Evaluation of transgenic insects for use in the control of insect-borne disease

Peter Winskill

Imperial College London
Department of Infectious Disease Epidemiology

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Abstract

The burden of many vector-borne diseases remains high and for some, such as dengue fever, continues to rise. It is estimated that up to half of the global population is at risk from dengue. Treatment of dengue fever is currently limited to case management and there are, at present, no licensed vaccines available. As a result, the front-line defence against dengue fever remains vector control. Modern approaches to vector control are attempting to push forward new techniques to target the mosquito vectors of dengue. One such technique is the release of transgenic insects that are genetically sterile due to a conditional dominant lethal gene. This modern adaptation of the traditional sterile insect technique is at the forefront of current new vector control solutions.

The success of a vector control effort using releases of transgenic insects relies on the technology being efficacious as well as effective in the field. To ensure the effectiveness of field-released sterile insects a deep knowledge of the mosquito biology and ecology must be combined with site-specific, logistical and cost considerations. In order to maximise the potential of this technology the field releases of these insects must be optimised. This work includes a specific focus on the exploration of the dynamics of releasing different life stages, investigations into the biology and ecology of the released insects and the development of applied methodology relating to the release and monitoring of transgenic insects.

Novel vector control techniques, such as the use of transgenic insects, have an important role to play in addressing the emergence and spread of dengue fever. In order to utilise these technologies to their full potential they must be optimised to maximise their effectiveness. In this thesis I present work towards this optimisation.
Declaration of originality

I declare this work to be my own, produced under the supervision of Prof. Christl Donnelly and Prof. Luke Alphey. Where applicable, any participants involved in collaborative work, which constitutes a large proportion of this thesis, have been duly acknowledged and studies referenced.

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List of abbreviations and acronyms

ACO Ant colony optimization
ACS Ant colony system
AIC Akaike information criterion
AUC Area under the curve
BIC Bayesian information criterion
CDC Centers for Disease Control and Prevention
CDF Cumulative distribution function
DHF Dengue haemorrhagic fever
DSP Daily survival probability
DSS Dengue shock syndrome
EIR Entomological inoculation rate
EM Expectation-maximisation
FR50 Flight range 50%
FR90 Flight range 90%
GFP Green fluorescent protein
GLM Generalised linear model
HEG Homing endonuclease gene
HLC Human landing catch
ITN Insecticide-treated bed net
LLIN Long-lasting insecticide-treated bed net
MASS Modern Applied Statistics with S
MDT Mean distance travelled
Medea Maternal effect dominant embryonic arrest
Medfly Mediterranean fruit fly
MLE Maximum likelihood estimate
MOPSO Multiple objective particle swarm optimization
MOPSO-ACS Multiple objective particle swarm optimization ant colony system
MRR Mark release recapture
PDF Probability density function
PSO Particle swarm optimization
R0 Basic reproduction number
RIDL Release of insects carrying a dominant lethal
RPP Rural postman problem
SD Segregation distorter
Semele Semen-based lethality
SIT Sterile insect technique
TE Transposable element
TSP Travelling salesman problem
tTA Tetracycline-repressible transactivator
ULV Ultra low volume
WHO World Health Organization
WT Wild type
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1 Introduction

1.1 Vector-borne diseases

Vector-borne diseases have been historically [1] and are currently responsible for a huge global public health burden. It was estimated that malaria was responsible for 627,000 deaths in 2012, the majority of which were young children [2]. Dengue, an arbovirus, has seen recent re-emergence and spread on a global scale [3] and is now responsible for an estimated 390 million infections annually [4].

Minimising the public health risk of vector-borne diseases often involves employing a combination of many tools. These can include treatment, prophylaxis, and vaccination alongside efforts to control the vector population associated with the disease. In some instances the toolkit is restricted; there are no case-specific treatments, licensed vaccines or prophylaxes currently available for the treatment or prevention of dengue fever. In such cases the reliance on effective methods of vector control is even more pronounced.

1.2 Vector control

Historically, vector control solutions have relied on a small number of approaches to target vector populations. Some of the most successful efforts target the adult life stage of the vector; early theoretical and modelling studies showed that increasing the mortality rate of the adult vector was a highly effective approach [5–7]. The formula for the basic reproduction number ($R_0$) derived by MacDonald (1957) and building upon earlier work by Ross on malaria is

$$R_0 = \frac{ma^2bp^n}{-r\ln(p)},$$

(1.1)

where $m$ is the mosquito density (assuming a constant human population), $a$ is the biting rate (the number of bites per mosquito per day), $b$ is the proportion of bites from an infected mosquito that lead to an infection in the human, $p$ is the mosquito daily survival rate, $n$ is the extrinsic incubation period and $r$ is the recovery rate in humans. The work elegantly demonstrates how reductions in the mosquito survival, $p$, will have large impacts on transmission due to the non-linear nature of

$$\frac{p^n}{\ln(p)}$$

(1.2)

in the model [5, 6, 8].
Insecticide-treated bed nets (ITNs) and long-lasting insecticide-treated bed nets (LLINs) have been a cornerstone for vector control of anthropophilic, endophagic mosquitoes. Proven to be highly efficacious in the prevention of Malaria [9], large efforts have been made to scale up the distribution of LLINs, with the aim of universal coverage [10].

Indoor residual spraying (IRS) has similarly been shown to be highly protective against malaria [11] and is recommended as a front-line control initiative by the World Health Organization (WHO) [2]. The success of the wide-scale distribution and use of LLINs and IRS campaigns is thought, in part, to be responsible for recent declines in malaria transmission [2]. However, the utility of LLINs and IRS lies in the prevention of indoor, night-biting mosquitoes, rendering them less effective in protection against outdoor or day-biting vectors, such as *Aedes aegypti*, the principal vector of dengue.

Insecticides have played an integral part in mosquito control for many decades and will continue to do so. For example, Paris-green was used to locally eliminate *Anopheles gambiae* from Brazil in the late 1930s [12] and spraying with the organochlorine insecticide dichlorodiphenyltrichloroethane (DDT) was hugely successful at reducing malaria transmission from the 1940s to the 1970s. However, the efficacy of DDT eventually began to wane as high coverage led to the emergence of resistance to the insecticide in the target populations [13]. This pattern of wide-scale use followed by the development of resistance has been seen and repeated a number of times with the organophosphate and pyrethroid classes of insecticides [14, 15], underlining the precarious nature of a reliance on insecticides for disease control. The small number of available compounds is emphasised by the fact that, in the light of emerging pyrethroid resistance, some vector control programmes have had to revert to the use of DDT for IRS [16]. Insecticidal resistance and concerning ecological outcomes such as toxicity and non-specificity emphasise the importance for a range of control methods to be used and developed for sustainable, effective vector control.

To try to overcome the problems associated with a ‘silver bullet’ insecticide methodology, integrated pest management (IPM) or integrated vector control (IVC) proponents champion a more holistic approach. This has included the addition of environmental management and biological control to the gamut of vector control solutions [17, 18]. Environmental management can operate and be effective over a wide range of spatial scales. Large-scale environmental management attempts, such as the draining of the Pontine marshes in Italy by the Mussolini regime, have been historically successful [19]. Modern examples include the manipulation and control of significant water courses or dams to manage breeding site dynamics [20] and an increased emphasis on waste management and urban planning [21]. These methods have, under certain circumstances, been shown to be efficacious but
often involve wide-ranging behavioural change or time-consuming, expensive infrastructure investment resulting in challenges to implementation.

Biological control has also been employed as a method to combat many insect vectors of disease. Pathogens, predators and parasites of the vector may all fall under the label of biological control and have been used to varying degrees of success. An example is the use of predacious copepods and fish in water storage containers in Vietnam to target the aquatic stages of *Ae. aegypti* [22]. Toxins from bacteria of the *Bacillus* genus are highly pathogenic to certain groups of insects. Some have target ranges specific to insect vectors of disease such as the mosquitoes, blackfly or Lepidoptera and are therefore useful as control agents [23]. The bacteria *Bacillus thuringiensis* subsp. *Israelensis* produces a mosquito-specific larvicidal toxin that has been widely used [24]. As with synthetic insecticides, the threat of resistance leading to a reduction in efficacy is ever present [25, 26], although multiple modes of action reduce this risk [27].

The sterile insect technique (SIT) was put forward with the aim to tackle the problem of vector-borne disease from a new angle. First proposed by R. Bushland and E. Knipling in the 1950s [28, 29], the technique has since been developed and successfully used in a number of contexts. SIT involves the sterilisation by irradiation and release of large numbers of insects into the environment. Sterile males mating with wild females will cause a reduction in the females’ reproductive potential. If sustained and conducted on a large enough scale over the required period of time, this can elicit population decline in the target species [30]. Given enough resources and time, local elimination of the target can be achieved and, in some instances, maintained. The tsetse fly, *Glossina austeni*, was eradicated from the island of Unguja, Zanzibar using this method. Over a period of three years, 8.5 million sterile males were released, flooding the natural population. Towards the end of the study there was a decline and crash in the natural population resulting in local elimination [31].

The most well publicised success story of SIT has been the sustained control of *Cochliomyia hominivorax*, the screwworm fly. This parasite is a livestock pest of the Americas and has been responsible for large losses in the livestock industry. In 1957 the screwworm eradication programme was initiated in the south-eastern United States with much success. The programme induced sterilisation via irradiation [32] and was successful, seeing eradication of the New World screwworm from the United States by 1966 [33]. Since then the programme has expanded towards the equator, now covering an area bordered by Panama which maintains a buffer zone protecting against reinvasion from the South [34]. SIT has been attempted, with less success, against mosquito vectors of human disease. Irradiation-induced sterilisation as well as chemosterilisation have been tested for mosquito SIT with mixed results [35].
1.3 Dengue fever

Dengue fever is the fastest growing vector-borne disease with up to half of the global population at risk [36], most of whom live in the tropics (Figure 1). Dengue fever, and the associated dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), are caused by one of four distinct, globally circulating, dengue serotypes (DEN 1-4). Primary infection is usually associated with subclinical or mild, flu-like illness. Subsequent infections, especially with a different serotype are more likely to result in much more severe clinical outcomes such as DHF and DSS [21].

![Figure 1. Dengue risk map. The probability of dengue occurrence, figure reproduced from Bhatt et al. (2013) [4].](image)

Treatment of dengue fever is currently limited to case management [3]. At present, there are no licenced vaccines although a number are in development and some are in the late stages of review [37]. Under present circumstances, the mainstay of dengue prevention has been vector control, combined with epidemiological surveillance and management [36]. The vector of dengue fever is the *Aedes* mosquito, with *Ae. aegypti* and *Ae. albopictus* responsible for the majority of disease transmission [38].

1.4 Aedes mosquito

1.4.1 Biology

The typical *Aedes* spp lifecycle proceeds as follows: a fertilised egg gives rise to the first-instar larvae. The larvae then proceed through a further three developmental larval stages, progressing from first- to second-, third-, and fourth-instar larvae. The fourth-instar larvae pupate and further development occurs within the pupal stage. Larvae and pupae are aquatic life stages. Adults emerge from pupae, a process also referred to as eclosion. Copulation between adult male and female insects leads to fertilisation of the female. After mating, the female will blood feed and oviposit (lay the fertilised eggs) [39]. *Aedes* mosquitoes favour oviposition in containers [3, 40], depositing eggs close to the
water where the aquatic stages will develop [41]. Urban habitats provide many potential oviposition sites; water storage containers, house plants and used tyres are all common and potentially productive breeding sites [42].

*Ae. aegypti*, historically referred to as the ‘yellow fever mosquito’, is the principal vector of dengue fever and is well adapted to the urban environment [43, 44]. *Ae. albopictus*, which has seen a vastly increased range in recent times, is an important secondary vector. The recent global re-emergence of dengue is thought, in part, to be due to the globalisation of trade, responsible for transporting the mosquito vectors across the globe, leading to rapid range expansion and wide distribution across the tropics [45]. For example, the global used-tyre trade has been implicated in the recent explosion in the range of *Ae. albopictus* [46]. Both *Ae. aegypti* and *albopictus* are highly anthropophagic [47, 48] which, combined with the close overlap in their habitat with areas of high human population density, is a key factor in their role as highly competent vectors. As well as dengue fever, *Aedes* spp are vectors of yellow fever, chikungunya, West Nile fever and eastern equine encephalitis.

1.4.2 *Aedes* vector control

Despite successful vector control targeting the adult life stages of other mosquito species, similar successes targeting adult *Aedes* mosquitoes have not been observed. The adult mosquitoes will rest outdoors [49] and are crepuscular or active throughout the day [50], limiting the effectiveness of IRS or LLINs. Other adult-targeted approaches, which circumvent these problems have been tried. Ultra-low-volume (ULV) spraying of insecticides, has not proved effective [51, 52] and scientific opinion of the efficacy of lethal oviposition traps (ovi traps), designed to kill ovipositing females, is mixed [53, 54].

The lack of efficacy of adult-targeting approaches has, by necessity, meant that control has been focussed against other life stages [55]. The elimination of breeding sites has been at the heart of *Aedes* control for many years, arguably being the defining enabling factor in the eventual completion of the ill-fated construction of the Panama canal which was blighted by the ‘yellow fever’ mosquito [55]. Modern, concerted efforts at larval source reduction, implemented by reducing the number of small breeding containers or rendering containers incompatible for oviposition or larval development, have also been made. This approach has been successful in a small number of cases [56] but is logistically complex and requires a continued and high level of compliance.

Community involvement in larval source reduction has been championed as a method by which *Aedes* control efforts may be sustained in the long-term [51]. Community involvement in a campaign using *Mesocyclops*, a predacious copepod that feeds on *Aedes* larvae, has been successful in
Vietnam [57], although results of community-focused approaches elsewhere have been less convincing [58].

Trials of traditional SIT against Aedes mosquitoes have been performed with encouraging results [59], but this technique has not been widely adopted.

1.5 Modern approaches

1.5.1 Genetic modification
More recently, techniques reliant on the genetic modification of the target vector species have been developed. Applications of these types of technique can enable the engineering of insects which are refractory to the target disease. A number of examples have been developed in insect vectors of human infections. Paratransgenic tsetse flies, harbouring symbionts engineered to express tissue-specific anti-parasitic gene products show reduced vector-competence [60]. Work has also moved forward in mosquito species. Specific peptides that inhibit Plasmodium ookinete movement through the gut lumen in Anopheline mosquitoes have been identified. Transgenic mosquitoes displaying site-specific peptide translation have been created as potential refractory lines against malaria [61, 62]. Transgenic Ae. aegypti mosquitoes refractory to dengue virus have also been developed. The production of hairpin RNA comprising a section of the of the DEN-2 virus genome is induced in the salivary gland of the mosquito. This can block viral replication or expression via RNA interference [63].

In addition to the engineering of genes conferring a desirable trait there must also be a mechanism to introduce and spread these genes into a wild population if they themselves do not confer a fitness advantage to the insect. One approach proposed involves an inundative release of insects carrying multiple copies of the transgene [64]. Many other methods put forward consist of linking a gene conferring a desirable trait to an element which shows non-Mendelian inheritance in order to drive it to useful frequencies in a wild population [65]. A number of naturally-occurring selfish genetic elements showing the necessary non-Mendelian inheritance patterns have been observed. These could potentially act as a driver for forcing a desirable gene into a wild population and are detailed in the following section. It is important to note that the use of gene-drive mechanism in the wild still faces significant regulatory hurdles and raise concerns regarding environmental security [66].

1.5.2 Gene-drive mechanisms
Homing endonuclease genes (HEGs) encode for restriction enzymes that recognise a specific DNA sequence where a cut is made on the homologous chromosome not containing the HEG. Recombinational repair of the cut chromosome, using the HEG-containing chromosome as a
template, ensures the HEG is copied across (Figure 2) [67]. This self-mediated copying within the genome ensures HEGs display the non-Mendelian inheritance required for a successful gene-drive candidate, with the potential to spread from very low frequencies [65]. Naturally-occurring HEGs usually show little or no effect as a consequence of insertion, having target sites located in self-splicing introns or inteins [68]. However, as HEGs are inserted into the middle of their own recognition site they could also be targeted to recognise and insert into an essential gene, disrupting its function with potentially fatal effects [67]. A gene drive system based on a synthetic HEG has been developed for a transgenic strain of *An. gambiae*, the principal vector of malaria [69].

Figure 2. Overview of homing endonuclease genes [67].

Meiotic drive occurs when the formation of certain gametes during meiosis is inhibited or disrupted. This causes a meiotic bias towards particular chromosomes, skewing the normal segregation frequency. A region on one chromosome, such as the segregation distorter (SD) in *Drosophila*, will target a specific susceptible region on its homologous chromosome. The chromosome with the active SD will have an inactive target region avoiding autocidal effects and will benefit from a positive bias during gamete formation (Figure 3) [70]. If the targeted chromosome is sex-determining, distinct sex ratio distortion can be brought about using meiotic drive mechanisms as observed in *Aedes* and *Culex* mosquitoes [71]. Endogenous meiotic drivers in *Ae. aegypti* have been observed to cause strong, male-biased sex distortion [72].
Maternal effect dominant embryonic arrest (*Medea*), killer-rescue and toxin-antidote systems are other classes of gene drive mechanism with potential for use in the control of disease vectors. In a *Medea* system a maternally produced toxin is conferred to all offspring. The toxin is lethal unless the zygotes express an antidote inherited from either or both parents. Consequently, from a cross of two heterozygotes all surviving offspring have at least one copy of the *Medea* element (Figure 4) [73]. *Medea* has the potential to spread from low frequencies. Other insect-based toxin-antidote mechanisms with more reserved dynamics have also been put forward. Semen-based lethality (*Semele*) where, in this case, the toxin is male derived and lethal to wild type (WT) females not carrying the antidote gene is an alternative. Approaches such as Semele have the potential advantage of needing a much higher initial threshold release frequency to drive the construct into a wild population [74]. This aspect may be desirable where safety and security of transgenic strains is paramount.
Transposable elements (TEs) are selfish genetic elements that replicate within the host genome. TEs are widespread and have been well documented in mosquitoes [75]. Two classes of TEs exist, differentiated by the method by which they copy themselves. Retrotransposons or Class I TEs replicate via a ‘copy and paste’ mechanism. First, there is transcription of the transposable element from DNA to RNA. This RNA fragment is then converted back into DNA mediated by reverse transcriptase. This DNA duplicate is then inserted into the genome at a new site [65, 76].

Class II, DNA transposons differ in that a ‘cut and paste’ method is used for replication within the host genome. The TE is catalysed and the excised element is inserted at a new location in the genome. The TE is then copied across to the unaffected chromosome via recombinational repair using the homologous chromosome as a template (Figure 5) [65, 76].

Figure 5. Transposable elements [65, 76].

1.5.3  Wolbachia
Wolbachia are parasitic bacteria that have been found naturally occurring in the reproductive tissues of a wide-range of insect species [77]. Wolbachia spp have evolved to bias the reproduction of their hosts to their own benefit. Cytoplasmic incompatibility, most importantly between infected and uninfected individuals is one of the mechanisms by which Wolbachia could spread to high frequencies in a population. Uninfected females mating with infected males are infertile crosses causing a Wolbachia-preferential ratio of infected to uninfected offspring. Cytoplasmic incompatibility is thought to occur via modification of an infected male’s sperm which can only be ‘rescued’ if the embryo has been infected by the mother with the same strain of Wolbachia (Figure 6) [65, 77]. Wolbachia has been demonstrated to induce cytoplasmic incompatibility in Ae. aegypti
The potential for Wolbachia to influence the field of dengue control has manifested in a number of ways. The wMel Wolbachia strain has been shown to reduce adult longevity and inhibit DEN-2 virus transmission in Ae. aegypti [78] and Ae. albopictus [79]. Wolbachia has also been shown to inhibit yellow fever and chikungunya infection in Aedes spp [80]. Field trials in Queensland, Australia demonstrated the ability of wMel Wolbachia to invade and reach near-fixation in natural populations of Ae. aegypti following releases of wMel infected individuals [81]. Following struggles to establish Wolbachia infections in Anophelines, achievement of Wolbachia infected lines of An. stephensi has shown that the infection induces a degree of refractoriness to Plasmodium infection in the mosquitoes [82].

![Wolbachia diagram](image)

**Figure 6. Wolbachia as a method of gene drive [65].**

### 1.6 Release of Insects Carrying a Dominant Lethal (RIDL)

One approach combines cutting edge genetic techniques with the sterile insect technique. This is the release of insects carrying a dominant lethal (RIDL). The aim is to replace the need for sterilisation by irradiation as occurs in traditional SIT operations. Here a dominant, repressible, lethal genetic system is constructed, first demonstrated in *Drosophila melanogaster*. In this system the transcriptional control elements of tetracycline-repressible transactivator fusion protein (tTA) are used as an on/off switch with which the expression of any gene controlled by a tetracycline-responsive element can be modulated. Using this system sex-specific and non-sex-specific promoter are linked to the expression of tTA which in turn can switch on expression of a toxic gene product.
Transformed insects harbouring this technology would be effectively sterile, allowing release and population suppression in a similar manner to SIT. The lethal gene is inherited by the RIDL insect’s offspring which will subsequently die before developing to adults. Sex-specificity provides the additional benefit of genetically-mediated sex sorting with the potential for male-only releases in certain circumstances. This is particularly relevant to control of insect vectors of disease where females are responsible for disease transmission.

1.6.1 Development in the fruit fly
Development and refinement of this technology continued and was applied to pest species. The Mediterranean fruit fly, *Ceratitis capitata*, (Medfly), is a major agricultural pest and a target for sterile-insect technologies. In the Medfly a RIDL system was developed as an alternative to traditional sterilisation techniques. A simplified version of the previous technology was implemented using tTA as both the transcriptional activator and the toxic product. In this situation in the absence of the repressor (tetracycline), basal expression of tTA leads to further expression of tTA in a positive feedback loop. High levels of tTA are deleterious to cells and therefore toxic to the insect. In the presence of tetracycline, tTA is only expressed at basal, harmless levels. A genetic marker was also included in the construct to allow discrimination between RIDL and WT insects.

In Medfly, it is the females which cause the agricultural damage and the subsequent economic impact. When undergoing a SIT or RIDL approach under such circumstances male-only releases are highly preferable. Further development of the Medfly RIDL insects included the engineering of a construct that would provide genetic sexing as well as effective sterilisation. In this instance the insects could be mass reared in the presence of the repressor. The release generation would then see the repressor removed allowing a male-only release population. By taking advantage of sex-specific splicing, a genetic sexing mechanism can be incorporated. In males the transactivator transcript is disrupted by splicing, this does not occur in females allowing a male-only release generation to be produced.

1.6.2 Development in mosquitoes
Similar advances have been made to develop a RIDL approach for mosquito vectors of human disease. A dominant lethal system was produced in *Ae. aegypti*, the primary vector of dengue fever, using constructs similar to those found in the Medfly products. Furthermore, a proportion of the transgenic *Aedes* produced displayed late-acting mortality at the larval-pupal stage of development. Modelling work examined and demonstrated the potential benefits of developing a strain with late acting mortality. It was shown that where there are density-dependent effects at the larval developmental stage, delaying mortality until after this time could be beneficial for a RIDL control.
programme. The ‘doomed’ larvae fathered by a RIDL male would compete with wild larvae, therefore also reducing the wild larvae’s chance of surviving to the adult life stage.

Sex-specific strains were also proposed and developed in Ae. aegypti [87]. Again, the benefit of this approach is to remove the need for mechanical or hand-sorting of individuals where male-only releases are required. A proportion of males surviving to the next generation in the wild will also provide a residual trickle-down effect from a single release. The developmental stage of release could also be altered from adults to eggs or pupae. The sex-specific strain was developed by taking advantage of a promoter from the Ae. aegypti actin-4 (Ae-Act-4) gene which drives sex-specific expression in female indirect flight muscles. In this line strong expression of tTA is promoted in the females’ indirect flight muscles, destroying them and rendering females flightless. An inability to fly is considered to be a lethal trait under field conditions. Females are also effectively sterile due to a reliance on wing-beat frequencies to attract a mate. The ability to coordinate a control programme via distribution of eggs or pupae could also be logistically beneficial under certain circumstances [87].

*Ae. aegypti* is the primary but not the sole vector of dengue fever. It is therefore important to consider secondary vectors, the most important of which is *Ae. albopictus*. A similar development approach to that used in *Ae. aegypti* has been followed, with germline transformation [88] and the development of a female-specific flightless phenotype [89] being demonstrated in *Ae. albopictus*.

The first open field release of transgenic sterile mosquitoes was performed using the OX513A strain of *Ae. aegypti* in Grand Cayman. The first stage of these field releases assessed the released insects’ performance in the field, demonstrating that the released transgenic males could successfully mate with wild females in the field [90]. Further field releases in Grand Cayman demonstrated that sustained released of the transgenic sterile males could successfully suppress the wild mosquito population [91]. Assessment of the field performance of the OX513A transgenic male *Ae. aegypti* has also been undertaken in Malaysia demonstrating that released transgenic males have similar longevity and dispersal behaviour compared with non-transgenic counterparts [92].

### 1.6.3 Pink Bollworm

RIDL constructs are linked to fluorescent markers. These are important as they allow distinctions to be made between transformed individuals or their offspring and wild or WT insects. An example of the benefits of an engineered heritable fluorescent marker is seen in the genetically engineered Pink Bollworm. The Pink Bollworm, *Pectinophora gossypiella*, is an important agricultural pest of cotton. Currently, control utilises a traditional SIT approach. Monitoring requires accurate distinctions to be made between wild and sterile moths that are caught. The identification is facilitated by the addition
of a dye to the larval diet which is apparent in the fatty tissue of adult moths [93]. However, this technique can be insensitive and an engineered heritable marker has been shown to be a viable and effective alternative [94].

1.6.4 Advantages over traditional SIT
As previously mentioned, SIT control programmes have been successful in a number of situations. There are however inherent disadvantages associated with the traditional application of this technique which limits its scope and usability. Integral to the success of SIT is the ability for the sterile males to compete with wild males to mate wild females. SIT produces sterile males by irradiating them. Irradiation is very damaging to the insect [95, 96] resulting in shorter-lived, less competitive individuals [97]. The effect of irradiation dramatically reduces the efficacy of a SIT release and therefore its applicability in many situations. A RIDL approach produces sterility by engineering dominant lethality as opposed to inducing dominant lethal mutations in insects via irradiation [86], with the potential to dramatically reduce the fitness costs associated with sterility, producing a fitter, more competitive sterile insect. This would in turn improve the efficacy and reduce costs associated with a control programme increasing the feasibility, success and appeal of such an approach [98, 99].

With the RIDL approach there is also the possibility of combining a genetic-sexing mechanism with the construct conferring sterility. In many cases male-only releases are preferential or compulsory. Even in cases where release of sterile females may not be directly harmful, single-sex releases may be more efficient at encouraging sterile-wild matings [100]. A RIDL approach can therefore benefit from the species-specificity and environmentally non-polluting characteristics of an SIT approach whilst improving the efficacy of the method.

1.7 Evaluation of transgenic insects
The fitness of a transgenic insect is integral to its worth as a potential tool in a sterile-insect control programme. In this respect, fitness can be broken down into two main components which must be assessed in any potential transgenic line, namely: competitiveness and longevity. An insect must be able to adequately compete for a wild mate and live long enough to do so. It should be noted that ‘fitness’, measured as the contribution to the gene pool in subsequent generations, for sterile insects is approximately zero and in this and following sections I refer to fitness when discussing the performance of sterile insects.

Transgenesis can impart fitness costs in a number of ways. The transgene can itself provide a burden on the insect. Proteins expressed by the transgene may affect the fitness of the insect. Any foreign protein expressed in the host cell may confer a cost. RIDL insects are engineered with very specific
promoters to ensure expression of deleterious proteins is tightly regulated within the target insect [101]. Insertional mutagenesis is a second problem which may reduce fitness in the transgenic line. Insertion of the transgene into an essential, transcriptionally active segment of the genome can disrupt gene function enough to reduce fitness [102, 103].

1.7.1 Mating-competitiveness

Accurately measuring and assessing the mating-competitiveness of an insect strain is difficult. Mating-competitiveness is an important measure for control-programme design and execution. The mating-competitiveness of the sterile or transgenic insect will determine the release ratio required to achieve population suppression or elimination. In more extreme cases it would be influential in determining if a control effort would be likely to succeed or fail. Techniques to provide estimates of mating-competitiveness were first developed in relation to SIT. One of the first attempts to formally quantify mating-competitiveness was made by Fried in 1971. First an expected egg-hatch rate is calculated based on a known ratio of wild to sterile males and hatch rates for mating between wild males and females and sterile males and females

\[
\text{Expected hatch rate } = E = \frac{N (Ha) + S (Hs)}{N + S} \tag{1.3}
\]

where \( N \) and \( S \) are the numbers of wild and sterile males respectively. \( Ha \), and \( Hs \) are their associated hatch rates. Equation (1.3) can then be rearranged to estimate the ratio of fully competitive sterile to wild males that would provide an observed hatch rate

\[
\text{Ratio of sterile to wild males } = \frac{S}{N} = \frac{Ha - E}{E - Hs} \tag{1.4}
\]

Finally, a point estimate of mating-competitiveness can be ascertained by comparing the calculated ratio in equation (1.4) with the actual ratio of sterile to wild males [104]. This method was developed further to allow the variance around this point estimate to be quantified. This allowed mating-competitiveness estimates from more than one experiment to be compared for significant differences [105]. These measures are important tools for monitoring and evaluating the merits of different lines of sterile or transgenic insects, allowing direct comparisons to be made. This is especially useful when comparing successful field trials. It can help to quantify the levels of mating-competitiveness needed for trials to be feasible, effective and successful. For example, mating-competitiveness has been shown to be a key parameter for assessment of the effectiveness and economic considerations of sterile screwworm eradication efforts. An in-depth evaluation of mating-competitiveness is important as, even for a single species such as the screwworm, the estimates of mating-competitiveness can vary dramatically [106].
Data to inform estimates of mating-competitiveness must be obtained from well-designed experiments and trials. Both indirect and direct competition experiments have been used to assess the mating-competitiveness of a transgenic line against that of a WT strain. One early example of a direct competition experiment was conducted to test the mating-competitiveness of two genetic control systems for Ae. aegypti. In this experiment, 100 genetically sterile males competed with WT males for WT female mates in a 1:1:1 ratio. Mated females were then isolated, blood-fed and had their egg batches assessed for viability to ascertain paternity. The ratio of mating could then be directly compared to the ratio of wild to sterile males, demonstrating the large variation in competitiveness between three strains of sterilised male. Estimated competitiveness in this study also varied dependent on the day egg batches were collected [107]. In some cases a threshold level of hatching is set to determine paternity based on previous hatching experiments [108].

Later examples of laboratory and cage experiments to assess the mating-competitiveness of transgenic mosquitoes follow a similar methodology. One difference is in the ability to assess paternity more accurately through a heritable fluorescent marker which is part of the RIDL transgene. Direct competition experiments of WT and transgenic An. arabiensis mosquitoes expressing a DsRed2 fluorescent marker are a good example of this approach. Here, a number of experiments were run to examine mating-competition in cages between transgenic and WT males for females of differing genetic backgrounds. The authors did not observe any significant differences in the mating competitiveness between transgenic and WT strains [109]. Indirect experiments have also been conducted with transgenic Anophelines. The mating-competitiveness of An. stephensi mosquitoes expressing transgenes reducing their vectorial competence for Plasmodium berghei was shown to be not significantly different from that of WT individuals in indirect cage experiments consisting of 250 females mixed with 250 males, either transgenic or WT [110].

