The BG-Sentinel™ trap as a suitable tool for *Aedes aegypti* surveillance in Far North Queensland, Australia

Thesis submitted by

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Statement on the Contribution of Others

Sections of the work presented in this thesis were completed with the assistance of other researchers. The contributions of others to this thesis and to future publishable outcomes of the research undertaken during my candidature are acknowledged below.

Nature of Assistance	**Contribution**	**Names, Titles, and Affiliations of Co-Contributors**
Intellectual support	**Editorial assistance**	Dr. Scott Ritchie, James Cook University, QLD
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_____ _____

Tamara S. Ball — January, 2010

Scott A. Ritchie — January, 2010

Declaration on Ethics

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National statement on Ethics Conduct in Research Involving Humans (1999)*, the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number H2250).

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In Memory of Professor Chris Curtis

List of Publications from this Thesis

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Abstract

Aedes aegypti is the vector of dengue fever in far north Queensland where dengue outbreaks occur each year. The most recent outbreak in 2008/2009 saw all four serotypes of the virus circulating in the region with 1025 reported cases. Surveillance of the vector population is an important component of vector control and therefore dengue management. There are several tools that are currently used to measure the *Ae. aegypti* population, and like any sampling method, these tools need to be better understood and refined in order to achieve measurable outcomes in the field. The value of these tools lies in their sampling efficacy, and ultimately in how well we use them and understand the data that they produce—our ability to accurately interpret data from a very limited subsample of the field population.

Each sampling tool presents certain biases. Once these biases are defined, methods used to estimate population size and structure can be calibrated accordingly, resulting in more accurate and complex estimates of the vector population. Currently there are control strategies being developed that involve manipulation of *Ae. aegypti* in the adult stage (e.g. the use of the bacterial endosymbiont *Wolbachia* to shorten the lifespan of the vector population). These novel strategies demand adult sampling tools to measure changes in population size, structure (age, sex ratio) and ultimately the success of the program. The BG-Sentinel™ trap (BGS) is a proven tool that successfully samples the *Ae. aegypti* adult population. A series of mark-release-recapture experiments with adult *Ae. aegypti* were conducted in a large outdoor flight cage and an indoor setting in far north Queensland, to investigate the sampling biases of this particular trap. Biases were investigated across several categories, including: i) mosquito age ii) sex iii) physiological status and iv) body size. Biases were not detected across age groups or body sizes. A significant bias was detected across physiological groups: nulliparous females were recaptured at a significantly lower rate than all other groups except blood-fed parous females which were also recaptured at a low rate by the BGS. Males were recaptured at a higher rate than all groups, but only a significant difference in recapture rates was observed between males and nulliparous females. The sampling bias of the BGS is measurable and can be used to

generate more accurate estimates of the adult population and its attributes when sampling the field population.

Once the sampling biases of the BGS were defined, the efficacy of the trap within a competitive visual environment was investigated. The impact of the visual environment on trapping efficacy was of particular interest due to the visual cues used by the BGS to attract *Ae. aegypti*. Four to five day old males and nulliparous females were released into a semi-controlled room to evaluate the effect of the presence, reflectance, and distribution of surrounding harbourage sites on BGS trapping efficacy. Low-reflective (dark) harbourage sites near the BGS had a negative effect on both male and nulliparous female recapture rates. However, a more pronounced effect was observed in males. The distribution (clustered vs. scattered) of dark harbourage sites did not significantly affect recapture rates in either sex. In a subsequent experiment, the impact of oviposition sites on the recapture rate of gravid females was investigated. Although gravid females went to the oviposition sites and deposited eggs, the efficacy of the BGS in recapturing gravid females was not compromised. *Aedes aegypti* sampling in the field will mostly occur in the urban environment, whereby the BGS will be amongst oviposition sites and dark harbourage areas in the form of household items and outdoor clutter. In addition to understanding sampling biases of the BGS, estimations of the adult population size and structure can be further adjusted based on an understanding of the impact of the visual environment on trap captures. Outcomes from this suite of experiments provide us with important considerations for trap deployment and interpretation of *Ae. aegypti* samples from the BGS trap.

In order to understand what the BGS field samples convey about the field population, BGS field data were correlated with field population estimates generated by a weather-driven container-inhabiting mosquito simulation model (CIMSiM). This model uses container data as well as weather data to create a life table that estimate the daily population size of *Ae. aegypti* from the number of eggs to the number of adults. This particular model was recently validated and calibrated for this region. A widespread survey of premises over an 11ha area was undertaken in

February (wet season) and August (dry season) of 2007. This area was divided into ten ~1ha sections for which individual data were generated both from the model and from the BGS. One hundred and fifty-nine and 152 of 176 premises were inspected for containers during the wet and dry season, respectively. Sixty-seven BGS traps were set out during the wet season and 72 during the dry season over a 24h period. BGS data were correlated with the outputs generated by the CIMSiM model. A positive correlation between the BGS and the model outputs was observed during the wet season, but not during the dry season. Widespread trapping with the BGS over longer periods of time may reveal a stronger or weaker relationship between trap and model. Whatever the relationship between trap and model may be, interpretation of adult sampling data with the BGS needs to be further pursued in order to achieve measurable outcomes that can assist in achieving long term success in dengue control strategies.

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CHAPTER 1.

Introduction and literature review

1. Introduction

Mosquitoes reach nearly every corner of the planet and are the vectors of many important disease-causing pathogens in humans. The malaria-causing parasite *Plasmodium* is a classic example of the potentially high morbidity and mortality associated with a mosquito-borne pathogen. Many factors determine one's vulnerability to mosquito-borne diseases, however today humans have been mostly successful at reducing their exposure to mosquitoes and the pathogens they carry through the development of better housing, infrastructure, personal protection, and vector control.

Today, dengue is the most prevalent mosquito-borne virus of humans (Reiter 2001). Approximately 2.5 billion people are at risk with an estimated 50 million cases each year (WHO 2009). Outbreaks, epidemic and endemic transmission occur even in the most developed countries in the tropical and subtropical parts of the world (e.g. Singapore, Australia, and the United States (Reiter 2001; Effler et al. 2005; Hanna et al. 2006; Koh et al. 2007). The health impacts of dengue are often amplified in areas of poor economic condition due to limited access to health care and vector control (VC). It is the rapid unplanned urbanization, failure of VC programs, and the global movement of people and goods that have had the greatest impact on the spread of the dengue virus and its vectors (Gubler 1997; Reiter 2001). Many biologically and socially complex factors support the global proliferation of dengue, making elimination of this arbovirus in the near future highly unlikely. A vaccine for dengue is not yet available, therefore focus on reducing transmission through VC is paramount.

Aedes aegypti (Linnaeus), also known as the "yellow fever mosquito," is the primary vector not only of yellow fever, but also dengue fever. *Aedes aegypti* belongs to the subgenus *Stegomyia* and includes other important vectors such as *Aedes albopictus* (Skuse) and *Aedes polynesiensis* (Marks). Vector control strategies involving vector manipulation in the adult stage, are currently being applied to several *Ae. aegypti* and dengue control projects. These include (i) the use of the bacterial endosymbiont *Wolbachia* to shorten the lifespan of the vector population (Brownstein

et al. 2003; McMeniman et al. 2009), and ii) the sterile insect technique (SIT) using female-lethal RIDL™ (Release of Insects carrying a Dominant Lethal) whereby released RIDL males mate with wild females and produce no female progeny (Oxitec ; Coleman & Alphey 2004). Such projects require surveillance methods that target adult *Ae. aegypti* to measure changes in size and structure of the population (e.g. age, sex ratio) to ultimately measure success of the program.

Vector surveillance with a proven sampling method allows us to make reasonably accurate estimations about transmission risk and success of the VC program through estimating vector abundance and distribution. These types of tools can provide a wealth of information about the vector population, including distribution, abundance, and transmission risk. Sampling methods differ in efficacy between different mosquito species and this is largely due to the behaviour and biology of different mosquito species as well as the environment.

Egg, larvae, and pupal sampling are the most common strategies for monitoring and sampling the *Ae. aegypti* population. However, these methods are limited in describing the adult population. Adult sampling provides a better estimate of vector density and transmission risk, at the same time providing specimens for analysis in spatial distribution, parity, ovarian development, identification of blood meal sources, susceptibility to pathogens, insecticide resistance, as well as molecular analyses such as genetic variability across space and time (Barata et al. 2001; Focks 2003; Ritchie et al. 2003). *Aedes aegypti* adult sampling has proved challenging from the beginning and therefore focus has remained primarily on sampling the immature stages of this mosquito. Traps that capture large number of other mosquito species, typically collect very few *Ae. aegypti* (Service 1993a).The standard Center for Disease Control (CDC)- and Encephalitis Virus Surveillance (EVS)-type light traps, baited with light, carbon dioxide, olfactory attractants or a combination of these baits, do not achieve high capture rates of *Ae. aegypti* (Russell 2004).

The most successful and popular methods for sampling adult *Ae. aegypti* have been the CDC backpack aspirator (CDC-BP) (Clark et al. 1994) and human landing catches (HLCs) (Service 1993a; Canyon & Hii 1997; Kroeckel et al. 2006). Even though both of these methods yield significant numbers of adults, they do have their challenges when applied in the field. The CDC-BP method is labour-intensive and requires entry into private households (Clark et al. 1994; Williams et al. 2006b). There are significant ethical considerations to be taken with human-landing catches as it places those involved at a greater risk of infection with dengue or other vector-borne pathogens circulating in the surveillance area.

There is a demand for the development and refinement in methods used to measure adult dengue vector abundance—to make them more efficient, standardized, and epidemiologically relevant (Focks 2003). In response to the search for more effective and efficient traps for adult *Ae. aegypti*, a very promising trap for adult *Ae. aegypti*, the BG-Sentinel™ trap (BGS) (Biogents), was recently developed by BioGents GmbH®. It is a compact, lightweight and easy to use trap that can be set up for surveillance over shorter or longer periods of time. In addition to *Ae. aegypti,* the trap has also been shown to successfully collect *Ae. albopictus,* and *Ae. polynesiensis* (Kroeckel et al. 2006; Meeraus et al. 2008; Schmaedick et al. 2008).

Aedes aegypti occur over the majority of Queensland, however dengue virus activity is limited to the northern regions (Queensland Health 2005) (Fig. 1.1). The area of far north Queensland (FNQ), the study area of my research, currently experiences annual outbreaks of dengue fever transmitted by *Ae. aegypti* (Ritchie et al. 2002; Hanna et al. 2006) (Table 1.1). Dengue activity has been reported in the region as far back as 1879 with the first fatalities attributed to dengue fever and dengue haemorrhagic fever reported in Charters Towers in 1885 and 1897, respectively (Hare 1898). A combined total of 1600 confirmed cases of dengue were reported in the 1990s (Queensland Health 2009), and the most recent outbreak in 2008/2009 saw all four serotypes of the virus circulating in the region with 1025 reported cases (Queensland Health 2009).

The BGS is used regularly as a sentinel trap in the region, and several studies have been done to determine the efficacy of the BGS trap as well as its role as a rapid assessment tool (Williams et al. 2006b; Williams et al. 2007a). We know that it is a highly effective tool in capturing both sexes and adult females across all physiological groups (Williams et al. 2006b), but what is not yet understood is what trap samples say about the age and physiological structure of the underlying vector population.

Each sampling method presents its own unique biases. It is important to define sampling bias to understand how the trap samples a population. If consistent biases of a trap can be identified, then methods used to estimate vector population size and structure can be calibrated accordingly, resulting in more accurate predictions of the vector population. In my doctoral research I attempt to define, interpret, and bring meaning to BGS trap samples, thus hopefully creating a role for the BGS as an efficient and valuable surveillance tool in FNQ.

To achieve this, first, I investigate and report on the sampling biases of the BGS trap across sex, age, and physiological groups; second, I measure the impact of the trap's surrounding environment on trapping efficacy; and third, in the field study, I attempt to correlate BGS collections of *Ae. aegypti* with population estimates generated by the recently validated and calibrated Container-Inhabiting Mosquito Simulation Model (CIMSiM) (Williams et al. 2008), a weather-driven, dynamic life table simulation model used to predict *Ae. aegypti* population size and structure based on weather and container data (Focks et al. 1993a). All of my doctoral research took place in Cairns, far north Queensland, Australia.

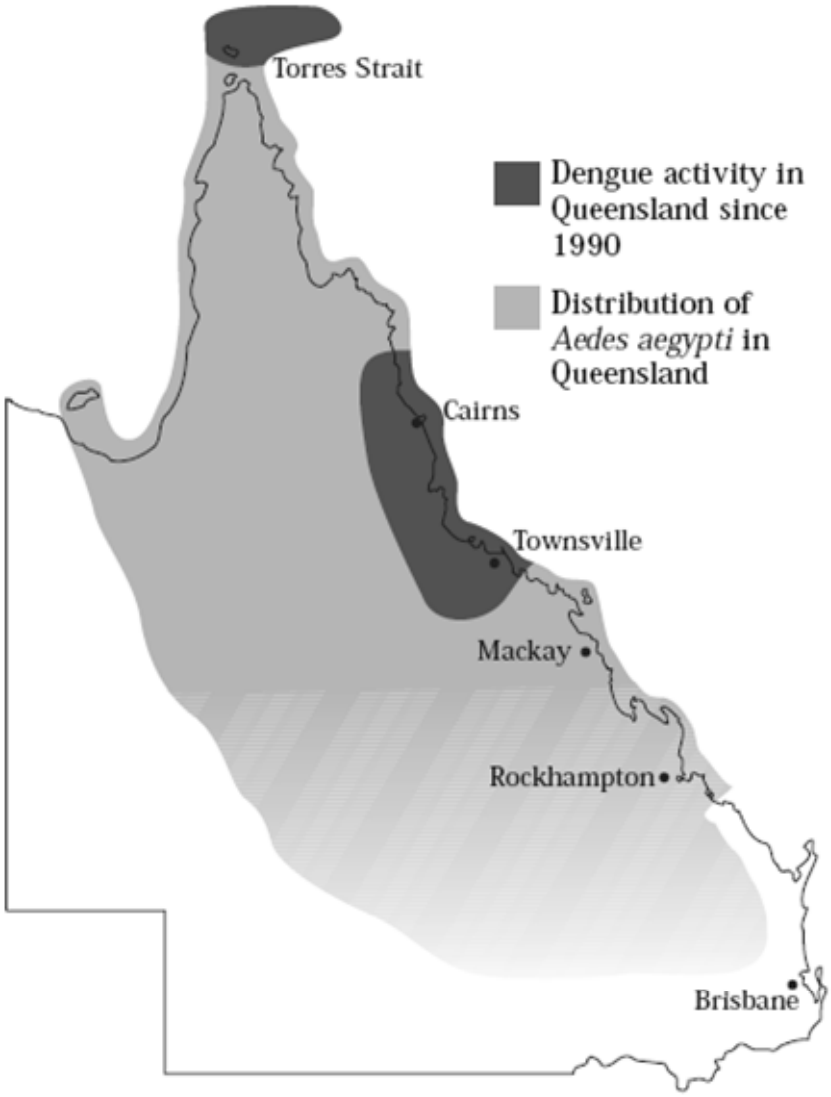

Figure 1.1. A distribution map of *Aedes aegypti* and dengue activity in Queensland, Australia. (Figure obtained from the Dengue fever management plan for North Queensland 2005-2010, Tropical Population Health Unit, Queensland Health, Queensland Government, 2005.)

Table 1.1. Dengue outbreaks in North Queensland from 1990-2005 (Table modified from the Dengue fever management plan for North Queensland 2005-2010. Tropical Population Health Unit, Queensland Health, Queensland Government, 2005).

Year	Location	Cases reported	Duration (weeks)	Serotype
1990-91	Cairns, Townsville, Torres Strait	27	8	DENV-1
1992-93	Townsville, Charters Towers	900	64	DENV-2
1995	Cairns	4	14	DENV-2
1996-97	Torres Strait, Cairns	208	28	DENV-2
1997-98	Cairns	12	11	DENV-2
1997-99	Cairns region	498	70	DENV-3
2000	Cairns	49	6	DENV-2
2001	Townsville	9	3	DENV-2
2002	Cairns region	21	10	DENV-2
2002	Townsville	2	2	DENV-1
2002	Cairns	2	3	DENV-4
2003	Cairns	3	2	DENV-1
2003	Cairns region	1	1	DENV-1
2003	Cairns	5	3	DENV-2
2003-04	Cairns, Townsville, Torres Strait	536	69	DENV-2
2003-04	Torres Strait, Cairns	356	41	DENV-2
2004	Torres Strait	1	1	DENV-2
2005	Torres Strait	56	10	DENV-4

2. Literature Review

There are many different strategies used to sample the *Ae. aegypti* population from the egg stage through to adult. A review of sampling tools and indices used for *Ae. aegypti* are described here in order to emphasise the limits of these tools in estimating the adult population.

2.1 Sampling the Egg Population

2.1.1 Standard Ovitraps

Ovitraps were first developed in 1964 as part of the United States *Aedes aegypti* Eradication Program (Schliessmann 1964) to detect *Ae. aegypti* (Fay & Perry 1965; Fay & Eliason 1966; Jakob & Bevier 1969a; Service 1993b). The trap was found to be highly sensitive for *Ae. aegypti* even when populations were low (Jakob & Bevier 1969b). The basic CDC ovitrap design involves a black jar with water and a wooden tongue depressor partly submerged in water and secured to the jar with masking tape or a paper clip (Fay & Eliason 1966). The efficacy of the trap was increased 8-fold through "enhanced pairing" of two traps respectively containing 10 and 100% hay infusion (Reiter et al. 1991).

Unlike some container-breeding mosquito species, *Ae. aegypti* does not oviposit its entire clutch of eggs at one site, but across several oviposition locations (Reiter et al. 1995; Colton et al. 2003; Reiter 2007). Through the use of DNA analysis to identify sibling eggs in Puerto Rico, it was estimated that female *Ae. aegypti* laid an average of 11 eggs per ovitrap, however the range of the number of eggs laid by each individual at each site ranged between 1 and 58 (Apostol et al. 1994), making it difficult to estimate the adult population unless a molecular analysis of the eggs from each deployed ovibucket was performed which would be a labour-intensive and costly method. Ovitrap data provides reliable information about the temporal (seasonal) and spatial distributions of *Ae. aegypti* but cannot be used to obtain dependable information that describes differences between blocks or neighbourhoods (Focks 2003). If for example, one block contains twice as many positive containers and therefore twice as many adults as the

other block, with one ovitrap set per block, then the average number of eggs per ovitrap is still nearly the same between blocks because the number of adults and daily oviposition is relative to the number of available containers (Focks 2003).

The CDC ovitrap is suitable for monitoring the efficacy of control programs (Focks 2003), research on dispersal studies (Reiter et al. 1995; Liew & Curtis 2004), and ovipositon behaviour (Allan & Kline 1995). A practical tool, however it provides limited information about the adult population (Reiter & Gubler 1997). There are two useful indices used to describe the level of infestation by *Ae. aegypti* using the ovitrap: the Ovitrap Positive Index (OPI) (number of positive traps / total number of inspected traps x 100), and the Egg Density Index (EDI) (number of eggs / positive trap x 100) (Gomes 1998). A study in Salvador, north eastern Brazil found the OPI and the EDI were a better method to assess infestation rates than the conventional House Index and Breteau Index (Morato et al. 2005).

Deployment of the ovitrap in the field is fast and simple but is not a rapid assessment tool since traps are usually required to remain in the field for several days. Due to the difficulty in egg identification, insectary facilities are often required for rearing of larvae and sometimes adults for identification, particularly where other container-breeding species of the *Aedes (Stegomyia) scutellaris* (Walker) group are present (Ritchie et al. 2003). Traps that are not collected in a timely fashion (1 week or less) or are left behind in the field, particularly in warmer weather, may potentially become a productive container for adult *Ae. aegypti* thus contributing to the vector population (Ritchie 1984).

