THE UNIVERSITY OF QUEENSLAND
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Assessing the risk of establishment by the dengue vector, *Aedes aegypti* (L.)(Diptera: Culicidae), through rainwater tanks in Queensland: back to the future.

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Abstract

In Australia, the re-introduction of large, permanent water storage containers (known as rainwater tanks) as a response to climate instability has increased the risk of establishment of mosquito invasions and therefore the transmission of disease. An estimated 300,000 rainwater tanks have been installed into south east Queensland since 2006. This project aimed to identify the risk they pose.

To understand the role that rainwater tanks played in past epidemics of vector-borne disease, such as dengue, this thesis reviewed the history and potential mechanisms for the disappearance of *Aedes aegypti* (L.) from its historical, southern range in Australia. Government surveillance records from Brisbane (the capital of Queensland, Australia) were collated from the period when *Ae. aegypti* was extant until its last detection in 1957. Several factors were likely responsible for the disappearance of the species including the introduction of reticulated water and the removal of rainwater tanks, and increased mosquito surveillance backed up by regulatory enforcement. This thesis demonstrates the value of historical data to help identify potential invasion risks and indicates the dramatic impact that mosquito surveillance, supported by government regulations, can have on *Ae. aegypti* persistence at the margins of its distribution.

Field observations and laboratory trials were conducted to measure the temperature buffered environment of rainwater tanks and determine growth and survival of *Ae. aegypti* in these containers during winter in Brisbane. Abiotic conditions in rainwater tanks were measured and data loggers were placed outdoors in buckets and rainwater tanks during winter and summer. For laboratory experiments daily temperature fluctuations were selected based on the coldest week of the year. Tropical and sub-tropical colonies of *Ae. aegypti* were established from material collected from different location in Queensland to test for their capacity to survive Brisbane winter temperatures. Greater than 48% survival (egg to adult) was observed in all treatments and mean adult development times (egg to adult) took 32 days. As such, a population of *Ae. aegypti* could survive in both buckets and rainwater tanks exposed to the coldest water temperatures in Brisbane.

To test the hypothesis that climate played a role in the disappearance of *Ae. aegypti* in south eastern Australia, I exploited an existing bio-climatic model (using CLIMEX). Survey records from the past 100 years were collated for the species and combined with a number of biological

parameters to predict: a) the historical distribution based on the presence of permanent water storage (rainwater tanks), and b) a natural distribution based on the absence of artificial and permanent water sources. Predictions for Australia were validated against a) international distribution records, and b) the annual population phenology of *Ae. aegypti* from Cairns, Botucatu and Sao Paulo state. If suitable water storage was present, such as rainwater tanks, climate was unlikely to have played a major role in the disappearance of *Ae. aegypti* in eastern Australia. However, when permanent water storage was absent, the majority of locations tested from the historical distribution were unfavourable for the persistence of the species.

To examine the role that rainwater tanks play in the presence and movement of *Aedes* species in urban environments, a mark, release and recapture experiment was designed and undertaken. Results suggested that roads act as barriers to male *Ae. aegypti* but not to female *Ae. notoscriptus* (Skuse), another disease vector that inhabits rainwater tanks. Female *Ae. aegypti* were four times more likely to be captured in traps placed proximal to rainwater tanks than in locations where rainwater tank were absent. Finally, an 'isotropic Gaussian dispersal kernel' was used to describe mosquito dispersal as a diffusion process of movement over time. This provides compelling evidence that permanent domestic water storage facilitated the historical persistence of *Ae. aegypti* across much of eastern Australia.

This thesis examined the role that a network of unsealed rainwater tanks may play in the spread of *Ae. aegypti* in Brisbane. Using a spatially explicit network model in Repast Simphony I simulated the movement of mosquito populations through known locations of rainwater tanks across 140 Brisbane suburbs. Movement was parameterized using an isotropic Gaussian dispersal kernel, a maximum distance of movement, average life expectancy and the probability of *Ae. aegypti* crossing a road over a two week period that was empirically derived. The model was run against a number of scenarios that examined population spread through rainwater tanks based on compliance rates (unsealed or not) and real road grids. Finally, this thesis described key network properties and produced risk maps to inform areas for future mosquito and rainwater tank surveillance.

The outcomes of this thesis demonstrate that large, permanent water storage containers present ideal larval habitat for the re-establishment and persistence of *Ae. aegypti* in areas south of its current distribution in Queensland, Australia. Large numbers of rainwater tanks have been present in south east Queensland for over a decade with little monitoring of their structural integrity or vector presence. Ongoing management of rainwater tanks in south east Queensland will be essential to prevent the establishment of disease vectors and the consequent risk of seasonal transmission of disease.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications & conference presentations during candidature

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Author Jansen C.	Edited paper (15%)
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Chapter 3

Brendan Trewin, Myron Zalucki, Greg Devine and Nancy Schellhorn were responsible for study conception. Expert advice on CLIMEX modelling and outputs were provided by Myron Zalucki. The experimental design, data collection, CLIMEX modelling, GIS mapping, data analysis and interpretation were conducted by Brendan Trewin. Lisa Wockner and David Smith developed the code for and performed the maximum likelihood evaluation of breakpoints. Lisa Wockner and Brendan Trewin provided R code and performed statistical analysis. Lisa Wockner provided guidance with implementation and interpretation of the LME model and outputs. Myron Zalucki, Jonathan Darbro and Greg Devine provided feedback for chapter preparation.

Chapter 4

Brendan Trewin, Jonathan Darbro, and Myron Zalucki were responsible for study conception and expert advice was provided by Greg Devine and Myron Zalucki. Collection of *Ae. aegypti* eggs from Gin Gin was performed by Brendan Trewin and collections from Cairns were by the Tropical Public Services, Queensland Health. All field work in Brisbane, including collection of *Ae. notoscriptus* eggs and container temperatures were performed by Brendan Trewin. Brendan Trewin maintained the laboratory colonies of *Ae. aegypti*. Brendan Trewin conducted the laboratory experiment, prepared larvae and food regimes. Jonathan Darbro provided technical assistance during daily counts and maintenance of larvae during experiment. Anne Bourne and Brendan Trewin interpreted the data and contributed to the statistical analysis for Experiment 1. Brendan Trewin interpreted the data and performed statistical analysis for Experiment 2. Jonathan Darbro, Cassie Jansen and Myron Zalucki provided feedback for chapter preparation.

Chapter 5

Brendan Trewin, Jonathan Darbro, Cassie Jansen, Myron Zalucki and Nancy Schellhorn were responsible for study conception. Expert advice and valuable insights were provided by Nancy Schellhorn, Cassie Jansen, Greg Devine and Jonathan Darbro. Cassie Jansen provided advice on public engagement and ethical approval. For experiment 1; Brendan Trewin, Greg Crisp and Jason Gilmore performed trapping. Brendan Trewin raised, identified and counted all larvae. Brendan Trewin performed and Anne Bourne provided advice for the statistical analysis. For experiment 2; Brendan Trewin, Catherine Liddington, Matt Elmer and a large number of staff from the Wide Bay Public Health Unit and North Burnett, Fraser Coast Bundaberg Regional Councils collected mosquitoes from traps. Brendan Trewin, Greg Crisp, and Jason Gilmore performed the rainwater tank survey. Brendan Trewin, Catherine Liddington and Matt Elmer sorted mosquitoes and Brendan Trewin performed all species identifications. All data sorting and statistical analysis was performed by Brendan Trewin. Brendan Trewin and Dan Pagendam developed the R code and concept of the isotropic Gaussian kernel framework. Mathematical description of the framework was written by Brendan Trewin and Dan Pagendam. Brendan Trewin performed all GIS and graphical outputs. Jonathan Darbro, Cassie Jansen, Nancy Schellhorn and Myron Zalucki provided feedback for chapter preparation.

Chapter 6

Brendan Trewin, Hazel Parry and Nancy Schellhorn were responsible for model conception. Expert advice was provided by Dan Pagendam, Hazel Parry and Nancy Schellhorn. Java code for the simulation model was written by Brendan Trewin and Hazel Parry. Dan Pagendam and Brendan Trewin provided R code for isotropic Gaussian model implemented within the simulation. Dan Pagendam provided the R code for probability of a mosquito crossing a road. Mathematical descriptions of the various equations were written by Brendan Trewin and Dan Pagendam. All statistical analysis, GIS outputs and model simulations were performed by Brendan Trewin. Hazel Parry, Dan Pagendam, and Nancy Schellhorn provided feedback for chapter preparation.

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Keywords

Aedes aegypti, arbovirus vector, dengue fever, elimination, rainwater tanks, water storage, establishment, growth and development, survival, dispersal, Brisbane, Australia.

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0103 Invasive Species Ecology, 60%
0808 Invertebrate Biology, 30%
0104 Landscape Ecology, 10%

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List of Abbreviations

ABS	Australian Bureau of Statistics
AIC	Akaike information criteria
ALE	average life expectancy
ANOVA	analysis of variance
BCC	Brisbane City Council
BGS	Biogents Sentinel trap
CDD	cooling degree days
CDF	cumulative density function
CI	confidence interval
CIMSiM	Container Inhabiting Mosquito Simulation
CSIRO	Commonwealth Science and Industrial Research Organisation
CT	constant temperature
DDT	Dichlorodiphenyltrichloroethane
DNM	dynamic network model
ECDC	European Centre for Disease Control
eDNA	environmental Deoxyribonucleic acid
EI	annual ecoclimatic index
FR_{50}	flight range over which 50% of recaptured mosquitoes have flown
FR_{90}	flight range over which 90% of recaptured mosquitoes have flown
FT	fluctuating temperature
GAT	Gravid *Aedes* Trap
GI	annual growth index

GIS	graphical information system
HDD	heating degree days
iGPDF	isotropic Gaussian probability density function
IIT	incompatible insect technology
LME	linear mixed effects
LSD	least significant difference
MDT	mean distance travelled
MI_W	weekly moisture index
NDM	native distribution model
NOAA	National Oceanic and Atmospheric Administration
MRR	mark, release, recapture
NRW	natural resources and water
NS	not significant
NSW	New South Wales
ODD	overview, design concepts, detail
OLE	optimal linear estimation
OR	odds ratio
PDF	probability density function
PDS	probability of daily survival
PMF	probability mass function
PWM	permanent water model
QIMRB	QIMR Berghofer Medical Research Institute
QLD	Queensland
RSVP	rapid surveillance for vector presence
SEQ	south east Queensland
SD	standard deviation
SI	stress indices

SIR	susceptible, infected, recovered
SIRS	susceptible, infected, recovered, susceptible
SIT	sterile insect technology
SLA	statistical local area
SNM	static network model
SX	stress interactions
TI_W	weekly temperature index
UV	ultra violet
U.S. C.D.C	United States Centre for Disease Control
VEDS	Vector-borne Disease Early Detection and Surveillance
VIF	variation inflation factor
WA	Western Australia
WHO	World Health Organisation

Chapter 1. Review of the Literature

Over the past century, the shift to urbanized living has dramatically increased the geographical spread and incidence of mosquito-borne diseases that are spread by vectors closely associated with urban environments (WHO, 2012). Despite the successes in the mid-1900s to eliminate infectious diseases associated with mosquitoes, the slow deterioration of public health infrastructure has resulted in a new era of epidemic dengue, chikungunya and Zika across the tropics (Gubler, 2011). Urbanization and a changing climate have increased the demands on public infrastructure (such as waste and sewer management, health services and the provision of clean water), demands which have been unable to match the pace of rapidly increasing human populations (Gubler, 2011). Subsequently, shifting lifestyles in developed and developing countries have provided an increasing number of domestic larval habitats (such as car tyres, discarded plastic containers and water storage vessels) that provide consistent population sources and a mechanism for establishment and geographical spread of insect disease vectors (Reiter, 2007, Gubler, 2011).

In Australia, the human response to drought in the early 2000s led to the mass installation of rainwater tanks, largely through government run rebate schemes, regulated water restrictions and mandated installations (Gardiner, 2010). The rapid re-expansion in the number of these large water storage containers has resulted in an estimated 300,000 rainwater tanks now installed throughout major urban centres in south eastern Queensland (Moglia et al., 2013b). *Aedes aegypti* (Linnaeus)(the yellow fever mosquito) is the only vector of dengue fever virus on mainland Australia and is also the primary vector of yellow fever, Zika and chikungunya viruses elsewhere in the world (Harrington et al., 2005, Russell et al., 2009). This species is highly associated with human water storage, and the re-introduction of permanent water holding containers has increased the risk of re-establishment by *Ae. aegypti* into areas south of its current distribution in Queensland (Kearney et al., 2009, Beebe et al., 2009, Trewin et al., 2013).

Consequently, the purpose of this study was to evaluate the importance of rainwater tanks for the re-establishment of *Ae. aegypti* in Brisbane. This thesis was written as a series of stand-alone chapters, each with its own set of questions and hypotheses that form a cohesive storyline around the importance of water storage on the distribution of *Ae. aegypti* in Australia. Chapter 2 provides the rationalization for this thesis by seeking an explanations for the historical elimination of *Ae. aegypti* and dengue transmission in Brisbane. In Chapter 3, I apply predictive CLIMEX models to estimate the potential distribution of *Ae. aegypti* in Australia based on current and historical surveillance records. From these models, I explore the role that climate may have played in the historical disappearance of the species from its southern distribution in Australia. To assess whether winter temperatures were limiting for *Ae. aegypti* in Brisbane, I assess the differences in larval development and survival between two Australian populations of *Ae. aegypti* and a population of *Aedes notoscriptus* (Skuse)(Chapter 4). Through a mark, release and recapture methodology, I assess the importance of rainwater tanks in the distribution and movement of disease vectors in an urban landscape (Chapter 5). In Chapter 6, I simulate *Ae. aegypti* movement between rainwater tanks in a spatially explicit network approach. Non-compliance rates of rainwater tanks are varied to understand which areas of Brisbane represented the highest risk for spread once established. Finally, a synthesis of this thesis is presented in Chapter 7, which contextualizes the broader risk, consequences and implications that the mass-installation of rainwater tanks now presents to the people of south east Queensland and elsewhere in Australia.

1.1 *Aedes aegypti* (Linnaeus)

Aedes aegypti was originally described by Linnaeus (1762) as *Culex aegypti* and has been known as *Culex bancrofti* (Skuse, 1889), *Stegomyia fasciata* (Theobald, 1901), *Mimeteomyia pulcherrima* (Taylor 1919) and *Aedes* (*Stegomyia*) *argenteus* (Edwards 1921). It was Dyar (1920) that first used the name *Aedes aegypti* which has been taken up as the valid nomenclature (Debenham and Leeson, 1987). An attempt by Reinert et al. (2004) to raise the *Aedes* subgenus *Ochlerotatus* to a generic rank meant a change from *Ae. aegypti* to *Stegomyia aegypti*. The change has attracted many criticisms and caused a great deal of controversy amongst medical dipterists (Savage and Strickman, 2004). The Journal of Medical Entomology and other organisations have clearly laid out the arguments for and against the name changes and state that more research needs to be done before they will accept the *Stegomyia* classification (JME, 2005). Recent analysis suggests a restoration of a simplified classification system to preserve stability within the Aedini classification (Wilkerson et al., 2015). There is still no consensus on the appropriateness of these name changes to the Aedine genera, so for the purpose of this thesis, *Aedes* will be used as the generic name.

1.1.1 Adult characteristics

The two crescent-shaped patches of white on black scales on the thorax of the adult mosquito form the species' most distinguishing characteristic. These patches of scales run along each side of the anterior half of the scutum. Two fine white lines then run straight toward the posterior of the thorax, nearly to the scutellum. Between the crescents are two narrow parallel white lines that, together with the other markings, form a characteristic ‘lyre’ pattern (Figure 1; Christophers, 1960). Other identifying features include a proboscis without banding, a white line on the anterior mid-femur and a patch of white scales on each side of the clypeus in the female but bare in the male (Ludlow, 1911). The wings are usually dark scaled with a small spot at the base of the costa (Christophers, 1960). The male genitalia have been described by a number of authors and include the following characteristics; a ninth tergite with two prominent conical lateral lobes with hairs and a deeply V-shaped embayment; style of the clasper regular in form, without a comb like row of spiniforms, but widened in the middle; lateral processes of the paraprocts nearly as long as the terminal processes and the lateral plates of the phallosome with fine teeth (Ross and Roberts, 1943, Iyengar and Menon, 1955, Christophers, 1960).

Figure 1. *Aedes aegypti* female. © Chen Wu 2017

1.1.2 Larval characteristics

Larval characters that uniquely identify *Ae. aegypti* include; smooth antennae with simple hair; a well-defined row of 8-12 comb teeth in a single row with strong basal lateral denticles; 15-20 pecten teeth on the siphon, not widely spaced; anal saddle without strong marginal spicules and meropleural and metapleural tubercles with thorn like spine twice as long as the basal portion of the tubercle (Christophers, 1960, Belkin, 1962). It is the 'pitchfork' shaped comb teeth that are displayed in a single row that distinguish the larval *Ae. aegypti* from *Ae. notoscriptus* (spatulate or rounded comb teeth) and *Aedes albopictus* (Skuse)(straight 'thorn like' comb teeth).

1.1.3 Pupal characteristics

Pupal characteristics that distinguish *Ae. aegypti* include; trumpets triangular and without transverse folds; paddles with a single hair at about one-quarter of the length; no accessory hair and abdominal segments II-VI with spine like chaeta. The unbranched condition of hairs and distinct denticles on the internal and external margins on the paddle distinguish this species from all South Pacific *Aedes/Stegomyia* (Christophers, 1960, Belkin, 1962).

1.1.4 *Aedes aegypti* as a vector of dengue fever

Mosquito-borne pathogens present a substantial threat to human health, exerting a large burden on economic and health systems globally. The re-emergence of the four dengue viruses over the past 25 years has led to much human morbidity, with major outbreaks in Asia, Oceania, Europe and the Americas (Gubler and Trent, 1993). By 2012 dengue had been declared the most important vector-borne viral disease of humans, and has likely contributed more in terms of morbidity and economic cost than malaria (Gubler, 2012). The World Health Organisation describes dengue fever as the most rapidly spreading mosquito-borne viral disease of the past 50 years (WHO, 2012). The global incidence of dengue fever and dengue haemorrhagic fever has increased 30-times since the 1960s, with an estimated number of worldwide infections ranging between 50 to 390 million cases and 20,000 deaths annually (WHO, 2012, Bhatt et al., 2013). The observed increase in disease cases is due largely to growth in urbanization, trade, international travel and increased chemical resistance and geographic spread of its major vectors, *Ae. aegypti* and *Ae. albopictus* (WHO, 2012).

Historically, much of northern Australia has experienced large dengue epidemics (Russell et al., 2009). The earliest published account of dengue fever in Australia dates to 1873 (Lumley and Taylor, 1943) and the first extensive epidemic was experienced in the goldfields of

Charters Towers in March, 1885 when up to 90% of the population was affected and the first official death attributed to the disease in Australia was recorded (Redmond, 1928). At this time the disease was called “Colonial Fever” in the north, and reports of the disease arriving on ships from New Caledonia, Fiji and Noumea were recorded in Sydney (Debenham and Leeson, 1987). Four large epidemics in 1897/98, 1905, 1911 and 1925/26 in Queensland and northern New South Wales were responsible for 733 deaths (Hare, 1898, Lumley and Taylor, 1943, Debenham and Leeson, 1987). These outbreaks swept through Brisbane, affecting between 70-90% of the population at the time. Deaths due to dengue were recorded in Queensland Health statistical records and the early medical literature (Hamlyn-Harris, 1931, Lumley and Taylor, 1943). The disease was not legally ‘notifiable’ in Queensland at the time, so many of the early case histories or infection rates can only be inferred by observations by medial entomologists (Derrick and Bicks, 1958). Based on a conservative infection rate of 75% during early epidemics, it has been estimated that a total number of 1,855,000 dengue infections have occurred in Australia since the introduction of *Ae. aegypti* in the 1800s (Canyon, 2008).

Currently 75% of the global population exposed to dengue fever is in the Asia-Pacific Region (WHO, 2012). Dengue fever is not endemic in Australia and outbreaks of the disease are reliant upon importations of the virus, the presence of the vector and a naive human population. A lack of herd immunity in human populations can result in a rapid and widespread epidemic of dengue fever (Halstead, 2008, Ritchie et al., 2013). Susceptibility and movement of a human population, effect of temperature, basic reproduction number (R_0), rainfall and the heterogeneity of *Ae. aegypti* spatial distribution can influence the explosive nature of the virus (Halstead, 2008). No large epidemics have occurred in Queensland since the 1955 (Doherty et al., 1967) and no effective vaccine or antiviral therapy is currently available to protect communities from outbreaks of dengue (Halstead, 2014). Thus it can be assumed that the majority of people in Brisbane in particular and Queensland in general are susceptible to dengue virus infection.

In 2009, Queensland experienced one of its largest outbreaks of dengue fever in 50 years, during which 931 cases and one death was confirmed (Queensland Health, 2009). Since this time, a rise in imported cases of mosquito-borne diseases has increased the risk of local transmission of dengue, chikungunya and Zika viruses in all regions of Queensland where *Ae. aegypti* is present (Knope et al., 2013, Huang et al., 2016) and represents a clear risk to the dengue-naïve population of Brisbane should the vector ever become established in the region.

1.2 *Aedes aegypti* distribution

1.2.1 Global: historical and current distribution

In the past, *Ae. aegypti* has spread out of Africa via merchant ships (where freshwater reservoirs could maintain populations of the species) and populations would establish at ports where these ships would stop to trade (Christophers, 1960). Populations of *Ae. aegypti* would then spread from these coastal ports throughout entire countries (Lumley and Taylor, 1943, Christophers, 1960). During and after the world wars, conditions favoured the expansion of *Ae. aegypti* populations throughout the world (Gubler and Clark, 1996). A large and coordinated campaign during the 1950s and 1960s, led by the Pan American Health Organisation, saw significant declines in the distribution of the species across the world and this included Australia (Soper, 1963, Soper, 1967). Elimination of the species from Europe, North Africa and most parts of the Americas was attributable to highly effective management and the widespread use of Dichlorodiphenyltrichloroethane (DDT)(Schliessmann and Calheiros, 1974). A re-emergence of the vector throughout its historical range has been observed since the 1970s, mainly due to a reduction in effort and the effectiveness of control efforts, resistance to chemicals and unplanned and uncontrolled urbanisation and trade (Gubler and Clark, 1995).

Currently, *Ae. aegypti* has a wide geographical distribution in tropical and subtropical areas which is documented extensively in the literature (Figure 2)(Hopp and Foley, 2001, Eritja et al., 2005, Honório et al., 2009, Russell et al., 2009, Beebe et al., 2009, Basso et al., 2012, Eisen and Moore, 2013, Kraemer et al., 2015b). The highly urban nature of the species means that human activity also greatly influences the distribution. Anthropogenic factors predicting distribution include urbanisation, socio-economic status, water supply quality, sanitation standards, vector management and public health services (Reiter, 2001, Higa et al., 2010). The increasing trend of high density urbanisation in developed and developing countries has dramatically altered the suitability of the landscape for mosquito vectors. Huge demands for critical infrastructure (such as reticulated water) in developing countries, have resulted in governments failing to provide reliable access to these services (Schmidt et al., 2011). Subsequent increases in domestic water storage have altered urban landscapes and provided more stable or numerous supplies of larval habitat. In developed countries, climate instability has resulted in water hoarding practices that reduce burdens on reticulated water supplies (Jansen and Beebe, 2010, Trewin et al., 2013, Moglia et al., 2013b, Eisen et al., 2014). The subsequent proliferation of larval habitat has increased the risk of vector persistence and disease transmission into areas where the species has historically been present (Jansen and Beebe, 2010, Trewin et al., 2013, Eisen et al., 2014, Chapter 2).

