectively (Welch's ANOVA, $F(2,68.66)=5.22, p=0.01$). However, its efficacy with *Ae. albopictus* was suboptimal compared to other treatments (Two-way ANOVA, $F=0.16, df=2, p>0.05$), highlighting the need for considering additional attractants. Notably, the placement of AMHS exhibited no discernible impact on its attractiveness for both mosquito species (T-test, $p>0.05$), underscoring the flexibility in trap deployment. The occurrence of simultaneous oviposition choices within the same replicates hinted at the possibility that the existing attractant in MHAQ did not significantly influence oviposition ($p>0.05$). Therefore, eliminating the attractant is suggested to reduce the cost of AMHS production. In the field study, AMHS traps significantly mitigated the Ovitrap Index (OI) in comparison to baseline traps ($p>0.05$). Treated traps demonstrated reduced Hatching Index (HI) and Emergence Rate (ER) without causing harm to non-target insects. Notably, autodissemination of pyriproxyfen was observed leading to a reduction in *Aedes* populations ($p>0.05$). Even though dengue infection rates cannot be quantified, these studies attest to the efficacy of AMHS in *Aedes* control, facilitated through direct contact and autodissemination, all while having minimal impact on non-target insects. The precision of ATA in egg counts consistently exhibited a low accuracy rate of 3.53±0.00% compared to manual counting. The three consecutive studies noted no discernible improvement in ATA's counting precision. Ongoing refinement is essential, especially in improving ATA precision and reducing its dependence on the internet. Overall, this investigation underscores the high potential of AMHS in controlling *Aedes* mosquitoes, substantiated by robust statistical evidence gleaned from controlled laboratory and field settings studies.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Approximately 390 million people are infected with dengue annually worldwide (Chong et al., 2020; Ebi & Nealon, 2016). In Malaysia, dengue is also recognized as a serious arbovirus and has been declared endemic due to persistently rising cases (Liew et al., 2016; Packierisamy et al., 2015). The country saw a 61.4% increase in dengue cases in 2019 compared to 2018 (idengue, 2023). However, between 2020 and 2021, there was a substantial decline of approximately 70.8% in the number of cases (idengue, 2023). This reduction could be attributed to various coronavirus disease of 2019 (COVID-19) containment measures that limited access to blood meals and decreased vector density (Jindal & Rao, 2021; Surendran et al., 2022). Despite this temporary respite, the incidence of dengue cases surged by 150.7% in 2022 compared to the previous year, and this upward trend persisted in 2023, with an 86.3% increase over 2022 (idengue, 2023; Ministry of Health [MOH], 2024a). The first week of 2024 alone saw 3,181 reported dengue cases with one fatality. In comparison, during the same week in 2023, there were 962 cases with one reported fatality (MOH, 2024b).

The prominent vectors for dengue in Malaysia, female *Aedes aegypti* and *Aedes albopictus*, have posed a significant problem in the national healthcare system during dengue epidemics, as stated by Selvarajoo et al. (2020). This disease, caused by DENV-1 to DENV-4, has led to the allocation of crucial resources such as time, finances, and hospital beds from other critical disease areas when dengue cases are on the rise, as mentioned by Hii et al. (2016) and Liew et al. (2016).

Although dengue is primarily known to affect urban areas, there has been an increment in cases in rural areas as well (Er & Abdullah, 2016). Dengue commonly affects individuals between the ages of ten to 30, as reported by Murphy et al. (2020) and Packierisamy et al. (2015). In Malaysia, the district health authority has implemented an online notification system mandating the reporting of all verified cases of dengue fever, whether they are suspected clinically or confirmed serologically by a medical officer (Liew et al., 2016).

Since its initial occurrence in 1901 in Penang, the illness has grown prevalent in the northern port city (Liew et al., 2016; Nazri et al., 2013; Packierisamy et al., 2015). The first major dengue outbreak recorded in Malaysia was in Penang in 1962 (Murphy et al., 2020). Dengue fever frequently occurs in the Southwest District of Penang Island, which includes Bayan Lepas, Pantai Jerejak, and Batu Maung(Hashim et al., 2019) including Sungai Nibong, Gelugor, Balik Pulau, Permatang Damar Laut, and Pintasan Bahagia (Hashim et al., 2019; Rahim et al., 2016).

Dengue fever in Malaysia can be transmitted by horizontal and vertical transmission (Pitchaimuthu et al., 2020; Rahman & Rosidi, 2022). Horizontal transmission of this virus can occur over the bites of infected female mosquitoes (Ferreira-De-Lima & Lima-Camara, 2018). Meanwhile, vertical transmission can occur during pregnancy from the mother to fetus and through the infected mosquito's parent, either male or female, to a part of their progeny (Ferreira-De-Lima & Lima-Camara, 2018; Selvarajoo et al., 2020). The transmission of the dengue virus is intricately tied to the geographic expansion and distribution of vector mosquitoes (Chang et al., 2014; Focks et al., 1993; Hopp & Foley, 2001). Moreover, changes in human behaviour (water storage, greater mosquito exposure when sleeping outside due to flood-damaged structures, a temporary halt in disease control activities, inappropriate waste disposal, and overcrowding) also elevate disease risk to the rest of society (Murphy et al., 2020; Watson et al., 2007).

In order to address the multifaceted nature of dengue transmission, numerous strategies have been implemented to combat the disease, and among these strategies, the utilization of a vector trap stands out as an effective approach that facilitates contact with disease-carrying vectors, leading to their eventual demise and reproductive impairment (World Health Organization [WHO], 2018). Traps are usually designed to target stages of mosquitoes such as eggs, larvae, pupae, or adults or focus on physiological stages such as gravid stages (WHO, 2018). Traps can be classified based on the employed strategy to eliminate vectors, such as the capture-kill approach, which encompasses two categories: physical kill or the utilization of fast-acting insecticides (Faierstein et al., 2019). Another strategy is the capture-release approach, which typically uses a slow-acting insecticide along with an autodisseminant, or just the autodisseminant alone (Buckner et al., 2017; WHO, 2018).