2.1.2 Lethal and Biodegradable Ovitraps

The lethal ovitrap (LO) is a modified version of the CDC ovitrap that employs an insecticide-treated oviposition strip to ovipositing *Ae. aegypti* (Zeichner & Perich 1999). Like the ovitrap, the LO can also be used to collect egg data. The percent of LOs positive for eggs and the mean number of eggs per trap provide an indicator for container mosquito abundance (Ritchie 2005;

Williams et al. 2007b). Field trials in Brazil illustrate the efficacy of the LO as a control method through successful trials that significantly reduced the adult *Ae. aegypti* population when deployed at a cluster of houses in large numbers (Perich et al. 2003). In north Queensland hay infusion is added to the LO to create a "lure-and-kill" effect (Reiter et al. 1991; Ritchie 2005; Williams et al. 2007b). This strategy is used in local dengue interventions in conjunction with interior spraying within a limited area, larval control with s-methoprene, and source reduction (Ritchie 2005). An area saturated with LOs in combination with source reduction increases the likelihood ovipositioning *Ae. aegypti* will visit the trap. In north Queensland 4 weeks is the maximum time for an LO in the field as the ovistrip loses its efficacy over time, and begins to disintegrate (Williams et al. 2007b). Overall the LO is limited to the same research scope as the standard ovitrap, but can remain for longer periods of time in the field.

Lethal ovitraps set out for control purposes need to be retrieved within 4 weeks once the lethality of the ovistrip is lost. The more LOs set, means a greater control effect, however retrieving deployed traps potentially becomes a logistical nightmare as traps are often moved and/or left behind in the field. The once lethal ovitrap now becomes a productive container thus negating the original control effect. In response to this problem a biodegradable version of the LO, the biodegradable lethal ovitrap (BLO) was developed as a "set it and forget it" strategy to control dengue (Ritchie et al. 2008). The trap is designed to breakdown over time so after 4 weeks when the bifenthrin-treated ovistrip is no longer effective, the trap does not hold water and can remain in the field to biodegrade (Ritchie et al. 2008). The BLO allows high numbers of traps to be deployed for a maximum control effect without the logistical problems of trap retrieval.

2.2 Larval / Pupal Sampling

2.2.1 Funnel Trap

The funnel trap was originally designed by Kay et al. (1992) for the purposes of sampling the immature stages of container-breeding mosquitoes in difficult to access sites, such as wells. The trap consists of a weighted, inverted funnel with a polystyrene 500 ml jar. The jar is filled with 400 ml of water to create an air space so it can float once inverted in the water (Kay et al. 1992). The larval and pupal stages of the mosquito are then guided by the inverted funnel into the jar once they swim toward the surface (Kay et al. 1992). Two versions of this trap exist, the Austrap (Kay et al. 1992) and the smaller Vietrap (Russell & Kay 1999). Both the Austrap and the Vietrap have proven to be highly sensitive in detecting *Ae. aegypti* in wells and were able to predict absolute immature population size with 70-95% and 84-97% accuracy, respectively (Russell & Kay 1999).

This trap is an important tool in areas where wells and large water storage containers are important habitats for *Ae. aegypti*. In far north Queensland (FNQ), the study area of this project, such containers are not important sources for *Ae. aegypti* production. Containers identified as important for *Ae. aegypti* production in FNQ include pot plant bases, indentations in tarpaulins, plastic buckets, and used car tyres which accounted for ~60% of containers and 73% of *Ae. aegypti* pupae (Williams et al. 2008). Such containers would not be suitable or necessary for use of the funnel trap. Risk of dengue transmission cannot be determined from funnel trap capture rates because a relationship between larval and pupal densities do not exist due to density-dependent larval survival (Focks 2003).

2.2.2 Estimating Productivity from Sampling Larvae and Pupae

Estimates of the adult population have been attempted through surveying the immature *Ae. aegypti* population. Focks et al. (1981) performed a larval/pupal container survey of *Ae. aegypti* of six city blocks in a residential area of Louisiana, New Orleans, U.S.A. They calculated the mean daily emergence of female *Ae. aegypti* per block per day as well as the absolute

population and the standing crop of vector competent females (12 days and older) through pupal numbers and assumption of pupal period (2 days), the sex ratio and daily survival rates (Focks et al. 1981; Service 1993c). Although the adult population size, and age structure cannot be verified, in the absence of suitable adult sampling tools, this is a high standard alternative that is very labour-intensive. The pupal stage, unlike the preceding larval stages, has a very low mortality rate, therefore the number of pupae is highly correlated with the number of adults (Focks et al. 1993a).

Pupal surveys, however labour-intensive, do provide a wealth of information in terms of abundance and identifying key container types responsible for high productivity of *Ae. aegypti* (Focks et al. 1981; Barrera et al. 2006). Key container information is important in focussing control efforts. In a nationwide pupal survey in Trinidad, Focks and Chadee (1997) found that 11 types of key containers were responsible for >90% of all *Ae. aegypti* productivity. Through source reduction based on key containers, and environmental sanitation targeted at removal of ubiquitous containers such as tyres, mosquito density could be reduced by 43% (Focks & Chadee 1997).

2.2.3 Larval / Pupal Indices

There are several indices developed to determine transmission risk in areas endemic for *Ae. aegypti*. These *Stegomyia* indices were first developed to determine, through source reduction, if prophylactic levels in *Ae. aegypti* population size had been achieved (Connor & Monroe 1923; Breteau 1954). The House Index (HI) refers to the percentage of houses infected with larvae and/or pupae and the Container Index (CI) refers to the percentage of water-holding containers positive with active larvae and/or pupae (Connor & Monroe 1923). The HI is a poor indicator of transmission risk, as it does not consider the number of positive containers, and the CI is also not very useful from an epidemiological perspective in that a particular site may have very few positive containers, however these few containers may be of very high epidemiological significance due their high productivity for *Ae. aegypti* (Chan 1985).

In 1954, Breteau (1954) developed the Breteau Index (BI), which refers to the number of positive containers per 100 houses. The World Health Organization (WHO) later developed the Density Figure (DF) which relates the above three indices to biting rates (the no. of *Ae. aegypti* biting a human collector per hour), calculating transmission risk on a scale of one (low) to nine (high) (WHO 1972).

In an attempt to develop a superior index to the Breteau, Tun-Lin et al. (1996) later developed and evaluated the Adult Productivity Index (API), which is the sum of container type frequency multiplied by a density figure of each type. An evaluation of the above CI, HI, BI, API plus three more indices, the larval density index (avg. immature *Ae. aegypti* per house), the *Stegomyia* Index (no. positive containers per 1000 persons), and the *Stegomyia* Larval Density Index (no. larvae per 1000 persons) were evaluated (Tun-Lin et al. 1996). The objective of this study was to find an index that best describes the abundance of adult female *Ae. aegypti* to assess dengue transmission risk (Tun-Lin et al. 1996). This study concluded that neither of these indices were superior to the Breteau, and that due to the labour-intensiveness of estimating immature indices, that new and cost-effective methods of adult surveillance be pursued. A subsequent study by Knox et al. (2007) evaluating net sampling and pumping/sieving methods, also acknowledges the undefined relationship between immature *Ae. aegypti* abundance and the emerging adult population.

2.3 Adult Sampling

2.3.1 Human Landing Catches

Human landing catches involve using human bait for collecting adult mosquitoes. Often considered the most sensitive method in monitoring the abundance of adult *Ae. aegypti* (Service 1993a; Marquetti et al. 2000; Schoeler et al. 2004), this is a very labour-intensive method with significant accompanying ethical considerations, placing those involved at a greater risk of infection with dengue or other vector-borne pathogens circulating in the surveillance area. This surveillance method is strongly biased to host-seeking females, although male *Ae. aegypti* do

occasionally land on a human host (Hartberg 1971). Teneral, gravid, or blood-engorged females are also underrepresented with this method as well (Edman et al. 1997)

2.3.2. Backpack Aspirators

In longitudinal studies performed in Thailand and in Puerto Rico, Scott et al. (2000) found that 77 and 90% of all *Ae. aegypti* were captured indoors with the CDC backpack aspirator (CDC-BP), respectively. The CDC-BP is considered an effective method for indoor collection of *Ae. aegypti* (Reiter & Gubler 1997; Scott et al. 2000), and collects comparable numbers as human landing catches (Schoeler et al. 2004). A modified version of the "AFS Sweeper" (Meyer et al. 1983) powered with a 12V battery, the CDC-BP weighs in total 12kg (Clark et al. 1994). The general sampling protocol described by Clark et al. (1994) for a premise involves access to the entire house, including all rooms and closets. Two people sample the premise; one operates the CDC-BP and the other assists with a flashlight and an insect net to collect any mosquitoes that escaped aspiration. Aspiration occurs on all walls, under and around furniture, lamps, and other household articles. Hanging clothing is also inspected and disturbed to locate mosquitoes. The mean collection time per premise is 0.5h in households in Puerto Rico (Clark et al. 1994) and Williams et al. (2006b) found that 10 minutes was sufficient time to aspirate the lower level of a house in Cairns, Queensland, Australia.

The Prokopack (Vasquez-Prokopec et al. 2009) is a recently developed lighter and smaller version of the CDC-BP, weighing 0.8kg. Unlike the CDC-BP, the Prokopack is not only lighter, but has an extendable pole which facilitates aspiration of mosquitoes at heights greater than 1.5m, and is considerably less expensive (Vasquez-Prokopec et al. 2009). The CDC-BP retails from $468-758 USD and the Prokopac is costs between $45-70 USD. In paired field tests in Iquitos, Peru, the Prokopac collected significantly more mosquitoes, including *Aedes aegypti*, than the CDC-BP (Vasquez-Prokopec et al. 2009).

Backpack aspirators are an excellent sampling tool for collecting both male and female adult *Ae. aegypti* across different physiological groups (Kroeckel et al. 2006; Maciel-de-Freitas et al. 2006; Williams et al. 2006b). The downside of using backpack aspirators is that they are generally labour-intensive, and require entry into households which can be intrusive (Clark et al. 1994; Kroeckel et al. 2006; Williams et al. 2006b). Williams et al. (2006b) found that in Cairns, sampling may be compromised in several ways including refused access to the house or to particular rooms, and inaccessibility to households when residents are not home during working hours.

2.3.3 Visual Traps

2.3.3.1 Sticky Ovitraps

The sticky ovitrap (SO) is a modified standard ovitrap designed to capture container-breeding ovipositing female mosquitoes. Both visual and olfactory signals are used to attract ovipositing *Ae. aegypti*. The idea behind the SO is that egg-laying female *Ae. aegypti* will stick to the adhesive surface on the inside of the modified ovibucket during oviposition (Ordoneez-Gonzalez et al. 2001). There are several versions of the SO, but all follow a general design where the inside top half is fitted with an adhesive strip and the bottom half is filled with water enhanced with an oviposition attractant such as hay infusion for ovipositing female *Ae. aegypti* (Ordoneez-Gonzalez et al. 2001; Ritchie et al. 2003; Facchinelli et al. 2007; Gama et al. 2007). Variation in SO-design occurs moderately in the size of the container, and the types of adhesives and attractants used (Ritchie et al. 2003; Gama et al. 2007; Gomes et al. 2007).

The SO is as efficient as standard ovitraps in detecting *Ae. aegypti*, however the SO directly targets adult gravid mosquitoes, eliminating the need for rearing facilities (Ritchie et al. 2003). Sticky ovitraps can also be used in quarantine areas for detection of exotic species (e.g. *Aedes albopictus*), to measure the efficacy of control programs, and monitor vector populations with the accompanying benefit of being adulticidal (Ritchie et al. 2003). The SO has been a useful tool in dispersal studies for *Ae. aegypti* in Mexico and Australia with 7.7 and 3.4% recapture

rates recorded respectively (Ordoneez-Gonzalez et al. 2001; Russell et al. 2005). Viral assays for pathogens such as dengue can be performed on captured adults (Bangs et al. 2001; Ritchie et al. 2004), and age-grading techniques can be applied to surviving adults (Cook et al. 2006; Hugo et al. 2006). The SO Index (SOI) developed by Ritchie et al. (2004) is the mean number of females *Ae. aegypti* per trap per week. Since it measures the abundance of gravid female abundance, it may be useful in determining transmission risk. The relationship between SO collections and the absolute abundance of adult *Ae. aegypti* is unknown (Ritchie et al. 2004). The SO "MosquiTRAP™" uses two indices to assess infestation levels: The MosquiTRAP Positive Index (MPI) (number of positive traps for *Aedes aegypti* / total number of inspected traps x 100) and the Adult Density Index (ADI) (total number of adult *Ae. aegypti* captured / total number of traps) (Gama et al. 2007). Both of these indices can be applied to any version of the SO.

Overall the SO is beneficial in that it targets the adult *Ae. aegypti* population, allowing for useful population indices to be developed (Ritchie et al. 2003). It is a non-intrusive and simple tool that can be used in both vector surveillance and research for *Ae. aegypti*. The trap captures a very small fraction of the adult population and is strongly biased towards gravid females (Favaro et al. 2006). Other groups including males, nulliparous, blood-fed and parous females are underrepresented if not excluded entirely in SO collections (Favaro et al. 2006).

2.3.3.2 Resting Boxes

Resting boxes were designed to exploit the preference of adult *Ae. aegypti* to rest in darker locations and on dark, non-reflective surfaces such as clothing (Fay 1968; Fay & Prince 1970; Muir et al. 1992a; Edman et al. 1997), and are meant to be used in conjunction with the CDC-BP. The purpose of these boxes is to accelerate the sampling process by concentrating resting *Ae. aegypti* on 2-4 "resting stations" within the household that can then be quickly aspirated using the CDC-BP instead of aspirating the entire home (Edman et al. 1997).

The design of the resting boxes is a simple box design made of medium weight cardboard with the surface area of the box is covered with black muslin cloth (Edman et al. 1997). Thirty-60% of the entire population of *Ae. aegypti* resting within a house were captured with 2-4 resting boxes, and 32% more were captured using 4 boxes instead of 2. The addition of an oviposition bowl lined with black cloth and filled with water increased the mean collection of female *Ae. aegypti* by 32% (Edman et al. 1997). Overall using resting boxes to reduce sampling time proved to be an efficient method for sampling *Ae. aegypti* resting indoors (Edman et al. 1997).

2.3.3.3 The Fay-Prince Trap (Bi- and Omni-Directional)

Fay (1968) designed a trap to capture male adult *Ae. aegypti* which exploited the attractiveness of dark surfaces to adult *Ae. aegypti*. A modified version of the trap that would have optimum field efficacy was created shortly thereafter, and is today known as the Fay-Prince trap (FPT) (1970) (Fig 1.2 A). The design has contrasting gloss black white panels with a wind-orienting cover, a cylinder that houses the motor which is powered by a 6V battery, and a cage to hold the trapped mosquitoes. Although originally designed to collect male *Ae. aegypti*, the FPT was found to collect females more successfully than any other adult mosquito trap (Jensen et al. 1994). Kloter et al. (1983) performed a trial comparing the FPT to the CDC-light trap, the CDC-light trap (without a light), an ultraviolet light trap, a near infrared light trap, and a black cylinder trap. They found that the FPT collected significantly more males and females day and night with or without CO_2 compared to any other trap except the near infrared trap which was found to be equally effective at capturing females at night.

Jensen et al. (1994) further modified the trap into the bi- and omni-directional (Fig. 1.2 B) FPT to increase trap efficacy by increasing access to the trap from multiple directions. In tyre yard experiments, the bi-directional collected more *Ae. aegypti* than the omni-directional FPT and the CDC light trap. In a subsequent study in Townsville, Queensland, Australia, attractants, including CO_2, 1-octen-3-ol (octenol), and lactic acid, were added to the bi-directional FPT and compared to human landing catches (HLC) (Canyon & Hii 1997). Addition of lactic acid and

octenol significantly decreased catches in both lab and field trials 50 and 100%, respectively relative to CO_2-baited FPTs. The authors concluded that HLCs were a much simpler, effective, and inexpensive alternative to expensive adult traps, particularly in areas where arbovirus activity isn't occurring. In Peru, HLCs and backpack aspirators were also found to be significantly more effective than the FPT, and they concluded that the FPT was not an acceptable alternative to these former methods (Schoeler et al. 2004). The FPT was also found to be less effective to HLCs, backpack aspirator collections, and a more recently developed visual trap, the BG-Sentinel Trap™ (BGS), in Belo Horizonte, Brazil (Kroeckel et al. 2006).

The FPT was the first adult trap design that successfully captured both male and female *Ae. aegypti*. The FPT being made from metal, bulky, and often requiring CO_2 in the form of dry-ice or gas cylinders to increase capture sizes, it is not the most convenient, reliable, or cost-effective collection strategy for *Ae. aegypti*. Today more effective, less costly, and more convenient visual traps such as the BGS, and the sticky ovitrap are available as alternatives to the FPT.

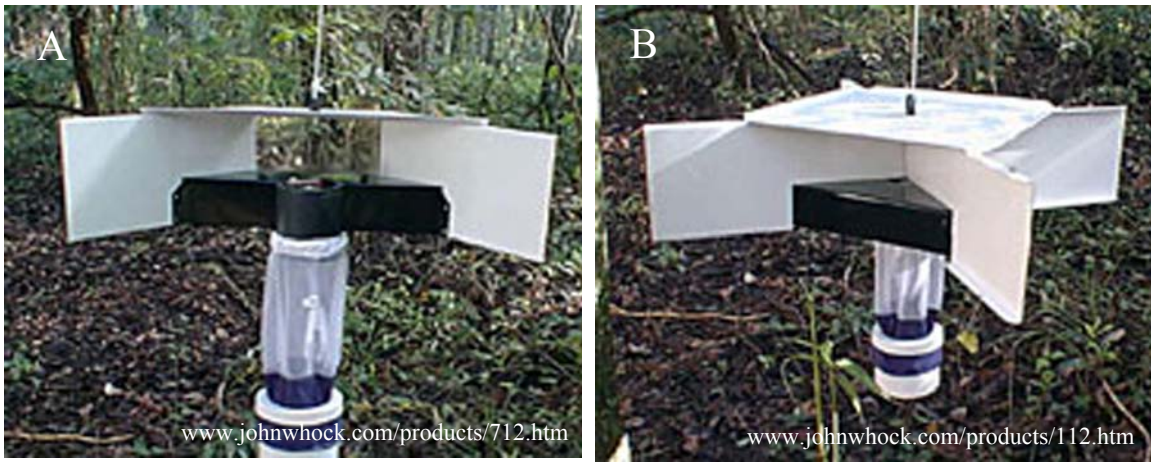

Fig 1.2. The bi-directional (**A**) and omni-directional (**B**) Fay-Prince traps (John W. Hock Co., Gainesville, FL).

2.3.3.4 The BG-Sentinel Trap™

The BG-Sentinel™ trap (BGS) is a recently developed trap that is increasingly being used to control and sample the adult stages of *Ae. aegypti* and other vectors of dengue belonging to the subgenus *Stegomyia* including *Aedes albopictus* (Ritchie et al. 2006; Meeraus et al. 2008) and *Aedes polynesiensis* (Schmaedick et al. 2008). This trap also successfully collects adult *Ae. aegypti* from both sexes and across many physiological stages (Maciel-de-Freitas et al. 2006; Williams et al. 2006b).

The main structure of the BGS is a collapsible white plastic cylindrical container with an open top which is covered and fitted with white gauze (Kroeckel et al. 2006). Suspended in the centre of the trap opening is a black cylinder with an attached catch bag where captured mosquitoes are held. Below the catch bag is a 12V fan which creates suction that sucks mosquitoes within a few centimetres of the trap opening into the catch bag of the trap located above the fan. The suction of air into the trap has a subsequent effect in that it creates an air current through and around the trap similar to convection currents generated by the human body (Fig. 1.3) (Kroeckel et al. 2006).

