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Title: Development of the Gravitrap: A Practical Sticky Ovitrap for the  
Surveillance and Control of *Aedes (Stegomyia)* Mosquito

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## **Abstract**

The Gravitrap is a novel tool for the surveillance and control of the container-breeding *Aedes* mosquito. The Gravitrap consists of two components, an outer and an inner container. Ten percent hay infusion is used as an attractant for the gravid female *Aedes* mosquito. The Gravitrap can be left in the field with minimal maintenance for at least 4 weeks without sacrificing trapping efficiency. From the timothy hay infusion headspace, 9 compounds were identified using gas-chromatography electroantennograph detector (GC-EAD). These compounds include cyclodecene, dodecanol, nonene and 3-ethyl-5-methylphenol. The Gravitrap proved valuable as a tool to monitor *Aedes* mosquito populations in dengue clusters in Singapore and has the ability to trap female *Aedes* mosquitoes to enable further molecular investigations into the prevailing dengue virus strains. While the *Aedes* population decreased in the test site after the introduction of the Gravitrap, this could not be completely attributed to the Gravitrap.

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# CHAPTER 1

## INTRODUCTION

The mosquito is a competent vector for various diseases throughout the world. These include viral fevers, such as dengue, chikungunya, yellow fever, Ross River fever, Barmah Forest fever etc., and parasitic infections, such as malaria, filariasis, etc. In the Southeast Asian country of Singapore, the most prevalent mosquito vector species is the *Aedes aegypti* mosquito, which is responsible for the transmission of the dengue, chikungunya and zika viruses. The *Aedes aegypti* mosquito is a highly anthropophilic mosquito that is highly adapted to the domestic and peri-domestic environment, breeding almost exclusively in artificial containers. The peak biting periods of the female *Aedes aegypti* mosquito are dawn and dusk.

Dengue is a flavivirus that remains a significant global healthcare issue. The symptoms of dengue include fever, headache, rash, severe joint pain, nausea and fatigue. Dengue Fever (DF) can lead to two more severe pathologies – Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). It is endemic in over 100 countries globally, ranging across Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific (WHO 2012). The World Health Organization (WHO) estimates that approximately 2.5 billion people (40% of the global population) are at risk of contracting dengue. The annual infection rate is 50-100 million, of which 500,000 are hospitalized and with a global mortality rate of 2.5% (Bhatt 2013).

Chikungunya is another febrile disease that occurs across Africa, Asia and the Indian subcontinent. In recent times, it has spread to Europe and the Americas (WHO 2008). Chikungunya symptoms include fever, swelling of joints, headache, muscle pain and rash. Chikungunya is caused by an RNA virus, which belongs to the alphavirus genus of the *Togaviridae* family.

Zika, caused by another member of the flavivirus family, was first discovered in Africa in the 1940s but was considered to cause only mild disease (Dick 1952). Zika has symptoms similar to dengue and chikungunya, including fever, rash, joint and muscle pain and headaches. Recently, Zika has been linked to more serious pathologies such as microcephaly and Guillain Barré syndrome (Musso 2016).

## **1.1 Attractants**

The attractants used in the traps endeavour to mimic the conditions that would attract female mosquitoes to either seek a host or oviposit, depending on the physiological state (Takken 1999, Zwiebel 2004).

### **1.1.1 Host-seeking attractants**

Traps that target female mosquitoes that are seeking hosts for a blood-meal will have attractants that mimic inorganic gases or volatile organic compounds (VOCs) secreted by the hosts. This would include carbon dioxide from respiration and VOCs found in breath and sweat, such as lactic acid, ammonia, etc (Geier 1999, Dekker 2005, Smallegange 2005).

### 1.1.2 Oviposition attractants

Traps that target female mosquitoes that are gravid and seeking oviposition sites will have attractants that mimic VOCs emitted by natural oviposition sites. The VOCs are typically the by-products of fermentation by bacteria of organic material found in the sites (Hazard 1967, Ponnusamy 2008).

Using plant infusions in ovitraps have been shown to increase the attractiveness of the ovitraps to ovipositing mosquitoes (Holck 1988, Reiter 1991, Obenauer 2009). Maple (*Acer buergerianum*), hay, bamboo, guinea grass (*Panicum maximum*), Bermuda grass (*Cynodon dactylan*), white oak (*Quercus alba* L.) and red oak (*Quercus rubra* L.) leaves have all been used successfully to enhance ovipositing (Chadee 1993, Allan 1995, Trexler 1998, Dieng 2003, Burkett 2004, Sant'ana 2006, Ponnusamy 2008, Zhang 2008).

The plant infusion makes the ovitraps more attractive to the female mosquito than other urban breeding sites, such as household containers, a majority of which would hold clean stagnant water (Trexler 2003). The peak of the plant infusion attractiveness is at 7 days, which also happens to be the peak for bacteria concentration and diversity (Ponnusamy 2010).

Studies have been done to isolate oviposition attractants that have been isolated from plant material. These compounds include phenol, 3-ethylphenol, 4-methylphenol (p-cresol), indole and 3-methylindole (skatole) (Fig. 1)(Bentley 1979, Millar 1994, Mboera 2000, Seenivasagan 2009, Barbosa 2010).

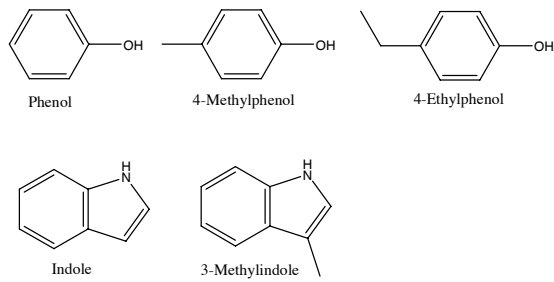


Fig. 1: Oviposition attractants isolated from plant extracts.

## 1.2 Methods of Adult *Aedes* Collection

### 1.2.1 The CDC Backpack aspirator



Fig. 2: CDC backpack aspirator ([www.johnwhock.com](http://www.johnwhock.com))

The CDC backpack aspirator (John W. Hock Co., Gainesville, FL, USA) is considered the “gold standard” of adult mosquito collection and has been used with reasonable success around the world (Morrison 2004, Maciel-de-Freitas 2008)(Fig. 2). It is used to collect resting and flying mosquitoes in the urban household environment. However, it is labour-intensive and in urban cities where access to homes can be limited, its use can prove to be quite challenging. The collection can also be biased by collector efficiency, site of collection,

house size, presence of furniture and the duration of collection (Clark 1994, Favaro 2006, Facchinelli, Valerio et al. 2007).

To overcome the issues of collection efficiencies of the aspirator, there has been a paradigm shift where instead of expending resources and time to actively search out mosquitoes and potential breeding habitats, the focus will be on deploying traps to lure and capture the mosquito. Instead of seeking the mosquitoes using aspirators, traps are being developed to entice the mosquitoes to seek them.

In general, mosquito traps can be divided into two categories – host-seeking and ovi-positioning.

### *1.2.2 Host-seeking traps*

Host-seeking traps target the *Aedes* mosquito ability to seek out its blood-meal via various sensory cues over distances. These cues include olfactory, visual, radiant heat, etc. Most of these trap focus on one or several aspects of the mosquito's attraction to humans.

#### **1.2.2.1 CDC Wilton Trap**



Fig. 3: CDC Wilton trap. ([www.johnhock.com](http://www.johnhock.com))

The CDC Wilton trap was developed for the capture of *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* adult mosquitoes. The hanging trap consists of a black cylinder with a screen and an electric fan at the bottom (Fig. 3). A collection cup is located on the top. Attraction to the trap is based on the mosquitoes' affinity for the black colour and carbon dioxide exhaled during breathing.

The advantages of the trap are its simplicity and sample preservation. Simplicity will allow for ease of trap deployment. Sample preservation is essential for physical entomological investigations, such as morphology, physiology and fecundity.

#### 1.2.2.2 CDC Light Trap



Fig. 4: CDC light trap ([www.johnwhock.com](http://www.johnwhock.com)).

The CDC Light Trap is a hanging trap used to capture various species of mosquitoes. The trap consists of a lid with a cylinder below it. Within the cylinder is housed an electric fan (Fig. 4).

The mosquitoes are then drawn through the fan into a catch bag attached to the bottom of the cylinder. There is an option of attaching a carbon dioxide source to increase the effectiveness of the trap.

### 1.2.2.3 Fay-Prince Trap



Fig. 5: Fay-Prince trap ([www.johnwhock.com](http://www.johnwhock.com))

The Fay-Prince trap is a host-seeking trap that is similar to CDC light trap. This directional trap is based on the attractive properties of contrasting black and white panels (Fig. 5). Similar to the CDC light trap, there is a cylinder with an electric fan below the panels. Mosquitoes attracted to the trap are drawn into the catch bag at the bottom of the cylinder. As with the CDC light trap, the attachment of a carbon dioxide source is an option.

### 1.2.2.4 BG-Sentinel trap

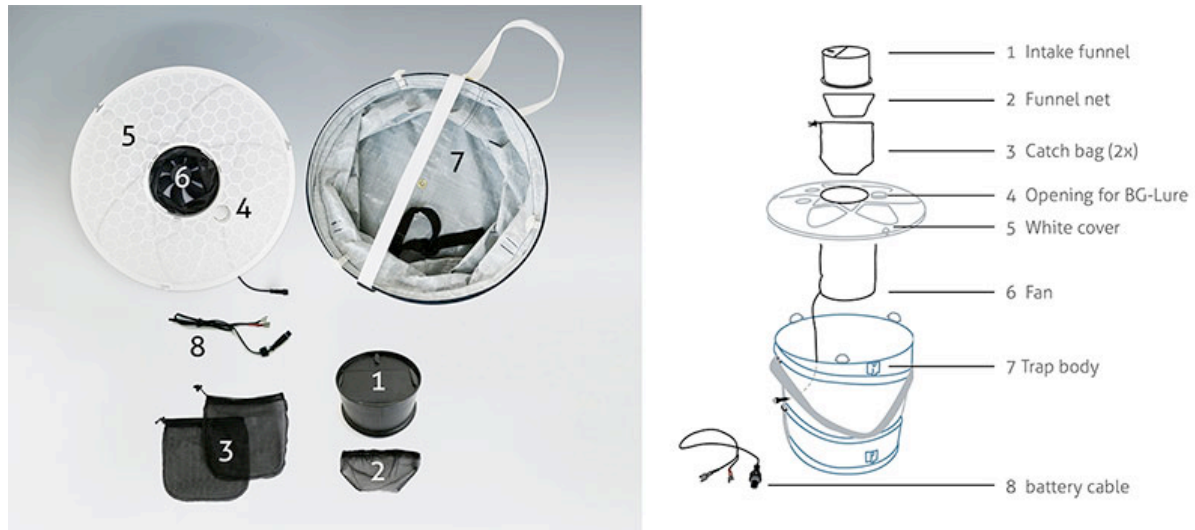


Fig. 6: Picture of the BG-Sentinel trap components (left) and diagrammatic representation of the assembly of the trap ([www.bg-sentinel.com](http://www.bg-sentinel.com)).

The BG-Sentinel trap is a powered fan trap developed by researchers from the University of Regensburg, Germany to capture female host-seeking *Aedes* mosquitoes (BioGents GmbH). It consists of a collapsible cylinder made from white plastic. The top of the trap is covered with a white gauze cover. In the middle of the trap, there is a black tube with a black mesh catch bag at the end. Below that is an electric fan that creates and pulls mosquitoes into the catch bag, while creating convection currents that carry the attractants out of the trap (Fig. 6).

The BG-Sentinel trap uses a lure known as the BG-Lure to attract mosquitoes to the trap. The trap consists of a specific blend of caproic acid, lactic acid and ammonia. These semio-chemicals mimic human skin emanations (Geier 1999, Bosch 2000).