The AedesTech Mosquito Home System (AMHS), which is a commercially available *Aedes* trap, has already proven itself as the top performer in the Prevention-Vector Control category of the Dengue Tech Challenge in 2016, effectively using autodissemination process (Lim Chee Hwa, personal communication 2020). The AMHS comprises three main components: a bucket with a lid, an OviTo (oviposition towel), and a Mosquito Home Aqua (MHAQ) (Mohd Ngesom et al., 2021). The bucket serves as the base and is designed in black opaque colour, while the lid is plum-coloured (Gopalsamy et al., 2021). Each trap is equipped with OviTo linen, a collection towel that facilitates mosquito oviposition (Mohd Ngesom et al., 2021) which allows the mosquitoes to deposit their eggs, enabling data collection for monitoring purposes (Yazan et al., 2020).

The MHAQ, on the other hand, contains pyriproxyfen (PPF), a substance that infects gravid female mosquitoes during oviposition (Mohd Ngesom et al., 2021). This infection ensures that the mosquitoes carry and spread the PPF to additional *Aedes* oviposition locations, effectively curbing their population (Man et al., 2020a). The design of the AMHS incorporates gravity to ensure the efficient flow of the solution, enhancing its functionality and effectiveness in controlling *Aedes* sp. (Mohd Ngesom et al., 2021). Remarkably, even at low doses, as a minimum of 1 ppm, PPF exhibits significant ovicidal activity across various (Suman et al., 2013).

According to Koama et al. (2015), exposure to PPF prevents the growth of ovaries in female mosquitoes even after multiple blood meals. In addition, the effectiveness of AMHS in reducing mosquito populations has been demonstrated in several locations, including 17th College, Universiti Putra Malaysia (UPM), as evidenced by studies conducted by Man et al. (2020c) and Yazan et al. (2020). The utilization of AMHS, as measured by the Ovitrap Index, has shown 100% effectiveness in controlling *Aedes* mosquitoes compared to conventional ovitraps, which achieved a rate of 90.63% (Yazan et al., 2020).

In the pursuit of controlling *Aedes* mosquitoes, it is essential to assess the impact of these control methods on non-target insects, aiming to minimize harm to other insect species while effectively reducing dengue transmission, as highlighted by studies conducted by Abeyasuriya et al. (2017) and Long et al. (2015). Specifically, the claims made by products employing traps or chemical release, such as fogging, for controlling dengue mosquitoes need to be validated by evaluating their potential impact on non-target insects, such as crucial pollinators (Abeyasuriya et al., 2017). Research has shown that non-target insects, including pollinators, can be highly sensitive to pesticide exposure, leading to both mortality and sublethal effects (Serrão et al., 2022), as demonstrated by studies conducted by Goulson et al. (2015), Mullin (2020), and Yang et al. (2008).

To gain a comprehensive understanding of the impacts of the controlling methods, such as the use of AMHS towards the *Aedes* and the diseases they transmit, studies in medical entomology encompass both laboratory and field investigations (Ferguson et al., 2008). In laboratory settings, researchers establish fundamental knowledge and explore cause-and-effect relationships (Devine et al., 2021). On the other hand, field studies provide valuable insights into vector behaviour in realistic ecological contexts (Resnik, 2017). By combining these two approaches, researchers gain a comprehensive understanding of vectors and the diseases they transmit, enabling the development of effective strategies for control and prevention (Moreno-gómez et al., 2021; Su et al., 2014).

Accordingly, the primary focus of this study is to meticulously simulate and recreate both indoor and outdoor conditions, implementing necessary modifications based on the esteemed guidelines set forth by the World Health Organization (WHO, 2018). Two study designs were employed in this research:(1) Laboratory room-sized cage trial and (2) Small-scale open-field trial. For laboratory room-sized cage trials, the trap was tested for the oviposition patterns based on trap placement and the effectiveness of attractants. Meanwhile, for small-scale open trials, the effectiveness of the AMHS trap was evaluated in the open field, including their impact on autodissemination. Furthermore, an assessment was conducted to investigate the impact of AMHS on non-target organisms, as well as to analyze the infection rates of mosquitoes in response to the usage of AMHS. In addition, the counting of *Aedes* eggs from the AedesTech Apps (ATA) was compared with manual counting methods in this study to understand the accuracy of this application.

1.2 Research Objective (s)

1.2.1 General Objective

This research aims to assess the potential of the AMHS as an innovative "lure-kill" trap, with the goal of diminishing *Aedes* mosquito populations and, consequently, mitigating dengue infections among humans.

1.2.2 Specific Objectives

(a) To examine the effectiveness of the AMHS trap efficacy under laboratory conditions.

(b) To examine the effectiveness of AMHS trap efficacy in open small-scale trials, and its impact on non-target organisms, including the mosquitoes' infection rates on dengue in response to AMHS usage.

(c) To evaluate the autodissemination impact of AMHS on the *Ae. aegypti* mosquitoes in the open small-scale field study.

(d) To evaluate the accuracy of the AedesTech Apps (ATA) in counting *Aedes* mosquito eggs.

1.3 Significance of the Study

In laboratory studies, there is presently insufficient information regarding the effectiveness of AMHS in attracting *Aedes* mosquitoes, including information on oviposition patterns based on time, trap placement, and the effectiveness of attractants. The impact on non-target organisms has yet to be demonstrated in published research papers. Meanwhile, the small-scale study and the infection rates of mosquitoes with the usage of AMHS are stated in a few papers (Gopalsamy et al., 2021; Man et al., 2020a, 2020b, 2020c; Yazan et al., 2020). The significance of this study is to test the efficacy of AMHS in the laboratory and field as well as evaluate the impact of the trap on non-target insects and the infection rates of mosquitoes. Additionally, this study also aims to assess the accuracy of the AedesTech Apps in counting mosquito eggs, thereby providing valuable insights and verification of their effectiveness. Consequently, drawing upon the results of this study, the AMHS trap is expected to be included among the recommended traps by the WHO.

1.4 Characteristics Required for a World Health Organization (WHO)-Recommended Ovitrap

According to the World Health Organization (2018), for a trap to be recognized as an autodissemination station utilizing pyriproxyfen, it must satisfy specific benchmarks derived from laboratory, small-scale, and large-scale field evaluations. In laboratory experiments, the trap should exhibit successful pyriproxyfen transfer to larvae, achieving lethal concentration values (LC50 or LC90) or emergence inhibition (EI) reflective of larval impact. Additionally, the pyriproxyfen must fulfil the necessary LC50 and LC90 standards. In small-scale field evaluations, the trap's effectiveness should be apparent through immediate and delayed mortality of adults and or larvae, or other metrics such as oviposition rates or adult EI. The trap must also sustain its potency over a specified period and be strategically deployed for optimal control outcomes.