The line of RIDL Ae. aegypti mosquitoes that would go on to be released in full field trials, OX513A, was also subject to cage-based mating-competitiveness trials. In these experiments, which were in preparation for a specific field trial, mating-competitiveness of the transgenic in relation to a local strain of Ae. aegypti was the specific indicator to be measured. Across five replicates, local females were mixed with local and transgenic males in a 1:1:1 ratio. Egg batches from mated females were allowed to hatch and a DsRed2 fluorescent marker used to assess paternity. These experiments yielded a competitiveness estimate of 0.56 (95% bootstrap CI: 0.032–1.97) for the transgenic males [90].

The relevance of mating-competitiveness indices estimated from cage data relies on the assumption that behaviour and conditions within the cage are not significantly different to those in the field.
try and minimise any variation between the cage and the field, larger cages in conditions mimicking those found in the field have been used. A competition experiment of a transgenic sexing strain of Medfly was conducted in large cages in green houses for this reason. Sweet Orange trees, *Citrus sinensis*, were placed in the cages to recreate the natural mating environment. In these experiments males of different strains were released in equal numbers to females. Mating pairs were collected to assess relative success of males from each strain, indicating that the relative competitiveness of the transgenic males was significantly reduced with respect to WT males [111].

The most relevant test of the mating-competitiveness of a transgenic strain is obtained through a field trial. There are however complications associated with calculating mating-competitiveness in this environment. As the ratio of transgenic to wild males is not known, it must be inferred from data collected in the field. It is also unlikely to be feasible to collect and individually assess all mated females to determine the background of their male mate. Where these studies have been conducted for conventional irradiation based SIT, values for field mating-competitiveness are substantially lower than those calculated in caged experiments [100, 106]. It is therefore important to estimate mating-competitiveness in the field despite the logistic and technical difficulties.

A mating-competitiveness study has to date only been performed once with transgenic mosquitoes in the field. This was with the aforementioned RIDL *Ae. aegypti* mosquitoes that were released over a period of four weeks in a study site on the Island of Grand Cayman. Nine BG-Sentinel adult traps (Biogents) were used to assess the ratio of wild to transgenic males in the treatment area. Forty-three ovitraps, a commonly used method for monitoring and surveillance of container breeding mosquitoes in the field, were used to collect eggs laid by mated females to assess the proportion of progeny from wild and transgenic fathers respectively. From these data, a mating competitiveness of 0.56 (95% bootstrapped CI 0.032-1.97) was estimated for the RIDL transgenic line being tested [90]. This is consistent with field estimates of mating competitiveness for other species, such as the New World screwworm (competitiveness ranging from 0.1-0.43), that have been used in successful sterile insect programmes [90].

### 1.7.2 Life history traits

Many different life history traits have been studied and measured. The variety examined gives an indication of the complexity of trying to best estimate the fitness of an insect. Perhaps one of the most widely recognised, easily quantifiable and important contributing factors to an insect’s fitness is longevity. Longevity is an integral factor when constructing life tables which form the backbone of many studies investigating life history traits.
The influence of green fluorescent protein (GFP) and two transposable elements on Ae. aegypti was studied by measuring a number of factors, including adult longevity, and constructing partial life tables, showing a significant impact of the transformation on demographic parameters [112]. This allows structured comparisons to be made between transgenic and control lines. In-depth investigation of this kind has also been used to assess potential fitness costs associated with RIDL transgenic lines. Comparisons were made between a transgenic Ae. aegypti and a non-modified counterpart [113]. Again, adult longevity was a key measure in this study and was shown to be marginally reduced in the transgenic line (20 and 24 days mean lifespan for transgenic and WT insects respectively). Measurement of survival is an important assessment to make with RIDL lines. For a line to be successful the lethal gene must be adequately silenced in the presence of a repressor. Therefore, the examination of longevity of the transgenic insects raised in the presence of the repressor is a vital stage in the RIDL development process. This process has been demonstrated in other RIDL species alongside Ae. aegypti; the longevity of Medfly RIDL lines has also been studied as part of a detailed investigation of the line’s prospects for use in a control programme. This study found no significant difference between the survival of transgenic and WT insects [84].

Often, life history studies of insects measure various indicators of size, such as wing length or pupal mass [112, 113]. In some instances these are used as a crude proxy for fitness. There is however conflicting evidence in the literature regarding the assumption that ‘bigger is better’. Some studies do suggest that being large confers certain advantages to the insect. For example, larger male Ae. aegypti were shown to live longer in Rio de Janeiro, Brazil [114]. This evidence would support efforts to rear large males for a RIDL control programme as maximising male fitness is paramount. There is scope to influence the size of males released via manipulation of the rearing conditions. Rearing densities, feeding regimes and many other aspects of the mass-rearing process may all be optimised to maximise insect fitness whilst minimising overheads. However, other studies in different mosquito genera have indicated that male size seems less important. There are arguments for stabilising selection associated with body size indicated by studies where mid-range sized An. gambiae were more successful in mating swarms than large or small males [115, 116]. The characteristics of a ‘fit’ insect and how these are best measured is likely to change with location, time, species and many other factors. The challenge therefore remains to identify the most robust and universal proxies for fitness, if such measures exist, and use these to maximise the success of transgenic-insect control programs.

1.7.3 Mark-Release-Recapture (MRR)
Independently conceived by Petersen in 1896 and Lincoln in 1930 [117], mark-recapture, capture-recapture or mark-release-recapture methodology (hereafter referred to as MRR) has since become
a key set of ecological methods. This set of methodological approaches allows inference to be drawn on a number of important ecological factors including the estimation of population size and quantification of dispersal and survival. The methods have been used across a diverse range of species, from whales [118] to fruit flies [119], and have seen extensive use in mosquito ecological studies.

MRR methods are the most common approach to estimating wild population size [120, 121]. In its most simple form, individuals are captured, marked and recaptured. A measure known as the Lincoln index or the Petersen-Lincoln index allows the true population size to be inferred from information of the proportion of marked to unmarked individuals recaptured, combined with knowledge of the initial release number [120]. Let $N$ be the population size, to be estimated, $K$ is the number captured and marked, $n$ is the total number recaptured and $k$ is the number of marked recaptures then

$$N = \frac{Kn}{k}.$$ \hspace{1cm} (1.5)

Many refinements and adjustments have been made to this basic approach allowing the methodology to be applied to many different scenarios. Common adjustments include making allowances for multiple markings, open or closed populations and mortality over time [120, 122].

The methodology extends well to the analysis of mosquito populations. Individuals can be marked or distinguished in a wide variety of ways: with paint, fluorescent dusts or pigments, trace elements, phenotypic mutations or radioactive or genetic markers [121]. RIDL insects are engineered to carry a heritable fluorescent genetic marker meaning that recognition of ‘marked’ individuals can be extremely specific and sensitive [123]. McDonald (1977) used paint to mark Ae. aegypti for MRR experiments and the Lincoln index to estimate the local population size [124]. Trpis et al. (1995) used the simple Lincoln method, as well as the more complex Jackson’s positive method and removal method to estimate the population size of Ae. aegypti in eastern Kenya [125]. An. gambiae s.l. population size in a village in Mali was estimated by Touré et al. (1998). This study used fluorescent dust to mark individuals before release and again used the Lincoln index, with an adjustment for small sample sizes, for population size estimates [126].

Estimates of the survival or mortality rates of individuals in a study population can also be inferred from MRR data. The study of the recapture of marked individuals through time is the basis of these analyses and was considered early in the history of MRR [127]. The estimation of survival is more robust to the restrictive assumptions associated with MRR analyses than methods to estimate
population size and there is a wide variety of published models to estimate survival from MRR data [128]. One consideration important for the analysis of mosquito MRR studies is the explicit inclusion of the removal of recaptured individuals from the analysis [128, 129]. Unlike with, for example, larger mammals, recaptured mosquitoes cannot be handled without significantly, detrimentally affecting the fitness of the individuals and therefore recapture individuals are not re-released.

Probabilistic formulation of the analysis of MRR data with respect to time allows for an explicit estimation of survival parameters. Likelihood-based approaches were proposed early [130, 131] and have been continually used and extended. A simple approach assuming no removal and constant mortality rate uses the exponential survival model, allowing recaptures with respect to time to be analysed with a log-linear regression model [132]. Modern analyses may be much more complex, for example Zheng et al. (2007) employed a Bayesian framework to estimate the survival of the Glanville fritillary butterfly (Melitaea cinxia) in a random effects model, accounting for age dependent survival and individual capture and survival probabilities [133].

Gillies (1961) published estimates of survival probabilities of An. gambiae in East Africa using the aforementioned log-linear regression analysis of MRR data [132]. Walker et al. (1987) used the log-linear regression method of Gillies to estimate the survival of Ae. triseriatus and Ae. hendersoni in India [134]. The loss rate of male Ae. aegypti in India, with respect to season, was estimated using MRR data by Reuben et al. (1975) [135]. More recent MRR studies with Aedes spp estimated survival of the insects in Missouri, USA (Daily survival probability (DSP) = 0.89) [136], Queensland, Australia (DSP = 0.57-0.91) [137] and Réunion Island (DSP = 0.93-0.96) [138], all using log-linear regression analyses proposed by Gillies. Buonaccorsi et al. (2003) have published an analysis which corrects the log-linear regression model to account for the removal of recaptured individuals. The work compares the results of the two models, analysing Ae. aegypti MRR data from Thailand and concludes that significant inaccuracy can result from not correcting for removal on recapture [139]. This method was later used by Maciel-De-Freitas et al. (2007) to assess survival of Ae. aegypti in Rio de Janeiro, Brazil (DSP = 0.32-0.73) [114] and Neira et al. (2014) to estimate survival probability for Ae. aegypti in Panama (corrected DSP = 0.65) [140]. There is some evidence for age-dependent mortality in mosquitoes [141, 142] which should be considered when drawing conclusions from the commonly used age-independent models.

Alongside population size and survival estimates, MRR is valuable in estimating the spatial dispersal of the organism under study. MRR techniques to estimate dispersal have seen particular focus in the field of entomology including studies of many vectors of disease. Examples include: the sandfly, Lutzomyia longipalpis, vector of leishmaniasis [143]; tsetse fly, Glossina palpalis gambiaensis, vector
of African trypanosomiasis [144]; and the triatomine, *Triatoma infestans*, vector of South-American trypanosomiasis, as well as many studies of mosquito vectors of disease, a number of which are detailed below.

In the 1970s Reuben et al. (1975) and McDonald (1977) studied the dispersal of *Ae. aegypti* using MRR [124, 135]. Morris et al. (1991) estimated the mean distance travelled (MDT) and maximum range of eleven different mosquito species in Florida, USA, in a study designed to highlight the merits of MRR for the evaluation of mosquito dispersal for control programmes [145]. MRR studies have also informed inference on the dispersal of *Aedes* spp in many other studies. Niebylski & Craig (1994) quantified *Ae. albopictus* dispersal in a tyre yard in Missouri, USA (maximum dispersal = 525m) [136]. Muir & Kay (1998) and Russell et al. (2005) have both estimated *Ae. aegypti* dispersal in northern Australia from MRR data (MDT = 35-56m and 78m respectively) [137, 146]. Three studies have provided a comprehensive overview of *Aedes* dispersal in and around Rio de Janeiro, Brazil [114, 147, 148]. Tsuda et al. (2001) used MRR methods to estimate the movement of *Ae. aegypti* on Hainan Island, China (MDT = 5-40m) [149], another island study saw Lacroix et al. (2009) estimate *Ae. albopictus* dispersal on Réunion [138]. Two studies have quantified *Ae. albopictus* dispersal in Italy using releases of marked individuals (MDT = 97-212m and 119m respectively) [150, 151].

Typically MRR approaches make a number of assumptions which must be considered when designing, conducting and analysing MRR studies. Common assumptions include: behaviour and survival are not affected by capture and marking; survival rate is not age-dependent; there is no immigration or emigration into or from the study population and recapture probability is equal for all individuals. Lebreton et al. (1992) refer to the iii assumption: the “independence of fates and identity of rates among individuals” [128].

These three uses of MRR data to estimate population size, survival and dispersal, have meant that MRR studies are an essential tool used throughout a RIDL-based control programme. Fundamental to the planning and implementation of a RIDL control strategy are accurate estimates of the wild population size. Estimates of population density in the area targeted for control using RIDL insects provide baseline information and are used to assess the magnitude of release of RIDL males that will be required. This estimate is therefore influential at the early stages of a project and fundamental to both cost and efficiency of target population suppression. As previously mentioned, fit, transgenic males are more able to compete with wild-males for a wild female mate. Released adult longevity is therefore a desirable character and MRR experiments are the best way by which these measures can be estimated under true field conditions. The dispersal ability of released insects may also be used as a proxy for fitness. Knowledge of the dispersal upon release of transgenic insects is also essential in
order to be able to accurately plan the spatial pattern of releases to ensure adequate coverage of the target area with transgenic insects is achieved.

The RIDL technique involves the mass rearing of insects which provides an excellent opportunity for large-scale releases and therefore also the potential for large-scale MRR experiments. Monitoring infrastructure also provides the means to recapture individuals in the field. As well as absolute measures of transgenic performance and fitness, simultaneous releases of transgenic and WT insects can aid relative comparisons of these measures. This allows the potential effects of the transgene, if any, to be quantified in the field. A case in point is demonstrated by the Lacroix et al. (2012) study in Malaysia. This study saw large releases of 6,045 and 5,372 transgenic (OX513A) and WT (My1) Ae. aegypti respectively. Recaptures, using BG-sentinel adult traps, were made over the subsequent 15 days post-release allowing absolute and relative inference on the survival and dispersal of the two strains to be drawn [92]. Similar performance comparisons have been made with other species. The transgenic OX1138B pink bollworm strain was assessed in a trial alongside a standard SIT strain (APHIS) in the first ever open-field experiment with genetically engineered insects. In this study preliminary experiments with small scale-releases of both OX1138B and APHIS strains was followed by large-scale releases (1-2 million per week) of the OX1138B strain. Again, this allowed field-based estimates of critical performance factors such as mating performance, dispersal and persistence to be made [94].

1.7.4 Penetrance
One evaluation stage particular to insects developed for the RIDL technique is to assess the penetrance of the lethal phenotype that has been engineered into an insect line. The ideal is for the transgene to be responsible for 100% lethality in the absence of the repressor and 0% lethality in its presence. Penetrance can be defined as 1 - the proportion surviving in the presence of the lethal phenotype, adjusted for background mortality. Measuring the penetrance of the lethal phenotype is one of the steps integral for the assessment of a potential transgenic line in the early stages of development. Although 100% penetrant lethality is the aim, modelling studies have shown that a degree of incomplete penetrance of lethality does not reduce the efficacy of a RIDL line a great deal. Lethality of 90% penetrance can still elicit population decline and elimination in the absence of immigration in modelled scenarios [86]. Levels of lethality much higher than this have been shown in the Grand Cayman field trial [90].

1.8 Monitoring
The following section presents an overview of the sampling and monitoring of a mosquito population. More specifically, I discuss the sampling and monitoring of a wild population that is the
target for control as well as monitoring of a RIDL control programme, that is, the assessment of the wild population under control alongside monitoring aims and goals of the control programme related to the transgenic insects.

The most immediate method of monitoring the adult population is by direct sampling of the adult population. The human landing catch (HLC) has been the gold standard in the malaria field for assessing the entomological inoculation rate (EIR) as a measure of malaria transmission [152]. However, when monitoring vectors of diseases with no prophylaxis or specific treatment, such as dengue, assessment via HLC is ethically questionable. Therefore, other methods of adult sampling have been developed. Adult traps are now widely used with perhaps the most well-known examples being the CDC (Centers for Disease Control and Prevention) light-trap and the BG-sentinel trap. These traps have been extensively used for mosquito sampling and sampling of Aedes adults specifically. Where the traps have been directly assessed alongside HLC methods, the traps have compared favourably and are widely considered to be a viable alternative to HLC methods [153, 154]. The adult traps have also been shown to outperform other, more labour-intensive approaches to adult sampling such as the CDC backpack aspirator [155, 156]. Efforts have been made to further increase the performance of adult traps with the addition of attractants. Ferriera de Ázara et al. (2013) showed an increased trap positivity rate for Ae. aegypti using a BG-sentinel trap plus a carbon dioxide (CO₂) attractant [157]. Lacroix et al. (2009) used a mouse-baited BG-sentinel to trap Ae. albopictus adults [158]. Biogents themselves produce a commercially available attractant, the BG-lure (Biogents, Regensberg, Germany) which has been shown to perform well when compared with an octenol attractant [159]. BG-sentinel traps were employed during field releases of transgenic sterile male Ae. aegypti on Grand Cayman. The adult sampling allowed estimates of the proportion of wild to transgenic insects in the target area to be estimated [90]. Lacroix et al. (2012) used a network of 45 BG-sentinel traps for sampling transgenic and WT adult mosquitoes during a MRR study in Malaysia [92].

Adult trapping is often expensive and logistically demanding. For example, BG-sentinel traps require a constant power source [160]. Therefore alternative trapping and sampling methods are widely employed, the most common being the ovitrap.

First described by Fay & Eliason (1966), the basic ovitrap consists of a small container, usually black in colour, containing water and a wooden paddle, upon which eggs can be oviposited [161] (Figure 7). This style of ovitrap, commonly referred to as the CDC ovitrap [162], has been widely adopted.
The presence of the vector, as determined by ovitrap sampling was shown to be a better predictor of dengue occurrence than larval surveying [163] in Belo Horizonte, Brazil. The spread of *Ae. albopictus* in Florida, USA, was monitored in part using ovitraps [164].

Many attempts have been made to improve the performance of the basic ovitrap. Reiter *et al.* (1991) used hay infusions in the trap water to significantly increase the attractiveness of the trap to *Ae. aegypti* mosquitoes [162]. Bellini *et al.* (1996) compared plastic, metal and glass ovitrap containers, concluding that plastic and glass traps performed significantly better than metal ones, with the hypothesised reason being the difference in the internal colour between traps [165].

Sticky ovitraps represent a combination of adult-trap and ovitrap, effectively using the potential oviposition site as a lure or attractant for ovipositing females. Sticky ovitraps have been shown to be as effective as the standard ovitrap at detecting *Ae. aegypti* presence [166]. Sticky ovitrap facilitated MRR experiments to quantify *Ae. albopictus* dispersal in Rome, Italy [151] and *Ae. aegypti* dispersal in North Eastern Mexico [167] and Cairns, Australia [146].

Factors beyond the design of the ovitrap or sticky ovitrap, such as trap placement, are also highly influential on the performance of the trap and have been explored by William *et al.* (2006) [168]. As previously mentioned, ovitraps have also been co-opted for use as a method of control as lethal-ovitraps [169].

Ovitraps have been used to monitor the releases of transgenic sterile male *Ae. aegypti*. An extensive ovitrap monitoring programme was used in field trials in Grand Cayman [90, 91] making use of the heritable fluorescent marker carried by the released transgenic males. This marker is present in the larvae resultant from a mating between a transgenic male and wild female and therefore inference
about the relative abundance of transgenic and wild males can be made from the proportion of fluorescent to non-fluorescent larvae collected [90, 91].

Standard measures of Aedes abundance or density are the House-, Container- and Breteau-Indices. These are the percentage of houses with Aedes larvae or pupae present, the percentage of containers with Aedes larvae or pupae present and the absolute number of containers with Aedes larvae of pupae per 100 houses respectively. However, due in part to density dependent effects [86], Aedes larvae experience high levels of mortality, pupal surveys have been put forward as a more epidemiologically relevant measure [170] and have been widely used as a monitoring tool [171]. The pupal survey method does not however provide scope for directly monitoring RIDL transgenic Ae. aegypti as some of the offspring of transgenic-mated females will die before the pupal stage [86].

1.9 Overview of project aims

Important research gaps exist in this field. The release of transgenic insects in the field is a recent challenge, providing much scope for improvement, refinement and optimisation. The process of a field release programme has many steps, from preparation and planning, through to releases and monitoring. Each of these stages has the potential to be optimised. Certain aspects of these knowledge gaps pertain to well examined problems that need further improvement and refinement, for example, assessing dispersal of mosquitoes in the field. Others are more specific and have received little, if any, attention in the literature, for example, planning the optimal route for releasing transgenic insects from a moving vehicle.

The aim of this thesis is to fill a number of these research gaps relating to the release of sterile insects in general and, more specifically, transgenic male Ae. aegypti. This thesis will address a range of topics affecting the use of transgenic mosquitoes in the field. Chapters are linked by the common thread of optimising the field releases, with the aim to provide applied recommendations that will help to improve the efficiency and efficacy of releases in the future. Throughout the thesis I aim to link existing and novel methods in computing, modelling and statistics to a wealth of Ae. aegypti field data to provide insights and expand our knowledge surrounding the release of these transgenic insects in the field. Although addressing field releases of transgenic Ae. aegypti, it is hoped that many of the methods used and conclusions drawn throughout will be applicable to a range of species and across the spectrum of traditional SIT and transgenic releases.

1.10 Outline of chapters

This thesis is divided into seven chapters, following this introductory chapter the structure of the thesis is as follows:
• Chapter 2: In this chapter I address questions concerning which is the best life stage of *Ae. aegypti* to release in the field.

• Chapter 3: The first of two chapters in which I aim to assess dispersal in the field, this chapter presents an evaluation of transgenic male *Ae. aegypti* dispersal.

• Chapter 4: The second dispersal-focussed chapter extends the dispersal analysis to transgenic-mated female *Ae. aegypti*.

• Chapter 5: I use dispersal simulations with optimisation algorithms to address the optimal release of transgenic *Ae. aegypti* in the field.

• Chapter 6: In this chapter I aim to inform monitoring practice. A spatial analysis assesses the quantification of spatial heterogeneities using ovitrap data.

• Chapter 7: A discussion chapter framing the work detailed in the thesis, summarising and analysing the findings whilst reflecting upon them in the wider context.
2 Genetic control of *Aedes aegypti*. Data-driven modelling to assess the effect of releasing different life stages and the potential for long-term suppression.


In this first data chapter I begin with an examination of the dynamics of releasing transgenic insects of different life stages. As for all following chapters I use field data to inform models that can help to answer specific research questions relating to the release of transgenic insects. Here, empirically supported models of the dynamics associated with releasing different life stages are used to compare adult-only, pupal-only and combined releases.

2.1 Introduction

Efficient implementation of a transgenic male *Ae. aegypti* based control programme in the field relies on effective release methods. Previous trials have released male mosquitoes at both the pupal and adult life stage [90, 91]. Adult-only releases are common in SIT control programmes, for example the screwworm control programme of the Americas releases 25-50 million sterile adult males per week, resulting in an estimated annual net benefit of more than $1 billion [172]. However, releases are not confined to the adult life stage. Releases at earlier life stages, such as the sterile pupal releases of the Queensland fruit fly, *Bactrocera tryoni*, have also been documented [173, 174]. An alternative approach is a combined release in which both adults and pupae are used. Releasing a specific life stage may have beneficial impacts on the dynamics of a release as well as affecting the logistic and technical aspects of rearing, transporting and releasing individuals. To inform the design of control programmes there is a need for a better understanding of the comparative dynamics and performance of pupal-only and combined releases relative to adult-only releases.

Modelling studies may serve a critical role in the optimisation of control programmes, allowing many potential approaches to implementing a release to be investigated at the theoretical level. In order for such studies to be accurate and applicable, it is vital that they are well informed with appropriate field data. In this study, data from a large-scale field study provide the foundation for a model describing the dynamics of a pupal release. The work goes on to use the data-driven model to investigate the dynamics of adult-only, pupal-only and combined releases of transgenic sterile insects, with the specific aim of testing the hypothesis that pupal-only or combined releases are a viable alternative to adult-only releases.
Different release methods and regimes are simulated and their ability to suppress a simulated wild population analysed. A successful vector control programme must be sustainable in the long-term. Furthermore, it must be able to withstand perturbations in wild population densities induced through immigration of wild individuals into the target population from neighbouring high-density or uncontrolled areas. Metapopulation structure can lead to persistence of a species even if local populations are unstable and prone to extinctions [175], or in the case of a sterile insect programme, under control. Such immigration pressures have the potential to seriously hinder a sterile-insect approach [176] and are therefore an important aspect to consider. Potential approaches that take advantage of releasing different life stages to maintain long-term population suppression in the presence of mosquito immigration into the control area are shown.

Many regions in the tropics, where Ae. aegypti are present, see highly seasonal rainfall. Changes in the amount of rainfall are known to be associated with seasonal fluctuations in vector abundance [177–179]. Seasonal dynamics can have a profound effect on the outcome of vector-control attempts and therefore their inclusion is an important component to consider in modelling studies [180–182].

2.2 Methods

2.2.1 Data
Pupal MRR data were collected during a field trial in Grand Cayman, a British Overseas Territory in the Caribbean, between September and October 2010 [91]. The study location was a 10 hectare, peri-domestic area consisting mainly of mixed brick and wooden housing [90]. Pupae (strain: OX513A) were distributed in pupal release devices that were placed in shaded locations across the study site. The release device consisted of two stacked deli pots (a clear plastic pot, base diameter=9cm, top diameter=10cm, base pot height=7cm, upper pot height=3.5cm). The lower pot housed the pupae in approximately 2cm of water. The upper pot consisted of an open meshed base and lid and was filled with polystyrene beads coated with the fluorescent dust. The device was placed in a water ant-trap to minimise the risk of predation. Pupae eclosed within the device where the resultant adult males may rest before being marked with fluorescent dust whilst exiting the device through the matrix of polystyrene balls [121]. During four independent MRR experiments 19,624, 38,968, 16,673 and 22,702 pupae were released at the field site. Recaptures were made on subsequent days using 15 BG-Sentinel traps (Biogents) distributed across the study area. Releases were conducted from September to October 2010, during the rainy season. It is assumed, as observed in preliminary data (not shown), that marked individuals do not transfer markings to unmarked individuals.
Pupal eclosion experiments were performed simultaneously to pupal MRR during the field trial. Pupae were sampled from the release generation and placed in small cages (25×25×25cm). Cages were stored overnight at 20°C and then transferred to an outdoor, semi-shaded location. The number of individuals that had eclosed was recorded at subsequent 24-hour intervals until eclosion ceased. Five replicates of the pupal eclosion experiment were conducted with 230, 252, 274, 292 and 267 individuals each.

### 2.2.2 Release model

A continuous-time compartmental deterministic model was developed to fit to pupal MRR data. The rate of change in the number of male pupae (P) decays logistically with respect to time

$$\frac{dP}{dt} = -\left(\frac{wP(k-P)}{k}\right)$$

where $w$ is the pupal eclosion rate and $k$ a pupal coefficient producing a sigmoidal decay in pupae numbers over time. A sigmoidal decay curve captures dynamics where there is a given period of time before eclosion occurs in the majority of individuals, after which most individuals eclose in a short time window. The model assumes that all pupae successfully eclose, all individuals are homozygous males and that males exit the release device immediately. Pupae can progress to being sexually immature newly eclosed adults (A). These individuals remain sexually immature for an average of $\sigma^{-1}$ days. Sexually immature adults may die or be recaptured with rates $\delta$ and $\gamma$, respectively. The mortality term ($\delta$) encapsulates both true mortality plus emigration from the study area, however, the size of the study area should minimise emigration effects. The rate of change in the number of sexually immature adults (A) with respect to time is given

$$\frac{dA}{dt} = \left(\frac{wP(k-P)}{k}\right)\sigma A - \gamma A - \delta A .$$

(2.2)

Those adults that have survived to sexual maturity are assumed to die or be recaptured with the same time-independent rates as sexually immature adults $\delta$ and $\gamma$, respectively. The rate of change in the number of sexually mature adults (M) with respect to time is

$$\frac{dM}{dt} = \sigma A - \gamma M - \delta M .$$

(2.3)

The rate of change in the number of recaptured (R) individuals is therefore dependent on the rate of recapture of both sexually immature and mature adults.
$$\frac{dR}{dt} = \gamma A + \gamma M.$$  \hspace{1cm} (2.4)

A model with an additional compartment, representing eclosed adults that had not left the pupal release device, was also considered. In this instance, equation (2.1) remains unchanged. The additional compartment represents the rate of change in the number of males that have eclosed, but not exited the release device (L)

$$\frac{dL}{dt} = \left( \frac{wP(k-P)}{k} \right) - \epsilon L,$$  \hspace{1cm} (2.5)

where $\epsilon$, represents the rate that eclosed males exit the release device ($\epsilon^2$ being the mean duration of rest within the release device). The rate of change in the number of sexually immature adults that have exited the release device now becomes

$$\frac{dA}{dt} = \epsilon L - \sigma A - \gamma A - \delta A.$$  \hspace{1cm} (2.6)

The rate of change in the number of individuals that are sexually mature adults (M) with respect to time is unchanged (equation (2.3)). The rate of change in the number of recaptured individuals is also unaffected (equation (2.4)).

The adult model used to simulate adult-only releases is a simplification of equation (2.3), where $A=0$. Adult males are released when sexually mature and are assumed to die or be recaptured with rates $\delta$ and $\gamma$, respectively, equal to those of adults in a pupal release. The rate of change in the total number of adult individuals with respect to time is

$$\frac{dM}{dt} = -\gamma M - \delta M.$$  \hspace{1cm} (2.7)

Pulsed releases can be simulated by summing multiple instances of single releases across release time points. The pupal model (equations (2.1)-(2.3)), parameterised from field data, alongside a more simple adult-only release model (equation (2.7)) was used to simulate pulsed releases of transgenic insects. Releases could be adult-only, pupal-only or combined.

### 2.2.3 Parameterisation of the model

Model fitting and parameter estimation were performed, using Berkeley Madonna [183], by minimising the sum of squared differences between model-predicted recapture estimates (equation (2.4)) and the observed recapture data from four pupal MRR experiments. Data describing pupal eclosion rates in the field allowed comparison of observed versus expected pupal eclosion rates.
from the best fit models as a means of validation. Poisson 95% confidence intervals (CIs) were calculated based on recapture data. CIs surrounding eclosion data were calculated using the product of variance from both eclosion and release data.

### 2.2.4 Population dynamics model

To assess the potential impact of adult-only, pupal-only or combined releases of transgenic insects a model of wild *Ae. aegypti* population dynamics [184] was used. The rate of change in the number of females \( F(t) \) with respect to time is

\[
\frac{dF}{dt} = QF(t-T)\exp\left[-\alpha\left(EF(t-T)\right)^{\beta}\right] - \omega F(t),
\]

where \( Q \) is the birth rate (egg to adult) in the absence of any density-dependent larval effects, \( T \) is the mosquito development time, \( \alpha \) the first larval density-dependent coefficient (set to determine the equilibrium number of females in the wild population in the absence of control, and therefore related to the carrying capacity of the environment), \( E \) is the female egg production rate, \( \beta \) the second larval density-dependent coefficient (set to = 1 throughout to ensure stability [185] but included for generality and consistency with previous published studies using this model) and \( \omega \) the adult mortality rate. The relationship between the density-dependent variable \( \beta \) and the birth rate \( Q \) influences the stability of the system. High values for both \( \beta \) and \( Q \) can lead to unstable dynamics [185].

This model has been altered to include the effect of a transgenic release with late-acting lethality on the wild population dynamics [86], giving

\[
\frac{dF}{dt} = \left(\frac{QF(t-T)}{F(t-T)+cD(t-T)}\right)\exp\left[-\alpha\left(EF(t-T)\right)^{\beta}\right] - \omega F(t).
\]

Parameters are as equation (2.8) with the addition of \( c \), the mating competitiveness of transgenic males and \( D(t) \) the number of transgenic males at time \( t \). In this instance the number of new females in the next generation is proportional to the ratio of wild to transgenic males in the population, adjusted for the relative competitiveness of transgenic males \( (c) \). A conservative value \((c=0.01)\) was assigned to male mating competitiveness and was assumed to be equal for all release types. The field estimate of mating competitiveness from the Cayman Islands was 0.059 (95% bootstrap CI 0.011-0.21) [91] and the lower end of this estimate was used. Assuming that mating competitiveness is equal for males released as adults or pupae, any variation in the value of \( c \) would impact the absolute number of transgenic individuals that would need to be released but would not impact the relative performance of releasing different life stages in the model. Other assumptions of this model
include: a closed population, a 1:1 sex ratio of wild individuals in the absence of control, random mating, all individuals taking the same average time to progress through each life stage [184, 186] and releases being of 100% homozygous RIDL male insects carrying a late-acting lethal construct [86].