The BG-Sentinel trap has been used successfully to capture host-seeking mosquitoes in the field (Maciel-de-Freitas, Eiras et al. 2006). A number of

studies have also shown the BG-Sentinel trap to have better catch rates than conventional traps (Krockel 2006, Meeraus 2008).

However, its use is limited by its high cost and the intense competition from human hosts in a densely population urban city.

#### **1.2.2.5 Disadvantages of host-seeking traps**

Along with the advantages of having traps that target host-seeking mosquitoes, there are several setbacks in using such traps. Most of these traps are sizable and are cumbersome to transport and deploy in large numbers. They also require a D.C. power source to provide sufficient power for the duration of the deployment. The cost of these would also limit the number of traps that could be deployed. In addition, the deployment and maintenance of the traps are highly labour-intensive. All these factors make the deployment of host-seeking traps for the surveillance and control of *Aedes* mosquitoes very challenging.

#### *1.2.3 Ovipositioning traps*

The *Aedes* mosquito is a container breeder, preferentially laying its eggs in natural and artificial containers, such as pots, discarded cans, tyres, pails, etc (Wongkoon 2007, Pamplona 2009, El-Badry 2010, Saleeza 2011). The *Aedes* mosquito adopts a skip-ovipositioning behaviour (Colton 2003, Reiter 2007), preferring not to lay its complete clutch of eggs in any one container. Instead, it tends to spread the batch across several containers to increase the chance of survival of its offspring.

The gravid female *Aedes aegypti* mosquito is attracted to breeding containers by organic material, such as decomposing plant material, other micro-organisms, etc (Merritt 1992, Ponnusamy 2008). This characteristic of the female *Aedes* mosquito is the basis of the use of hay infusion to increase the attractiveness of ovipositioning traps.

Ovipositioning traps target the female *Aedes aegypti* mosquito after it has taken a blood meal and have become gravid. In this physiological state, the female mosquito is looking for a suitable place to lay their eggs. These traps are also known as ovitraps. Ovitrap traps can be characterized into two groups – non-lethal and autocidal – and have been used successfully throughout the world for surveillance and control (Ordóñez-Gonzalez 2001, Polson 2002, Facchinelli 2007, Lourenço-de-Oliveira 2008, Ritchie 2008, Rapley 2009).

### **1.2.3.1 Non-lethal Ovitrap**

The function of a non-lethal ovitrap is to sample the mosquito population as part of surveillance. The trap does not remove the adult mosquito from the population. It will remove some eggs from the population but is unlikely to have an adverse effect on the population due to skip-oviposition behaviour exhibited by the *Aedes* mosquito. In addition, to surveying the population, the trap can also collect eggs for the purpose of colonizing a field strain of the mosquito in the laboratory.

An example of such a trap is the autocidal ovitrap (Tan 2000). The autocidal ovitrap consist of a black container with a floatation device. The floatation device has a wire mesh and wooden paddles, upon which the female mosquito would lay its eggs (Fig. 7). The hatched larvae would fall through the wire mesh and when they emerge as adults, will be trapped by the same wire mesh, causing them to drown (Tan 2000). The main drawback is that infected female mosquitoes are not caught by the trap and are allowed to continue being vectors for arboviruses. When used as a research tool, the ovitraps require a large number of field officers to inspect and maintain. It also requires a few staff to hatch, grow and speciate the larvae that emerge to quantify the *Aedes* species that laid eggs on the paddles.

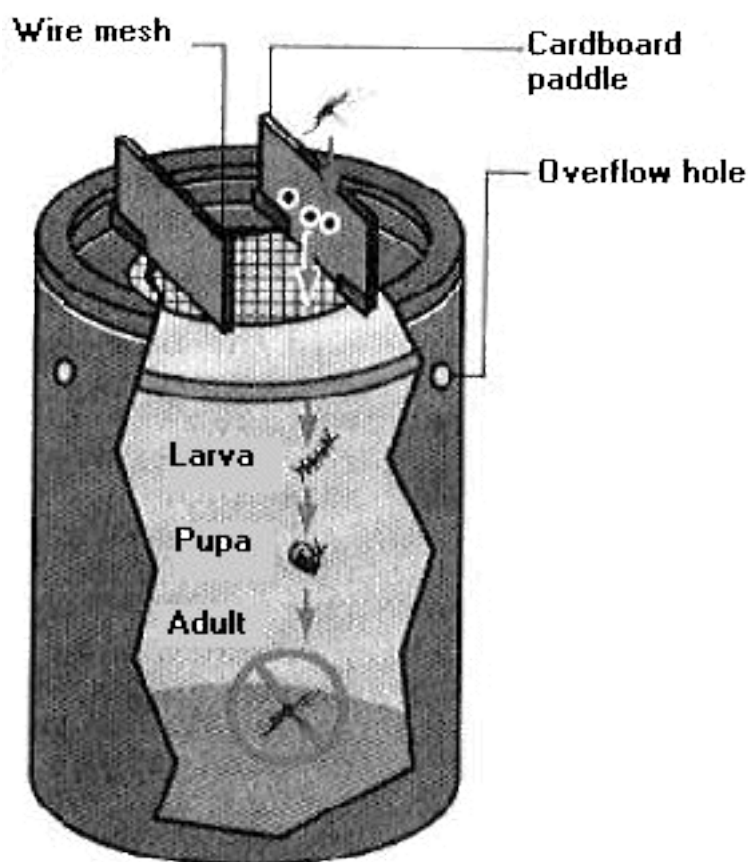


Fig. 7: The autocidal ovitrap (Tan 2000).

### **1.2.3.2 Lethal Ovitrap**

The mode of action of a lethal ovitrap involves attracting the mosquito to land within the trap and pick up a lethal agent, such as an insecticide, a larvicide or entomopathogenic fungus (Williams 2007, Farenhorst 2008, Barbosa 2010).

The mosquito will then leave the trap and spread the agent to any mosquito or environment it comes into contact.

### **1.2.3.3 Sticky Ovitrap**

A sticky ovitrap traps mosquitoes attempting to lay eggs within. This is usually achieved by means of an all-weather adhesive on the inner surface of the trap.

The use of a sticky ovitrap has many advantages. In addition to the surveillance of a mosquito population, it allows for the removal of mosquitoes from the population. This will contribute to a reduction of the population (Barrera 2014).

Mosquitoes that are trapped can assist in further analyses. These include epidemiological data collection, such as infection status, serology, etc., and entomological analyses, such as population genetics, phenotypic studies, etc.

The Australian sticky ovitrap consists of a black, 1.2litre plastic bucket. On the inside of the bucket is a plastic adhesive strip that is shaped into an arc. A polybutylene adhesive (Atlantic Paste and Glue Co. 170 53<sup>rd</sup> Street, Brooklyn, NY) is applied on one side of the strip. The adhesive strip covers the top half of the inside of the bucket. The bottom half of the bucket is filled with water.

The water contains 50 % hay infusion and 0.4 g of lucerne (alfalfa) pellets to enhance the effectiveness of the ovitraps (Reiter 1997, Ritchie 2001). A black plastic mesh of mesh size  $1.7 \times 1.7$  cm is secured over the top of the ovitrap to prevent animals from coming into contact with the glue (Ritchie 2003).

The Brazilian MosquiTrap™ (Ecovec Ltd., Belo Horizonte, MG, Brazil) consists of a robust black container that is 16 cm tall with a diameter of 11 cm and a volume of 1.2 litres. Housed within is a removable sticky card measuring 31cm by 9cm, on which the ovipositioning mosquitoes are captured. It can hold approximately 280 ml of water (Fig. 8)(Honorio 2009). It uses a patented synthetic oviposition attractant called AtrAedes™ (Ecovec Ltd), to lure gravid female mosquitoes into the trap. It has been used to estimate temporal distribution of *Ae. aegypti* in Brazil

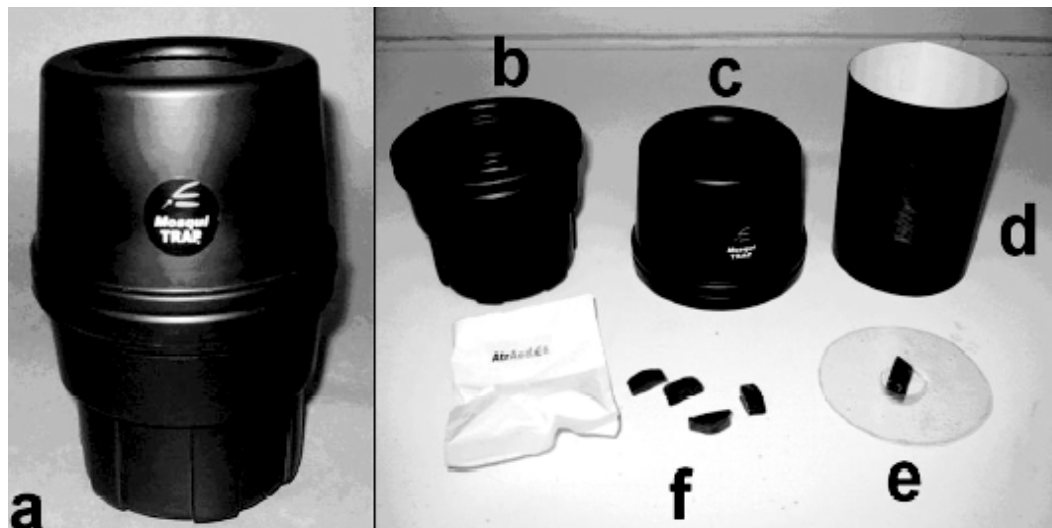


Fig. 8. Assembled MosquiTrap™ autocidal ovitrap (left) and its components (right) – including the base of the trap (b), the top of the trap with funnel (c), the sticky card for capturing the mosquitoes (d), AtrAedes attractant (f) and the mesh to prevent the emergence of mosquitoes (e).

### 1.3 The development of the Gravitrapp

Dengue has been an important disease in Singapore since its appearance in the 1960s. Source reduction and environmental management through inter-sectoral coordination, community participation and a penalty regime are the hallmarks of the nation's vector control strategy. While the comprehensive vector control

programme that began in 1966 has reduced the *Aedes* premise index to a low level of less than 1% (Ooi 2006), Singapore remains vulnerable to outbreaks of dengue due to urbanization, high population density, low herd immunity, intense travel pattern, geographical expansion of the *Aedes aegypti* mosquito (Ng, Tan et al. 2009) and possibly natural adaptation/selection of dengue viruses to overcome the pressure of low *Aedes* population (Lam, Burke et al.).

To further suppress the *Aedes* mosquito population in Singapore, there needs to be a paradigm shift to a more targeted approach to vector control. To this end, a trap is required to complement the current field operations practices for surveillance and control.

### *1.3.1 Trap design*

The sticky adult Gravitrapp consists of two components, an outer and an inner container (Fig. 9). The outer container is a black cylindrical container with two overflow holes on opposite sides, 7 cm from the bottom. Ten percent hay infusion is added into the cylinder and excess infusion solution is removed by the two holes. The smaller inner cylindrical container is lined with an environmentally-stable adhesive glue and has wire-mesh netting at the base. When inserted into the outer cylinder, the netted base reaches the level of the two holes, such that water level remains below the net. Gravid mosquitoes seeking oviposition sites will be caught by the sticky lining. Adults emerging from any eggs laid prior to the adult being caught will be contained below the wire mesh. A laboratory trial was conducted where 60 gravid female *Ae. aegypti* were released in a cage ( $1.0 \times 0.5 \times 0.5 \text{ m}^3$ ) containing a Gravitrapp. After 24 hrs, it was observed that 80% of the mosquitoes were attracted to the trap, and were stuck on the sticky surfaces as they landed on the inner lining.