The trap uses a combination of visual cues, olfactory signals (excluding CO_2), and convection currents (similar to those generated by humans) to attract *Ae. aegypti* (Kroeckel et al. 2006). The olfactory attractants used with the trap are delivered by the BG-Lure®, a dispenser within the trap consisting of lactic acid, ammonia, and caproic acid, a combination of compounds attractive to *Ae. aegypti* found on human skin (Geier et al. 1999; Bosch et al. 2000; Steib et al. 2001).

Many comparative studies of the BGS have been performed since its development with very positive outcomes for the trap. The BGS has successfully outperformed other traps designed for capturing adult mosquitoes, including the Fay-Prince Trap, and the EVS trap (Rohe & Fall 1979)—a miniature light trap baited with CO_2 (Kroeckel et al. 2006; Williams et al. 2006b).

Human landing catches (HLCs) and CDC backpack aspirator (CDC-BP) provide a "gold standard" in terms of their efficacy in capturing adult *Ae. aegypti*. Field trials in Belo Horizonte, Brazil compared the BGS to HLCs and found no significant difference in capture rates between the two methods (Kroeckel et al. 2006). Williams et al. (2006b) found that the BGS collected significantly more *Ae. aegypti* than the CDC-BP, and that the BGS was more specific for *Ae. aegypti*. In a mark-release-recapture study in Rio de Janiero, Brazil, Maciel-de-Freitas et al. (2006) compared the efficacy of the BGS with the CDC-BP and found that the BGS collected more *Ae. aegypti* and concluded that it is an efficient tool for monitoring adult populations.

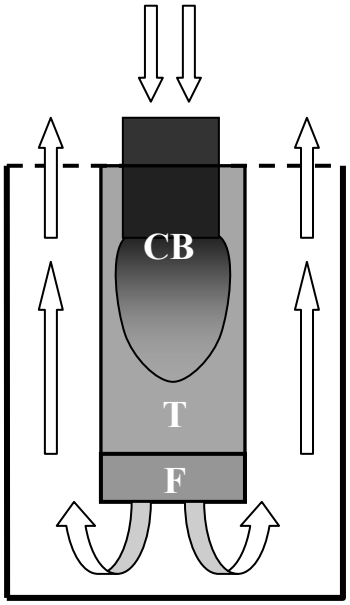

Fig 1.3. Diagram of the BG-Sentinel™ trap showing components and airflow through the trap. **CB** catch bag; **T** down flow of air through tube; and **F** fan.

2.4 Conclusions

Many methods have been developed to sample and gain insight into the *Ae. aegypti* adult population, however very few of these methods have been successful. The BGS is the first visual trap developed for *Ae. aegypti* that shows significant promise in the field. Due to the efficacy of the BGS, it can potentially be used in replacement of labour-intensive methods such as aspiration and human landing catches. In order to define itself as an informative, efficient, and valuable surveillance tool in FNQ, the BGS requires further investigation in order to bring meaning to trap samples, allowing for more accurate estimates of the size and structure of the underlying adult vector population.

CHAPTER 2.

Sampling biases of the BG-Sentinel™ trap with respect to physiology, age, and body size of adult *Aedes aegypti*.

1. Introduction

Dengue is the most prevalent mosquito-borne virus of humans (Reiter 2001). Approximately 2.5 billion people are at risk with an estimated 50 million cases each year (WHO 2009). Endemic and epidemic transmission occurs even in the most developed countries in the tropical and subtropical parts of the world (e.g. United States, Singapore, and Australia) (Reiter 2001; Reiter et al. 2003; Effler et al. 2005; Hanna et al. 2006; Koh et al. 2007). The health impacts of dengue are often amplified in areas of poor economic condition due to limited access to health care and vector control. Rapid unplanned urbanization, failure of vector control programs, and the global movement of people and goods have had the greatest impact on the spread of the dengue virus and its vectors (Gubler 1997; Reiter 2001). There are many factors which support the global proliferation of dengue which are biologically and socially very complex and make elimination of this arbovirus in the near future highly unlikely. Therefore, focus on reducing transmission through vector control and vaccine development is paramount.

Aedes aegypti (L.), also known as the 'yellow fever mosquito,' is the primary vector not only of yellow fever, but also dengue fever. *Aedes aegypti* belongs to the subgenus *Stegomyia* that includes other important vectors such as *Aedes albopictus* and *Aedes polynesiensis*. Currently there are vector control strategies that involve manipulation of the vector population in the adult stage. These strategies are being applied to several *Ae. aegypti* and dengue control projects including i) the use of the bacterial endosymbiont *Wolbachia* to shorten the lifespan of the vector population (Brownstein et al. 2003; McMeniman et al. 2009), and ii) Sterile insect technique (SIT) using female-lethal RIDL™ (Release of Insects carrying a Dominant Lethal) whereby released RIDL males mate with wild females and produce no female progeny (Oxitec ; Coleman & Alphey 2004). Such projects demand surveillance methods that target adult *Ae. aegypti* to measure changes in size and structure of the population (e.g. age, sex ratio) to ultimately measure success of the program.

Aedes aegypti sampling has primarily been focused on the immature stages (e.g. larval/pupal surveys). Human landing catches and the Center for Disease Control (CDC) backpack aspirator collections (Clark et al. 1994) are the two most common adult *Ae. aegypti* sampling methods, and both are successful at collecting significant numbers of adults (Canyon & Hii 1997; Focks 2003; Williams et al. 2006b). The CDC backpack aspirator method is labour-intensive and requires entry into private households, and there are significant ethical considerations to be taken with human-landing catches as it places those involved at a greater risk of infection with dengue or other vector-borne pathogens circulating in the surveillance area. Therefore, there is a demand for more efficient adult trapping methods. Recently, the BG-Sentinel™ trap (BGS) was developed by Biogents GmbH® which uses a combination of visual cues, olfactory signals (excluding CO_2), and convection currents (similar to those generated by humans) to attract *Ae. aegypti* (Kroeckel et al. 2006). This trap has also been used successfully with other vectors belonging to the subgenus *Stegomyia* including *Aedes albopictus* (Ritchie et al. 2006; Meeraus et al. 2008) and *Aedes polynesiensis* (Schmaedick et al. 2008).

In combination with age determination methods, mathematical modeling, and the development of a reliable adult surveillance method such as the BGS, information on population size, age and its physiological structure can potentially guide us towards more accurate interventions. In the following suite of experiments our aim was to determine consistent sampling biases of the BGS. Here we quantify collection biases of the BGS across different age, physiological groups, sex, and body sizes of *Ae. aegypti*.

2. Materials and Methods

2.1 Physiological Bias - Outdoor Flight Cage.

Site. This experiment was conducted in a large outdoor flight cage measuring 15 x 6 x 5m (volume 450 m^3) at James Cook University (JCU) in the suburb of Smithfield, Cairns, Queensland (16.9º S, 145.8 º E) from 20 September to 13 December, 2006. The flight cage was covered with 80% shade cloth to minimize heating and to prevent mosquitoes from escaping

from the cage (Fig. 2.1A). Shade cloth with shade factors 50, 70, and 80% were tested prior to the experiment, and we found that *Ae. aegypti* could escape through the 50 and 70%, but not through the 80% shade cloth. Soil, mulch and golden cane palms (*Dypsis lutescens*) were added to the cage to increase relative humidity and provide harbourage sites for mosquitoes (Fig. 2.1B). The set-up within the flight cage was designed to simulate the backyard of a typical residence found in far north Queensland where *Ae. aegypti* and dengue occur. A small shelter (2 x 2 x 2m) made from timber and 70% shade cloth was built within the cage to create an outdoor-accessible room/shelter such as a laundry area typical to the elevated houses found in this area. It is within this type of space that a BGS would be set in a residential property. Within the shelter two chairs, one cardboard box, one stool, and used articles of clothing were placed within the shelter to provide harbourage and resting areas. The BGS was set within the shelter amongst the furniture and clothing articles. Oviposition sites were not made available within the flight cage. Temperature and humidity data for the flight cage were recorded throughout the 24h period of each experiment using a digital hygrometer/thermometer.

Mosquitoes. All mosquito-rearing for the following experiments took place within an insectary at JCU in small (40 x 30 x 30cm) cages. *Aedes aegypti* eggs were collected from ovibuckets set in the suburb of Machans Beach in Cairns, Queensland (16.9º S, 145.8 º E). The F2 generation was used. Adults were provided with a 10% honey solution until 24h before release, and 48h if receiving a blood meal. The honey solution had a fructose/glucose concentration of 82%. Adults were reared into six different physiological groups: 1-2d nulliparous females (teneral), 8-9d gravids, 8-9d blood-fed gravids, 15-16d parous females, 15-16d blood-fed parous females and 3-4d males. Females belonging to a cohort where holding a blood meal was required during the experiment, were fed to repletion from a human leg and visually inspected for a full blood meal 6-7h prior to release (Human ethics approval from James Cook University, H2250).

Marking. Mosquitoes were placed in a 1-litre sealed container and lightly dusted with one of six fluorescent colours. Bulb dusters were used to apply the fluorescent dust (Radglo RS series,

HCA Colours Australia Pty. Ltd., Kingsgrove, Australia) to distinguish between groups. Using bulb dusters with fluorescent dust was successfully used in an *Ae. aegypti* mark-release-recapture (MRR) study by (Russell et al. 2005). Dust was applied two hours prior to commencing the experiment and morbid and dead mosquitoes discarded before release.

Release & Recapture. Approximately 110 individuals from each cohort were released simultaneously for each replicate. A total of 3,213 mosquitoes across six physiological groups were released over five replicates (540 nulliparous, 534 gravid, 527 blood-fed gravid, 537 parous, 538 blood-fed parous, 537 males). Marked mosquitoes were released in the middle of the flight cage at 1300h every seven days. The BGS remained covered during the initial release to allow time for mosquitoes to naturally distribute themselves throughout the flight cage. One hour after the initial release the trap was uncovered and mosquitoes were collected 24h later. Recaptured mosquitoes were identified to their physiological cohort in the lab. The BGS remained running and no nutritional sources were made available for the next week to ensure the cage was empty for the following replicates. Mosquitoes were not found recaptured in the BGS beyond 72h after the initial release.

Data Analysis. Data were tested for normality using a Shapiro-Wilks test. A main-effects analysis of variance (ANOVA) was run across replicates, followed by a post-hoc pair-wise comparison using a Fisher's least significant difference (LSD) test to look for differences between physiological groups. Data were analysed in *STATISTICA 7.1* (Stat Soft Inc. 2300 E 14th St., Tulsa, OK, U.S.A.).

2.2 Age Bias.

Site. This experiment was conducted in a semi-controlled room within a two bedroom apartment in the suburb of Manoora, Cairns, Queensland, Australia from 17-18 April, 2007. Each of the two rooms had white walls and contained natural outdoor light from one large window. The first and second room measured 3.8 x 2.8 x 2.45 m (volume 26.1m^3) and 3.0 x 2.6

x 2.45m (volume 19.1 m^3), respectively. The room windows and doors were screened and sealed to prevent escape of adult mosquitoes. The floors within the rooms were covered with white fabric to facilitate locating dead or resting mosquitoes. A black suitcase, chair, blue box, and a black box were scattered within each room as alternative harbourage sites to the BGS. The BGS was set in one corner of each of the rooms, approximately 60cm away from the corner walls. Temperature and RH data were originally collected in the apartment with the Tiny Tag Extra TGX-3580 data logger.

Mosquitoes. The F3 generation *Ae. aegypti* eggs from Machans Beach were used in this experiment. Mosquitoes were reared into 3 different age groups of parous females: 10-12d, 24-26d, and 32-34d that had completed one, two, and three gonotrophic cycles, respectively. Rearing occurred in small cages (40 x 30 x 30cm) and females were provided with blood meals from a human leg. Small plastic containers (100ml) half-filled with 10% hay infusion and a piece of sandpaper (8 x 4 cm) adhered to the container were provided as ovipositon sites within the cage. The oviposition substrate was changed when it was densely filled with eggs or after 24h, whichever occurred first. Adults were considered parous once oviposition activity had ceased within the cage for a minimum of 48h. All mosquitoes were provided with a 10% honey solution until 24h before release.

Release and Recapture. Mosquitoes were marked with fluorescent dust as described previously in the flight cage study. Due to the significantly smaller size of the rooms relative to the flight cage (~95% smaller), a smaller cohort of mosquitoes was released for each replicate. Approximately 25 female mosquitoes were released for each age group per replicate. A total of 425 mosquitoes were released (146 (10-12d), 147 (24-26d), 132 (32-34d)). The duration of each replicate was three hours. Ultimately if the duration of each replicate occurred for longer periods of time, there would be a very high recapture rate due to the size and restriction of space in each room. The BGS remained switched off and covered with a 60 x 60cm piece of white gauze for ten minutes after the release of the mosquitoes to allow a dispersal and settlement

period for the mosquitoes, reducing influence by the trap. Attached to the gauze was a length of fishing line leading under the door to the outside of the room so the trap could be uncovered without human disruption in the room. Mosquitoes collected in the trap were removed and all mosquitoes remaining in the room were removed immediately using a sweep net. Replicates occurred at 0900h and 1600h on the first day and 0900h on day two for a total of six replicates.

Dissections. Both 'recaptured' and 'non-recaptured' mosquitoes were dissected under a dissecting scope in the Insectary Lab at James Cook University, Cairns. Ovaries were removed and eggs counted.

Data & Analysis. Data were tested for normality using a Shapiro-Wilks test for normality followed by a one-way ANOVA to test for an effect of age. A main-effects ANOVA was run across replicates and age groups, followed by a post hoc analysis using a Fishers LSD test. Data were analysed in *Statistica 7.1*.

2.3 Body Size Bias.

Site. This experiment occurred on the 23-24 April, 2007 between 0900h and 1800h within the same rooms, set-up, and location as the 'age bias' experiment. Temperature and RH data were originally collected in the apartment with the Tiny Tag Extra TGX-3580 data logger.

Mosquitoes. The F3 generation *Ae. aegypti* eggs from Machans Beach were used in this experiment. To create two discrete body sizes (small & large) in both male and female *Ae. aegypti*, mosquitoes were reared at two different temperatures in two separate controlled temperature cabinets. An inverse relationship exists between wing-length and temperature between 20°C and 30°C in *Ae. aegypti* (Tun-Lin et al. 2000). The 'small' body size group was reared at 30°C and the 'large' body size group was reared at 20°C. *Aedes aegypti* eggs were initially flooded with 10% hay infusion. First instar larvae were then distributed into containers (17 x 11 x 5 cm) of 500ml of distilled water. Larval density started at 25 larvae per container. In

a preliminary study, the wing-length of both treatments were measured and it was found that rearing *Ae. aegypti* at 20°C and 30°C created two discrete body sizes (large and small) for both sexes, similar to the results of Tun-Lin *et al.* (2000) (Fig. 2.2). All adult mosquitoes were provided with a 10% honey solution until 24h before release. Four to five day old nulliparous females and males were released.

Release, Recapture & Data Analysis. Approximately 20 males and 20 females were released from each 'large' and 'small' treatment group reared at 20°C and 30°C, respectively. Overall, a total of 119 small and 136 large females, 132 small and 107 large males were released for the 20°C and 30°C reared groups, respectively across six replicates. The commencement, frequency and duration of this experiment as well as mosquito collection were as previously described in the 'age bias' experiment. Mosquitoes were not marked with fluorescent dust. The wing-length of 'recaptured' and 'non-recaptured' males and females were measured. A logistic regression was used in *Statistica 7.1* to determine if the body size of the mosquito influenced the likelihood of the individual to be captured by the BGS.

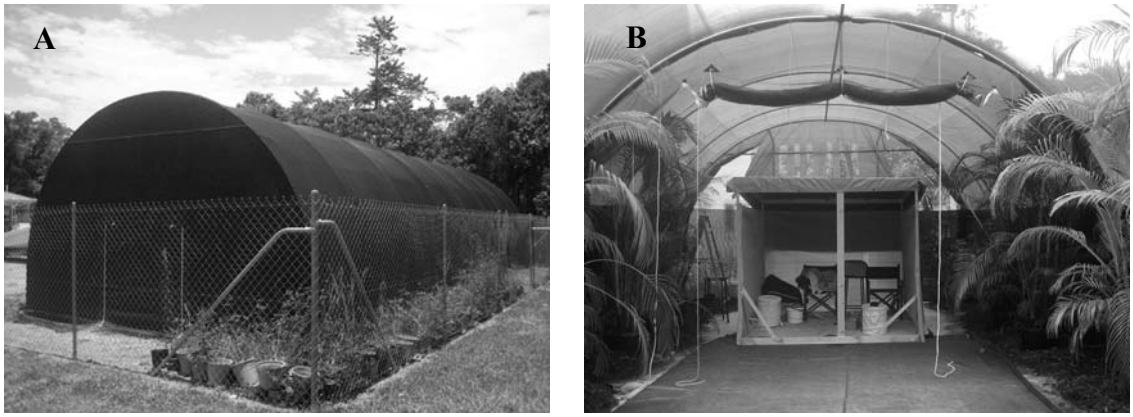

Figure 2.1. A. Exterior of the outdoor flight cage at JCU. **B.** Interior of flight cage showing internal shelter, foliage, and competing resting sites.

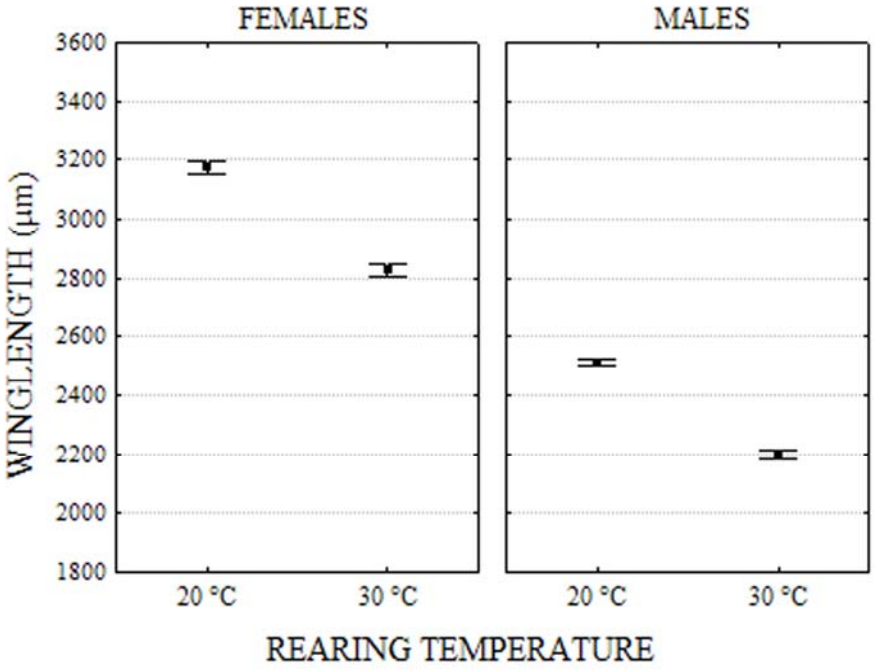

Figure 2.2. Preliminary data of mean wing-lengths (±SE) indicate two discrete body size groups within both female and male *Ae. aegypti* reared from egg to adult at 20°C (♀ n=66, ♂ n=88) and 30°C (♀ n=56, ♂ n=71).

3. Results

3.1 Physiological Bias - Outdoor Flight Cage.

The mean temperature and relative humidity (RH) for the experimental period were 28ºC (range 20-36) and 76% (range 49-100). Data were normally distributed (Shapiro-Wilks test: $W = 0.97$; $P = 0.60$).