2.2.5 Seasonality
The general model for wild-population dynamics under the influence of a transgenic release can be modified to include a seasonal element [187]. Seasonal forcing of the $\alpha$ parameter has been used to facilitate the assessment of releasing different life stages in the presence of seasonal dynamics in the wild population. The $\alpha$ parameter relates to the carrying capacity of the environment and seasonally forcing this parameter causes seasonal fluctuations in the carrying capacity of the environment such as may be observed with fluctuating numbers of water bodies caused by seasonal rains. Equation (2.8) is modified with a cosine curve to force the seasonal dynamics resulting in

$$\frac{dF}{dt} = QF(t-T) \exp \left[ -\frac{\alpha}{1 + \chi \cos \left( \frac{2\pi t}{\phi} \right)} (EF(t-T))^\beta \right] - \omega F(t),$$

(2.10)

where $\chi$ is the amplitude of seasonality and $\phi$ determines the periodicity. Equation (2.9) can be modified in the same manner to give

$$\frac{dF}{dt} = \left( \frac{QF(t-T)}{F(t-T) + cD(t-T)} \right) \exp \left[ -\frac{\alpha}{1 + \chi \cos \left( \frac{2\pi t}{\phi} \right)} (EF(t-T))^\beta \right] - \omega F(t),$$

(2.11)

All analyses that include seasonality use a hypothetical, generalised seasonal profile which is not fitted to represent specific seasonal dynamics at the Grand Cayman field site.

2.2.6 Measuring performance
Measurement of the effectiveness of a given control approach involves a comparison between the population in the absence and presence of control. Previous studies have quantified the release effect for single releases [185], I employ a similar approach for multiple releases that allows a measurement of treatment effect relative to the wild population in the absence of control. The treated area under the curve (AUC) in the controlled population is
\[ \text{Treated AUC} = \frac{\int_{t_1}^{t_1+400} F_c \, dt}{\int_{t_1}^{t_1+400} F_0 \, dt} \]  

(2.12)

where \( t_1 \) is the first release day, \( t_n \) the last release day, \( F_c \) the wild population of females in the presence of control and \( F_0 \) the wild population in the absence of control. The control effect is measured 400 days after the last release to capture population recovery dynamics.

Where two different control methods or regimes are being compared, a relative measure of their respective effectiveness is used

\[ \text{Relative effect size} = \frac{\text{Treated AUC}_a}{\text{Treated AUC}_b}, \]  

(2.13)

where \( a \) denotes one control method or regime and \( b \), the other. In this instance, values of relative effect size <1 occur when control \( a \) outperforms control \( b \), values >1 occur when control \( b \) outperforms control \( a \), values and relative effect size=1 when the two approaches perform equally.

All simulations were programmed and implemented in the statistical program R [188] using the deSolve package [189], with default settings used for the differential equation solver. All parameters were set to the default values as specified in table 1 throughout unless otherwise stated.
Table 1. Pupal- and adult-release and wild population model variables and parameters. State variable and parameter definitions and default parameter values.

<table>
<thead>
<tr>
<th>State variable</th>
<th>Definition</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Number of Pupae</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Number of sexually immature adult males</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Number of sexually mature adult males</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Number of individuals that are recaptured</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Number of females in the wild population at time t</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Number of sexually mature transgenic adult males at time t</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wild parameter</th>
<th>Definition</th>
<th>value</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>Number of offspring produced by each adult per day that survive to adulthood in the absence of density-dependent mortality</td>
<td>0.7</td>
<td>[186]</td>
</tr>
<tr>
<td>α</td>
<td>Density-dependent coefficient (set to determine the equilibrium number of females in the wild population in the absence of control)</td>
<td>1.21e^05</td>
<td>-</td>
</tr>
<tr>
<td>β</td>
<td>Density-dependent coefficient</td>
<td>1</td>
<td>[184]</td>
</tr>
<tr>
<td>E</td>
<td>Female daily egg production</td>
<td>16</td>
<td>[184]</td>
</tr>
<tr>
<td>ω</td>
<td>Wild mosquito mortality rate (conservative value chosen) (days⁻¹)</td>
<td>0.1</td>
<td>[136, 139, 190]</td>
</tr>
<tr>
<td>T</td>
<td>Mosquito generation time (days)</td>
<td>18.5</td>
<td>[184]</td>
</tr>
<tr>
<td>χ</td>
<td>Amplitude of seasonal forcing</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>φ</td>
<td>Periodicity of seasonal forcing (chosen to produce two high seasons per year)</td>
<td>182.5</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RIDL parameter</th>
<th>Definition</th>
<th>value</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>w</td>
<td>Eclosion parameter (relates to the rate at which pupae eclose) (days⁻¹)</td>
<td>1,2,4,8</td>
<td>estimated</td>
</tr>
<tr>
<td>k</td>
<td>Eclosion coefficient</td>
<td>1.01×release size</td>
<td>estimated</td>
</tr>
<tr>
<td>δ</td>
<td>Mortality rate (days⁻¹)</td>
<td>0.5</td>
<td>estimated</td>
</tr>
<tr>
<td>γ</td>
<td>Recapture rate (days⁻¹)</td>
<td>0</td>
<td>estimated</td>
</tr>
<tr>
<td>c</td>
<td>Transgenic male mating competitiveness (conservative value chosen)</td>
<td>0.01</td>
<td>[90, 106, 191]</td>
</tr>
<tr>
<td>σ</td>
<td>Sexual maturation rate (days⁻¹)</td>
<td>1</td>
<td>[192]</td>
</tr>
<tr>
<td>ε</td>
<td>Rate that eclosed males exit the release device (days⁻¹)</td>
<td>-</td>
<td>estimated</td>
</tr>
</tbody>
</table>

2.2.7 Comparing release methods and regimes
The efficacy of adult-only, pupal-only and combined release methods was tested on a large simulated wild population set to reach a stable equilibrium at 10,000 female *Ae. aegypti* in the absence of any control.

Simulations of releases were made for a number of scenarios varying the weekly production capacity (production from 2×10⁷ to 3.5×10⁷ males per week) and release frequencies (time between releases from 1 to 20 days). Individual release sizes were calculated as
\[ \text{release size} = \frac{\text{weekly production}}{7} \times \text{days between release} . \]  

Releases were started following 500 days of simulation without control and concluded after 100 days. All RIDL parameters were set as default (Table 1) except the pupal eclosion rate, \( w \), which was varied for pupal-only release scenarios (\( w=1,2,4,8 \text{ days}^{-1} \)). For combined releases only one pupal eclosion rate (\( w=2 \text{ days}^{-1} \)) was considered. This rate was at the lower end of estimates from the data and emphasised observed differences between adult and pupal dynamics. Adult and pupal releases were assumed to occur simultaneously and in a 1:1 ratio when in combination.

### 2.2.8 Long-term suppression

The population dynamics model was further altered to assess the potential for various release strategies to successfully suppress the population in the long-term. A term that included stochastic increases in wild population numbers was added to represent external immigration pressures. Immigration was modelled as a random negative binomial process, with constant dispersion parameter, \( z=1 \), and probability of success, \( p=0.05 \). An independent immigration event was set to occur at the start of each day (mean = 1.9 individuals day\(^{-1} \), max=148).

The potential benefits of adult-only, pupal-only or combined releases for the long-term maintenance of suppression of a population in the presence of immigration were studied. An initial intensive control effort was simulated to suppress the wild population down to a low level (100 days of adult-only releases every two days). The ability of adult-only, pupal-only or combined releases of low frequency (releases every 7 days) to maintain population suppression for 5 years was then examined. The effect of varying adult-to-pupae ratios in a combined release (ranging from all adults to all pupae) on the reduction in AUC was calculated. The ratio of adults to pupae used in the combined releases was set at the optimum, maximising the estimated level of suppression as a function of the adult to pupal ratio.

### 2.3 Results

#### 2.3.1 Experimental data

From the four independent MRR experiments 46, 146, 32 and 56 marked adult males were recaptured in the field. Recaptures were conducted from one to thirteen days post-release. Recapture numbers peaked between two and four days post release, the latest recapture occurred on day nine. Pupal eclosion in the cage study was observed over a period of three days.
2.3.2 Pupal dynamics model

Model fit of the pupal dynamics model to pupal MRR data from Grand Cayman was qualitatively good with predicted values falling within the 95% CIs for all but two time points (Figure 8) allowing estimates of mortality and recapture rates to be made (Table 2).

![Figure 8. Pupal MRR data and model fit. The number of marked recaptured individuals with respect to time (points, with 95% Poisson CIs for the underlying rate) and estimated model fit (lines) for four (A-D) MRR experiments.](image)

The pupal eclosion model given in equation (2.1) reproduced the observed trends in pupal eclosion data but had an associated lag (Figure 9). When explicitly modelled, this lag, attributed to time spent within the release device, was estimated as being between 12 and 18 hours.

<table>
<thead>
<tr>
<th>Release date</th>
<th>Number released</th>
<th>Mortality rate ($\delta$ in days$^{-1}$)</th>
<th>Recapture rate ($\gamma$ in days$^{-1}$)</th>
<th>Eclosion rate ($w$ in days$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/09/2010</td>
<td>19,624</td>
<td>1.25</td>
<td>0.0027</td>
<td>3.82</td>
</tr>
<tr>
<td>24/09/2010</td>
<td>38,968</td>
<td>6.95</td>
<td>0.025</td>
<td>3.40</td>
</tr>
<tr>
<td>08/10/2010</td>
<td>16,673</td>
<td>0.64</td>
<td>0.0014</td>
<td>2.22</td>
</tr>
<tr>
<td>13/10/2010</td>
<td>22,702</td>
<td>1.40</td>
<td>0.0034</td>
<td>5.86</td>
</tr>
</tbody>
</table>
Figure 9. Model validation using pupal eclosion data. Pupal eclosion data (points, with 95% CIs for the underlying rate) and predicted numbers of pupae with respect to time (lines) from models fitted to recapture data for A-D) simple model (equations (2.1)-(2.4)), E-H) the model including a lagged exit from the release device (including equations (2.5)-(2.6)).

The pupal dynamics model and the adult model were used to simulate pulsed releases of transgenic males (Figure 10).

Figure 10. Multiple, pulsed releases of transgenic insects. Examples of the number of RIDL individuals with respect to time resulting from pulsed releases of A) adults only and B) pupae only. The resulting, C) sexually mature adult males from a pupal release and D) sexually mature adults from a combined release. Arrows denote release days, all parameter values as default, \( w = 2 \) days\(^{-1}\). Frequent releases show a cumulative effect.

2.3.3 Release method comparisons
Trends in the relative effect of different release methods remained consistent for the range of release sizes and timings considered, therefore results assuming a production \( \times 3.5 \times 10^7 \) males week\(^{-1}\) are shown (Figure 11). The maximum departure from 1 of the relative effect size measure observed was 0.78. For all comparisons the relative effect size tended towards 1 with less frequent releases (time between releases of >15 days).
Figure 11. The relative effectiveness of releasing different life stages. The relative effect size (eq. (2.13)) of A) adult-only against pupal-only ($w=1,2,4,8$ days$^{-1}$), B) adult-only against combined releases ($w=2$ days$^{-1}$) and C) combined against pupal-only release ($w=2$ days$^{-1}$) for a range of release frequencies. All parameter values (except $w$) as default, production=$3.5\times10^7$ males per week.

Adult-only releases were universally more effective compared with the four pupal-only release scenarios and combined releases when release frequency was every 4 days or more frequent. The relative performance of adult-only releases was greatest when releases occurred daily. The relative performance of pupal-only releases was strongly influenced by the rate of eclosion ($w$). For slow eclosion rates ($w=1,2$ days$^{-1}$) pupal-only releases strongly outperformed adult-only releases when releases were at least 5 days apart. The strength of this relative advantage waned with increasing eclosion rates. The highest rate of pupal eclosion considered ($w=8$ days$^{-1}$) saw nearly all advantage of pupal-only releases over adult-only releases at infrequent releases periods diminished. Combined release methods ($w=2$ days$^{-1}$) were more effective than adult-only release methods for releases at least 4 days apart and outperformed all pupal-only releases ($w=2$ days$^{-1}$) considered.

Examples of how these relative effect sizes may translate to wild-population suppression in a RIDL-based control programme are shown in Figure 12. Here, two scenarios are considered, one where releases are daily ($7\times1,000,000$ release week$^{-1}$) and one where releases are every 7 days ($1\times7,000,000$ release week$^{-1}$). The release programme is simulated for a period of 100 days. Daily adult-only releases outperform daily pupal-only releases and, marginally outperform the combined releases. Infrequent (every 7 days) pupal-only releases are marginally more successful at suppressing the wild population than adult-only releases at the same frequency, whilst the combined releases perform most effectively.
Figure 12. Examples of control with frequent and infrequent releases of transgenic *Ae. aegypti*. *Ae. aegypti* population dynamics in the absence of control (dotted line) and under adult-only, pupal-only and combined RIDL release regimes (pink, blue and green lines respectively). With A) frequent releases (time between releases = 1 day) adult-only releases achieve the best levels of population suppression. With B) infrequent releases (time between releases = 7 days) wild population recovery between releases can be observed and is most apparent with adult-only releases which are outperformed by combined and pupal-only releases. All parameter values as default, $w=2$ days$^{-1}$.

### 2.3.4 Long-term suppression

For a release frequency of every seven days the optimum ratio of pupae to adults was close to 1:1 (55% pupae, 45% adults) (Figure 13).
Figure 13. Optimising the adult-to-pupae ratio. The level of suppression of a wild population achieved as a function of varying adult-to-pupae ratios in a combined release. The optimum ratio of pupae to adults for releases every seven days is (55% pupae, 45% adults).

Scenarios with infrequent releases indicated that combined releases may be a more efficient way of maintaining suppression than adult- or pupal-only releases in the long-term. Maintenance of suppression was achieved when releasing 1.9 million individuals (1,387,000 pupae + 513,000 adults) every seven days in combined releases. Adult- or pupal-only releases at these numbers failed to maintain suppression in the target population (Figure 14) requiring releases of 2.8 and 2.7 million individuals per week respectively to maintain suppression.
2.3.5 Seasonality

The efficacy of the RIDL control programme, regardless of life stage released, was dependent upon the onset of releases of transgenic insects in relation to seasonal peaks in wild population abundance. All three life stages performed best when releases began shortly prior to the lowest wild
female abundances. The precise optimal time point at which releases started was dependent on the life stage released and the dynamics of the life stage.

**Figure 15.** The effect of seasonal dynamics on releases. The A) seasonal dynamics of the wild population in the absence of control and the relative effect size of starting releases throughout the season with respect to the life stage released (coloured lines) for releases every B) 3 days or C) 7 days. Peaks in effect size indicate that the most effective timing of the release start day is a short period prior to the natural seasonal trough in wild population numbers. Seasonal dynamics have little influence on the rank of each release method for frequent or infrequent releases; adult releases remain most effective for frequent releases (B) whilst combined releases are preferable for infrequent releases (C).
2.4 Discussion

This study makes use of large-scale pupal MRR data to inform models that allow comparisons between adult-only, pupal-only and combined releases of transgenic *Ae. aegypti* over a range of scenarios. Adult-only releases are most beneficial when releases are frequent whilst pupal-only and combined releases may outperform adult-only releases in scenarios when releases are less frequent. Potential approaches to maintaining long-term suppression of the vector population have been explored. Combined releases can provide increased effectiveness for a long-term vector control programme.

The pupal release model provides a method by which adult male numbers from transgenic pupal-only pulsed releases can be simulated over time. The fit of the model-predicted recaptures to recapture data was good (Figure 8); however, there were differences between observed and predicted pupal numbers over time (Figure 9, A-D). The predicted pupal numbers show similar but lagged trends to numbers predicted from eclosion experiments. One potential cause for this lag would be eclosed males resting in the pupal release device before exiting. The number of males predicted by the model would be those functional males that had exited, not eclosed males within the release device. A model explicitly including this lag improved the fit to pupal eclosion data (Figure 9, E-H), at the expense of reduced fit to the recapture data. The more simple model and the sigmoid functional form of pupal eclosion was chosen for all simulations as they provide a superior fit to recapture data. Assuming recapture rates were constant, both models predicted similar numbers of mature males over time, the critical factor for control efficacy. A second potential explanation may be disparities in the recapture rates of sexually immature and sexually mature males, which may manifest if there are differences in the drivers affecting dispersal between those two groups. A similar lag may be explained by newly eclosed males being less likely to be recaptured. Throughout this study the use of appropriate field data as the basis of model design and use has been championed. Even in this scenario, where very large-scale MRR experiments were used to parameterise the model, there is scope for further field studies to improve the understanding of the early-stage dynamics of a pupal release. This emphasises the iterative process by which data inform models that in turn can be used to influence the design and direction of future field studies.

The ability of adult-only releases to consistently outperform both pupal-only and combined releases at high release frequencies is a very clear outcome of this analysis. The benefits of frequently releasing adults have also been shown in other studies [185]; to date, most programmes using RIDL *Ae. aegypti* have involved relatively frequent adult-only releases of males, e.g. three releases per week [91]. Adult-only releases introduce males that are already sexually mature. Pupal-only releases
will always be disadvantaged in comparison as newly eclosed males will almost certainly experience higher mortality rates in the period it takes them to sexually mature in the field than individuals that have sexually matured prior to release. Frequent releases of adults perform well due to the initial spike in RIDL numbers immediately after release. Numbers remain relatively high until the next release (e.g. the next day) where a new pulse of sexually active transgenic males is introduced into the population allowing very high densities of sexually active transgenic males to be maintained over time. A high transgenic-to-wild-male ratio increases the chances of a wild female mating a transgenic male resulting in increased numbers of infertile matings, thus improving control. When releases are less frequent the effectiveness of adult-only releases is reduced. Troughs in transgenic numbers between releases allow wild population numbers to increase, reducing the suppressive effect (Figure 12 B).

The peak in sexually active males from a pupal-only release is delayed and prolonged compared with that from an adult-only release (Figure 10). This is advantageous if releases of transgenic insects are less frequent. Here, the less peaked distribution of sexually mature adults over time provides better coverage of transgenic males in the population when releases occur less often. Our results reflect this, with pupal-only releases outperforming adult-only releases when the time between releases becomes greater (Figure 11 A).

The relative performance of a pupal release is highly dependent on the rate at which pupae eclose. The relative benefit of pupal releases decreases with increasing pupal eclosion rates when releases are infrequent. Increasing eclosion rates produce a shorter and more intense pulse of adult insects into the population, akin to an adult-only release. The actual pupal eclosion rates may be highly dependent on external variables, such as temperature [182, 193, 194]. The lowest estimate of $w$, 2.22 (Table 2), would lead to considerably different dynamics compared with an adult release, however, eclosion rates as low as 1 day$^{-1}$ are unlikely without some external manipulation. The ability to predict and manipulate the eclosion rates for a given target area may significantly affect the performance of pupal-only releases compared with the adult-only release method. These effects may be even more pronounced if earlier life stages, such as eggs [195], were distributed.

Combined releases have the potential to benefit from both the initial peak produced by the adult component of the release as well as the secondary peak of transgenic males from the pupal component. Whilst undoubtedly being logistically more challenging, combined releases outperformed both pupal-only and adult-only releases for the majority of scenarios considered. Combined releases show the strongest suppression of wild population recovery between releases.
when time between releases was seven days (Figure 12 B). Combined releases are only marginally outperformed by adult-only releases when release frequency is <every 4 days (Figure 11 B).

The comparative performance of releasing different life stages are consistent in the presence of seasonal fluctuations in wild population density. For frequent releases, releasing the adult life stage remains the most effective option. Likewise, for less frequent releases releasing a combination of adults and pupae again performs best. The timing of the start of release with respect to seasonal fluctuations is important. Starting releases just prior to the peak of the dry season (lowest wild population abundance) is most effective for all life stages. The exact timing of the start of releases again is dependent on the development time when pupae are used. Seasonal dynamics could impact the system in a number of ways other than through a varying environmental carrying capacity. For example, seasonal fluctuations in temperature may impact development times [182, 193, 194] and could lead to more pronounced relative differences between the release times as pupal-based approaches would be more sensitive to changes in temperature-driven development time than adult releases.

It is important to note that throughout these analyses any spatial element has been omitted. This approach was chosen to allow clear comparisons between the release types examined. Field and suppression programme release of RIDL individuals, be it pupal or adult, are likely to encounter spatial heterogeneity and metapopulations, which can lead to reduced efficacy of a RIDL-based approach [195]. All of the approaches considered have benefits and drawbacks unrelated to population dynamics that will also determine their feasibility and use. Production costs for adult-only releases would include the storage and maintenance of ecling adults, an additional stage not required for pupal-only releases. The distribution of adult-only releases may be logistically the most simple, with releases potentially able to be performed from a moving vehicle or even aircraft. Pupal release would involve the distribution of pupal release devices and may be prone to problems of disturbance, theft or predation. However, pupae are a more robust life stage than adults making them more amenable to long-distance travel between production facilities and release sites. The gradual appearance of adult mosquitoes in the control area from pupal releases may also reduce the perception of public nuisance.

Releases of RIDL Ae. aegypti have been shown to successfully suppress wild populations in a short period of time [91]. To maximise the potential vector control and public health benefits of a vector control programme such suppression must be maintained in the long-term. Maintenance of suppression must be conducted in a cost-effective manner in the face of immigration pressures from external populations. When population numbers have been driven to very low levels, random events
may have relatively large impacts on population dynamics. I included this stochastic term into the model to emphasise the potential for single, sporadic immigration events to disrupt maintenance of suppression of a wild population. Immigration may also be expected to vary seasonally, however as this would not change with respect to release-type it should not affect the relative performance of the specific life stage released. Optimising low-intensity maintenance releases of transgenic insects will be vital to achieve the goal of long-term suppression. Low-frequency releases of a combination of adults and pupae may be the most effective method of maintaining suppression in the long-term (Figure 14 C). This method allows fewer insects to be released at low-frequency compared with adult- or pupal-only releases. These benefits could outweigh the disadvantages of the logistical demands of a combined release and help to provide an optimal cost-effective, long-term solution.

2.5 Conclusion

This study demonstrates the process by which field data can be successfully used to design models to inform future studies and practical approaches. Parameterised models show that adult-only, pupal-only and combined releases of transgenic insects all demonstrate a good ability to suppress a simulated wild population of *Ae. aegypti*. When releases are frequent adult-only releases are superior to pupal-only and combined releases and have in their favour more simple logistical implementation in the field. Under certain circumstances, such as when releases are more infrequent, pupal-only and combined releases can outperform adult-only releases. The relative benefit of using combined releases when releasing infrequently suggests they could be utilised to maintain long-term suppression in a sustainable manner.

2.6 Acknowledgements

I would like to thank the Cayman Islands Mosquito Research and Control Unit and Oxitec staff, especially Angela Harris, Siân Morgan, Jessica Stevenson and Norzahira Raduan for their work in the original field trials described here. I am grateful to the community of East End, without whose support and participation field work evaluations would not have been possible.
3 Estimating field dispersal of transgenic male *Aedes aegypti* mosquitoes.

In the previous chapter I showed that frequent, intensive release of adult life stage transgenic male *Ae. aegypti* to control a simulated wild population was efficacious. As a proven strategy for transgenic insect releases, it is therefore advantageous to further our understanding of the dynamics of adult releases to allow them to be conducted in an optimal manner. In this chapter I aim to improve our knowledge surrounding the spatial dynamics of adult releases by characterising the dispersal of transgenic male *Ae. aegypti* in the field.

3.1 Introduction

SIT using irradiated [28, 196] and, more recently, genetically engineered [99] insects is a valuable vector control tool. Understanding the ability of the released insects to disperse, and their behaviour whilst doing so, is an important step in designing robust, efficient and effective releases. Attaining adequate coverage of released sterile insects across a given area is a major operational challenge of a sterile insect control effort [197]. Knowledge of the distribution of dispersal distances of released insects will improve our ability to target releases, obtain required coverage densities and confidently predict the potential spatial range of a release. In order for a release programme to be successful there must be coverage of males above a target density across the target area. To be able to ensure adequate male densities are achieved across the spatial extent of the target area we must have detailed knowledge of the distribution of males we can expect to be achieved from a given set of release points.

MRR studies have been undertaken to assess the dispersal ability of male *Ae. aegypti* lab [114, 135, 137, 149] and transgenic [92] strains. However, these studies often document only the MDT [92, 114, 135, 137, 149] or range [92, 137] of dispersal of released insects. Common measures of range are the flight range 50% and flight range 90% (FR$_{50}$ and FR$_{90}$ respectively) which are estimates of the distance within which 50% or 90% of all insects are expected to disperse [92, 145]. To accurately predict coverage the distribution of dispersal with respect to distance, corrected for potential confounding variables must be better understood. Dispersal kernels allow a more in-depth assessment of dispersal ability over the whole flight range. They can take a wide range of forms with the flexibility to represent dispersal for a diverse range of species [198]. Incorporating dispersal kernel theory, popular in studies of population spread [199] and seed dispersal [200], into a generalised linear model (GLM) framework is one such way to achieve this [201, 202]. As well as distance and time post-release (capturing mortality and emigration) we would hypothesise that other covariates may significantly influence recapture number. Mosquitoes are known to cluster in
households [203–205] so we would expect that locations where large numbers of wild mosquitoes are captured would also see large marked-male recaptures. Meteorological variables may affect overall recapture numbers but would not be expected to influence released and wild males differently, having little influence once the number of wild-recaptures is controlled for.

This study attempts to accurately determine the dispersal ability of transgenic male *Ae. aegypti* mosquitoes using data from large-scale MRR experiments carried out at an urban field site in Brazil. The analysis facilitates the quantification of dispersal through the parameterisation of a dispersal kernel for the released insects. Many summary measures of interest, such as the MDT, FR\textsubscript{50} and FR\textsubscript{90}, relating to dispersal may be drawn from such a kernel. To enable a comparison of both the biological outcomes and methodology employed, the analytical methods are also used to re-analyse published data on the dispersal of transgenic male *Ae. aegypti* at an uninhabited forested site in Pahang, Malaysia [92]. The dispersal ability of the transgenic insects was previously analysed in the Malaysian study using methods detailed in Morris et al. (1991) and evaluated the MDT to be 52m (95% CI: 42m, 61m) [145]. The aim of the re-assessment of dispersal ability is to provide an indication of how robust the estimate of dispersal from Brazilian data may be to habitat and locational heterogeneities, to explore potential differences in dispersal behaviour between sites and to assess the applicability of the methods in comparison with more common approaches to estimating and quantifying dispersal.

### 3.2 Methods

#### 3.2.1 Study site

The field site is located in Itaberaba, a suburb of the city of Juazeiro, Bahia, Brazil (Latitude: -9° 26' 59", Longitude: -40° 28' 53") (Figure 16 A). The site is located in a semi-arid part of Brazil and consists mainly of low-socioeconomic status residential housing. The habitat across the sampled region was a homogenous urban environment.
Figure 16. Study site sampling locations. A) The study site, Itaberaba, a suburb of the city of Juazeiro, Bahia State, Brazil. B) MRR release points (numbered circles) and sampling locations (pink circles) within households distributed across the sampling grid at the study site.

3.2.2 Mark-Release-Recapture
A total of 19,164 transgenic male *Ae. aegypti* formed three releases. Individuals from each release were marked with the same coloured fluorescent powder. Release one (red release) and release two (blue release) were performed on 21 February 2011 and consisted of 5,349 and 5,465 individuals released from points one and two (Figure 16 B) respectively. Release three (yellow release) was performed on 25 February 2011 and saw 8,350 individuals released from point one (Figure 16 B). February is typically the rainy season at this location.

Aspiration sampling was used to recapture marked adults. Sampling locations were distributed across the study site (Figure 16 B) and sampling was conducted for up to nine days post release. Resampling locations were chosen by randomly sampling a household from each of the 47 (60m by 60m) grid squares each day (with the exception of day five for the red and blue release and day one for the yellow release where, for logistical reasons, the number of households sampled was lower). Each mosquito collected was assessed to determine the (i) origin (transgenic or wild as indicated by the presence/absence of fluorescent powder respectively), (ii) sex and (iii) genus (*Aedes* or non-*Aedes*). Weather variables (daily maximum temperature and maximum humidity) were recorded from a local weather station (situated approximately 10.2km north-west of Itaberaba).
An analysis of the Malaysian MRR data was undertaken to obtain a comparative second estimate of the transgenic insect's dispersal ability and associated density kernel. In this study a total of 6,045 marked transgenic males were released at a forested site in Pahang, Malaysia. For a detailed description of the study site and MRR methods please see Lacroix et al. (2012) [92].

3.2.3 Model framework
All multivariable analyses were performed within a GLM framework. The count of recaptured marked males, per collection is used as the response variable in the model, detailed below. In instances where the number of recaptures is small relative to the total releases, the Poisson regression model may be used as an appropriate approximation [206].

I assume that the count response variable (recaptures) is Poisson distributed with mean $\mu$ and variance $\mu$

$$Y_i \sim \text{Pois}(\mu_i).$$

(3.1)

The response must be $\geq 0$. Therefore a log link function is used to link the mean to the explanatory variables

$$\ln(\mu_i) = x_i \beta,$$

(3.2)

where $x_i \beta$ is a linear predictor

$$x_i \beta = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{ip},$$

(3.3)

where $\beta$ denotes the unknown parameters to be estimated and $x_i$, the explanatory variables.

Parameter estimates were obtained by maximising the log-likelihood ($\ell$) of the data:

$$\ell(\beta \mid Y) = \sum_{i=1}^{n} \left( y_i \ln \mu_i - \mu_i - \ln(y_i!) \right)$$

$$= \sum_{i=1}^{n} \left( y_i x_i \beta - \exp(x_i \beta) - \ln(y_i!) \right).$$

(3.4)

In the situation where the response variable is overdispersed (variance>>mean) a Poisson GLM, where the variance is assumed to equal the mean, would be misspecified. In this instance, the negative binomial GLM, detailed below, may be used [207–209].
I assume that the count response variable follows a negative binomial distribution. A Poisson model is used for the count, conditional on the mean value, $Z_i$, where $Z_i$ is assumed to have a gamma distribution, with mean, $\mu_i$, and constant scale parameter, $\theta$

$$Y_i \sim \text{Poisson}(Z_i), \quad Z_i \sim \text{gamma}(\mu_i, \theta).$$ (3.5)

Therefore the expected value of $Y$ and the variance of $Y$ are as follows

$$E(Y_i) = \mu_i, \quad \text{Var}(Y_i) = \mu_i + \frac{\mu_i^2}{\theta}. \quad (3.6)$$

The mean response, $\mu_i$, may be linked to a linear combination of explanatory variables using the log link function.

$$\ln(\mu_i) = x_i' \beta,$$ (3.7)

where $x_i' \beta$ is a linear predictor

$$x_i' \beta = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{ip}, \quad (3.8)$$

where $\beta$ denotes the unknown parameters to be estimated and $x_i$ denotes the explanatory variables. Parameters were estimated by maximising the log-likelihood ($\ell$) of the model:

$$\ell(\beta, \theta | Y) = \sum_{i=1}^{n} \theta(\ln(\theta) - \ln(\theta + \mu_i)) + \ln(\Gamma(\theta + y_i)) - \ln(\Gamma(\theta)) - y_i(\ln(\mu_i) - \ln(\theta + \mu_i)), \quad (3.9)$$

where the gamma function, $\Gamma$, is

$$\Gamma(n) = (n-1)! \quad (3.10)$$

### 3.2.4 Dispersal kernels

Considerable inconsistencies abound regarding different interpretations of the term ‘dispersal kernel’ [198, 210, 211]. Two, often confused, kernel types are:

1. The probability density function (pdf) of the dispersal distance of each disperser. Referred to as the distance kernel [198] or the distance pdf [211].
2. The density of probability of a given bearing and dispersal distance from the source (the probability in a very small area of the potential dispersal space). Referred to as the location kernel [198] or the density pdf [211].
I adopt the terminology of Cousens et al. [211], henceforth referring to kernel type 1 as the distance pdf and type 2 as the density pdf. Both kernel types are true pdfs, integrating to 1 (the density pdf is integrated over the whole 2d space). Both kernel types are closely related. The distance pdf can be derived by multiplying the density pdf by $2\pi d$ where $d$ is the distance from the source [198] (assuming radial symmetry). Examples of these kernel types are shown in Figure 17.