In large-scale entomological field evaluations, primary efficacy measures include a substantial reduction in local adult *Aedes* mosquito population density in treated zones compared to control areas and a notable decrease in the proportion of older female mosquitoes. Secondary measures encompass a shift in the sex ratio favouring males, reduced oviposition rates, fewer blood-fed females, and a diminished infection rate. These assessments ensure that the autodissemination station effectively diminishes mosquito populations and alters population dynamics in both controlled and field settings. However, this research exclusively catered to laboratory and small-scale field evaluations, excluding large-scale trials.

1.5 Study Framework and Graphic Illustrations

The research is underpinned by a straightforward study framework, as depicted in Figure 1.1, which delineates the sequential progression of the investigation in both laboratory and field settings. Figure 1.2 provides a clear representation of the procedural steps and data collected during the laboratory study, while Figure 1.3 visually presents the deployment of traps and data collection in the field setting study.

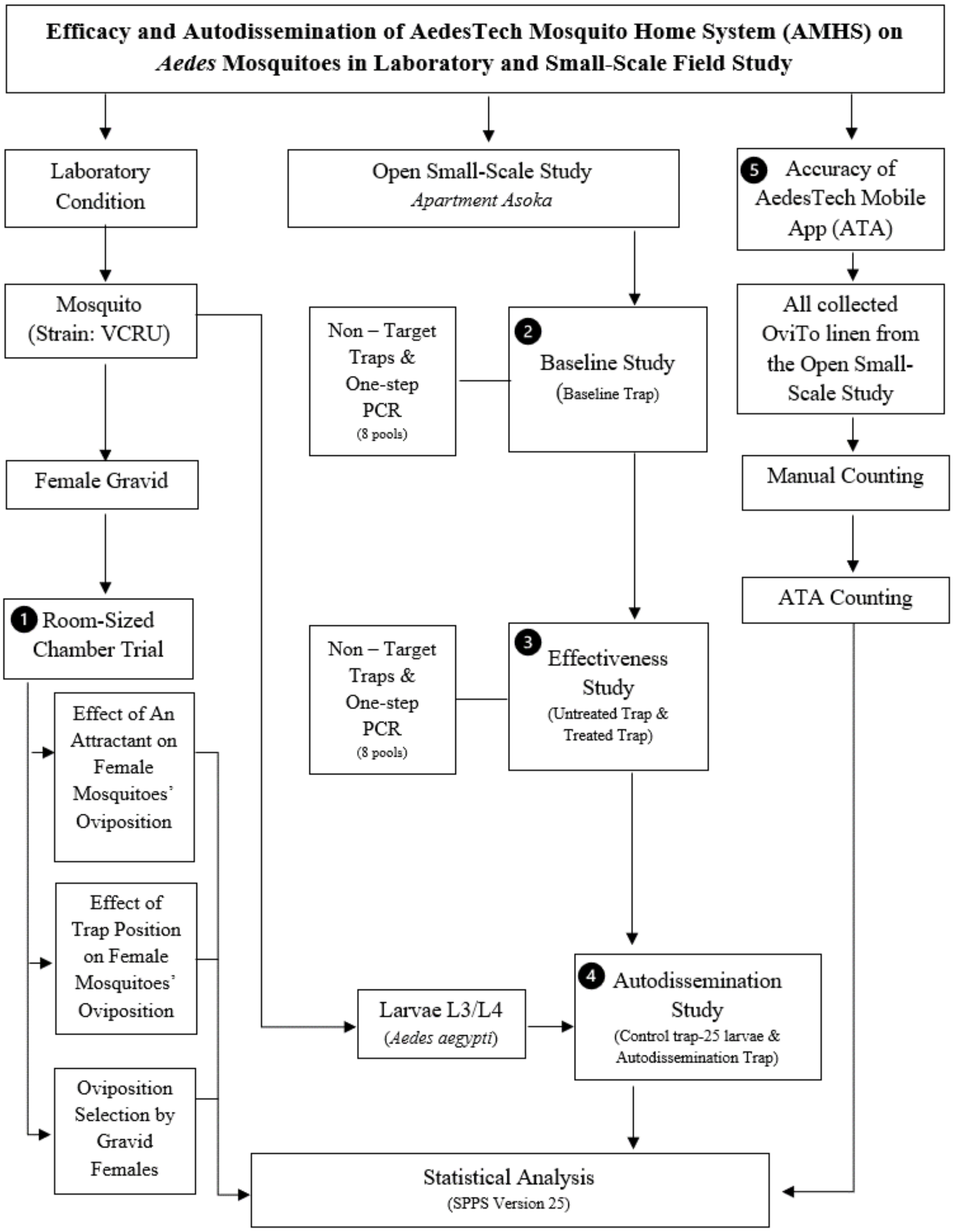

Figure 1.1 The illustration for the study framework of this research included the first part in laboratory settings, parts two to four in the field setting, and the fifth part involved the OviTo linen collection from field setting and counting in the laboratory