A total of 3,213 *Ae. aegypti* were released across six physiological groups over five replicates. Within a 24h period, nulliparous females were recaptured at a mean (±SE) rate of 12.3% ± 3.6; gravids and blood-fed gravids were both recaptured at a mean (±SE) rate of 27% ± 4.9; males, parous females, and blood-fed parous females were recaptured at a mean (±SE) rate of 30% ± 3.4, 26% ± 4.6, and 18% ± 4.7, respectively.

A main-effects ANOVA run initially across replicates found no significant difference within groups between replicates ($F = 0.60$; df = 4, 20; $P = 0.67$). A significant effect of 'physiological group' for *Ae. aegypti* was observed ($F = 2.79$; df = 5, 24; $P = 0.04$). A post-hoc pair-wise comparison using a Fisher's LSD test revealed no significant difference between nulliparous females and blood-fed parous females ($P = 0.27$). Nulliparous females were recaptured at a significantly lower rate than all remaining physiological groups ($P < 0.05$). No significant difference in recapture rates was observed between blood-fed parous females and all other groups. A marginally insignificant difference was observed between males and blood-fed parous females, whereby males were recaptured at a higher rate ($P = 0.059$) (Fig. 2.3).

3.2 Age Bias.

The mean daily (0830h – 1900h) temperature and RH for the 17-18 April 2007 were 25.7 ºC (range 24.7-26.3), and 69% RH (range 61-79).

Data were found to be normally distributed (Shapiro-Wilks test: $W = 0.91$; $P = 0.08$). A one-way ANOVA revealed that mosquito age was not a significant predictor in recapture rates by

the BGS (F = 3.52; df = 2, 15; P = 0.06). The mean (±SE) recapture rates for the 10-12d, 24-26d, and 32-34d groups were 50% ± 4.3, 67% ± 6.7, and 42% ± 8.3, respectively. A slightly higher recapture rate was observed in the middle age group (Fig. 2.4A). To further investigate this outcome both 'recaptured' and 'non-recaptured' mosquitoes were dissected and examined for the presence or absence of eggs. Mosquitoes were then categorized into two groups, those with eggs and those without. A logistic regression (logit model) found that the presence or absence of eggs was a significant predictor of capture with the BGS (n = 405; W = 28.47, P<0.001), whereby females without eggs are more likely to be captured with the BGS than those with eggs. This same model again confirmed that there was no statistically significant effect of age (W=5.38, P = 0.06) and no interactive effect between age and the presence or absence of eggs (W = 3.92, P = 0.14). The higher recapture rate observed in the middle age group can potentially be explained by the overall higher number of mosquitoes released that had no eggs in this group (Fig. 2.4).

3.3 Body Size Bias. The mean daily (0900h-1900h) temperature for the 23-24 April, 2007 were 25.9ºC (range 24.7-26.4), and 67% RH (range 61-76).

The observed wing-lengths of both 'recaptured' and 'non-recaptured' mosquitoes confirmed the release of two discrete body sizes in both males and females throughout the experiment (Fig. 5A). No observed significant difference was observed between the body size of 'recaptured' and 'non-recaptured' females and males (Fig. 2.5B). A logistic regression (logit model) indicated that overall, wing-length is not a significant predictor of recapture of *Ae. aegypti* with the BGS for males (n= 157, W = 0.20, P = 0.66) or females (n=223, W = 0.38, P = 0.54).

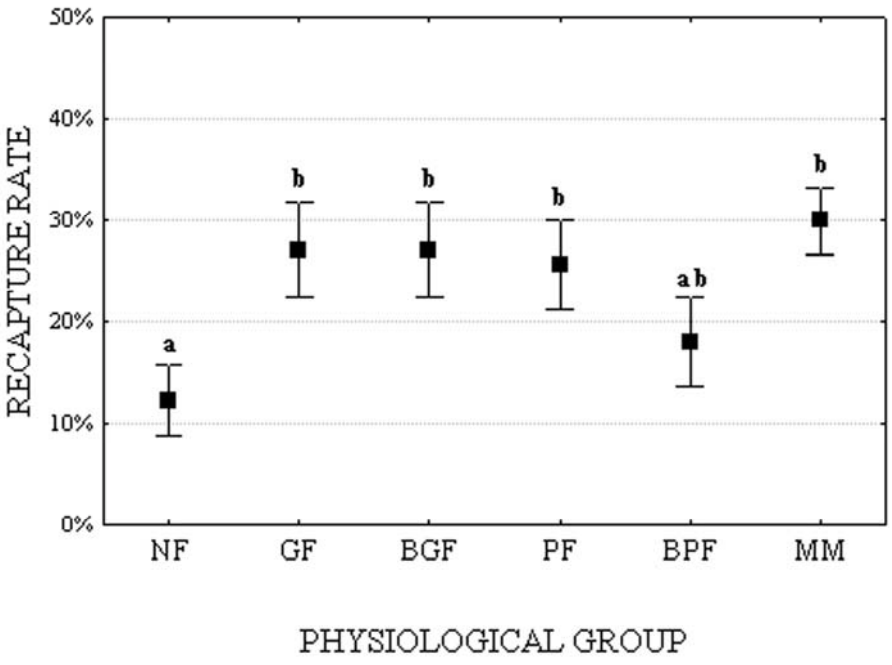

Figure 2.3. Mean number (±SE) of individuals recaptured by the BGS trap within a flight cage over a 24h period. Individuals from the NF group were recaptured at a significantly lower rate than all other individuals excluding BPF. The same letter is allocated to groups which are not statistically different from each other. **NF** (nulliparous), **GF** (gravid), **BGF** (blood-fed gravid), **PF** (parous), **BPF** (blood-fed parous), and **MM** (males).

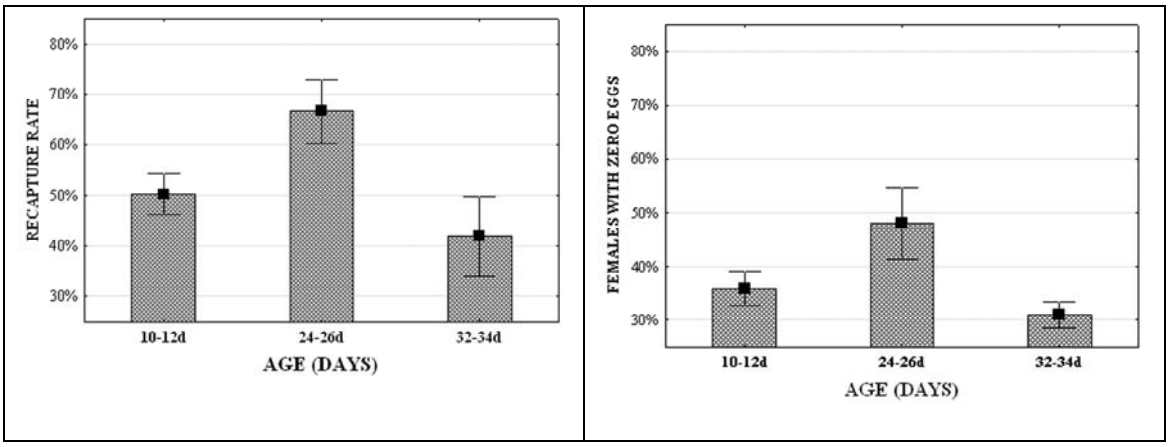

Figure 2.4. A. Mean recapture rate of parous females across 3 age groups. **B.** The percentage of females with zero eggs from each age group. The higher recapture rate (mean ± SE) observed in the 24-26d group **(A)** is potentially explained by the higher percentage of total females within this group released without eggs.

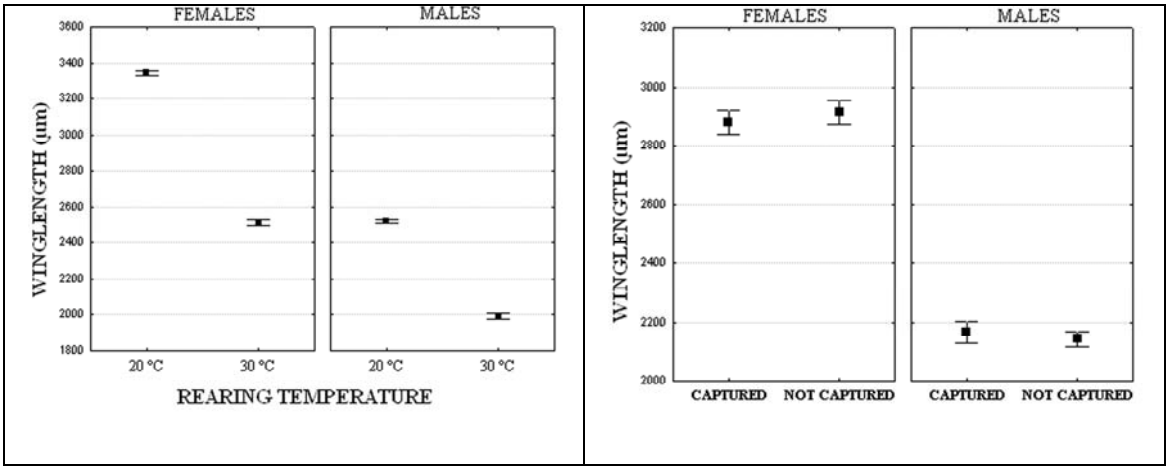

Figure 2.5. A. Mean wing-lengths (±SE) of released mosquitoes reared at 20 and 30 °C, verifying the two discrete body sizes of males and females used in the experiment. **B.** No significant difference in the mean (±SE) body size observed between recaptured and non-recaptured males and females.

4. Discussion

Sampling biases of the BGS for capturing *Ae. aegypti* across age, physiology, and body size were investigated. Sampling biases were not detected across age and body size groups, however in the 'physiological bias' experiment a clear bias was observed across different physiological groups. Nulliparous females were recaptured at a significantly lower rate than all other groups except blood-fed parous females which were also recaptured at a low rate by the BGS.

Males were recaptured at a higher rate than all groups, but only a significant difference in recapture rates was observed between males and nulliparous (teneral) females. A higher recapture rate of males relative to females is consistent with the capture rate observed in a field study by Williams et al. (2006b) in Cairns, Australia. Five day-old nulliparous female and male *Ae. aegypti* were recaptured at a similar mean rate of 77.7 and 70.6%, respectively within an empty room containing only the BGS (Ball & Ritchie 2010a). In a room containing dark harbourage areas, males were recaptured at a significantly lower mean rate (25.3%) than 5d nulliparous females (47.3%) (Ball & Ritchie 2010a). If in the presence of dark harbourage sites the BGS recaptures males at a lower rate relative to nulliparous females, then the higher number of males occurring in the BGS relative to nulliparous females in Williams et al. (2006b) may then be explained in two ways: 1) the BGS in their experiment was set in an area without dark harbourage sites where greater or equal numbers of males relative to nulliparous females were exposed to the trap or 2) the BGS was set amongst dark harbourage sites and exposed to more males than nulliparous females.

A higher recapture rate of nulliparous *Ae. aegypti* relative to other female physiological groups was observed in a MRR study using the BGS by Maciel-de-Freitas et al. (2006) in Rio de Janiero, Brazil, whereby non-teneral nulliparous females were recaptured at a higher rate than blood-fed parous, blood-fed nulliparous, and gravid females. Williams et al. (2006b) collected higher numbers of nulliparous and gravid females relative to parous, and blood-fed groups with the BGS. The high recapture rate of gravid females in the 'physiological bias' experiment

appears to reflect what was observed by Williams et al. (2006b), however in the MRR study of Maciel-de-Freitas et al. (2006), gravid females were recaptured at a very low rate and might be indicative of trap location and the availability of oviposition sites nearby the BGS.

Gravids were recaptured at a significantly higher rate than nulliparous females in the 'physiological bias' experiment. A potential explanation for this result is that the nulliparous (1-2d) mosquitoes used in this experiment were too young (teneral) to possess strong motivation and engage in significant flight activity associated with host-seeking behaviour. The steady low level of activity in teneral mosquitoes occurs for longer periods in females than in males (Gillett 1983). According to Gillett (1983) during the first day following eclosion, the muscles required by the mosquito for swimming are being transformed into flight muscles. Generally not until the third day following eclosion are female *Ae. aegypti* fully active in flight, mating, and host-seeking. If older non-teneral nulliparous females were used, we might assume that they would have been recaptured at a higher rate relative to other physiological groups, and at a more similar rate to males.

Although a statistically insignificant result ($P = 0.06$) was observed across age groups in the 'age bias' experiment, a higher recapture rate in the middle age group (24-26d) prompted further investigation. What we did find unexpectedly in this middle age group was a higher occurrence of females released with zero eggs, where the young (8-10d) and old (32-34d) groups had fewer females with zero eggs, potentially explaining the higher recapture rate of the middle age group (Fig. 4). We found that parous females without eggs were more likely to be captured than females holding eggs. This may be explained by host-seeking inhibition that occurs in female *Ae. aegypti* retaining more than four eggs (Klowden 1981).

Parous females were captured nearly half as frequently as nulliparous and gravid females by the BGS in Williams et al. (2006b), but this may not describe the abundance of each physiological group relative to one another outside the trap. Even though half as many parous females as nulliparous females were captured by the BGS, the ratio occurring within the trap may not be

reflective of their relative abundance outside the trap and therefore relative abundance within the trap has no meaning unless the differences in trapping efficacy of the BGS across physiological groups is applied.

Across the six physiological groups used in the 'physiological bias' experiment, we expected to observe a lower recapture rate by the BGS in blood-fed groups compared to all other groups as we would expect groups blood-fed to repletion to be less active. Although insignificantly different, a slightly lower recapture rate was observed in the blood-fed parous females relative to blood-fed gravids. This could be explained by the difference in the size of the blood meal taken by a parous female versus a gravid female. Assuming that a parous female could ingest a higher volume of blood, 'distention-induced host-seeking inhibition' (Klowden & Lea 1979) caused by a larger blood meal may explain the lower recapture rate observed in blood-fed parous females relative to blood-fed gravids. According to Klowden and Lea (1978), no effect on host-seeking is observed until a volume of blood greater than 2.5 µl is ingested. Above this threshold, a sharp decline in host-seeking occurs, and when more than 4 µl is ingested, host-seeking ceases (Klowden & Lea 1978). Termination of host-seeking and blood-feeding occurs once a critical volume of blood is ingested by the mosquito into the mid-gut (Klowden 1983).

Adult *Ae. aegypti* across all physiological groups are attracted to the BGS, however their motivations for going to the BGS are likely very different between them. Attraction to the BGS could be explained by several different motivations experienced by the mosquito. Male *Ae. aegypti* attraction to the BGS can be explained by their attraction to dark objects as harbourage sites (Muir et al. 1992a; Edman et al. 1997) as well as their tendency to host-seek to exploit the blood-feeding behaviour of females, therefore occurring around human hosts for a mating opportunity (Hartberg 1971). Male *Ae. aegypti* are known to land on human hosts and have been collected in human landing catches (Hartberg 1971; Kroeckel et al. 2006). Gravid females could potentially be seeking a host for a blood meal however host-seeking may not always be an explanation for the capture of blood-fed groups. The BGS does mimic convection currents

generated by humans, but the visual attraction of the BGS may also represent other 'attractive' sites to *Ae. aegypti*, including oviposition sites, harbourage areas for mating and periods of inactivity, thus potentially explaining the capture of *Ae. aegypti* across a broad spectrum of physiological stages.

Well-defined sampling biases of the BGS for *Ae. aegypti* permit more rigorous analysis and interpretation of *Ae. aegypti* data obtained in the field. The physiological biases observed in the BGS are measurable and consistent. Since the differing efficacies of the BGS for *Ae. aegypti* across sex and physiological status have been defined, we can potentially describe the unobserved population in terms of size, and physiological structure (e.g. age and sex) within a given space and time, providing a 'snapshot' of the population (e.g. 1 household over a 24h period). Application of these known biases can be applied in control projects where a particular physiological group is targeted and/or used as an indicator to measure efficacy of control efforts.

CHAPTER 3.

Evaluation of BG-Sentinel™ trap trapping efficacy for *Aedes aegypti* in a visually competitive environment.

1. Introduction

The BG-Sentinel™ trap (BGS) is a recently developed trap that is increasingly being used in controlling and sampling the adult stage of *Aedes aegypti* L. (Williams et al. 2006b; Williams et al. 2007a) and other vectors of dengue belonging to the subgenus *Stegomyia* including *Aedes albopictus* (Skuse) (Ritchie et al. 2006; Meeraus et al. 2008; Farajollahi et al. 2009) and *Aedes polynesiensis* (Marks) (Schmaedick et al. 2008).

The BGS uses a combination of visual cues, olfactory signals, and convection currents (similar to those generated by humans) to attract *Ae. aegypti* (Kroeckel et al. 2006). The olfactory attractants used with the trap are delivered by the BG-Lure, a dispenser within the trap consisting of lactic acid, ammonia, and caproic acid, a combination of compounds attractive to *Ae. aegypti* found on human skin (Geier et al. 1999; Bosch et al. 2000; Steib et al. 2001). In an earlier study, experiments including a BGS without BG-Lure showed that the addition of kairomone lures did not significantly increase recapture rates of *Ae. aegypti* in field trials in Cairns, Queensland, Australia, suggesting that despite the attraction of these compounds to *Ae. aegypti*, the visual cues play a primary role in attracting *Ae. aegypti* to the BGS (Williams et al. 2006a).

Muir et al (1992b) demonstrated that *Ae. aegypti* are capable of limited colour discrimination, with an overall high sensitivity to light and very poor visual acuity. In a subsequent study, it was shown that both male and female *Ae. aegypti* display an attraction to objects of dark and solid colour with low reflectance, discriminating between surfaces of 34 and 30% reflectance, indicating an overall total sensitivity of the eye (Muir et al. 1992a). Additionally, Fay and Perry (1965) found that a black-coloured beaker was most attractive to ovipositing female *Ae. aegypti*. The attraction of *Ae. aegypti* to dark surfaces was further illustrated in the development of the ovitrap (Fay & Eliason 1966) and trials in Thailand whereby black resting boxes were used to attract and concentrate indoor *Ae. aegypti* populations to two or more sites for rapid sampling (Edman et al. 1997). The white (high reflectance) body of the BGS in a low-light

environment may highlight the black cone (low reflectance) centred in the trap, potentially explaining in part its attractiveness to *Ae. aegypti*.

The BGS attracts adult *Ae. aegypti* from both sexes and across many physiological stages (Maciel-de-Freitas et al. 2006; Williams et al. 2006b). Depending on the sex and physiological stage of the mosquito, this dark surface area could appear to the mosquito as a general cue that represents very specific needs (e.g., oviposition, harbourage/resting, host-seeking, or mating).

Each sampling tool presents certain biases. The efficacy of each sampling tool is also potentially further influenced by its surrounding environment (e.g., temperature, humidity, and light intensity). In an earlier study on BGS sampling bias, significant biases were detected across sex and physiological groups in *Ae. aegypti* (Ball & Ritchie 2010b). The interaction of the trap's sampling biases and the influence of its surrounding environment on trapping efficacy are important considerations to be taken in interpreting data generated from these sampling tools.

Visual cues of the BGS are an integral component in attracting *Ae. aegypti* to the trap. In the following suite of experiments we explored the potential effect of the surrounding visual environment on BGS trapping efficacy. Firstly, we investigated the influence of the presence/absence and reflectance (low-reflectance vs. high-reflectance) of surrounding harbourage areas on the efficacy of the BGS. Since we know that *Ae. aegypti* are attracted to solid black/dark surface areas of low reflectance (Muir et al. 1992a), we predicted that the presence of dark harbourage areas would attract *Ae. aegypti*, thus decreasing the proportion of mosquitoes attracted to and recaptured by the BGS. Secondly, we investigated the distribution of competing black harbourage areas on trapping efficacy. We suspected that the distribution of visual cues throughout the room may strengthen the effect of the dark harbourage areas on BGS efficacy by interrupting the continuity in the visual and physical path between the mosquito and the BGS, thus reducing the number of mosquitoes collected in the trap. Thirdly, we investigated

the influence of surrounding oviposition sites on the recapture rate of gravid *Ae. aegypti* by the BGS. Gravid females comprised 31.6% of female *Ae. aegypti* collected in the BGS in Cairns (Williams et al. 2006b). Thus, it is important to know if gravid females are selectively attracted away from the trap in the presence of oviposition sites. We predicted that gravid females will visit and deposit eggs at the provided oviposition sites, however due to their tendency for 'skip-oviposition' (Reiter et al. 1995; Reiter 2007) behaviour, we expect gravid females will eventually move to and be captured by the BGS, ultimately having no negative effect on recapture rates.