Figure 17. Dispersal kernels. Examples of different kernel interpretations for the negative exponential (A, B and C) and exponential power (D, E and F) kernels. The distance pdf is shown in panels A and D. The density with respect to distance is shown in panels B and E and the density pdf is illustrated in panels C and F (after Cousens et al. [211]). Kernels in A, D, C and F integrate to unity (in 1 dimension for the distance pdfs and 2 dimensions for the density pdfs).

The density pdfs, assuming radial symmetry, used in this analysis are defined by the following functions

\begin{align*}
\text{Negative exponential kernel} & = \frac{1}{2\pi a^2} e^{-\frac{d}{a}} \quad a > 0, \quad (3.11) \\
\text{Exponential power kernel} & = \frac{b}{2\pi a^2 \Gamma \frac{a}{b}} e^{-\frac{d}{a}} \quad a, b > 0, \quad (3.12)
\end{align*}

where $d$ is the distance (metres), $a$ and $b$ are kernel parameters and $\Gamma$ the gamma function (Equation (3.10)) [198]. The associated MDT functions are

\begin{align*}
\text{Negative exponential MDT} & = 2a, \quad (3.13)
\end{align*}
\[
Exponential\ power\ MDT = a \left( \frac{\Gamma \frac{3}{b}}{\Gamma \frac{2}{b}} \right).
\]

Estimates of \( FR_{50} \) and \( FR_{90} \) are made by assessing the cumulative distribution of the distance pdf at the 50% and 90% levels.

### 3.2.5 Variables

The outcome variable was the number (count) of marked transgenic male \textit{Ae. aegypti} recaptured. Potential explanatory variables included in the Itaberaba analysis were:

- **Spatial measure**:
  - Distance – the measured distance (m) between release and recapture.
  - Distance density – the density as calculated by a parameterisation of a given density pdf.
- **Number of days post release** – the effect of which is assumed to be linear.
- **Wild \textit{Aedes} spp.** – the number of wild \textit{Aedes} individuals collected.
- **Wild other spp.** – the number of wild non-\textit{Aedes} individuals collected.
- **Maximum temperature** – maximum temperature (°C) on the day of collection.
- **Maximum relative humidity** – maximum humidity (%) on the day of collection.
- **Quadrant** – the directional quadrant, North, South, East or West (relative to release point) that the collection was made in.

Due to the relatively low recapture number, data from all three MRR experiments were combined for analysis.

For the Malaysian analysis the outcome variable was the number (count) of marked transgenic male \textit{Ae. aegypti} recaptured. Potential explanatory variables included in the analysis were:

- **Spatial measure**
  - Distance – the measured distance (m) between release and recapture.
  - Distance density – the density as calculated by a parameterisation of a given density pdf.
- **Number of days post release** – the effect of which is assumed to be linear.
- **Wild \textit{Aedes} spp.** – the number of wild \textit{Aedes} individuals (specifically: \textit{aegypti}, \textit{albopictus} and \textit{togoii}) collected.
- **Wild \textit{Culex} spp.** – the number of wild \textit{Culex} individuals collected.
• Altitude – A categorical variable indicating if the recapture location was uphill or downhill from the release site.

Three models were evaluated to compare different transformations of the distance explanatory variable:

**Model 1.** All explanatory variables including distance.

**Model 2.** All explanatory variables including distance density (negative exponential kernel).

**Model 3.** All explanatory variables including distance density (exponential power kernel).

For model 1 the full model was fitted using maximum likelihood techniques, utilising the GLM and Negative binomial GLM function of the statistical software package R [188] with the MASS package [207]. All explanatory variables were included in the initial model as well as an interaction term between distance and day post release. Model selection by minimising the Akaike information criterion (AIC) was then performed using the package MASS [207]. The AIC is calculated as

$$AIC = 2k - 2\ln(\mathcal{L}),$$

where $k$ is the number of parameters and $\mathcal{L}$ the maximised likelihood value.

For models 2 and 3 fitting was performed using the following process. The distance density was estimated using the assigned kernel. The GLM was fitted using the same process as for model 1, as a function of the transformed distance explanatory variable. This process was then optimised over the kernel parameter space allowing identification of the optimal combination of explanatory variables and kernel parameters as indicated by the AIC. The best overall model was judged to be the one with the minimum AIC value.

### 3.2.6 Kernel confidence intervals

Following model estimation, 95% confidence intervals were calculated for the maximum likelihood kernel parameter estimates using the profile likelihood method. The maximised log-likelihood with respect to $\beta$, $a$ and $b$, i.e. that corresponding to the maximum likelihood estimates (MLEs) of $\beta$, $a$ and $b$, is defined as $\ell(\hat{\beta}, \hat{a}, \hat{b} \mid Y)$.

First kernel parameter $a$ was increased or decreased in small increments whilst $\beta$ was held at the MLE ($\hat{\beta}$) and kernel parameter $b$ was optimized conditional on $\hat{\beta}$ and the assumed value of $a$. 

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(a_0), giving the log-likelihood:

\[
\ell(\hat{\beta}, a_0, \tilde{b} | Y),
\]

(3.16)

where \( \tilde{b} = \text{MLE}(b | a_0, \hat{\beta}) \).

Secondly, kernel parameter \( b \) was increased or decreased in small increments whilst \( \beta \) was held at the MLE (\( \hat{\beta} \)) and kernel parameter \( a \) was optimized conditional on \( \hat{\beta} \) and the assumed value of \( b \) (\( b_0 \)), giving the log-likelihood:

\[
\ell(\hat{\beta}, \tilde{a}, b_0 | Y),
\]

(3.17)

where, \( \tilde{a} = \text{MLE}(a | b_0, \hat{\beta}) \).

After each change the log-likelihood of the model was recalculated and a corresponding test statistic assessed. For example, evaluating for \( a \), the \( G^2 \) statistic was calculated

\[
G^2 = 2\left(\ell(\hat{\beta}, \tilde{a}, \tilde{b} | Y) - \ell(\hat{\beta}, a_0, \tilde{b} | Y)\right),
\]

(3.18)

The \( G^2 \) statistic was compared to the \( \chi^2 \) distribution (with 1 degree of freedom) for the (1-\( \alpha \)) percentile. Thus for 95% confidence intervals the critical \( G^2 \) value is 3.84. The log-likelihood surface was calculated with respect to kernel parameters of the optimal model for exploration and visualisation of the parameter space for both the Brazilian and Malaysian analyses.

### 3.2.7 First differences and conditional first differences

First differences provide a convenient and intuitive representation of the output from a GLM. An explanatory variable of interest is selected. The GLM predicted response is assessed with the explanatory variable of interest at its minimum and maximum, whilst all other explanatory variables are held at their mean. The first difference quantifies the difference between the two response predictions. This process can be repeated for all explanatory variables.

In the presence of interaction terms first difference calculations may be misleading. In this instance a conditional first difference followed the methodology above except that the relevant interaction terms are also analysed at their minimum and maximum, not held at their mean as was the case previously. For example, for a model with two covariates and an interaction term the corrected conditional standard errors are calculated as
\[ SE_{\beta_1 + \beta_2 X_1 X_2} = \left( \text{var} \left[ \beta_1 \right] + X_1^2 \text{var} \left[ \beta_2 \right] + 2X_1 \text{cov} \left[ \beta_1, \beta_2 \right] \right)^{\frac{1}{2}}, \]  

(3.19)

where \( X_1 \) and \( X_2 \) are our two covariates of interest and \( \beta_1 \) and \( \beta_2 \) are the coefficient estimates for \( X_1 \) and the interaction respectively [212].

CIs for first differences were calculated by parametric bootstrapped resampling (100,000 resamples) of the GLM coefficient estimates, \( \beta_s \), assuming that they were normally distributed with standard deviation equal to the estimated standard errors.

### 3.3 Results

#### 3.3.1 Primary Analysis - Brazil

Recaptures for the three MRR experiments are summarised in Table 3. The locations of recaptures are shown in Figure 18.

Table 3. Summary data of the three MRR experiments.

<table>
<thead>
<tr>
<th>Release</th>
<th>Release date</th>
<th>Number released</th>
<th>Release point</th>
<th>Number (%) recaptured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>21-Feb-2011</td>
<td>5,349</td>
<td>1</td>
<td>22 (0.4)</td>
</tr>
<tr>
<td>Yellow</td>
<td>25-Feb-2011</td>
<td>8,350</td>
<td>1</td>
<td>17 (0.2)</td>
</tr>
<tr>
<td>Blue</td>
<td>21-Feb-2011</td>
<td>5,465</td>
<td>2</td>
<td>30 (0.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>-</strong></td>
<td><strong>19,164</strong></td>
<td><strong>-</strong></td>
<td><strong>69 (0.36)</strong></td>
</tr>
</tbody>
</table>

The mean count of recaptured marked males (the response), per sample, per day was 0.077 (variance = 0.73). Over the recapture period the maximum daily temperature ranged between 25.4°C-34.6°C and the maximum relative humidity between 66%-92%.
Figure 18. Recaptures for three MRR experiments at the field site in Brazil. Numbered circles represent the two release points for MRR experiments. Coloured circles indicate the location and size of recaptures for three separate MRR releases (insects marked with red, yellow and blue fluorescent powder).

A summary of model performance using the untransformed- and transformed-distance explanatory variable is shown in Table 4. Combining all available data (from the red, yellow and blue releases) the best fitting model (lowest AIC) incorporated the exponential power kernel. The maximum likelihood exponential power density pdf has an associated MDT of 52.8m (95% CI: 49.9m, 56.8m), FR$_{50}$ of 52.4m (95% CI: 50.6m, 54.7m) and FR$_{90}$ of 83.0m (95% CI: 74.8m, 93.9m).

Table 4. Summary of model performance. The estimated model performance (minimum AIC indicated in bold) and kernel parameters for different transformations of the distance explanatory variable for the Brazilian analysis.

<table>
<thead>
<tr>
<th>Distance transformation</th>
<th>AIC</th>
<th>Explained variance (%)</th>
<th>Number of covariates</th>
<th>Kernel parameter estimates (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untransformed</td>
<td>293.5</td>
<td>46.7</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Negative exponential</td>
<td>286.5</td>
<td>48.6</td>
<td>6</td>
<td>97.8 (57.8, 238.3)</td>
</tr>
<tr>
<td>Exponential power</td>
<td>276.2</td>
<td>51.4</td>
<td>5</td>
<td>75.3 (66.1, 85.0)</td>
</tr>
</tbody>
</table>

For all three models the distance or distance density and the number of days post release were strongly associated with recapture number. There was no evidence of an interaction between distance (untransformed or transformed) and the number of days post release in any of the models considered. Other significant explanatory variables were the number of non-Aedes mosquitoes recorded from the sample and the maximum humidity. There was evidence of a lack of radial
symmetry in dispersal from the release point as the quadrant explanatory variable was also associated with recapture number.

A summary of the parameter estimates from the optimal model, using the exponential power transformation of distance as an explanatory variable is shown in Table 5.

Table 5. Brazilian model summary. GLM coefficient estimates and associated standard errors, z-value and p-values from the optimal model for the Brazilian analysis. Distance was transformed using the exponential power kernel.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimate</th>
<th>Standard error</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.35</td>
<td>1.18</td>
<td>-1.16</td>
<td>0.25</td>
</tr>
<tr>
<td>Transformed distance</td>
<td>83.140</td>
<td>7.015</td>
<td>11.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of days post release</td>
<td>-0.62</td>
<td>0.11</td>
<td>-5.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wild other spp</td>
<td>0.021</td>
<td>0.0079</td>
<td>2.62</td>
<td>0.0088</td>
</tr>
<tr>
<td>Maximum humidity</td>
<td>-0.035</td>
<td>0.016</td>
<td>-2.22</td>
<td>0.027</td>
</tr>
<tr>
<td>Quadrant*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>South</td>
<td>-1.88</td>
<td>0.80</td>
<td>-2.36</td>
<td>0.018</td>
</tr>
<tr>
<td>East</td>
<td>0.76</td>
<td>0.37</td>
<td>2.07</td>
<td>0.039</td>
</tr>
<tr>
<td>West</td>
<td>1.40</td>
<td>0.35</td>
<td>3.97</td>
<td>0.00071</td>
</tr>
</tbody>
</table>

*Overall significance level p<0.0001 (χ²=46.50, 3df).

First difference evaluations of significant explanatory variables in this optimal model are shown below (Figure 19). The plot indicates the change in predicted recapture count when evaluating an explanatory variable set at its minimum and maximum observed value, whilst all other explanatory variable are held at their mean.
Figure 19. First difference plot from the optimal Brazilian GLM. Ticks indicate best estimate. Thick and thin bars indicate 75% and 95% bootstrap CIs respectively. CIs for first differences were calculated by parametric bootstrap resampling (100,000 resamples) of the GLM coefficient estimates, $\beta$s, assuming that they were normally distributed with standard deviation equal to the estimated standard errors. The plot indicates the change in predicted recapture count per sample when evaluating an explanatory variable at its minimum and maximum, whilst all other explanatory variables are held at their mean. For example increasing the (transformed distance) from its minimum (furthest from release point) to its maximum (nearest to release point) increases the predicted marked male recaptures by 0.6 per sample. The magnitude of differences is low due to the low recapture numbers.

The maximum-likelihood estimate (MLE) kernel, log-likelihood surface and examples of kernels drawn from 95% CI parameter values are shown in Figure 20.
3.3.2 Secondary analysis - Malaysia
The MRR performed in Malaysia saw consistently higher recaptures than the MRR experiments in Brazil. Of 6,045 release transgenic males 3,034 (50.2%) were recaptured over the 15-day course of the experiment. The count of recaptured marked males (the response) was very overdispersed (mean=2.6 per sample per day, variance=523), and therefore a negative binomial GLM was fitted. A summary of the model performance using the untransformed and transformed distance explanatory variable is shown in Table 6.
Table 6. Summary of model performance. The estimated model performance (minimum AIC indicated in bold) and kernel parameters for different transformations of the distance explanatory variable for the Malaysian analysis.

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>Explained variance (%)</th>
<th>Number of covariates</th>
<th>Kernel parameter estimates (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untransformed</td>
<td>728.7</td>
<td>7.9</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Negative exponential</td>
<td>668.4</td>
<td>54.1</td>
<td>3</td>
<td>31.3 (27.7, 34.9)</td>
</tr>
<tr>
<td>Exponential power</td>
<td>668.0</td>
<td>46.8</td>
<td>3</td>
<td>48.1 (45.3, 52.1) 1.4 (1.3, 1.5)</td>
</tr>
</tbody>
</table>

*Including the interaction term

Again, the optimal model, as determined by AIC, used the exponential power density pdf, although the negative exponential density pdf produced only marginally inferior fit. The MLE exponential power pdf estimates a MDT for the transgenic release of 58.0m (95% CI: 51.1m, 71.0m), FR$_{50}$ of 51.8m (95% CI: 47.9m, 58.7m) and FR$_{90}$ of 105.7m (95% CI: 86.5m, 141.1m). The results from the negative binomial model using the exponential power transformed distance explanatory variable are shown in Table 7 and visualised in a first difference plot (Figure 21). This second analysis again implicates distance as an important significant predictor of the expected count of recaptures. The number of days post release was also significantly associated with recapture number. Unlike the analysis for Brazil there was evidence of an association between the distance and the number of days post release explanatory variables.

Table 7. Malaysian model summary. GLM coefficient estimates and associated standard errors, t-values and p-values from the optimal model for the Malaysian analysis. Distance was transformed using the exponential power kernel.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimate</th>
<th>Standard error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.013</td>
<td>0.79</td>
<td>-0.016</td>
<td>0.98</td>
</tr>
<tr>
<td>Transformed distance</td>
<td>96,300</td>
<td>17,500</td>
<td>5.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of days post release</td>
<td>-0.46</td>
<td>0.22</td>
<td>-2.10</td>
<td>0.036</td>
</tr>
<tr>
<td>Interaction ( Transformed distance × Days post release)</td>
<td>-24420</td>
<td>6467</td>
<td>-3.78</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Figure 21. Conditional first difference plot from the optimal Malaysian GLM. Ticks indicate best estimate. Thick and thin bars indicate 75% and 95% bootstrap CIs respectively. CIs for first differences were calculated by parametric bootstrap resampling (100,000 resamples) of the GLM coefficient estimates, $\beta$s, assuming that they were normally distributed with standard deviation equal to the estimated standard errors. The plot indicates the change in predicted recapture count per sample when evaluating an explanatory at its minimum and maximum. In this instance, as there was a positive significant interaction between distance and the number of days post release, conditional first differences are shown. The parameter not being examined is not set at its mean but at its minimum and maximum respectively. For example increasing the (transformed distance) from its minimum (farther from release point) to its maximum (nearest to release point), whilst number of days post release=1, increases the predicted marked male recaptures by approximately 500. The lower 95% limit for number of days post release (Transformed distance = max) = -11,924.

The MLE kernel, log-likelihood surface and examples of kernels drawn from 95% CI parameter values are shown in Figure 22.
Figure 22. Dispersal kernel summary for the Malaysian analysis. A) Maximum likelihood estimate density with respect to distance for Malaysian data. B) Maximum likelihood distance pdf. C) The log-likelihood surface with respect to kernel parameters $a$ and $b$, coloured points highlight the MLE (black, log-likelihood $= -328$) and examples of extreme 95% CI (green, light blue, dark blue and mauve) kernel parameter combinations. The dotted line demarks the 95% CI contour. Solid black contour lines are at intervals of 10 log-likelihood. Examples of D) distance densities and E) distance pdf from the 95% CI range corresponding to the coloured points shown in panel C.
For a direct comparison the distance pdf and density with respect to distance for the optimal kernels estimated from the Brazilian and Malaysian MRR experiments have been overlaid (Figure 23).

Figure 23. Dispersal kernel comparison. A comparison of the A) distance pdf and B) density with respect to distance for estimates using Brazilian (solid blue line) and Malaysian (dashed pink line) MRR data. The comparison highlights the similarity in estimated kernels for experiments conducted on different continents, in different habitats.

3.4 Discussion

An in-depth analysis of the dispersal ability of released transgenic male Ae. aegypti mosquitoes in the field has been conducted. The primary analysis, of MRR data from Brazil, indicates distance from the release point to be an important predictor of the expected number of recaptures. The relationship between the recapture number and the distance from the release point is highly non-linear. The regression model performed optimally when an exponential power dispersal kernel was used to transform distances. The analysis methodology was also used to re-analyse MRR from Malaysia, where again an exponential power kernel provided best model fit.

The optimal Brazil GLM performed well, explaining around half of the variation observed in the data. Transformed distance and the number of days post release were the most influential, highly significant predictors of recapture number. The decline in numbers temporally after release is considered to be predominantly due to the effect of mortality. Emigration from the study area may further reduce numbers.

Distance was highly significantly correlated with recapture number in all models considered. The exponential power dispersal kernel provided the optimum model fit. This kernel parametric form is slightly more flexible than the negative exponential. The kernel produced showed a high and consistent level of dispersal from 0-33m from the release site. After this point the density falls fairly steeply, reaching very low levels shortly after 100m (FR90 = 83.0m), indicating that coverage decreases quickly at increasing distances more than 33m from a release point. The MDT estimated using the best-fit kernel parameters was 52.8m (95% CI: 49.9m, 56.8m). This is consistent with a
number of published field studies of male *Ae. aegypti* dispersal which estimate mean distance travelled ranging from 10m to 100m (Table 8). It is however, worth noting that for skewed distributions of dispersal distances the MDT as a measure of central tendency should be interpreted with some caution.

Table 8. Summary of a literature review of male *Ae. aegypti* dispersal estimates.

<table>
<thead>
<tr>
<th>MDT/MDT range (m)</th>
<th>Location</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-30</td>
<td>Hainan Island, China</td>
<td>Released in the centre of a village</td>
<td>[149]</td>
</tr>
<tr>
<td>15-39</td>
<td>Sonepat, India</td>
<td>-</td>
<td>[135]</td>
</tr>
<tr>
<td>32</td>
<td>Ilha do Governador, Brazil</td>
<td>Raised on poor diet</td>
<td>[114]</td>
</tr>
<tr>
<td>35</td>
<td>Pentland, Australia</td>
<td>-</td>
<td>[137]</td>
</tr>
<tr>
<td>35-60</td>
<td>Hainan Island, China</td>
<td>Released at the edge of a village</td>
<td>[149]</td>
</tr>
<tr>
<td>42</td>
<td>Ilha do Governador, Brazil</td>
<td>Raised on rich diet</td>
<td>[114]</td>
</tr>
<tr>
<td>52</td>
<td>Jalan Tentera, Malaysia</td>
<td>Transgenic</td>
<td>[92]</td>
</tr>
<tr>
<td>100</td>
<td>Jalan Tentera, Malaysia</td>
<td>Laboratory strain</td>
<td>[92]</td>
</tr>
</tbody>
</table>

The number of non-*Aedes* mosquitoes was significantly positively correlated with the number of recaptured transgenic mosquitoes. This explanatory variable is thought to be a proxy for the house-attractiveness or accessibility of a house to mosquitoes; large numbers of other mosquito *spp* may indicate the household is situated in a favourable location or particularly susceptible to mosquitoes. Clustering of *Aedes* mosquitoes at the household level is a commonly observed phenomenon [203–205]. An alternative explanation could also lie in differences in the abilities of operators to sample mosquitoes.

The humidity and directional quadrants were significantly associated with the number of recaptured transgenic mosquitoes but had relatively small effect sizes. It would be expected that any directional differences are attributable to site- and time-specific heterogeneities in terrain, habitat type, wind direction or other external factors [213, 214]. Humidity saw a small significant positive relationship with recapture number, which could be due to an increased tendency for *Ae. aegypti* to seek shelter with increasing humidity [215]. These explanatory variables must be interpreted with some caution as there is the potential for selection by AIC to overfit models [216, 217]. The covariates may therefore be included in the final models despite their relatively small influence on model fit.

One limitation of this study was the low number of recaptures in the Brazil dataset. For this reason there was little power to analyse individual releases separately, necessitating the analysis of the combined datasets and the assumption that influences not accounted for would be similar for both release points. The proportion of individuals recaptured may have been improved by a more targeted, or higher intensity sampling effort or increased survival of the released individuals. Alternatively, the absolute number of recaptures could have been increased with larger release
numbers. The dataset could also have been further improved with household-specific monitoring of climatic factors to give greater resolution to observations on the relationship between weather variables and recapture numbers. The standard errors of the GLM coefficients (Table 5, Table 7) were estimated conditional upon the MLEs for kernel parameters $a$ and $b$ whilst the confidence intervals for the kernel parameters were obtained conditional upon the MLE for the GLM coefficients. Thus the reported standard errors are likely to be smaller than if we had been able to compute the unconditional standard errors and confidence intervals for all of the parameters. Analysis of the residuals indicated little residual spatial autocorrelation with perhaps the exception of some under-estimation of recapture numbers at further distances (>150 m), potentially indicating the influence of long-distance dispersers [218], although, due to the small number of recaptures, this is difficult to verify.

The optimal GLM associated with the Malaysian data also explained approximately 50% of the variation observed in the recapture data. For the optimal model only two covariates, number of days post release and distance, plus their interaction term were included. The optimal model again used the exponential power dispersal kernel, with a corresponding MDT estimate of 58m (95% CI: 51.1m, 71.0m) that was in good agreement with (and within the 95% CI of) the MDT estimate (52.4m) from the previously published analysis of these data [92]. The FR$_{50}$ estimate of 51.8m was substantially different from the previously published estimates of 16.2m, a product of the different underlying models for dispersal with respect to distance used in each analysis. This deviation further highlights the need for metrics above and beyond MDT to accurately characterise dispersal. The number of days post release covariate, as expected, was significantly negatively associated with recapture number. The coefficient indicated a smaller effect size than seen in the Brazilian MRR data, implying improved survival, less emigration or a combination of the two for individuals in the Malaysian releases. The Malaysian MRR experiment saw a very high recapture rate, approximately 50% of all individuals released were recaptured. This may bias the results and could violate the underlying assumption that the negative binomial distribution approximates proportions when recapture numbers are small relative to the release size.

The lack of significant interaction between the number of days post release and distance in the Brazil data provides evidence for a single main dispersal event on release (the probability of travelling a given distance is not influenced by the number of days post-release). The significant interaction term indicates a more continual dispersal process over time in this Malaysian context, possibly due to the lack of favourable habitat across the whole range of the study site at this location. However the influence of the interaction term on predicted recaptures is very small; the majority of released
individuals have died (or emigrated) before the interaction term becomes influential. For both locations the majority of recaptures are predicted spatially and temporally close to the release location and date respectively. There is published evidence to support either the occurrence of a single dispersal ‘event’ [124, 219, 220] or a more continuous dispersal process upon release [135, 137, 149].

For experiments carried out in different habitats, on different continents, the estimated dispersal kernels were very similar (Figure 23). The Brazilian dispersal kernel is slightly fatter tailed (larger $b$ parameter) but in general there is evidence for a degree of consistency in the dispersal ability of transgenic male *Ae. aegypti* across a range of environments. Consistent dispersal may facilitate more generalised release procedures for sterile insect releases across a range of release locations and scenarios.

### 3.5 Conclusion

Accurately measuring and assessing the dispersal of released transgenic male *Ae. aegypti* in the field is a vital component necessary to optimise vector control using these ‘genetically sterile’ individuals. A successful control program using transgenic male *Ae. aegypti* would maximise transgenic insect density over the target area. Knowledge of the released insects’ ability to disperse is vital in predicting their density with respect to specific release points or routes. An ability to predict the coverage of dispersed individuals will facilitate the design and implementation of more efficient control and monitoring programs in the future. I will broach a number of these aspects of release in following chapters.

### 3.6 Acknowledgements

Many thanks to Oxitec for providing data and facilitating the analyses in this chapter. Thanks to Projeto Aedes Transgênico and Moscamed Brasil for their collaboration and support. I also thank the participants of the field studies in Itaberaba, Brazil.
4 Estimating field dispersal of female *Aedes aegypti* using data from large-scale releases of transgenic mosquitoes

In this chapter I continue to develop work relating to the spatial aspects of transgenic *Ae. aegypti* releases. I extend the study of *Aedes* dispersal in the field to wild females, specifically those that have mated released transgenic males. This chapter incorporates methodology set out in the previous chapter and presents a novel approach to estimating female dispersal, further improving our knowledge of the spatial dynamics associated with releases of transgenic mosquitoes.

4.1 Introduction

Estimating the dispersal of mosquitoes in the field in an unbiased manner is non-trivial. Obtaining objective estimates for female mosquitoes that have mated in the wild is harder still. Previous efforts to estimate mosquito dispersal have relied heavily on MRR techniques [92, 124, 135–138, 145, 146, 148–151, 167, 204, 221] which involve a number of assumptions [41] that, if violated, may bias the results. Released individuals must be identifiable on recapture and it is assumed that physical marking of the released individuals does not affect their dispersal [121], supporting arguments in favour of the use of genetic or radioactive markers [148, 220, 222, 223]. However, released individuals are often lab-reared and may present divergent physiological or behavioural responses in comparison to wild individuals resulting in different dispersal patterns and results that are not generalisable. In all instances the very fact that individuals must be released into the environment may significantly alter their natural behaviour patterns. Methods based on monitoring the dispersal of emerging individuals from known oviposition sites [202, 224, 225] can overcome these limitations of MRR. However, these studies rely on the presence and knowledge of isolated source areas from which dispersing individuals are known to emerge.

Many dispersal studies produce single summary estimates of dispersal, for example the mean MDT [114, 137, 146, 149, 167, 204, 226] or the observed range [151, 223]. These estimates must be interpreted with caution, especially when the observed distribution of dispersal distances is highly skewed. In this instance, the mean distance poorly represents dispersal, being prone to influence from extreme values which may be observed if the true distribution has a long tail. The range may better highlight these extreme values but gives no information of the general shape of the distribution of dispersal distances.

Dispersal kernels, which represent the statistical distribution of dispersal distances, have long been used in ecological studies of dispersal [198]. Popularised through studies of seed dispersal (for examples see [210, 227, 228]), dispersal kernel theory has now been used to assess the dispersal of a
wide range of species [201, 229–233], including the mosquito Culex erraticus [202]. Dispersal kernels provide an estimate of the probability of dispersal for the full range of potential dispersal distances. Thus, inference regarding characteristics such as the shape of the tail or the skew of the distribution as well as the mean and median dispersal distance can be made.

In this study I have adopted approaches, based on dispersal kernel theory, to estimate the dispersal kernel of transgenic-mated female Ae. aegypti in the wild. A heritable fluorescent marker [86] allows the identification of the offspring resultant from a mating between a released transgenic male and a wild female and provides an end-point for female dispersal, which is known to be driven by a search for suitable oviposition sites [221]. Knowledge of this end-point combined with the expected distribution of released transgenic males provides a novel opportunity by which the dispersal of transgenic-mated female mosquitoes in the wild may be studied without artificial rearing, dye-marking or handling of these females.

Changes in the habitat and boundaries where these changes occur may be expected to have a significant influence on the dispersal of Ae. aegypti which have been shown to preferentially restrict dispersal to urban and peri-urban areas [124, 147, 148, 170, 204, 234]. For this reason we can hypothesise that analyses of dispersal adjusting for these changes in habitat may have more predictive power than those assuming that habitat does not influence dispersal.

The spatial distribution of the transgene, as indicated by the presence of transgenic larvae in the field, is a combination of the dispersal of both released transgenic males and the wild females that they mate. Knowledge of the expected distribution of the transgene is valuable, informing trial design and allowing the range of monitoring to be demarcated as well as the monitoring effort to be concentrated at the appropriate scale. Combining inference of the transgenic-mated-female dispersal kernel with previous estimates of released male-dispersal allows the estimation of their combined kernel as well.

In this study I aim to characterise the dispersal of transgenic-mated female Ae. aegypti mosquitoes in the field. I use a large-scale field-data set, GLMs and stochastic simulation techniques to produce novel estimates of the dispersal kernel of transgenic-mated females in the wild. I have combined estimates of transgenic male and transgenic-mated female dispersal to make inference on the expected range and distribution of distances that a transgene may be observed from a release site.
4.2 Methods

4.2.1 Data
The field site is located in Itaberaba, a suburb of the city of Juazeiro, Bahia, Brazil (Latitude: -9° 26' 59'', Longitude: -40° 28' 53'') (Figure 24). The site is located in a semi-arid part of Brazil and consists mainly of low-socioeconomic status residential housing. The location was chosen due to the high year-round abundance of *Ae. aegypti* in the area, despite some seasonality in rainfall (with the dry season running from June to October). This analysis considered 173 releases conducted between 07 May 2011 and 16 July 2012 in which a total of approximately 16,395,232 OX513A male *Ae. aegypti* were released. Releases consisted of transgenic male *Ae. aegypti* mosquitoes being distributed from the back of a moving vehicle driving one of two release routes, illustrated in Figure 24.