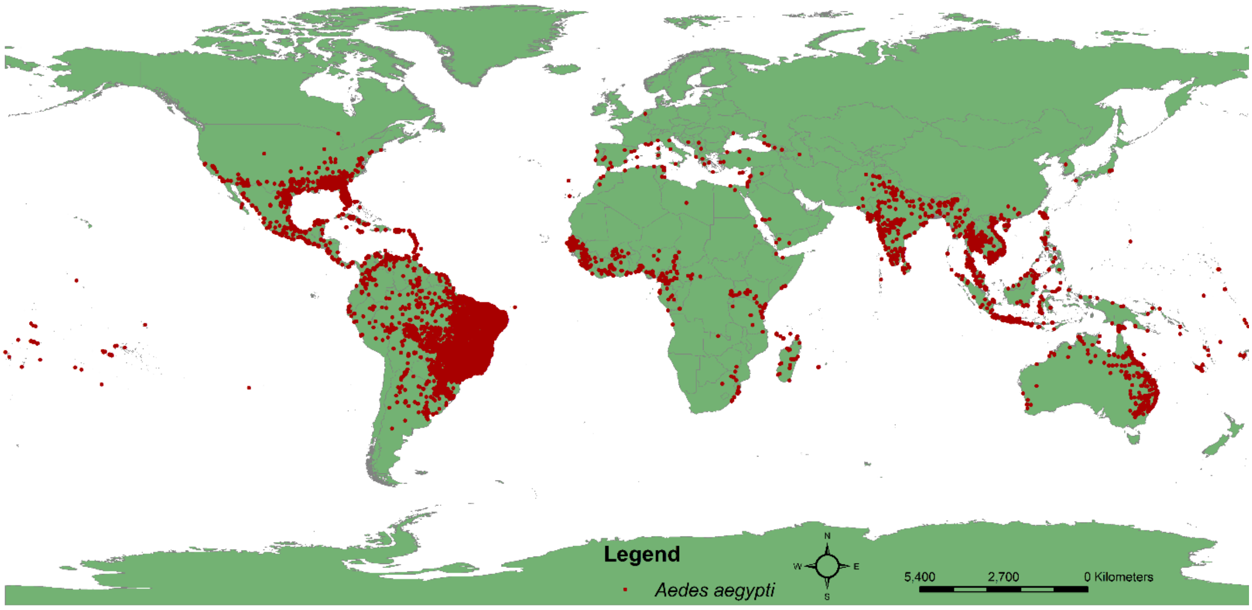

Figure 2. Global distribution of *Aedes aegypti* including historical records. Presence (red dots) sourced from Kraemer et al. (2015b), Beebe et al. (2009) and Appendix 1. Map base layer sourced from GADM (University of California, Davis, 2012) digital boundary data.

1.2.2 The invasion biology of *Aedes aegypti*

Invasive species have a major influence on native species, natural ecosystems and human fragmented landscapes and these effects have been documented for decades (Lodge, 1993, Mack and D'Antonio, 1998, Elton, 2000). Invasive species are now a major element of global change science and attempts to quantify the costs of these invasions worldwide suggest that they require hundreds of billions of dollars to manage annually (Pyšek and Richardson, 2010). The invasion by *Aedes* mosquitoes into Europe, the Americas, Asia and Australia, whether due to climate change or human mediated transport, has emerged as one of the most important topics for vector management in developed countries (Benedict et al., 2007, Medlock et al., 2012). Australia currently faces the risk of potential invasion by both *Ae. albopictus* and further range extension by *Ae. aegypti* (Cooper et al., 2005, Russell et al., 2009, Williams, 2012).

Biological invasions have traditionally been characterized by three essential stages which include an introduction phase, the establishment phase and subsequent dispersal and spread (Williamson, 1996, Freckleton et al., 2006). Originating from Africa, *Ae. aegypti* is highly invasive and has established itself across most of the northern and southern hemispheres, between the January and July 10°C isotherms respectively (Christophers, 1960). The biology of *Ae. aegypti* is unique amongst mosquito species, in that it is closely associated with human habitation and it exhibits certain life history traits that predispose it to long distance introductions into new environments (Christophers, 1960). *Aedes aegypti* primarily feeds on humans, while other vertebrate species only represent a small proportion of blood meals (Jansen et al., 2009). Oviposition is preferentially undertaken in artificial containers that may include buckets, pot plant bases, tyres and water storage (like rainwater tanks) in and around domestic dwellings (Christophers, 1960). Eggs are resistant to desiccation and, thus, have the ability to survive dry periods of many months and produce viable offspring if temperatures are optimal (Christophers, 1960, Sota and Mogi, 1992, Eisen et al., 2014). These traits have enabled *Ae. aegypti* to exploit human mediated transport and successfully invade the majority of tropical and sub-tropical regions across the world (Figure 2; Jansen and Beebe, 2010).

In Australia extensive work has categorized container types associated with the presence of *Ae. aegypti*. Barker-Hudson et al. (1988) define eight categories of domestic habitats. The authors found water storage contributed to significant larval habitat in rural areas, while garden accoutrements such as pot plant bases and striking pots provided the majority of sites in more urbanized environments (Barker-Hudson et al., 1988). Tun-Lin et al. (1995) were the first to suggest that certain container types (such as rainwater tanks) contributed disproportionately to the *Ae. aegypti* population and that these ‘key containers’ should be preferentially targeted during control campaigns. North Queensland surveys by Montgomery and Ritchie (2002) concluded that roof gutters were an uncommon but productive source of *Ae. aegypti* larvae in Cairns, while Ritchie et al. (2002) identified rainwater tanks as primary larval habitat in surveys of Torres Strait during a dengue outbreak in 2002.

Like all insects, *Ae. aegypti* establishment occurs within physiological and climate suitability gradients where previous generations have successfully reproduced in. These gradients range from near optimal at the core of its distribution to borderline suitable and unsuitable at the margins of geographical distribution (Eisen et al., 2014). *Aedes aegypti* has adapted to a relatively wide range of climates and domestic environments and this is supported by the wide geographic distribution of the species (Figure 2; Brown et al., 2011). This wide distribution may be due to a combination of life-history traits like rapid colonization (Kay, 1999), and genetic characteristics that allow for phenotypic plasticity (Baker, 1965, Gray et al., 1986) or rapid evolutionary change (Sakai et al., 2001). Brown et al. (2011) has described *Ae. aegypti* as a "rich in genetic, morphological and ecological variation" and suggest that rapid adaptation to human environments has led to the successful establishment of the species in new locations over the past few centuries. Genetic studies have shown variants of Asian *Ae. aegypti* to be less genetically diverse than African strains (Tabachnick, 1991, Brown et al., 2011) and Australian strains to be less diverse than Asian strains (Beebe et al., 2005, Endersby et al., 2009). *Aedes aegypti* has been present in Australia for over one hundred years and some populations show distinct genetic variation divided by geographic distance (Endersby et al., 2009). Endersby et al. (2009) show a distinct genetic divide between coastal and inland populations and results suggest that mixing between populations is rare. They also suggest that Australian *Ae. aegypti* populations have a lower allelic richness than those from other south east Asian countries, likely due to a bottleneck associated with colonization of Australia (Endersby et al., 2009). Two separate *Ae. aegypti* establishments (and subsequent eliminations) that recently occurred in Tennant Creek, Northern Territory, in 2005 and 2011 were genetically similar to populations from Townsville, Queensland (Beebe et al., 2005). These invasions are an example of the species overcoming Allee effects, and establishing in semi-arid areas where climate is sub-optimal to that found at the source population. Persistence of *Ae. aegypti* populations in arid and semi-arid environments is a common global occurrence (Angel and Joshi, 2008, Walker et al., 2011, Khormi et al., 2014, Metzger et al., 2017). Limited mixing between populations in Australia means it is unknown how long *Ae. aegypti* have been present in southern Queensland, and therefore, it is possible that these populations may have adapted to regional climatic conditions over a long period of time.

In regions where climate is suitable, establishment of a self-sustaining population is driven primarily by the landscape into which populations are introduced. Close association with domestic dwellings means these drivers are primarily the availability of oviposition sites and adult access to blood meals (Christophers, 1960, Brown et al., 2011). Once established, *Ae. aegypti* readily invades suitable habitat as it spreads through the urban environment (Reiter, 2007). *Aedes aegypti* is capable of seeking out subterranean and cryptic habitats that enhance persistence after periods of low rainfall (Kay et al., 2000, Barrera et al., 2008). Despite a keen interest in controlling mosquito related diseases, the pervasiveness of *Ae. aegypti* has meant that attempts at eliminating the species have failed due to a lack of economic resources and the ineffectiveness of current methods of control (Gubler, 1989).

1.2.3 Historical Australian distribution

Aedes aegypti is estimated to have been introduced to Australia during the second half of the 19th century through increased immigration (Marks, 1975), although some have theorized its arrival with the First Fleet (Debenham and Leeson, 1987). Historically the distribution of *Ae. aegypti* has included Western Australia, Queensland, New South Wales, the Northern Territory and possibly Victoria (O'Gower, 1956, Russell, 2009). Maps of the historical distribution of *Ae. aegypti* in Australia can be found in Russell et al. (2009) and Kearney et al. (2009)(Figure 3). The most southern reported distribution in eastern Australia includes Calcairn (36° S) and Natimuk (37° S) near the Victorian border and, in Western Australia, Harvey (33° S)(Debenham and Leeson, 1987, Russell et al., 2009). There is substantial evidence for the historical presence of the mosquito in Brisbane (27.4698° S, 153.0251° E) the largest city Queensland, through entomological surveys and dengue fever outbreaks (Bancroft, 1906, Scott, 1911, Cleland et al., 1918, Cooling, 1923, Hamlyn-Harris, 1927b, Hamlyn-Harris, 1931). However, a comprehensive collation and analysis of historical surveys undertaken by government organisations has never been completed. From an east coast perspective, the historical southern (cold limiting) and western (moisture limiting) distribution of the mosquito is thought to have been facilitated by the buffering of temperature and desiccation stress that rainwater tanks provided (Lumley and Taylor, 1943, Russell et al., 1984).

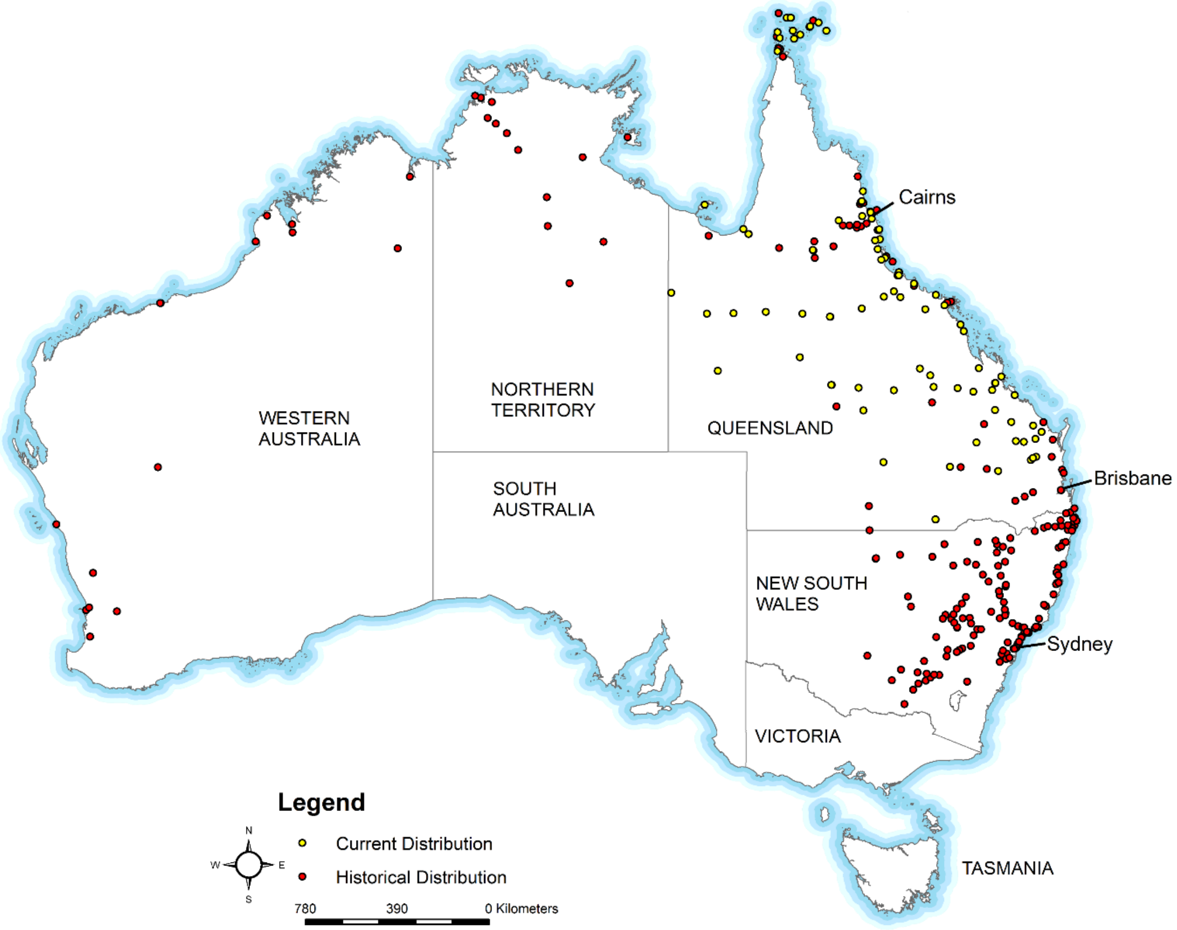

Figure 3. Historical distribution of *Aedes aegypti* in Australia 1887-1981. Current distribution is documented from records between 1982 and 2016. Location records from Beebe et al. (2009) and Appendix 1. Primary urban centres of concern are Brisbane (27.4698° S, 153.0251° E), Sydney (33.8688° S, 151.2093° E) and Cairns (16.9186° S, 145.7781° E). Most southerly location of confirmed presence is Calcairn, New South Wales (35.65° S, 147.03° E). Map base layer sourced from Australian ABS digital boundary data (Australian Bureau of Statistics, 2016) and licensed under Creative Commons 2.5 (2016).

The spread of *Ae. aegypti* into western areas of eastern Australia can be attributed to the massive increase in steam engines across the country and their attendant water storage (Hamlyn-Harris, 1931, Lumley and Taylor, 1943). By federation in 1901, all states except Western Australia were linked by 20,000 km of railways (Figure 4; Australian Government Website, 2014) and in 1930 Hamlyn Harris stated "*A. argenteus* (*Ae. aegypti*) is making steady inroads into all inland towns in Queensland and most assiduously follows the railway lines. Its presence in any community is evidence of the neglect of Local Authorities in carrying out the provisions of the Health Acts" (Hamlyn-Harris, 1931). In 1943, Lumley and Taylor (1943) noted that "(he) had never travelled on long-distance train in both northern New South Wales and Queensland without finding adults of this mosquito". In Australia, it is common for *Ae. aegypti* surveillance to occur only after local disease transmission (Ferguson, 1926, Kay et al., 1983, Kay et al., 1984). Ferguson (1926) noted that *Ae. aegypti* had been present in Newcastle, NSW for 20 years and that its presence in inland towns may have been overlooked until the attention of authorities was drawn by an outbreak of dengue fever in 1925. Kay et al. (1983) noted a substantial decrease in the distribution of *Ae. aegypti* in eastern Australia during the mid-to-late 20th century, likely due to population decline or waning interest by local authorities. Until the recently recorded incursion in Tennant Creek, populations of the species were last identified in the Northern Territory in 1956. Further, populations were last recorded in Western Australia in 1970, and New South Wales in 1948 (Debenham and Leeson, 1987). Queensland saw a similar range reduction to localised populations in northern Queensland by the mid-1960s (Marks, 1975, Russell et al., 2009). Hypotheses for this range reduction have been related to a number of factors and these are discussed in section 1.2.5.

1.2.4 Current distribution in Australia

In Australia, the core distribution of *Ae. aegypti* is restricted to the north and central (including southern) parts of Queensland where conditions of temperature and rainfall are favourable for year-round presence of all life stages (Williams et al., 2010). Surveys for *Ae. aegypti* since 2010 have identified eastern Australian populations as far south as Charleville in the west of Queensland and Wondai and Goomeri in the east (Figure 5; VEDS, 2014). Current surveillance efforts are patchy and historical surveillance records are incomplete. The origins of this apparent expansion in range are therefore unknown. The mosquito may be actively expanding southwards, or these populations may be remnant of the original distribution (Marks, 1975, Kay et al., 1984, Sinclair, 1992). Wondai is approximately 150 kilometres from Brisbane, and represents the closest population of the mosquito to a city of 2 million people. Entomological surveys of Brisbane in from 1995 to 1997, 2007 to 2009 and a broad 5,000 premises survey from 2010 to 2013 did not detect *Ae. aegypti* (Kay et al., 2008, Trewin et al., 2013, Heersink et al., 2015). There have been no locally-acquired cases of dengue fever in the city since 1948 (Brisbane City Council, 1948, Queensland Health, 2017) and it seems probable that A*e. aegypti* has been absent from Brisbane for some decades.

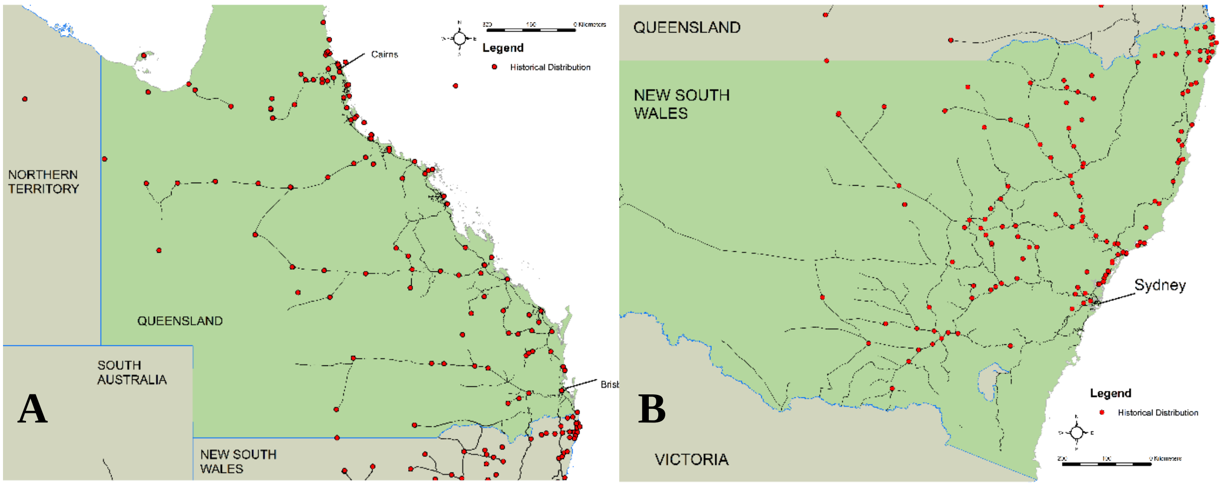

Figure 4. Historical *Aedes aegypti* distributions (red spots) in Queensland (A) and New South Wales (B) showing relationship with train lines (black lines). Location records from Beebe et al. (2009) and Appendix 1. Map base layer sourced from Australian ABS digital boundary data (Australian Bureau of Statistics, 2016) and licensed under Creative Commons 2.5 (2016).

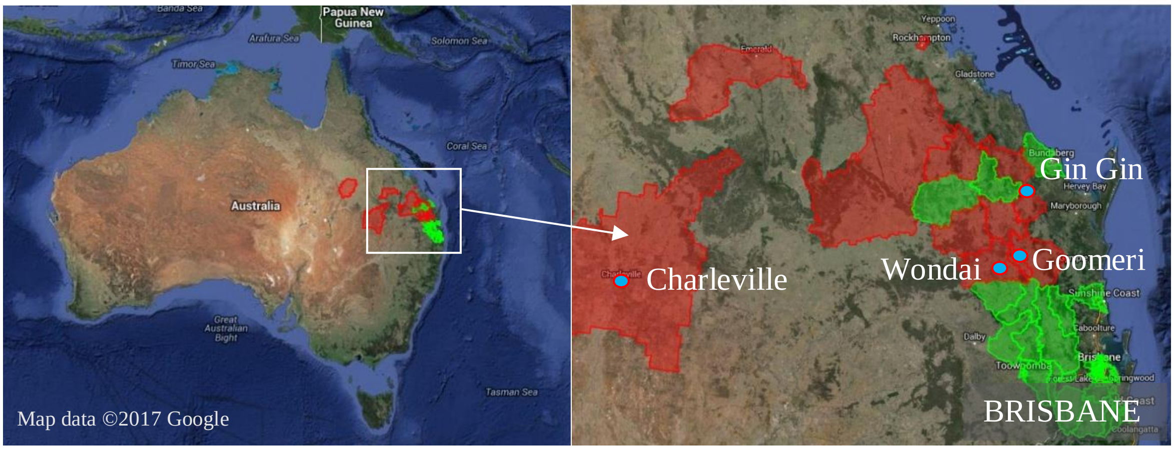

Figure 5. Southern range of *Aedes aegypti* in relation to Brisbane, including identifications in Charleville (2011), Gin Gin (2011) Wondai (2013) and Goomeri (2012)(VEDS, 2014). Red represent local governments where surveys between 2010 and 2013 revealed *Aedes aegypti* as present, green where not present and non-coloured as not-surveyed. Map data: Google, DigitalGlobe.

1.2.5 Absence of *Aedes aegypti* since the mid-1900s. Hypotheses for a range retraction

Hypotheses offered by medical entomologists for the southern range retraction of *Ae. aegypti* have been related to social and technological change. These include; the decline of steam rail and its attendant water storage infrastructure, cleaner yards after the invention of the motor mower, the widespread use of residual insecticides such as DDT (Dichlorodiphenyltrichloroethane), increased formalisation of vector control operations and anti-mosquito legislation, an increase in the prevalence of household reticulated water, and most importantly for Brisbane, the removal of domestic larval habitat such as domestic food storage cabinets and rainwater tanks, a primary breeding site for *Ae. aegypti* (Marks, 1957, Russell et al., 2009, Williams et al., 2010).

There has been little empirical study on the influence of these changes on mosquito distribution but each has been proposed by medical entomologists as a significant factor (Marks, 1966, Hooper, 1967, Kay et al., 1984, Russell et al., 2009). It is likely that technological improvements such as the introduction of refrigerators and the motor mower led to the removal of larval habitat (Marks, 1957), while the extensive use of DDT in Brisbane for flies, cockroaches and swamp mosquitoes occurred around the period that *Ae. aegypti* disappeared (Brisbane City Council, 1946). The decline in steam rail and attendant water storage may have influenced the retraction in range. Dispersal and invasion of *Ae. aegypti* is often the result of accidental movement of eggs, immatures or adults by human action and has been observed in container-inhabiting mosquitoes many times in both historical and contemporary contexts (Debenham and Leeson, 1987, Moore et al., 2007, Hoffmann et al., 2011, Fischer et al., 2011). Large water tanks and fire-buckets at railway stations may have provided ideal larval habitat (Lumley and Taylor, 1943, Russell et al., 1984) and the removal of such steam train infrastructure may have played a role in minimising the spread and re-establishment of the species during the period when it disappeared from inland and southern Australia.

The introduction of reticulated water into the urban landscape of Australia may also have contributed to the disappearance of *Ae. aegypti* from Brisbane (Kay et al., 1984). Kay et al. (1984) state that the most important factors in this local extinction were the vigorous campaign by Brisbane City Council (BCC) to eliminate breeding sites and the reduction in rainwater tanks after reticulated water was introduced. Mackenzie et al. (1996) also suggested that the change of urban water supplies from household rainwater tanks to a reticulated system was the single most important factor in the decline of *Ae. aegypti* in Australia. That view is repeated by numerous authors (Kearney et al., 2009, Beebe et al., 2009, Russell et al., 2009, Williams et al., 2010) although none present quantitative data supporting the assertion. I explore these hypotheses in Chapter 2.