Due to the observations by Williams et al. (2006a) whereby no significant difference was observed between a BGS baited with the BG-Lure and one without, the BG-Lure was not used in this set of experiments. Using the BGS without the lure further allows us to focus on the visual properties of the trap.

2. Materials and Methods

The BG-Sentinel Trap. The main structure of the BGS is a collapsible white plastic cylindrical container with an open top which is covered and fitted with white gauze (Kroeckel et al. 2006). Suspended in the centre of the trap opening is a black cylinder with an attached catch bag where captured mosquitoes are held. Below the catch bag is a 12V fan which creates suction whereby any mosquito within a few centimetres of the trap opening would be sucked into the catch bag of the trap located above the fan. The suction of air into the trap has a subsequent effect which creates an air current through and around the trap similar to convection currents generated by the human body (Kroeckel et al. 2006).

2.1. Effect of Presence/Absence/Reflectance of Competing Harbourage Sites

Site. This experiment was conducted in a semi-controlled room within a two bedroom apartment in the suburb of Manoora, Cairns, Queensland, Australia from 31 March – 6 April, 2007. Each of the two rooms had white walls and contained natural outdoor light from one large

uncovered window. The room measured 3.8 x 2.8 x 2.45 m (volume 26.1m^3). The room windows and doors were screened and sealed to prevent escape of adult mosquitoes. The floors within the rooms were covered with white fabric to facilitate locating dead or resting mosquitoes.

Temperature and relative humidity (RH) data were originally collected at the site with the Tiny Tag Extra TGX-3580 (Gemini Data Loggers, Sussex House, Terminus Rd, Chichester, W. Sussex, PO19 8UJ, UK) data logger. Data files obtained during the period 25 March – 30 April, 2007 became corrupt and unusable. To estimate the temperature and RH for this missing period, a simple linear regression (95% CI) was performed using half-hourly temperature and RH data from the Australian Bureau of Meteorology (BOM) Cairns Station 31011 (~5km from the site) and temperature and RH data from the TGX-3580 set within the apartment from the 21 May – 10 June 2007 (21 d) (Temp: $R = 0.617$, $P < 0.001$, $Y = 16.784 + 0.3304X$) (RH: $R = 0.734$, $P < 0.001$, $Y = 25.685 + 0.723X$). From this, estimates of the minimum, maximum, and mean temperature and RH were generated.

Mosquito Rearing. Mosquito-rearing took place within an insectary at James Cook University, Cairns campus. *Aedes aegypti* eggs were originally collected from ovibuckets set in the suburb of Machans Beach in Cairns, Queensland (16.9º S, 145.8 º E). The F3 generation was used. Adults were housed in small (40 x 30 x 30cm) cages within an insectary and fed on a 10% honey and water solution.

Experimental Design, Release, and Recapture. In the first of three treatments, ten identical 70-litre black plastic storage crates (low-reflectance) were placed along two walls of the room with the BGS centred 1 meter in front of the crates (Fig. 3.1a). In the second treatment, ten 70-litre white plastic crates (high-reflectance) were arranged in the same manner as the first treatment (Fig. 3.1b). The purpose of the second treatment was to determine whether or not the reflectance of surrounding harbourage sites influenced recapture rates by the BGS. The third

treatment was a control whereby the BGS was set in the room without any crates or competing harbourage sites added. Crates had no known previous exposure to pesticides. The interior and exterior of all the crates were textured by rubbing thoroughly with sandpaper, making it easier for the mosquitoes to rest on the walls of the containers as well as matting the plastic to avoid unwanted reflectance or "shininess" created by the plastic material of the crates.

Twenty-five 5d nulliparous females and 25 males were released for each treatment for a period of 3h at 0900h, 1230h, and 1600h for six days for a total of six replicates. A total of 900 mosquitoes (450 ♀, 450♂) were released. Treatments were rotated equally through the 3h release periods to nullify a potential "time-of-day" effect. After the mosquitoes were released in the room, the BGS remained switched off and covered with a 60 x 60 cm piece of white gauze for 10 minutes after the release of the mosquitoes to allow a dispersal and settlement period for the mosquitoes, reducing influence by the trap. The BGS was connected to a power source outside of the room where it was switched on 10 minutes post-release. Attached to the gauze was a length of fishing line leading under the door to the outside of the room so the trap could be uncovered after 10 minutes without human disruption in the room. The catch bag from the BGS was collected after the 3h trapping period, and all non-captured mosquitoes in the room were removed immediately using a sweep net and accounted for. All mosquitoes were positively identified and counted in the insectary at JCU.

Data Analysis. Recapture rate data were tested for normality using a Shapiro-Wilks test and arcsin transformed. A factorial ANOVA was performed followed by a post-hoc analysis using a Fisher's LSD test in *STATISTICA 7* (Stat Soft Inc. 2300 E 14th St., Tulsa, OK, U.S.A.).

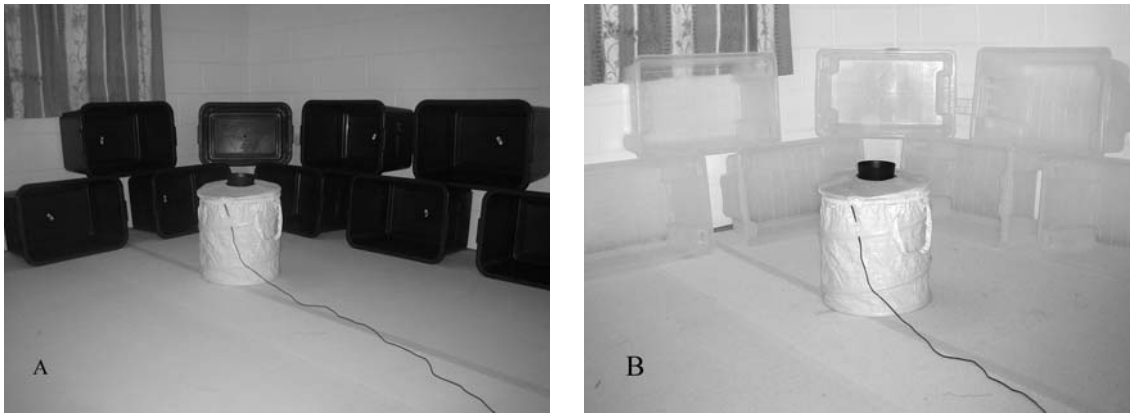

Fig. 3.1. Low (**A**) and high-reflectance (**B**) harbourage sites in the surrounding environment of the BGS.

2.2. Effect of the Distribution of Competing Harbourage Sites

Site. This experiment was conducted in one semi-controlled room within an apartment nearby James Cook University in the Cairns suburb of Smithfield, Queensland, Australia from 7 – 12 November 2007. The room had white walls and contained natural outdoor light from one large uncovered window. The room measured 3.5 x 3.57 x 2.45 m (30.6 m^3). The window and door in the room were screened and sealed to prevent escape of adult mosquitoes. The floors within the room were covered with white fabric to facilitate locating dead or resting mosquitoes. Temperature and RH data were recorded using the Tiny Tag Extra TGX-3580 data logger.

Mosquito Rearing. Mosquitoes were the F4 generation of eggs collected at Machans Beach, a northern suburb of Cairns. Mosquitoes were reared and housed as described in previous study.

Experimental Design, Release, and Recapture. In the first of three treatments seven 70-litre black plastic storage crates were placed along two walls of the room behind the BGS to create a "cluster" of dark harbourage area (Fig. 3.2a). In the second treatment the same seven 70-litre crates were "scattered" around the trap 1-2 m apart from each other (Fig. 3.2b). The position of the crates for each treatment remained the same across all replicates. The third treatment was a

control where the BGS was set within the emptied room without any competing harbourage areas.

Twenty-five 5d nulliparous females and 25d males were released for each treatment for a period of three hours at 0830h, 1200h, and 1530h for six days for a total of six replicates (one replicate per day). A total of 900 mosquitoes (450 ♀, 450♂) were released. Treatments were rotated equally throughout the 3h release periods to nullify a potential "time-of-day" effect. Mosquitoes were released, collected, and identified as described in previous experiment.

Data Analysis. Recapture rate data were analysed as described in the previous experiment in *STATISTICA 7*.

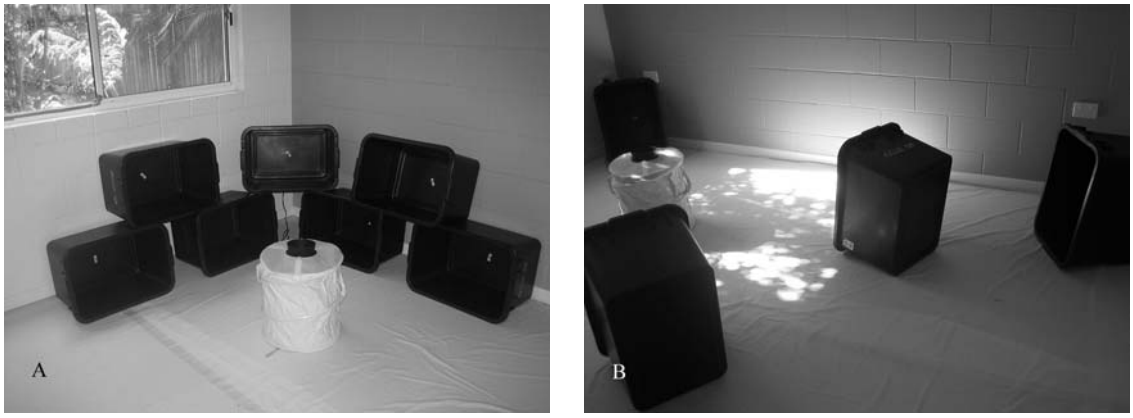

Fig. 3.2. A. BGS and 'clustered' harbourage area treatment. **B.** BGS and scattered' harbourage area treatment.

2.3. Effect of Oviposition Sites on Recapture of Gravid Females

Site. This experiment was conducted at the same site as described in the previous experiment from 27-29 February and 3-5 March 2008. Temperature and RH data were recorded using the Tiny Tag Extra TGX-3580 data logger.

Mosquito Rearing. The F4 generation *Ae. aegypti* eggs from Machans Beach were used. Mosquitoes were reared as described in previous study and provided with a blood meal at 3d.

Experimental Design, Release, and Recapture. In this experiment, two 1.2-litre red ovibuckets (Ritchie 2001) were used and placed in opposite corners of the room with the BGS. In addition, four black crates (4 x 70 litres) were "scattered" within the room to create additional harbourage sites. The red colour of the ovibucket appears black to the *Ae. aegypti* eye due to the limits of their visual spectrum and is in accordance with the attractive low-reflectance properties of attractive surfaces and oviposition sites for *Ae. aegypti* (Snow 1971; Muir et al. 1992a; Muir et al. 1992b). An ovistrip (14 x 6 cm) made of red flannel cloth was fastened to the inside of each ovibucket with a paper clip. The ovistrip provides a substrate for mosquitoes to deposit eggs upon. The ovistrip provided an indicator of activity in the room by observing the number of eggs deposited during a 3h period.

In the first treatment group, 500 ml of 50% concentration of hay infusion, made by soaking 5 g yeast, 500 g hay in 120 litres of water for seven days, was used in two ovibuckets to attract gravid females (Reiter et al. 1991). The hay infusion used was 8-9d old. The second treatment was the control whereby two clean and empty ovibuckets were placed in the room with the BGS. For each treatment, approximately twenty 10d gravid *Ae. aegypti* were released into the room for a period of 3h at 0830h and 1600h each day for 6d for a total of 6 replicates (1 replicate per day). A total of 238 mosquitoes across 6 replicates were released.

Data Analysis. Recapture rate data were tested for normality using a Shapiro-Wilks test and arcsin transformed. A one-way ANOVA was performed in *STATISTICA 7* to evaluate the effect of attractive ovipositioning sites on BGS recapture rates.

3. Results

3.1. Effect of Presence/Absence/Reflectance of Competing Harbourage Sites

The mean daily temperature and RH generated from a linear regression for the period 27 March – 6 April 2007 (0900h-1900h) was 25.9 ºC (range 24.5-26.9 ºC), and 73.5% RH (range 55-94%).

In the presence of low-reflectance (black) harbourage areas, female and male *Ae. aegypti* were recaptured at a mean (±SE) rate of 47.3% ± 0.12 and 25.3% ± 0.04 respectively. Females and males were recaptured at a mean rate of 68% ± 0.08 and 60.7% ± 0.08 respectively in the presence of high reflective (white) harbourage areas, and in the control (empty room) females and males were recaptured at a mean (±SE) rate of 77.7% ± 0.06 and 70.6% ± 0.1, respectively (Fig. 3.3).

Recapture rate data were arcsin transformed and found to be normally distributed for both females and males ($SW = 0.95$, $P = 0.44$, and $SW = 0.95$, $P = 0.41$, respectively). A factorial ANOVA found that an overall significant effect of treatment ($F = 9.74$; df = 2, 30; $P < 0.001$), and no interactive effect between sex and treatment was observed ($F = 0.43$; df = 2, 30; $P = 0.66$). A post-hoc pair-wise comparison using a Fisher's LSD test revealed a significant difference between treatments for each sex. An increase in harbourage areas of high-reflectance (white) had no effect on the recapture rate of females or males ($P = 0.44$, and $P = 0.45$, respectively). Females and males were recaptured at a significantly lower rate in the presence of low-reflectance (black) harbourage sites ($P = 0.02$, and $P = 0.001$, respectively). The negative effect of surrounding low-reflectance harbourage sites was more pronounced in male recapture rates (Fig. 3.3).

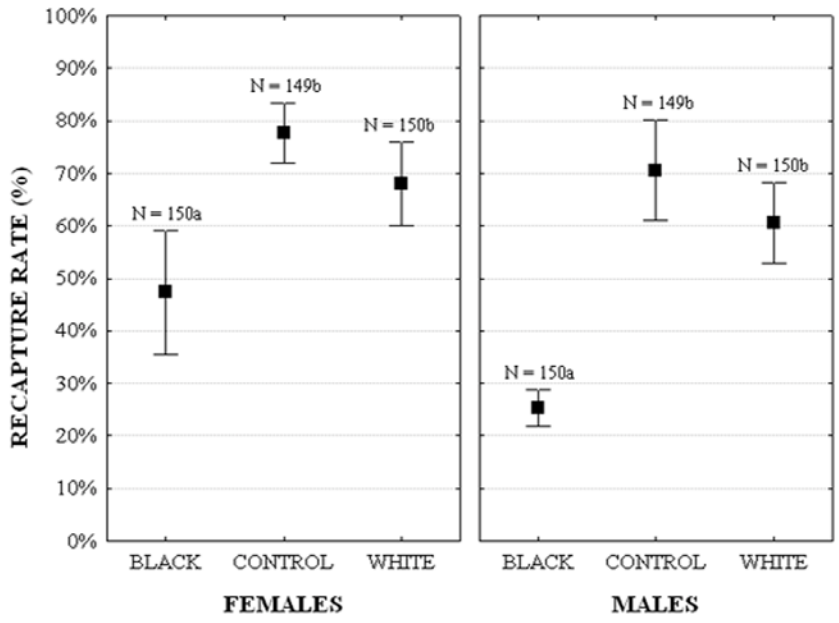

Fig. 3.3. BGS recapture rates (±SE) of female and male *Ae. aegypti* released in a room in the presence of low-reflectance (black) and high-reflectance (white) harbourage areas. Values followed by different letters are significantly different ($P < 0.05$) by ANOVA. Both males and females are recaptured at a significantly lower rate in the presence of black (low-reflectance) harbourage sites.

3.2. Effect of the Distribution of Competing Harbourage Sites

The mean daily (0830h-1830h) temperature and relative humidity within the room during this period was 28.7 ºC (range 23.5 - 31.4 ºC), and 73.7% RH (range 44.5 - 91.3%).

When low-reflective (black) harbourage areas were 'clustered,' female and male *Ae. aegypti* were recaptured at a mean (±SE) rate of 44.2% ± 0.05 and 23.3% ± 0.08 respectively. Females and males were recaptured at a mean (±SE) rate of 38.1% ± 0.07 and 11.7% ± 0.03 respectively when harbourage areas were 'scattered' around the BGS (Fig. 3.4). Recapture rate data were arcsin transformed and found to be normally distributed ($SW = 0.97$, $P = 0.37$). A factorial ANOVA showed that treatment was a significant predictor of recapture in both males and females ($F = 52.67$; df = 2, 30; $P < 0.001$). A significant interaction between treatment and sex

was also observed (F = 7.89, df = 2, 30; P = 0.002). A post-hoc pair-wise comparison using a Fisher's LSD test revealed a significant difference between treatments for each sex. Both females and males were recaptured at a significantly lower rate in the presence of low-reflectance harbourage areas regardless of its distribution ($P \leq 0.001$ and $P < 0.001$, respectively) (Fig. 3.4). No significant difference in recapture rates for female or male *Ae. aegypti* was found between the 'clustered' and 'scattered' distribution of the harbourage areas (P = 0.49 and P = 0.09, respectively); however, scattered crates tended to reduce recapture rates in comparison to crates that were clustered. Females were recaptured at an overall higher rate than males in both 'scattered' and 'clustered' treatment groups (F = 15.12; df = 1, 22; $P <$ 0.001).

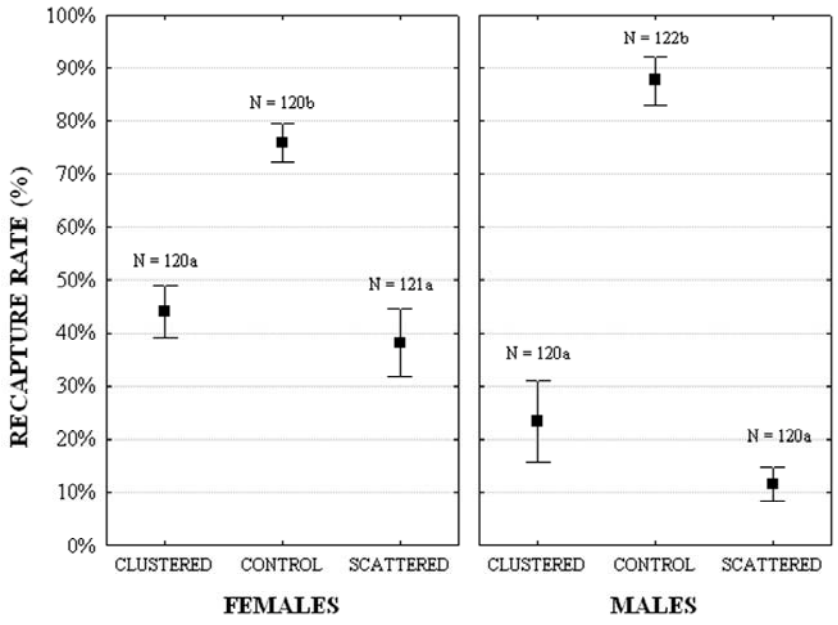

Fig. 3.4. BGS mean recapture rates (±SE) of male and female *Ae. aegypti* amongst clustered and scattered attractive low-reflective (black) harbourage areas. Values followed by different letters are significantly different (P < 0.05) by ANOVA. Distribution of the harbourage areas has no significant effect on recapture rates for males or females, however recapture rates are negatively affected by the presence of low-reflective (black) harbourage areas.