A total of 263 unique ovitraps locations were used over the course of the study (Figure 24). Ovitraps consisted of a small, black plastic container partially filled with water in which sits a wooden paddle which acts as a site for oviposition [161]. Traps were located inside and outside residential buildings across the study site. Collections occurred weekly, although not all ovitraps were sampled each week (mean number collected = 112 per week). The count of transgenic larvae per trap, per collection is used as the response variable in the model, detailed below.

For count data a Poisson log-linear GLM may be used. In the instance of an over-dispersed response variable (variance >> mean) a negative binomial log-linear GLM [206–208, 235], further detailed below, is more appropriate. In this instance we assume that the count response variable follows a negative binomial distribution. A Poisson model is used for the count, conditional on the mean value, $Z_i$, where $Z_i$ is assumed to have a gamma distribution, with mean, $\mu_i$, and constant scale parameter, $\theta$

$$Y_i \sim \text{Poisson}(Z_i), \quad Z_i \sim \text{gamma}(\mu_i, \theta),$$

for $i = 1, ..., n$. Therefore the expected value of $Y$ and the variance of $Y$ are as follows

$$E(Y_i) = \mu_i, \quad \text{Var}(Y_i) = \mu_i + \frac{\mu_i^2}{\theta}.$$  (4.2)

The mean response, $\mu_i$, is linked to a linear combination of explanatory variables using the log link function.

$$\ln(\mu_i) = x_i \beta,$$  (4.3)
where $x_i$ is a linear predictor

$$x_i \beta = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{ip},$$

(4.4)

where $\beta$ denotes the unknown parameters to be estimated and $x_i$ denotes the explanatory variables. Parameters were estimated by maximising the log-likelihood, $\ell$, of the model:

$$\ell(\beta, \theta | Y) = \sum_{i=1}^{\infty} \theta (\ln(\theta) - \ln(\theta + \mu_i)) + \ln(\Gamma(\theta + y_i)) - y_i \ln(\mu_i) - \ln(\theta + \mu_i),$$

(4.5)

where the gamma function, $\Gamma$, is

$$\Gamma(n) = (n - 1)!.$$  

(4.6)

Models were implemented using the statistical software R [188] and the MASS [207] package.
Figure 24. An overview of the field site. The field site, release routes (blue and pink lines), ovitraps locations (white circles) and habitat boundary (dashed red line). Areas to the east of the boundary were scrubland, to the north-west and south, industrial and to the west barren fields.

4.2.2 The explanatory variables
Each GLM has three explanatory variables \((x_{i1}, x_{i2}, x_{i3})\) described below.

1. **Wild-type larvae** \((x_{i1})\). The number of wild-type larvae per trap, per collection. Wild-type larvae were distinguished from transgenic larvae by an absence of fluorescence.
II. Trap position ($x_{i2}$). Traps could be located indoors or outdoors. A binary variable for each trap was included to indicate the trap position.

III. Spatial ($x_{i3}$). One of a choice from a set of spatially-explicit, distance-related explanatory variables. Members of this set, distinguished by the varied methods for calculation and differing underlying assumptions, all serve to relate the response variable to the spatial distribution of released transgenic males. A general overview of the calculation for members of this set is outlined below.

Consider a count response variable, $Y_i$, that equals the number of fluorescent larvae in trap $i$ at time $t$. The associated explanatory variable, $x_i$, may be

1. Equal to the sum of distances, $D$, between the location, $XY$, of trap $i$ and the locations of all $j$ transgenic male mosquitoes alive on the day the trap was set out, $M_{j,t-7}$

$$x_i = \sum_j D(XY_i, XY_{M_{j,t-7}}). \quad (4.7)$$

2. Equal to the sum of the distance density, $D^*$, as determined by a specific density pdf, between the location, $XY$, of trap $i$ and the locations of all $j$ transgenic male mosquitoes alive on the day the trap was set out, $M_{j,t-7}$

$$x_i = \sum_j D^*(XY_i, XY_{M_{j,t-7}}). \quad (4.8)$$

3. Equal to the count of all sterile male mosquitoes alive on the day the trap was set out, within a given radius of the trap.

Dispersal simulations are used to obtain estimates of the number and location of sterile male mosquitoes upon release. For each released male the dispersal location coordinates are determined by sampling a grid square from a lattice with a probability determined by the dispersal kernel. Probabilities may be radially symmetric or adjusted for habitat effects. Starting coordinates for released individuals, assuming a constant release rate, are assigned by distributing male mosquitoes uniformly along one of two release routes driven during the field trial. The released male mortality rate was assumed to be 0.6 day$^{-1}$ (equivalent of a DSP = 0.55), informed from previous studies (Chapter 3).

We may impose the assumption that the boundary between the urban and other (scrubland/major roads/industrial) habitats, henceforth referred to as the habitat-boundary, affects the dispersal of
released mosquitoes. In this instance, the dispersal kernel density estimates must be altered to correct for these effects. The first stage of this process begins with the creation of, for a given origin with coordinates (XY), a 1500m x 1500m lattice (resolution = 30m) with (XY) as the centre (Figure 25A). Densities are assigned to grid squares based on the distance from the origin and a given kernel transformation (Figure 25B). Initially the kernel is considered to be radially symmetric. In the second stage adjustments to predicted densities, to correct for habitat heterogeneities (Figure 25C), may be made via one of three approaches, detailed below:

**Normalised**
Each grid square on the lattice outside of the urban area is assigned a 0 density. The kernel is then normalised, assuring that the probability of dispersal to locations within the habitat-boundary sums to 1 (Figure 25D). This kernel has no biological mechanistic explanation but was included as a computationally simple, baseline approach to kernel-adjustment.

**Reflection**
An alternative assumption of mosquito behaviour when reaching the habitat-boundary is to ‘reflect’ the individuals dispersal back into the urban area (Figure 25E). This is performed using the following process:

1. Identify those grid squares that lie on the habitat-boundary. Specifically, these are grid squares which lie within the habitat-boundary but that are adjacent (diagonally, horizontally or vertically) to grid squares outside of the habitat-boundary.
2. For each grid square outside of the habitat-boundary:
   a. Calculate the centroid-to-centroid distance, $d$, to the closest boundary grid square lying on a vector between the grid square and the origin.
   b. Identify grid squares within the urban area at $d$ distance from the boundary point.
   c. Distribute the density equally across these points.
3. Assign all grid squares falling outside of the urban area a density = 0.
4. Normalise the densities to correct for approximation on a lattice (to ensure that densities sum to 1).

**Boundary retention**
Mosquitoes that, on dispersal, reach the habitat-boundary may be assumed to halt their dispersal at this point. Under this assumption the density associated with points outside the urban area is reassigned to those points within the urban area, along the habitat-boundary (Figure 25F). This is performed using the following process:
1. Identify those grid squares that lie on the habitat-boundary (as for reflected dispersal).
2. For each grid square outside of the habitat-boundary add the density to the closest boundary point that lies on a vector between the grid square and the origin.
3. Assign all grid squares falling outside of the urban area a density = 0.
4. Normalise the densities to correct for approximation on a lattice (to ensure that densities sum to 1).

Figure 25. Transforming the dispersal kernel in the presence of habitat heterogeneities. A) The lattice with central origin marked. B) Densities can be assigned to grid squares within the grid dependent on the distance to the origin and a specific density kernel. For scenarios where boundary effects are assumed to occur: C) gives an example where the area outside of the habitat-boundary area is highlighted in grey. Grid squares within the habitat-boundary are assigned zero densities. The remaining densities are transformed by D) normalising the kernel, E) reflecting dispersing mosquitoes back into the urban area, D) retaining dispersing mosquitoes at the habitat-boundary.

In the following section the six different members from the set of potential spatial explanatory variables are outlined in detail. The distinguishing assumptions and methodological differences associated with each member are highlighted. Numbers (1-6) assigned to members of the set of spatial explanatory variables are used to refer to them in the following sections.

1. **Distance, no boundary effects.**
   In the most simple configuration of the spatial explanatory variable, distance is left untransformed. I assume that the habitat-boundary has no effect on dispersal, analogous to assuming a homogenous landscape across the whole field site. Under this assumption the relationship between distance and the probability of travelling that distance is independent of locations factors (viz. the start and finishing points).
2. **Threshold distance, no boundary effects**
   In this instance the spatial explanatory variable consists of the sum of the number of transgenic males within a given range of each trap. As for 1, I assume no boundary effects.

3. **Distance density, no boundary effects**
   A density pdf, with given parameters, is applied to all distances before the spatial explanatory variable is calculated, using the methodology detailed above. Once again, as for 1 and 2, I assume that there are no boundary effects. This assumption again renders the relationship between distance and probability independent of spatial location. Under this assumption dispersal from any starting point is generalised by a single dispersal kernel. The kernel is radially symmetric around the origin, exhibiting identical properties in all directions.

4. **Distance density, boundary effects, normalised**
   Member four of the set of spatial explanatory variables is the first to allow for a boundary effect. Specifically, I assume that dispersing mosquitoes will not disperse past the habitat-boundary. Under this assumption dispersal kernels are location dependent and radially asymmetric. In this instance each kernel is adjusted by removing any density from non-urban habitat and normalising the resulting kernel (Figure 25D).

5. **Distance density, boundary effects, reflection**
   As for 4, a boundary effect is assumed. In this situation densities outside of the urban habitat are reflected back into the urban habitat (Figure 25E).

6. **Distance density, boundary effects, boundary retention**
   The final approach for creating a spatial explanatory variable again assumes a boundary effect. In this instance the density that would be outside the urban area is accumulated on the habitat-boundary (Figure 25F).

The density pdf used for these analyses is the exponential power kernel [227]

\[
\text{exponential power kernel} = \frac{b}{2\pi a^2 \Gamma \left( \frac{2}{b} \right)} \exp \left( \frac{-a^d}{2} \right) a, b > 0,
\]  

(4.9)

where, \(a\) and \(b\) are kernel parameters, \(d\) is the distance (m) and \(\Gamma\) the gamma function (equation (3.10)).

When estimating models using members 2-6 from the set of spatial explanatory variables, model estimation must be repeated to allow the kernel parameters or threshold distance to be estimated.
alongside GLM parameters. As detailed in Figure 26 an optimisation procedure was used to maximise the log-likelihood of the GLM with respect to the kernel parameters or threshold distance.

The simulation of dispersal of transgenic male *Ae. aegypti* mosquitoes is a stochastic process. The resulting distance-based explanatory variables used in the GLM are reliant on this stochastic process and are therefore themselves inherently variable [236]. To account for this stochasticity in the resulting parameter estimation the output from multiple (n=100) simulated runs have been summarised. A summary of the model estimation and repeated simulation procedure is shown in Figure 26.

**Figure 26. Model process overview.** An overview of the model estimation and repeated simulation procedure used to obtain summary parameter estimates.

### 4.2.3 Likelihood-based confidence intervals

Following model estimation, 95% confidence intervals were calculated for the maximum likelihood kernel parameter estimates using the profile likelihood method. The maximised log-likelihood with respect to $\beta$, $a$, $b$ and $\theta$, i.e. that corresponding to the maximum likelihood estimates (MLEs) of $\beta$, $a$, $b$ and $\theta$ is defined as $\ell(\hat{\beta}, \hat{a}, \hat{b}, \hat{\theta} | Y)$.

First kernel parameter $a$ was increased or decreased in small increments whilst $\beta$ was held at the MLE ($\hat{\beta}$) and kernel parameter $b$ was optimized conditional on $\hat{\beta}$, $\hat{\theta}$ and the assumed value of $a$ ($a_0$), giving the log-likelihood:

$$\ell(\hat{\beta}, a_0, \hat{b}, \hat{\theta} | Y),$$  \hspace{1cm} (4.10)

where $\hat{b} = MLE(b | a_0, \hat{\beta}, \hat{\theta})$.

Secondly, kernel parameter $b$ was increased or decreased in small increments whilst $\beta$ was held at the MLE ($\hat{\beta}$) and kernel parameter $a$ was optimized conditional on $\hat{\beta}$, $\hat{\theta}$ and the assumed value of $b$ ($b_0$), giving the log-likelihood:
where, $\tilde{a} = MLE(a | \hat{b}, \hat{\theta})$.

After each change the log-likelihood of the model was recalculated and a corresponding test statistic assessed. For example, evaluating for $a$, the $G^2$ statistic was calculated

$$G^2 = 2 \left( \ell(\hat{\beta}, \tilde{a}, \tilde{b}, \hat{\theta} | Y) - \ell(\hat{\beta}, a_0, \tilde{b}, \hat{\theta} | Y) \right).$$

(4.12)

The $G^2$ statistic was compared to the $\chi^2$ distribution (with 1 degree of freedom) for the $(1 - \alpha)$ percentile. Thus for 95% confidence intervals the critical $G^2$ value is 3.84.

Comparisons of GLMs using different spatial explanatory variables were performed using the AIC, a penalised likelihood criterion. The AIC is calculated as

$$AIC = 2k - 2 \ln(L),$$

(4.13)

where $k$ is the number of parameters and $L$ the maximum likelihood value.

4.2.4 Convolution

To estimate the kernel associated with the transgene we combine estimates of released male dispersal with estimates of mated female dispersal. Statistically, this can be performed by the convolution of the released-male and mated-female dispersal kernels.

Given the exponential power density kernel, $f(d, a, b)$ parameterised for dispersal of transgenic males, $f(d, a_M, b_M)$, and females, $f(d, a_F, b_F)$, a distance pdf, $g(d)$, to describe the combined dispersal can be calculated using the process of convolution [198, 237]. The probability of the combined dispersal being a distance of $d$ metres from the male release points is as follows:

$$g(d) = 2\pi d \int_{-\infty}^{\infty} f(d(initial_M, final_M), a_M, b_M) f(d(final_M, final_F), a_F, b_F) \, dd, \quad (4.14)$$

where $d(initial_M, final_M)$ denotes the distance between the males’ release and final location and $d(final_M, final_F)$ the distance between the males’ final location and the females’ final location.

4.3 Results

Between 07 May 2011 and 13 June 2011 releases were small (mean = 10,256 individuals per release). After this period releases were scaled up (mean = 104,584 individuals per release). Due to
the very large size of the transgenic male releases, simulated male numbers per release were reduced by a factor of ten. This was found to provide considerable improvements to the computational time without subsequent degradation to estimated GLM coefficient parameters. The count of fluorescent larvae per trap per collection (the response variable) was overdispersed (mean=1.3, variance=61) and therefore the negative binomial GLM was used for analyses. For each sampling period not all traps were used; the mean number of traps collected each week was 165.

Summaries of the median coefficient estimates from 100 simulations for the six GLMs with each spatial explanatory variable are presented in (Table 9). For all models the number of wild larvae in an ovitrap and the spatial measure were highly significantly associated with the number of transgenic larvae in an ovitrap (Figure 27).

Table 9. Model coefficient summaries. Median GLM coefficient estimates from 100 simulated runs for six models with different distance treatments (1-6). The estimates are presented for GLMs using six different spatial explanatory variables.

<table>
<thead>
<tr>
<th>Model</th>
<th>Explanatory variable</th>
<th>Median coefficient estimates (median 95% confidence limits)</th>
<th>Median p-value (95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Distance</td>
<td>Intercept</td>
<td>0.18 (-0.09, 0.46)</td>
<td>0.182 (0.180, 0.183)</td>
</tr>
<tr>
<td></td>
<td>log(wild + 1)</td>
<td>0.96 (0.83, 1.08)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Outdoors</td>
<td>-0.38 (-0.68, -0.08)</td>
<td>0.0140 (0.0138, 0.0141)</td>
</tr>
<tr>
<td></td>
<td>Spatial</td>
<td>-0.0085 (-0.0101, -0.0070)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td>2. Threshold</td>
<td>Intercept</td>
<td>-1.79 (-2.10, -1.48)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>log(wild + 1)</td>
<td>1.03 (0.89, 1.17)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Outdoors</td>
<td>-0.42 (-0.75, -0.08)</td>
<td>0.0157 (0.0152, 0.0162)</td>
</tr>
<tr>
<td></td>
<td>Spatial</td>
<td>0.0011 (0.0000, 0.0013)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td>3. No boundary</td>
<td>Intercept</td>
<td>-2.13 (-2.49, -1.77)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>log(wild + 1)</td>
<td>0.98 (0.83, 1.13)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Outdoors</td>
<td>-0.32 (-0.69, 0.04)</td>
<td>0.0847 (0.0828, 0.0865)</td>
</tr>
<tr>
<td></td>
<td>Spatial</td>
<td>1107904 (919360, 1296446)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td>4. Normalised</td>
<td>Intercept</td>
<td>-2.16 (-2.37, -1.94)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>log(wild + 1)</td>
<td>0.99 (0.90, 1.08)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Outdoors</td>
<td>-0.35 (-0.56, -0.13)</td>
<td>0.00168 (0.00164, 0.00173)</td>
</tr>
<tr>
<td></td>
<td>Spatial</td>
<td>858 (771, 944)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td>5. Reflected</td>
<td>Intercept</td>
<td>-2.14 (-2.36, -1.93)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>log(wild + 1)</td>
<td>0.99 (0.90, 1.08)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Outdoors</td>
<td>-0.34 (-0.56, -0.13)</td>
<td>0.00193 (0.00185, 0.00201)</td>
</tr>
<tr>
<td></td>
<td>Spatial</td>
<td>847 (762, 932)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td>6. Border retention</td>
<td>Intercept</td>
<td>-1.96 (-2.17, -1.75)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>log(wild + 1)</td>
<td>1.00 (0.91, 1.09)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Outdoors</td>
<td>-0.35 (-0.57, -0.13)</td>
<td>0.00158 (0.00135, 0.00181)</td>
</tr>
<tr>
<td></td>
<td>Spatial</td>
<td>672 (605, 740)</td>
<td>&lt;0.0001 (&lt;0.0001, &lt;0.0001)</td>
</tr>
</tbody>
</table>
Figure 27. The relationship between the number of transgenic larvae and the number of wild larvae per trap per collection. The number of transgenic larvae per trap increases with increasing numbers of wild larvae per trap except for at very high densities of wild larvae (>200 larvae per trap per collection) where the trend may be reversed.

Traps positioned indoors were associated with marginally (but significantly) higher number of transgenic larvae (Figure 28) in all models except for the model with spatial explanatory variable 3, which assumed no boundary effects. Coefficient estimates for non-spatial explanatory variables were robust to the range of spatial explanatory variables used, remaining consistent across all models.
Figure 28. The relationship between the number of transgenic larvae per trap per collection and the trap position.

Kernel estimates and the MDT associated with the spatial explanatory variables 3-6 are summarised in Table 10 and further explored in Figure 29, Figure 30, Figure 31. These figures explore the level of variation across all 100 simulated runs and illustrate the stability of the relationship between kernel parameters for all simulations. These plots demonstrate the robust nature of kernel estimates in relation to simulation stochasticity and lend support to using mean estimates as kernel-parameter summary measures.

An example likelihood surface for one run is shown in Figure 32.

Table 10. Model dispersal kernel summary. Median kernel parameter estimates from 100 simulated runs and the associated MDT. The best fit kernel is highlighted in bold.

<table>
<thead>
<tr>
<th>Model</th>
<th>Median kernel parameter estimate (median 95% profile CIs)</th>
<th>Mean distance travelled (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>3. No boundary</td>
<td>39.7 (35, 44.6)</td>
<td>0.74 (0.71, 0.78)</td>
</tr>
<tr>
<td>4. Normalised</td>
<td>186.5 (126.6, 238.2)</td>
<td>1.64 (1.25, 2.64)</td>
</tr>
<tr>
<td>5. Reflected</td>
<td><strong>84.2 (55.8, 115.2)</strong></td>
<td><strong>0.95 (0.82, 1.19)</strong></td>
</tr>
<tr>
<td>6. Boundary retention</td>
<td>90.0 (70.7,123.7)</td>
<td>168.44 (2.04,∞)</td>
</tr>
</tbody>
</table>
Figure 29. Summary of kernel parameter estimates for 100 runs of normalised model. A) Parameter $a$ MLE and CI. B) Width of CI for parameter $a$. C) Association between the MLE of $a$ and the CI width. D) Parameter $b$ MLE and CI. E) Width of CI for parameter $b$. F) Association between the MLE of $b$ and the CI width.
Figure 30. Summary of kernel parameter estimates for 100 runs of reflected model. A) Parameter $a$ MLE and CI. B) Width of CI for parameter $a$. C) Association between the MLE of $a$ and the CI width. D) Parameter $b$ MLE and CI. E) Width of CI for parameter $b$. F) Association between the MLE of $b$ and the CI width.
Figure 31. Summary of kernel parameter estimates for 100 runs of boundary retention model. A) Parameter $a$ MLE and CI. B) Width of CI for parameter $a$. C) Association between the MLE of $a$ and the CI width. D) Parameter $b$ MLE and CI, the upper limits of $b$ tend to infinity and therefore estimates of width are not shown.

Figure 32. Example likelihood surface for kernel parameters for the best-fit reflected model. The log-likelihood (colour) for combinations of kernel parameter estimates ($a$ and $b$). The black dot is the maximum likelihood estimate (MLE). The dotted line indicates the 95% confidence boundary.
The associated kernel distance pdfs, for 100 simulated runs are shown in Figure 33. The threshold spatial explanatory variable produced estimates of the threshold shown in Table 11.

![Figure 33. Summary of estimated dispersal kernels. Estimated distance pdfs for the GLM models with four kernel-related spatial explanatory variables. Each model summary consists of 100 separate estimates demonstrating the variation across 100 simulated runs. Kernels for no boundary (red lines), normalised (green lines) and reflected (purple lines) models showed a degree of similarity. The kernels for the boundary retention (blue lines) were dissimilar, a symptom of the poor fit and likely misspecification of the distance adjustment in this model.](image)

Table 11. Frequency of threshold distance for 100 simulated runs.

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>76</td>
<td>2</td>
</tr>
<tr>
<td>78</td>
<td>2</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>81</td>
<td>4</td>
</tr>
<tr>
<td>82</td>
<td>25</td>
</tr>
<tr>
<td>83</td>
<td>1</td>
</tr>
<tr>
<td>84</td>
<td>6</td>
</tr>
<tr>
<td>86</td>
<td>27</td>
</tr>
<tr>
<td>88</td>
<td>16</td>
</tr>
<tr>
<td>90</td>
<td>15</td>
</tr>
</tbody>
</table>

Examples of the estimated transgenic male and associated transgenic-mated female densities, were made using the predicted kernels (Figure 34).
Model comparison, using AIC, allowed the performance of different models to be compared. A comparison of the AIC values for each model type, over 100 simulated runs is shown in Figure 35. The model using the untransformed spatial explanatory variable (1) performed most poorly. The boundary retention and threshold models (2 and 6 respectively), performed substantially better than the untransformed model but will still outperformed by the model assuming no boundary effects (3) and the normalised and reflected boundary models (4 and 5 respectively). Of these three, the reflected boundary model (5) had the lowest AIC for all 100 runs, although there was little to distinguish between the reflected and normalised models (mean AIC of 13747 and 13749 respectively).

The effect size of the explanatory variables used in the reflected boundary model are demonstrated using a first difference plot (Figure 36). The plot represents the change in the predicted number of transgenic larvae when moving an explanatory variable from the lowest to the highest observed values whilst keeping all other explanatory variables at their mean values.

The convolution of the male and estimated transgenic-mated female dispersal kernel produces a distance pdf of the relationship between the transgene (transgenic male, up to the point of mating, + transgenic-mated-female dispersal) and distance (Figure 37 A) in a homogeneous environment. The MDT associated with this kernel is 191 metres. The cumulative distribution function (cdf) of this function may be used to estimate the distance within which a given percentage of all transgenic larvae may be expected to be found in an environment with no habitat boundaries, the 50%, 95% and 99% levels (158m, 451m and 637m respectively) are illustrated (Figure 37 B).
Figure 34. Density estimate examples. Examples of density estimates (colour) for released transgenic males (A-D) and resultant transgenic-mated females (E-H). The models are no-boundary (A,E), normalised (B,F), reflected (C,G) and boundary retention (D,H). Density estimates are in relation to the Itaberaba field site shown in Figure 24. Black lines represent the driven release route. Red lines represent the habitat boundary.
Figure 35. A comparison of model AIC values for models with six different spatial explanatory variables. Points demonstrate the AIC for individual simulation runs, the horizontal black lines represent the median AIC (1. Untransformed=13,938.7, 2. Threshold=13,772.0, 3. No boundary=13,753.1, 4. Normalised=13,748.5, 5. Reflected=13,746.8, 6. Border retention=13,768.9).

Figure 36. A first difference plot for the GLM model with the reflected boundary spatial explanatory variable. Thick and thin lines represent the 75th and 95th percentiles respectively. CIs for first differences were calculated by parametric bootstrap resampling (100,000 resamples) of the GLM coefficient estimates, $\beta$, assuming that they were normally distributed with standard deviation equal to the estimated standard errors.
Figure 37. Estimates of the combined released-male plus mated-female dispersal represented by a convoluted kernel and cumulative distribution function. The convolution of transgenic male and transgenic-mated female dispersal kernels represented as a A) pdf and 8) cdf with associated distances for the 50%, 95% and 99% quantiles (grey lines, 158m, 451m and 637m respectively).
4.4 Discussion

I present results demonstrating the estimation of the dispersal of transgenic-mated female *Ae. aegypti* mosquitoes in the field. Using data of consecutive releases of over 16 million transgenic male *Ae. aegypti*, I have simulated released male dispersal from complex release routes. Simulated dispersal data were used as an explanatory variable in a range of GLMs to assess the spatial relationship between released transgenic males and the ovitrap data collected in the field.

These methods provide a novel way of inferring wild female dispersal behaviour without the need for artificial, and potentially confounding, rearing, handling and marking of the insects. I assume that transgene-mated females are reasonably representative of the female population as a whole and that mating a transgenic male does not affect behaviour relative to mating a wild male. Transgenic-mated female dispersal was best represented when the spatial explanatory variable was transformed using an exponential power density pdf and was corrected for potential effects of the habitat-boundary. The predicted dispersal of released transgenic males and transgenic mated females was used to estimate the transgene distance pdf and make inferences on the potential distribution of distances at which larvae resulting from a mating between a transgenic male and wild female may be observed.

The three best performing members of the set of spatial explanatory variables considered, as measured by model AIC, all used kernel transformations of distance. The poorest performing member used untransformed distance, suggesting that transgenic-mated female dispersal is highly non-linear; the majority of females disperse relatively short distances whilst a small number may disperse much further. The convolution of male and transgenic-mated-female dispersal kernels also exhibits this skewed distribution. The shape of these distributions can directly inform monitoring practices. The spatial extent and limits of monitoring can be informed by the tail of the convoluted distribution. Knowledge of the expected distribution of distances at which the transgene may be observed can also be used to effectively design further field trials, increasing the accuracy by which control and treated areas may be defined and trial areas demarcated. Well characterised barrier zones surrounding areas where transgenic mosquitoes are released are an important consideration when dealing with controlled releases of transgenic organisms into the environment [238].

All models, regardless of the spatial assumptions, saw highly significant positive correlations between the number of wild and transgenic larvae in an ovitrap (Figure 27, Table 9). It is likely that the wild larvae explanatory variable is acting as a complex proxy for a number of trap-specific biological determinants of catch number. Large wild larval catches suggest that the trap may be readily accessible, attractive to ovipositing females, in preferential habitat and/or located in a pocket.
of high mosquito density, all characteristics that would also increase the likelihood of transgenic-mated females ovipositing. The inclusion of wild larval catches in the model may also act to control for site-wide fluctuations in the wild population density that could occur due to changes in meteorological factors or ongoing control. The relationship between wild larvae and the transgenic catch (Figure 27) may possibly have been better captured with a non-linear term. A small negative correlation between outdoor traps and transgenic catch was observed (Figure 28, Table 9); it is not uncommon for *Ae. aegypti* to preferentially oviposit indoors [239, 240], however it is not clear why this relationship would be apparent after adjusting for wild catch and the effect size (Figure 36) is so small that it might indicate a level of over-fitting in the model.

The two best performing models used dispersal kernels that corrected for assumptions of habitat-boundary effects. Under these conditions, it is assumed that dispersing mosquitoes will preferentially stay within the urban/peri-urban environment and will not cross boundaries demarking transitions to less favourable habitats. A number of subspecies or populations of *Ae. aegypti* are known to be well adapted to the urban environment [43, 44, 241]. These anthropophilic, domesticated populations are highly competent at surviving and reproducing in the urban and peri-urban environment [170, 234]. There is evidence that domestic forms of the mosquito may preferentially constrain dispersal to within an urban habitat when challenged with an urban-sylvatic habitat-boundary. Harrington et al. (2005) observed a very low frequency (4 out of 834 recaptures) of inter-village movement during *Ae. aegypti* MRR experiments in Thailand, even when villages were in close proximity (~300m) [204]. Lourenço-de-Oliveira et al. (2004) and Maciel-de-Frietas et al. (2006) noted an absence of released *Ae. aegypti* dispersing more than 100m into the forest fringe in Brazil, unlike *Ae. albopictus*, which readily dispersed further [147, 148]. Similar evidence is seen in Kenya where *Ae. aegypti* were rarely observed dispersing more than 200m from a village [124]. The model that did not assume any habitat-boundary effects was only marginally outperformed by the two best performing models (median AIC no boundary = 13,753, median AIC for normalised and reflected = 13,749 and 13,747, respectively). It is likely that some dispersal does occur in and across these regions but at reduced or diminishing rates compared with more favourable habitats. Ovitrapping, or adult-sampling, on the periphery and outside of the habitat-boundary would be the best way to test the validity of such assumptions.

The reflected and normalised corrections to predicted densities performed much better than the boundary retention. The reflection and normalised corrections adjusted the predicted densities in similar ways, redistributing those individuals that would have dispersed outside of the habitat-boundary more evenly over possible locations within the habitat-boundary. The resulting kernel
parameter estimates were very similar, producing MDT estimates of 187m and 192m for the reflected and normalised analyses respectively, which are within the range of the published estimates of female *Ae. aegypti* dispersal in the literature (range 5-279m, Table 12).

The assumption of boundary retention did not provide a good fit with the data. The maximum likelihood kernel parameters for the boundary retention example resulted in a highly positively skewed kernel, minimising mid- and long-distance dispersal which, in turn, minimised the effects of any correction to densities, indicating that this assumption of mosquito dispersal behaviour and associated density correction was not appropriate.

A further assumption of this analysis is that the released transgenic males can be expected to undertake a single large dispersal event soon after release. This was observed during MRR studies conducted at this location (Chapter 3). A review of the literature highlights further studies that provide supporting evidence for this behaviour. McDonald (1977) found that dispersal of released *Ae. aegypti* males and females did not increase after the second day post release [124].
**Table 12. Female *Aedes* dispersal estimates.** A summary of a literature review of female *Aedes* mosquito dispersal.