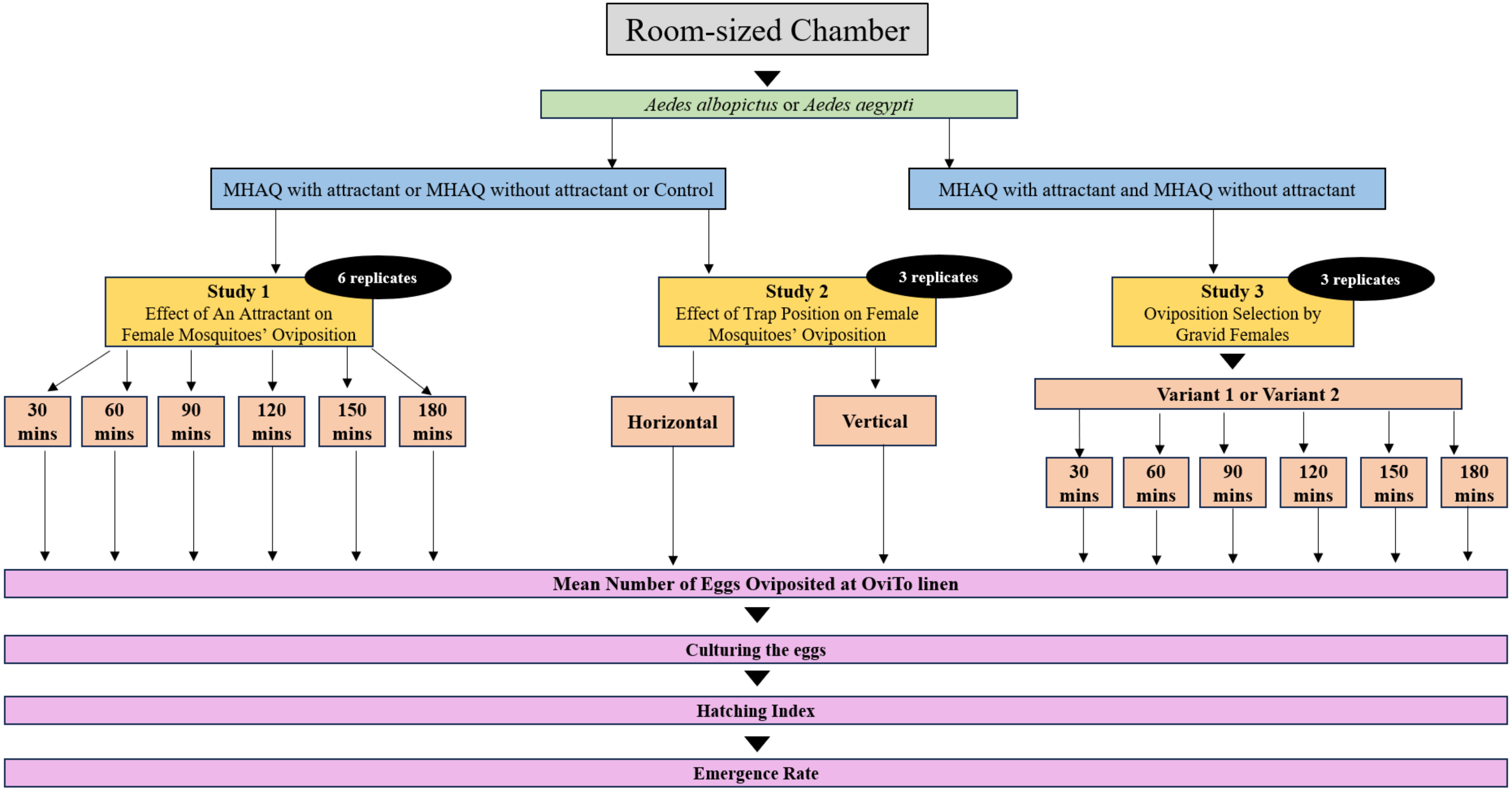

Figure 1.2 Graphic illustration of procedures conducted in the room-sized chamber within a laboratory setting for Chapter 3

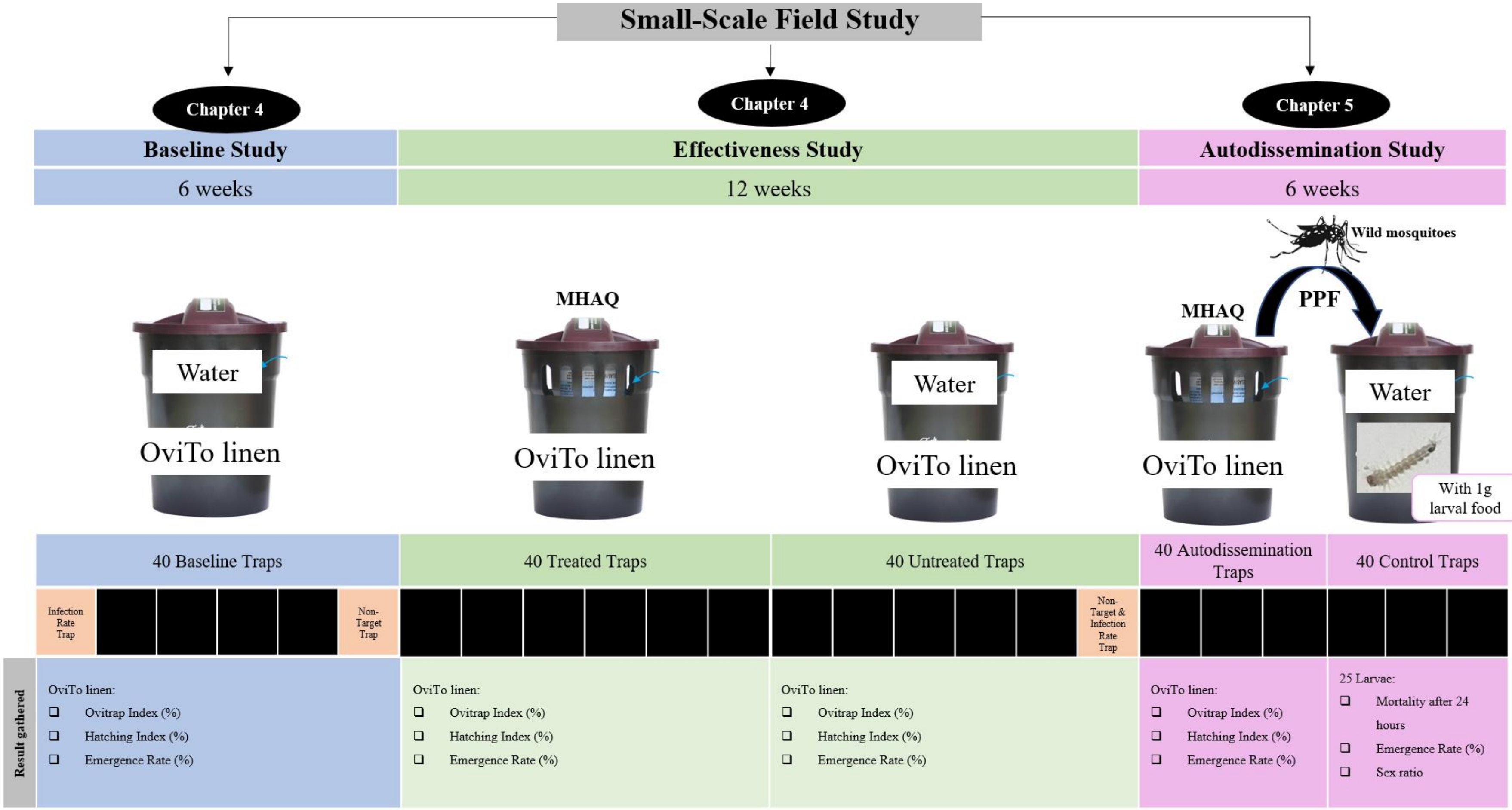

Figure 1.3 Graphic illustration of small-scale field study involving the deployment of traps in Chapter 4 and Chapter 5