3.3. Effect of Oviposition Sites on Recapture of Gravid Females

The mean daily (0830h-1900h) temperature and relative humidity within the room during this period was 25.8 ºC (range 23.2 – 30.1 ºC), and 85.5% RH (range 68 – 98%).

In the absence of oviposition sites gravid females had a mean (±SE) recapture rate of 58.2% ± 0.14. In the presence of oviposition sites gravid females had a mean (±SE) recapture rate of 59.1% ± 0.13. Oviposition activity was observed in the room during the treatment replicates by recording the number of eggs deposited on the ovistrips. An average (±SE) of 491 eggs (246 / container) ± 115.1 were oviposited during a 3h period when ovibuckets containing hay infusion (treatment) were present. Eggs were not deposited during the control replicates as ovibuckets were dry.

Data were arcsin transformed and found to be normally distributed ($SW = 0.88$, $P = 0.09$). A factorial ANOVA found no significant difference between repetitions ($F = 0.16$; df = 5, 5; $P = 0.97$) and detected no significant difference in recapture rates between the presence and absence of surrounding oviposition sites ($F = 0.001$; df = 1, 5; $P = 0.97$).

4. Discussion

The impact of the surrounding visual environment on trapping efficacy of the BGS was investigated. The presence of harbourage areas alone, represented by high-reflectance (white) containers, had no significant effect on recapture rates. However, low-reflectance (black) containers significantly reduced recapture rates of male and female *Ae. aegypti*. Females were recaptured at a higher rate than males in the presence of low-reflectance harbourage area, suggesting a higher level of flight activity and movement in females. This could potentially be explained by observations made by Jones (1981) that illustrate the marked variation in flight activity between gravid, blood-fed, virgin and inseminated female *Ae. aegypti* whereby virgin

and inseminated females prior to a blood meal and post oviposition display a high level of spontaneous activity.

The distribution of low-reflective harbourage areas (clustered vs. scattered) was found to have no significant effect on recapture rates for male or female *Ae. aegypti*. However both females and males were observed to be recaptured at a lower rate in the "scattered" treatment versus the "clustered" treatment which may be related to our initial predictions of an interrupted visual path between the mosquito and the BGS, which may reduce recapture rates.

Ovibuckets containing a [50%] hay infusion were used to investigate the impact of nearby oviposition sites on BGS efficacy. The presence of ovipostion sites near the BGS appeared to have no effect on BGS efficacy with respect to gravid *Ae. aegypti*. Eggs were detected on the ovistrips, an indication that ovipositioning activity occurred throughout the treatment components of the experiment. Although ovipositioning behaviour did occur during the 3h period, evidenced by the presence of eggs on the ovistrips, the presence of these sites near the trap did not affect the recapture rate of *Ae. aegypti*. As predicted, the attraction of the surrounding oviposition sites may have been a distraction for *Ae. aegypti*, but only temporarily as ovipositing females continued to move throughout the room and were recaptured by the trap.

In the field, the BGS is set in a variety of locations, each imposing its unique attributes onto the efficacy and biases of the trap. The visual attractiveness of the BGS is a short-range signal, available to mosquitoes only within direct line-of-sight of the trap. Within the visual environment, objects of low reflectance with a solid dark surface area, are the most attractive sites for *Ae. aegypti* (Fay & Eliason 1966; Muir et al. 1992a), potentially representing a variety of micro environments such as harbourage sites, oviposition sites, hosts, or mating opportunities. Visually, the BGS could represent any one of these opportunities, which may explain the success of this trap in capturing males and females across a broad spectrum of physiological groups (Maciel-de-Freitas et al. 2006; Williams et al. 2006b).

Models such as the Premise Condition Index (PCI) use variables such as 'tidiness' and 'shade' to estimate premises that are likely to have flooded artificial containers that could produce *Ae. aegypti* (Tun-Lin et al. 1995). Similarly, an index based on 'clutter' within the room or area where the BGS is placed, could be applied to correct collection data and obtain a more accurate population estimate. As discovered with the PCI, untidy premises are 2.5 times more likely to be positive for immature *Ae. aegypti* than a tidy premise (Tun-Lin et al. 1995). Here, we discussed how low-reflectance harbourage areas surrounding the trap create competition for the BGS, thus reducing trapping efficacy. However, what we must also consider is that it is the presence of harbourage areas or 'untidiness' that is ultimately one of many variables that attracts adult *Ae. aegypti* to a particular premise and, therefore, the BGS, potentially negating the reduction in trapping efficacy observed in these experiments. We would assume that the number of *Ae. aegypti* captured by the BGS would still be relative to the amount of surrounding harbourage sites.

The initial experiments investigating the impact of the presence, absence, reflectance, and distribution of harbourage areas on trapping efficacy do not include mosquitoes belonging to other physiological groups (e.g. gravid, blood-fed). Flight activity varies between physiological groups (Judson 1968; Jones 1981) (Ball & Ritchie 2010b), therefore more complex interactions between mosquito physiology and the visual environment may exist. The limits of these experiments did not allow for these observations to occur. Outcomes from this suite of experiments provide us with important considerations for trap deployment and interpretation of *Ae. aegypti* samples from the BGS trap. Future studies involving different physiological stages and variations of the visual environment may be useful in the field application of this trap.

CHAPTER 4.

Does the BG-Sentinel™ trap reflect absolute abundance of *Aedes aegypti*: correlations between trap collections and simulation model population estimates.

1. Introduction

Novel control strategies have been developed for *Aedes aegypti* that target the adult population. Control strategies that use population replacement with *Ae. aegypti* with reduced vectorial capacity require sampling and modeling methods that can accurately describe the absolute field population. These include the use of the bacterial endosymbiont *Wolbachia* to shorten the lifespan of the vector population (Brownstein et al. 2003; McMeniman et al. 2009), and the sterile insect technique (SIT) using female-lethal RIDL™ (Release of Insects carrying a Dominant Lethal) whereby released RIDL males mate with wild females and produce no female progeny (Oxitec ; Coleman & Alphey 2004). Accurate estimates of the size, age and physiological structure of the adult vector population are paramount in facilitating successful vector control where population replacement is required.

The BG-Sentinel™ trap successfully traps *Ae. aegypti* across different physiological stages, ages, body sizes, and sexes. What is yet to be understood is what BGS collections in the field say about the abundance of *Ae. aegypti* in a given area. Through monitoring the immature stages, the most common sampling method for *Ae. aegypti*, certain attributes about the population can be described, particularly presence/absence, distribution, preferred breeding habitat ('key containers'), and abundance of the immature population. These methods are extremely labour-intensive, and are limited in their capacity to describe adult abundance.

The container-inhabiting mosquito simulation model (CIMSiM) is a weather-driven life table simulation model designed to estimate abundance of container-breeding mosquitoes such as *Ae. aegypti* (Focks et al. 1993a, 1993b). It is considered to be the most detailed tool available for understanding the population dynamics of *Ae. aegypti* (Magori et al. 2009). Recently, Williams et al. (2008) validated CIMSiM in the field in the Cairns region, the location of this study. What they found was that CIMSiM could be used to accurately estimate the pupal crop of four key containers in a timely manner through simple container surveys of an area. From the number of pupae, estimates of the adult population size can be generated. Focks et al. (1981) estimated the

number of vector competent *Ae. aegypti* (females >12d) in New Orleans, Louisiana, U.S.A. based on pupal numbers, and assumptions of the pupal period, sex ratio, and survival. It is these same assumptions that are used in CIMSiM where a life table is generated to estimate, in addition to the number of pupae within an area, the number of eggs, larvae, and adult males and females.

In addition to weather data, CIMSiM requires container density values to estimate *Ae. aegypti* productivity in a given area. In order to obtain container density, widespread surveys are required to record the various types of container that produce or could potentially produce *Ae. aegypti*. Container surveys are less intensive than larval and pupal surveys, but are still labour-intensive. In this study, a less labour-intensive sampling strategy to estimate adult abundance, the BGS, is investigated as a suitable tool to estimate adult *Ae. aegypti* abundance within a given area. Correlations between adult estimates generated in CIMSiM with the mean number of adults collected by the BGS are used to determine the potential of the BGS to estimate *Ae. aegypti* abundance. The CIMSiM model was chosen as a suitable comparison model due to its recent validation in the field, whereby actual pupal numbers per hectare highly corresponded with the estimated number of pupae generated by the model (Fig 4.1) (Williams et al. 2008). An area-wide container survey and 24h adult sampling with the BGS was performed over an 11 hectare area in February (wet season) and August (dry season) 2007. We expect to see a positive linear correlation between the per hectare adult data derived from the BGS and the per hectare adult abundance estimates generated by CIMSiM.

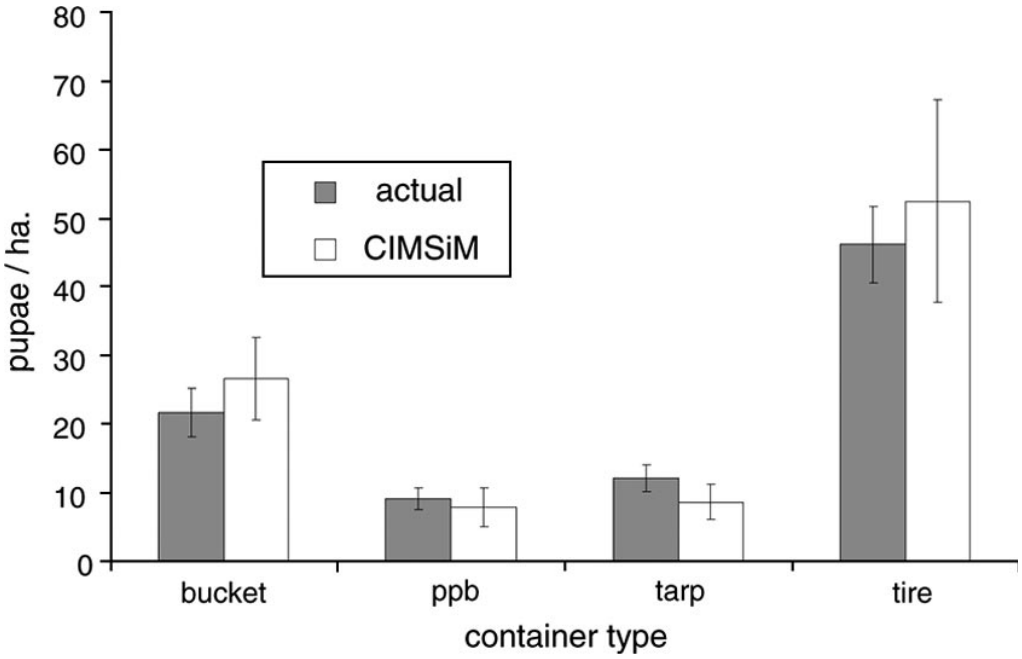

Figure 4.1. Figure from Williams et al. (2008) illustrating the validation of the CIMSiM model calibration of pupal productivity of four sentinel key containers (buckets, plant pot bases, tarp, and tyres) in greater Cairns, Australia.

2. Materials and Methods

Site. This study was conducted over an 11ha area within the suburb of Parramatta Park, Cairns, Queensland, Australia (16.9º S, 145.8 º E) from the 30 January – 3 February 2007 (wet season) and from the 22-25 August, 2007 (dry season). Parramatta Park was the site of the index case within a dengue virus serotype-2 outbreak in February, 2003 (Ritchie et al. 2004; Hanna et al. 2006). This suburb is particularly vulnerable in dengue outbreaks due to the high occurrence of Queenslander-style homes that are open and unscreened, allowing ease of movement between homes of potentially infected *Ae. aegypti.*

Meteorological Data. Daily temperature, rainfall, and relative humidity data were collected for the years 2006 and 2007 from the Australian bureau of meteorology's (www.bom.gov.au) Cairns Airport weather station, located ~5km from the study site.

Container Survey. With the assistance of the Dengue Action Response Team (DART) of Queensland Health, a widespread survey of the outdoor area of premises within the study site was performed. The first survey occurred during the wet season from the 30 January – 3 February 2007, and a subsequent survey during the dry season from the 22-25 August 2007. The number and types of containers holding water or that could potentially hold water were recorded. Even though the immature stages of *Ae. aegypti* occur in a variety of different container types, plastic buckets, pot plant bases, tyres, and indentations in tarpaulins made up ~60% of all containers and 73% of *Ae. aegypti* pupae in a recent survey within this study site (P.H.J., unpublished data) (Williams et al. 2008). These containers will be referred to as 'key containers' for the study site. All other container types were excluded from the analysis.

Adult Sampling. Adult sampling was performed using the BGS trap. Each household within the study site was visited and inhabitants were asked for permission to set-up a trap on their property for a 24h period. Trapping occurred over a 3d period due to an insufficient number of traps to be set at one time. Traps were set-up typically underneath houses, on the veranda,

outdoor laundry areas, or in open garages/sheds. Trap location was often determined by the availability of a power point. If a power point was not available, a 12V battery was used. Traps were set out between 0900 and 1000 and collected 24h later. Bags were removed from the BGS traps, labelled and placed within a refrigerated box. The sex and species of the mosquitoes for each premise were determined in the insectary at James Cook University, Cairns.

The mean number of males and females per trap was generated for each of the ten study sections. A one-way ANOVA was used to detect seasonal differences in BGS captures for females and males in *STATISTICA 7* (Stat Soft Inc. 2300 E 14th St., Tulsa, OK, U.S.A.)

Population Simulation. CIMSiM (MS-DOS version 1.4, USDA, Gainesville, FL) generates life tables based on 'per hectare' data. The 11ha study site was divided into 10 study sections, ranging in area from 0.9 – 1.6 ha (Fig. 4.2), creating 10 replicates for each season (wet/dry). The productivity of the four identified key container types being used in the model were formerly validated within CIMSiM in the Cairns region by Williams et al. (2008). The density of each of the four key container types per hectare was then calculated for each of the ten sections and included in the model. All model simulations were run over a two-year period (2006-2007). Even though the container survey occurred in 2007, the first year is an equilibration year (Focks et al. 1993b), whereby the *Ae. aegypti* population is stabilized (Williams et al. 2008). Within the model, adjustments were made to the container database as well as to survivorship as per Williams et al. (2008). All containers in the model were started with a set number of eggs on 1 January, 2006: 20 eggs each in pot plant bases, plastic buckets, and tarpaulin indentations, and 50 eggs in used car tyres. Daily adult survivorship was adjusted from the default value of 0.89 to 0.83 based on estimates from locally conducted mark-release-recapture studies and cage experiments (P.H.J. and L.P.R., unpublished data) (Williams et al. 2008).

Analysis. The model generates a life table from which an estimate of the daily population size of adult males and females is produced. The food delivery for the buckets was set to 'random' for each container and the model was run a total of six repetitions for each of the ten sections, from which a daily mean was obtained. Random food delivery produces a different outcome each time the simulation is run by generating random numbers from zero to twice the daily food value already stated within the model. Williams et al. (2008) used random food delivery to calibrate the CIMSiM model in this area. A fixed food delivery was also used for each section, whereby food is added to the containers at a fixed rate of every four days within the model. From the model simulations, the daily population size of adult females and males was recorded over a 14-day period, commencing the first day of the container survey— two days prior to the initial adult trapping date using the BGS. A mean population size of males and females was obtained from the 14-day period with both the fixed and random food delivery settings. The number of adult females generated by the model is equal to the number of adult males under the model's assumption that the sex ratio of emerging male and female adults is 1:1 (Williams et al. 2008).

To determine if model outputs differed significantly between seasons and between the two food delivery settings, an ANOVA was run. Subsequently, a correlation analysis was performed between the BGS mean trap captures for each section and the CIMSiM model outputs for the same corresponding sections. The mean number of females, mean number of males, and combined mean of females and males were each correlated to the model outputs from both the random and fixed food delivery settings. The purpose of this was to find out which settings in the model created outputs that correlated best with the BGS trap results. All correlation analyses were run in *STATISTICA 7*.

Fig 4.2. Parramatta Park study site in Cairns, QLD divided into ten sections, ranging in area from 0.9-1.6 ha.

3. Results

The mean temperature and relative humidity (RH) for the wet season (30 January – 3 February 2007) and dry season (22-25 August, 2007) sampling periods were 27ºC (range 25-32), 91% (range 75-100) and 22 ºC (range 16-28), 73% (43-100), respectively. Total rainfall for the months of January and August 2007 was 339 and 16.9mm, respectively (www.bom.gov.au).

Adult Survey. Of 176 houses in the study area, permission to set BGS traps was granted for 67 (38%) and 72 (41%) houses during the wet and dry season, respectively. A total of 225 and 65 female and 158 and 42 male *Ae. aegypti* were captured during the wet and dry season, respectively. Individual male and female per hectare data for each season is shown in Tables 4.1 and 4.2. The mean number of females and males captured by the BGS was 3.4 (range 0-24, SE ±0.54) and 2.4 (range 0-22, SE ±0.49) during the wet season, and 0.92 (range 0-11, SE ±0.19) and 0.59 (range 0-4, SE ±0.11) during the dry season. A one-way ANOVA found that there was a significant seasonal difference in both female ($F = 18.97$, df = 1,136, $P < 0.001$) and male ($F = 18.97$, df = 1,136, $P < 0.001$) capture rates with the BGS, whereby both sexes were captured at a higher rate in the wet season, an expected result due to an increase in abundance of *Ae. aegypti* during this time of year.

Population Simulation. A total of 159 and 152 premises out of 176 were inspected for containers during the wet and dry season, respectively. The density of each of the four key containers was calculated per hectare for each of the 10 sections (Table 4.3). These densities were then entered into the model. The model produced a 'per hectare' population estimate for males and females for random food delivery and fixed food delivery within each section for both the wet and dry season (Figs. 4.3 & 4.4). The mean predicted population size of the area for males or females during the wet season was 41.8 (11-106, SE ±9.3) and 36 (15-84, SE ±7.7) at random and fixed settings, respectively. During the dry season, the mean predicted population size was 36.7 (range 11-81, SE ±8.2) and 16.5 (range 5-28, SE ±2.8) at random and fixed settings, respectively. An ANOVA found no significant difference between using random

food delivery and fixed food delivery model settings during the wet season ($F = 0.219$, df = 2, 18, $P = 0.65$). However a significant difference was observed between these two food delivery settings in model outputs generated during the dry season ($F = 5.589$, df = 2,18, $P = 0.03$). A seasonal difference in the predicted *Ae. aegypti* abundance was observed between the wet and dry season estimates when food delivery was fixed ($F = 5.69$, df = 1, 18, $P = 0.03$) No seasonal effect was observed when food delivery in the model was random ($F = 0.15$, df = 1, 18, $P = 0.70$).

Correlation. A linear correlation analysis was performed between the random and fixed food delivery setting model outputs and the BGS mean trap captures for each of the ten sections. The mean number of females, mean number of males, and combined mean of females and males were each correlated to the model outputs from both the random and fixed food delivery settings. The strongest positive linear correlations between the BGS adult data and the CIMSiM model were observed when the food delivery method was fixed within the model (Table 4.4). Although statistically insignificant, positive linear correlations between fixed CIMSiM outputs and the BGS were observed during the wet season for females ($r^2 = 0.215$, r = 0.463, $P = 0.178$), males ($r^2 = 0.350$, r = 0.592, $P = 0.072$) and combined females and males ($r^2 = 0.275$, r = 0.524, $P = 0.119$) (Table 4.4) (Figs. 4.5, 4.6, and 4.7). No linear correlation was observed between CIMSiM model outputs and BGS data during the dry season alone (range, $r^2 = 0.0001 – 0.012$, r = -0.111 – 0.069). A significant linear relationship was observed between the CIMSiM and the BGS mean number of females ($r^2 = 0.322$, r = 0.567, $P = 0.009$), males ($r^2 = 0.420$, r = 0.648, $P = 0.002$), and combined males and females ($r^2 = 0.371$, 0.609, $P = 0.004$) when the wet and dry season were both included in the correlation (Table 4.4) (Figs. 4.8, 4.9, and 4.10).