1.2.6 Climate suitability and range retraction in Australia

Climate broadly influences mosquito biology in ways that significantly alter core and range margin distributions. Eisen et al. (2014) recently classified the presence of *Ae. aegypti* under four scenarios: 1) year-round activity and potential for high abundance; 2) year-round activity but potential for high abundance only during the most favourable period of the year; 3) distinct seasonal activity where active stages can reach moderate to high abundance during part of the year, are absent during some seasons but have an egg bank that continues to produce viable larvae each spring; and 4) distinctly seasonal activity where mosquitoes are found in low numbers during the warm part of the year but are otherwise absent with no viable egg bank. The distribution of these scenarios was predicted using relative climate data and the published literature (Eisen et al., 2014). These predictions have relevance to the types of establishment seen in Australia.

The largest, well mixed and genetically diverse populations of *Ae. aegypti* in Australia are found in the major urban areas of north Queensland including Cairns and Townsville. Cairns is the only location covered by Eisen et al. (2014) and they define it as scenario 2, with year round activity but only potential for high abundances during the most favourable time of year. Cairns has an annual temperature and rainfall average of 24.7°C (monthly averages of 21.1°C - 27.5°C; Table 1) and 2,064mm (average monthly rainfall 22 - 449mm; Table 2) respectively. In comparison, Brisbane has an annual average temperature of 20.6°C (9.5 – 21.1°C; Table 1) and rainfall of 1,186mm (35mm - 175mm; Table 2). This puts Brisbane in Eisen et al. (2014) suboptimal scenario 3. This scenario is supported by historical observations and current predictive modelling (Cooling, 1923, Hamlyn-Harris, 1931, Beebe et al., 2009, Kearney et al., 2009). Comparing Australian cities to the temperature data within the Eisen et al. (2014) scenarios, it seems that Sydney varies little in comparison with Brisbane or Neuquen City (Argentina) and would be placed into scenario 3 or 4 (Eisen et al., 2014). Although temperatures in Adelaide differ only slightly from Sydney, annual rainfall varies considerably to the other Australian cities, and that might place Adelaide into scenario 4. *Aedes aegypti* has been noted in Sydney previously (Lumley and Taylor, 1943) but never in Adelaide (Russell et al., 2009).

Table 1. Temperature statistics of key Australian cities in relation to Eisen et al. (2014) climate scenarios that may influence potential *Aedes aegypti* distributions in Australia (Bureau of Meteorology, 2015). Star (*) represents estimated Eisen et al (2014) scenario based on temperature alone.

City	Average Annual Temperature (°C)	Lowest Average Monthly Air Temperature (°C)	Highest Average Monthly Air Temperature (°C)	Difference (°C)	Eisen et al. (2014) Climate Scenario
Cairns	24.9	17.0	31.5	14.5	2
Brisbane	20.6	9.5	29.1	19.6	3*
Sydney	17.8	7.2	26.5	19.3	3*
Adelaide	16.5	7.0	28.1	21.1	4*

Table 2. Rainfall statistics of key Australian cities in relation to Eisen et al. (2014) climate scenarios that may influence potential *Aedes aegypti* distributions in Australia (Bureau of Meteorology, 2015). Star (*) represents estimated Eisen et al (2014) scenario based on rainfall alone.

City	Average Annual Rainfall (mm)	Lowest Average Monthly Rainfall (mm)	Highest Average Monthly Rainfall (mm)	Difference (mm)	Likely Eisen et al. (2014) Climate Scenario
Cairns	2,008	27	449	422	2
Brisbane	1,186	35	175	140	3*
Sydney	1,083	60	123	63	3/4*
Adelaide	447	17	60	43	4*

Changes in the seasonal abundance of *Ae. aegypti* due to variations in rainfall are hard to predict due to the domestic nature of the species, its highly variable oviposition strategy and desiccation resistant eggs (Jansen and Beebe, 2010). In recent years there has been renewed interest in predicting the spread and re-invasion of *Ae. aegypti* into its cool range margins, primarily due the increased impacts of climate change and the importance of the species as a disease vector (Eisen et al., 2014). Rising temperatures and increased variability in rainfall may impact the distribution of the species, daily activity patterns and peak abundance in its historically cool ranges (Jetten and Focks, 1997, Kearney et al., 2009, Beebe et al., 2009, Russell et al., 2009, Bader and Williams, 2012, Khormi and Kumar, 2014). The degree to which temperature will affect *Ae. aegypti* seasonality will be affected by other climate and habitat variables and by the potential adaptation of the mosquito as it expands (Kearney et al., 2009). In Australia, temperature is limiting for the species in both a north/south gradient and a coastal/central gradient (Kearney et al., 2009). These gradients limit the southern distribution of *Ae. aegypti* in Australia (Kearney et al., 2009, Williams et al., 2010, Richardson et al., 2011).

1.2.7 Modelling the potential Australian distribution of *Aedes aegypti*

The primary goals of ecology are to understand the abundance and distribution of organisms and how these fundamental characteristics change through time (Jackson et al., 2009). Predictions of future distributions of *Ae. aegypti* are contentious because they rely on a quantitative assessment of the interactions between environment, population dynamics and mosquito and human behaviours (Christophers, 1960, Gubler and Clark, 1995, Jansen and Beebe, 2010). In particular the human environment provides abundant blood resources, protected resting sites and reliable aquatic habitats in areas where the mosquito could not otherwise endure (without human habitation). Moreover, human-mediated transport of eggs or adult forms provides a means of dispersal between these optimized “islands” and constant colonisation pressure in new areas. In that context, climate models alone cannot predict potential distributions (Jansen and Beebe, 2010).

At the heart of a species’ abundance and distribution lie two concepts of the ecological niche. The first is the Eltonian niche which is based on the availability of resources. The second is the Grinnellian niche which centres on environmental influences (abiotic and biotic factors) on the growth, reproduction and survival of individuals (Chase and Leibold, 2003, Soberón, 2007, Soberón and Nakamura, 2009). The thermal niche is a part of the latter and determines those locations where ectothermic species can survive (Huey and Kingsolver, 1989, Gilchrist, 1995). This niche is conceptualized as a single response curve with an optimum range and thresholds beyond which individuals cannot survive regardless of life cycle stage. Physiological data including optimum developmental temperatures, total range of temperatures and critical limits to development are crucial to modelling the thermal niche. Mechanistic species distribution and population models such as CLIMEX (Sutherst, 1999), CIMSiM (Focks et al., 1993b, Williams et al., 2008), Skeeter Buster (Magori et al., 2009) and Niche Mapper (Kearney et al., 2009, Kearney and Porter, 2009) explicitly incorporate the thermal niche of organisms to determine suitable habitats across the landscape (Richardson et al., 2011). They allow coarse climatic data such as rainfall, temperature, wind and solar radiation to be translated into biological factors relevant to an organism's microclimate (Richardson et al., 2011).

CIMSiM and Skeeter Buster are weather driven, dynamic life table models that simulate the dynamics of *Ae. aegypti* populations inhabiting natural and artificial containers (Focks et al., 1993a, Magori et al., 2009). These models have been used extensively in the literature to simulate *Ae. aegypti* populations (Focks, 1992, Focks et al., 1993a, Richardson et al., 2011, Williams et al., 2013, Williams et al., 2014, Rasic et al., 2014) and are designed to provide a mechanistic account of the factors that influence population dynamics (Focks et al., 1993a). Life stage development and survival estimates within the models are defined by a set of temperature-compensated differential equations based upon enzyme kinetics and incorporate information on egg predation, nutrient availability and the number, size and metabolism of larvae present in containers (Focks et al., 1993b). Individual models can be calibrated for regional variability, although a detailed knowledge of the mosquito population and the containers they exploit is essential (Williams et al., 2008, Ellis et al., 2011). CIMSiM has been field validated and used to estimate *Ae. aegypti* population size, structure and persistence although validation occurred in areas where year-round activity of *Ae. aegypti* occurs (Focks et al., 1993a, Williams et al., 2008, Williams et al., 2010, Eisen et al., 2014).

Scenarios provided by Eisen et al. (2014) support the hypothesis that, based on long term climate averages, Brisbane is capable of supporting a population of *Ae. aegypti*. Weather, however, is considerably more varied than long term climate averages, and fluctuations may have occurred in the past that did not favour the physiology of *Ae. aegypti* in Brisbane. The only climate based modelling paper that has attempted to explore the historical distribution of *Ae. aegypti* in Australia is by Williams et al. (2010). In this paper, the authors used CIMSiM to simulate the extinction of *Ae. aegypti* life cycle stages in 13 locations across Australia, including Brisbane. They conclude that *Ae. aegypti* larvae and adults go extinct in Brisbane from July to September, leaving only eggs during this period (Williams et al., 2010). The authors propose that the observed extinction event that occurred in Brisbane was likely caused by cool air temperatures during winter periods (adults) and the removal of continuously wet containers (rainwater tanks) associated with increased reticulated water (Williams et al., 2010). In another finding, they suggest that smaller container types would have similar productivity between the locations of Charters Towers and Brisbane. They also suggest the slightly increased temperatures found in Charters Towers would allow greater persistence there (Williams et al., 2010). This observation has implications for Brisbane as rising temperatures due to climate change may increase the potential persistence of *Ae. aegypti* (Mweya et al., 2016). Likewise, by incorporating temperature data from rainwater tanks in the CIMSiM model, the authors might have forecast a much wider southern range than was suggested in the paper. Unfortunately, as acknowledged by the authors, the conversion factors in CIMSiM do not model rainwater tanks accurately, so conclusions cannot be drawn about this particular microenvironment (Williams et al., 2010). The authors do suggest that field productivity values from rainwater tanks in Australia for *Ae. aegypti*, would be useful for future simulation modelling (Williams et al., 2010).

CIMSiM does not simulate water temperatures accurately when temperatures drop below, or rise above, the thermal optimum for *Ae. aegypti* development and because of this productivity values can be over-or-underestimated. Kearney et al. (2009) point out that this type of climatic model assumes that larval and adult mosquitoes experience ambient air temperatures and results are based on a correlation between weather conditions and container environments. In this way, the variation in temperature experienced by containers is assumed to follow that of air temperatures. Eisen et al. (2014) demonstrate how CIMSiM inaccurately predicts survival and growth in the cooler range margins of *Ae. aegypti* in two ways. Firstly, the plotted data used in CIMSiM predicts a gradual linear relationship between survival and temperature in the 10-19°C range but data presented by Eisen et al. (2014) suggest that this linear relationship might be incorrect, as development is arrested steeply between ~10 to 14°C. CIMSiM may also make inaccurate assumptions about the survival of eggs and immatures at lower temperatures. Focks et al. (1993b) imply a 100% survival rate for eggs over 0°C and adults and larvae ≥10°C but Eisen et al. (2014) show that, in the 15 to 16°C range, survival drops to ~80% in eggs, 10 to 77% in larvae and ~90% for pupae. In the 10 to 14°C range, survival drops from ~25% at 14°C and in the 10 to 13°C range there was no survival for larvae or pupae (Eisen et al., 2014). Thirdly, the non-linear enzyme kinetics model used by Focks et al. (1993b) does not incorporate the positive or negative impact that fluctuations in temperature have around the lower or upper development threshold (Eisen et al., 2014). The effects of temperature fluctuations are reviewed in section 1.3.1.

The Hutchinsonian niche concepts of fundamental and realised niche have been used to estimate species distributions for many decades (Hutchinson, 1957). The fundamental niche is a concept relating to the range of environmental conditions in which a species (without interference) could survive and reproduce. The realised niche is the actual range of environmental conditions that a species is observed surviving and reproducing in, usually as a result of other limiting factors such as competition, availability of resources and predation (Griesemer, 1992). CLIMEX is a hybrid bioclimatic modelling tool for exploring the relationships between the fundamental and realised niche of a species (Sutherst, 1999). It predicts the distribution of a species based on two approaches; first it can infer a distribution based upon actual occurrence data and then matches meteorological data among these locations to predict the potential range of a species; and second it can infer a distribution from available empirical data on a species' response to temperature, moisture or both (Rogers et al., 2007, Webber et al., 2011). The benefit to using this modelling approach is the flexibility in using either of these methods to predict a species' distribution, especially in areas where occurrence records are insufficient (Sutherst et al., 2007). This flexibility has allowed CLIMEX to successfully estimate the potential distribution of invasive weeds, insects and pathogens in Australia and to match climates where potential bio-control agents may be sourced (e.g. Yonow et al., 2004, Sutherst and Maywald, 2005, Rafter et al., 2008, Van Klinken et al., 2009). CLIMEX has been used to successfully estimate the potential range of *Ae. albopictus* if it were to establish in Australia (Ritchie et al., 2005). For these reasons, CLIMEX is suited for modelling the potential or historical distribution of *Ae. aegypti* in Australia.

There has been one attempt to use CLIMEX to model the potential world-wide distribution of *Ae. aegypti*. Khormi and Kumar (2014) examine the potential risk posed by climate change on the abundance of the mosquito species. They assembled a database describing the global distribution of *Ae. aegypti* and used it to develop distribution models under current and future climate scenarios (Khormi and Kumar, 2014). Their model is flawed due to inaccurate occurrence data and because it assumes that dengue case location predicted *Ae. aegypti* distribution. This is not the case: *Ae. albopictus* is also a competent dengue vector and dengue outbreaks do not necessarily occur in the presence of *Ae. aegypti*. In 2014, *Ae. albopictus* was declared the primary vector of a dengue outbreak in Japan, a location where *Ae. aegypti* is not currently present (Saho et al., 2015). Likewise, it is likely that *Ae. albopictus* is also a major vector of dengue in China (Xiao et al., 2016). Yang et al. (2014) also demonstrated a spatial gradient between the two mosquito species in Taiwan, and implicated *Ae. albopictus* as the only vector in an epidemic of dengue where *Ae. aegypti* did not occur. Further, *Ae. aegypti* persists in locations that do not experience dengue transmission, such as through much of central and southern Queensland (Queensland Government, 2010). Secondly, the Khormi and Kumar (2014) database does not include accurate mosquito distribution data from Australia even though it is available in the literature (Beebe et al., 2009, Russell et al., 2009).

Biological parameters (lower limiting temperature 18°C, lower optimal 25°C, upper optimal 32°C and upper limiting 38°C) were chosen to reflect the geographic location of the foci: the Arabian Peninsula (Khormi et al., 2014). Lowest limiting moisture (set at 0), heat stress accumulation (0.9 week^{-1}), dry stress threshold (0.001) and dry stress rate (0.001 week^{-1}) were also based on observations from Saudi Arabia. Those parameters do not represent the true limits for the species (Eisen et al., 2014). In selecting a lower limiting temperature of 18°C, the authors do not take into consideration the capacity of eggs and larval stages to overwinter in areas where cold is limiting for adult stages. These areas include locations where the species is known to currently exist in Europe (ECDC, 2017) and where it has previously existed in North America and south eastern Australia (Gubler, 2005, Russell et al., 2009). The selection of dry stress threshold parameters that suit the authors' experience in Saudi Arabia tend to overestimate the eco-climatic index in potential current and future areas of high desert, such as Australia and northern Africa. The eco-climatic indexes suggested in this paper would be best described as predicting the future potential of dengue fever transmission if the vector was present.

Some authors suggest that ecology has struggled to bridge the gap between the dynamics of population patterns and the small scale functional traits of individual organisms (Kearney et al., 2010). This is exemplified by the challenge of linking broad climatic trends with the micro-climate niche of a species and an example of this is to consider the difference between the buffered microclimate of a rainwater tank and the ambient air temperature recorded by a meteorological station. Kearney et al. (2009) addressed this discrepancy by using a mechanistic, niche-based model called Niche Mapper (Mitchell and Porter, 2006) to combine information on behaviour, physiology, morphology and the environment to describe the micro-climate niche of the mosquito. They then predict the past historical and potential future distribution of *Ae. aegypti* in Australia based on the availability of that niche (buckets and rainwater tanks). They modelled the temperatures of containers based on transient-state energy mass balance equations under different micro-climatic environments of solar radiation, convection, conduction and evaporation (25% and 75% shade). These temperatures are used to predict the growth and survival of egg and larval stages based on physiological parameters and container properties presented by Focks et al. (1993b). Their results for rainwater tanks fit well with the historical and current distribution of *Ae. aegypti* in Australia and interestingly, predict that Adelaide is capable of supporting approximately 8-10 life-cycle completions at 25% shade (16-20 weeks at a 2 week cycle) per year. This is similar to results observed in Sydney (Kearney et al., 2009). Under the climate change scenarios they examined, the greatest change occurred throughout the northern parts of Australia where life-cycle completions increased by 1-2 cycles across the range (Kearney et al., 2009). Due to the fact that rainwater tank prevalence in Adelaide is now almost as high as Brisbane, this observation may suggest a greater risk of *Ae. aegypti* establishment in southern cities than previously thought (Australian Bureau of Statistics, 2013). Their results indirectly support the hypothesis that the removal of large water storage containers contributed to the range retraction of *Ae. aegypti* in Australia (Kearney et al., 2009).

Limitations to the Niche Mapper approach include the reliance upon accurate biological data from experiments, lack of GIS output, and difficulty for researchers to access its complicated programming structure. The reason the Kearney et al. (2009) paper approximates an *Ae. aegypti* distribution relatively accurately, is due to the large amount of experimental physiological data available for this species (Focks et al., 1993b). One might criticise the authors for using physiological data that was derived from an African population (Gilpin and McClelland, 1979, Focks et al., 1993b) and applied to an Australian distribution of *Ae. aegypti*. Eisen et al. (2014) suggest that survival curves of *Ae. aegypti* are very similar between geographic populations of *Ae. aegypti*, however, as seen in Chapter 4 development rates may vary. Secondly, the prediction that the population of *Ae. aegypti* discovered in Tennant Creek (2004 to 2006, 2011 to 2014)(Cooper et al., 2005, Kurucz and Pettit, 2014) sit on the edge of the potential distribution with a yearly prediction 0-1 life cycles completed. Although the second invasion (2011 to 2014) occurred after the paper was published, the model fails to predict the potential for *Ae. aegypti* to successfully colonize warmer, dryer regions, even though their model simulates both small and large containers in these areas. This is may be due to the reliance of the model on natural rainfall, and not artificial water sources like reticulated water. A final limitation of the Kearney et al. (2009) paper is that the dated data used in the survival curves for egg, larval and adult data were adopted from Focks et al. (1993a)(see CLIMEX section above). Depending on what temperature values Kearney et al. (2009) simulated in rainwater tanks in southern Australia, their predictions may have overestimated the potential distribution of *Ae. aegypti* in its southern range due the higher predicted survival rate in colder temperatures implied by the Focks et al. (1993b) survival data. Because of this, it is important to validate the temperatures inside rainwater tanks in Brisbane, Sydney and Adelaide and rerun the model with updated physiological data to improve the accuracy of outcomes.

1.3 Limits to establishment

There are numerous barriers to the invasion of a mosquito into a new environment. These include: climate, the ability to initiate a founding event, predation, competition and resource limitation (blood-feeding, resting and oviposition habitat)(Sakai et al., 2001). *Aedes aegypti* is a highly invasive species, found throughout the tropical regions of the world (Christophers, 1960). As such, limits to establishment occur primarily in temperate regions and at higher elevations where temperature and climate play a role (Eisen and Moore, 2013).

1.3.1 The effects of temperature on the development and survival of *Aedes aegypti*

Insects are small ectotherms and their development and survival is closely linked to environmental temperatures. Until the 1970s it was common to study insect development at near-constant water temperatures (CT)(Colinet et al., 2015). Scientists recognized that these studies were observing development in abnormal conditions and began conducting experiments that accurately reflected what was observed in the field. These studies used fluctuating temperatures (FT), which reflected the natural daily and monthly cycles experienced by insects in the environment (Colinet et al., 2015). It became evident that FT had a number of effects that improved the thermal tolerance and fitness of insects when compared to studies using constant temperature (Colinet et al., 2015).

Differences between CT and FT experiments, called Jensen’s inequality, are linked to the performance of temperature related processes on either end of an insect’s thermal performance curves (Ruel and Ayres, 1999). Maximum rates of performance occur at the peak of the curve and the efficiency of temperature related processes change depending on where the temperature sits on each side of the peak. In the convex (warmer) part of the curve, small fluctuations in temperature can quickly pass the upper critical temperature and performance can drop quickly due to inefficiencies associated with temperature related process such as the denaturing of proteins. In this part of the curve, the performance of a temperature related process in insects exposed to fluctuating temperatures will exceed that exposed to CT. In the concave (cooler) part of the thermal performance curve, rates occur more slowly and have less chance to suddenly hit lower critical temperatures. In the cooler part of the curve the opposite effects of FT and CT on temperature related processes occur. The performance of processes exposed to FT in the cooler part of the curve will be less than those exposed to constant temperatures. The thermal sensitivity of the temperature related process is also important when studying the effect of variation in the amplitude of each temperature cycle. Larger amplitudes will have a greater impact the further the temperature lies towards each extreme of the curve. Therefore development will be faster under FT than CT, although the cost of this increase is more energy demanding than in static environments (Dallwitz, 1984, Ratte, 1985, Hagstrum and Milliken, 1991, Liu et al., 1995, Eisen et al., 2014).

Research on the bionomics of *Ae. aegypti* has traditionally focused on the effects of near-constant temperatures, probably due to the inherent difficulties associated with designing lab and field experiments with disease vectors. Experiments have examined hatching success, survival and development rates of immature forms, at near-constant water temperatures. These studies have provided valuable quantitative data and there is an extensive literature (Eisen et al., 2014). This bionomic data has been used to predict population growth rates, vector competence and the geographical distribution of the species (Watts et al., 1986, Kearney et al., 2009, Barbazan et al., 2010, Hartley et al., 2012). Eisen et al. (2014) have reviewed the bionomic literature on *Ae. aegypti* and defined a number of critical thresholds to development and survival, known as the developmental zero point. This threshold represents the theoretical point where development and survival in *Ae. aegypti* no longer occurs (Table 3). The authors' focus was on clearly defining the lower critical thresholds due to knowledge gaps in cool range margins. These thresholds may be important in understanding the limit to the southern cold range margin of *Ae. aegypti* in Australia and whether or not these temperatures contributed to the local extinction of *Ae. aegypti* in Brisbane.

Table 3. *Aedes aegypti* critical development thresholds as given by Eisen et al. (2014). Upper thresholds are estimates due to a focus on lower critical thresholds.

Life Stage	**Lower Critical Threshold (°C)**	**Upper Critical Threshold (°C)**
Egg	14.0	36-38
Larvae	11.8	36-42
Pupae	10.3	38-42

Studies have rarely focused on the effects of FT on the bionomics of *Ae. aegypti* adults and immatures. Reed and Carroll (1901) observed that eggs held at high temperature then placed at 20°C for 2 hours daily, when compared with eggs held at a constantly high temperature, resulted in delayed adult emergence from the cooled eggs. The first studies to compare the direct effects of various amplitudes of fluctuating temperatures (± ~3°C, ~5.5°C and ~8°C) were Headlee (1940), Headlee (1941), Headlee (1942) and Keirans and Fay (1968). Experiments involving both CT and FT see similar results, with lower development time and highest survival to adult between a moderate temperature range of 15 to 32°C. However the strongest impact of fluctuating temperatures were observed in temperature ranges that crossed the upper and lower developmental zero temperatures. Compared with the moderate range, exposure to the lower range (10 to 26.7°C) resulted in reduced survival and longer development time and exposure to the high range (21.1 to 37.8°C), resulted in minimal survival and the longest development time. Mohammed and Chadee (2011) show that fluctuations between 24.5 to 34.5°C show minor reductions in larval survival and increases in development time for *Ae. aegypti* in fluctuating water temperatures, when compared with constant temperatures.