Fig. 9: The adulticidal Gravitrapp.

The objectives of subsequent chapters include the optimization of the deployment protocol for the Gravitrapp and an analysis into the headspace of the hay infusion to isolate the volatile organic compounds (VOCs) that elicit a response to an electroantennogram preparation of the female *Aedes aegypti* mosquito. Deployment of the Gravitrapp in dengue clusters to determine the efficacy of using the trap to capture dengue-infected mosquitoes and to complement existing vector control operations.

## CHAPTER 2

# STUDY OF THE EFFECTIVENESS OF AGED HAY INFUSION SOLUTION

### 2.1 Introduction

The hay infusion is a critical component of the Gravitraps. The presence of hay infusion increases the attractiveness of the Gravitraps to ovipositing female *Aedes aegypti* over other ovipositing sites in the surrounding environment (Service 1993). Hay infusion provides organic plant matter for the larvae to consume during development through the instar stages. During the fermentation of the organic material in the hay infusion, volatile organic compounds (VOCs) are released into the surrounding air.

The hay infusion is the gold standard as an attractant for ovipositing *Aedes* mosquitoes. However, there are studies that have shown that the effectiveness of the infusion changes as time passes (Isoe 1995, Sant'ana 2006). This could be due to the variability of bacterial fermentation process as time passes or the reduction of VOCs being emitted over time (Maw 1970, Isoe 1995). The objective of the first part of this study is to determine the effect of aged hay infusion on the catch rate of the *Aedes aegypti* mosquitoes in the Gravitraps.

### 2.2 Materials and Methods

Four sites were selected for the deployment of Gravitraps. Eight blocks were randomly chosen from each of the four sites. Each block had 6 traps deployed on the 2<sup>nd</sup>, 6<sup>th</sup> and 10<sup>th</sup> floors. The traps at one side of the block were left at the site for the entire duration of the trial. The traps were checked every week and

mosquitoes found were removed. The hay infusion in the trap was refilled up to the overflow holes. The traps at the other side of the block were exchanged for fresh traps on a weekly basis for the duration of the tests. The total duration of the study was 8 weeks.

### 2.3 Results

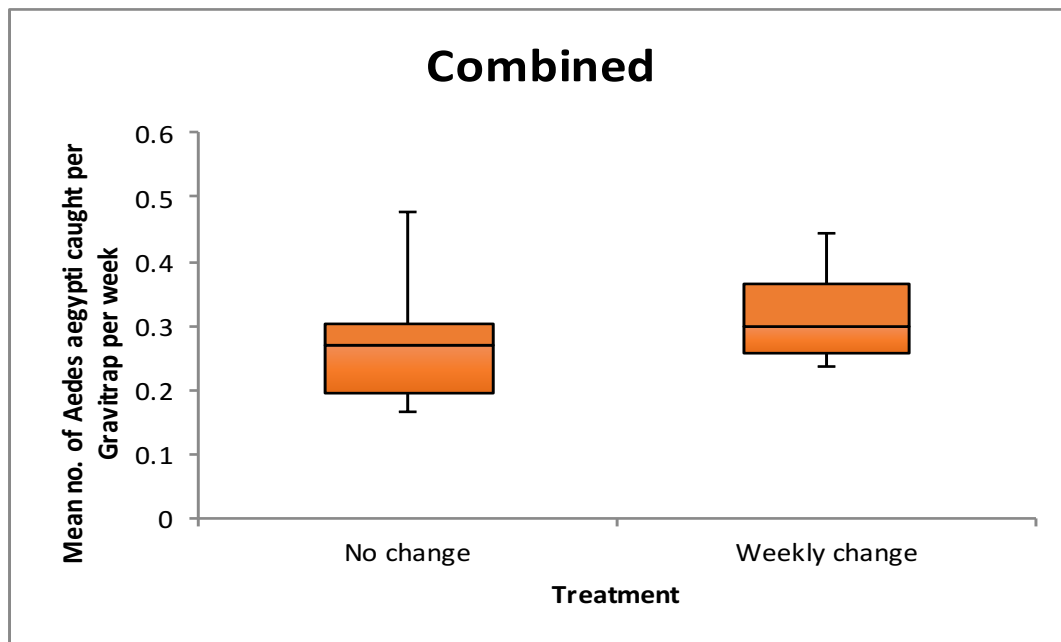


Fig. 10: Comparison of mean female *Aedes aegypti* caught per Gravitrapp per week between the two treatment regimes.

In general, there is no significant difference ( $p>0.01$ ) between the mean *Aedes aegypti* catch rates with and without the weekly fresh trap replacement (Fig. 10). Three of the four sites showed no significant difference ( $p>0.05$ ) between the catch rates of the two testing regimes – Commonwealth Close, Serangoon Central and Jurong East. One of the four sites, Toa Payoh, showed that traps that were exchanged for fresh traps had a significantly higher catch rate ( $p<0.005$ ) than traps that were left at the site for the entire test.

## **2.4 Discussion**

The results of the aged hay infusion experiments showed that the Gravitrap can be left in the field for at least a month without affecting *Aedes aegypti* catch rate. However, the results from the Toa Payoh site did show a significant difference ( $p < 0.005$ ) between the two treatments. This could be attributed to unknown conditions that were present in the site but not in the other sites. It could also indicate that the traps in Toa Payoh accumulate debris at a higher rate as compared with the other sites, which would lead to a loss in catch rate efficiency. That said, Toa Payoh is likely to be the exception, rather than the rule.

## **2.5 Conclusion**

Overall, the Gravitrap can be left in the field with minimal maintenance for at least 4 weeks without sacrificing trapping efficiency. This would provide a useful guide in the development of Gravitrap deployment and maintenance protocols.

## CHAPTER 3

# ISOLATION OF SEMIO-CHEMICAL ATTRACTANTS FROM THE HEADSPACE OF HAY INFUSION SOLUTION USING THE ELECTROANTENNOGRAM DETECTOR (EAD)

### 3.1 Introduction

In addition, to studying the efficacy of aged hay infusion, it is also prudent to determine the VOCs that are directly involved in the attraction of gravid female *Aedes aegypti* mosquito to the ovipositioning sites. Analysis of these VOCs have been shown in different studies to include *n*-heneicosane, 3-methylindole, tetradecanoic acid, nonanoic acid, tetradecanoic acide methyl ester, propyl octadecanoate, indole, 3-methylindole, 4-methylphenol (Reiter 1991, Zwiebel 2004, Seenivasagan 2009, Barbosa 2010, Bohbot 2011, Seenivasagan 2012). That said, the semio-chemicals that induce the ovipositioning behavior of the local *Aedes aegypti* population is relatively unknown. The objective of the second part of the study is to identify the VOCs that attract the female gravid *Aedes aegypti* mosquitoes to oviposit from Timothy hay infusion headspace.

### 3.2 Materials and Methods

#### 3.2.1 Testing of hay infusion headspace via gas chromatography-mass spectrometry (GC-MS)

##### 3.2.1.1 Hay infusion preparation

The container used to ferment the hay consisted of a glass bottle with a plastic lid that was fitted with a rubber septum. The addition of the septum enables the sampling. The hay infusion was prepared by mixing Timothy hay (Tallahassee)

with distilled water to a concentration of 10g/L. Three bottles were prepared concurrently to form a set. A total of three sets were used in this experiment.

### 3.2.1.2 Sampling of hay infusion headspace via Solid-Phase Micro-Extraction (SPME)

Sampling was performed with a Solid-Phase Micro-Extraction (SPME) fibre (Agilent part no. 39186301). The fibre was pre-conditioned in the injection port of the GC-MS for 5 minutes to remove all impurities that might have been bound to the stationary phase. The SPME needle was inserted into the septum on the lid of the bottle. After ensuring that the tip of the needle was halfway in the headspace, the fibre was exposed. The fibre was exposed for five minute in the headspace. After exposure, the fibre was retracted into the needle before the needle is withdrawn from the septum. The needle was then inserted into the injection port of the GC-MS. After insertion, the fibre was exposed. The exposure period was 5 minutes. The fibre was then retracted and the needle removed from the injection port. Sampling was performed every week starting from the end of the first week of fermentation. Sampling was done for 4 weeks. The stationary phase of the fibre was composed of polydimethylsiloxane (PDMS). Each bottle was sampled 3 times with 3 different fibres.

### 3.2.1.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The identification of volatiles organic compounds (VOCs) from the headspace was performed using a 6890N gas chromatograph coupled with a 5975 mass spectrometer (Agilent Technologies). Compounds were separated on a polar capillary column DB-1 (30m × 0.25mm inner diameter coated with chromatographic film with 0.25um film thickness)(Agilent Part No: JW-DB-

1MS). The carrier gas used was helium (36cm/s). The oven temperature was held at 50°C for 3min and then increased to 280°C at a rate of 10°C/min and held for 5min.

The identity of the VOCs was determined using references from mass spectral libraries (NIST2.0, Agilent Technologies)

### 3.2.2 Testing of compounds using electro-antennogram detector (EAD)

#### 3.2.2.1 Preparation of the mosquito head

F2-5 generation female *Aedes aegypti* mosquitoes were colonized in the insectary at 28°C ± 1°C and 80% ± 10% relative humidity. The female mosquitoes were fed with 10% sucrose with Vitamin B. The female mosquitoes used for the analysis were 5-7 days old and bloodfed 48h prior. The female mosquitoes were anaesthetized using carbon dioxide gas. After immobilization, the heads were pulled from the body. The proboscises were removed and the tips of the antennae were excised using an iris scissors.

#### 3.2.2.2 Mounting of the head

Electrode gel (Spectra 360, Parker Laboratories, Orange, NJ. USA) was applied on the two prongs of the probe. The base of the head was mounted onto the gel on the indifferent electrode. The tips of the antennae were inserted into the gel of the recording electrode.

#### 3.2.2.3 Stimulus preparation

A piece of filter paper (Whatman, GE Healthcare, Maidstone, Kent, UK) measuring 1cm by 5cm was folded in a zig-zag manner. The folded filter paper was partially inserted into the tip of a disposable Pasteur pipette. Test compounds were diluted with hexane to concentrations of 0.1ppm, 10<sup>2</sup>ppm and

10<sup>4</sup>ppm. 100uL of the test compound diluted in hexane solvent was applied onto the tip of the filter paper. The solvent was allowed to evaporate before the filter paper was inserted completely. Control pipettes included a clean Pasteur pipette, a pipette with filter paper only and a pipette with filter paper and solvent. The airflow was delivered by a stimulus controller (Syntech GmbH, Buchenbach, Germany), through a humidifier at 120ml/min over the antennal preparation that was inserted into a borosil glass tube. Stimulation was achieved by introducing a stimulus puff through the tip of the Pasteur pipette introduced into a side port along the airflow.

#### 3.2.2.4 Electroantennogram experiments

Each replicate consisted of a new mosquito antennal preparation. Seven replicates (n=7) were performed for each concentration. Each recording session started with the introduction of a hexane control. The test compounds were then introduced in random order. The interval between stimulations was 10 seconds. Stimuli were introduced into the air stream via a side port 10cm from the borosil glass tube.

The electroantennogram signals were imported into a computer via an intelligent data requisition controller (IDAC). Signals were processed using EAG software version 4.4 (Syntech, Hilversum, The Netherlands). The EAG signals were zeroed and compared to solvent controls based on maximum EAG amplitude.