CHAPTER 2

LITERATURE REVIEW

2.1 *Aedes* sp. Mosquitoes

The word *Aedes* is from the Greek word that means ‘unpleasant’ which suits well the portrayal of this genus (Sohipah et al., 2020). The unpleasantness associated with these mosquitoes arises from their anthropophilic behaviour, as they preferentially seek humans as blood hosts. This not only causes itchiness but also poses a public health risk, as they serve as vectors for multiple diseases (Kahamba et al., 2020; Muhammad et al., 2020; Ohtsuka et al., 2001).

It is even more intriguing that the genus *Aedes* has over 100 mosquito species and can be found in all places except Antarctica (Ghosh & Ghosh, 2022; Rai, 1991; Rodríguez-Martínez et al., 2020). They have become an important genus that is helping to spread dengue disease in more than 100 countries (Bhatt et al., 2013; Peña-García et al., 2016). Malaysia, which is included in that list, has recorded more species from this genus, such as *Aedes aegypti, Aedes albopictus, Aedes butleri, Aedes cranceraedes,* and *Aedes niveus* (Hashim et al., 2019; Nurin-Zulkifli et al., 2015).

Aedes aegypti, originating in Africa, is the primary vector of dengue (Andreo et al., 2021; Souza-Neto et al., 2019). Another species of *Aedes* sp. that is responsible for dengue transmission is *Ae. albopictus* (Lwande et al., 2020; Reinhold et al., 2018). Both species are prevalent in Penang, along with other species such as *Culex gelidus, Culex pipiens* and *Toxorhynchit*es sp. (Kamal et al., 2020).

Global trade, unsystematic growth, the proliferation of slums, insufficient water storage, sewage, water storage practices, overcrowding, international travel, inadequate waste management systems, and global warming all contribute to the efficient spread of this virus, particularly in low- and middle-income countries like Malaysia. Heavy rains and river overflows create stagnant water, which provides mosquito vectors, notably *Aedes* mosquitoes, plenty of fresh and ideal hatching grounds (Chang et al., 2014; Focks et al., 1993; Hopp & Foley, 2001; Roiz et al., 2018).

2.1.1 Aedes aegypti

The *Aedes aegypti* mosquito, also known as the "yellow fever mosquito" because of its association with yellow fever, is the most common carrier of the dengue virus. Furthermore, Powell (2016) claims that this species is the deadliest of all animals, and it is a well-known fact. In addition to their role as vectors spreading dengue and yellow fever, they also contribute to the spreading of Chikungunya and the Zika viruses (Gratz, 2004; Louise et al., 2015; Mundim-Pombo et al., 2021; Powell & Tabachnick, 2013).

As a holometabolous insect, *Ae. aegypti* goes through four stages of development throughout its life cycle, including the egg, larva, pupa, and adult stages (Mundim-Pombo et al., 2021). The egg stage is vital as the *Ae. aegypti*'s control efforts are severely hampered by the fact that eggs are resilient to desiccation and can persist for up to a year. But, when they come into touch with water, they will immediately hatch (Farnesi et al., 2015; Lima-Camara, 2016; Mundim-Pombo et al., 2021; Rezende et al., 2008; Scott et al., 2000).

The egg of *Ae. aegypti* is cigar-shaped, lustrous jet black, and has a little dorsoventral curvature. It is decorated with polygon exterior chorionic cells, which encase the whole layer of the egg and are slender at the ends (Supriyono et al., 2023). The chorion, often called chorionic cells, served as a shield, a location for gas exchange, and a means of preventing water loss. The exterior layer of the chorion (exochorion), is typically characterized by different ornamentation, making it an effective marker for identifying key distinctions across species (Mundim-Pombo et al., 2021).

Varying exochorion details also contribute to different degrees of egg desiccation resistance. Consequently, the viability of the egg will be impacted by this. In a dry environment, *Anopheles aquasalis* and *Culex quinquefasciatus* can stay viable for one day and a few hours, respectively. However, *Ae. aegypti* eggs have the potential to persist in the same environment for months (Farnesi et al., 2015).

The larvae of *Ae, aegypti* have anal papillae with a strong, curved comb spine and apical plus subapical denticles (Supriyono et al., 2023). Each instar marks an increase in the number and length of pecten teeth. During larval development, four anal papillae lengthen and widen (Andrew & Bar, 2013).

Morphologically, adult *Ae. aegypti* has white, lyre-shaped scales on the upper surface of the thorax (Meena, 2022). Adults have a black or brown background scutum with a pair of sub-median longitudinal white stripes (Rueda, 2004; Supriyono et al., 2023). According to Andrew & Bar (2013), the membrane of the wing lacks white scales. It has a distinctive venation with scales that are flat. In the meantime, the claws of the front, middle, and rear leg of an *Ae. aegypti*, whether it be a male or female, will always have a distinct appearance, as shown in Figure 2.1.