Carrington et al. (2013) examined the effects of different amplitudes of daily fluctuations (daily temperature range or DTR) around a mean of 26°C on immature stages and observed that a large DTR (18.6°C) resulted in an extended mean development time to adult than a smaller DTR (7.6°C) or the baseline DTR (0°C). This paper was also the first to associate the concept of degree days in fluctuating temperatures with *Ae. aegypti*. Degree day models have been used to forecast the population dynamics of pest insects in agriculture and forensic entomology (Sutherst and Maywald, 1985, Rafoss and Sæthre, 2003). Degree days can estimate the time insects spend developing between critical developmental thresholds (heating or cooling degree days), time below the lower critical threshold (degree days cold stress) or time above the upper critical threshold (degree days heat stress). Once known, these threshold estimates can predict rates for *Ae. aegypti* population growth and survival in fluctuating temperatures.

In Australia, it is thought that temperature and desiccation are limiting factors for *Ae. aegypti*, in both a north/south gradient and a coastal/inland gradient (Kearney et al., 2009). The current geographic distribution of *Ae. aegypti* is restricted to the north and central parts of Queensland where conditions of temperature and rainfall are favourable for year-round presence of all life stages (Williams et al., 2010). Historically, however, this species has been collected to the southern border of New South Wales, thousands of kilometres from its current southern distribution. In Brisbane, Williams et al. (2010) suggest that containers are important for persistence, with only eggs surviving the winter period (July until September). Kearney et al. (2009) looked at potential life-cycle completions per year in buckets and rainwater tanks across Australia. This model suggested that south east Queensland could support a maximum of two *Ae. aegypti* life-cycle stages a year in buckets and ~10-16 stages in rainwater tanks (Kearney et al., 2009). Richardson et al. (2011) applied a similar modelling approach using CIMSIM (Focks et al., 1995) and found *Ae. aegypti* unlikely to complete a life-cycle in buckets, while rainwater tanks were highly suitable.

1.3.2 The effects of fluctuating temperatures in cool range margins

An understanding of what occurs in cool ranges is important when predicting the effects of climate change on mosquito bionomics and pathogen transmission (Eisen et al., 2014). As the scale of observation decreases, it is the interaction between climate and human water containers that determines the microclimate experienced by mosquito immatures (Richardson et al., 2013). The growth rate of larval mosquitoes is defined by the microclimate found within each container, as they have little physiological or behavioural ability to avoid stressful conditions in their aquatic environment (Richardson et al., 2013). This microclimate is determined by the type (volume, colour) and location (often shade) of each container and these factors influences the thermal stability of breeding sites. The thermal stability of containers is important when determining the short-term acute stress (degree days heating or cooling stress) and the long term developmental suitability (growing degree days) caused by the fluctuating temperatures experienced in the environment (Richardson et al., 2013). Rainwater tanks represent ideal larval habitat for mosquitoes; they provide reliable food resources, a protected environment, and a large permanent or semi-permanent aquatic habitat and it is unlikely that larval density in these containers will be restrictive to development in the majority of cases (Timmermann & Briegel, 1993, Arrivillaga et al., 2004, Barrera et al., 2006).

In eastern Australia, little is known about the FT experienced in breeding containers along the southern distribution of *Ae. aegypti*. Richardson et al. (2011) compared developmental times for immatures kept at CT and compared with those placed under natural daily fluctuations during October in Cairns, Australia. They reported small differences in mean time to pupation or survival to adult between field conditions and those predicted by a Schoolfield model based on constant temperature data (Schoolfield et al., 1981). The importance of these later studies is that they focused on fluctuations around the mean temperature typical of the optimal (scenario 1) or near-optimal (scenario 2) geographic distribution of the species (Eisen et al., 2014). However, these mean temperatures and the fluctuations around them are not based on those observed in the cool range margins of the species, such as those in Brisbane, Sydney or Adelaide, nor do they contain the temperature gradients observed in containers found in these locations.

Richardson et al. (2013) examine the thermal and hydric regimes for *Ae. aegypti* immatures development and thermal stress between multiple container types observed inside the core range (Cairns: 28th November until 11th December 2008) and outside (Melbourne: 12th-17th September and 2nd-16th February 2008). The authors present their data in terms of temperatures experienced by containers in the sun and shade that corresponded with favourable conditions or if developmental thresholds were crossed (Richardson et al., 2013). They analyse their temperature data as upper and lower safety margins relevant to the thresholds they present (lower 13.8°C, upper 40°C) through a statistical model that gives measures of suitability (Richardson et al., 2013). They also convert their temperature data into potential productivity by applying a model (CIMSiM) that is based on development in CT regimes. Their findings indicated that containers fluctuated from between 10 to 15°C internally and digressed considerably from air temperatures (Richardson et al., 2013). Smaller containers were more closely tied to air temperature than larger objects, and larger volume containers exhibited more thermal inertia and dampened fluctuations in temperature. The variation in fluctuations observed in the largest containers (35L and 100L) was minimal, although they both had higher relative means to other container categories. The authors suggest that the higher means were due to their thicker boundary layer and stronger coupling to solar radiation (when compared to convection)(Richardson et al., 2013). Observations linking shade with thermal stress indicated that no containers between Cairns or Melbourne went above the upper critical temperature in open sun containers and because of this the authors suggest that hotter temperatures would not be a limiting factor in southern Australian regions (Richardson et al., 2013). In fully shaded conditions in Melbourne, most containers sat close to or below the lower critical temperature during the September period observed.

These results are the first attempt to measure the FT observed in containers in the cold range margin of *Ae. aegypti* in Australian (Richardson et al., 2013). They are informative because they provide an estimate of potential heat stress experienced in containers in the cold range margin. However they fail to provide quantitative evidence to support one of their conclusions: that the persistence of *Ae. aegypti* in Melbourne is limited by cold temperature. Firstly their observations were made during spring time and not at the coldest time of year, which is in July (Bureau of Meteorology, 2015). Secondly, their assignment of 13.8°C as the lower critical temperature is 2°C more than that determined by Eisen et al. (2014). This temperature difference would make the results of their statistical analysis and development predictions unreliable. Finally, their container categories do not contain a water volume equivalent to the container category most relevant to the persistence of *Ae. aegypti* in Melbourne: the rainwater tank. Like Brisbane, this city has installed a large number of rainwater tanks in recent years (Australian Bureau of Statistics, 2013) and their observations would have been more informative for risk of establishment if they had included this container category.

1.3.3 Competition

Many mechanisms exist that may influence the dynamics between mosquito species including larval resource competition (Reiskind and Lounibos, 2013), predation (Steffan and Evenhuis, 1981) and adult interactions that may involve satyrization (Tripet et al., 2011) or cytoplasmic incompatibility induced by intracellular bacteria (Zabalou et al., 2004). The competitive exclusion principle positions that if two species occupy identical niches, then competition between individuals will inevitably drive one population to extinction, particularly when resources are limited (Leisnham and Juliano, 2009). Larval competition is suggested as one mechanism for the large decrease in the distribution of *Ae. aegypti* in the United States after the introduction of *Ae. albopictus* in the 1980s (Juliano & Lounibos, 2005). However recent larval competition studies between *Ae. albopictus* and *Ae. notoscriptus* suggest this may not necessarily occur in Australia (Nicholson et al., 2015).

It has been hypothesised that *Ae. notoscriptus* acts as a competitor in southern areas of Australia, potentially excluding *Ae. aegypti* from these regions (Russell et al., 2009). Russell (1986) was the first to show differences in development rates at different temperatures between the two species but larval competition experiments designed around the optimal region of the *Ae. aegypti* thermal performance curve were not conclusive. The author found the effect was marginal, with *Ae. notoscriptus* developing quicker at lower temperatures (22°C) and *Ae. aegypti* at higher temperatures (28°C). As the two species often share larval and adult habitat, it is still possible that *Ae. notoscriptus* may retain a competitive advantage over *Ae. aegypti* through other means (Russell, 1986).

Alternatively it has been hypothesised that species can coexist, while sharing resources, by forming independent spatial aggregations (Leisnham and Juliano, 2009). This type of interaction is can occur when competitors are attracted to different environmental characteristics or conditions that have spatial independence (Ives, 1988, Chesson, 2000b). Chesson (2000a) have explored this phenomenon and suggest that random dispersal would lead to a Poisson distribution in space, where emigrants from any particular area are equally distributed over all areas. Non-random dispersal may occur when organisms become clumped in space as aggregations that relate to local conditions, and this type of behaviour is best described using a negative binomial distribution where k is a clumping parameter (Chesson, 1998). Aggregations between species are more likely to occur when each is attracted to different spatially independent cues, which is effectively a type of resource partitioning. Spatial segregation is another term used to describe resource partitioning of competing species at larger spatial scales (Shigesada et al., 1979). Both independent aggregations and spatial segregations, have been observed occurring between invading populations of *Ae. albopictus* with *Ae. aegypti* (Lounibos, 2002, Juliano & Lounibos, 2005) and *Aedes japonicus japonicas* (Theobald) with *Ae. albopictus* and *Toxorhynchites rutilus* (Coquillett) in the eastern United States (Freed and Leisnham, 2014). In Florida, *Ae. aegypti* and *Ae. albopictus* have exhibit both spatial segregation and independent aggregations. *Aedes aegypti* was found to have a higher spatial correlation with urban environments, while *Ae. albopictus* was associated with canopy vegetation (Reiskind and Lounibos, 2013). In a study of vases in cemeteries, it was shown that each species oviposited independent of one another, and authors suggest that environmental variation between vases may favour either species differently (Leisnham and Juliano, 2009).

In Australia, historical observations suggest *Ae. aegypti* and *Ae. notoscriptus* show both spatial segregation and independent aggregations when in coexistence. Hamlyn-Harris (1928) observed independent aggregations of *Ae. aegypti*, *Ae. notoscriptus* and *Culex quinquefasciatus* (Say), with only 0.6% - 16% of containers and graves showing coexistence between any of the species from three cemeteries and five sample periods. The same study observed spatial segregations between the two species, with *Ae. notoscriptus* more likely to be found in tree holes and bromeliads away from human habitation, while *Ae. aegypti* was only associated with containers close to domestic dwellings (Hamlyn-Harris, 1928, Hamlyn-Harris, 1930, Hamly-Harris, 1933). Tun-Lin et al. (1999) showed a significant relationship between the presence and absence of *Ae. aegypti* and *Ae. notoscriptus* in northern Queensland, with the *Ae. notoscriptus* preferring to fill larval habitat not occupied by *Ae. aegypti*. These apparent spatial segregations of the species are likely due to differences in adult preference for blood meals (Hamlyn-Harris, 1930, Jansen et al., 2009).

1.4 *Aedes aegypti* establishment facilitated by rainwater tanks

1.4.1 Rainwater tank design and use

Since their introduction in the mid-late 1800s, rainwater tanks have been used as a reliable source of potable water in residential and commercial premises of the middle and upper classes (Ferguson, 1926, Hamlyn-Harris, 1931). However, the mass-installation of rainwater tanks into modern Australian cities has led to a prevalence approaching ~40% of properties in Brisbane (Moglia et al., 2013a). The basic design of rainwater tanks has remained similar. The structure is intended to capture and store large volumes of water, where guttering and pipes direct flows off rooves and into a large entrance. Early designs were a simple cylinder of galvanised iron on a stand, with entrances exposed to inflows and outflows of water and mosquitoes (Figure 6). Modern rainwater tanks are designed with volumes ranging from 500L to >100,000L and constructed of durable materials such as ultraviolet (UV) stabilized polyethylene, rolled metal and polymer skin and re-enforced concrete. These materials are expected to last 50 years, however warranties are approximately for 25 years (Stratco, 2017). Rainwater tank shapes are generally round designs for larger volumes (Figure 7A) or slimline when space is an issue within smaller properties (Figure 7B). In large urban areas, water from rainwater tanks is typically used outdoors for garden irrigation, such as on lawns, trees or pot plants, topping up swimming pools or cleaning cars and boats (Gardiner, 2010). Although not common, indoor uses include toilet flushing, clothes washing and for drinking and cooking purposes (Gardiner, 2010).

Figure 6. Historical design of a rainwater tank made of galvanized iron where water is gravity-fed from a tank stand.

Figure 7. Modern circular design 10,000L rainwater tank with electric pump and first flush diverter (both to the left side)(A). Slimline 3,000L rainwater tank designed to fit into smaller spaces (B).

1.4.2 Infrastructure associated with rainwater tanks

There are a range of devices designed to ensure mosquitoes and detritus do not enter (or exit) rainwater tanks. These devices also prevent the eutrophication of rainwater tanks, improve water quality for drinking and prevent blockages in pumps and piping. Rain heads are designed to prevent blockages of pipes by deflecting leaves and debris away from rainwater tank inlets (Figure 8A). Downpipe "first flush" diverters are the next level of potential filtering (Figure 8A). These are sections of downpipe that collect smaller sediments that settle on a roof and are flushed during the first downpour. These sediments may include dirt, bird droppings and insects that may pass through the rain head (if present). A floating ball can be installed within the section of pipe that may prevent mosquitoes emerging if installed correctly (Figure 8A). In gravity fed rainwater tanks, where water is pushed into above-ground rainwater tanks, there is often a portion of piping that remains underground or below the entrance level. The outlet of these pipes are supposed to be sealed with flaps to prevent emergence of mosquitoes (Figure 8B). The most important level of mosquito protection on rainwater tanks are the mesh sieves that cover the entrance in the roof of the tank, and the overflow (Figure 8A). Overflows ensure that water does not flow back through and over the entrance sieve. Development of sieves first began in 1911 once it was discovered that *Ae. aegypti* were using rainwater tanks for larval habitat (The Brisbane Courier, 1908). Meshes were originally made of iron which rusted quickly, then replaced by brass and copper and finally stainless steel is used in modern sieves (Cooling, 1923). UV inhibitors are used to stop light entering the tank and prevent algal growth, a source of potential nutrition for mosquito larvae. Finally, mesh can be placed over roof gutters to prevent leaves (and mosquitoes) from entering guttering (Figure 8C/D), as gutters are supposed to be clear of overhanging tree branches and leaves (Coffey, 1942). Montgomery and Ritchie (2002) describe roof gutters as key larval containers in Cairns, Australia, observing large numbers of *Ae. aegypti* and *Ae. notoscriptus* pupae.

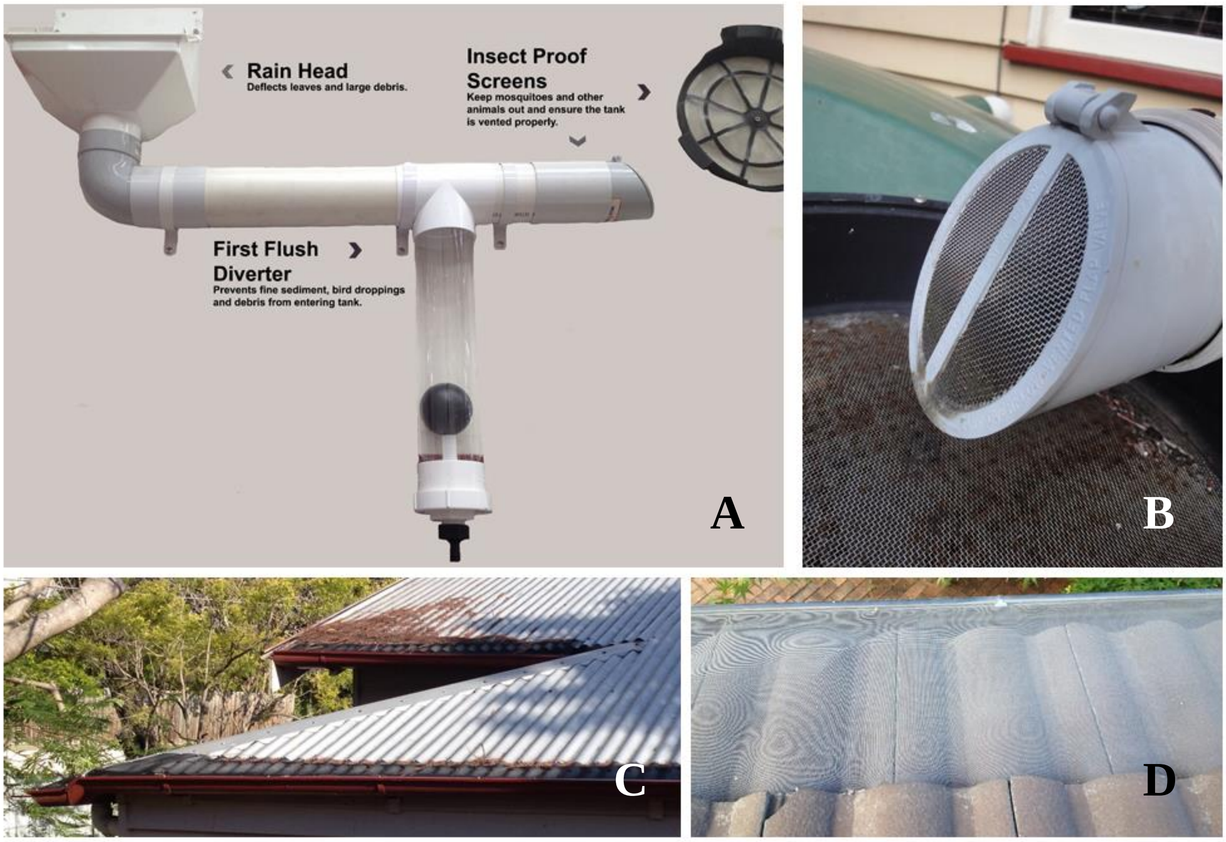

Figure 8. Infrastructure associated with rainwater tanks to remove debris from sieves. Rain head with first flush diverter and tank sieve (A). Vent to prevent mosquitoes and other animals entering pipes where water is gravity fed and pools underground (B). Mesh to prevent leaves, mosquitoes and other debris from clogging roof gutters (C and D).

1.4.3 Contemporary installations of rainwater tanks and legislation in Queensland

The use of rainwater tanks is still standard through most of Australia, especially in areas without a centralized reticulated water system (Moglia et al., 2013a). However, severe drought conditions in Queensland during the early 2000s enforced a culture of water harvesting through government educational campaigns and legislated water restrictions (Queensland Health, 2008). With dam levels in some council areas reaching less than 15% in 2006 (S.E.Q. Water, 2014), the Queensland government passed legislation restricting mains water use, promoted rebates to all homeowners that retroactively installed rainwater tanks on their properties and mandated the installation of rainwater tanks into new houses (Queensland Government, 2006, Queensland Health, 2008). Water restrictions banned watering of gardens and washing of cars and conditioned homeowners to hoard rainwater in storage containers and rainwater tanks (Gardiner, 2010, Trewin et al., 2013). Governments justified the installation of rainwater tank infrastructure as a way of augmenting mains water supplies through decentralisation (Gardiner, 2010). Current management of rainwater tank infrastructure in south east Queensland is through regulation at the federal, state and local levels. A summary of these can be found on the Queensland Health website at (Queensland Health, 2011). The most relevant legislation to this literature review are as follows:

Public Health Act (2005a): Provides a definition of the public health risks and requirements for preventing risks (includes mosquito regulations).

Public Health Regulation (2005): Details on the prevention of mosquito presence in rainwater tanks.

Building Act (1975): Provides information on the Building Code of Australia and Queensland Development Code.

The manufacture of urban rainwater systems has advanced considerably over the past decade. Innovations occurred in the mass production of tanks using long lasting laminated polymer designs and plumbing. Design changes include: multiple types of cylindrical or elongated shapes which retain structural stability and allow large volumes of water to fit into the compact nature of the urban environment; trickle top-up systems that automatically back up with mains supply (meaning the tanks are never empty) and internal and irrigation plumbing connectivity (Gardiner, 2010). These improvements, however, do not mean the technology is at a mature stage. As such, there is potential for design or manufacturing flaws, or homeowner mismanagement or neglect to cause the potential for decay or dysfunction of these systems and result in exposure to potential mosquito breeding. The most relevant Australian Standards are as follows:

AS/NS 1397:2001 (2001) – Provides requirements for 5mm steel sheet and strip used in construction.

AS/NS 3735:2001 (2017) – Provides requirements for concrete structures retaining liquids.

AS/NS 4020:2005 (2005) – Provides requirements for the suitability of products for use in contact with drinking water.

AS/NS 2070:1999 (1999) – Provides materials and procedures to be used in the various stages of production of plastics for food contact.

AS/NS 4766:2006 (2006) – Provides requirements for the design and manufacture of cylindrical, ribbed or straight walled polyethylene storage tanks capable of containing water.

Queensland Development Code MP 4.2 (2013) – Water savings targets, and provides a framework which consolidates Queensland specific standards of the construction and installation (including signage) and allowable end uses (such as laundry and toilet connections).

Queensland Development Code MP 4.3 (2013) – Specifies the standards of construction and installation of rainwater tanks as well as suitable uses of rainwater.

In Queensland, rebates were offered by local councils from July 2006 until February 2013 and the state government for the installation of new rainwater tanks (Natural Resources and Water, 2012). Homeowners often paid little or nothing for the installation of their tank due to multiple rebates being offered (Moglia et al., 2013a). Due to these large rebate schemes, various government agencies and councils have extensive databases of tank installations across the state. A Queensland government Natural Resources and Water (NRW) database contains records of 222,594 rainwater tank installations across Queensland. This database contains details on installation address, capacity and local council (Natural Resources and Water, 2012). A BCC database contains details on 93,986 rainwater tank installations (Figure 9). This database includes details on the installation address, date of approval, capacity and internal connection status to laundry and toilet (Brisbane City Council, 2012). Modern rainwater tanks vary in size from 500 litres to over 100,000 litres in volume, and the stock of tanks in south east Queensland represent a significant contribution to the water budget of the region (Moglia et al., 2014). The size of both the NRW and BCC databases indicate the huge and rapid increase in the number of rainwater tanks now installed across Queensland.

It is unknown exactly how many tanks have been installed into the south east Queensland (SEQ) landscape. The first published numbers of rainwater tank installations were by Gardiner (2010) who estimated that a total of 200,000 had been installed based upon the Queensland government database mentioned above. This study estimated that 30,000 tanks had been compulsorily installed into new houses with reticulated water auto-top-up devices, with many homeowners having no experience of rainwater collection. The same report estimated that 36% of detached houses in south east Queensland had recently installed a rainwater tank (Gardiner, 2010). Further work by Moglia et al. (2012) suggest that 300,000 tanks have been installed into SEQ, with approximately 59,000 of these into new homes and at a cost of $1 billion to the community.