Table 4.1. BGS trap data for females in February (wet season) and August (dry season) 2007.

BGS Trap Results for Female *Aedes aegypti* (Wet/Dry)						
Section ID	**No. Traps**	**Total ♀**	**Mean No. Per Trap**	**Max**	**Min**	**SE**
H1	7 / 5	58 / 2	8.3 / 0.4	24 / 1	0 / 0	3.21 / 0.25
H2	3 / 4	38 / 2	12.7 / 0.5	20 / 2	8 / 0	3.71 / 0.50
H3	9 / 8	28 / 22	3.1 / 2.8	4 / 11	0 / 0	0.72 / 1.32
H4	4 / 4	11 / 3	2.8 / 0.8	7 / 2	0 / 0	1.38 / 0.48
H5	8 / 9	24 / 14	3.0 / 1.6	6 / 4	0 / 0	0.85 / 0.44
H6	3 / 6	6 / 5	2.0 / 0.8	5 / 2	0 / 0	1.53 / 0.31
H7	10 / 12	31 / 7	3.1 / 0.6	9 / 4	0 / 0	0.90 / 0.36
H8	7 / 10	8 / 4	1.1 / 0.4	3 / 2	0 / 0	0.51 / 0.22
H9	8 / 7	9 / 3	1.0 / 0.4	4 / 2	0 / 0	0.44 / 0.30
H10	7 / 7	12 / 4	1.71 / 0.6	4 / 2	0 / 0	0.64 / 0.30

Table 4.2. BGS trap data for males in February (wet season) and August (dry season) 2007.

BGS Trap Results for Male *Aedes aegypti* (Wet/Dry)						
Section ID	**No. Traps**	**Total ♂**	**Mean**	**Max**	**Min**	**SE**
H1	7 / 5	31 / 2	4.4 / 0.4	13 / 1	0 / 0	1.72 / 0.25
H2	3 / 4	29 / 4	9.7 / 1.0	15 / 3	5 / 0	2.91 / 0.71
H3	9 / 8	30 / 7	3.3 / 0.9	22 / 3	0 / 0	2.41 / 0.40
H4	4 / 4	9 / 6	2.3 / 1.5	4 / 4	0 / 0	1.03 / 0.87
H5	8 / 9	10 / 8	1.3 / 0.9	7 / 4	0 / 0	0.84 / 0.16
H6	3 / 6	4 / 6	1.3 / 1.0	2 / 2	1 / 0	0.33 / 0.37
H7	10 / 12	34 / 4	3.4 / 0.3	10 / 1	0 / 0	1.12 / 0.14
H8	7 / 10	2 / 3	0.3 / 0.3	2 / 1	0 / 0	0.29 / 0.15
H9	8 / 7	5 / 1	0.6 / 0.1	3 / 1	0 / 0	0.34 / 0.14
H10	7 / 7	4 / 1	0.6 / 0.1	2 / 1	0 / 0	0.30 / 0.14

Table 4.3. Density of each key container type across ten study sections (Container density per hectare = no. containers*area).

Density of Key Containers per Hectare (Wet/Dry)										
Section ID (Area ha)	**H1 (1.0)**	**H2 (1.0)**	**H3 (1.6)**	**H4 (1.0)**	**H5 (1.0)**	**H6 (0.8)**	**H7 (1.6)**	**H8 (0.8)**	**H9 (1.0)**	**H10 (1.0)**
Tarpaulin Indentations	1/0	1/1	2/4	1/2	1/1	0/1	15/2	0/0	1/0	2/3
Used Tyres	1/0	2/0	1/0	0/0	1/4	0/0	0/0	0/0	1/0	0/2
Plant Pot Bases	2/14	24/38	7/20	19/29	7/37	3/6	18/12	38/19	9/14	6/29
Plastic Buckets	6/3	16/7	9/5	3/5	12/7	6/5	2/2	13/23	2/3	2/2

Table 4.4. Correlation between CIMSiM per hectare population size estimate and the corresponding hectare data of the mean number of adults collected by the BGS. Italicized rows are not statistically significant, but indicate a strong positive linear correlation. Significant correlations indicated in bold.

CIMSiM : Food Delivery Method Random (R), and Fixed (F)	BGS: Males (M), Females (F), Males and Females (MF)	**N**	**R^2**	**R**	***P***	**Y**
WET SEASON						
R	F	10	0.033	0.181	0.618	=36.22 + 1.43X
F	*F*	*10*	*0.215*	*0.463*	*0.178*	*=24.29 + 3.05X*
R	M	10	0.089	0.299	0.402	=32.91 + 3.18X
F	*M*	*10*	*0.350*	*0.592*	*0.072*	*=21.51 + 5.24X*
R	MF	10	0.055	0.234	0.516	=34.61 + 1.08X
F	*MF*	*10*	*0.275*	*0.524*	*0.119*	*=22.76 + 2.00X*
DRY SEASON						
R	F	10	0.001	0.038	0.918	=35.44 + 1.43X
F	F	10	0.003	0.057	0.876	=15.72 + 0.73X
R	M	10	0.012	-0.111	0.759	=54.45 – 16.97X
F	M	10	0.001	0.034	0.927	=14.72 + 1.72X
R	MF	10	0.0001	0.010	0.977	=36.09 + 0.42X
F	MF	10	0.005	0.069	0.849	=14.55 + 0.94X
WET & DRY SEASON						
R	F	20	0.027	0.164	0.489	=1.766 + 0.02X
F	**F**	**20**	**0.322**	**0.567**	**0.009**	**=0.311 + 0.08X**
R	M	20	0.056	0.236	0.316	=1.18 + 0.02X
F	**M**	**20**	**0.420**	**0.648**	**0.002**	**=0.156 + 0.07X**
R	MF	20	0.039	0.197	0.406	=2.951 + 0.04X
F	**MF**	**20**	**0.371**	**0.609**	**0.004**	**=0.467 + 0.15X**

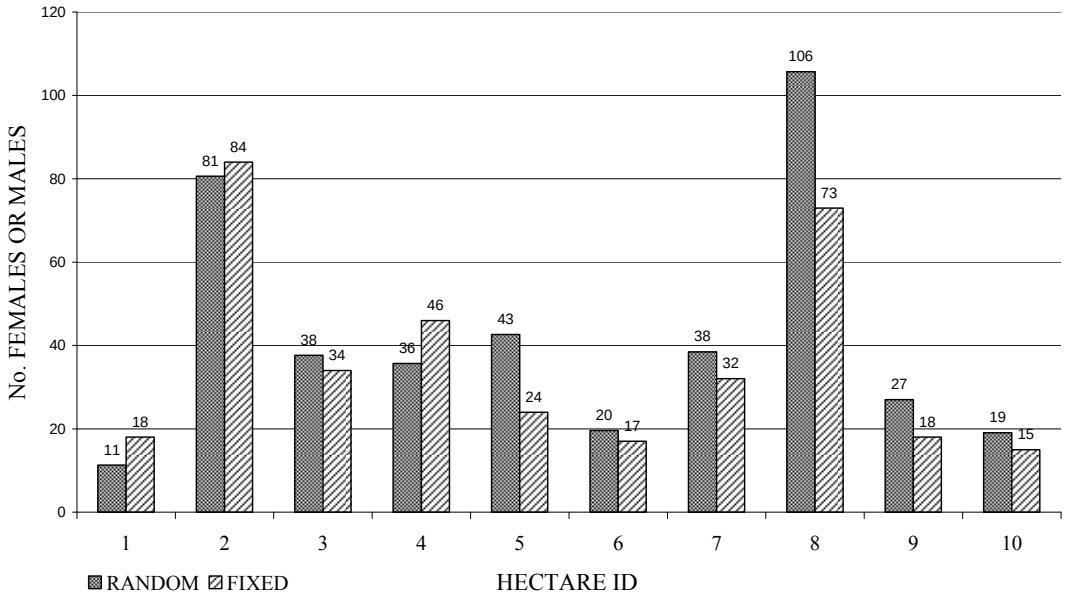

Fig. 4.3. Mean wet season population estimates for male or female *Aedes aegypti* with random and fixed food delivery settings.

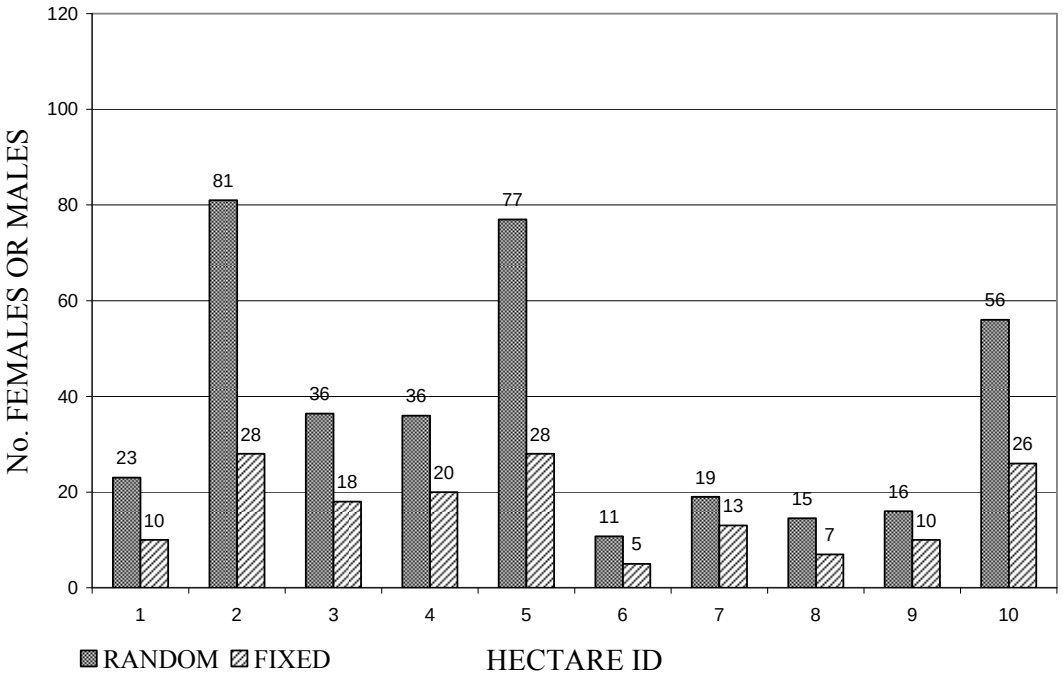

Fig. 4.4. Dry season population estimates for male or female *Ae. aegypti* with random and fixed food delivery options.

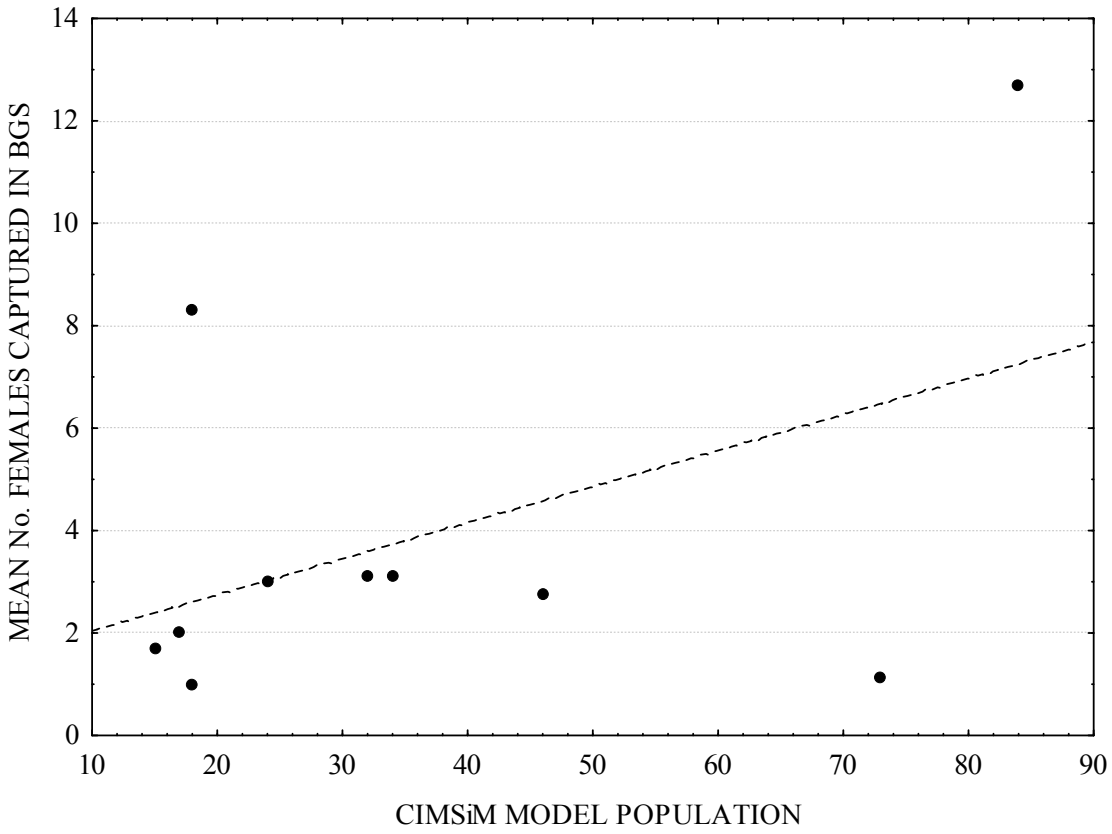

Fig. 4.5. CIMSiM predicted population outputs of *Ae. aegypti* correlated with the mean number of females captured by the BGS for each of the 10 study sections during the wet season.

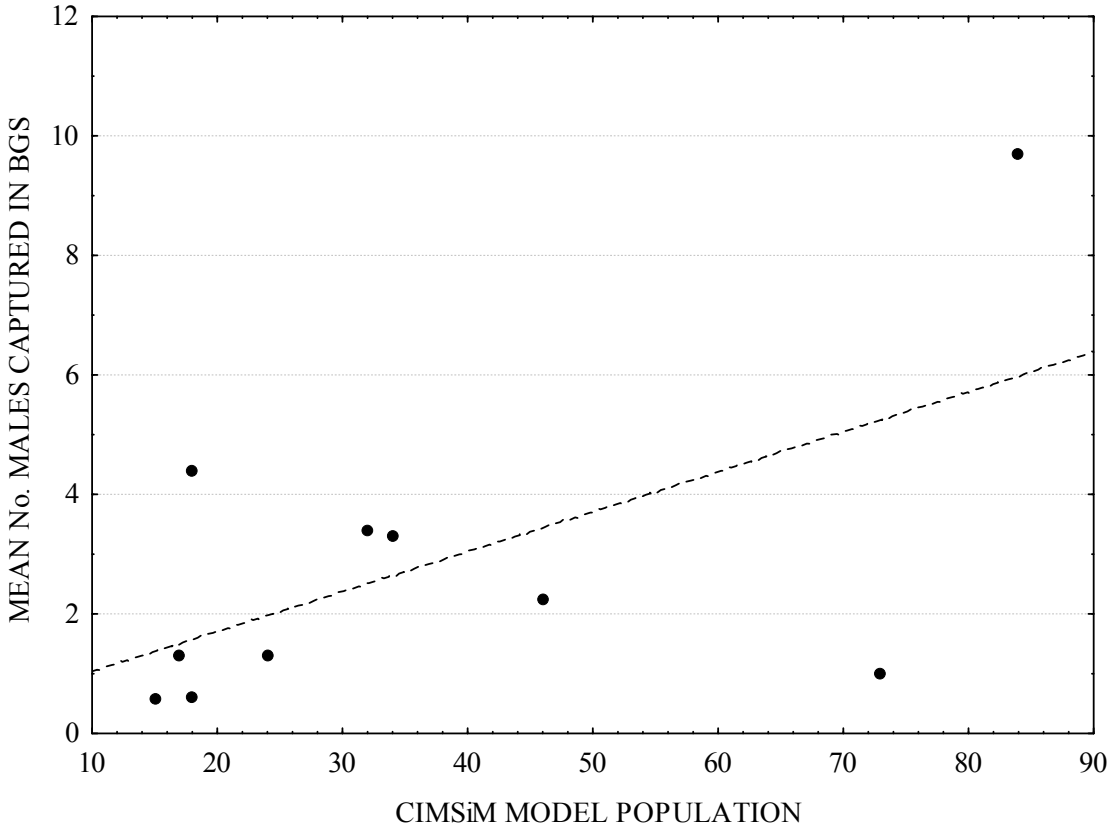

Fig. 4.6. CIMSiM predicted population outputs of *Ae. aegypti* correlated with the mean number of males captured by the BGS for each of the 10 study sections during the wet season.

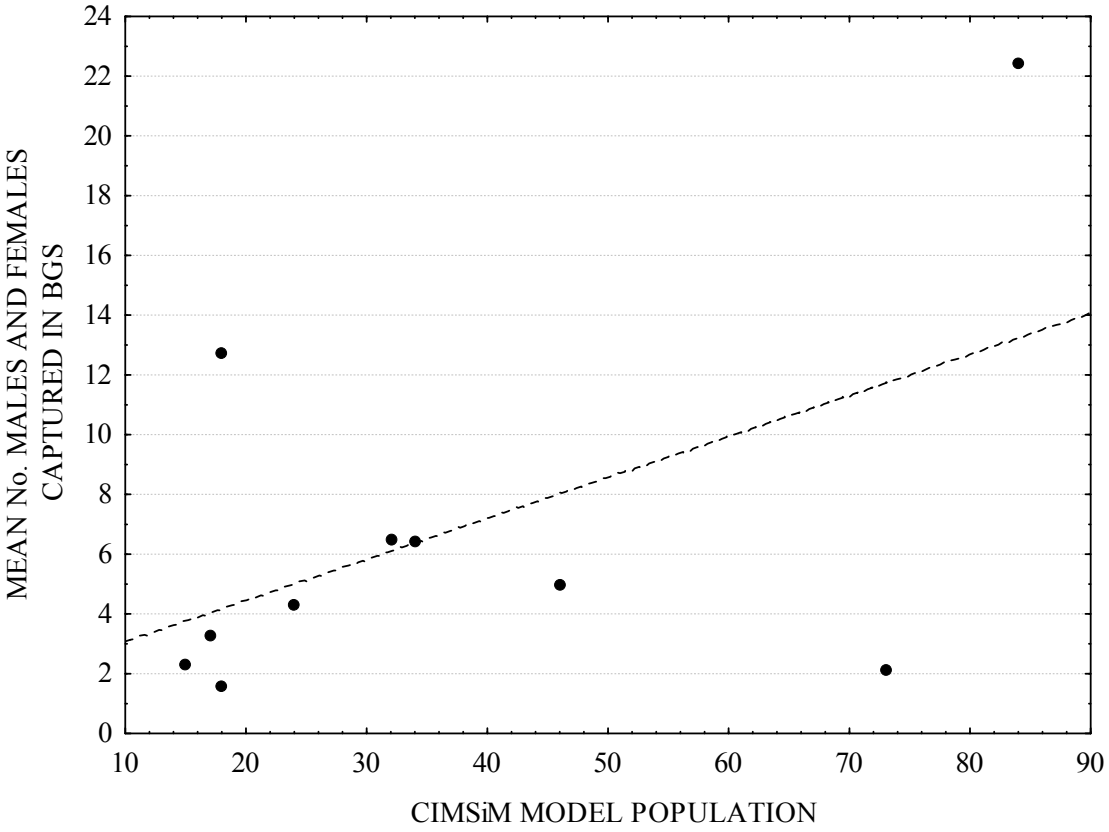

Fig. 4.7. CIMSiM predicted population outputs of *Ae. aegypti* correlated with the mean number of males and females captured by the BGS for each of the 10 study sections during the wet season.