### 3.3 Results

#### 3.3.1 Testing of hay infusion headspace via gas chromatography-mass spectrometry (GC-MS)

Compound	MW	Chemical formula	R <sub>t</sub>
2-Tetradecene, (E)-*	196	C <sub>14</sub> H <sub>28</sub>	7.589
2-Dodecene, (Z)-	168	C <sub>12</sub> H <sub>24</sub>	7.596
Cyclopropane, 1-methyl-2-octyl-	168	C <sub>12</sub> H <sub>24</sub>	7.599
1-Undecanol*	172	C <sub>11</sub> H <sub>24</sub> O	7.602
7-Hexadecene, (Z)-	224	C <sub>16</sub> H <sub>32</sub>	7.602
3-Hexadecene, (Z)-	224	C <sub>16</sub> H <sub>32</sub>	7.605
5-Undecene	154	C <sub>11</sub> H <sub>22</sub>	7.605
Cyclopropane, 1-heptyl-2-methyl-	154	C <sub>11</sub> H <sub>22</sub>	7.608
1-Tetradecene	196	C <sub>14</sub> H <sub>28</sub>	7.611
1-Dodecanol*	186	C <sub>12</sub> H <sub>26</sub> O	7.622
Cyclododecane*	168	C <sub>12</sub> H <sub>24</sub>	7.627
Phenol, 2-ethyl-4-methyl-	136	C <sub>9</sub> H <sub>12</sub> O	7.635
Phenol, 3-(1-methylethyl)-	136	C <sub>9</sub> H <sub>12</sub> O	7.635
Phenol, 4-ethyl-2-methyl-	136	C <sub>9</sub> H <sub>12</sub> O	7.635
Phenol, 2-(1-methylethyl)-	136	C <sub>9</sub> H <sub>12</sub> O	7.640
Phenol, 2-ethyl-5-methyl-	136	C <sub>9</sub> H <sub>12</sub> O	7.644
Benzene, 1-ethyl-4-methoxy-	136	C <sub>9</sub> H <sub>12</sub> O	7.648
Phenol, 3-ethyl-5-methyl-*	136	C <sub>9</sub> H <sub>12</sub> O	7.651
1-Undecene*	154	C <sub>11</sub> H <sub>22</sub>	7.660
1-Nonene*	126	C <sub>9</sub> H <sub>18</sub>	7.668
Phenol, 4-ethyl-3-methyl-	136	C <sub>9</sub> H <sub>12</sub> O	7.671
Cyclopropane, 1-pentyl-2-propyl-	154	C <sub>11</sub> H <sub>22</sub>	7.709
2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.)-	194	C <sub>13</sub> H <sub>22</sub> O	10.237
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	204	C <sub>15</sub> H <sub>24</sub>	11.714
1H-3a,7-Methanoazulene, octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	204	C <sub>15</sub> H <sub>24</sub>	11.891
Epizonarene	204	C <sub>15</sub> H <sub>24</sub>	11.899
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	204	C <sub>15</sub> H <sub>24</sub>	12.399
Butylated Hydroxytoluene*	220	C <sub>15</sub> H <sub>24</sub> O	12.758
Phenol, 2,4,6-tris(1-methylethyl)-	220	C <sub>15</sub> H <sub>24</sub> O	12.765
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	204	C <sub>15</sub> H <sub>24</sub>	12.911
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	204	C <sub>15</sub> H <sub>24</sub>	12.914
Diethyl Phthalate*	222	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	13.485

Table 1: List of 34 compounds isolated from Timothy hay infusion solution. 9 compounds to be tested indicated with (\*).

From the headspace of the Timothy hay infusion solution, 34 compounds were identified using the gas chromatograph-mass chromatograph (GC-MS)(Table 1). Of these 34 compounds, 9 were selected for testing in the electroantennogram detector (EAD) based different factors including, commercial availability, isomeric stability, complexity of synthesis, etc. These 9 compounds are 2-tetradecene ( $R_t = 7.611$ min), 1-undecanol ( $R_t = 7.602$ min), 1-dodecanol ( $R_t = 7.622$ min), cyclododecane ( $R_t = 7.627$ min), 3-ethyl-5-methylphenol ( $R_t = 7.651$ min), 1-undecene ( $R_t = 7.660$ min), 1-nonene ( $R_t = 7.668$ min), butylated hydroxytoluene ( $R_t = 12.758$ min) and diethyl phthalate ( $R_t = 13.485$ min) (Fig 11).

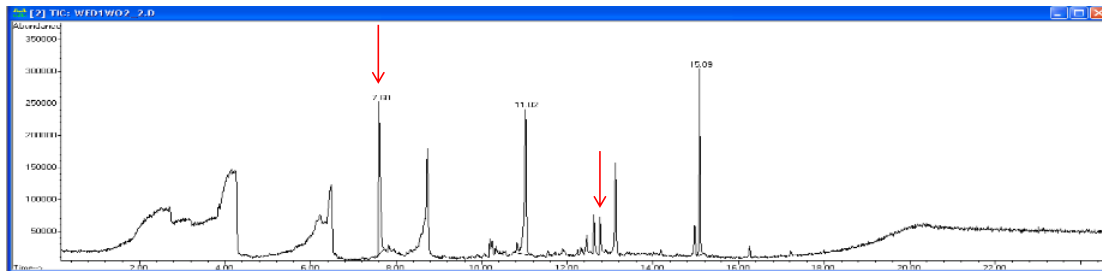


Fig. 11: Sample of gas chromatograph of Timothy hay infusion headspace. Red arrows indicate the peaks where the compounds were found.

### 3.3.2 Testing of compounds using electro-antennogram detector (EAD)

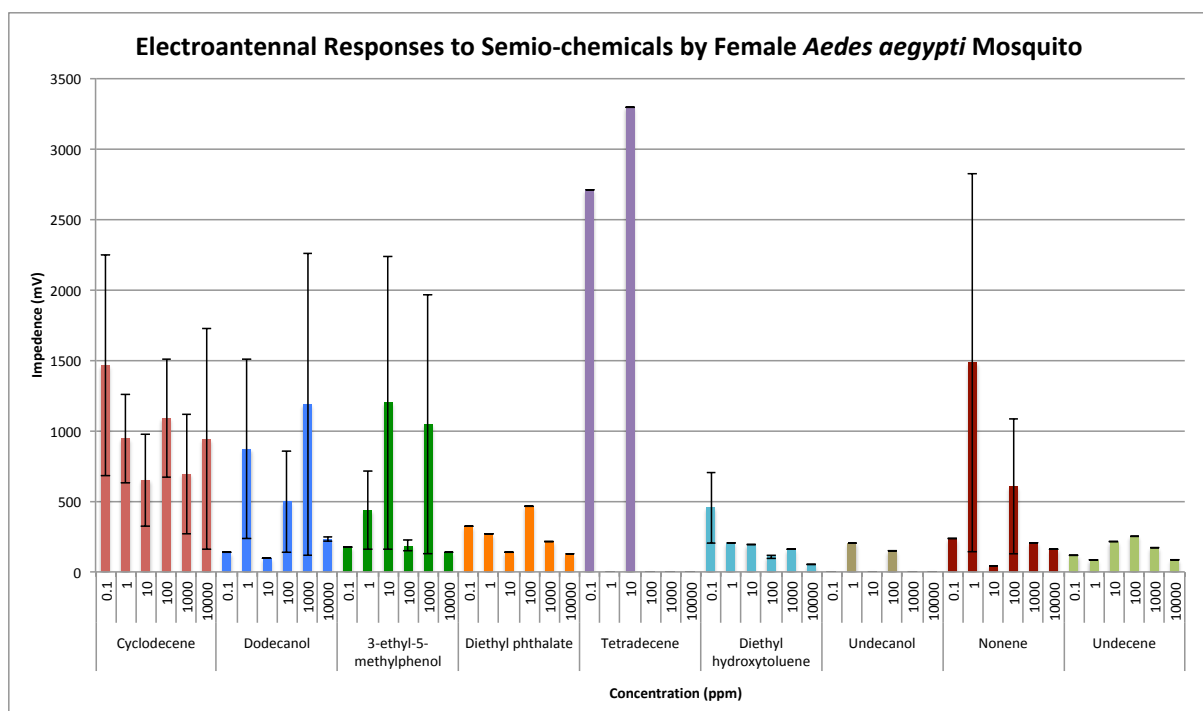


Fig. 12: Electroantennogram response of *Aedes aegypti* to the 9 compounds identified in Timothy hay infusion headspace.

The most consistent responses recorded from the antennal preparations were from the compounds cyclodecene, dodecanol, nonene and 3-ethyl-5-methylphenol, with cyclodecene elucidating the highest responses (Fig. 12). The highest responses from the *Aedes aegypti* antennal preparation were for the compound tetradecene at concentrations of 0.1ppm and 10ppm. Unfortunately,

these responses were only observed for one antennal preparation and could not be repeated with the other six.

### **3.4 Discussion**

Out of 34 compounds detected from the headspace of the Timothy hay infusion, nine were tested. The various compounds elicited a variety of responses at different concentrations. There was no common concentration at which all the compound elicited the maximum response. Some studies have detected responses at slightly lower concentrations (Ganesan 2006, Seenivasagan 2012). Whilst others have detected responses at similar concentrations (Seenivasagan 2009). As there are genotypic differences between mosquitoes of the same species from different regions of the world (Gloria-Soria 2016), one could expect local mosquito species to be more sensitive to semio-chemicals that are typical to the local environment.

None of the nine compounds isolated in this study have been previously described. Of the nine, only cyclodecene, dodecanol, nonene and 3-ethyl-5-methylphenol elicited a consistently significant response across the various concentrations. While tetradecene elicited the highest response, the result could not be replicated. This suggests that the responses were anomalous. Further testing will be required to determine if the electrophysiological responses will translate to behavioural attraction in the mosquito. Although the other compounds did not elicit significant responses from the antennal preparations, they might still offer clues to other possible attractants. These compounds could serve as pre-cursor or intermediate compounds of the bacterial fermentation that will eventually give rise to the VOCs that entice the gravid female to oviposit.

Each of the nine compounds tested possess unique characteristics and different industrial applications. 1-nonene is highly flammable and used in the production

of detergents. 1-undecanol is naturally found in fruits (apples, bananas), butter, eggs, cooked pork and has a floral citrus odor with a fatty taste and is commonly used as food flavorings. 1-dodecanol possesses a floral odor and is obtained from palm kernel or coconut oil. It has industrial uses that include the making surfactants, lubricating oils, pharmaceuticals and as food additive, emollient. Cyclododecane is used in production of flame retardants, detergents and as a volatile binding medium. Butylated hydroxytoluene is used in food additives, to prevent oxidation, household product additive, industrial additive, personal care product, pesticide ingredient, plastic/rubber ingredient and medical/veterinary science. Diethyl phthalate has low toxic potential and is commonly used as binders of cosmetics and fragrances and as plasticizers, detergent bases and aerosol sprays.

It should be noted that some of the compounds isolated – 1-undecanol, 1-dodecanol, cyclododecane, 3-ethyl-5-methylphenol and butylated hydroxytoluene – do carry a risk of bio-accumulation in aquatic systems (Sigma-Aldrich, St. Louis, Missouri, USA). Most of the compounds will cause irritation to the skin, eyes and respiratory tract at stock concentrations. These attributes should be taken into consideration during the integration into the Gravitrap.

### **3.5 Conclusion**

Of the 9 compounds tested, consistent responses were obtained from the antennal preparation of the female *Aedes aegypti* mosquito for the compounds cyclodecene, dodecanol, nonene and 3-ethyl-5-methylphenol. Further investigations with oviposition *Aedes aegypti* mosquitoes will determine if these responses translate to attraction.