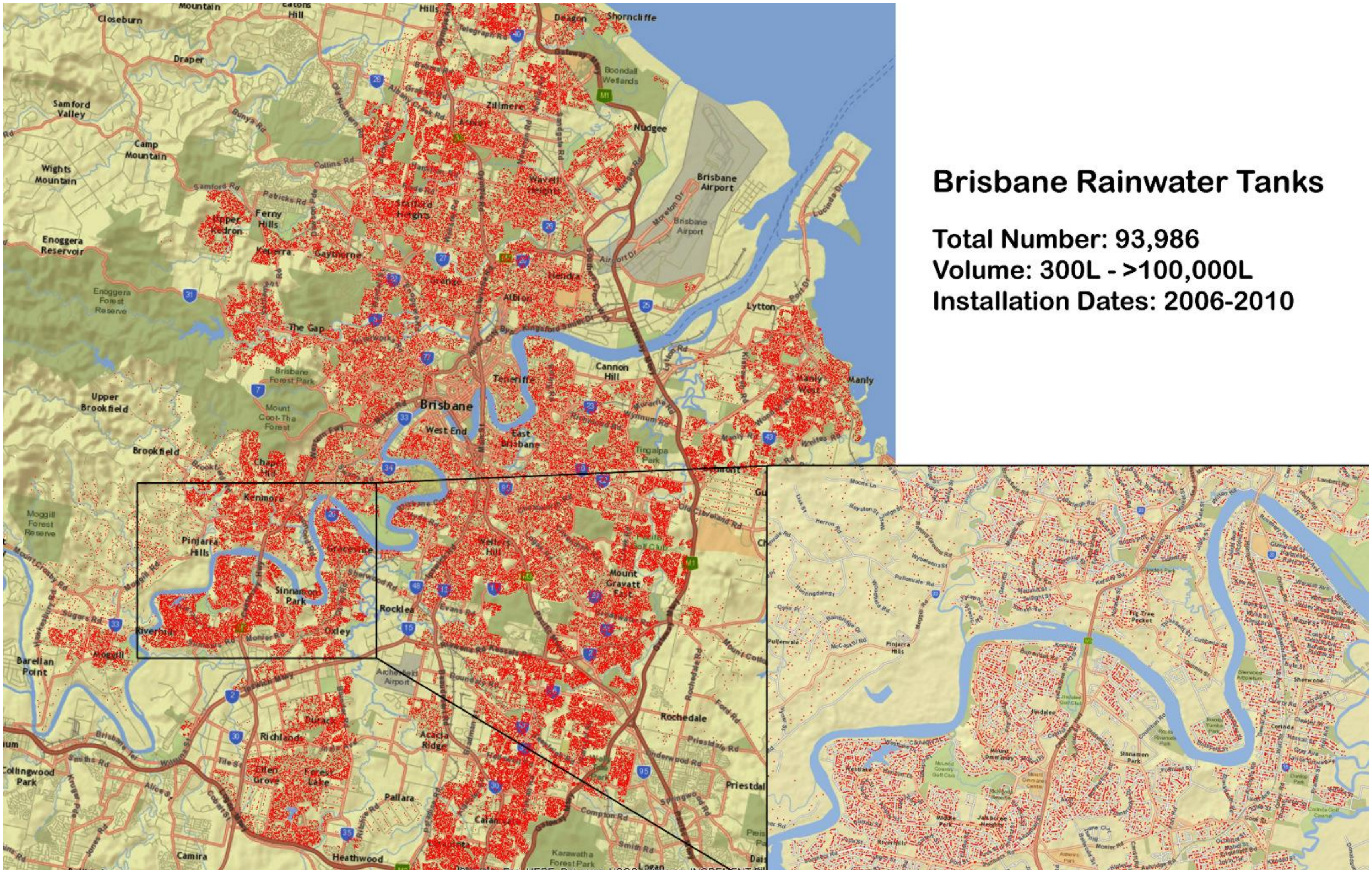

Figure 9. Map showing the extent of rainwater tank installations across the Brisbane City Council (Brisbane City Council, 2012). Data is sourced from Brisbane City Council (2012)

1.4.4 Rainwater tanks as permanent larval habitat

The installation of large numbers of rainwater tanks into the Brisbane urban landscape has provided *Ae. aegypti* with an ideal habitat for establishment. Kearney et al. (2009), Beebe et al. (2009) and Richardson et al. (2013) have attempted to model the micro-climate within large water bodies and how this niche relates to *Ae. aegypti* distribution. There is little other scientific literature on the micro-climate of a rainwater tank and this is a knowledge gap of high importance. Rainwater tanks have previously been recognized as key containers as they potentially provide ideal larval habitat due to their large water volume and reliability as a source for oviposition (Tun-Lin et al., 1995b, Ritchie et al., 2002).

Little has been documented in the historical literature on the role that rainwater tanks have played in disease transmission and mosquito prevalence in Australia. Mosquitoes were first implicated as the vector of dengue fever in 1906 and findings led to the Queensland Health Act of 1911 (The Brisbane Courier, 1912). Priority was given to the destruction of mosquitoes and their breeding locations through Brisbane and Queensland. During this early period, tanks were identified as a primary source of mosquito breeding in Queensland and surveys were undertaken to identify the prevalence of tanks and the mosquitoes found in them (Taylor, 1915). There is little Australian scientific literature on mosquitoes and rainwater tanks at this time. It is thought that rainwater tanks were commonly used for oviposition by *Ae. aegypti* due to a preference for larval habitat in the immediate vicinity of a food source and the reliability of the water they contained (Hamlyn-Harris, 1927b). The first mosquito surveys of Brisbane were documented in government reports by health authorities in 1912/13. They revealed that from 1,386 houses and 1,832 tanks examined, 37% (679/1,832) had *Ae. aegypti* present and six in total were screened (Elkington, 1913). It is implied that rainwater tanks were acting as a major source of mosquitoes at the time when dengue outbreaks were affecting 75-90% of the population in Brisbane (Lumley and Taylor, 1943).

Rainwater tanks have also been implicated in disease outbreaks and as important larval habitat for populations of *Aedes* mosquitoes elsewhere (Tun-Lin et al., 1995b, Hanna et al., 1998, Ritchie et al., 2002). Tun-Lin et al. (1995b) showed rainwater tanks in northern Queensland comprised 13.5-29.6% of positive containers, while supporting 60-63% of all *Ae. aegypti* immatures surveyed, with a mean of 466 larvae per tank (± 1,196; with one sample contained 6,601 larval forms). Hanna et al. (1998) observed rainwater tanks contributing to >50% of total containers surveyed with *Ae.*

aegypti present. They suggested that rainwater tanks were the major source of vectors contributing to the dengue outbreak that occurred in 1996 (Hanna et al., 1998).

The temperature buffering effect of rainwater tanks could be the mechanism that protects mosquito larval stages from extremes in cold temperature and desiccation in Brisbane and regions further south (Kearney et al., 2009). Water is known for its high specific heat capacity, a property that allows it to store a large amount of heat energy. Large volumes of water have another property called thermal mass. This is the ability to resist fluctuations in temperature, or low thermal inertia (Maréchal, 2006). A high specific heat capacity and low thermal inertia are two properties that provide the mechanism by which rainwater tanks buffer the potentially large fluctuations in temperature that occur in the Australian environment. In doing so, rainwater tanks could provide the ideal thermal niche in southern Australian regions, where climatic conditions are not favourable for the persistence of *Ae. aegypti* throughout the year (Kearney et al., 2009).

Eisen et al. (2014) identified key knowledge gaps on the population dynamics of *Ae. aegypti* in cool range margins, specifically related to key container types that contribute to the overwintering of mosquito populations. Modelling by Williams et al. (2010) suggest that populations of *Ae. aegypti* in Brisbane would be reduced to eggs (only) during winter periods if rainwater tanks did not exist environment. A small increase of 5% in egg survival predicted increased survivorship and persistence in Brisbane. Rainwater tanks now existing in the urban landscape may provide this increase in survival during the winter months, potentially due to their capacity to buffer temperature fluctuations and desiccation stress. In this way, rainwater tanks may be defined as sources, sinks and potentially pseudo-sinks to mosquito populations in the urban landscape, depending on the time of year and compliance status. Little is known about how rainwater tanks influence mosquito populations in urban environments. Theoretically tanks exposed to the egress of adult mosquitoes could act as sources to a population at all times of year if nutrition was not limiting and temperatures were within thermal limits for development.

1.5 Movement and distribution of *Aedes aegypti* in urban environments

1.5.1 Measurements of *Aedes* population abundance, movement and spread

With growing interest in the implementation of incompatible insect (IIT) and sterile insect technologies (SIT), understanding the influence of urban environments on movement and the population dynamics of disease vectors has become increasingly important. Urban environments are often heterogeneous and comprised of features which obstruct or facilitate the movement of disease vectors. The first movement studies on *Aedes* mosquitoes in Australia focused on female dispersal distances and survival rates, to understand the potential for disease spread. Muir and Kay (1998) measured the movement of male and female *Ae. aegypti* within a single urban block. They observed that shady habitat and accessibility to hosts were characteristic features of the urban environment that attracted adults (although these were not tested quantitatively), and that wind affected mosquitoes released outdoors (Muir and Kay, 1998). Russell et al. (2005) released marked female *Ae. aegypti* in Cairns, Australia, in a study that examined movement within large urban blocks. The authors observed that small, quiet roads had little influence on female movement, however, they observed a significant difference between the number of mosquitoes crossing a busy road than those that didn't. Their conclusions suggest that unshaded areas and roads could act as barriers to movement (Russell et al., 2005). Hemme et al. (2010) used molecular analyses to show limited mixing of *Ae. aegypti* populations across large urban roads in Trinidad, West Indies, concluding that roads 80-130m wide act as barriers to dispersal. Recent work with *Wolbachia* infected *Ae. aegypti* suggests that population movement through a landscape is slow at a rate of 100-200m per year and is influenced by barriers such as parklands (Schmidt et al. (2017). Other recent contradictory work in Australia on the establishment of *Wolbachia* infected *Ae. aegypti*, observed movement across a large highway and results suggest that long distance dispersal may still occur (Hoffmann et al., 2011). Unfortunately long distance dispersal via human mediated transport is suggested to be common (Lumley and Taylor, 1943, Jansen and Beebe, 2010, Guagliardo et al., 2014) though studies rarely attempt to discriminate between human mediated or via individual based, long distance movement events.

Aedes notoscriptus is often associated with *Ae. aegypti* in rainwater tanks. Vector competence experiments and mosquito collections have implicated *Ae. notoscriptus* as a potential Ross River and Barmah Forest vector (Ritchie et al., 1997, Watson and Kay, 1998, Watson, 1999). This species has wider host feeding preferences and larger dispersal than *Ae. aegypti* and therefore has the potential to contribute to the spread of diseases in urban environments (Watson et al., 2000b). Watson et al. (2000b) were the first to examine the movement of *Ae. notoscriptus* in an urban setting. They observed that natural barriers (trees, creeks and bushes) and artificial barriers (fences and roads) did not restrict dispersal, and that females were attracted to urban features such as harbourage sites and shade. Furthermore, they estimated that marked females lived long enough to potentially transmit both Ross River and Barmah Forest (Watson et al., 2000b).

Traditional methods for estimating the movement of mosquito vectors involves trapping and removing individual insects at discrete distances from release sites, and then applying a correction for unequal trapping density within each annulus (Lillie et al., 1981, White and Morris, 1985, Morris et al., 1991). The statistic used in these studies, mean distance travelled (MDT) is considered less biased for inconsistencies in trap location or size of study area. The flight range where 50% (FR_{50}) and 90% (FR_{90}) of the marked population were recaptured is then extrapolated using the antilog of the $\log_{10}$(distance + 1) over the cumulative number of expected flies (White and Morris, 1985). Calculations of MDT and flight range are summary measures, and due to their discrete nature are unable to accurately characterise skewed distributions with long tails (Winskill et al., 2015). Likewise, methods for marking, release and recapturing mosquitoes are often limited in their ability to accurately measure movement rates as marks can influence mosquito mortality, decrease mobility and interfere with sensory organs (Hagler and Jackson, 2001).

Recently, the study of mosquito movement has focused on the development of mathematical models called dispersal kernels, where the probability of recapture is estimated using density functions. Unfortunately, the development of insect dispersal kernels has suffered from inconsistencies in the terms and methodologies used to describe movement in mathematical terms (Winskill et al., 2015). The term dispersal kernel can be used interchangeably as the probability density function (PDF) of a) the dispersal distance of each disperser or b) the probability density of a given distance and bearing from a source (Cousens et al., 2008). Currently, these methods for measuring insect dispersal are based on plant propagule movement, where kernels use discrete distances, and assume that the location where an insect enters a trap is as far as it would have moved in its lifetime. As such, potential distance travelled by an individual over a lifetime is confused with distance travelled until capture.

Traditional measurements of *Ae. aegypti* populations have focused on immature abundance and *Stegomyia* indices due to the use of oviposition site removal in the reduction of dengue risk (Connor and Monroe, 1923, Focks, 2004). However, the collection of immatures for *Stegomyia* indices has drawn increased criticism due to the labour-costs associated with sampling and a lack of evidence that it works in estimating disease risk (Focks, 2004, Bowman et al., 2014). Instead, attention has moved to counts of eggs and adult mosquitoes as an estimation of local population and disease risk. Ovitrapping of eggs have been using extensively for more than 50 years to detect the presence of adults and their spatiotemporal distribution and dispersal (Maciel-de-Freitas et al., 2006, Honório et al., 2009, Chadee and Ritchie, 2010, de Melo et al., 2012). Although this method has the benefit of being inexpensive and easy to operate, it requires a laboratory for rearing of larvae and identification, and ovitraps are a poor proxy for adult abundance due to “skip oviposition behaviour” observed in *Ae. aegypti* (Reiter, 2007, Chadee and Ritchie, 2010). BG-Sentinel traps are the preferred method for adult surveillance of *Ae. aegypti* in recent years. This trap has been proven as the most effective design because it collects both sexes across a range of different physiological groups of adults. The costs associated with this trap type are, unfortunately, inhibitory for sampling large spatial areas because electricity is required to power the fan motor that draws mosquitoes into the trap (Williams et al., 2007, Eiras et al., 2014). Recently Eiras et al. (2014) developed a tool for capturing gravid adult mosquitoes called a Gravid *Aedes* Trap (GAT). Their method uses a simplistic bucket containing visual and olfactory cues to attract adult females in search for an oviposition site. Adults enter the trap and are killed by a pyrethroid insecticide within 3-15 minutes, allowing easy identification (Eiras et al., 2014). When tested in semi-field conditions, GATs performed significantly better than double sticky ovitraps and only resulted in minimal escapes from the trap (Eiras et al., 2014). The low costs associated with GATs (~$30 as opposed to ~$300 for BG Sentinal traps) could allow for a greater number of traps for sampling adult populations.

1.6 Network models and simulating mosquito populations

Modelling of mosquitoes is typically done as large-resolution, meta-populations as the digital environment is less computationally intensive when applied at these resolutions (Dufourd and Dumont, 2012, Reiner et al., 2013, Dommar et al., 2014). Mosquito vectors typically represent populations within habitat patches and it is assumed that the size of a mosquito population influences pathogen transmission to humans (Moulay and Pigné, 2013, Mniszewski et al., 2014, Dommar et al., 2014, Manore et al., 2015). Models involving mosquitoes are typically used to understand gene flow (Gonçalves da Silva et al., 2012), pathogen transmission dynamics (Reiner et al., 2013), population dynamics (Focks et al., 1993b, Williams et al., 2008, Aldstadt et al., 2011, Wang et al., 2016) and are not typically designed to simulate individuals or populations moving through a landscape. Otero et al. (2006) developed a dynamic population model for *Ae. aegypti* applied at the level of a city. Otero et al. (2008) furthered this work by developing a stochastic, spatial, dynamic model to simulate aspects of population movement and explore ideas around persistence and the colonization processes. As computer power has increased and digital tools have improved, so has the ability of modellers to increase the complexity and sophistication of models, and to explore complex biological processes.

Almeida et al. (2010) were the first to simulate an *Ae. aegypti* population with an agent-based modelling approach. Authors used the Repast (Collier and North, 2011) modelling environment which allows for the simulation of individual mosquitoes and their movement. Authors compared real experimental data to a simulated mosquito population where mortality was related to location of traps (Almeida et al., 2010). A difficulty in simulating fine resolution movement in this way is the lack of literature detailing the decision-making process of individual mosquitoes and the accuracy of parameters applied (Almeida et al., 2010). Recently, simulations of individual mosquitoes have been used to study contact rates between vectors and hosts (Cummins et al., 2012). This model was designed to study movement in spatial scales of metres and time scale of minutes and was unable to simulate movement at the population level (Cummins et al., 2012). Thus, simulating individual mosquito movement as part of a larger meta-population is a rarely done and there are a number of knowledge gaps not addressed by previous studies. Further work is required to describe and understand movement parameters of mosquitoes at finer temporal and spatial scales.

Network theory has undergone enormous growth in recent years, offering scientists a way of displaying interacting entities within populations and food webs, diseases between organisms, proteins in cells, cells in organisms and traffic through the internet (May, 2006). Empirical and theoretical work on the structure and dynamic behaviour of networks is increasingly being used by ecologists to explain spatiotemporal patterns of organisms within landscapes. The theory provides a number of approaches and measures to quantify biological systems that are consistent with the ecological processes of dispersal, disease transmission and flows of energy between habitat patches or metapopulations (Brooks et al., 2008, Ferrari and Lookingbill, 2009, Ferrari et al., 2014). A network perspective provides powerful leverage on ecological applications involving connectivity or ecological fluxes (Van Langevelde et al., 1998). The strength of this perspective lies in its ability to represent discrete entities (known as nodes or vertices) on a heterogeneous topology, while characterizing and measuring the connectivity between these nodes (as links or edges) at broad spatial scales. Areas representing nodes in a landscape are typically ecological resources and are distinguished from areas of unsuitable habitat. Normally this consists of a collection of pixels aggregated spatially onto a digital map to form habitat patches and for invasive species these locations represent areas where population growth is occurring or could expand to in the future (Ferrari et al., 2014). Nodes are typically connected by dispersing organisms or gene flow, and this movement is represented as either a true distance between nodes or a functional distance, with links being a binary variable (0/1) or a transitional probability (0 to 1, depending on likelihood)(Ferrari et al., 2014). Once formed, complex networks have a number of statistical ways to measure flows of information.

The simplest statistic used to describe the number of links (i) a node has formed with surrounding nodes is its degree. Within a large network, nodes are characterized by a 'degree distribution' or the probability (P) that a randomly chosen node will have degree i with other nodes (Rosen, 2011). The degree distribution of some networks can represent Poisson or binomial distributions where the probability of finding large i values diminish rapidly past the mean (May, 2006). Other network degree distributions can represent fat tails which decrease slowly as i increases (small world networks) and even fatter (scale-free networks) where degree distributions follow a power-law with no characteristic number of links per node (May, 2006). The clustering coefficient is one statistic property of large networks that gives a sense of how 'clumpy' a network is (Bollobás, 1998, Strogatz, 2001). This is the average probability that two neighbours of a node are also neighbours of each other, or the ratio between the total number of links connecting a node's nearest neighbour, and the total of all possible links between these nearest neighbours (May, 2006, Dorogovtsev and Mendes, 2013). Measurements of centrality are a relevant measure for invasive networks, as nodes that contribute most to the spread or persistence of an organism can be identified and these are relevant to management approaches (Ferrari et al., 2014). Freeman (1979) defined three measurements of centrality; degree or the number of nodes connecting to the focal node; closeness or the inverse sum of the shortest distance to all other nodes from the focal node; and betweeness measures the degree to which a nodes lies on the shortest route between two other nodes. Ferrari et al. (2014) use degree centrality and a further measure called 'source strength' to measure which nodes acts as high sources of infection to other nodes and therefore promote further spread through the network.

Until recently, network theory was rarely used to simulate mosquito movement or population dynamics. Yakob and Yan (2010) presented a novel network model of malaria vector population dynamics to examine the effect of a patchy vector distribution on population growth after the extended dry season in Africa. Through simulations based on a theoretical environment of 500 pools (or nodes) the authors were able to show that dispersal was important for population persistence if adults had a patchy distribution after the dry season; that population growth was more rapid if mosquitoes continued to breed through the dry season; and that different dry-season survival strategies had distinctive influences on population dynamics. While this model is useful in directing future control activities, results are formulated solely on a theoretical basis. A more useful model would have used field data and attributes (such as dispersal or measured population growth) based on the biology of the mosquito they were simulating to provide firmer evidence for their conclusions.

Networks of invading organisms have typically been designed as static systems which focus on a structure that effects stability and connectivity. In static network models (SNM), nodes and links are fixed in space and time. These type of networks are used to study interpatch connectivity as a function of distance. Here a species' dispersal ability can be used to define the underlying links and therefore connectedness within the network (Ferrari et al., 2007, Lookingbill et al., 2010). One of the strengths of SNM is their ability to predict locations relevant to targeted management practices such as where large numbers of connections occur, their importance within the network and possible pathways of future spread. Ferrari and Lookingbill (2008) used a SNM with a cellular automaton to track a simulated invasion across a fragmented landscape. They found that the site of initial establishment can significantly affect the temporal dynamics of an invasion and that traditional measurements of degree distribution and measures of distance were insufficient for predicting the direction and speed of an invasion. Ferrari et al. (2014) criticize static models as they offer no insight into the probability of when connections are likely to occur (or disappear) and evolve throughout an invasion. The authors propose an alternate framework for modelling range expansion: dynamic network models (DNM). DNMs share similar features with static models, in that they both represent connectivity between interacting entities as links and nodes respectively. However, DNMs create connections that incorporate temporal dynamics such as when connections arise or disappear and their probability of doing so (Ferrari et al., 2014).

Chapter 2. The eradication of the dengue vector, *Aedes aegypti* from Brisbane, Australia: the role of surveillance, larval habitat removal and policy.

Chapter published in PLOS Neglected Tropical Diseases and formatted for that journal.

2.1 Introduction

Dengue fever is the 21st Century's most important mosquito-borne viral illness, exerting a huge economic and health burden in the tropics and sub-tropics [1]. The incidence of dengue has increased 30 fold over the past five decades and is now estimated to affect up to 390 million people each year [1, 2]. This increase in prevalence is due to substantial growth in urbanization, trade, international travel, and the spread of its major vectors [3]. Historically, the high infection rate of the disease has exerted a severe toll on development and economic progress through lost productivity and costs of mosquito control activities [3-5].

In Australia, four large dengue epidemics that swept through Queensland and New South Wales between 1897 and 1926, were responsible for a reported 733 deaths, and affected up to 90% of the urban population in each outbreak [6]. Dengue was first isolated in Australia around 1956 [7, 8] and until this time, all dengue cases in Australia were based on clinical diagnosis. More recently, a rise in imported cases of mosquito-borne diseases into Australia have increased the risk of local transmission of dengue, chikungunya and Zika viruses in all regions where the primary vector, *Aedes aegypti* (L.) is present [9, 10].

Aedes aegypti is most abundant in northern Queensland and some parts of central and southern Queensland, but a number of populations have recently been discovered in the South East of the state, 170km north of Brisbane, the state capital [11]. It was in Brisbane that Thomas Bancroft first implicated *Ae. aegypti* as an agent in dengue transmission in 1906 [12], and this was later confirmed by Cleland in 1917 [13]. At the start of surveys for *Ae. aegypti* in 1911, Brisbane was ~77 km^2 ($30mi^2$) and by 1923 had grown to ~$163km^2$ ($63mi^2$)[14]. Currently, Brisbane is the largest urban centre of the state with a population of 1.18 million inhabitants [15], an area of 1,338 km^2 (516mi^2) and lies just outside of the southern margin of *Ae. aegypti* in eastern Australia (Figure 2) [16].

Adult female *Ae. aegypti* feed predominantly on humans and use many types of artificial containers for oviposition that are found in urban locations [20, 21]. This mosquito species is closely associated with the domestic environment and the many microclimates found there [22]. Climate alone does not determine the geographical distribution of *Ae. aegypti*, and as such, presence is not entirely dependent on rainfall for the provision of suitable aquatic larval habitats and its persistence is facilitated by its use of artificial water-holding containers [23, 24]. Eggs are desiccation-resistant and can stay dormant during unfavourable periods [21]. The unscreened house design typical of Queensland in the early 1900s represented highly favourable habitat for *Ae. aegypti* adults, providing unrestricted access to a human blood meals and enhancing disease transmission [25]. Once established, *Ae. aegypti* disperses readily through the urban environment and suitable habitat is constantly being invaded [26].