<table>
<thead>
<tr>
<th>MDT/MDT range (m)</th>
<th>Location</th>
<th>Mated*</th>
<th>Blood fed</th>
<th>Notes</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-22</td>
<td>Hainan Island, China</td>
<td>-</td>
<td>N</td>
<td>Released at the village centre</td>
<td>[149]</td>
</tr>
<tr>
<td>25</td>
<td>Amorim, Brazil</td>
<td>-</td>
<td>N</td>
<td></td>
<td>[226]</td>
</tr>
<tr>
<td>31</td>
<td>Guadalupe, Mexico</td>
<td>Y</td>
<td>N</td>
<td></td>
<td>[167]</td>
</tr>
<tr>
<td>31</td>
<td>Lao Bao, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>38</td>
<td>Amorim, Brazil</td>
<td>-</td>
<td>N</td>
<td></td>
<td>[226]</td>
</tr>
<tr>
<td>40</td>
<td>Lao Bao, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>40</td>
<td>Hainan Island, China</td>
<td>-</td>
<td>N</td>
<td>Release at the village edge</td>
<td>[149]</td>
</tr>
<tr>
<td></td>
<td>Ilha do Governador, Brazil</td>
<td>-</td>
<td>N</td>
<td>Raised on rich diet</td>
<td>[114]</td>
</tr>
<tr>
<td>45</td>
<td>Lao Bao, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>56</td>
<td>Pentland, Australia</td>
<td>Y</td>
<td>N</td>
<td></td>
<td>[137]</td>
</tr>
<tr>
<td>62</td>
<td>Pai Lom, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>62</td>
<td>Amorim, Brazil</td>
<td>-</td>
<td>N</td>
<td></td>
<td>[226]</td>
</tr>
<tr>
<td>63</td>
<td>Tubiacanga, Brazil</td>
<td>-</td>
<td>N</td>
<td></td>
<td>[226]</td>
</tr>
<tr>
<td>68</td>
<td>Lao Bao, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>71</td>
<td>Lao Bao, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>75</td>
<td>Mae Kasa, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>78</td>
<td>Cairns, Australia</td>
<td>Y</td>
<td>N</td>
<td></td>
<td>[146]</td>
</tr>
<tr>
<td>78</td>
<td>Cairns, Australia</td>
<td>Y</td>
<td>Y</td>
<td></td>
<td>[146]</td>
</tr>
<tr>
<td>79</td>
<td>Ilha do Governador, Brazil</td>
<td>-</td>
<td>N</td>
<td>Raised on poor diet</td>
<td>[114]</td>
</tr>
<tr>
<td>93</td>
<td>Lao Bao, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>105</td>
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<td>-</td>
<td>N</td>
<td></td>
<td>[226]</td>
</tr>
<tr>
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<td>-</td>
<td>N</td>
<td></td>
<td>[226]</td>
</tr>
<tr>
<td>124</td>
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<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>151</td>
<td>Mae Kasa, Thailand</td>
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<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>156</td>
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<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>160</td>
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<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>199</td>
<td>Mae Kasa, Thailand</td>
<td>Y</td>
<td>Y</td>
<td>Released indoors</td>
<td>[204]</td>
</tr>
<tr>
<td>181-279</td>
<td>San Juan, Puerto Rico</td>
<td>Y</td>
<td>Y</td>
<td>Rubidium marked eggs</td>
<td>[223]</td>
</tr>
</tbody>
</table>

* dashed cells indicate the mated status of release females was not specified.

Sheppard *et al.* (1969) also observed the major component of *Ae. aegypti* male and female dispersal occurring within the first 24 hours after release [219]. A study assessing the distribution of rubidium-marked eggs by released *Ae. aegypti* females saw no significant evidence for continuous, progressive dispersal [220].

The GLM coefficient standard errors were estimated conditional upon the MLEs for kernel parameters *a* and *b* whilst the confidence intervals for the kernel parameters were obtained conditional upon the MLE for the GLM coefficients. Thus the reported standard errors are likely to be smaller than if we had been able to compute the unconditional standard errors and confidence intervals for all of the parameters.
The convolution of released male and transgenic-mated female dispersal kernels provides an estimate of the expected distribution of fluorescent larvae (those sired by released transgenic males). The shape of the kernel is predominantly driven by the estimate of transgenic-mated-female dispersal which is, on average, further than the estimate of released male dispersal. There is considerable uncertainty (the combined uncertainty in our estimates of released-male and mated-female kernels) associated with this convolution. One of the most effective methods of monitoring mosquito populations and sterile-mosquito releases is through the use of ovitraps in the field [90, 91, 162]. Improved knowledge of the expected distribution of fluorescent larvae will aid targeted and efficient ovitrap monitoring.

4.5 Conclusion

This work presents a novel method to estimate the dispersal of transgenic-mated female *Ae. aegypti* in the field using monitoring data from a large-scale release of transgenic mosquitoes. Ovitrap field data, combined with GLM, dispersal kernel and stochastic simulation techniques allow a transgenic-mated-female dispersal kernel to be estimated. The predicted dispersal of transgenic-mated females at the field site sits within the range of published dispersal estimates derived by alternative methods. The dispersal kernel approach allows us to produce a more detailed picture of female dispersal than single-summary measures. Combining the estimated female dispersal with male dispersal allows us to estimate the kernel for the transgene, with the potential to improve efficiency and data quality of current monitoring techniques.

4.6 Acknowledgements

Many thanks to Oxitec for providing data and facilitating the analyses in this chapter. Thanks to Projeto Aedes Transgênicos and Moscamed Brasil for their collaboration and support. I also thank the participants of the field studies in Itaberaba, Brazil.
5 Computational optimisation with “virtual insects” to inform transgenic insect release.

This chapter presents a methodological approach aimed at optimising the implementation of the release stage of a transgenic insect vector control programme. I present a flexible computational methodology by which multiple objectives, relating to the cost or effort associated with a release and the coverage of transgenic insects achieved, can be optimised. The chapter builds upon work in chapter 3, using estimates of the released male dispersal kernel to simulate dispersal from complex sets of release points or driven routes.

5.1 Introduction

Field releases of sterile insects are complex, involving many logistic, biological, economic and social considerations. Increasing the scale, number and diversity of release programmes will lead to further complexity in the system. In order to manage these complicated and often competing factors we may turn to automated procedures that can provide optimal or near-optimal solutions for specific aspects of a release across a range of scenarios. One aspect of the release process that lends itself to automation and computational optimisation is the allocation of spatial locations at which releases occur.

Releases of transgenic sterile Ae. aegypti have been conducted from discrete points or continuously from a moving vehicle [90, 91] (Chapter 4). Both approaches aim to deliver transgenic males to the target area in a systematic manner. Male coverage should achieve a pre-determined density which may or may not be homogeneous across the target area(s). Releases should aim to minimise patchiness or hotspots in released male density. Patchiness, areas of lower than target density, may provide refugia within which adequate control is not being achieved [195, 242], potentially jeopardising the control effort as a whole. Hotspots, areas of higher than target density, are less problematic from a control standpoint but are associated with ‘wasted’ coverage which could be more efficiently deployed elsewhere. Further to these biological aims, economic considerations must be taken into account. In competition with biological optimisation are costs associated with releases, such as the time or monetary cost of servicing release points or driving a release route. For an efficient, economical programme these costs must be managed and minimised without detriment to the biological objectives.

Spatial optimisation of a sterile insect release is a computationally intensive and complex procedure. Engineering problems that attempt to efficiently distribute wireless sensors or mobile communication stations in a network [243–247] are, in part, analogous to the distribution and
placement of transgenic insect release points. Attempts to solve these problems have used an optimisation procedure known as particle swarm optimisation (PSO). The PSO algorithm was first introduced as a result of efforts to model the social behaviour of swarming organisms, such as bird flocks, shoals of fish [248, 249] or insects [250]. Since its inception, PSO has been adapted for use in solving a range non-linear optimisation problems. Kennedy and Eberhart’s [248, 249] original formulation of the algorithm involves a virtual multidimensional ‘environment’ in which the particles, our computational organisms, exist. The environment represents the solution space for a given optimisation problem. Thus, each particle, occupying a point in this space represents a potential solution to the problem. Particles move around the solution space, with their movements directed by three factors:

1. A random element – to encourage exploration of the solution space.
2. Their own previous best position.
3. The previous best position found by any particle in the whole swarm.

Particles swarm in this manner and may converge on a point in the solution space which can indicate a potential optimal or near-optimal solution. Further modifications have been applied to adapt the procedure for discrete problems [244, 251, 252]. As mentioned previously, optimising the release of transgenic insects involves a trade-off between multiple factors such as coverage and cost. Alvarez-Bentitez et al. (2005) demonstrate how these trade-offs may be incorporated into a PSO approach by using Pareto dominance concepts, adapting the PSO to a multiple-objective particle swarm optimisation (MOPSO) [253]. A Pareto-dominant or Pareto-efficient solution is one where it is impossible to improve the measured outcome in relation to one variable without making a second variable worse off and vice-versa (If there are ten apples in total, I have six and you have four, then I cannot increase my number of apples without decreasing your number of apples).

In the context of this work, I harness the power of a swarm of computational ‘insects’ to find solutions to challenges faced when dealing with field releases of their real-world counterparts.

Coverage from a point associated with a transgenic insect release can be expected to decrease non-linearly with respect to distance from the release point. Therefore, unlike the aforementioned engineering applications of the PSO, the coverage (in this instance, of released insects) from a release point will not be homogeneous across the full range of influence of the release. A parameterised dispersal kernel can be used to approximate the distribution of dispersal distance of transgenic male Ae. aegypti from a release point or route (Chapter 3). Measures of coverage across the target area must also be adapted accordingly.
For large-scale releases, sterile insects can be distributed from a moving vehicle. In this scenario an efficient, economical approach may aim to minimise driving distance or driving time whilst maintaining the required levels of coverage. This adds further complexity to the process of optimising release procedures. Route finding problems of this nature are known to be NP-hard [254, 255] (these are problems where there is no simple solution by which we can resolve the answer in reasonable computational time), and researchers have attempted to solve them near-optimally using a range of heuristic algorithms. The rural postman problem (RPP) attempts to find the minimum-cost, closed circuit solution that traverses all required edges in a graph [256]. The name derives from a description of this problem whereby a postal worker attempts to find the shortest route to service all roads on their delivery round. In this instance the graph represents a road network and required edges the release locations. A number of heuristic solutions [257–260] have been proposed to solve the RPP problem approximately in reasonable computational time [254].

One of these solutions, [260], involves transforming the RPP, an arc routing problem, into the travelling salesman problem (TSP), a vertex routing problem. The TSP is a famous problem which challenges us to find the shortest route for a salesman to travel so that they have visited all required cities and have returned back to their starting point. The TSP can be solved using a variant of ant colony optimisation (ACO) with the resulting solution converted back into a RPP solution [260]. The Ant Colony System (ACS), a version of ACO, is a heuristic, based on the natural foraging behaviour of ants, that has been used to solve the TSP [261]. The system makes use of the ants’ ability, facilitated by pheromones, to find the shortest distance between their nest and a food source. The ACS approach presented by Dorigo & Gambardella (1997) [261] uses an iterative process by which artificial ‘ants’ construct tours of the graph guided by:

1. The cost of potential edges (determined by their length).
2. The amount of pheromone on potential edges.

Each ant lays down pheromone on the edges it has traversed which subsequently evaporates at a constant rate. The cooperative learning of the colony can therefore converge on an optimal route.

Once again, I use “virtual entomology” to inform its field counterpart.

For this work MOPSO and ACS heuristics have been adapted, combined and applied to problems regarding the release of transgenic sterile insects. Specifically, these problems consist of a set of spatially-explicit multiple-objective optimisation challenges. All members of this set require an optimal balance to be established between levels of coverage obtained by a release, or set of releases, and the cost (or effort) of achieving it. The optimisation procedures have been tested
against a number of theoretical scenarios. The MOPSO and ACS systems have been applied to real field sites where a set of release points or driven route must be designed to conduct the release of transgenic sterile male *Ae. aegypti* mosquitoes.

### 5.2 Methods

#### 5.2.1 Basic PSO

A general outline of the PSO [248, 249, 262, 263] is presented in the following section. Formally, consider a problem in $d$ dimensions. For $N$ particles each particle $i = 1, 2, 3, ..., N$ is associated with a linear vector of length $d$ denoting the position, $x_{id}^t$, and velocity, $v_{id}^t$, of particle $i$, in dimension $d$ at time-step $t$.

Each particle is assigned an initial random starting position

$$x_{id}^0 = \text{rand}(x_{\text{min}}, x_{\text{max}}),$$  \hspace{1cm} (5.1)

and velocity

$$v_{id}^0 = \text{rand}(v_{\text{min}}, v_{\text{max}}).$$  \hspace{1cm} (5.2)

Here $\text{rand}(a,b)$ represents a random number drawn from the interval $(a,b)$. At each time-step the velocity and position of each particle is updated according to the following rules

$$v_{id}^{t+1} = \omega v_{id}^t + c_1 \text{rand}(0,1)(p_{id} - x_{id}^t) + c_2 \text{rand}(0,1)(p_{gd} - x_{id}^t),$$  \hspace{1cm} (5.3)

$$x_{id}^{t+1} = x_{id}^t + v_{id}^{t+1},$$  \hspace{1cm} (5.4)

where $\omega$ is the inertia factor, used to control divergence, and $c_1$ and $c_2$ determine the relative influence of the particle’s own best position, $p_i$, and the swarm’s best position, $p_g$, respectively. Individual and global best positions are determined by a problem-specific fitness function which evaluates a given solution with respect to the target goal. The sequence of velocity-updating, position-updating and fitness evaluation is iterated until a pre-determined level of convergence has been achieved [248, 249, 262, 263].

#### 5.2.2 Discrete PSO

Here the particle position consists of a binary string of length $d$. Particle velocity, calculated as in equation (5.3), is converted to a value in the interval $(0,1)$ using a hyperbolic function
\[ S_{id}^t = 2 \left( \frac{1}{1+e^{-v_{id}}} - 0.5 \right), \quad (5.5) \]

after which particle position can be updated

\[
x_{id}^{t+1} = \begin{cases} 
\text{exchange}(x_{id}^t), & \text{if } S_{id}^t > \text{rand}(0,1) \\
x_{id}^t, & \text{if } S_{id}^t \leq \text{rand}(0,1). 
\end{cases} \quad (5.6)
\]

In the discrete case a high velocity will elicit a change in state of the binary integer \((0 \rightarrow 1 \text{ or } 1 \rightarrow 0)\) at lower velocities the binary integer will remain the same. By using this kind of transformation the PSO concepts can be applied to discrete problems [244]. Discrete solutions are desirable in this instance, allowing specific release points or segments of road from which releases occur to be switched on or off.

### 5.2.3 Multiple Objective PSO (MOPSO)

Commonly, the optimal solution to a problem may be a trade-off between more than one fitness measures. Alvarez-Bentitez et al. (2005) used Pareto dominance concepts to adapt the PSO approach to a MOPSO [253]. This methodology is outlined in the overview below.

For a given MOPSO problem, the aim is to maximise \(R\) objectives of ‘fitness functions’

\[
f_{i(x)}, \quad i = 1, 2, 3, ..., R. \quad (5.7)
\]

One solution, \(u\), is said to strictly dominate another, \(v\), if

\[
f_i(u) \geq f_i(v) \quad \forall i
\]

and

\[
f_i(u) > f_i(v) \quad \text{for some } i. \quad (5.8)
\]

Solution \(u\) is said to weakly dominate solution \(v\) if

\[
f_i(u) \geq f_i(v) \quad \forall i. \quad (5.9)
\]

A set of solutions consisting of members where no member dominates any other is referred to as a non-dominated set. The true Pareto front, \(\varphi\), is a set of non-dominated solutions. No member of \(\varphi\) can be dominated by any feasible solution [253].

Converting the PSO procedure to a MOPSO one involves the addition of an archive \(A\), in which are stored all non-dominated solutions found from time \(t = 0\) to \(t = t\). At each time step all, \(R\), fitness
functions are evaluated. Any solutions not weakly dominated by any member of \( A \) are added to \( A \).

Any members of \( A \) dominated by a current solution are removed from \( A \) [253].

The particle’s own best ever position, \( p_i \), is initially set as the starting location, \( x_i^0 \). At each time step, \( p_i \), is re-evaluated

\[
p_i = \begin{cases} x'_i & \text{if } x'_i \text{ weakly dominates } p_i, \text{ or } x'_i \text{ and } p_i \text{ are mutually non-dominating} \\ p_i & \text{otherwise} \end{cases} \tag{5.10}
\]

The global best position for each particle, \( p_{ig} \), is initially set as the starting location, \( x_i^0 \). At each time-step \( p_{ig} \), for each, \( i = 1, 2, 3, ..., N \), particles is selected, at random, with equal probability, from those members of \( A \), that dominate \( x_i^t \) [253]. A schematic of the process is shown below (Figure 38).

![Schematic diagram](image)

**Figure 38.** Schematic of the multiple objective particle swarm optimisation (MOPSO) used to determine Pareto-dominant solutions. Particle positions are initialised and iteratively updated based on two fitness functions. Dominant solutions are stored in an archive.

### 5.2.4 Parameter selection

The parameters \( \omega \) and the acceleration coefficients, \( c_1 \) and \( c_2 \), correspond to the inertia weight and the influence of the particle’s own experience and the swarm experience respectively. It is customary for \( c_1 = c_2 \) [264].

The inertia weight can be used to control the divergence of velocity with respect to time. If \( \omega > 1 \) particle velocity will \( \rightarrow \infty \) over time. If \( \omega < 1 \), particle velocity will \( \rightarrow 0 \) over time. A value of
\( \omega = 0.7298 \) was recommended by Eberhart and Shi to ensure convergent dynamics, [265], and has been commonly used. However, the appropriateness of these empirically derived estimates should be used with caution for specific optimisation problems. It has also been shown that, in order to observe convergent behaviour [266]

\[
\omega > 0.5(c_1 + c_2) - 1. 
\]  

(5.11)

An approach using an adaptive, time-varying, inertia value has been suggested [265]. In these cases the value decreases with each additional time-step. This has the effect of shifting the particle dynamics from global- towards local-exploration as the number of time-steps increases. The parameters used for the following analyses are outlined in Table 13.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>References</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Particles</td>
<td></td>
<td>100</td>
<td>[253]</td>
<td></td>
</tr>
<tr>
<td>Inertia factor</td>
<td>( \omega )</td>
<td>0.9-0.4</td>
<td>[265]</td>
<td>Time varying: -0.0001 per iteration (from min to max, then remaining at min)</td>
</tr>
<tr>
<td>Acceleration coefficient 1</td>
<td>( c_1 )</td>
<td>1.49</td>
<td>[265]</td>
<td></td>
</tr>
<tr>
<td>Acceleration coefficient 2</td>
<td>( c_2 )</td>
<td>1.49</td>
<td>[265]</td>
<td></td>
</tr>
<tr>
<td>Stopping criteria (number of iterations with no change)</td>
<td>-</td>
<td>2000</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>The true Pareto front</td>
<td>( \varphi )</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>A set of current, non-dominated solutions</td>
<td>( A )</td>
<td>-</td>
<td>[253]</td>
<td></td>
</tr>
</tbody>
</table>

5.2.5 Grid – theoretical arena

All theoretical applications of these procedures were performed on a 1km\(^2\) lattice grid, an area of similar scale to the Brazil field site introduced in chapters 3 and 4. Sterile male mosquito dispersal is simulated for released males. For each released male, dispersal location coordinates are determined by sampling a grid square from a lattice with a probability determined by the dispersal kernel relative to the starting coordinates (release point) of the male. All dispersal simulations used the exponential power kernel [198]:

\[
\text{exponential power kernel} = \frac{b}{2 \pi a^2 \Gamma} \left( \frac{d^b}{a^b} \right) e^{-\frac{d^b}{a^b}} \quad a, b > 0, 
\]  

(5.12)

where, \( a \) and \( b \) are kernel parameters, \( d \) is the distance (metres) and \( \Gamma \) is the gamma function:
All simulations set $a=75.2$ and $b=3.75$, informed by released male dispersal kernel parameterisation estimated in chapter 3. This method of simulating dispersal uses centroid to centroid approximation of continuous dispersal on a lattice [267], density estimates are normalised to adjust for approximation to a grid. I assume isotropic dispersal from a radially symmetric kernel.

### 5.2.6 Fitness functions

I have formulated a number of fitness functions, specific to the release of transgenic sterile insects, for use within the MOPSO framework. Broadly, these fitness functions all fit into one of two categories: 1) Measures of coverage and 2) Measures of cost. As a general rule, functions in 1 will conflict with functions in 2 (increasing the fitness as measured by a function from 1 will lead to a corresponding decrease in the fitness as measure by a function from 2, i.e. more coverage=more effort).

<table>
<thead>
<tr>
<th>1. Measures of coverage (aim = maximise)</th>
<th>2. Measures of cost (aim = minimise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells receiving any coverage</td>
<td>The number of release points (release size equal)</td>
</tr>
<tr>
<td>Number of cells receiving coverage above that of a required threshold</td>
<td>The number of mosquitoes released (from points)</td>
</tr>
<tr>
<td>Number of target cells receiving any coverage</td>
<td>The number of mosquitoes released (from road segments, assuming constant release rate)</td>
</tr>
<tr>
<td>Number of target cells receiving coverage above that of a required threshold</td>
<td>The distance driven to complete a release route</td>
</tr>
<tr>
<td>Number of target cells receiving coverage above that of a required threshold (multiple thresholds)</td>
<td>The number of non-target cells receiving coverage</td>
</tr>
</tbody>
</table>

### 5.2.7 Specific formulation of the fitness functions

The most basic fitness function from set 1, sums the number of cells in the lattice that achieve a sterile male density $>0$

\[
\text{Count } S_{xy} > 0, \tag{5.14}
\]

where $S_{xy}$ represents the density of sterile males at cell with coordinates $(x, y)$.

A more useful variant of equation (5.14) involves quantifying the number of cells in which we observe a sterile male density above a given threshold

\[
\text{Count } S_{xy} > H, \tag{5.15}
\]

where $H$ represents a target threshold sterile male density.
The target areas may represent a subset of our total area, in which case the fitness function becomes

\[
\text{Count } S_{vw} > H \quad \forall w \in xy, \quad (5.16)
\]

where \( S_{vw} \) represents the sterile male density at the cell with coordinates \((v, w)\) where \( v \) and \( w \) are from \( x \) and \( y \) respectively. Finally, target areas may require differing target densities

\[
\text{Count } S_{vw} > H_{vw} \quad \forall w \in xy, \quad (5.17)
\]

where \( H_{vw} \) represents a cell-specific, and therefore target area-specific, target density. One fitness function from set 2, the cost functions is also closely related where

\[
\text{Count } S_{mn} > H \quad \forall w \in xy, \quad (5.18)
\]

where \( S_{mn} \) represents the sterile male density at a cell with coordinates \((m, n)\).

The release route distance proves to be the most challenging cost function to estimate, being a version of the rural postman problem (RPP). Due to the complexity of this cost function it is further detailed in an individual section below.

### 5.2.8 Route distance

The route distance fitness function aims to calculate the minimum distance required to complete a circuit that covers all road segments highlighted for release. Non-release road segments may be traversed to complete the circuit in the most parsimonious manner. To formalise and analyse this problem it is converted to a graph theory problem, specifically the RPP.

Consider a connected, undirected graph, \((V, E)\), with a set of vertices, \( V \), and edges, \( E \). Each edge represents a road segment in the release area and each vertex a road junction. A subset of edges are designated as required, \( E_r \), for traversal, these road segments must be included in our release route. The edges, \( E_r \), represent road segments along which releases are conducted.

Outlined below is a general overview of the ACO, the application of ACO to the RPP problem and finally details of our inclusion of ACO into a MOPSO framework.

I start with a given graph \( G = (V, E) \) with vertex set \( V \) and edge set \( E \). Each edge has an associated cost where \( \sigma(a, b) \) denotes the cost for edge \((a \rightarrow b)\). An instance where, for all edges, \( \sigma(x, y) = \sigma(y, x) \) is denoted as symmetric. Converesely if, for any edge in \( E \), \( \sigma(x, y) \neq \sigma(y, x) \) the
problem is asymmetric and has been termed the windy rural postman problem [268]. The TSP tasks us to find the least cost closed tour of the graph that visits each vertex once and only once.

Following the ACS approach presented by Dorigo [261], an ant, \( s \), located at vertex \( i \) is assigned its next destination, \( j \), via a random-proportional rule. Let \( q \) be a random draw from the uniform distribution on the interval \((0,1]\):

If \( q \leq Q \)

\[
p^s_{ij} = \begin{cases} 1 & \text{if } j = \text{arg max}_{k \in C_s(i)} \left\{ \tau_{ik}^\alpha \cdot \eta_{jk}^\beta \right\}, \\ 0, & \text{otherwise} \end{cases}
\]  

(5.19)

If \( q > Q \)

\[
p^s_{ij} = \begin{cases} \tau_{ij}^\alpha \cdot \eta_{ij}^\beta \sum_{k \in C_s(i)} \tau_{ik}^\alpha \cdot \eta_{jk}^\beta & \text{if } j \in C_s(i) \\ 0, & \text{otherwise} \end{cases}
\]  

(5.20)

where \( p^s_{ij} \) is the probability that ant \( s \) will move from vertex \( i \) to vertex \( j \). The function \( C_s(i) \) returns the set of vertices available to ant \( s \), positioned at vertex \( i \) that have not been visited before. The amount of pheromone between vertex \( i \) and vertex \( j \) is denoted as \( \tau_{ij} \). The visibility of a connection between vertex \( i \) and vertex \( j \) is denoted \( \eta_{ij} \) and is defined as \( \sigma(i,j)^{-1} \), so that closer vertices are more visible. Parameters \( \alpha \) and \( \beta \) determine the influence of the pheromone and the influence of the cost (distance) respectively. The parameter \( Q \) is used to determine the relative importance of exploration (choosing unexplored routes) to exploitation (choosing routes based on the prior experience of the ant colony).

When traversing the edge \((i,j)\) an ant will deposit pheromone via the local updating rule

\[
\tau_{ij} = \rho_l \tau_{ij} + (1 - \rho_l) \tau^0,
\]  

(5.21)

where the parameter \( \rho_l \) represents the local persistence of pheromone and \( \tau^0 \) the amount of pheromone that is laid down. Furthermore, at the end of each iteration, when all ants have constructed a complete tour, the pheromone levels are again updated. The level of pheromone associated with the globally best tour (the least cost tour found by any ant throughout all iterations) is updated using the global updating rule

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where the parameter $\rho_g$ represents the global persistence of pheromone. The set $T_g$ contains the edges visited of the global least cost tour, with the associated cost denoted as $L_g$ [261]. The pheromone is a method by which the combined experience of the colony (be it physically for the ants or algorithmically in this analysis) is shared and can positively influence future choices, aiding the discovery of the shortest route.

The ACS is summarised below (Figure 39).

**Figure 39. Schematic of the Ant-colony system (ACS) approach used to inform driven routes for releases.** Ants are initialised at starting positions then proceed, through an iterative process, to complete a full tour. Throughout iterations pheromone levels are updated. The procedure is repeated until a stopping rule is satisfied.

Pérez-Delgado (2007) has applied the ACS algorithm to the RPP [260]. To facilitate this application the RPP must first be transformed from an edge-routing problem to a vertex-routing problem [255]. Let $G_r = (V_r, E_r)$ be the subgraph of $G$ containing the subset of required edges $E_r$ and the associated induced vertices $V_r$. The transformation, demonstrated in [255] and used in [260], proceeds as follows

$$S_i = \{ s_j \mid j \in N(i) \} \quad \forall i \in V_r ,$$

(5.23)

for each vertex $i$ in $V_r$ a set is created containing all, $N(i)$, neighbouring vertices to vertex $i$. A complete, weighted graph, $G' = (V', E', c')$ is constructed

$$V' = \bigcup_{i \in V_r} S_i .$$

(5.24)

Edge costs, $c'$, are calculated based on the following rules
\[c'(s^h_i, s^k_j) = 0 \quad \forall i \in V_r \text{ and } h, k \in N(i), \; h \neq k\]  \hspace{1cm} (5.25)

\[c'(s^h_i, s^k_j) = \begin{cases} 
-M & \text{if } i = k \text{ and } j = h, \; \forall i, j \in V_r, \; i \neq j, \; h \in N(i), \; k \in N(j) \\
\text{d}(i, j) & \text{otherwise}
\end{cases},\]  \hspace{1cm} (5.26)

where \( d(i, j) \) represents the cost of the shortest path, found using Dijkstra’s algorithm, between vertex \( i \) and vertex \( j \) in the original graph, \( G \). The value \( M \) is calculated as the sum of all connections in the original graph, \( G \). Under this cost framework, edges in the original graph that reciprocally link two independent sets of required edges are assigned a very low cost, \(-M\), encouraging the sets of required edges to be connected. Linked required edges in the original graph can be traversed with zero cost and are therefore attractive. All other paths are assigned the distance cost, favouring shorter routes.

A RPP on \( G \) can be reconstructed from the TSP solution on \( G' \). The TSP solution with \( N \) steps consists of the sequence of vertices, \( s^h_{i_1}, s^{i_2}_{i_2}, \ldots, s^{i_N}_{i_N}, \; \forall i, j \in V_r, \) from \( G' \). The first step in the associated RPP \( = i_1 \). For \( n = 2, 3, \ldots, N \) subsequent steps are constructed using the following rules:

1. If \( i_{n-1} = j_n \) and \( i_n = j_{n-1} \) stop \( j_{n-1} \) is added.
2. If \( i_{n-1} = i_n \) and \( j_{n-1} \neq j_n \) no stops are added.

otherwise, stops along the shortest path, on \( G \), between \( i_{n-1} \) and \( i_n \) are added.

5.2.9 Combining the MOPSO and ACS (MOPSO-ACS)
I have nested the ACS optimization routine within the MOPSO framework to allow the release route length to be used as the cost-based fitness function. Under this arrangement, for each iteration of the MOPSO, one ACS optimisation procedure is performed for each particle to provide the cost-based fitness of each particle. An overview of this combined system is illustrated below (Figure 40).
To summarise how each process is used: firstly the discrete PSO is used to turn release points or road segments from which we release on or off. Next, the coverage of males is estimated from simulations of male dispersal from those active release points or road segments. Finally, the MOPSO is used to maximise the coverage we achieve for a given amount of effort, where the effort taken to service a driven route is estimated using the ACS.
Table 15. Ant colony system (ACS) variables and parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Ants</td>
<td>-</td>
<td>10</td>
<td>[261]</td>
<td>-</td>
</tr>
<tr>
<td>Graph</td>
<td>$G$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vertex set</td>
<td>$V$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Edge set</td>
<td>$E$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cost of edge $i \rightarrow j$</td>
<td>$\sigma(i, j)$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Subgraph</td>
<td>$G'$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Required vertex set</td>
<td>$V_r$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Edges associated with required vertex set</td>
<td>$E_r$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cost of edge $i \rightarrow j$ in the subgraph</td>
<td>$c'(i, j)$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Random draw from the uniform distribution on the interval (0,1)</td>
<td>$q$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exploration weight</td>
<td>$Q$</td>
<td>0.9</td>
<td>[260, 261]</td>
<td>-</td>
</tr>
<tr>
<td>The visibility of a connection</td>
<td>$\eta_{ij} = \sigma(i, j)^{-1}$</td>
<td>-</td>
<td>[261]</td>
<td>Inverse of edge cost</td>
</tr>
<tr>
<td>Initial pheromone level</td>
<td>$\tau_0$</td>
<td>-</td>
<td>[261]</td>
<td>Graph dependent</td>
</tr>
<tr>
<td>Pheromone level on edge $i \rightarrow j$</td>
<td>$\tau_{ij}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Local persistence of pheromone</td>
<td>$\rho_l$</td>
<td>0.9</td>
<td>-</td>
<td>Set by observation</td>
</tr>
<tr>
<td>Global persistence of pheromone</td>
<td>$\rho_g$</td>
<td>0.1</td>
<td>[260, 261]</td>
<td>-</td>
</tr>
<tr>
<td>Influence of pheromone</td>
<td>$\alpha$</td>
<td>1</td>
<td>[260, 261]</td>
<td>-</td>
</tr>
<tr>
<td>Influence of distance</td>
<td>$\beta$</td>
<td>2</td>
<td>[260, 261]</td>
<td>-</td>
</tr>
<tr>
<td>Sum of all connections on the subgraph</td>
<td>$M$</td>
<td>-</td>
<td>[260][254]</td>
<td>-</td>
</tr>
<tr>
<td>Stopping criteria (number of iterations with no change)</td>
<td>-</td>
<td>75</td>
<td>-</td>
<td>Set by observation</td>
</tr>
<tr>
<td>Edges associated with the global least-cost tour</td>
<td>$T_g$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cost of the global least-cost tour</td>
<td>$L_g$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

5.2.10 The Pareto front

On completion, the relationship between the fitness of all non-dominated solutions, $A$, as determined by the coverage fitness function and cost fitness function, can be compared. Plotting the coverage fitness with respect to cost fitness of dominant solutions produces the Pareto front (Figure 41). Assuming that the optimisation heuristic has been successful in finding true optimal solutions, the following statements are true: (i) solutions on the Pareto front are optimal in that the fitness of either fitness function cannot be improved upon without detriment to the fitness as measured by the other function, (ii) the area below the Pareto front represents sub-optimal solutions. In this situation the fitness of either one of the fitness functions can be improved without detriment to the other. These positions represent inefficiency in the system and (iii) the area above the Pareto front represents unachievable scenarios as measured by one or both fitness functions.
Figure 41. A Pareto front example. A hypothetical Pareto front (dotted line) inferred from dominant solutions (points). The area below the Pareto front represents inefficient solutions. The area above represents unobtainable solutions.