## CHAPTER 4

### ROLE OF GRAVITRAPS IN THE MANAGEMENT OF DENGUE CLUSTERS

\*The work in this Chapter was published in the American Journal of Tropical Medicine and Hygiene (Lee, C., Vythilingam, I., Ching, C.-S., Razak, M. A. A., Tan, C.-H., Pok, K.-Y., Ng, L.-C. Gravitrap for Management of Dengue Clusters in Singapore, 2013)

#### 4.1 Introduction

Most (85%) of the population in Singapore dwells in high-rise apartments (Liew C. 2004). This population faces regular outbreaks of dengue because of its high level of urbanization, high population density, low herd immunity, intense travel pattern, presence of *Aedes* vectors (Ng 2009) and possibly natural adaptation/selection of dengue viruses (DENVs) under the pressure of small populations of *Aedes* mosquitoes (Lam 2012). Source reduction and environmental management are the hallmarks of the nation's vector control strategy, which decreased House Index (HI) from more than 50% in the 1960s and 1970s to 0.3% in the 2000s. Dengue control programs are challenged by the lack of cost effective tools that could trap adult *Aedes* mosquitoes and provide a reasonable population estimate of *Aedes* mosquitoes in the environment.

This study sets out to determine their effectiveness in collecting dengue-infected mosquitoes and to complement source reduction efforts in controlling transmission.

#### 4.2 Materials and Methods

A total of 551 Gravitrap were set up in 11 dengue cluster areas (June–November 2010). A cluster is defined as two or more cases of dengue that

reside within 150 meters of each other and have disease onset dates that are within 14 days of each other. All Gravitraps were set up in corners where mosquitoes were likely to be resting or breeding (i.e. cool, shaded areas or around potted plants), along the common corridors of the apartments or backyards of landed houses. The Gravitraps were placed on the ground level, 2nd and 6th floors, which have long stretches of corridors that span across the block, and 4–6 traps were placed in each apartment block with reported dengue cases.

The Gravitraps were checked and hay infusion water was replaced every 3–4 days. Abdomens of trapped *Aedes* mosquitoes were pooled into groups of five and screened for DENV by using the Dengue NS1 Ag Strip (Bio-Rad, Marnes-la-Coquette, France). Individual mosquitoes from NS1-positive pools were tested for DENV by using a real-time polymerase chain reaction (Lai 2007).

### 4.3 Results

*Aedes* mosquitoes were caught in all but one of the clusters (Table 2). Dengue virus–infected *Aedes aegypti* were detected in half ( $n = 6$ ) of the clusters (Figure 13). Gravitraps set near or before the peak of dengue cluster trapped at least one DENV-infected *Aedes aegypti* in each cluster area. Conversely, placing of Gravitraps towards the end of the cluster usually failed to trap any DENV-infected *Aedes aegypti*, as seen in the Clementi West Ave 2, Lorong M Telok Kurau, Holland Hill, and Serangoon Ave 4 clusters. All clusters, except Telok Blangah and Serangoon Ave 2, closed within five weeks of the Gravitrapp deployment.

A total of 382 mosquitoes were trapped and *Aedes aegypti* was the predominant

species (73.3%), and *Aedes albopictus* comprised 18.6%. Only three *Culex quinquefasciatus* mosquitoes were caught. The rest were damaged *Aedes* specimens that could not be speciated. Among trapped *Aedes aegypti*, 15 were infected with DENV, and positivity rates ranged from 2.8 to 13.6% for each location (Table 2). No infected *Aedes albopictus* was found, confirming the primary role of *Aedes aegypti* in dengue transmission in Singapore. Real-time reverse transcription–polymerase chain reaction results for the mosquitoes also concurred with the dengue serotype in infected patients from the same cluster (Table 2). In the Bishan St 24 and the Serangoon Ave 2 clusters, DENV-positive mosquitoes were detected beyond the period of reported transmission, suggesting that active transmission could still be ongoing despite no additional reporting of cases.

Cluster Area	No. of dengue cases (Dengue serotype of patients)	No. of traps deployed	No. of weeks deployed	Number of mosquitoes caught					No. of mosquitoes positive (Dengue virus serotype)	Percentage of <i>Ae. aegypti</i> positive
				<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Ae. sp</i>	<i>Cx. sp</i>	Total		
<i>Central Singapore</i>										
Cairnhill	106 (Den-2)	40	9	9	25	0	0	34	1 (Den-2)	11.1
Telok Blangah	149 (Den-2)	104	7.5	44	14	10	2	70	6 (Den-2)	13.6
<i>Eastern Singapore</i>										
Lorong M Telok Kurau	36 (Unknown)	10	3.5	0	0	0	0	0	0	
Livia Pasir Ris	57 (Unknown)	14	3	7	0	0	0	7	0	
Pasir Ris St 52	52 (Unknown)	45	4	10	3	0	0	13	1 (Den-2)	10.0
<i>Western Singapore</i>										
Clementi West Ave 2	46 (Unknown)	36	7	13	3	5	0	21	0	
Holland Hill	40 (Unknown)	20	4.5	2	1	0	0	3	0	
<i>North-eastern Singapore</i>										
Serangoon North Ave 4	36 (Unknown)	44	6	20	8	2	1	31	0	
Serangoon Ave 2	71 (Den-1)	85	10	97	14	3	0	134	4 (Den-1)	4.1
Ang Mo Kio Ave 8	17 (Den-2)	58	5.5	36	1	1	0	38	1 (Den-2)	2.8
Bishan St 24	49 (Den-2)	41	6.5	24	1	4	0	29	2 (Den-2)	8.3

\*No. of traps deployed in Telok Blangah increased from 57 to 104 over 7.5 weeks. No. of traps deployed in Cairnhill increased from 25 to 40 over 9 weeks. Table shows final numbers achieved.

Table 2: Summary of 11 dengue cluster areas where the Gravitraps were deployed and the number of mosquitoes trapped

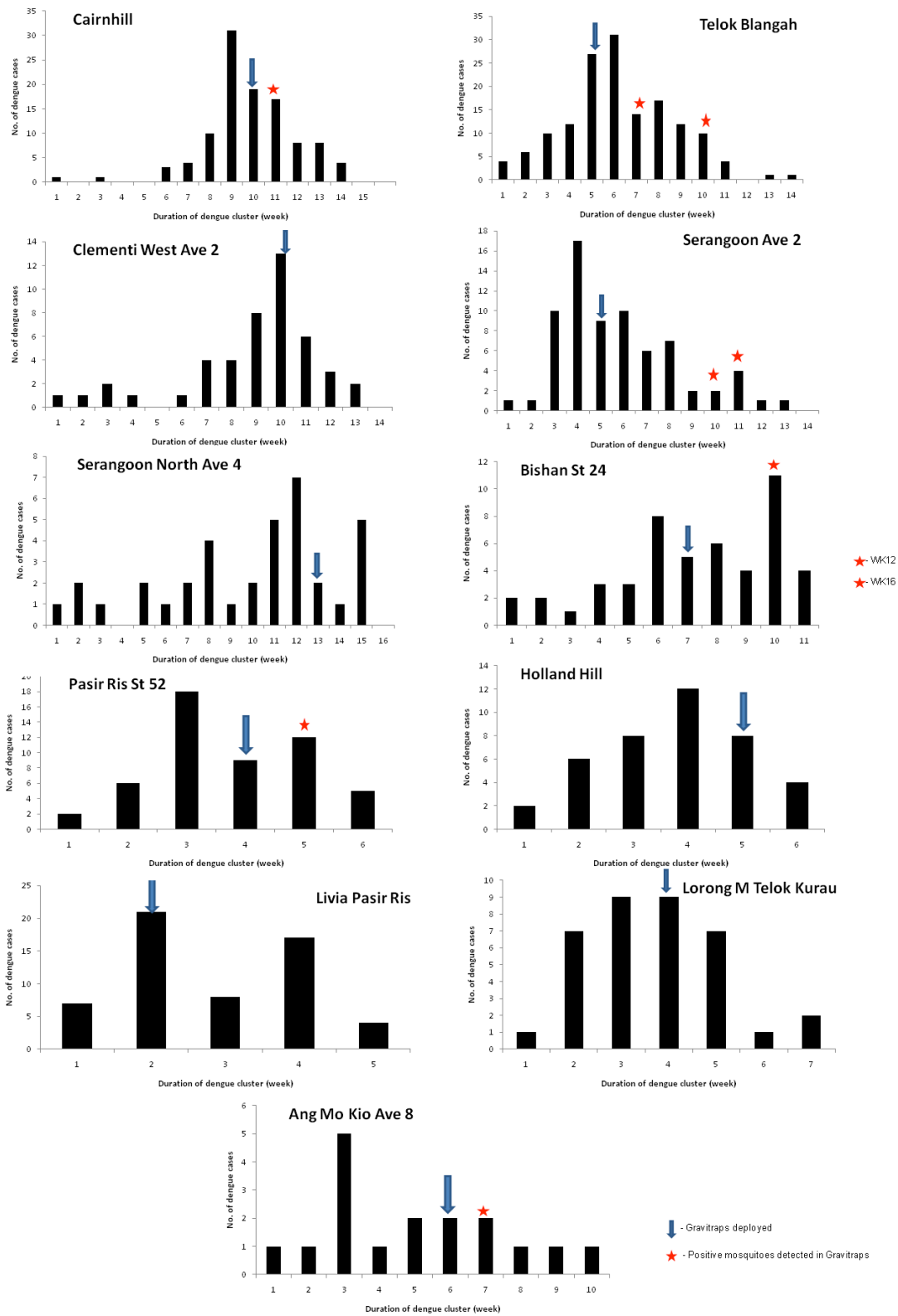


Figure 13: Charts of dengue cases for the duration of each cluster where Gravitraps were deployed. Arrows represent when the Gravitraps were introduced into the cluster. Stars represent weeks where dengue-positive mosquitoes were caught in Gravitraps.

## 4.4 Discussion

This result highlights the need for continued vigilant surveillance of an area even after transmission has apparently ceased. Because of the complex interplay of multiple factors in dengue transmission and the variability in ongoing cluster control measures, it is unlikely that we can definitively demonstrate the contribution of the Gravitraps in the intervention of any dengue transmission.

Nevertheless, because female *Aedes aegypti* are known to take multiple blood meals during each gonotrophic cycle, removal of a single infected mosquito could result in the prevention of at least 3–5 persons from becoming infected with dengue, and the avoidance of subsequent generation of cases. Moreover, because one *Aedes* female mosquito could lay approximately 80–100 eggs after a single blood meal (Woke 1955), every female mosquito trapped could also prevent emergence of tens or hundreds of progenies.

Globally, current transmission control relies on insecticide sprays, which are plagued by many issues: development of insecticide resistance, toxicity to non-target insects, and limited accessibility of homes to target indoor dwelling *Aedes aegypti*. The Gravitraps are an attractive, environmentally friendly, and convenient tool to lure and remove *Aedes* mosquitoes. The challenge in its use for control of dengue transmission lies in the timing of deployment. Early deployment would result in resources being thinly spread, and late deployment could render it ineffective. The chance and rate of trapping infected *Aedes* mosquitoes is influenced by factors that include the extent and duration of transmission (reflected by number of dengue cases), location of the Gravitraps, time of deployment (Figure 8), competition from cryptic oviposition sites in the environment, and success of community participation in source reduction. Nevertheless, a large number of the Gravitraps (more than 40 or at least five

traps in each apartment block) are needed for trapping of any infected adult *Aedes* mosquito. The Gravitrap may also provide a measure of the *Aedes* population, and the infective status of trapped *Aedes* mosquitoes could assist in situational risk assessment and operational decision-making in controlling outbreaks. In Singapore, these traps have served as an evaluation tool to ascertain effectiveness of ground mosquito control measures. Although there have been a number of sticky traps developed and reported, such as the MosquiTrap™ (Gama R.A. 2007) and the CDC gravid trap (Reiter 1983), the hallmark of the Gravitrap is its simplicity and a physical barrier to prevent emergence of adult mosquitoes in case eggs are laid before trapping of gravid females. The simplicity and fail-safe design are particularly important to enable it to be deployed by the community, an approach consistent with Singapore's advocacy for community participation.