In the past, these characteristics have allowed *Ae. aegypti* populations to persist throughout much of Australia. Accordingly, its historical distribution includes Queensland, the Northern Territory (NT), Western Australia (WA; including the south) and New South Wales (NSW; Figure 2), with unconfirmed reports in Victoria [27, 28]. This range decreased dramatically in the mid-twentieth century, with the last recorded collections in NSW in 1948, and WA in 1970 [29]. In the NT the last recorded collection was in 1957 [27], but there were some temporary establishments in 2004 and 2011 which were subsequently eliminated [30, 31]. Herein we refer to contemporary term "elimination" as the intentional action by humans to remove *Ae. aegypti* from a defined geographical area [32]. Historically, this action has been referred to as "eradication" by medical entomologists [33-37]. We refer to the elimination of dengue as the reduction of disease transmission to zero incidence in a defined geographical area [32]. In Brisbane during the early 1900s, *Ae. aegypti* was highly abundant and government agencies set out to eliminate it based on the control successes (that targeted larval habitat) observed during the construction of the Panama Canal, [38-44]. At some time after the last outbreak of dengue in 1943, the mosquito was assumed to have disappeared from Brisbane. There are a number of hypotheses suggested by medical entomologists for the wide-spread geographic disappearance of the species, however there are little quantitative data to support these assertions and whether or not they apply to Brisbane [29, 45].

Rainwater tanks have historically been a conspicuous feature of the Australian rural and urban landscape. Since their introduction in the mid-to-late 1800s, these large structures have provided a reliable source of potable and non-potable water to households and commercial premises and inadvertently acted as key larval habitat for *Ae. aegypti* [27, 29, 43, 46]. In Brisbane, rainwater tanks were first identified as habitat for mosquitoes during the early 1900s [47, 48], and regulations were introduced in 1911 to prevent mosquitoes from exploiting rainwater tanks and dwellings [49]. Permanent water storage containers provide a reliable population source by acting as refugia for mosquitoes during times of sub-optimal climatic periods such as drought or extreme seasonal temperatures [23, 27, 50, 51]. Recent modelling suggests the presence of rainwater tanks was an important factor in the historical distribution of *Ae. aegypti* by potentially buffering the effects of climatic extremes on biological processes at geographical range limits [52].

Despite a keen interest in controlling mosquito related diseases in tropical and subtropical countries around the world, there are few documented examples of elimination and little understanding of the factors that have led to localized *Ae. aegypti* extirpation [53-55]. Here we review data collected from a range of historical sources and evaluate the role of anti-mosquito regulations, surveillance and the removal of rainwater tank infrastructure in the elimination of *Ae. aegypti* from Brisbane.

2.2 Methods

We acquired historical records compiled from a range of scientific, government and media sources describing anti-mosquito legislation, mosquito surveillance, regulation enforcement and the presence of dengue and *Ae. aegypti* in Brisbane. We then asked a series of questions. Firstly, what evidence is there to test the hypothesis that *Ae. aegypti* is still present in Brisbane? Secondly, if local elimination occurred, what quantifiable evidence was there to test the hypotheses that the removal of rainwater tanks contributed to the elimination of the species? Finally, what were the drivers that led to the removal of rainwater tank infrastructure? To answer these questions, we created a time-line of events and linked direct observations recorded by government surveillance and regulatory enforcement.

Scientific publications and annual council and state government reports provide quantitative and subjective measures of a number of factors that potentially affected the historical presence of *Ae.*

aegypti in Brisbane. We collected data from entomological surveys that estimated the prevalence of rainwater tanks on premises and incidence of mosquitoes within these tanks. From the first surveys in 1911, anti-mosquito policy deemed rainwater tanks non-compliant if they allowed the ingress and egress of mosquitoes [49]. Through enforcement of this legislation, residents found to have larval habitat on their property (including non-compliant rainwater tanks) were warned that they were in violation of mosquito regulations, and given a "notice of breach". Residents were asked to comply within two months, after which patrol members would return and re-survey the property [56]. If the return survey was again in breach of regulations, the resident could be in default, fined and prosecuted. We only included initial surveys (and excluded re-surveys) in estimations of total dwellings surveyed and for prevalence and compliance rates of rainwater tanks as a reflection of conditions at that time point. Survey results allowed us to identify temporal trends in the outcomes of entomological inspections; prevalence of tanks on properties, rates of rainwater tank compliance with regulations, mosquito presence in tanks, the number of notices given for a breach of mosquito regulations and the number of notices complied with. This is the first time those data have been collated to demonstrate the role of government in the elimination of *Ae. aegypti* from Brisbane.

To test the hypothesis that *Ae. aegypti* was still extant in Brisbane over the period from 1887 until 2016, we applied the method of Jaric and Ebenhard [57]. Briefly, this method extends the work of Solow [58] who provided an equation for inferring extinction based on sighting records over time. Here sightings during the observation period are arranged from first to last and used to express the probability of species presence in relation to the number of time units in which the species was recorded [58]. Authors using this method use $P = 0.05$ as the probability threshold, below which the species can be regarded as extinct [47].

Next, we examine the evidence for whether the mosquito population is extinct by first estimating the probability, p, that mosquitoes are observed each year (based on 27 sightings over the first 69 years), and secondly, estimating the probability that there would be 59 subsequent years of no sightings given the estimated value of p. The probability of observing mosquitoes from one year to the next, p, was estimated as $\hat{p}$ using maximum-likelihood, and a 99% confidence interval, $[p_l, p_u]$, was created by inverting a likelihood ratio test-statistic. The confidence interval includes all the values of p for which

$$l(p; x) \geq l(\hat{p}; x) - 3.317, \tag{1}$$

where $l(p; x)$, is the log-likelihood function evaluated with a yearly sighting probability of p, x denotes the observed historical data, and 3.317 is half the 99th percentile of a χ^2 random variable with one degree of freedom.

Finally, we use the optimal linear estimation (OLE) function from the R package sExtinct() to estimate the date of extinction, with 95% confidence intervals. The OLE method infers time to extinction from a temporal distribution of species sightings [59]. All models and simulation code were created and run in R v3.3.2 [60].

All *Ae. aegypti* 'sightings' were based on yearly observations from scientific or government publications from individual authors or organisations. Multiple reports by the same author were not double counted in a single year. Identification to species level was done by qualified entomologists. We estimated rates of dengue during historical outbreaks in Brisbane using minimum infection rates documented in the literature during epidemics (70%), and population data obtained from government statistics [6, 13, 61-63]. Deaths associated with dengue during epidemics were taken from historical medical literature and government population statistics [6, 13, 61]. As case data were not technically confirmed, it is possible that outbreaks may have been undiagnosed cases of chikungunya or other viral diseases [6, 64, 65].

Brisbane was first declared a city in 1902 and by 1911 contained 26,645 private dwellings [66]. In 1925, the city amalgamated with a number of other local authorities to become Greater Brisbane and expanded from 43,935 dwellings in 1921 to 68,096 by 1933 [67, 68]. We used government statistics describing the number of dwellings within the council boundary of Brisbane annually from 1895 until 1971 to estimate the total number of tanks (proportion of dwellings in surveys that had a tank present multiplied by total dwellings in Brisbane for relevant year), total non-compliant tanks (proportion of non-compliant tanks surveyed multiplied by total estimated number of tanks), the number of non-compliant tanks with mosquitoes present (estimated non-compliant tanks multiplied by proportion of tanks with mosquitoes present in surveys) and the proportion of non-compliant tanks to total dwellings (estimated total non-compliant tanks divided by total dwellings)[61].

2.4.1 Results

2.4.1 What evidence is there for the presence of *Aedes aegypti* in Brisbane?

Noting that historic records refer to *Ae. aegypti* by multiple names (*Culex bancroftii*, *Stegomyia fasciata*, *Aedes aegenteus* and *Stegomyia calopus*; see [69]), the first published record of *Ae. aegypti* in Brisbane was in 1887 [70] and we recorded a total of thirty-seven references from historical records (Table 4, A1). Entomological, house-to-house surveys for the species did not commence until anti-mosquito regulations were introduced in 1911 and, at this time, *Ae. aegypti* was highly prevalent across the city [14, 71].

Table 4. Estimated locally acquired dengue cases based on a 70% incidence of dengue, recorded deaths due to dengue and number of references to the presence of *Aedes aegypti* in Brisbane from historical records.

Decade	**Dengue cases***	**Deaths#**	**Reference to *Ae. aegypti*†**
1890-1899	77,200	40	1
1900-1909	87,970	108	4
1910-1919	Not recorded	62	12
1920-1929	184,597	66	8
1930-1939	2	0	4
1940-1949	664	0	7
1950-1959	0	0	1
1960-1969	0	0	0
1970-1979	0	0	0
Total	**350,438**	**276**	**37**

* Number of dengue cases estimated from a minimum of 70% incidence rate within the Brisbane metropolitan area. Incidence rate in the literature was estimated between 70-90% in historical medical records during large outbreaks [6, 13, 61-63]. # Deaths recorded from scientific literature and government vital statistics [13, 61]. † References to *Aedes aegypti* in Brisbane from scientific and government reports [Appendix 1]. The reader should note that *Ae. aegypti* was first implicated as a vector of dengue in 1906 [12] and confirmed by 1917 [13].

By 1932, mosquito control was decentralized to local health inspectors and little species-specific data was recorded in house-to-house surveys [56]. A dengue outbreak during 1943 re-intensified mosquito surveys with identification to species (Table 4)[72]. However, once the Second World War was over the role of mosquito surveillance was, again, taken over by generalist local government officers, and council reports after 1947 no longer identified or recorded mosquito presence to species level classification. Subsequent recordings of *Ae. aegypti* were made by Elizabeth Marks who recorded distributions from 1957 until 1981, documenting the last accounts of larval *Ae. aegypti* in Brisbane in 1944 [73], 1948 [74] and 1957 (Table 4)[75].

When inferring extinction likelihood, the total length of the observation period (T) was 128 years (1887 until 2016), the total number of years where *Ae. aegypti* was observed (n = 27), and the period between the last positive survey and last observation was 59 years (1957 until 2016). Results suggest the probability that the species is still present based on the mean frequency of sightings was calculated as $P = 0.042$. This value was below the threshold level ($P = 0.05$) and suggests the population does not exist. Using the maximum-likelihood and inverted ratio test-statistic approach, we estimated $\hat{p} = 0.391$ and assuming the true value of $\hat{p}$ is in the 99% range, we estimate $[p_l, p_u] = [0.257, 0.546]$. The probability of observing 59 consecutive years with no sightings were estimated from the probability mass function of the binomial distribution as 2.45E-8 and 5.83E-21 respectively. Therefore, based on the inferred sighting probabilities, there is very little evidence to suggest that the mosquito population is extant given the 59 consecutive years of absence. Finally, using OLE we estimate that from 37 total observations in Brisbane, the species went extinct in 1961 (upper CI = 1973, lower CI = 1961).

2.4.1 Historical records of dengue in Brisbane

A series of dengue outbreaks occurred in Brisbane during the late 19th and early 20th century that took a severe toll on the population of Brisbane and subsequent business and commercial activities [6]. Deaths due to dengue peaked in 1905 and infection rates of 70-90% were reported during all epidemics until 1926 (Table 4)[13, 63, 76, 77]. The last outbreak in Brisbane during 1942/43 resulted in 646 cases [72] and was precipitated by epidemics occurring in northern Queensland cities [78]. The last recorded locally acquired case of dengue noted in the records was in 1948 [74].

2.4.2 Rainwater tanks as potential larval mosquito habitat

Shortages in reticulated water in Brisbane during the 1800s and early 1900s meant rainwater tanks provided the most reliable source of potable drinking water for use around the home [79]. The mean number of water tanks declined from over 1 per dwelling in 1912 to less than 1 in 10 in 1971 (Figure 10). House-to-house surveys were conducted systematically after the introduction of the Brisbane Entomological Section in 1926, with the city divided up into 88 blocks and house-to-house patrols responsible for a number of blocks per year [42]. During the severe dengue epidemic years from 1897 to 1927, the mean number of rainwater tanks per dwelling were at the highest recorded (Figure 10; mean ± *SD* = 0.94 ± 0.3). Following the introduction of a large water reservoir to supply Brisbane in 1954, the mean number of rainwater tanks per dwelling consistently fell below 0.50 tanks. Based on the number of dwellings within the city area, we estimated the total number of rainwater tanks at 39,513 (95% *CI* ± 0) in 1912, before reaching a maximum of 70,794 (95% *CI* ± 515) in 1950, and declining to 24,647 (95% *CI* ± 935) by 1971 (Figure 10).

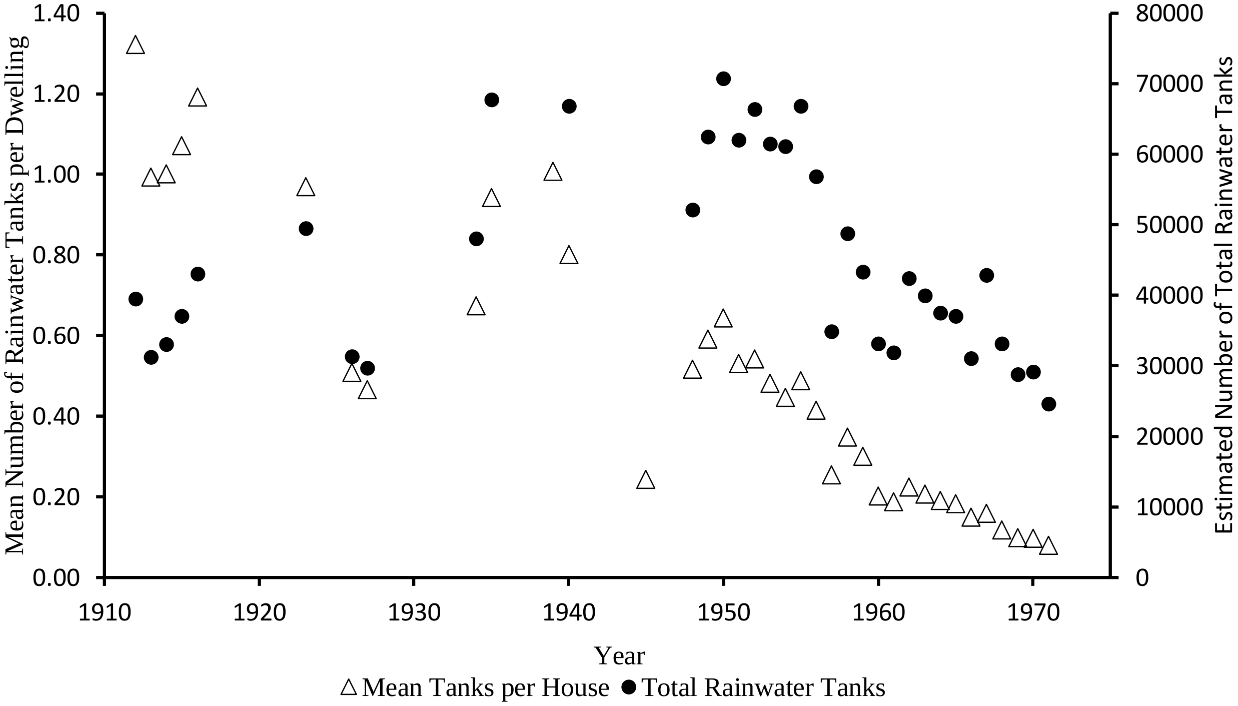

Figure 10. Estimated mean number of rainwater tanks per dwelling (primary axis) and estimated total number of domestic rainwater tanks (secondary axis) in Brisbane, 1912-1971.

2.4.3 The role of regulations and compliance on the prevalence of mosquito habitat

The total number of house-to-house surveys to monitor compliance and prevalence of mosquitoes per year by entomologists and inspectors fluctuated greatly (Figure 11). The period after World War 2 is of most interest, because this was when the city council focused the most effort on the elimination of *Ae. aegypti* [80]. During this period, there was a large increase in annual house-to-house surveys, from 11,158 in 1948, to 91,127 in 1964. The number of surveys decreased after 1964 with the mean dropping to 37,436 (95% *CI* ± 8,041) per year until 1989. Surveys no longer recorded entomological data after 1971, although inspectors continued to give notices of breaches in regulations until surveys were discontinued in 1989.

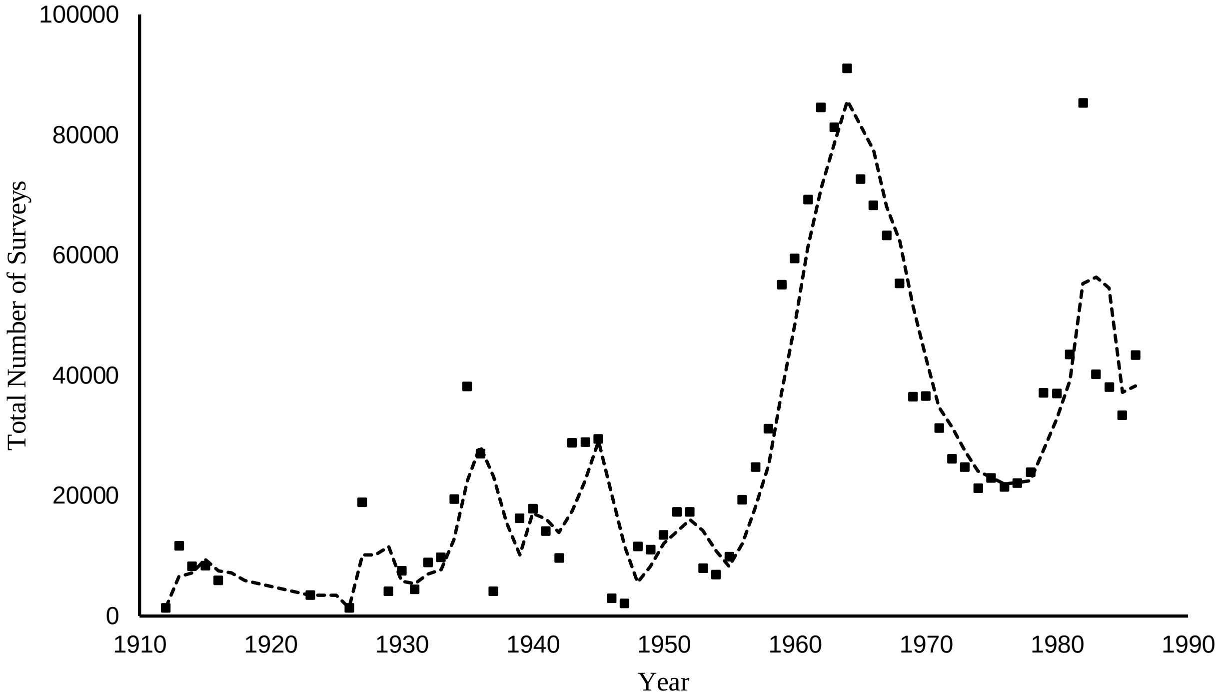

Figure 11. Dwellings surveyed for breaches in mosquito regulations from 1912 until 1989 in Brisbane, Australia. Trend line represents a three year moving average.

The original anti-mosquito regulations defined mosquitoes as noxious, what constituted a breach in regulations, and prescribed ways that the resident must prevent mosquito presence [49]. City Ordinances introduced in 1921 [81] and amended in 1933 [82], were implemented to allow the enforcement of regulations by local authorities and to set penalties for a breach in these regulations (Table 5)[83]. The system of notices provided data on breaches with regulations and "notices complied with" provided measurement of the success of surveillance programs. Compliance with anti-mosquito regulations would involve the resident sealing or removing rainwater tanks that were at risk of breeding mosquitoes and removing or treating other potential containers. Between 1913 and 1954 many surveyed dwellings were in breach of regulations (Figure 12; squares; mean ± *SD* = 0.27 ± 0.18). After that time however, the proportion of surveys resulting in a notice for a breach in regulations dropped (Figure 12; squares; 0.05 ± 0.05), and reached 0.03 by 1974. Compliance with notices was relatively low between 1934 and the end of World War 2 (Figure 12; triangles; 0.80 ± 0.09) but during the 1950s and 1960s (the period when *Ae. aegypti* disappeared) most notices resulted in compliance (Figure 12; triangles; 0.95 ± 0.04).

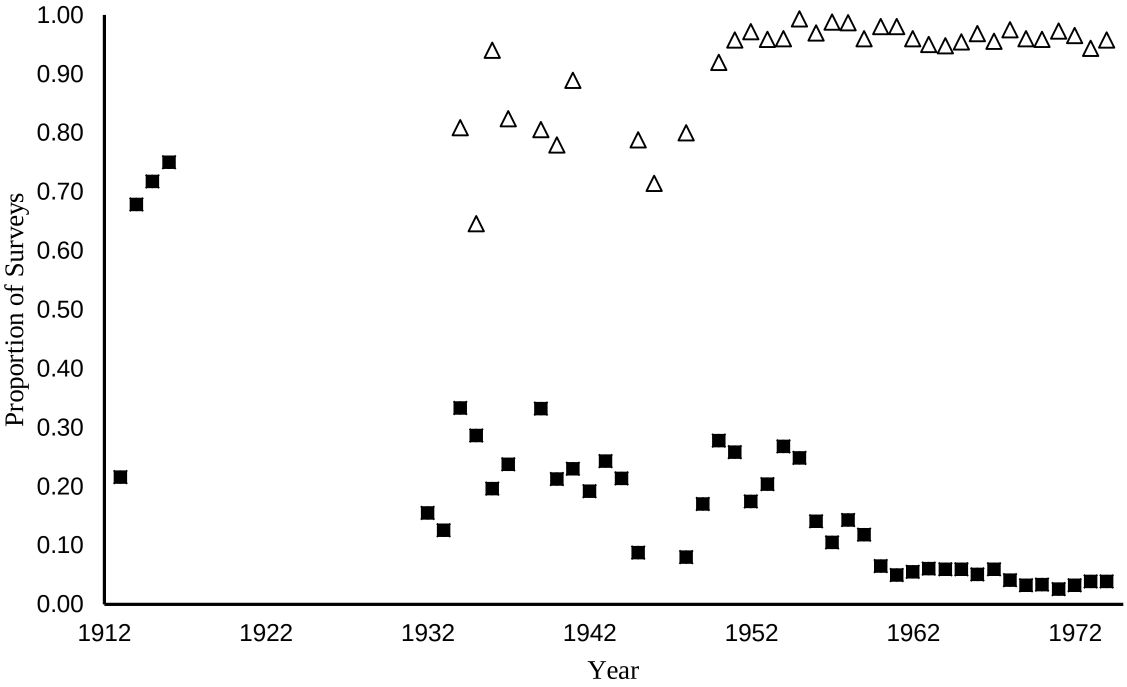

Figure 12. Proportion of surveyed houses resulting in a notice of breach in regulations (squares), and the subsequent proportion of those notices that resulted in compliance (triangles) from 1912 until 1974.

Table 5. Key changes in local government legislation to prevent container inhabiting mosquitoes in residential dwellings, Brisbane from 1911 to 1942.