5.2.11 Theoretical scenarios
A number of theoretical scenarios have been designed to test the MOPSO approach for optimising releases of sterile insects. These are summarised in the table below (Table 16). Each scenario is characterised by a unique combination of operational aims, formalised by the fitness functions, and approaches to sterile insect release.

The theoretical arena in which scenarios to optimise sterile insect releases were conducted consists of a 1km$^2$ area. The arena is covered by a lattice of 10m × 10m grid squares upon which mosquito densities were approximated (Figure 42).

Three spatially unconnected target zones were demarcated within the arena (Figure 43). These zones represent the areas targeted for coverage with sterile male insects. The threshold target coverage could be varied between zones. A target coverage density of 10 males per grid square within all three zones was used for scenarios 1-4. For scenario 5, the target density was, 25, 10 and 1 mosquitoes per grid square for zones, 1, 2 and 3 respectively (Figure 43).

Releases were considered from release points for scenarios 1-3 and 5. A total of 50 release points were available, 25 distributed randomly in the target area and 25 distributed randomly in the non-target area. The number of mosquitoes released from each point could be fixed (as in scenarios 1 and 3) or left to vary (as in scenarios 2 and 5).
Table 16. Summary of theoretical test scenarios for the multiple objective particle swarm optimisation (MOPSO).

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Target area</th>
<th>Release method</th>
<th>Fitness function 1 (coverage-based, maximise)</th>
<th>Fitness function 2 (cost-based, minimise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Three zones, equal target density</td>
<td>Point, constant release size</td>
<td>Number of grid squares within target area above a given threshold</td>
<td>Number of active release points</td>
</tr>
<tr>
<td>2</td>
<td>Three zones, equal target density</td>
<td>Point, varying release size</td>
<td>Number of grid squares within target area above a given threshold</td>
<td>Number of mosquitoes released</td>
</tr>
<tr>
<td>3</td>
<td>Three zones, equal target density</td>
<td>Point, constant release size</td>
<td>Number of grid squares within target area above a given threshold</td>
<td>Number of mosquitoes released</td>
</tr>
<tr>
<td>4</td>
<td>Three zones, varying target density</td>
<td>Driven, constant release rate</td>
<td>Length of route segments where releases occur</td>
<td>Number of mosquitoes released</td>
</tr>
<tr>
<td>5</td>
<td>Three zones, varying target density</td>
<td>Point, varying release size</td>
<td>Number of grid squares within target area above a given, zone-specific thresholds</td>
<td>Number of mosquitoes released</td>
</tr>
</tbody>
</table>

Figure 42. The theoretical area and lattice upon which sterile insect releases were simulated. A 1km² area made up of a lattice of 10m × 10m grid squares.

The fixed release number was set at 2,000 per point, giving a total potential release size, if all points were used, of 100,000 individuals. Varying release sizes were limited to a maximum of 2,000 individuals per point and increased in steps of size 250 from 0 to the maximum.
Figure 43. The three zones targeted for coverage with sterile insects within the theoretical arena. Zones could, for example, represent neighbourhoods in which releases of transgenic insects were planned.

For scenario 4 a simple grid was used to represent a road network (Figure 44). Each section of this grid could be set to an active or inactive state. In an active state releases would occur from the road segment. Releases were assumed to occur at a constant rate; the total number of individuals released along a segment is therefore proportional to the segment length.

Figure 44. Simple grid road-network within the arena from which continuous sterile insect release are simulated.
5.2.12 Field-site application, Itaberaba

During a field trial in Itaberaba, a suburb of the city of Juazeiro, Bahia, Brazil (Latitude: -9° 26' 59", Longitude: -40° 28' 53") a set-release route was driven whilst releases of transgenic insects were conducted. This release route has been examined in the context of the Pareto front, as determined by the MOPSO-ACS.

The following procedure, also shown in (Figure 45), was followed to allow the application of MOPSO-ACS to an applied situation.

1. **Digitising the field site.**
   - A digitised road network was created using satellite imagery in ArcGIS (ESRI, Redlands, CA), combined with local and personal knowledge of the field site.

2. **Creating a road network graph**
   - The digitised road network was split into discrete segments which correlated with edges, $E$, in the graph, $G$. Vertices, $V$, were assigned as intersections on the road map, corresponding to the termini of the edges. Each edge was assigned a cost, $c$, the length of the road segment.

3. **Matrix representation**
   - The graph, $G(E, V, c)$, was represented as an $i \times j$ adjacency matrix where by a direct link was represented by $c(i, j)$.

![Figure 45. Producing the adjacency matrix. The workflow demonstrating the process of taking the field site satellite image, digitising the road network, converting the network to a graph and the resultant adjacency matrix (representing the cost (distance) of moving between all connected vertices (road junctions) on the road network).](image)

The graph representation of the Itaberaba release-site was used as the focus of the MOPSO-ACS.

Optimisation of two scenarios was considered, these were:

1. Continuous driven releases, low target threshold and
2. Continuous driven release, high target threshold.

Releases were simulated at a rate of 30 sterile males per metre. If all road segments saw active releases this would result in a maximum release size of 98,481 individuals (the mean release size at
the Brazil field site for the study described in chapter 4 was 94,770. Low and high thresholds considered were 10 and 40 mosquitoes per 10m\(^2\) respectively (equivalent of 100,000 and 400,000 mosquitoes per km\(^2\)). The resultant Pareto front was compared to the true-release route (driven distance optimised using the ACS) to examine potential inefficiencies in the system.

5.2.13 Field-site application, Panama
A second field site, located in Panama (Latitude: 8° 57' 09", longitude: 79° 41' 51"), was also analysed using the MOSPSO and MOPSO-ACS approaches detailed previously. As for the analysis of Itaberaba, the field site in Panama was digitised to facilitate the analysis. Optimisation of four potential release scenarios was conducted. These were:

1. Point releases, low target threshold.
2. Point releases, high target threshold.
3. Continuous driven releases, low target threshold.

A total of 60 potential release points were included in the point release analysis, the majority of which (57) were located along roads. The remaining three were situated on a sports pitch in the centre of the field site. Each active release point saw a release of 1000 sterile males (maximum release size = 60,000 individuals). Again, for continuous releases a rate of 30 sterile males per metre was used (maximum release size = 62,418 individuals). Low and high thresholds considered were 10 and 40 mosquitoes per 10m\(^2\) respectively (equivalent of 100,000 and 400,000 mosquitoes per km\(^2\)).

5.3 Results
In the following results section I will work through the five theoretical test pieces for the application of MOPSO to sterile insect release. For each scenario the arena, inferred Pareto front and selected examples of estimated optimal solutions will be presented.

I will then go on to present results of the application of the MOPSO-ACS to the Itaberaba and Panama field sites, including the inferred Pareto fronts, a comparison with operational procedures and comments on potential improvements that could be made.

5.3.1 Scenario 1
In Figure 46 the randomly assigned configuration of 50 potential release points across the theoretical arena (split equally so that 25 points were within and 25 points outside of the target areas) is shown.
Figure 46. Scenario 1 arena. The arena, target areas for coverage (grey shaded areas) and randomly assigned potential release locations (purple points) for scenario 1.

The MOPSO produced 56 unique solutions for scenario 1. The estimated optimal solutions and inferred Pareto front associated with scenario 1 are shown in Figure 47. There was no increase in target coverage achieved with more than 30 releases and very small gains associated with increases in the number of release points after approximately 18 releases.
Figure 47. The estimated optimal solutions and inferred Pareto front for scenario 1. Target coverage increases linearly with increasing number of release points to around 20 active release points where the additional coverage achieved with addition releases begins to plateau.

Examples of the coverage obtained from two solutions estimated using the MOPSO are given. The two examples demonstrate solutions with low (few active release points; Figure 48 A) and high (many active release points; Figure 48 B) cost.

5.3.2 Scenario 2
The second scenario considered allowed the number of mosquitoes released at active points to vary. The arena and potential release locations are the same as considered in scenario 1 (Figure 46). The estimated optimal solutions and inferred Pareto front are shown below (Figure 49). Many more
potential unique solutions are available than in scenario 1; the curve is densely populated with points.

![Graph showing number of individuals released vs target coverage](image)

**Figure 49. The estimated optimal solutions and inferred Pareto front for scenario 2.** Many more unique potential solutions exist than in scenario 1, producing a much more densely populated curve.

A total of 280 unique solutions were found by the MOPSO when challenged with scenario 2. Below, two examples of solutions from the inferred Pareto front are demonstrated. The first shows an estimated solution for a small release (Figure 50 A), whilst the second demonstrates an estimated optimal for a large release (Figure 50 B).

![Examples of coverage of target areas](image)

**Figure 50. Examples of the coverage of target areas obtained from solutions estimated by the multiple objective particle swarm optimisation (MOPSO).** Examples are shown for A) a small release of 1,750 individuals and B) a large release of 30,000 individuals.
There is the potential for marginal gains from allowing some heterogeneity in release size from point releases. To assess this the inferred Pareto front for scenarios 1 and 2 may be directly compared (Figure 51). Areas where the Pareto front is higher for a given release size show small gains (maximum of approximately 43 extra grid squares of target coverage for the same number of individuals released) in coverage obtained from the extra flexibility allowed by heterogeneous release sizes.

![Figure 51. Comparison of Pareto fronts. An overlay of the inferred Pareto fronts estimates for scenario 1 (black points) and scenario 2 (grey squares). The additional flexibility of the variable release sizes in scenario 2 provides few incremental gains over the more simple fixed release size approach in scenario 1.](image)

5.3.3 Scenario 3

The third scenario challenged the optimisation procedure to minimise the ‘wasted’ coverage of non-target areas whilst maximising the coverage of target areas. The arena, target areas and potential release points are identical to the previous two scenarios (Figure 46). The inferred Pareto front for this scenario demonstrates the minimum expected wasted coverage for a given number of active release points (Figure 52). The front rises steeply to a plateau; increasing target coverage quickly comes at the expense of increased non-target coverage.
Figure 52. The estimated solutions and inferred Pareto front for scenario 3. Target coverage is measured as the number of grid squares within the target area with mosquito density above a given threshold. Non-target coverage is the same measure for non-target areas. Increasing target coverage quickly comes at the expense of non-target coverage.

The MOPSO produced 56 unique solutions for scenario 3. Examples of the estimated maximum coverage achievable with very low (Figure 53 A) and higher (Figure 53 B) levels of non-target coverage are shown below. Low levels of target coverage can be achieved with very little ‘wasted’ non-target coverage. Figure 53 A demonstrates that all coverage is within the target area boundaries. High levels of coverage, as seen in Figure 53 B, are associated with higher levels of non-target coverage outside of the target areas.

Figure 53. Examples of the coverage of target areas obtained from solutions estimated by the multiple objective particle swarm optimisation (MOPSO). Examples are shown for A) very low non-target coverage (0) and B) higher levels of non-target coverage (371).
5.3.4 Scenario 4
In scenario 4 the analysis progresses from point releases to continuous releases along segments of a route. The arena and associated road network used for the scenario have been shown previously (Figure 44).

The Pareto front, estimated by the MOPSO when challenged with this scenario is shown below (Figure 54).

![Chart showing Pareto front and dominant solutions](image)

*Figure 54. The estimated optimal solutions and the inferred Pareto front for scenario 4.* The inferred Pareto front is less smooth and exhibits more steps under this scenario as turning on further sections of road leads to larger increases in the release number in comparison to additional single release points.

The MOPSO produced 86 unique solutions for scenario 4. Once again, two examples are provided to demonstrate the coverage obtained by solutions with low (Figure 55 A) and high (Figure 55 B) release numbers.
Examples of the coverage of target areas obtained from solutions estimated by the multiple objective particle swarm optimisation (MOPSO). Examples are shown for A) a small release (short length of release segments, 100m) and B) a larger release (longer length of release segments, 2,040m).

5.3.5 Scenario 5
Analysis of the final theoretical scenario, where the coverage thresholds differ across the three target areas, also produces estimated optimal solutions (Figure 56).

A total of 264 unique solutions were found by the MOPSO when challenged with scenario 5. Two associated examples, of a small (Figure 57 A) and large (Figure 57 B) release are shown below. In the high coverage example (Figure 57 B) the differing coverage thresholds in the three target areas can be clearly observed.
Figure 57. Examples of the coverage of target areas obtained from solutions estimated by the multiple objective particle swarm optimisation (MOPSO). Examples are shown for A) a small release of 2,250 individuals and B) a large release of 26,000 individuals. The second plot clearly demonstrates the required heterogeneous levels of coverage achieved across the three target areas.

5.3.6 Field Site example, Itaberaba
The digitisation of the road network at Itaberaba yielded a graph consisting of 19 vertices with 28 associated edges (appendix 1, appendix 2).

The results of the MOPSO-ACS applied to the Brazilian field site are presented in this section. Firstly the inferred Pareto front for this scenario is shown (Figure 58). This figure compares the estimated optimal solutions with the shortest route for an operational release. For the low target threshold coverage used in this analysis (10 mosquitoes per 10m$^2$) it can be seen that the operational point lies far to the right of the Pareto front. In comparison, the operational route for a high target threshold (40 mosquitoes per 10m$^2$) lies closer, but still in a sub-optimal position in relation to the inferred Pareto front.
Figure 58. Comparison of Pareto fronts for the Brazilian field site. The inferred Pareto dominant solutions for low and high target thresholds (circles and squares respectively) and associated Pareto fronts (dashed lines) for the field site at Itaberaba. The operational routes for the low and high target thresholds (pink and blue points respectively) highlight potential inefficiencies in the system (where drivers have to complete longer routes to obtain the same level of coverage as possible from shorter routes).

A comparison of the operational route (Figure 59 A) and estimated optimal routes for low and high target thresholds (Figure 59 B and C respectively) is shown below.

Figure 59. Brazil release route coverage examples. Examples of coverage obtained from A) the operational route at the field site, B) an estimated route with a low target threshold and C) an estimated route with a high target threshold.

5.3.7 Field site example, Panama
The digitisation of the road network at Panama produced a slightly smaller graph than the Itaberaba site consisting of 18 vertices with 23 associated edges (appendix 3, appendix 4).
Firstly, the results for discrete release points are presented. Two scenarios, one with a low target threshold (10 transgenic males per 10m$^2$) and one with a high target threshold (40 transgenic males per 10m$^2$) were considered. The Pareto fronts for these two target thresholds show asymptotic and linearly increasing forms respectively (Figure 60).

![Figure 60](image_url)

**Figure 60. Comparison of Pareto fronts for the Panamanian field site (release points).** The inferred Pareto fronts and dominant solutions for the low- (circles) and high- (squares) target threshold scenarios. Return for increased effort is linear for the high-threshold scenario (more releases always improves coverage by a similar amount). For more than 20 active releases little extra coverage is achieved by increasing the number of release points for the low-threshold scenario.

Examples of low and high coverage dominant solutions for low and high target thresholds are shown in Figure 61.
Figure 61. Panama release point coverage examples. Examples of low (A,C) and high (B,D) coverage solutions for low (A,B) and high (C,D) target thresholds.

As for point releases, Pareto fronts may also be inferred for releases conducted along a route (Figure 62).
Figure 62. Comparison of Pareto fronts for the Panamanian field site (release routes). The inferred Pareto fronts and dominant solutions for the low (circles) and high (squares) threshold scenarios when releasing along a route.

Examples of low and high coverage dominant solutions for low and high target thresholds for releases from a route are shown in Figure 63.
5.4 Discussion

I have presented an adaptation and combination of MOPSO and ACS optimisation techniques applied to spatial and cost components of a sterile insect release. The techniques have been applied to a range of theoretical and field examples, using heuristics to attempt to maximise the coverage of sterile insects over the target area whilst minimising various measures of cost. The methods have proved flexible to a range of different challenges and may provide direct, location-specific, as well as more generalisable rules which can be applied to the planning of operational procedures for future sterile insect releases.

The dispersal of transgenic male *Ae. aegypti* has been simulated from both discrete release points and continuous driven routes. Both of these approaches are flexible, allowing tailoring to specific scenarios. The number, location and magnitude or rate of release from points or route segments may all be varied without disruption to the analytical procedure. This is important, as for the methodology to be a useful applied tool a level of flexibility is vital. It must be noted here that the simulated dispersal of released individuals is simplified. I approximate dispersal on a lattice and
assume a homogeneous environment. In reality, a host of landscape, biotic and other external factors [213] (chapter 4) may influence dispersal, and therefore coverage, and must be considered.

A key feature of the MOPSO and MOPSO-ACS output is the Pareto front. The front can be used to aid key decision making procedures when planning a release in a number of ways. Firstly given a pre-determined amount of effort or cost which may be expended, the front can indicate the optimal level of coverage that could be achieved. The inverse may also be useful, allowing an estimate of the cost required to achieve a desired level of coverage. Secondly, the shape of the front may itself be informative for decision making. A linear relationship between the two objectives indicates a constant return for increased effort (cost). Many of the fronts demonstrated here display diminishing returns (Figure 47, Figure 49, Figure 52, Figure 56 and Figure 60) or step-like functions (Figure 54, Figure 58, and Figure 62) with increasing cost. In these examples, changes in the slope may highlight “decision points”. The points can be used to highlight thresholds above which increased effort does not generate worthwhile increases in coverage. For step-like fronts the “decision points” indicate areas where effort must be further increased over a threshold level to see any advances in coverage.

The non-dominated solutions provided by the optimisation procedure can aid the provision of specific recommendations for spatial aspects of a release. For a given level of cost the optimal distribution of active release points or the optimal release route can be approximated. The approach allows a range of different scenarios to be considered, being adaptable to many different situations. I have demonstrated a range of potential fitness functions (Table 14), however the examples given are non-exhaustive and target-specific fitness functions could be custom-designed to help realise specific goals. Functions may specifically take into account heterogeneity in the target densities as well as fragmented target patches. The third theoretical scenario considered demonstrates a different set up with the aim of maximising coverage within target areas whilst minimising coverage of non-target areas. The situation may be particularly applicable to the design of field trials, where clearly defined treatment and control areas are often required.

The analysis highlights the problems involved with attempting to obtain a homogenous coverage of sterile insects over the target area (assuming uniform wild population densities). With non-uniform release points or routes, as seen with field examples, there will always be hotspots and patchiness in the coverage of released insects. Although sterile male Ae. aegypti released are capable of seeking out wild females and therefore may be better suited to reaching uncovered areas than conventional vector control methods [30], there may still be patchiness in coverage due to the dispersal limitations of released individuals. Computational optimisation of the release procedure provides a
means by which these areas of missed coverage can be minimised. In instances where patchiness is a problem the methods can be used to highlight areas where coverage may be low enough to jeopardise a control effort which may then be specifically targeted for separate point releases. Minimising the hotspots obtained by over-coverage can lead to marked improvements in efficiency (Figure 58, Figure 59).

Further general rules may be taken from these analyses. Assuming radially symmetric dispersal from a point, the optimal arrangement of release points in a homogeneous environment resembles circle-packing problems. Thue’s theorem of circle packing implicates hexagonal arrangements of circles as being most efficient [269] and, under permissible circumstances, we see these solutions begin to materialise in the output. For example, in Figure 48 B, and Figure 50 B we see hexagonal arrangements of points in zone 2. A hexagonal grid is therefore a better foundation for release point location planning instead of the more commonly adopted square grid. However, site-specific heterogeneities must be thoroughly considered and explored before general rules are applied. For instance, assumptions of radially symmetric dispersal or homogenous habitat are likely to be very site-specific and also influence dispersal and therefore coverage, requiring more customised approaches to be explored.

These efficiency gains are further aided by the use of the ACS heuristic to minimise the driven distance required to service road segments marked for active release and inform routing. Even in the case where releases are scheduled for the entire network (Figure 63 D) the ACS heuristic can aid optimal route planning to service the required area, suggesting the most parsimonious route to cover all road segments.

The MOPSO analysis can facilitate comparisons between approaches whereby all release points are of constant size or where size may vary. The comparison between scenario 1 and scenario 2 (Figure 51) indicates that, at the resolution considered, there are only very small gains from varying the release size compared with keeping it constant. Any benefits, in this case, would be greatly outweighed by the increased logistical complexity of a release programme with varying release sizes.

There are a number of limitations associated with these approaches to the optimisation of complex problems. Firstly, the procedures used are heuristics. Solutions produced by these heuristics are attempts to solve the problems to near-optimality in a realistic timeframe. As such, output must be interpreted with this in mind. The individual elements of the MOPSO-ACS have been challenged with various test problems that have known solutions to assess their accuracy and computational speed, performing well in most cases [253, 260, 261]. However, the application I present here sees the
approaches combined and applied to novel, complex scenarios of which there are no standardised
test problems with known solutions. As a result, the performance of solutions relative to the true
optimum remains unknown. However, the computational procedures are likely to systematically
produce solutions much nearer to the true optimum than more ad-hoc human-mediated
approaches, as demonstrated in the Itaberaba field-site example.

A direct outcome of the complexity of these problems is that they can also be computationally
intensive and time-costly. Computational time is dependent on the complexity of the problem, as
well as the resolution of the solution required and the stopping requirements, both of which can be
altered. Furthermore formulation of the algorithms in more efficient computational languages than
R, which was used throughout, could also provide significant time savings. These restraints however
do limit the complexity of potential scenarios, restricting the feasibility of adding in extra layers of
complexity such as habitat heterogeneities, spatial variability in mosquito dynamics or immigration.
Increased target area size will be associated with increased complexity. However, with a
Corresponding relaxation in the resolution of the solution required the methods presented could be
applied at larger spatial scales than demonstrated here.

5.5 Conclusion
For vector control using sterile insects to be successful sufficient coverage of the target area with
released insects must be achieved. Designing effective and efficient release strategies that take into
account the dispersal behaviour of the released insects, target areas and release methods requires
multiple factors to be optimised. I have adapted and applied computational optimisation techniques
using virtual ‘insects’ to optimise releases of sterile insects in the field. The methods presented allow
point-, or driven-releases to be optimised for a range of scenarios. Simultaneous optimisation of
target coverage alongside cost considerations can provide recommendations for site specific release
practice, maximising the chance of a successful release programme.

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Panama field data.
6 An expectation-maximisation approach to quantify spatial heterogeneity in *Aedes aegypti* density with recommendations for ovitrap monitoring.

Ovitraps are a common tool for monitoring mosquito populations and were integral in the collection of data analysed in chapter 4. In this chapter I present methodologies for exploring spatial heterogeneities in mosquito densities from ovitrap data. I then use the methods to examine the influence of trapping effort and the strength of spatial differences on our ability to identify clusters of different mosquito density. The aims of this chapter are twofold: i) to present a novel method for identifying clustering within the *Ae. aegypti* ovitrap data and ii) to give recommendations and provide a critique of using ovitrap field data for the estimation of spatial heterogeneities in *Ae. aegypti*.

6.1 Introduction

The spatial distribution of *Ae. aegypti*, the principal vector of dengue fever, is known to be heterogeneous. Spatial heterogeneities arise at a range of spatial scales, from household [205, 270] to the neighbourhood and city scale [271, 272] and can be influenced by many biotic and abiotic factors such as meteorological variables [273, 274], the level of urbanisation [43, 234, 271], socio-economic status [275] and the presence of available breeding sites [221]. The spatial distribution of the vector also plays an important role in the associated dynamics of dengue fever in humans [276] and may be a significant obstacle to control.

Accurate monitoring and quantification of these spatial heterogeneities in the distribution of *Ae. aegypti* is receiving increased attention due to the importance of accurate targeting and implementation of vector control strategies [55]. With the advent of modern vector control techniques, such as the release of transgenic sterile male mosquitoes [83, 91], there are further added incentives to being able to target and monitor control programmes with a high degree of precision. Currently, a range of monitoring tools is available for quantifying the density and distribution of *Ae. aegypti* populations. The most commonly used approaches include ovitraps, adult traps, sticky-ovitraps and container surveys [277]. Each method has relative pros and cons associated with the ease of use, cost and logistics involved as well as the resultant data quality.

Ovitraps are perhaps the most financially and logistically economical approach to monitoring and have been used extensively [81, 91, 278], but do have limitations, for example biases associated with the availability of alternative breeding sites [53, 279] and the skip-oviposition behaviour (a gravid female laying eggs from a single egg batch at multiple oviposition sites) of the vector [41, 280].
As with many biological systems [281], sampling of mosquito populations is often associated with aggregated, or overdispersed data [282]. This has the potential to cloud inference if not dealt with appropriately. As demonstrated in chapters 3 and 4, clustering at the trap level due to the trap position/attractiveness/accessibility is common and must be considered when assessing for spatial patterns such as spatial autocorrelation in trap catches. Different monitoring methods may be associated with varying levels of overdispersion, violating the assumptions of many simple parametric models and affecting the conclusions which can be drawn from the data [283]

A range of methodological approaches has been used to quantify the spatial heterogeneities associated with Ae. aegypti populations. Geostatistical and GIS-based methods have been widely adopted and are attractive as densities may be visually examined, interpolated and linked to predictive models [271, 284]. However, in many operational vector-monitoring programmes collection of these detailed environmental covariate models may not be feasible as it requires considerable effort to characterise and collect site specific-data for a region. Furthermore, available covariates may not always be able to explain the observed spatial patterns [285]. The identification of hotspots or clusters of significantly high or low densities has been performed using spatial scan statistics [163], such as the Kulldorff SatScan [286]. Spatial scan statistics work by repeatedly drawing spatial windows of varied size and location which represent potential clusters. The observed density within the window is compared statistically with the expected density for the area as a whole to identify windows with significantly high (or low) densities [286]. Both methods rely on the quantity and quality data provided by the monitoring approach to inform inference. A paucity of good quality data, as a result of insufficient sampling, low catch/positive rate or significant overdispersion will lead to considerable uncertainties in predicted densities. To date, there are few published statistical recommendations for Aedes spp sampling regimes [287–290], and considerations relating to sampling effort, sample size and sampling density for ovitrap monitoring of Aedes spp populations are scarce [291].

I present an implementation of the expectation-maximisation (EM) algorithm [292] to estimate mosquito density with respect to spatial heterogeneities. The EM algorithm has been used across a wide range of subject areas for clustering, incomplete-data and latent-variable models, and allows the estimation of maximum likelihood parameters in the presence of a latent variable (for examples see: [293, 294]). This statistical, likelihood-based, approach is designed to identify and quantify areas of differing underlying mosquito density from monitoring data, explicitly accounting for the overdispersed nature of the data.
I use the EM method to investigate the applicability of ovitrap and sticky-ovitrap data for identifying areas of differing mosquito density. I examine the effect of increased trapping effort, characterised by an increased number of traps or trapping time, on the ability to accurately predict spatial heterogeneities. I investigate how the strength of heterogeneities affect predictions and highlight influential limitations associated with attempting to infer true spatial heterogeneities from ovitrap and sticky-ovitrap data.

6.2 Methods

6.2.1 Process overview
An overview of the process by which data were simulated, clustered using an EM algorithm and the performance of the resulting analysis assessed is shown in Figure 64. Specifics of each step are detailed in the following text.
1. Areas and sub-areas
A square area (100×100 grid squares) was divided into $A$ sub-areas using a Voronoi diagram. The seeds for areas were produced by randomly sampling $A$ pairs of XY coordinates. Each grid square was then assigned to the nearest seed, as determined by Euclidean distance. This process was repeated until all resulting sub-areas have areas >10% of the total area.

Each sub-area was assigned a true density characterised by the mean trap catch. Given a baseline mean trap catch, $\sigma_b$, estimated from the data and a pre-determined level of variation between sub-areas, $\lambda$, the assigned area means, $\sigma$, for $A$ sub-areas are
\( A = 1, \quad \sigma = \{ \sigma_b \} \)
\( A = 2, \quad \sigma = \left\{ \sigma_b - \frac{\lambda}{2}, \sigma_b + \frac{\lambda}{2} \right\} \)
\( A = 3, \quad \sigma = \{ \sigma_b - \lambda, \sigma_b, \sigma_b + \lambda \} \)
\( A = 4, \quad \sigma = \left\{ \sigma_b - \frac{3\lambda}{2}, \sigma_b - \frac{\lambda}{2}, \sigma_b + \frac{\lambda}{2}, \sigma_b + \frac{3\lambda}{2} \right\} \)
\( A = 5, \quad \sigma = \{ \sigma_b - 2\lambda, \sigma_b - \lambda, \sigma_b, \sigma_b + \lambda, \sigma_b + 2\lambda \} \).

It follows that all members of a given set, \( \sigma \), must be \( \geq 0 \) and therefore \( \lambda \) must be \( \leq \frac{\sigma_b}{2} \).

Three contrasting datasets were used for baseline estimates \( \{ \sigma_b \} \) characterising data from ovitraps used in areas of high and low mosquito density as well as data from sticky-ovitraps. The ovitrap data were collected throughout a field trial located in Itaberaba, a suburb of the city of Juazeiro, Bahia, Brazil (Latitude: -9° 26' 59", Longitude: -40° 28' 53"). High and low mosquito density trapping was associated with pre- and post-control trapping data, respectively. Sticky-ovitrap data were collected from three urban sites during a field trial in Panama (Latitudes and longitudes: 8° 57' 09", 79° 41' 51"; 8° 57' 35", 79° 41' 52" and 8° 57' 54", 79° 42' 07").

Maximum likelihood estimates and 95\% CIs of baseline mean trap catches and baseline dispersion parameters were obtained using the MASS and fitdistrplus packages in R [207, 295].

2. Trap distribution

Trap locations were distributed randomly across the whole area. Trap coordinates were selected by randomly sampling \( N \) pairs of XY coordinates from the area coordinates.

3. Simulating trap catch

Trap catches were simulated by randomly drawing from the negative binomial distribution with sub-area specific mean \( \in \sigma \) and a fixed dispersion parameter, \( r \), based on the baseline dispersion parameter.

By this process the complete data set \( X_{1Y_1}Z_{1i}A_i,...,X_{nY_n}Z_{ni}A_n \) was produced, where \( X_{iy} \) are the spatial coordinates of trap \( i \), \( Z_i \) is the trap catch of trap \( i \) and \( A_i \) represents the sub area that trap \( i \) belongs to. In the following sections parameters and area-assignments are assumed unknown and to be estimated. This leaves the observed, incomplete data, XYZ, for which we assume a complete dataset, XYZC, exists. The complete dataset includes the latent variable \( C_i,...,C_n \), where \( C_i \) represents the cluster to which trap \( i \) belongs.
4. Estimating the dispersion parameter

The dispersion parameter was estimated from the data \( Z ' s \) and simply assumed to be

\[
r = \frac{\bar{Z}^2}{\text{Var}(Z) - \bar{Z}},
\]

(6.2)

where \( \bar{Z} \) is the mean trap catch and \( \text{Var}(Z) \) is the variance of the trap catch.