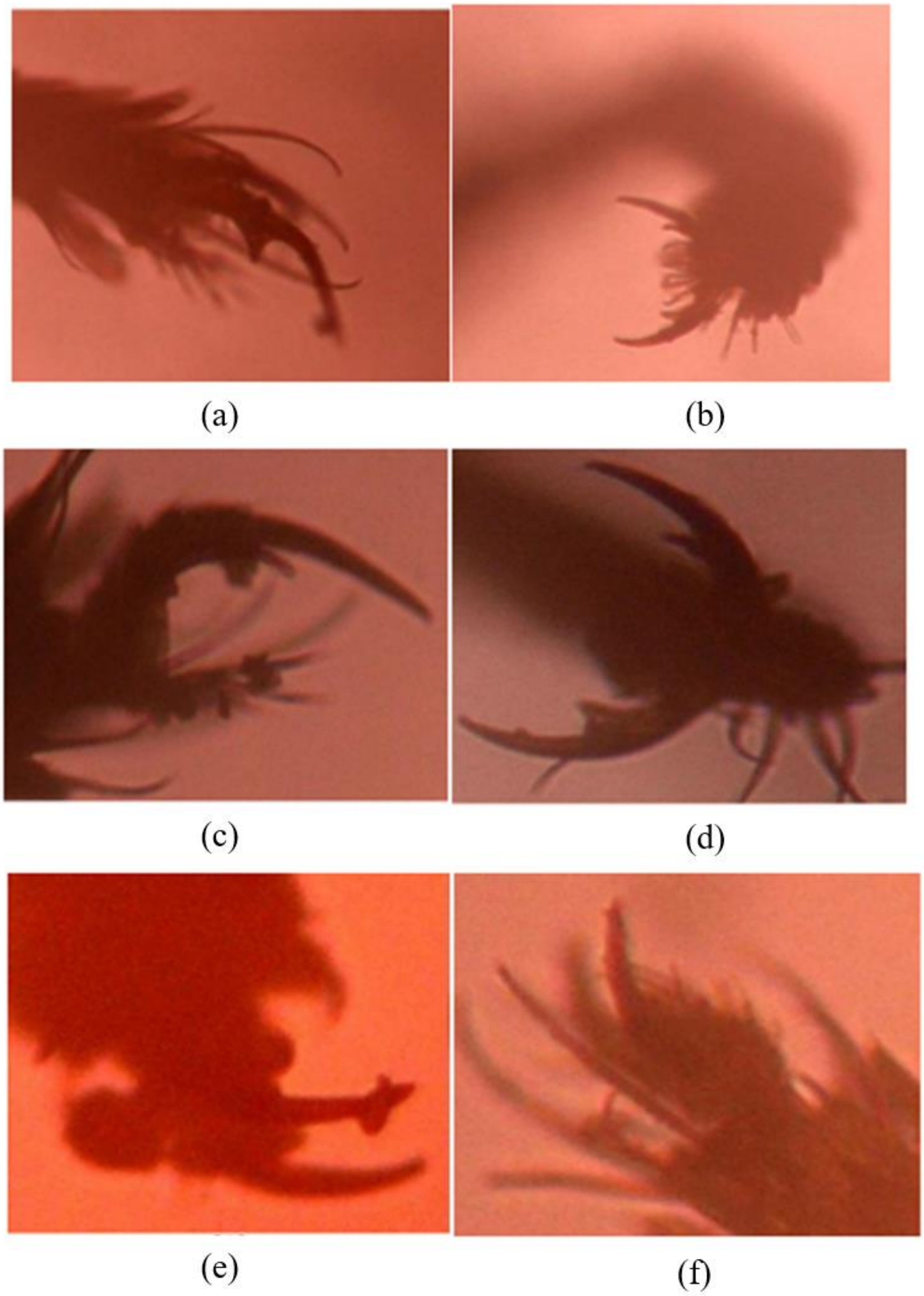

Figure 2.1 Figure shows some claw parts of *Aedes aegypti* in 196x magnification (a) Claw of fore leg of male (b) Claw of fore leg of female (c) Claw of middle leg of male (d) Claw of middle leg of female (e) Claw of rear leg of male (f) Claw of rear leg of female (Source: Andrew & Bar, 2013)

The feeding habits of *Ae. aegypti* are most likely anthropophilic, endophagic, and endophilic, which means that they prefer to bite humans, prefer to feed, stay and rest indoors after sucking the blood while ingested blood is metabolised and the eggs mature (Muktar et al., 2016). They actively seek their prey and feast on blood throughout the day, especially during dawn and dusk (Yin et al., 2019). They also typically have many feeding occasions between egg-laying periods (Scott et al., 2000).

Experimentations on the behaviour and physiology of *Ae. aegypti* show that this mosquito is attuned to sound frequencies between 150 and 500 Hz, and its audibility range extends up to 10 meters. This range is aligned with the vowel sounds that are used in human speech, which helping for them to detecting us as their prey (Menda et al., 2019).

2.1.2 Aedes albopictus

Within the context of Asia, *Aedes albopictus* is known as a secondary vector that responsible for transmitting dengue fever. It is hypothesized that, *Ae. albopictus* has helping the spreading of dengue to more than half of the states in the United States and more than 25 countries in the European Region. The global trade in used tyres potentially serves as a breeding ground for the *Ae. albopictus*, has been identified as a significant contributor to the widespread transmission of dengue. Additionally, other commodities like lucky bamboo have also been implicated in facilitating the dissemination of the disease. Despite not being regarded as the primary vector, this mosquito species exhibits remarkable adaptability to its surroundings and the regions it inhabits. Its ability, both as an egg and an adult to survive in colder environments has contributed significantly to its geographic distribution (Medlock et al., 2006; Paupy et al., 2010; Romi et al., 2006).

It is postulated that the species already started the act by snatching the throne of *Ae. aegypti* as the primary vector in Penang, Malaysia, because now it can be found in most parts of Penang, including urban areas (Kamal et al., 2020). *Aedes albopictus* has been identified as the leading vector in a dengue outbreak in the majority of the islands in Torres Strait, Australia, where *Ae. aegypti* is previously present but already absent or scarcely present (Muzari et al., 2019).

The thorax of an adult *Ae. albopictus* is marked with a silverish-white line (Meena, 2022). Their scutum has a thin white stripe running down the middle of it longitudinally (Rueda, 2004). They also have a mesepimeron and a clypeus without any patches of white scales. In terms of length ratio, when comparing the proboscis at the mouthpart to the femur in the forelegs, the proboscis is shorter. On the posterior mesonotum or supra-alar region, some scales are white. Each segment of the dorsal abdomen, also known as the tergite, is covered in white scales with subtly rounded corners. There is a predominance of white colour in the fifth tarsomere of the hind legs. Moreover, the scutellum (Figure 2.2) of the *Ae. albopictus* contains three sections (Supriyono et al., 2023).