1911 – The Health Acts Noxious Vermin (Mosquitoes) order of Governor in Council, Metropolitan Area of Brisbane[1]	**1921** – Mosquito Prevention and Destruction Regulations, Brisbane[2]	**1933** – Amendments to Ordinances Greater Brisbane[3]	**1942** - Mosquito Prevention and Destruction Regulations, Brisbane[4]
Declares mosquitoes to be noxious	Prescribes that Local Authorities will be given power to make house-to-house visits to ensure regulations are being complied with, as required by the Director General	Declares type of mosquito habitat that the *resident* is responsible for managing	Declares penalties for breaches in regulations
Prescribes that every tank, cistern or similar receptacle used for the storage of water will be kept protected against the ingress and egress of adult mosquitoes through the use of mesh at every opening (18 meshes to the inch)	Prescribes that a penalty of up to 50 pounds may be given to owners or occupiers interfering with, obstructing, damaging or destroying gutters or drains.	Declares precautionary measures *residents* must apply to mosquito container habitats	Declares officers of Local Authorities to be authorized to act upon the regulations.
Prescribes the use of: kerosene, fish, wire netting or emptying any container (dried and cleaned) every seven days.	Prescribes a penalty for owners or occupiers causing and failing to fill in excavations.	Prescribes the duty of all *residents* to apply the appropriate precautionary measures in respect of all mosquito container habitats: prescribes penalty for default	Prescribes a penalty for a breach in regulations (those mentioned here) of up to 20 pounds, besides cost and expenses for proceedings.
Declares Owners or occupiers must not allow rubbish/containers that could serve as habitat for juvenile mosquitoes		Prescribes the giving of notice of default only in cases in which the resident may reasonably be presumed not to be aware of the default	Prescribes a penalty for failure to comply with a breach in regulations to be no more than 20 pounds.
Declares Owners or occupiers must repair drainage to prevent holding water and habitat for juvenile mosquitoes		Prescribes that in the obstinate default of *residents*, Ordinances may be carried out by the Local Authority at the expense of the resident involved	Prescribes every tank, cistern or similar receptacle used for the storage of water to be kept protected against the ingress and egress of adult mosquitoes through the use of mesh at every opening (16 meshes to the inch, with 28 gauge wire)
Declares Owners or occupiers must use all available means to prevent the presence of mosquitoes on their properties		Prescribes the Chief Inspector shall administer the oversight of the regulations	Prescribes the resident or owner to cut down overhanging vegetation that would deposit leaves in gutters or on tank-tops, at the request of an Inspector Prescribes a report to be made of all inspections and forwarded to the Director General.

[1] [38]. [2] [84]. [3] [85]. [4] [86].

2.4.4 Non-compliant rainwater tanks as larval mosquito habitat

Reports by government entomologists provided the first qualified evidence that *Ae. aegypti* was present in rainwater tanks from 1912 [71, 87]. Surveys in 1923 and 1927 provide further evidence for the presence of *Ae. aegypti* in rainwater tanks [14, 43]. Using survey data, we estimated the total number of non-compliant tanks in Brisbane (Figure 13). When regulations were first introduced in 1911, the majority of tanks were non-compliant [71] and we estimate a total of 39,341 (95% *CI* ± 182) non-compliant rainwater tanks in Brisbane at this time (Figure 13). This was followed by a period of steady decline to 1940 when we estimate 11,900 (95% *CI* ± 293) non-compliant tanks were present (Figure 13). The marked increase in the estimated number of non-compliant tanks observed post World War 2 (with a maximum of 33,931; 95% *CI* ± 788) was due to shortages in labour and materials to make repairs during and shortly after the war (Figure 13)[88]. A rapid decline in the estimated number of non-compliant tanks after 1955 corresponds to the increase in surveillance effort (Figure 11) after which rates returned to levels observed before the war. By 1971 there were only an estimated 4,627 (95% *CI* ± 324) non-compliant tanks left in Brisbane (Figure 13).

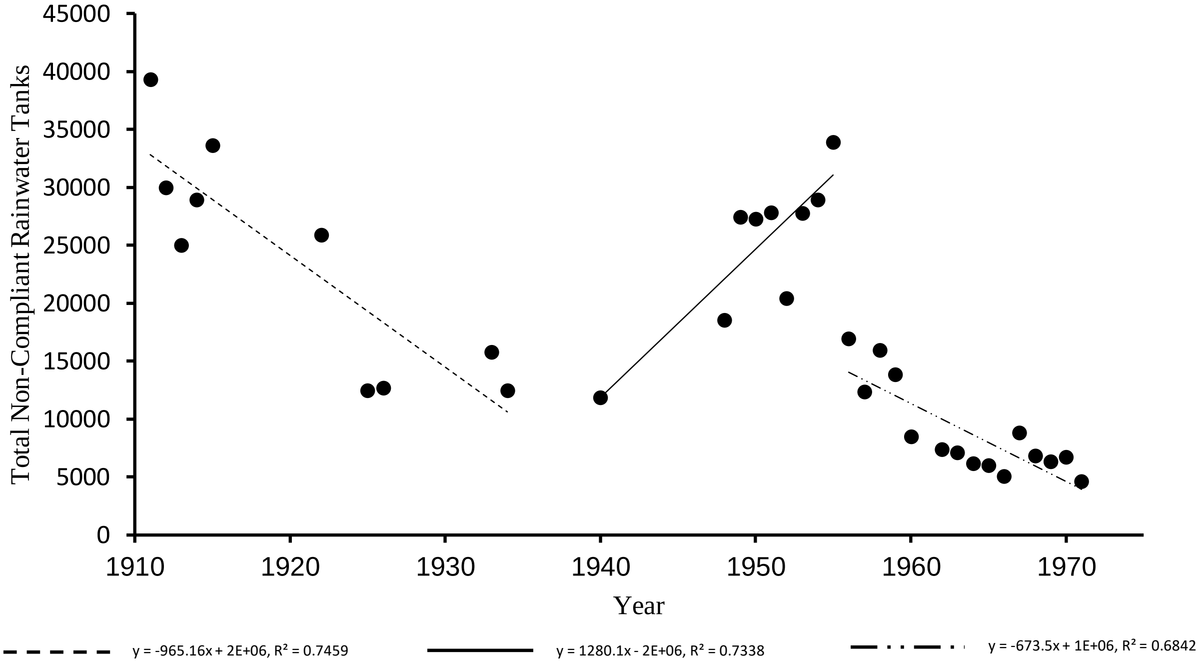

Figure 13. Estimated total number of non-compliant rainwater tanks in Brisbane from 1912 until 1971. Regression lines represent trends in tank compliance during three time periods. Of interest is the second around World War 2 when materials were unavailable for tank maintenance and number of surveys was low (see Fig 3).

2.4.5 Presence of mosquitoes in non-compliant rainwater tanks

Early entomological surveys recorded the number of non-compliant rainwater tanks that contained *Ae. aegypti* [71]. The highest proportion containing this species was observed during years when dengue epidemics were most severe (Figure 14), and a high proportion of non-compliant rainwater tanks harboured *Ae. aegypti* (0.37: 679/1,832, *SE* ± 0.011; Figure 14). After the large dengue epidemic in 1925/26, which was associated with 66 deaths attributed to dengue infection, the proportion of non-compliant tanks with *Ae. aegypti* was highest at 0.51 in 1927 (1,940/3,768, *SE* ± 0.008; Figure 14)[43]. The proportion of non-compliant rainwater tanks containing all mosquito species had decreased to 0.12 in 1945 (284/2,344, *SE* ± 0.007) and 0.07 in 1952 (20/2,902, *SE* ± 0.002; Figure 14). The subsequent rise in mosquito prevalence, to a peak of 0.15 in 1955 (359/2,443, *SE* ± 0.007; Figure 14), is likely due to increases in the total number of non-compliant rainwater tanks present during the same period (Figure 13). As surveys did not identify to species at this time, we cannot determine these were *Ae. aegypti* or the native container inhabiting species, *Ae. notoscriptus.* After 1955, the proportion progressively decreased until the end of entomological surveys in 1974 where container inhabiting mosquitoes were no longer in high prevalence (Figure 14) and non-compliant rainwater tanks were not a common feature in Brisbane's urban landscape (Figure 13).

Figure 14. The proportion of non-compliant rainwater tanks surveyed with *Aedes aegypti* (black diamonds) and mosquito presence only (white diamonds) from 1912 until 1974.

To estimate the density of larval habitat provided by domestic rainwater tanks, we calculated the ratio of non-compliant tanks to dwellings in Brisbane from 1912 until 1971 (Figure 15). High ratios of non-compliant tanks per dwelling (between 1.32 to 0.83 during 1912 and 1923 respectively) relate to periods where epidemic dengue occurred throughout the city. Although the density of non-compliant tanks decreased to 0.2 by 1925/26, the large dengue epidemic during these years may have been facilitated by the high proportion of non-compliant rainwater tanks with *Ae. aegypti* present (Figure 14). From 1926, the ratio of non-compliant tanks to dwellings remained constant, at around 0.2 non-compliant tanks per dwelling until 1955. As Brisbane continued to grow and the number of rainwater tanks started to decrease, the ratio of non-compliant tanks per dwelling decreased to 0.08 by 1971 (Figure 15).

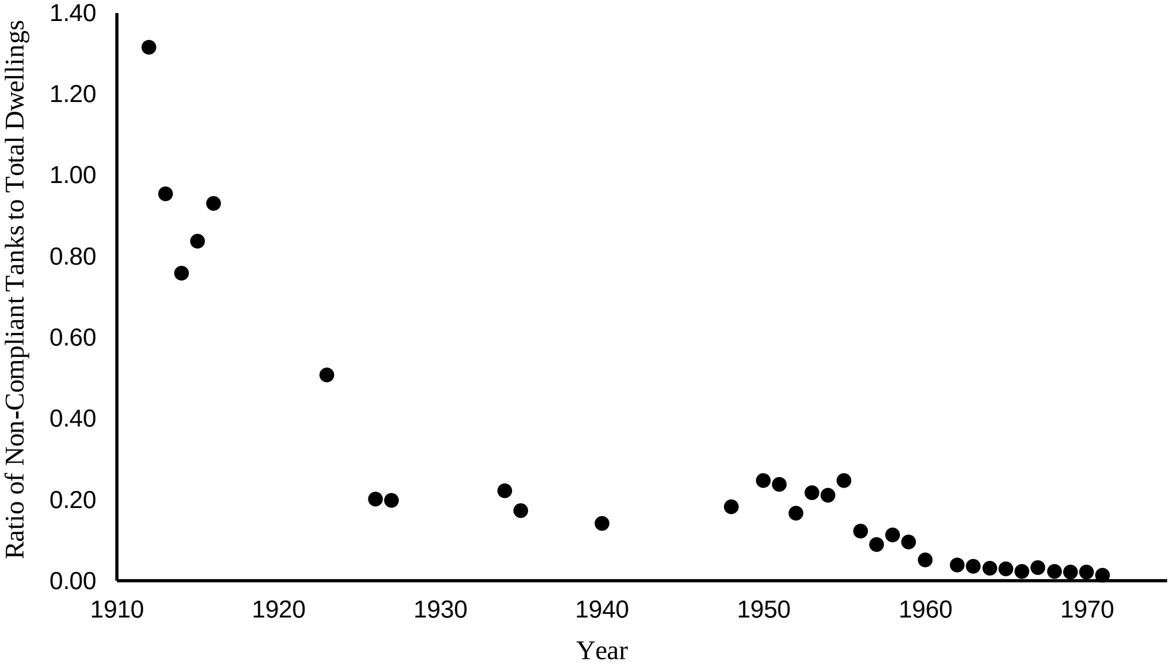

Figure 15. Ratio of the total number of non-compliant tanks to the total dwellings in Brisbane from 1912-1971.

2.5 Discussion

2.5.1 Evidence that *Aedes aegypti* was eliminated and remains absent.

The extirpation of invasive species and the decision on whether an elimination program can be declared successful is challenging. Techniques for detecting invasive mosquitoes are imperfect, and failure to detect a species does not confirm absence with certainty. In Brisbane, despite a constant mosquito management and surveillance program, there have been no recorded specimens of *Ae. aegypti* outside of first ports of entry or quarantined facilities since 1957. Surveys conducted by Elizabeth Marks (Brisbane) and local health authorities from 1965 until 1980 did not detect the species, and larval surveys in 1995 until 1997 and 2007/08 also failed to isolate the vector in high risk residential areas in Brisbane [69, 73, 89-91]. An extensive larval survey of 4,983 premises from 2010 until 2012 likewise did not detect *Ae. aegypti* [92]. Contemporary surveillance efforts have not detected *Ae. aegypti* in the Brisbane City Council region to date, though the species has been intercepted during routine surveillance at first ports (C. Jansen, Queensland Health, unpubl. obs.).

The absence of locally-acquired dengue cases in Brisbane since 1948, despite being a notifiable disease, suggests that any population of *Ae. aegypti* if established, is no longer sufficient in distribution and abundance to vector the virus. The large decrease in dengue fever cases from the 1926 epidemic when compared to the outbreak in 1943 (Table 4) indicates that the mosquito population had likely been reduced substantially prior to the outbreak. As such, the limited cases observed during the 1943 outbreak suggest there was little opportunity for local transmission (Table 4). Following the large outbreak in Townsville in 1955 (approximately 15,000 cases), no cases of locally acquired dengue were recorded in Brisbane, as noted by Doherty [93] who stated that in 1955, “Brisbane remained unaffected, although each of the seven previous outbreaks of dengue in North Queensland since 1895 had been followed by a high incidence in Brisbane” and from a council annual report, “University collectors have tried in vain to obtain specimens of the vector (*Ae. aegypti*). This position is a direct result on the active eradication policy of the Council” [94]. These suggest that by 1955, *Ae. aegypti* was no longer in numbers large enough to vector the virus amongst the population of Brisbane. Between 1948 and the present day (despite high numbers of imported cases in recent years) there have been no locally-acquired cases of dengue fever, suggesting dengue has been eliminated from Brisbane.

Calculations (based on frequency of sighting records) suggest the species is unlikely to be present in Brisbane and it likely went extinct around 1960. Results from the Jaric and Ebenhard [57] method were not highly significant, suggesting there is a small probability of presence. However our maximum likelihood method, where the probability of detection after the last survey date is the same as before it, suggests it is highly unlikely that the species is present after a period of 59 years. We propose, therefore, that *Ae. aegypti* is currently either absent from the city or below our ability to detect the mosquito. As *Ae. aegypti* is just north of the region (Figure 2) there is high risk of future re-establishment, thus discontinuing ongoing surveillance could lead to increased future costs in mosquito management and higher disease risk.

2.5.2 Factors driving the elimination of *Aedes aegypti* in Brisbane.

Historically it is likely *Ae. aegypti* relied on particular larval habitat features to persist through periods of lower rainfall and for populations to subsequently re-establish [27, 52]. We believe that three major factors contributed to the disappearance of *Ae. aegypti* from Brisbane soon after the 1940s.

2.5.2.1 Replacement of rainwater tanks. The removal of large domestic water storage containers (rainwater tanks) and their replacement with reticulated water has been suggested as the principle reason for the elimination of *Ae. aegypti* in the city [29, 45]. O'Gower [27] suggested the species might be eliminated in areas of low rainfall by ‘a continuous mosquito control program, and complete replacement of rainwater tanks by a reticulated water system’. It is likely that a similar process led to the species disappearance in the Mediterranean region, where commonly-used basement cisterns were gradually replaced with reticulated water [95]. Although Brisbane had reticulated water from the creation of its first dam in 1866, the crude reservoir systems had no purification and the reservoirs had limited capacity and were prone to the effects of drought. Rainwater tanks were heavily relied upon as a dependable, clean source of drinking water. The first purification system was created in 1919 but tank use continued to increase into the 1950s [79]. It was not until the creation of Somerset Dam in 1954 that a reliable, unrestricted and clean water supply was available to the whole city. This coincided with the period where elimination of *Ae. aegypti* occurred. We conclude that it was the gradual introduction of a clean, reliable source of reticulated water that led to the disuse and removal of rainwater tanks throughout the city. Although the removal of these large water storage containers may be considered a tipping point in the process of extirpation, it is unlikely to have been the primary reason for the elimination of the species. Large numbers of rainwater tanks were still present in the city by the end of entomological surveys in 1974.

2.5.2.2 Removal of non-compliant rainwater tanks. More importantly, the removal of non-compliant rainwater tanks from the early 1930s appears to have coincided with a decline in *Ae. aegypti* presence and therefore, reduced the risk of dengue transmission. Non-compliant rainwater tanks can provide essential refugia for eggs and larvae and act as population sources after unfavourable climatic periods (such as droughts, dry seasons and winter; Trewin et al. unpubl. data). It is possible that non-compliant rainwater tanks reflected the general disordered nature of backyards in the early 1920s. The removal of large water storage containers and other larval habitat during house surveys would have reduced opportunities for population growth and persistence. From the peak of dengue fever cases in the 1920s, where ~50% of non-compliant rainwater tanks had *Ae. aegypti* present, it appears surveillance and regulations began to influence the abundance of the species in the city. This is reflected in the proportion of non-compliant rainwater tanks containing mosquitoes after World War 2 (Figure 13). By the early 1930's, Hamlyn-Harris [96] observed that "never in the history of the mosquito campaign had there been fewer domestic mosquitoes" in the city, although he did not directly record the presence of *Ae. aegypti* in any of his surveys at this time. This is also the period where the ratio of non-compliant tanks to dwellings plateaued and this likely influenced the abundance of the species. As such, it is possible that the decrease in non-compliant tanks from 1912 until 1940 led to a reduction in the numbers of container-inhabiting mosquitoes (Figures 13 and 15), and contributed to the lower incidence of dengue in the 1943 outbreak. The shortage of materials during World War 2 contributed to the rapid rise in non-compliant tanks from 1940 until 1955 and there was a need for increased surveillance and enforcement after the war [88]. The deployment of experienced health inspectors after this time [28] and the considerable number of dwellings surveyed from 1948 until 1964 (Figure 11), likely ensured the number of non-compliant tanks dropped rapidly from 1955. Health authorities understood that *Ae. aegypti* had been "effectively reduced in numbers to the point where it was no longer a capable link in the chain of [dengue] transmission by 1952 [97], the campaign continued to "be vigorously waged" [98] until 1964, and by 1966 the species had been "virtually eliminated" [99]. During 1964 the number of surveys undertaken represented approximately half the number of dwellings in the city and exemplifies the effort by government officials to eliminate *Ae. aegypti* from the city. By 1971, the proportion of non-compliant rainwater tanks compared to total dwellings in Brisbane was minimal. We conclude the drop in total non-compliant rainwater tanks, during the 1930s and a sustained reduction after 1957 would have dramatically reduced the availability of rainwater tanks suitable for mosquito habitat during extended periods of unfavourable rainfall.

2.5.2.3 Alternate larval habitat. Starting in 1912, teams applied a variety of larval suppression techniques to target other species in swamps, sewers, cemeteries and gully-traps [71, 100, 101]. It is likely that the intensive mosquito program run in parallel to house-to-house surveys (see DDT section below) contributed to the removal of alternate larval habitat, such as subterranean sites. Likewise, the objective of dwelling surveys was to "eliminate all mosquito breeding places" [56] which included containers such as rubbish, water barrels, jugs and flower-pot saucers [71]. By the time *Ae. aegypti* had disappeared from Brisbane, other oviposition sites may have been too transient or too rare to allow the species to maintain an established population, particularly in an era without disposable plastic containers.

2.5.2.4 The role of regulatory enforcement. The extirpation of *Ae. aegypti* is extremely rare and successful large scale elimination campaigns have focused primarily on top-down approaches, funded by international organizations and the use of chemical control agents [33]. The majority of these elimination campaigns were abandoned due to their unsustainable cost, the development of resistance in mosquito populations and results that cannot be reliably predicted [55, 102]. Other successful attempts have focused on very local, bottom up approaches by engaging local communities to take ownership of the problem, and provide affordable tools for villages to maintain control for long periods of time [36, 103]. These have not proven applicable to many other environments and so have not been widely adopted.

From the inception of anti-mosquito regulations in Brisbane, it was the role of the resident to ensure that mosquitoes were not present on their property. High proportions of breaches in regulations during the period from 1913 until 1955 indicates the initial poor condition of the rainwater tanks in use at that time, and the considerable effort required to reduce the threat they posed for the presence of *Ae. aegypti*. Early in this period (1912 until 1933), surveys for mosquitoes in and around rainwater tanks in Brisbane were performed by trained public health inspectors and entomologists [43, 71, 14]. Although these efforts developed the mosquito control program (that was later to be so successful in the suppression of a number of species within the city), they were unable to scale their workforce effectively or enforce regulations in the newly amalgamated city [56]. With the formation of the Brisbane City Council mosquito control section, the role of surveillance was undertaken by District Health Inspectors who, by 1942, had the legislated fiat to enforce regulations [56]. The ability to compel householders to rectify breaches in regulations was necessary to justify surveillance activities and control a very real public health threat. Compliance with anti-mosquito regulations were variable before and during World War 2, but by 1955 compliance with notices had reached almost 100%. We infer that it was the removal of non-compliant rainwater tanks as key larval habitat through the continued enforcement of regulations that played a substantial role in the disappearance of *Ae. aegypti* from Brisbane.

2.5.3 Potential alternate mechanisms influencing elimination.

2.5.3.1 Improvements to living standards. Broad social trends and improved living standards after World War 2 changed the perception of what the domestic dwelling and yard meant to the Australian public. O'Gower [27] stated that in the early 20th century ‘the yard was depicted as a service yard complementary to the house in which the pseudo-suburban farmer generated resources for living’. These conditions were a reflection on the social and economic circumstances of the early 1900s which saw two depressions (1890 and 1930) and two World Wars [104]. By the 1940s, improving societal utilities and disposable personal wealth made the traditional uses of the backyard obsolete. Improvements included sewerage systems to remove waste, wash houses moved inside, the popularity of the Hills Hoist, the efficient and mass production of animal farming systems, the motorized lawn mower for keeping lawns tidy, domestic appliances such as the refrigerator replaced food cabinets and, the introduction of reliable reticulated water to urban areas that effectively replaced rainwater tanks. The primary purpose of the yard or ‘outdoor living area’ had become a space for recreation; an area that was ordered, designed and purposeful and often displayed to one’s peers [104]. By the 1970s, the society and resident (knowingly or not) had effectively removed the majority of larval habitat from around the domestic dwelling. This is particularly important in marginal areas of lower rainfall, such as Brisbane, where containers are essential for the persistence of the species.

2.5.3.2 Use of residual chemicals. Government records lack data on the use of DDT during house-to-house patrols in Brisbane. DDT was first used by the Brisbane Mosquito Control Section in tests on mosquitoes in September 1946 [105]. At the time, surveys for Anopheline mosquitoes were being undertaken after local transmission of malaria occurred, and these mosquitoes were the primary target for DDT application [106]. After the initial trial period in Brisbane, DDT was approved for use in swamps and was used to target freshwater and saltmarsh mosquitoes such as *Ae. vigilax* [105]. For mosquitoes targeted in gully traps and freshwater bodies such as water-holes, drains, sewers and swamps, council reports documented the use and volumes of DDT applied [107, 108]. There is a record for the use of DDT on *Culex fatigans* (*Cx. quinquefasciatus*) in 1947 which may be used to indicate its use in drains and sewers to control mosquitoes [109]. By 1950, truck mounted Tifa thermal fogging machines were being used to treat a range of insects including lice, cockroaches and flies [108]. It is possible that the use of DDT in sewers, garbage tips and commercial premises may have removed potential cryptic and subterranean sites. Although underground septic tanks were identified as habitat for other mosquito species, health officials ensured they were sealed from mosquitoes [42] and by 1975 the majority of Brisbane had a domestic sewerage system [110]. By the mid-1950s, the negative environmental and resistance effects were becoming apparent and the final mention of DDT in council records was in 1954 [111] and it was replaced by pyrethrum and malathion sprays by 1958 [112]. There are no government records of DDT being used in residential buildings, no volumes or number of houses sprayed by inspectors. As such, there is no suggestion that DDT was commonly used indoors by inspectors (where *Ae. aegypti* commonly rests [25]) and it was unlikely used in rainwater tanks as authorities were aware of its negative health effects [113]. If it had been used widely at the household level in Brisbane, DDT may have played a major role in the elimination of *Ae. aegypti*, however there is no evidence of a large scale peri-focal spray strategy that was used so successfully in the Americas.

2.5.3.3 The influence of climate. The role that climate plays on the distribution of *Ae. aegypti* in Australia is a complex subject and it remains unclear how long term weather patterns influenced the elimination from Brisbane. The city sits outside the southern range of the current distribution of *Ae. aegypti* and authors have suggested that climate may not be suitable for the long term persistence of the species [63,17]. The relative suitability of the region remains debatable however, as there are more complex factors (such as the type and frequency of larval habitat in domestic environments) which influence the distribution of *Ae. aegypti* [114, 115]. In any case, Brisbane represents marginal habitat for the species when compared to some other cities in northern Australia, such as Cairns. We conclude, therefore, that local extirpation was likely facilitated by both the lower suitability of the region and human agency.