5. The expectation-maximisation (EM) algorithm

The implementation of the EM algorithm follows Dempster et al. (1977) [292]. In our case, the observed data are from the density

\[
f(XY_i Z_1, ..., XY_i Z_n) = \prod_{i=1}^{n} \sum_{k=1}^{K} \pi_k f_k(XY_i | \mu_k, \Sigma_k) f_k(Z_i | m_k, r),
\]

(6.3)

where \( \pi_k (k = 1, ..., K) \) is the mixing parameter, the proportion of observations that belong to the \( k \)th cluster, where the \( \sum_{k=1}^{K} \pi_k = 1 \). The \( f_k(XY_i | \mu_k, \Sigma_k) \) component is the multivariate density

\[
f_k(XY_i | \mu_k, \Sigma_k) = \frac{1}{\sqrt{(2\pi)^K | \Sigma_k |}} \exp \left( -\frac{1}{2} (XY_i - \mu_k)^T \Sigma_k^{-1} (XY_i - \mu_k) \right),
\]

(6.4)

with mean \( \mu_k \), covariance \( \Sigma_k \) and \( | \Sigma_k | \) the determinant of \( \Sigma_k \). Finally, the \( f_k(Z_i | m_k, r) \) component is the negative binomial density

\[
f_k(Z_i | m_k, r) = \left( \frac{r}{r + m_k} \right)^r \frac{\Gamma(r + Z_i)}{Z_i! \Gamma(r)} \left( \frac{m_k}{r + m_k} \right)^{Z_i},
\]

(6.5)

with mean \( m_k \) and dispersion parameter \( r \). The gamma function, \( \Gamma \), is

\[
\Gamma(n) = (n-1)!. 
\]

(6.6)

The log-likelihood (of the observed data) is

\[
\ell(\theta | XYZ) = \sum_i \log \left( \sum_{k=1}^{K} \pi_k f_k(XY_i | \mu_k, \Sigma_k) f_k(Z_i | m_k, r) \right),
\]

(6.7)

where \( \theta \) contains the parameters, \( \mu_k ' s \), \( \Sigma_k ' s \) and \( m_k ' s \).
The E-step of the EM algorithm consists of calculating the conditional distribution of missing variable (probability of cluster $i$ being $j$ given the data $XY_iZ_i$ and the current parameter estimates $\theta^t$).

$$T_{ji}^t = p(C_i = j \mid XY_iZ_i, \theta^t) = \frac{\pi_j^t f(XY_i \mid \mu_j^t, \Sigma_j^t) f(Z_i \mid m_j^t, r)}{\sum_{k=1}^{K} \pi_k^t f(XY_i \mid \mu_k^t, \Sigma_k^t) f(Z_i \mid m_k^t, r)}.$$  

(6.8)

For each $i$ the $\sum_j T_{ji}^t = 1$. The $T_{ji}^t$'s are also known as membership probabilities.

For the M-step of the EM algorithm parameter estimates are updated by maximizing the weighted log-likelihood

$$\theta^{t+1} = \arg \max_{\theta} \sum_i T_{ji}^t \ln p(XY_iZ_i \mid \theta).$$  

(6.9)

Maximum likelihood estimates of $\pi, \mu, \Sigma$ and $m$ may all be estimated independently as they appear in separate linear terms, this is detailed in equations (6.10)-(6.13)

$$\pi_j^{t+1} = \frac{1}{n} \sum_{i=1}^{n} T_{ji}^t,$$  

(6.10)

$$u_j^{t+1} = \frac{\sum T_{ji}^t XY_i}{\sum T_{ji}^t},$$  

(6.11)

$$\Sigma_j^{t+1} = \frac{\sum T_{ji}^t (XY_i - u_j^{t+1})(XY_i - u_j^{t+1})^T}{\sum T_{ji}^t},$$  

(6.12)

$$m_j^{t+1} = \frac{\sum T_{ji}^t Z_i}{\sum T_{ji}^t}.$$  

(6.13)

The EM algorithm consists of iteration of the E-step and the M-step:

1. Start with an initial guess for $\theta$ for a given number of clusters.
2. Repeat iterations of the E-step and M-step until convergence or termination criteria are met.

Whilst the EM algorithm is associated with a monotonically increasing likelihood, it may converge to local optima and is therefore not guaranteed to converge to the global optimum. For each run, to increase the probability of finding the global optimum, the algorithm is repeated 20 times with different starting parameters and the maximum likelihood solution chosen.
6. Choosing the best-fit run
For each data set the EM algorithm was run assuming, in turn, 1-6 clusters and the likelihood recorded. In order to compare models the Bayesian information criterion (BIC), a penalised likelihood measure, was used

\[ BIC = -2 \ln(\mathcal{L}) + k(\ln(n) - \ln(2\pi)), \]

where \( \mathcal{L} \) is the maximised likelihood, \( k \) is the number of parameters to be estimated and \( n \) is the number of data points. The run with the lowest BIC was chosen.

7. Assessment of the analysis
All unique coordinates in the 100\times100 area were assigned to the most probable cluster where the probability of point \( i \) being in cluster \( j \) given the coordinates \( XY_i \) and the maximum likelihood parameter estimates \( \hat{\Theta} \) is

\[ p(C_i = j \mid XY_i, \hat{\Theta}) = \frac{\hat{\pi}_j f(XY_i \mid \hat{\mu}_j, \hat{\Sigma}_j)}{\sum_{k=1}^{K} \hat{\pi}_k f(XY_i \mid \hat{\mu}_k, \hat{\Sigma}_k)}. \]

All coordinates were now assigned an associated cluster and predicted mean density. The predicted mean density was compared to the true density. If the predicted density was within \( \pm \frac{\lambda}{2} \) of the true density the assignment was considered correct. The value of \( \pm \frac{\lambda}{2} \) represents an arbitrary, user-defined, level of precision by which the performance of the algorithm is measured. The resulting performance score indicates the percentage of correct assignments. The score is a combined measure of both the ability to correctly rank areas by predicted density as well as the accuracy of the density estimation and is bounded between 0 and 100.

6.2.2 Investigations
The previously detailed process was used to investigate the relationship between trapping effort (number of traps or number of weeks trapping) and the ability to correctly classify areas of differing underlying mosquito density. For each study four trapping scenarios were considered, ovitraps in a high density area, ovitraps in a low density area, sticky-ovitraps and a positive control. The positive control represents fake, desirable data characterised by a high mean catch (\( \sigma_h = 20 \)) and no overdispersion (\( \rho = 1000 \), as \( r \to \infty \) the negative binomial \( \to \) Poisson). For each trapping scenario the number of true sub-areas was also varied from 1 to 5. To take into account the stochasticity in trap location and simulated trap catch each run was repeated 100 times.
The effect of increased trapping density was investigated by increasing the number of traps from 25 to 500 traps in increments of 25 and assessing the resultant performance score at each step. Increasing the number of weeks of trapping data that were combined was also investigated. In this instance 100 traps were used and the sum of trap catches from 1 to 16 weeks was investigated. This takes advantage of the fact that an independent sum of random negative binomial variables is also negatively binomially distributed, leaving the EM-algorithm essentially unchanged.

I also examined the performance of the algorithm whilst varying the strength of heterogeneity between cluster means. Increasing the similarity between cluster means was implemented by reducing the $\lambda$ parameter when simulating means. For each run $\lambda$ was varied in ten steps from $\lambda$ to $\frac{\lambda}{10}$ in order to model sets of clusters with increasingly similar mean trap catches.

Traps were assumed to remain in the original locations throughout the sampling period and weekly trap catches are assumed to be independent conditional on the underlying mean. Sub-area spatial boundaries and mean densities were also assumed to remain constant for the whole time period.

### 6.3 Results

An overview of the parameterised trapping datasets is shown in Table 17. A comparison of the simulated data with the true data is shown in Figure 65.

**Table 17. Summary of ovitrap datasets.** The datasets were used to parameterise the negative binominal distribution to simulate trap catches. CIs are estimated using parametric bootstrapping with 1,000 resamples.

<table>
<thead>
<tr>
<th>Trap type</th>
<th>Outcome measure</th>
<th>Location</th>
<th>Time period</th>
<th>Sample size</th>
<th>Parameter estimates (95% bootstrapped CI)</th>
<th>Mean</th>
<th>Dispersion parameter (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sticky-ovitrap</td>
<td>Captured female Ae. aegypti</td>
<td>Panama</td>
<td>25/Feb/14 – 11/Mar/14</td>
<td>266</td>
<td>0.67 (0.31, 3.69)</td>
<td>0.25</td>
<td>0.18, 0.33</td>
</tr>
<tr>
<td>Ovitrap (low density)</td>
<td>Ae. aegypti eggs</td>
<td>Brazil (end of suppression trial)</td>
<td>04/Jul/12 – 24/Oct/12</td>
<td>7459</td>
<td>8.72 (7.78, 9.59)</td>
<td>0.045</td>
<td>0.042, 0.047</td>
</tr>
<tr>
<td>Ovitrap (High density)</td>
<td>Ae. aegypti eggs</td>
<td>Brazil (pre suppression trial)</td>
<td>22/Jul/10 – 11/May/11</td>
<td>1179</td>
<td>18.87 (15.57, 22.12)</td>
<td>0.10</td>
<td>0.095, 0.12</td>
</tr>
</tbody>
</table>
Figure 65. Ovitrap data simulation. Comparison of simulated (grey bars) and true data (black bars) from A) sticky-ovitraps, B) ovitraps in a low density region and C) ovitraps in a high density region.

Examples of successful and unsuccessful cluster assignments are shown in Figure 66. Panels A and C in Figure 66 show the true sub-areas (as defined by the Voronoi diagram) as well as the randomly distributed traps. Panel B shows a successful cluster assignment of the trapping data, accurately estimating the number and spatial boundaries of the sub-areas as well as the mean trap catch within each sub area. The algorithm fails with poor quality data, as seen in panel D, in this instance the number of sub-areas, as well as the sub-area spatial boundaries and mean trap catch are all poorly estimated.

Visual summaries of the algorithm performance for the different trap-types and number of sub-areas with respect to the number of traps (Figure 67), number of weeks of trapping (Figure 68) and the level of heterogeneity between sub-areas (Figure 69) have been produced. For all summaries the top row represents results using desirable high quality data, acting as a positive control, to highlight the ability of the algorithm to estimate cluster parameters correctly. The remaining three rows represent analyses of simulated data based on field collections using sticky-ovitraps and ovitraps. Columns show the effects of increasing the number of true sub-areas, from 1 to 5.
Figure 66. Clustering algorithm output examples. Examples of A-B) a successful cluster assignment and C-D) an unsuccessful cluster assignment. Panels A and C show the true area divided into five sub-areas with differing mean trap catch (coloured polygons) and the trap distribution (black crosses). Panels B and D show the predicted sub-areas and mean trap catch (coloured polygons) as determined by the EM algorithm in comparison to the true areas (black outlines).
Figure 67. Algorithm performance for a range of scenarios with respect to the number of traps. The ability to accurately discriminate sub-areas with respect to the number of traps, trap type (rows) and number of sub-areas (columns). Boxplots represent the output from 100 simulation runs. Blue points and lines represent the mean score and smoothed (loess smoothing, span=0.75) mean score respectively.
Figure 68. Algorithm performance for a range of scenarios with respect to the number of weeks trapping. The ability to accurately discriminate sub-areas with respect to the number of weeks of trapping, trap type (rows) and number of sub-areas (columns). Boxplots represent the output from 100 simulation runs. Blue points and lines represent the mean score and smoothed (loess smoothing, span=0.75) mean score respectively.
Figure 69. Algorithm performance for a range of scenarios with respect to the level of heterogeneity between sub areas. The ability to accurately discriminate sub-areas with respect to the level of heterogeneity between sub-areas, trap type (rows) and number of sub-areas (columns). Boxplots represent the output from 100 simulation runs. Blue points and lines represent the mean score and smoothed (loess smoothing, span=0.75) mean score respectively.
6.4 Discussion

I have designed and implemented an EM algorithm to identify heterogeneities in mosquito density. When challenged with identifying true sub-areas of differing mosquito density the algorithm performed well given simulated high-quality data. In these, positive-control scenarios, the algorithm achieved consistently high performance scores (Figure 67 A-E, Figure 68 A-E) even when challenged with limited data, characterised by a small number of traps or a small amount of trapping-time. A good ability to differentiate the sub-areas was maintained with an increasing number of sub-areas, or decreasing variation between the mean trap catch of sub-areas. High performance scores are indicative of the algorithm’s ability to accurately discern the spatial characteristics, rank and absolute mean trap catch of each sub-area as well as the number of different sub-areas.

The ability of the algorithm to accurately distinguish true sub-areas was greatly diminished when provided with simulated data representing ovitrap and sticky-ovitrap field data-sets. In comparison with the positive-control data, these field data, and associated simulated datasets, were all highly overdispersed (Table 17). The overdispersion in ovitrap and sticky-ovitrap data is associated with a large number of negative traps (zero counts). Approximately 72%, 79% and 59% of all traps for the sticky-ovitrap, low density ovitrap and high density ovitrap respectively returned zero counts, compared with close to 0% for the positive control. In all instances, regardless of the scenario being tested, this difference in the data resulted in dramatic decreases in the performance scores (frequently reduced by more than half). For most scenarios these reduced scores indicated the algorithms failure to accurately identify true sub-areas to a satisfactory level. For example, a performance score of 50% would be associated with the misclassification of half of the area under investigation. Misclassification may arise via a number of routes: the spatial extent and shape of sub-areas may be poorly approximated, the number of clusters misclassified or the mean trap catch of sub-areas under- or over-estimated. I see this primarily as a data-quality issue; positive-control runs indicate the algorithms ability to overcome these issues when the data quality is very high. The multivariate-normal distributions used to estimate the spatial extent and shape of sub-areas are flexible and accurately map to sub-area boundaries when data are good (Figure 66 B). Likewise in positive-control runs the algorithm consistently estimated the mean trap catch of sub-areas and the number of sub-areas with a high degree of accuracy. These results should be interpreted as a call for a level of caution when trying to identify hotspots, clusters or areas of different densities from highly overdispersed data.

For all data-types (positive control, sticky-ovitrap and ovitrap) increasing the number of traps (at unique coordinates) was associated with an improved ability to accurately identify sub-areas (Figure
Increasing the number of traps was associated with diminishing returns with regards to increases in performance score. Initial increases (25 traps to 200 traps) led to strong improvements in performance score in all positive-control scenarios (Figure 67 A-E) as well as some field-data scenarios (Figure 67 F, G, K, L, M, P and Q) after which improvements in performance score with increasing number of traps diminished or the score plateaued. There are very shallow increases in performance score with respect to increasing number of traps for field-data scenarios with higher numbers (4-5) of true sub-areas.

Combining data from multiple-weeks of trapping was associated with stronger improvements in performance score than increasing the number of traps. Performance score in the positive-control was consistently high. Increasing trapping-time was associated with improvements in performance score for field-data scenarios (Figure 68 A-E). Once again the number of true sub-areas influenced the performance of the algorithm, but trends of increasing performance score with increasing trapping-time were consistent throughout. Increasing the time period over which trapping data is combined is only appropriate if other time-dependent variables that may influence mosquito density can be assumed to be constant for the time period in question.

As expected, performance score consistently decreased with decreasing levels of heterogeneity between true sub-areas (Figure 69). As sub-areas became more alike the failure-rate of the algorithm increased. This is not surprising as, for all data types, there is a limit to the resolution at which two different groups can be differentiated given a finite sample size. The effect of changing levels of heterogeneity became less pronounced as the number of sub-areas decreased as, for high number of sub-areas, the performance score is already very low. In practice, this may not prove influential. When the mosquito densities of sub-areas become more similar operational approaches to mosquito control in those areas will also converge. Therefore, a threshold of resolution for discerning differences between sub-areas is not concerning, as long as sub-areas with mosquito densities that are different at an operationally-relevant level can be identified.

A number of applied, operational recommendations arise from this analysis. Firstly, provided with high-quality data, the EM algorithm can be used efficiently and reliably to identify sub-areas of differing mosquito density. However, as demonstrated, the field data quality from ovitraps and sticky-ovitraps may not be high enough to draw these inferences in a dependable manner. A recent study in Cairns, Australia has also demonstrated the difficulties of using ovitrap data, showing the presence of spatial autocorrelation in adult-trap catches but not in sticky-ovitrap catches [296]. Therefore, of primary importance, is the improvement of the trapping data. This could be achieved by using a different sampling method completely (adult traps, pupal surveys), or by improving the
trapping methods used here. Attempts to enhance ovitraps and sticky-ovitraps have been wide ranging, with improved design [165], placement [168] and attractants [162, 297] all being considered. With reference to the specific question of identifying sub-areas, improvements must aim to reduce the level of overdispersion observed in the data without increasing any bias. Increasing the trap-positive rate, through improved placement, better trap design or attractants may be a good starting point in this regard.

Beyond improvements in the data quality, I have shown that increased trapping effort can be influential on our ability to accurately capture heterogeneities. With the assumption that spatial heterogeneities in cluster shape, size or mosquito density are temporally stable, combining trapping data from multiple weeks may be the most efficient way to improve the outcome of such an analysis.

Increasing the number of traps improves our ability to discern sub-areas but is associated with direct increases in the logistical and financial cost of a monitoring effort and this approach may therefore be restricted in scope. An upper threshold to the required trap density is useful when considering the design of a monitoring programme. The simulations are essentially scale free, in that the size of the area and sub-areas as well as the trap density are all relative and therefore the analysis is appropriate from the neighbourhood- to the city-scale, depending on the level of resolution required. Therefore the investigation could be used to specifically tailor a monitoring programme to a given area.

The results from these analyses must be interpreted with a number of limitations in mind. Throughout I have assumed that the mean trap catch is a reliable proxy for the true mosquito density in an area or sub-area. This assumes that the potential biases associated with ovitraps and sticky-ovitraps, such as the availability of alternative breeding sites and the level of skip oviposition, are homogeneous across all sub-areas. I also assume that sub-areas are distinct, with well-defined borders. In practise this is unlikely. Sub-areas are likely to be less well defined with dispersal across borders and temporal heterogeneities leading to a more ephemeral picture than portrayed in these simulations. It is also assumed that individual trap catches are independent across multiple weeks when, in reality, there may be clustering at the trap level, with the same traps being attractive across multiple weeks.

6.5 Conclusion
This chapter illustrates the potential for identifying sub-areas of differing mosquito densities using an EM algorithm and also the difficulties associated with using ovitrap data to do so. The algorithm
performed well with data that were not overdispersed but more poorly with ovitrap and sticky-ovitrap data that are commonly highly overdispersed. Performance could be improved by combining multiple weeks’ worth of trapping data or increasing the number of traps. The analysis highlights the difficulties in identifying true clusters from overdispersed data.

6.6 Acknowledgments

Many thanks to Oxitec for providing data and facilitating the analyses in this chapter. Thanks to Projeto Aedes Transgênico and Moscamed Brasil for their collaboration and support. I also thank the participants of the field studies in Itaberaba, Brazil and Nuevo Chorrillo, Panama. Thanks to Dr. Roya Haghighat-Khah for sharing the sticky-ovitrap data.
7 Discussion

The common thread and driving motivation throughout this thesis has been to improve our understanding of transgenic sterile insect releases. In turn, the work aims to provide evidence for applied approaches by which the efficacy (performance) and efficiency (cost-effectiveness, where costs may be time/labour/logistical as well as monetary) of releases can be maximised. Optimising releases is a multi-disciplinary challenge, requiring intimate knowledge of the biology and ecology of the released and wild insects, the human-factors and epidemiology in the target location as well as logistical considerations. Improving and maximising the potential of these releases to impact human public health outcomes therefore must be an iterative process. The aim of my work throughout this thesis is to contribute to this feedback loop, providing applied, empirically-supported recommendations for future releases.

The narrative of the five data-chapters follows that of a field release. I have explored early stages of the release process and which life stage to use, made inference about dispersal in the field, as well as analysing monitoring and large-scale field release operations.

7.1 Summary of findings

In chapter 2, and published work [298], I assessed the potential and relative benefits of releases at the adult- or pupal-life stage. The choice of which life stage to release has fundamental implications on the logistical approach to a release programme. Adults and pupae would require different rearing times at the rearing facility and specific transport considerations as well as life stage-specific release methods, protocols and timings. It is therefore crucial to understand, in depth, the dynamics and relative merits of releasing different life stages. As with all the data chapters in this thesis, I applied modelling, computational and statistical techniques to field data to provide insight into the particular research questions of interest. In this chapter, I made use of MRR data from pupal releases to model the dynamics of such a release. A deterministic, compartmental model, parameterised using field data, was used to simulate releases of transgenic pupae. I compared and contrasted these with simulated adult releases using a simple adult model. To assess the ability of each release type, as well as releases of a combination of both life stages, to suppress the wild population the simulations were linked with a model of wild-female dynamics. Using this approach I showed that, for frequent releases, releasing adults was most effective. In certain circumstances, for instance infrequent maintenance releases to sustain suppression, releasing pupae or a combination of adults and pupae was a preferable option.

In chapter 3, I addressed the dispersal of released male insects in the field. The movement of mosquitoes in the field, alongside other fundamental ecological measures such as survival, remains a
key subject central to our understanding of a transgenic insect release. The infrastructure put in place for a release programme provides an opportunity to perform large-scale MRR studies that can inform our understanding of the dispersal of released individuals. In this chapter I fitted dispersal kernels to MRR data within a GLM framework. Fitting a dispersal kernel is a method by which the non-linear relationship between distance and the probability of dispersal can be accurately captured. This allowed a much more finely resolved understanding of the distribution of insects from a release point to be inferred. The parameterised dispersal kernel also produced estimates of key measures such as the MDT, 52.8m (95% CI: 49.9m, 56.8m), to be extracted for comparison with other analyses. In this chapter I repeated this analysis on MRR data from Malaysia which produced a similar kernel, indicating a level of consistency in transgenic male dispersal from a release point across different situations.

Chapter 4 built upon the understanding of male dispersal, and dispersal kernel theory to estimate the dispersal of mated-females in the field. The novel approach presented allowed inference about the dispersal behaviour of mated-females in the wild to be made. Importantly, the method negates the need to rear, handle, mark or release these females, avoiding these intrusive and potentially confounding factors, whilst estimating female dispersal. The analysis performed in this chapter linked ovitraps positive for eggs resultant of a mating between a transgenic male and wild female with estimates of male dispersal. Male dispersal was simulated from a complex release route using the dispersal kernel estimated in the previous chapter. The predicted spatial distribution of released males was then linked to the empirical distribution of ovitrap data at the field site. This allowed the dispersal kernels to be parameterised within a GLM framework as in chapter 3. In this analysis I assessed a number of ways to adjust estimates of dispersal for potentially influential habitat boundaries. The best fit model indicated that dispersing individuals may be ‘reflected’ back into areas of favourable habitat when reaching a boundary between favourable and unfavourable habitats. Females appear, on average, to disperse further than males (MDT=187m) with dispersal probability again being non-linear with respect to distance.

Dispersal simulations are a key component of Chapter five where I used computational optimisation heuristics to try and optimise complex logistical aspects of releases. In this chapter I addressed the challenge of optimising the positioning of release points or the release route driven. I used a number of computational optimisation techniques in order to do this. The MOPSO technique allows two, potentially competing objectives, to be optimised simultaneously. Examples in the chapter include maximising the target coverage of released insects whilst minimising the number of release points that are required. I extended the analysis a step further by nesting a route-finding computational
algorithm, ACS, within the MOPSO approach. By this means, highly efficient driven routes navigating a given road network that maximise the target coverage of released insects could be estimated. Throughout the chapter I used proof of principal theoretical examples as well as field-site case studies to demonstrate the power of the methods.

In the final data chapter, chapter 6, I proposed a method to identify and quantify spatial heterogeneities in *Ae. aegypti* abundance from trapping data. The method used an EM technique to fit trapping data into clusters, estimating the spatial extent and the mean trap catch for each cluster. I demonstrated the ability of the approach to discern clusters using simulated high-quality data. I also tested the performance of the approach against simulated data based on three field-datasets from ovitraps and sticky-ovitraps. The algorithm performed very well with high-quality data but failed regularly with highly overdispersed field-data. Improving the quality of data by combining multiple weeks’ worth of trapping or increasing the trap number could increase accuracy in the ability to discriminate between areas of differing mosquito density. Decreasing the levels of heterogeneity between areas was associated with corresponding decreases in the performance of the algorithm.

### 7.2 Future work

There are a number of areas into which the work presented in this thesis could be extended. I propose three main avenues of future work: studies to advance and further our understanding of the ecology and biology of released and wild mosquitoes, improvement of applied tools and the refinement and further development of the methodological techniques presented. I discuss these options below.

Whilst analysing MRR data relating to mosquito dispersal a number of future research questions of interest have presented themselves. These relate to the further refinement of our understanding of the complex dynamics associated with the dispersal of both released and wild mosquitoes in the field. The methodology presented in chapter 4 corrects and adjusts for potential effects of heterogeneous habitat when considering the dispersal of mosquitoes. In the future, our understanding of these dynamics could be greatly improved through efforts to trap at high intensity across clear habitat boundaries, such as those observed at the field site shown in chapter 4. This could be a stand-alone study or conducted as part of the general implementation of MRR studies at a field site associated with a transgenic mosquito release. Improving our understanding of these boundary-dynamics would improve our ability to confidently predict and simulate the dynamics associated with a field release.
Further improvements to the methodology presented in chapters 3 and 4 could also be made. For instance, inference of the dispersal kernels as well as our general understanding of the drivers of dispersal could be improved by including more specific and detailed explanatory variables into the GLM framework. The collection and analysis of high-resolution meteorological data and spatial explanatory variables alongside the explanatory variables presented could greatly improve our understanding of dispersal at a given field site.

One aim throughout this PhD has been to use my relationship with the industrial collaborator, Oxitec Ltd., to focus on applied problems. In future work the approaches presented here, which have been developed in response to applied questions, could be further developed with an applied end-goal in mind. A suite of software tools that could be used out of the box by teams in the field would be the ultimate goal of this exercise. As an example, the complex methodology applied to route-finding for releases presented in chapter 5 would greatly benefit from a user-friendly software interface to facilitate users to be able to infer efficient release routes from GPS information collected at a field site. Less complex improvements could also be made through the development of a set of very simple to use tools based on basic recommendations supported by evidence from this thesis. These guidelines may act as useful starting-points and general rules-of-thumb for the planning and implementation of a release programme in situations where time, resources or data are scarce.

There are a number of methodological refinements to the techniques I have used which could be pursued in future work. The inclusion of immigration into the ODEs presented in chapter 2 could be performed more elegantly and perhaps with improved realism with a full meta-population model, allowing for specific controls that are heterogeneous in time and space to be implemented across the meta-populations. In chapter 4, multiple simulation runs are performed to allow for stochastic heterogeneity in the dispersal of released males. This is computationally intensive and therefore time-consuming. The inclusion of the estimated male dispersal kernel into a statistical, likelihood-based model that did not require multiple-runs would be of benefit. This would allow inference on the mated-female dispersal to be made in a much more efficient manner.

7.3 Implications of research

The research presented in this thesis has all been conducted with applied objectives in mind. The aim of each chapter is to improve the release of transgenic *Ae. aegypti* as a tool for vector control. The work attempts to facilitate these improvements in a number of ways.

Some chapters, for example chapters 3 and 4, represent my contribution to our knowledge of fundamental aspects of the biology of the vectors with which we work. Vector control remains a vital
tool in the control of many of the world’s most deadly diseases. However knowledge gaps still exist relating to many fundamental aspects of vector biology and ecology [180]. The infrastructure put in place as part of a programme of transgenic Ae. aegypti releases, alongside specific biological characteristics of the released insects, provides unique opportunities to add to the pool of knowledge relating to the biology of disease vectors. Release programmes are associated with releases of very large numbers of insects, providing a perfect foundation for large-scale MRR studies. Furthermore, the monitoring infrastructure of ovitraps and adult traps, combined with transgenic elements such as the heritable fluorescent marker, allow for very high resolution data relating to field behaviour of these insects to be collected. In this way, novel approaches can be applied to data-rich scenarios to allow new and in-depth understanding relating to key biological characteristics of the vector to be made. These advances in our understanding of the vector biology are valuable for almost all control efforts relating to the vector, be it traditional approaches such as spraying with insecticides, SIT or transgenic releases.

Other chapters, for example chapters 2 and 6, use rigorous mathematical modelling alongside statistical and computational techniques to give recommendations on specific field-related research questions. This allows detailed recommendations for future field releases of transgenic Ae. aegypti, using data from previous releases to inform future ones as part of the process of iterative improvement mentioned previously. Furthermore, these recommendations are applicable to a wide range of vector-borne disease applications. Chapter 2 makes recommendations relating to the specific life stage to release. The methods used and conclusions drawn in this chapter may be pertinent to a wide range of vector and insect-pest species that are potential targets for SIT or transgenic insect control. Likewise, the analysis presented in chapter 6 is valid for many Ae. aegypti monitoring efforts. This chapter provides specific recommendations on the limitations of trapping data, characterises ‘ideal data’ and recommends ways by which field data may be improved.

Finally, I have produced chapters, for example chapters 5 and 6, which lay down methodological approaches that can be applied to future releases of transgenic Ae. aegypti. These methodological examples provide specific additional tools that can be used to increase our understanding of the dynamics of a release, plan future releases and analyse the resulting data. Once again, the work presented can be applied to a range of vector control scenarios outside of transgenic Ae. aegypti releases.

### 7.4 Conclusions

Despite decades of effort to control vector-borne diseases many are still a huge public health burden. Indeed, some, such as dengue, are increasing in incidence and range [3]. Despite some
successes, such as the use of LLINs to prevent malaria [9], there remains a scarcity of effective tools to target and control the vectors of disease. As such, the development and refinement of novel vector-control technologies are of upmost importance. Improving our understanding of how these technologies work and the systems that they target is a vital step in bringing new approaches to the forefront of modern vector control efforts. The release of transgenic, ‘genetically sterile’, insects is one such approach and this thesis represents my attempt to contribute to the success of this technology.
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Appendices

Appendix 1. Itaberaba road network graph. $G$ represents the graph consisting of $V$ vertices (road junctions) and $E$ edges (road segments) joining vertices $\{a, b\}$.

$G = (V, E)$

$V = \{1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19\}$

$E = \{\{1, 2\}, \{2, 3\}, \{2, 12\}, \{3, 4\}, \{4, 5\}, \{4, 8\}, \{5, 6\}, \{6, 7\}, \{6, 15\}, \{7, 8\},$

$\{7, 14\}, \{8, 9\}, \{9, 10\}, \{9, 14\}, \{9, 17\}, \{10, 3\}, \{10, 11\}, \{11, 12\},$

$\{11, 13\}, \{12, 13\}, \{13, 19\}, \{14, 16\}, \{15, 16\}, \{15, 18\}, \{16, 17\},$

$\{17, 18\}, \{18, 19\}, \{19, 10\}\}$
Appendix 2. The Itaberaba distance-adjacency matrix (costs are in metres). Values represent the distance between one junction (row number) and another (column number). Dashes indicate that no direct link between the two junctions was present.

<table>
<thead>
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Appendix 3. The Panama road network graph. $G$ represents the graph consisting of $V$ vertices (road junctions) and $E$ edges (road segments) joining vertices $\{a, b\}$.

$G = (V, E)$

$V = \{1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18\}$

$E = \{\{1, 3\}, \{2, 3\}, \{3, 4\}, \{4, 5\}, \{4, 15\}, \{4, 18\}, \{5, 6\}, \{5, 7\},$

$\{7, 8\}, \{7, 18\}, \{8, 9\}, \{8, 10\}, \{10, 11\}, \{10, 17\}, \{11, 16\}, \{11, 12\},$

$\{12, 13\}, \{13, 14\}, \{13, 16\}, \{14, 15\}, \{15, 17\}, \{16, 17\}, \{17, 18\}\}$
Appendix 4. The Panama distance-adjacency matrix (costs are in metres). Values represent the distance between one junction (row number) and another (column number). Dashes indicate that no direct link between the two junctions was present.

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