Figure 2.2 The scutellum of (a) *Aedes albopictus* and (b) *Aedes aegypti*
(Source: Andrew & Bar, 2013)

The larval stages of *Aedes* sp. are also intriguing in terms of the morphology features. There are no subapical denticles present on the anal papillae of the larval stage of the *Ae. albopictus*, in contrast to the larval stage of the *Ae. aegypti*. Meanwhile, the eggs laid by *Ae. albopictus* have a posterior end that is more pointed compared to that of to the *Ae. aegypti* (Supriyono et al., 2023). Plus, the size of the *Ae. albopictus* eggs were substantially smaller than those of *Ae. aegypti*. Several other traits may also improve the desiccation resistance of *Ae. albopictus* eggs deposited in vessels. While *Ae. aegypti*'s micropylar disc was broader and featured incomplete circular sections, *Ae. albopictus's* was a slenderer polygon with no sectors. Specifically, in *Ae. albopictus*, they were elongated, conspicuous, and solid-wall-like, whereas *Ae. aegypti* has exochorionic networks that were intricately reticulated and widely spread out (Suman et al., 2011).

According to Bonizzoni et al. (2013) and Mohiddin et al. (2015), *Ae. albopictus* mosquito is frequently encountered in remote regions and can be discovered deposited their eggs in environmental conditions such as tree hollows, graveyard urns, or even bromeliads. However, as the *Ae. albopictus* has adapted well to urban environments, it is also prevalent in both urban and suburban areas (Baker et al., 2022; Kraemer et al., 2015). On Penang Island, female *Ae. albopictus* is currently detected indoors and are currently shifting to colonising within human residences, thereby enhancing the opportunity for sanguivorous feeding (Mohiddin et al., 2015). There is also a report that showed that in Rome, gravid females were captured indoors (Valerio et al., 2010).

2.2 The Life Cycle of *Aedes* sp.

The complete life cycle of *Ae. aegypti* requires about 28.5 days. Nevertheless, for *Ae. albopictus* only took a shorter time of 22.5 days compared to *Ae. aegypti* (Anoopkumar et al., 2017). As a holometabolous insect, *Aedes* sp. life cycle comprises four distinctive phases: egg, larva, pupa, and adult (Carvalho & Moreira, 2017).

2.2.1 Egg

Both water and oxygen are necessary for the development of a mosquito egg; a newly laid egg can become larger and heavier depending on the amount of water it receives, but the opposite is true if it desiccates (Farnesi et al., 2015; Rezende et al., 2008). But, it is well-known that the desiccation-resistance of the eggs produced by members of the genus *Aedes* and these eggs typically function as an overwintering strategy through diapause (Bova et al., 2019).

2.2.2 Larvae

During the larval stage of mosquitoes, they require an aquatic habitat of standing or moving water in order to develop successfully. In most cases, the larvae of most mosquito species will remove organic debris and other microbes from water while feeding on them. Some of the organic materials that they consume are found in the environment, notably microorganisms such as bacteria, protozoa, and algae. They also consume crustaceans, plant debris, and insect exuviae in addition to these things (Anoopkumar et al., 2017; Souza et al., 2019). As holometabolous insects, they provide the impression that well-fed larvae will develop into healthier adults (Zeller & Koella, 2016).

Mosquito larvae, including those of the *Aedes* genus, go through four distinct developmental stages known as instars, with temperature significantly impacting their growth rate (Anoopkumar et al., 2017). Instars are the distinct developmental stages that insect larvae go through between each moult. Variations in physical characteristics, such as body size, shape, colouration, segmentation, and appendage dimensions, are commonly observed between instars (Kaleka et al., 2019). For example, the siphon structure of *Aedes* larvae becomes more sclerotized and darker in colour as the larva matures, with the dimensions increasing proportionally to overall larval growth as illustrated in Figure 2.3. Specifically, the siphon of the 4th instar larva measures approximately 0.79 mm in length and 0.39 mm in width, demonstrating the siphon length is roughly double the width (Hossain et al., 2022).

Focusing on *Ae. aegypti*, larval body size progressively increases with each successive moult, reaching nearly 8 mm in length by the fourth instar stage (Schaper & Hernández-Chavarría, 2006). Specifically, the average lengths of *Ae. aegypti* larvae are 1.745 mm in the first instar, 2.935 mm in the second instar, 4.343 mm in the third instar, and 7.202 mm in the fourth instar (Bar & Andrew, 2013). Meanwhile, in the fourth larval instar of *Ae. albopictus*, the eighth abdominal fragment is relatively diminutive and pentagonal, with 12 scales that are shaped like a comb arranged along its sides (Yamany & Abdel-Gaber, 2024)

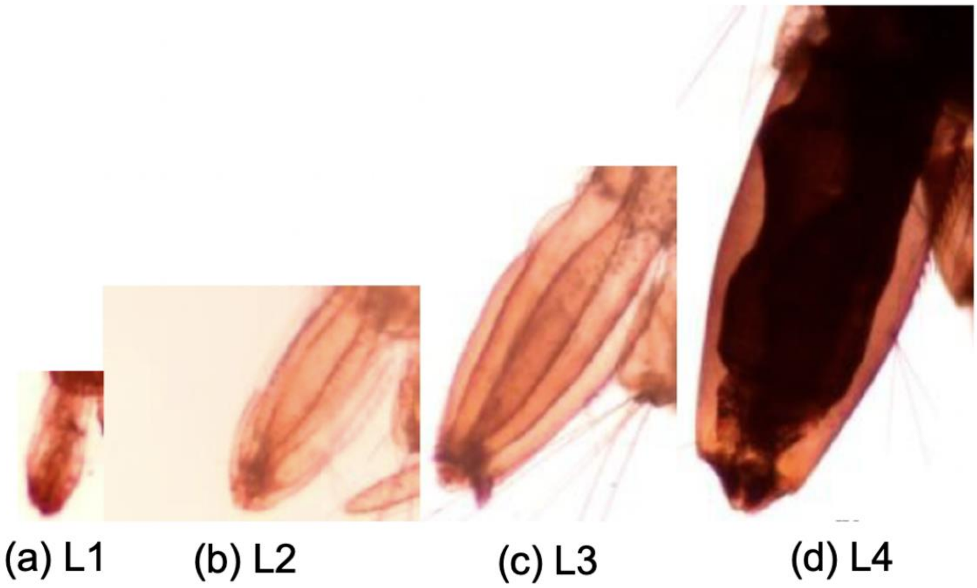

Figure 2.3 The proportional growth of siphon dimensions for each larval development stage in *Aedes* mosquitoes. (a) L1 - First instar larvae, (b) L2 - Second instar larvae, (c) L3 - Third instar larvae, and (d) L4 - Fourth instar larvae (Source: Bar & Andrew, 2013)