#### **4.5 Conclusion**

This study shows the value of the Gravitrap as a tool to monitor *Aedes* mosquito populations in dengue clusters in Singapore. At the same time, it has the ability to trap female *Aedes* mosquitoes to enable further molecular investigations into the prevailing dengue virus strains. The study also highlighted the need for constant vigilance in areas where clusters have closed.

## CHAPTER 5

### SUPPRESSION OF *Aedes* POPULATIONS USING GRAVITRAPS

#### 5.1 Introduction

The spread of mosquito-borne diseases around the globe has led to many governments and institutions focusing their efforts into controlling the vectors of these diseases. One of the strategies employed to achieve this is the use of oviposition traps.

Oviposition traps target gravid female mosquitoes that have taken a blood meal and are ready to lay their clutch of eggs. These traps are typically smaller in size to the host-seeking traps and have no mechanical parts, making them cheaper to produce. They use water with organic matter that act as olfactory cues for the female mosquitoes. Oviposition traps have been used in many parts of the world effectively (Zeichner 1999, Williams 2007, Maciel-de-Freitas 2008, Ritchie 2008).

The recent push to suppress the mosquito population has led to the development of two distinct types of lethal oviposition traps. The types are the lethal ovitraps and the autocidal ovitraps.

The lethal ovitraps lure the gravid female mosquito into a trap laced with an insecticide, such as deltamethrin (Perich 2003, Sithiprasasna 2003) & bifenthrin (Rapley 2009). They operate by luring the mosquito to lay eggs in the ovitraps. While doing so, they pick up a lethal dose of the respective insecticides which will kill them and any other mosquitoes they come into contact with.

In the autocidal ovitraps, the gravid female mosquito gets caught on an adhesive surface whilst trying to lay eggs within the trap (Barrera 2014, Degener 2015). The Gravitrap used in this study falls into this category.

The successes of the various trapping regimes have been varied. Some studies have reported reductions of up to 88% (Barrera 2014), while others have not found any significant reduction in populations (Degener 2015, Cornel 2016).

The objective of this study is to determine the impact of mass deployment of Gravitrap on the local *Aedes* populations.

## **5.2 Materials & Methods**

### *5.2.1 Mosquito control study*

In this experiment, non-autocidal ovitraps were used as a surveillance tool to determine mosquito population density. The ovitraps used consisted of a container with a float place inside. Two wooden paddles were placed vertically on the float. The *Aedes* mosquitoes lay their eggs on these floats (Lok 1977).

The sticky adult Gravitrap is a black adulticidal sticky trap that targets gravid female *Aedes* mosquitoes using an infusion of hay (Lee 2013). It has a sticky inner lining on which to trap the ovipositioning *Aedes* mosquito.

Hay-infusion water is used in both traps as an attractant for the egg-laying female mosquitoes. The hay was obtained from a local grass farm. The hay infusion stock solution was prepared by soaking the hay in a plastic tub of tap water to a concentration of 10g/L. The plastic tub was covered with a lid and sealed with cellophane wrap and left to ferment for 5-7 days before use. After

fermentation, the stock solution was filtered through a wire mesh strainer to remove most of the plant matter. The filtered stock solution was then diluted to a ratio of 1 part of stock solution to 4 parts of tap water to produce the working solution. This working solution was used in the traps.

### 5.2.2 Study site

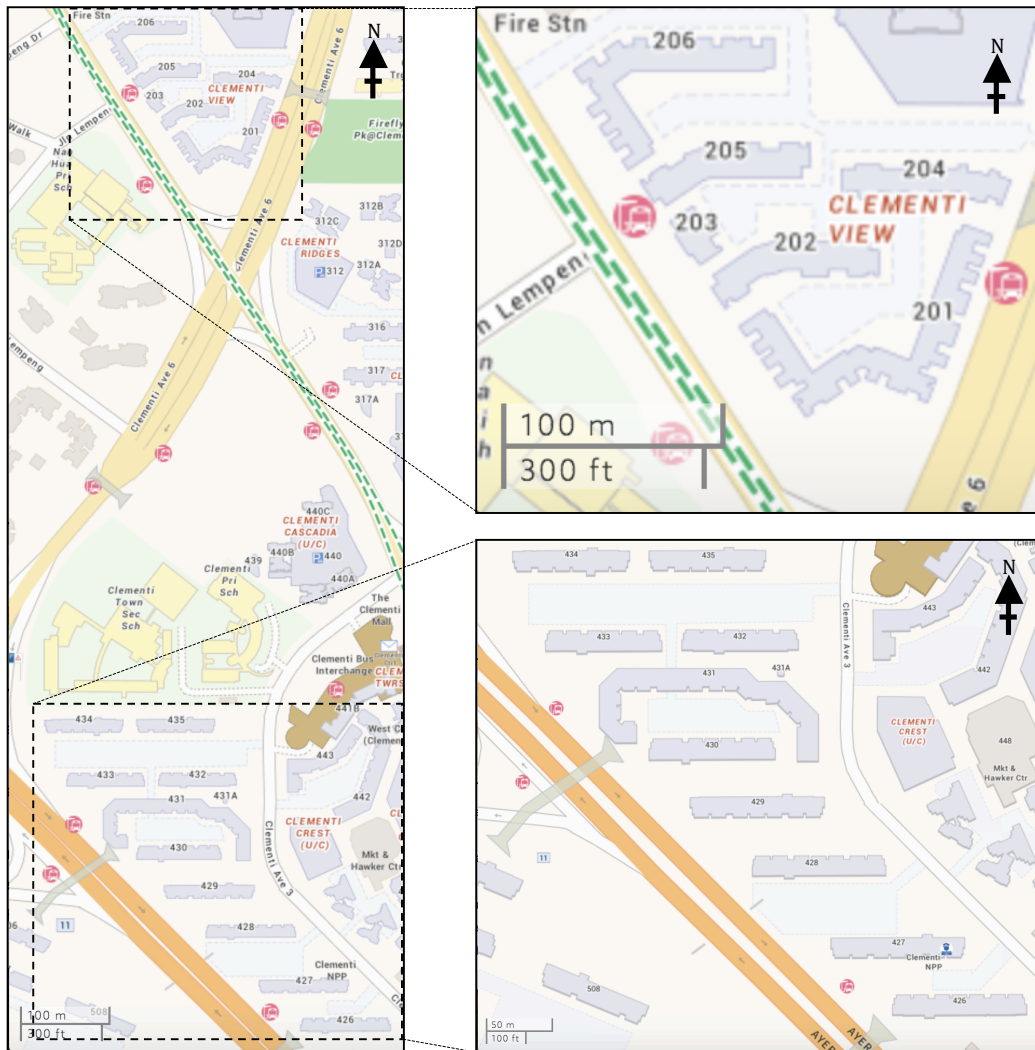


Fig. 14: Location of control and test sites in Clementi, Singapore.

In the 1960s, the Housing & Development Board (HDB) was established to build and provide sanitary and affordable housing for the population of

Singapore. To date, with each flat housing an average of 5 individuals, more than a million flats have been built to house over 80% of the population of Singapore ([www.hdb.com.sg](http://www.hdb.com.sg)). It is, therefore, a priority to focus on the suppression of the *Aedes* population in such an area.

An area of about 0.07 km<sup>2</sup> consisting of nine HDB blocks were selected in the western region of Singapore (Clementi Ave 3) for this experiment, hereby known as the test site. These blocks were chosen based on their relative similarity in layout and relatively high mosquito breeding incidence. Each of these blocks were 12-13 storeys high, comprising 12-13 apartment units per floor. Another location (Clementi Ave 6) in the same region of about 0.03 km<sup>2</sup>, consisting of six blocks, 0.7 km from the test site was chosen. This site will hereby be known as the control site (Fig. 14). The test and control sites are approximately 850m apart.

The entire duration of the experiment was seven months, with the pre-deployment phase occurring between May and June and the deployment phase lasting from July to November. Ovitrap were placed on both control and test blocks at a ratio of one trap per 25 residential units. Ovitrap were placed throughout the duration of the experiment in both sites. The Gravitrap were placed in the test site two months (pre-deployment phase) after the ovitrap were introduced. The Gravitrap were placed only in the test blocks at a ratio of one trap per four residential units. The Gravitrap were placed on every level along the corridors. Three Gravitrap were placed on each level - one at each end of the block and one in the middle. No Gravitrap were placed on the ground level as this was a common area with no residential units. This adds up to three traps per storey and 33 traps per block and amounts to a total of 303 Gravitrap.

The ovitraps and the Gravitraps were checked weekly. Paddles from the ovitraps were removed back to the laboratory for counting of eggs. Larvae from the ovitraps were also removed. Mosquitoes that were stuck to the Gravitraps were also removed and brought back to the laboratory for identification. Traps that had insufficient hay-infusion water were re-filled. Traps that had been tampered with or are missing were replaced.

### 5.2.3 Data analysis

All data analysis were performed using IBM SPSS Version 21 (Corporation 2012).

## 5.3 Results

### 5.3.1 Correlation of mosquitoes caught and eggs laid

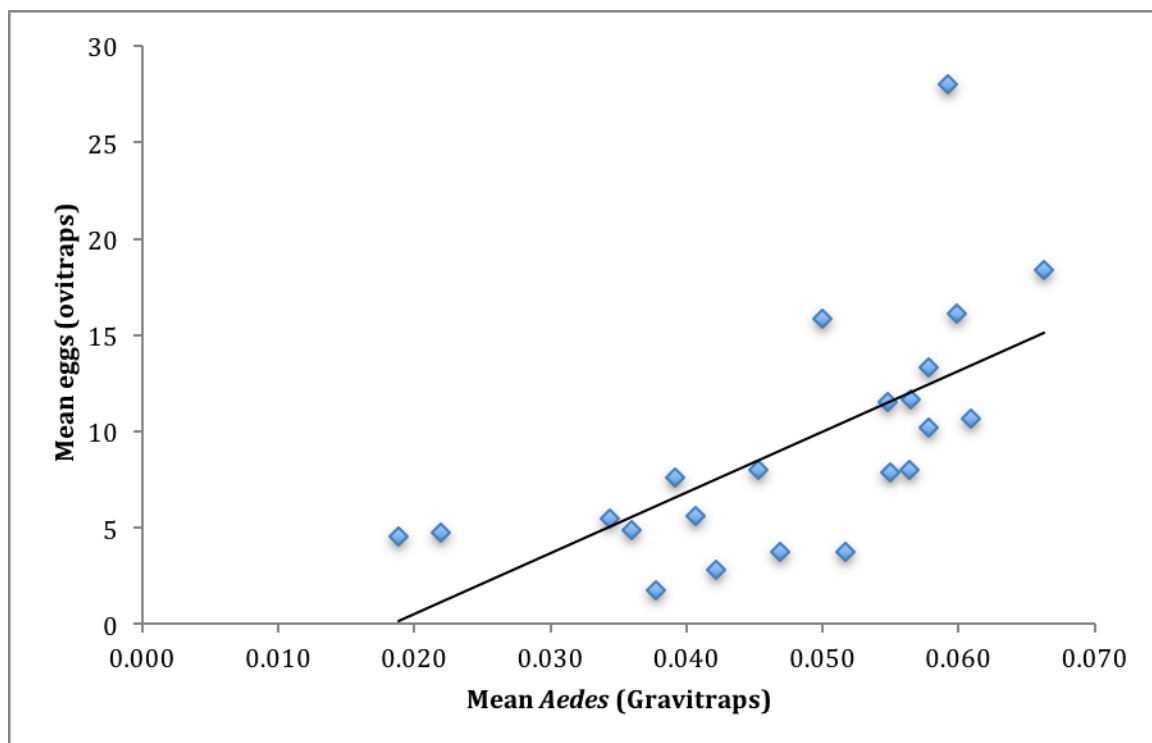


Figure 15: Scatterplot of the correlation of mean eggs in ovitraps and mean *Aedes* in Gravitraps ( $r = 0.64$ ).