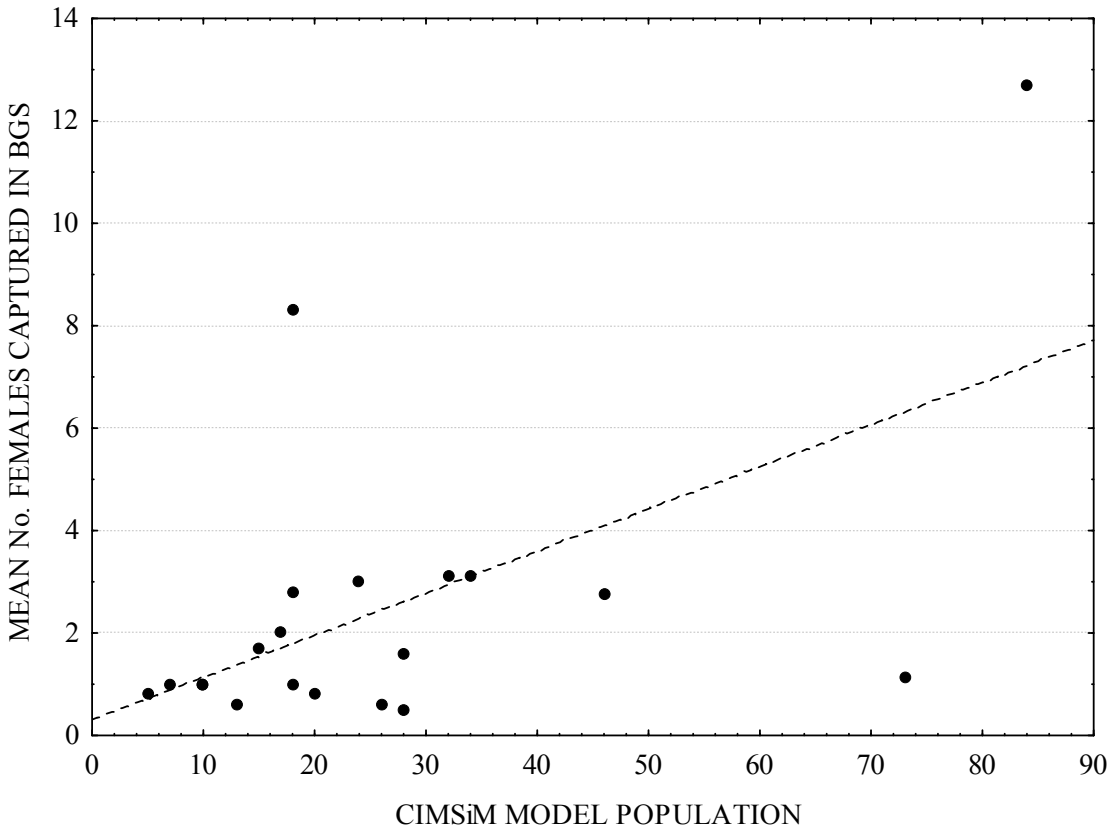

Fig. 4.8. CIMSiM predicted population outputs of *Ae. aegypti* correlated with the mean number of females captured by the BGS for each of the 10 study sections during the wet and dry season.

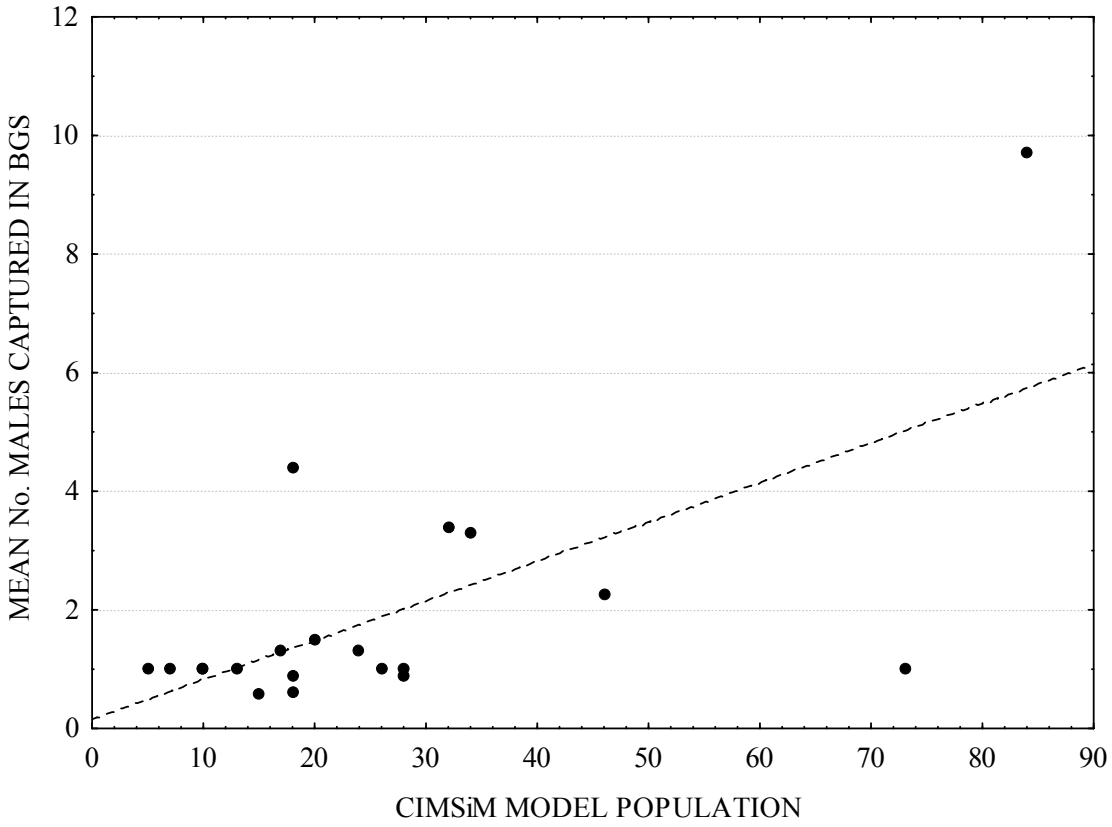

Fig. 4.9. CIMSiM predicted population outputs of *Ae. aegypti* correlated with the mean number of males captured by the BGS for each of the 10 study sections during the wet and dry season.

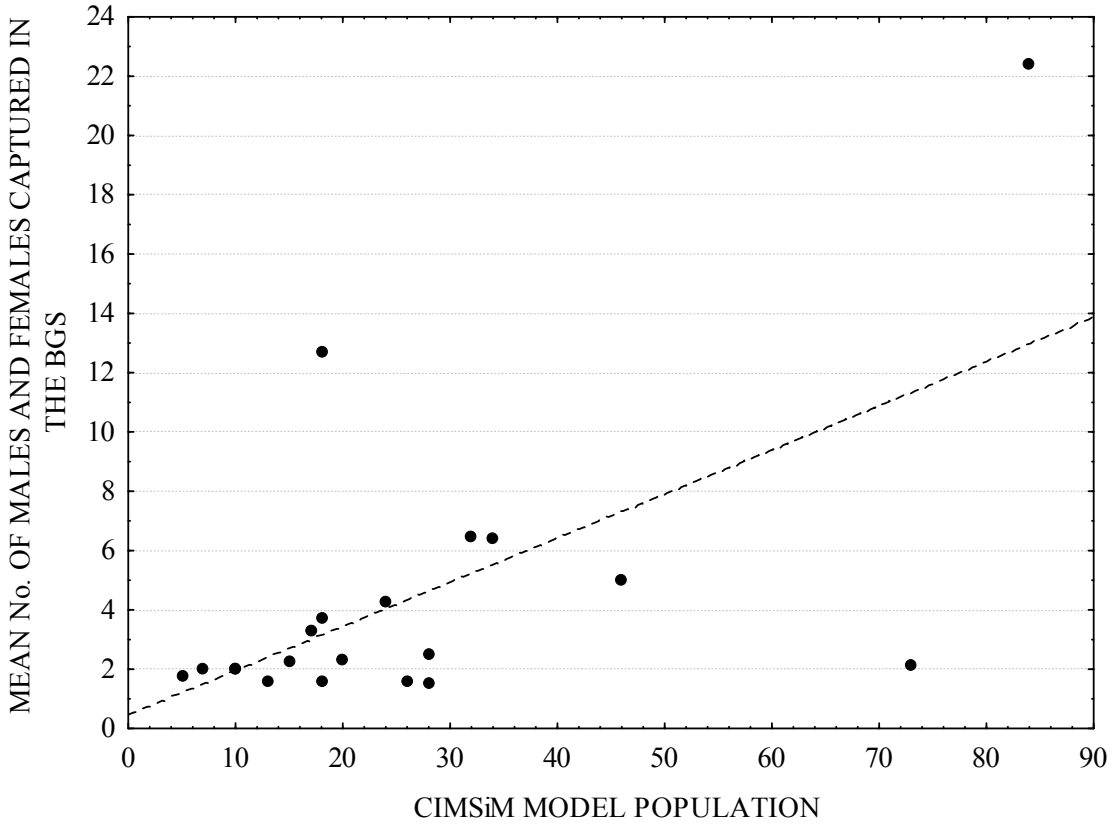

Fig. 4.10. CIMSiM predicted population outputs of *Ae. aegypti* correlated with the mean number of combined males and females captured by the BGS for each of the 10 study sections during the wet and dry season.

4. Discussion

In the wave of development of new strategies in vector control for *Ae. aegypti*, novel methods to estimate the complex structure of the adult population must also be developed. Here, the potential use of the BGS to estimate adult *Ae. aegypti* abundance was investigated. Although an insignificant correlation between the CIMSiM adult estimates and BGS data was observed, a positive linear relationship does exist between trap and model based on data obtained during the wet season. No evidence of a positive relationship was observed in the dry season alone. However combined seasonal data produced significant linear relationships between CIMSiM outputs and the mean number of males, females, and combined males and females. Although a general trend for a linear relationship does exist, the presence of several outliers within the data set may have produced the significant result, whereby without the outliers a lesser correlation may have been observed (Figs 4.8-4.10).

Underestimation of the CIMSiM model for a given hectare was much more easily detected than overestimation. Within 'Section 1,' the CIMSiM model predicts that a total of 11 (random) or 18 (fixed) females and the same number of males are within the same area. However, in the BGS 24h collection for the same section, one trap produced 24 females and 13 males. The total number of females and males collected from 7 traps in 'Section 1' was 58 and 31, respectively. The data from the BGS suggest that the CIMSiM outputs for the same section underestimated the adult population. What is not taken into consideration in the CIMSiM model, and is clearly illustrated here, is the movement of adults. In a mark-release-recapture study by Russell et al. (2005) they found that the mean distance travelled by adult males and females over a 15d period was 78m, and by day 15, 23% of recaptured females were recorded beyond 100m. What we cannot account for in this model is the addition of mosquitoes from outside the area, and the departure of individuals to a neighbouring area.

We assume that lower numbers of *Ae. aegypti* are being produced in the dry season, a function of cooler temperatures and a significant decrease in rainfall. A distinct seasonal difference in

abundance is detected in the BGS between the wet and dry season. Significantly higher numbers of adults were captured by the BGS during the wet season relative to the dry season. A seasonal difference between the wet and dry season in CIMSiM was only observed when the food delivery setting was fixed, otherwise no difference was detected.

The purpose of any model is to simplify a very complex system. The CIMSiM model attempts to make accurate estimations based on the known values of a few variables shown to affect the abundance of *Ae. aegypti*. Estimating the immobile population of *Ae. aegypti* for a given area in a model is likely to be more accurate than estimating the adult population, whereby adults are free to move from one area to another. Models are not perfect, and more complex models are being developed to make more accurate population of estimates of the *Ae. aegypti* population. Skeeter Buster, a newly developed model, builds on the CIMSiM model, and adds stochasticity, explicit spatial structure and genetics (Magori et al. 2009).

In conclusion, even though CIMSiM has been validated in the field as a reliable predictor of the pupal crop size in a defined area, it is not necessarily a reliable predictor of adult *Ae. aegypti* abundance. For this reason, we can only say that it is unknown whether or not BGS trap collections reflect absolute abundance. The number of adults captured between properties was highly variable, similar to observations made by Williams et al. (2007a) whereby BGS trap captures were highly variable between premises, however within a premise, day-to-day variability was low. Estimating absolute adult abundance for a large inhabited area based on BGS data obtained from one or two households, would not likely produce an accurate estimation. What the BGS collections are more likely to provide, is a more accurate estimate of the abundance within a given premise. A mean number of adult *Ae. aegypti* per trap value for a defined area may be useful if a sufficient density of traps are deployed.

CHAPTER 5.

General discussion and future directions.

1. General discussion and future directions

Novel control strategies for *Aedes aegypti* that rely on population replacement require knowledge of the absolute adult field population size and structure (e.g. sex ratio, age distribution, physiological structure). Egg, larval, and pupal surveys of *Ae. aegypti* attempt to describe the population primarily in terms of productivity and distribution. Such information is useful in measuring the effect of vector control efforts that focus on significantly reducing the abundance of *Ae. aegypti* within a given area. If the control strategy used does not focus on reducing abundance, but rather reducing vectorial capacity, sampling methods need to be directed toward the adult stages to monitor the success of the penetration of the modified mosquitoes' genes or accompanying pathogen (e.g. *Wolbachia* wMelPop) into the wild population.

The use of the BG-Sentinel (BGS) trap for sampling the adult population of *Ae. aegypti*, has proven to be a highly effective tool in both an experimental setting and in the field in far north Queensland. Unlike other adult sampling methods for *Ae. aegypti*, the BGS is a sensitive, non-labour intensive, and highly effective tool. Its efficacy and the ease of use for both deployment and collection, make it a suitable tool for routine surveillance. All sampling methods have limits in terms of their ability to collect a sample that is representative of the absolute population. It is important that these limits are clearly defined in order to obtain more accurate population estimates based on limited data, and this is precisely what this research attempted to achieve.

Trapping bias is inevitable with any sampling tool. However, despite a significant bias detected in the BGS, whereby teneral nulliparous females were captured at a lower rate than all other physiological groups, the trap successfully captures all females and males despite their physiological status, body size, and age (Chapter 2). The BGS has negligible biases in sampling the adult *Ae. aegypti* population, which make it a suitable sampling tool in population replacement control strategies. Although biases of the BGS have been defined within the field population, these biases would need to be defined within the modified population and between

the modified and field population. It is possible that the BGS is more effective at capturing field populations of *Ae. aegypti* than modified mosquitoes or vice versa. The efficiency with which the BGS captures a variety of different physiological groups in the field may change in the modified population as well. Particularly in the instance of *Wolbachia*-infected *Ae. aegypti*, where the lifespan of the mosquito is significantly reduced, we may find that an age or 'fitness' bias does exist, in that older and less fit mosquitoes are more or less likely to be recaptured by the BGS. It is important before embarking on any form of population replacement of *Ae. aegypti* which intend on using the BGS as a sampling tool, that these biases are defined.

The presence of low-reflective harbourage sites nearby the trap did have a negative effect on trapping efficacy for both males and females, with a more pronounced effect in males (Chapter 3). However, it can be argued that the presence of harbourage areas or 'untidiness' may ultimately be one of the variables that attracts adult *Ae. aegypti* to a particular premise and therefore, the BGS, potentially negating the reduction in trapping efficacy observed in these experiments. In Edman et al. (1997) low-reflective resting boxes were used to concentrate the household *Ae. aegypti* population to a particular space for more efficient collection with the CDC backpack aspirator. The addition of low-reflective harbourage sites nearby the BGS or deliberately locating the BGS within a household in areas with attractive resting sites may be the most productive site for the BGS in a given premise instead of the most convenient. The effect of the visual environment on modified populations of *Ae. aegypti* as well as their responsiveness to the BGS relative to field populations should be defined.

Measuring absolute abundance of any species of mosquito is a very challenging task. Models have been developed to estimate population size based on defining 'key' variables believed to be important predictors for the proliferation of a particular species. CIMSiM (Focks et al. 1995a, 1995b) is a weather-driven simulation model used to predict *Ae. aegypti* abundance in a given area. This model was recently calibrated and validated in the Cairns region (Williams et al. 2008). One of the key variables that need to be defined in CIMSiM is density of container

types in an area. Container surveys may not be as labour-intensive as a pupal survey, in that it does not require rearing or identification of samples, however this is a very labour-intensive component of running the model. These surveys also require entry into private properties where the details and number of every container (including plants) that could potentially be a breeding site for *Ae. aegypti* must be recorded and then entered into the model. Not a suitable tool for routine surveillance unless container conditions are presumed to be static. The model does not tell us much about the adult population for an area. It may successfully predict the number of adults produced within an area, but it cannot determine adult abundance in area because it does not take into consideration the movement of adults from one area to the next.

In comparison to CIMSiM, between seasons the BGS appeared to be more sensitive to detecting changes in absolute abundance of the population (Chapter 4). Although a very useful tool, CIMSiM is extremely limited in describing the adult population. It is important to understand absolute abundance in terms of the data provided by the BGS trap captures. To facilitate this, the BGS in conjunction with a tool like Prokopac (Vasquez-Prokopec et al. 2009), a modified version of the CDC backpack aspirator, may provide further insight into the meaning of BGS trap collections with respect to absolute abundance and structure of the adult *Ae. aegypti* population.

Williams et al. (2007a) observed that between households BGS collections varied significantly, however very little variation was observed within a premise over a few days. This outcome could be explained in two ways: i) the location of the BGS for each household is not standardised, therefore trap efficacy varies between houses based on trap location (e.g. back veranda vs. carport) and/or ii) the absolute adult population varies between each household. Standardising trap location and assessing the environment within which the trap is set (e.g. the amount of clutter in its immediate surroundings) could increase the value of the BGS trap data. We need to understand if the variation in the numbers collected between premises is a function of trap location or absolute abundance.

Based on the results from Williams et al. (2007a) and the results achieved in the CIMSiM correlation experiments, whereby a significant difference in the number of mosquitoes captured between the wet and dry season was observed (Chapter 4), the BGS appears to be an excellent tool in detecting rises and falls in absolute abundance. To truly observe changes in abundance, traps would need to be set at fixed locations, continuously sampling the population (sentinel surveillance). Since no 'trap out' effect by the BGS was observed (Williams et al. 2007a), whereby the constant running of the BGS trap does not reduce the household population, this trap is an excellent candidate for determining household risk and the effect of vector control strategies that focus on reducing vector abundance. If a vector control program which reduces adult abundance is initiated in a given area, the impacts should be detected from fixed sentinel BGS traps.

It appears that the limits of using the BGS as a surveillance tool, whereby traps are set on private premises, are limited by areas where the trap is easily accessed, and where permission is granted by the proprietors. This typically means that the trap is located outside the household, adjacent to an accessible power point, and not all households are sampled. In Chapter 4, permission was requested from all households in the study area either through verbal communication or a letter. Of 176 households, 72 (41%) was the maximum number of households where permission was granted to set-up a BGS. The distribution of the traps throughout the area was not randomly or evenly distributed, but determined by access granted. In the event of a release of modified populations of *Ae. aegypti*, some, if not all of these limits will need to be expanded or vanquished in order to achieve sufficient data about the adult population.

Further research with the BGS with respect to absolute abundance of the field population of *Ae. aegypti* should be further investigated through both field and experimental trials. Understanding how the effect of sampling bias and the impact of the visual environment on BGS efficacy in the field is the next step from these controlled sets of experiments. How these biases and the

environmental effects apply to modified populations is also crucial if this trap is to be used in these important and expensive vector control strategies.

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