2.2.3 Pupae

After larvae have completed their fourth moult as larvae, they transform into pupae, also referred to as tumblers. Pupae do not eat and can remain alive for one to three days before transforming into an adult. In response to external stimuli, such as mechanical or visual cues, they demonstrate a level of responsiveness that triggers a vigorous dive escape response. Subsequently, their positive buoyancy allows them to resurface (Anoopkumar et al., 2017; Montell & Zwiebel, 2016). The productivity of pupae produced is highly sensitive to the size and type of container used. The productivity of pupae is highest in containers with a volume of 50 L or more. Pupae were more abundant in pots with some shade than those without (Islam et al., 2019).

2.2.4 Adult

Adult male mosquitoes obtain most of their energy from plant nectar, whereas adult females rely on a blood meal to produce eggs that will survive as the next generation. In most cases, female mosquitoes must feed once every three to five days. They are diurnal feeders; while they give priority to preying on large mammals, they also consume blood feasts from birds. In terms of size, males are often of a more diminutive stature than their female counterparts (Anoopkumar et al., 2017). However, because of the influence of other environmental conditions on size, the body size is not the best criterion to utilise to differentiate between the sexes in this species (Carvalho & Moreira, 2017).

2.2.5 Female Mosquitoes' Behaviour

While both male and female mosquitoes usually rely on plant nectar and sap for nutrition, it is the female mosquitoes that can adapt their feeding behaviour in times of limited sugar sources, opting to partake more the blood meals to meet their energy needs for egg laying (Barredo & DeGennaro, 2020; Jové et al., 2020; Swan et al., 2021). The development of eggs in female mosquitoes necessitates the acquisition of protein from the blood of a host (Duvall et al., 2019).

Duvall et al. (2019) also stated that the process of egg maturation in female mosquitoes relies on the presence of proteins derived from blood, and the inability of female mosquitoes of this species to obtain a blood meal results in their inability to reproduce. Although blood is vital for providing the necessary proteins for egg production, an interesting study has shown that a straightforward protein mixture sourced from bovine can boost egg production in female *Ae. albopictus* mosquitoes. Surprisingly, this protein formulation outperforms the use of whole human blood, offering a more cost-effective and practical alternative (Pitts, 2014).

In their quest for a blood meal, female *Ae. aegypti* mosquitoes exhibit a remarkable ability to actively search human hosts, employing a range of sensory cues to precisely locate and target humans. Carbon dioxide sensing is essential for these mosquitoes to activate their attraction towards the heat emanating from humans (Liu & Vosshall, 2019). Various factors can influence the host-seeking behaviour of *Ae. albopictus*, and one of these factors is the observed delay in their host-seeking behaviour following nectar consumption. Furthermore, the feeding behaviours exhibited by *Ae. albopictus* can be influenced by the vitellogenin genes. Meanwhile, in certain social insects, such as bees and ants, vitellogenin genes assume a vital responsibility in regulating the foraging and brood-care behaviours specific to different castes (Dittmer et al., 2019). The biting behaviour is also influenced by timing. In suitable times, female *Ae. albopictus* mosquitoes exhibit frequent biting behaviour, peaking around dawn and dusk (Yin et al., 2019).

The production of eggs in most mosquitoes, such as *Ae. aegypti*, is contingent upon the obligatory act of hematophagy, wherein they partake in the consumption of blood from a vertebrate host. The intricate process of egg cell development, scientifically termed oogenesis, encompasses a sequential progression comprising two well-defined stages: the pre-vitellogenic stage preceding blood sucking and the subsequent vitellogenic stage following blood sucking (Valzania et al., 2019). For entering vitellogenic stage, a process induced by ecdysone, it is heavily relies on the presence of EcI-4, a significant ecdysone importer, specifically in adult female mosquitoes (Hun et al., 2022). Upon achieving complete maturation, the eggs will be oviposited, characterized by a meticulously regulated temporal window of 18 hours, during which the process is efficiently concluded (Jahangir et al., 2008).

2.3 Transmitted Diseases by *Aedes* sp.

Both the *Ae. aegypti* and the *Ae. albopictus* mosquito species are common mosquito species found both inside and outside of buildings. They are unable to go vast distances in the air, and as a result, they live out their entire lives nearby to one another (Gopalsamy et al., 2021). They are responsible for a variety of human vector-borne illnesses all over the world, including Zika, yellow fever, and dengue (Andreo et al., 2021; Centers for Disease Control and Prevention [CDC], 2016; Souza-Neto et al., 2019).

Besides the act of *Aedes* mosquitoes directly biting humans, there are alternative pathways through which these diseases can be transmitted to humans. One of these additional routes is vertical transmission, also known as maternal transmission, which can transmit diseases like dengue fever from a mother to another human. In this case, a pregnant mother can pass the infection to her fetus, potentially leading to the transmission of the disease from generation to generation (Basurko et al., 2018). Fortunately, the consequence of vertical transmission, which is associated with the timing of dengue infection during pregnancy, appears to be low (Basurko et al., 2018). However, if a gravida contracts the dengue virus during pregnancy, it can lead to detrimental consequences for the baby, such as premature birth, reduced birth weight, and complications during fetal development (Ribeiro et al., 2016).

Furthermore, it should be noted that the act of transfusing blood from an infected individual to another person can serve as an additional avenue for the transmission of these diseases. Numerous cases have been documented where seemingly healthy blood donors were discovered to be infected with the dengue virus, thereby posing the risk of transmitting the virus to individuals who receive blood from them (Perera et al., 2020). Gimenez-Richarte et al. (2022) found that during outbreaks, a notable number of blood