A comparison was done to see if there was any correlation between the ovitraps and the Gravitraps (Fig. 15). A significantly positive correlation ( $r = 0.64$ ) was found between the mean eggs found in the ovitraps and the mean *Aedes* mosquitoes found in the Gravitraps

### 5.3.2 Control site

After a sampling period of seven months, a total of 31 ovitrap collections were made on a weekly basis. This yielded a total of 21,341 eggs, with an average ovitrap positivity rate of 62% and an average of 15.1 eggs per ovitrap.

### 5.3.3 Test site

During the pre-deployment period, the ovitraps collected a total 25,048 eggs over eight collections. The average number of eggs per ovitrap was 27.57, with an average ovitrap positivity rate of 88.30%. In comparison, the control site yielded a total of 8,430 eggs, an average of 23.36 eggs per ovitrap and an average ovitrap positivity rate of 74.14% over the same period.

During the deployment phase, the ovitraps collected a total of 25,504 eggs over 22 collections. The average number of eggs per ovitrap was 9.48, with an average ovitrap positivity rate of 53.28%. In comparison, the control site yielded a total of 12,804 eggs, an average of 12.69 eggs per ovitrap and an average ovitrap positivity rate of 59.42% over the same period.

The Gravitraps were able to catch a total of 682 mosquitoes over 22 collections, out of which 550 were *Aedes aegypti* and 100 were *Aedes albopictus*. 23 were *Aedes* mosquitoes that could not be speciated because of age or damage. There were only nine *Culex* mosquitoes caught during the entire duration of the

experiment. The maximum number of mosquitoes caught in a Gravitrap in a week was three.

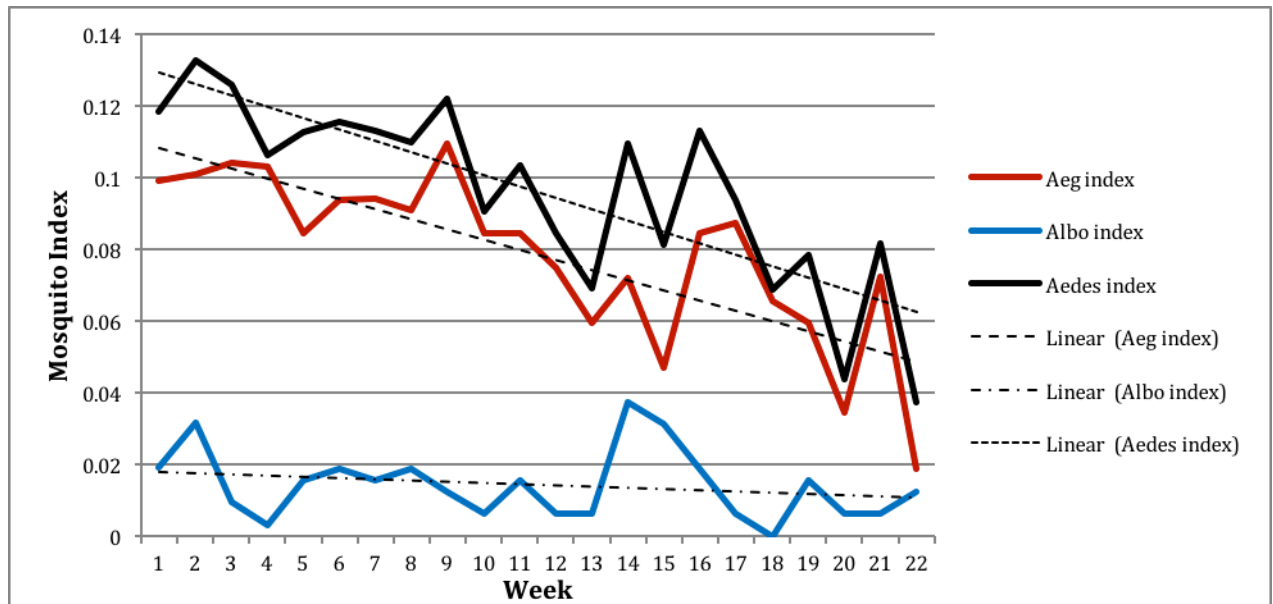


Fig. 16: Comparison of Gravitrap *aegypti* index and Gravitrap *albopictus* index with the overall31 Gravitrap *Aedes* index.

A comparison was made between the overall Gravitrap *Aedes* index ( $GI_{Aed}$ ) and the Gravitrap *aegypti* index ( $GI_{aeg}$ ) and the Gravitrap *albopictus* index ( $GI_{albo}$ ) (Fig. 16). It was observed that there was a correlation between the overall  $GI_{Aed}$  and the  $GI_{aeg}$ .  $GI_{albo}$  had no correlation with either of the other indices. During the deployment period, a distinct decline in both the  $GI_{Aed}$  and  $GI_{aeg}$  was observed.  $GI_{albo}$  did not show any marked decline over the same period.

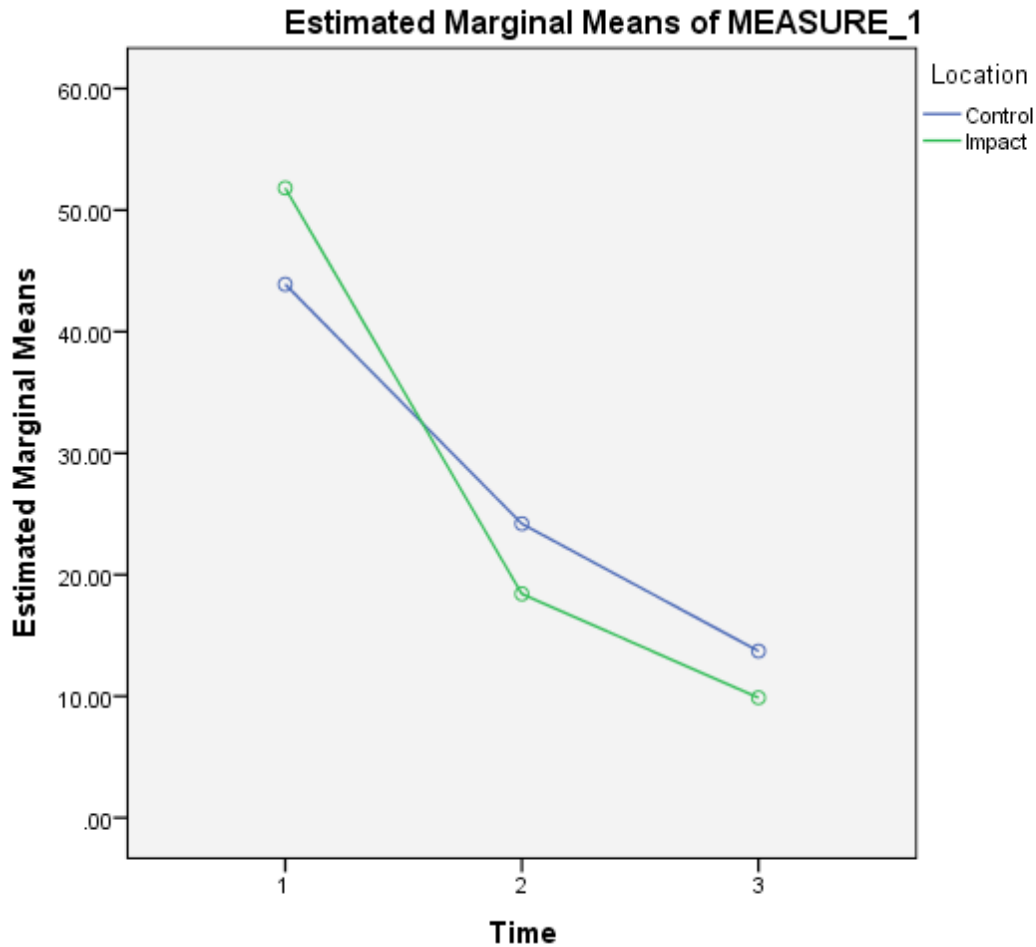


Fig. 17: Repeated measure ANOVA analysis of Control site vs Impact site.

A repeated measures ANOVA analysis revealed that there were significant differences in mean number of eggs between the pre-deployment, during deployment and post-deployment periods ( $p < 0.001$ ) for both control and test. Separate univariate ANOVA analyses of the control and test sites yielded similar results ( $p < 0.001$ ). Although the mean number of eggs caught at the test site was higher than the control site during the pre-deployment period and subsequently became lower, the differences in mean number of eggs was not significantly different between the two sites at all three time-points (Fig. 17).

### 5.3.4 Weather correlations

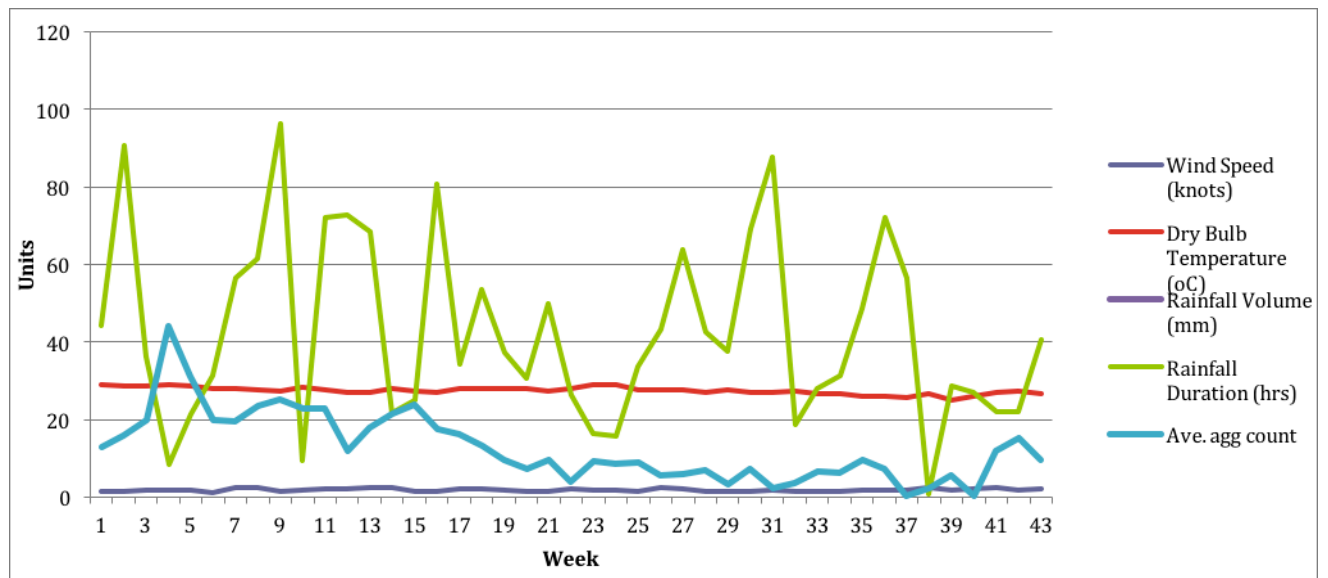


Fig. 18: Comparison of environmental factors – wind speed, dry bulb temperature (DBT), rainfall duration (RF Dura.) and rainfall volume (RF Vol) - with average ovitrap egg counts. Units for each factor are stated in brackets in the legend.

The average egg counts of the ovitraps in the control site was compared to various weather data, such as wind speed, dry bulb temperature, rainfall volume and duration (Fig. 18). Spearman's Rank Order correlation analysis determined that there was a significant correlation between the average egg counts from the ovitraps and dry bulb temperature ( $r_s = 0.498$ ,  $p = 0.001$ ). There was no significant correlation with wind speed, rainfall volume or duration.

### 5.3.5 Vertical stratification

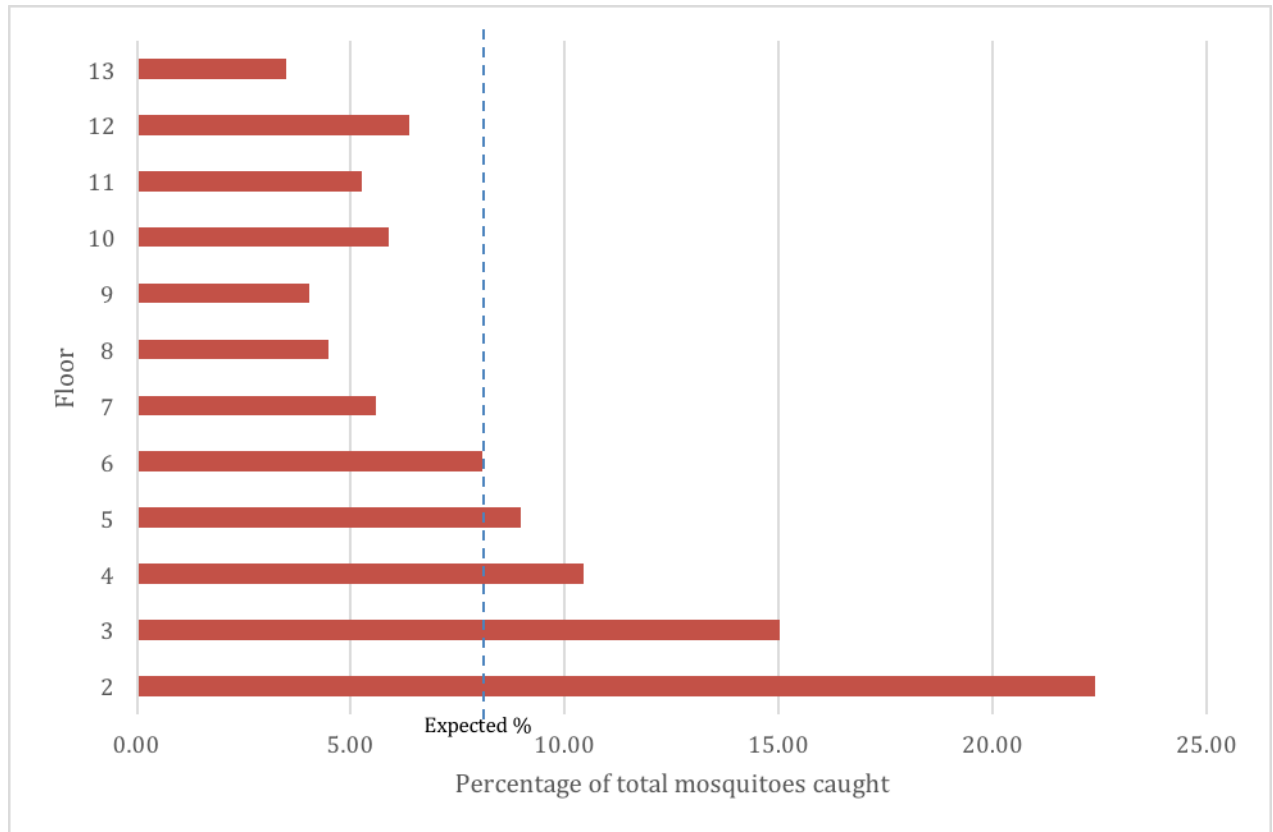


Fig. 19: Vertical distribution of mosquitoes caught by percentage. Dashed line represents the expected percentage (8.33%) if distribution were to be equal.

Analysis on the vertical distribution of mosquitoes caught in the Gravitraps was performed to determine if the distribution was even on all floors (Fig. 19). The Gravitraps on the lower floors (2-6) (64.91%) were observed to have a higher catch rate than the Gravitraps on the higher floors (7-13) (35.09%). This uneven distribution of mosquitoes was found to be significantly different (Fig. 3;  $\chi^2 = 188.5$ ,  $d.f. = 11$ ,  $p < 0.001$ ).

## 5.4 Discussion

The *Aedes aegypti* mosquito is vector for arboviruses such as dengue, chikungunya and zika and remains the prevalent species in Singapore. Despite a vigorous vector control program and a relatively low premise index, dengue clusters continue to be a regular occurrence in Singapore throughout the year (Ooi 2006). There is, therefore, a need for a paradigm shift in targetting the container-breeding *Aedes* mosquito. To augment existing source reduction efforts, the adulticidal Gravitrap can lure gravid female *Aedes* mosquitoes to oviposit and become ensnared.

While there was an observable reduction in mosquito populations during a four-month deployment of the Gravitraps in a cluster of apartments, this could not be solely attributed to the presence of the Gravitraps as the control site observed a similar reduction in mosquito population. This is similar with other studies that show that trapping regimes alone do not lead to reduction of mosquito populations (Degener 2015). This eludes to the fact that there are more native breeding sites in the local vicinity than Gravitraps deployed. And the skip oviposition behaviour of the *Aedes* mosquitoes allow the perpetuation of the progeny as the female mosquito might already laid some eggs in other breeding sites before getting trapped in the Gravitrap. It is possible that the fact that the decrease in the control site just happened to coincide with the deployment of Gravitraps in the test site. It is also possible that a significant reduction might be observed if the Gravitraps were left for a longer period.

While it is true the majority of the mosquitoes (64.91%) were found on the lower half of the apartment block, it would be ill-advised not to deploy Gravitraps in the higher floors to get a sense of the mosquito prevalence on the higher floors and to highlight areas of concern. This study followed a

deployment ratio of one Gravitrap per four apartments. This was repeated on every floor of the apartment block. This resulted in a Gravitrap maintenance regime that proved to be very labour-intensive, requiring a dedicated team of six to eight persons for the maintenance and upkeep of the Gravitraps. The accuracy of the identification and removal of the captured mosquitoes also relied heavily on the expertise of the staff involved. Despite this, the Gravitrap has proved to be a useful tool for the surveillance of mosquito populations. Some studies have performed pre-deployment source reduction, larviciding and oviciding to eliminate competing oviposition sources and increasing the effectiveness of the traps (Rapley 2009, Barrera 2014, Barrera 2014). Further improvements on the deployment protocols and trap design are required to streamline the surveillance and control program. This could include deployment and maintenance protocols mentioned in earlier chapters.

Predominant mosquito species caught in this study was *Ae. aegypti*, which is highly adaptable to human settlements. The low positive index (0.02-0.1) of the Gravitraps in Singapore is reflective of the low *Ae. aegypti* adult population in Singapore. This has also been reflected in the trapping of adult mosquitoes using the BG sentinel traps (unpublished data). With only 2-10 positive Gravitraps out of every 100 deployed per week, it would require the deployment of a few hundred Gravitraps in each site to achieve significant resolution for a robust surveillance system.

The current findings agree with previous studies that had found that *Ae. aegypti* prefer to breed close to the ground level. This agrees with similar finding in Australia and USA (Williams 2006, Obenauer 2009), suggesting that strong winds make it difficult for mosquitoes to locate ovipositioning sites at a higher elevation. These findings could help to optimize future deployment protocols.

The correlation found between the mean eggs in the ovitrap and the mean *Aedes* mosquitoes in the Gravitrap indicate that the Gravitrap can be used to monitor the fluctuations mosquito populations.

Along with mosquitoes, other organisms were also found caught in the Gravitrap from time to time. These include lizards, geckos, flies, moths, spiders, ants, etc. The Gravitrap also trapped environmental debris such as dust, leaves, etc. As such, the sticky paper required regular replacement throughout the period of the experiment. This could limit the use of the Gravitrap in areas such as construction sites, forests, rural communities, etc.

It was also interesting to note that there was a correlation between the eggs found in the ovitrap and the ambient temperature, which has been observed in other studies (Rueda 1990, Alto 2001). In contrast, there was no observable relationship between mosquito population and rainfall. This contradicts findings in other parts of the world (Gama R.A. 2007). This could be due to the fact that the *Aedes* mosquito is a container breeder and in Singapore, where the environment is predominantly urban, the man-made breeding sources have less to do with rainfall and more to do with human behaviour.

## **5.5 Conclusion**

While the *Aedes* population decreased in the test site after the introduction of the Gravitrap, this could not be completely attributed to the Gravitrap. In addition, the deployment ratio of one gravitrap to four apartments has proved rather tedious, future investigations could analyze the minimum ratio of Gravitrap needed to affect a reduction in the mosquito population. Further modification could be done to the Gravitrap to improve the efficiency and

reduce the amount of maintenance required. One factor that has been highlighted regularly is the need for a simpler oviposition attractant. This is due to the fact that hay infusion attractant is rather tedious to prepare and deploy.

There was a correlation found between the number of eggs found in the ovitraps and the temperature. It was also noted that there was a higher concentration of mosquitoes caught in the lower floors (2-6). This information could be used in the future for the modelling of mosquito populations and effective deployment of Gravitraps.

## CHAPTER 6

### CONCLUSION

A new lethal adult mosquito ovitrap has been developed to target the container-breeding *Aedes aegypti* mosquito – the Gravitrap. The Gravitrap has the advantage of being inexpensive to manufacture and easy to deploy in the urban residential setting of Singapore. This enables the mass deployment of Gravitraps for the surveillance of *Aedes* mosquito populations in the urban environment.

A key component of the Gravitrap is the hay infusion solution. It serves as an attractant to entice the gravid female mosquito to oviposit in the Gravitrap, and in the process, become trapped on the sticky lining (Reiter 1991, Trexler 1998, Ritchie 2001). While the hay infusion allows the Gravitrap to maintain a good catch rate for a period of at least four weeks with minimal maintenance, there is a need to isolate and identify semio-chemicals that cause the attraction (Chapter 2). The identification of 3-ethyl-5-methylphenol, cyclodecene, nonene and dodecanol as possible attractants will aid in the development of a consistent and long-lasting lure for use in the Gravitrap (Chapter 3). Such a lure would ensure a consistent catch rate of mosquitoes throughout the trapping period. It would also enable the simplification of logistics involved in the deployment and maintenance of the Gravitraps. However, care must be taken to ensure that these compounds do not pose a threat to the environment.

The Gravitrap has proven useful in managing dengue clusters (Chapter 4). The Gravitraps have been shown to provide an efficient way to monitor mosquito populations in dengue clusters around the island. In doing so, the Gravitraps can help provide vital information on the effectiveness of vector control measures and to help focus operations. The capture of dengue-positive *Aedes* mosquitoes

in Gravitrap placed in closed cluster highlights the risk of dengue transmission in non-cluster area. This emphasizes the importance of continued vigilance in mosquito control.

In high enough numbers, the Gravitrap have proved to be an efficient tool in reducing mosquito populations in a given area. The short-comings of such a trapping regime is that it is highly labour-intensive and logistically challenging (Chapter 5). A possible way to overcome this would be to equip all households with Gravitrap to reduce the mosquito populations, while maintaining a minimum amount of Gravitrap in each area for the monitoring of local mosquito populations.

Further development of the Gravitrap would allow it to be deployed for longer durations in the field without needing maintenance. The Gravitrap could also be used in an integrated vector surveillance system similar to those used in Brazil and Australia (Eiras 2009, Rapley 2009), where real-time data on the *Aedes* mosquito population can assist in stream-lining the efficiency of vector control operations.

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