2.6 Conclusion

The elimination of *Ae. aegypti* from Brisbane incorporated elements of a top-down approach. Unlike the Soper campaigns in South America which used large scale residual chemical spraying to target larval habitat, the strategy in Brisbane was to intensively survey residential areas, and target larval habitat by insisting on screening rainwater tanks and removing all water-holding rubbish [43]. During the decade leading up to the 1964, the majority of dwellings within Brisbane were surveyed for compliance with anti-mosquito regulations and ~8% received notices for breaches. This top-down intervention was necessary to identify key mosquito production hotspots, difficult and recalcitrant residents, and implement the appropriate legal framework. It is possible this method of elimination may be applicable to small towns in arid and semi-arid areas where rainfall is limited to one period a year [27]. However, with the modern proliferation of plastic containers and cryptic subterranean sites (like underground street drainage and telephone pits), the elimination we have documented here may remain an artefact of history.

When scaled up to the size of a modern city, top-down elimination approaches are doomed to fail if all stakeholders are not engaged and integrated into the management approach. The public plays a crucial role in mosquito control and elimination campaigns. In 1927, Hamlyn-Harris stated, “No mosquito campaign which does not include the question of publicity can ever hope to be successful, and this together with the education of the public has become a very important part of daily activities” and “It’s (*Ae. aegypti*) control is only possible provided an educated and sympathetic public cooperate” [43].

It is important that we continue to value the health initiatives that have safeguarded our cities in the past. The methodologies developed in Brisbane over many years show how a successfully integrated regulatory framework, with appropriate public engagement and enforcement can be applied to prohibit residents from producing larval mosquito habitat on their premises. Our literature review and analysis demonstrates the value of historical data in identifying key risks and shows the dramatic impact that surveillance and government regulations can have on invasive mosquitoes like *Ae. aegypti*. These historical data sets are rare, and this example shows how *Ae. aegypti* can be dramatically impacted at the margins of its distribution if key larval habitats are managed appropriately and supported by effective policy and legislation.

Drought conditions during the early 2000s have resulted in the installation of over 300,000 rainwater tanks in Queensland (>41% of properties in Brisbane) and was driven by a culture of water harvesting via educational campaigns and legislated water restrictions [116]. Likewise, large numbers of water barrels and cisterns have been installed as a human response to drought throughout southern California and are likely to be an ongoing concern for local health authorities monitoring the spread invasive mosquito species [117]. As such, these regions now face the danger of the introduction or reintroduction and spread of *Ae. aegypti* populations.

A clearer understanding of the threat posed by past epidemics and the enormous effort required to eliminate such a threat provides justification for ongoing surveillance by local and state authorities to ensure Australian cities remain vector free. With the current distribution limit of *Ae. aegypti* just north of Brisbane, ongoing mosquito surveillance and rainwater tank monitoring is essential. Although regulations are still in place, little is being done to inspect the condition of rainwater tanks, enforce anti-mosquito regulations, or educate residents on proper tank management within the Brisbane council area. The emergence and re-emergence of arboviruses including dengue, chikungunya and Zika viruses across the globe, further highlights the importance of early detection and response to invasive urban vectors. The successful campaign that led to the elimination of *Ae. aegypti* from Brisbane offers insights into the challenges we may face in the future, and provides an important source of knowledge from which to plan for and combat invasions of disease vectors internationally.

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Chapter 3. Global predictive CLIMEX models for *Aedes aegypti*: the implications of artificial water sources at the margins of distribution.

3.1 Introduction

Recent epidemics of Zika, chikungunya, and dengue fever throughout the Americas has re-ignited the need to understand the range limits and distribution of mosquito vectors such as *Ae. aegypti* and *Ae. albopictus*. The lack of management options, including effective mosquito vector control or vaccines, means large naïve human populations are at risk of disease epidemics and significantly increased disease burdens, particularly within developing countries (Gubler, 2011, WHO, 2012). Understanding the potential distribution of *Ae. aegypti* will enable health authorities to plan for future mosquito surveillance and control activities, and define the spatial limits of autochthonous disease transmission. The rapid spread of Zika across central and southern America resulted in the World Health Organisation declaring a Public Health Emergency of International Concern due to clusters of microcephaly and other neurological disorders (WHO, 2016). Fears that the disease could spread to Australia and the United States have resulted in authorities seeking information to ascertain where vectors can persist and to identify gaps in vector surveillance (U.S. C.D.C, 2016, Queensland Health, 2016).

During and after the two World Wars, conditions favoured the expansion of *Ae. aegypti* populations throughout the world (Gubler and Clark, 1995). Historical distribution data summarized in Christophers (1960), suggests the species distribution is limited by 10°C winter isotherms. A large and coordinated campaign during the 1950s and 1960s, led by the Pan American Health Organisation, saw significant declines in the distribution of the species across the world, and this included Australia (Soper, 1963, Soper, 1967, Russell et al., 2009, Chapter 2). Elimination of the species from Europe, North Africa and most parts of the Americas was attributed to highly effective management and the widespread use of DDT (Schliessmann and Calheiros, 1974). The re-emergence of the vector throughout its historical worldwide range has been observed since the 1970s, mainly due to both a reduction and a decline in the effectiveness of control efforts, in part due to resistance to chemicals and the expansion of urbanisation and trade (Gubler and Clark, 1995).

Currently *Ae. aegypti* has a wide distribution in tropical and subtropical areas (Beebe et al., 2009, Kraemer et al., 2015a, Chapter 2). Non-biological factors often used to 'predict' this distribution include climate, urbanisation, socio-economic status, water supply quality, sanitation standards, vector management and public health services (Reiter, 2001, Higa et al., 2010). Increasing high density urbanisation in developed and developing countries has dramatically altered the suitability of the landscape for mosquito vectors (Jansen and Beebe, 2010). Rapid population expansion and demands for critical infrastructure (such as reticulated water in developing countries) have resulted in governments failing to provide reliable access to these services (Schmidt et al., 2011). In developed countries, climate instability has resulted in water hoarding practices that reduce burdens on reticulated water supplies (Jansen and Beebe, 2010, Trewin et al., 2013, Moglia et al., 2013a, Eisen et al., 2014). Increases in domestic water storage have altered urban landscapes and provided more numerous supplies of larval breeding sites. This has subsequently increased the risk of vector persistence and disease transmission (Jansen and Beebe, 2010, Eisen et al., 2014).

Predicting the potential future distribution of *Ae. aegypti* is a contentious area due to the complex nature of the species' behaviour, life cycle and close association with humans (Christophers, 1960, Gubler and Clark, 1995, Jansen and Beebe, 2010). Climate broadly influences insect biology in ways that can significantly alter distributions (Colinet et al., 2015) and like all species of insects, *Ae. aegypti* persists within climate suitability gradients where previous generations have successfully reproduced (Eisen et al., 2014). These gradients vary from optimal at the core of the geographic distribution, to borderline suitable and unsuitable at range margins (Eisen et al., 2014). *Aedes aegypti* readily exploits a multitude of man-made containers and avoids extreme temperature and desiccation stress in the micro-climates found within domestic environments (Kearney et al., 2009). Thus, the presence of water storage containers, which provide oviposition sites and larval habitat, enable *Ae. aegypti* to extend its distribution into regions independently of rainfall and to some extent temperature; large storage containers buffer against temperature extremes (Chapter 4). Relying entirely on climate models makes predicting potential distributions difficult at a local scale and it is apparent that any prediction of the future distribution of *Ae. aegypti* will need to consider micro-habitats within urban environments that provide temperature conditions suitable to the species (Kearney et al., 2009, Jansen and Beebe, 2010).

CLIMEX is hybrid bioclimatic modelling tool for exploring the relationships between the fundamental and realised niche of a species (Kriticos et al., 2015a). It predicts the distribution of a species based on two approaches; first it can infer a distribution based on occurrence data and then matches long term meteorological data among these locations to predict the potential range of a species; and second it can infer a distribution from known physiological data on a species and how it would respond to changes in temperature, moisture or both (Zalucki and Furlong, 2005, Rogers et al., 2007, Webber et al., 2011, Zalucki et al., 2012). The benefit of using a CLIMEX modelling approach is the flexibility to use either of these methods to predict a species' distribution, especially in areas where occurrence records are scarce (Sutherst et al., 2007). This flexibility allows for the estimation of locations where the permanent establishment of invasive weeds, insects and pathogens could occur or to match climates where potential bio-control agents may be sourced (Yonow et al., 2004, Sutherst and Maywald, 2005, Rafter et al., 2008, Van Klinken et al., 2009, Sims-Chilton et al., 2010). Although an effective method for estimating species distributions, any model that uses climate data averaged over 30 years is, by definition, generalized in time and space. CLIMEX has other limitations which include an inability to model micro-climates, consideration of abiotic factors (e.g. pollution, salinity, pH), competitors and predators, and inaccuracies in surveillance data. The flexibility of CLIMEX makes it an ideal candidate to model the historical and potential distribution of *Ae. aegypti*.

This chapter estimates the potential global distribution of *Ae. aegypti* from historical records and physiological data using CLIMEX v4.0 software (Kriticos et al., 2015a). It assembles and adds to extensive occurrence datasets from published literature, entomological surveys, and government reports, and it combines these with bio-physiological data gleaned from the published literature. I was particularly interested in developing predictive *Ae. aegypti* distribution maps for cold and dry range margins, with a focus in identifying areas where water storage may influence the likelihood of presence. Two distribution maps are generated from these datasets; 1) the potential distribution without artificial containers, the Native Distribution Model (NDM) and 2) the potential distribution where containers acting as sources of water are available all year round, the Permanent Water Model (PWM). The PWM was then used to test the hypothesis that climate played a role in the historical disappearance of *Ae. aegypti* from New South Wales and southern Queensland in Australia. As storage of water in large tanks continues to increase across the developed and developing world due to climate instability, this chapter identifies key areas that will need surveillance and planning activities for invasions of *Ae. aegypti*.

3.2 Materials and methods

CLIMEX assumes that species experience favourable and unfavourable seasons where growth can be positive or negative. Growth within the different temperature and moisture regimes experienced during extremes in climate variation can be described by four stress indices (hot, cold, wet and dry) and all interactions between these. The growth index (GI_W) is equivalent to population growth rate, is scaled between 0 and 1, and is calculated on a weekly temperature index (TI_W) and moisture index (MI_W) where,

$$GI_W = TI_W \times MI_W, \tag{2}$$

and the annual growth index (GI),

$$GI = \frac{\sum_{i=1}^{52} GI_{W_i}}{52}. \tag{3}$$

The temperature and moisture indices represent the species response to favourable conditions. In general there are a range of temperature and moisture gradients where growth is maximal (eq. 2) and decline to zero at lower and upper critical thresholds. Stress indices (SI) and interactions (SX) are included to form an annual index of suitability, the annual Ecoclimatic Index (EI);

$$EI = GI \times SI \times SX \tag{4}$$

The EI represents the favourability of a location, with values under 20 considered unsuitable for long term persistence (Sims-Chilton et al., 2010). Stress indices (hot, cold, dry and wet stress) are weekly cumulative effects when a population may experience adverse conditions, and each is accumulated non-linearly with respect to time (Sutherst, 1999). For details on how the parameters that define a species response to climate are estimated see Sutherst and Maywald (1985) and Sutherst (1999).

3.2.1 Occurrence records

Entomological occurrence data came from a range of published literature sources from 1887 until 2015. Incorporating historical entomological data is essential due to the reliance of large water storage containers during these times, and thus our ability to predict *Ae. aegypti* distributions. For occurrence data within the core geographic distribution and after 1960, I relied on the dataset compiled by Kraemer et al. (2015a). For the majority of Australian historical presence data before and after 1960, the dataset from Beebe et al. (2009) was used. Additional records in cold range margins were compiled from an extensive literature search covering a range of contemporary and historical datasets from Australia, Asia, the United States, Europe and Argentina (Appendix 1). Occurrence records are defined by presence only at a spatial location during a survey. As historical records can vary in their spatial resolution, I attempted to include the finest scale resolution possible. In the case of the United States, I have historical surveys at the county level, whereas more recent surveys are at the town or city level.

3.2.2 Eco-physiological data

All physiological parameter estimates (DV1 to DV3, DVCS) used in CLIMEX modelling were based on growth estimates from Eisen et al. (2014). While the semi-mechanistic approach used by CLIMEX does not model each life-cycle stage, I incorporated data from the summary published for upper and lower critical thermal thresholds for key limiting lifecycle stages (Focks, 1992, Eisen et al. 2014, see Table 6 below).

3.2.3 Potential distribution development

The potential world distribution of *Ae. aegypti* was generated using the Compare Locations function in CLIMEX using methods described in Sutherst (2004). A current Australian range NDM model for *Ae. aegypti*, was initially developed by using the wet tropics template provided by CLIMEX, threshold levels from the literature (Focks, 1992, Eisen et al., 2014). Using the physiological data allowed for an iterative process to be used for estimating dry and cold stress parameters in the NDM (the current distribution of *Ae. aegypti* in Australia). First, cold stress parameters were set, then the NDM distribution was modified by incorporating the cold/dry stress interaction parameter. The unknown cold and dry stress parameters were iteratively adjusted until the CLIMEX prediction conformed to the current distribution of *Ae. aegypti* in Australia (the NDM). The PWM was then created using the ability of CLIMEX to model artificial habitats and microclimates found in these by incorporating artificial rainfall (Sutherst and Yonow, 1998, Kriticos et al., 2015a). The PWM simulated the addition of permanent water storage into the NDM by iteratively adding 2.5mm, then 5mm and finally 10mm per day to rainfall in all locations. At 10mm of irrigation, the PWM conformed to the historical distribution in Australia. With the addition of irrigation comes the implicit assumption that this "artificial" rainfall is occurring and subsequently lowers desiccation stress through higher humidity. For instance, this could represent higher humidity found in human dwellings, the space above water in rainwater tanks or simply rainwater tanks with water providing adult and larval habitat throughout the year. Once an irrigation scenario fitted the historical distribution, I fine-tuned the cold/dry desiccation stress interaction to match the "known" distribution as closely as possible. This fitted Australian distribution was first validated against independent, international datasets of *Ae. aegypti* distribution in cold range margins. Then I returned to the NDM to validate it using monthly population abundance data from Cairns, Australia (Duncombe et al., 2013), Botucatu, Brazil (Campos et al., 2012), and Hanoi, Vietnam (Tsunoda et al., 2014). To do this, trends in yearly abundance data (Compare Years function) were used to compare known population abundances (above) to the mean monthly GI output from locations in CLIMEX. I considered the model validated if yearly trends in GI outputs were similar to trends in abundance data from each location. All maps were developed using the 10' fishnet (1961-1990) from the CliMond dataset centred on 1975 (Kriticos et al., 2012).

3.2.4 Statistical analysis

To determine whether climate had an effect on the disappearance of *Ae. aegypti* from eastern Australia, a linear mixed effects (LME) model was applied to EI values from 1889 until 2015. I hypothesised that favourability (EI) would decrease at a range of sites during the period where the species disappeared from its southern range. This would have occurred when rainwater tanks were present in both Queensland and New South Wales, thus use of the PWM was justified. Historical climate data (1889-2015) was sourced from the SILO database (Jeffrey et al., 2001) for twenty sites in Queensland and New South Wales where historical surveys had identified the presence of *Ae. aegypti* (Beebe et al., 2009). Within the LME model, year was a fixed effect and site and year (given site) were independent random effects. This allowed each site to have a random intercept, as well as allowing the slope of EI to vary at each site. We further defined the residuals in the time series to have an autoregressive correlation structure of order 1 (AR1) when compared with a model with no autocorrelation in errors, which is standard practice for time-series analyses. The arcsine transformation of EI was taken so that the residuals of the fitted model were approximately normally distributed and variances were homoscedastic across sites.

If climate was responsible for the disappearance of the species, a consistent drop in EI would have resulted in a negative slope in the LME model. We set “break points” in the LME model as points in time where EI would have changed. Thus, breakpoints were set at times when authors had previously suggested *Ae. aegypti* had gone extinct in New South Wales (Russell et al., 1984) and Queensland (Marks, 1957, Chapter 2). As these break points were not informative, it was hypothesised that the EI was subject to abrupt change and not necessarily consistent across years. All years from 1900-2000 were trialled as potential break points in the linear relationship with year by including an interaction term between year and the candidate breakpoint on both the PWM and NDM. The suitability of the breakpoints was assessed by comparing log-likelihoods evaluated by maximum likelihood. A LME version of a piecewise regression analysis was performed once breakpoints had been determined. The statistical programme R version 3.3.2 (R Core Team, 2017) and the ‘nlme’ library was used for all statistical analysis.

3.3 Results

3.3.1 Potential distribution in Australia

Potential distributions for *Ae. aegypti* were different to those predicted by the CLIMEX parameter set developed by Khormi and Kumar (2014)(Table 6). The NDM depicted the population dynamics of *Ae. aegypti* from three locations where conditions for the species are suitable for year-round presence (Figure 16 A/D, B/E, C/F). Predictions of potential distributions conform well with a) current distribution records (NDM, Figure 17) and b) to historical and current distribution records (PWM, Figure 18). Under the NDM the species has the potential to spread south into New South Wales and west into the Northern Territory and Western Australia, particularly along the coastal strip (Figure 17). Under the PWM, the species has the potential to persist across the majority of the continent if it successfully spreads to locations where permanent water storage is present (Figure 18). The locations unlikely to maintain a population are in Tasmania, the Great Southern

Region of Western Australia, the highlands of New South Wales and Victoria and parts of the Eyre Basin in South Australia (Figure 18).

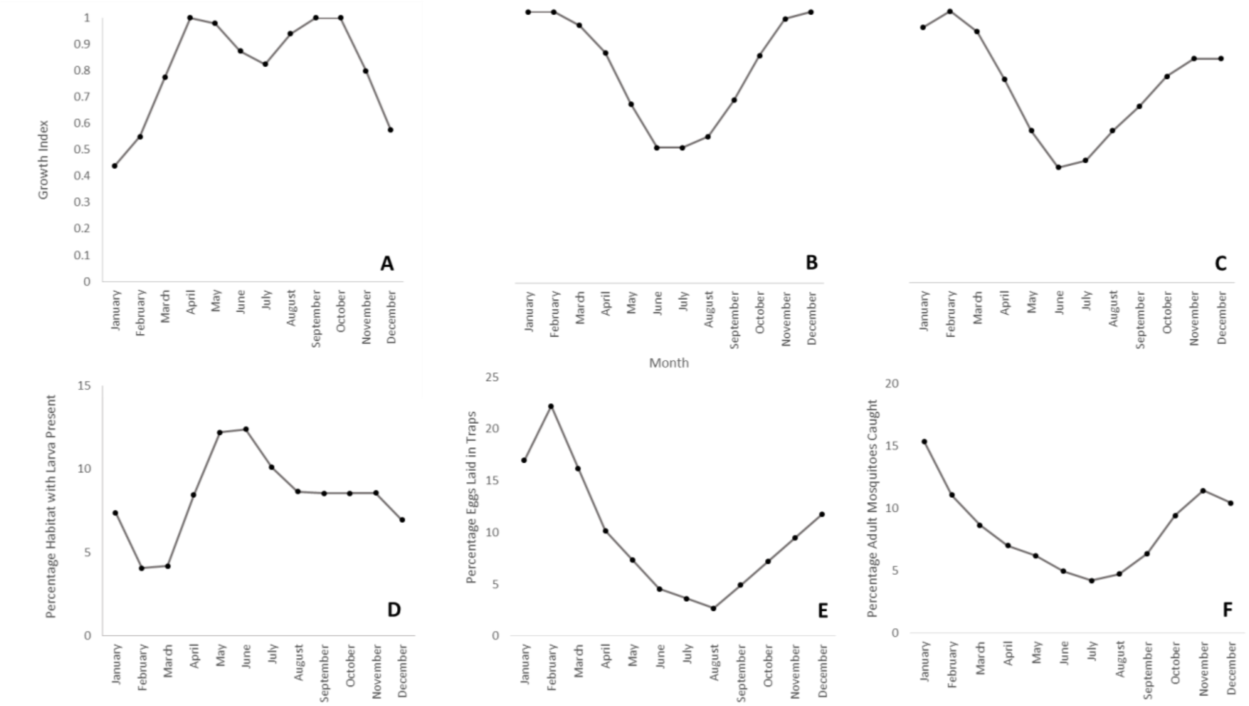


Figure 16. Model comparison of monthly Growth Indexes with mosquito abundance data in similar locations. Growth Indexes predicted by the Native Distribution Model (NDM) for regions: (A) Hanoi, Vietnam (B) Botucatu, Brazil and (C) Cairns, Australia. The percentage *Aedes aegypti* larvae caught monthly from published data for: (D) Hanoi (Phong and Nam, 1999), percentage eggs laid by *Aedes aegypti* in traps, (E) Botucatu (Campos et al., 2012) and percentage adult *Aedes aegypti* caught in traps, (F) Cairns (Duncombe et al., 2013)(F).

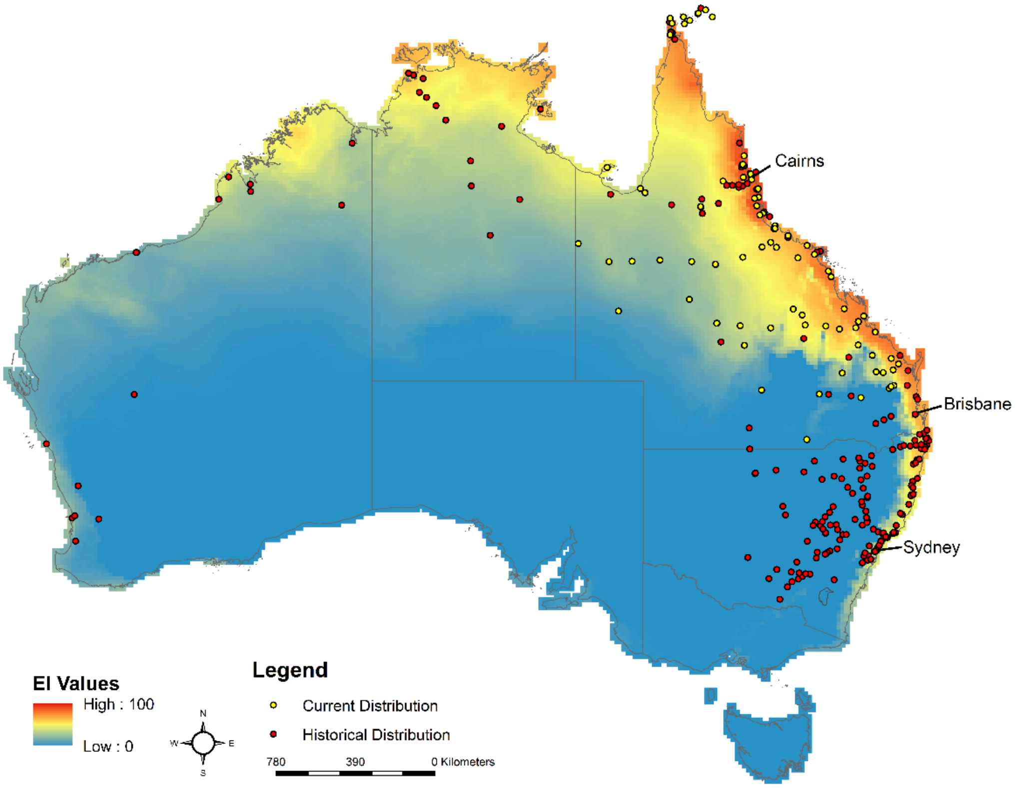


Figure 17. Australian *Aedes aegypti* distribution as predicted by the CLIMEX Native Distribution Model (NDM). Red spots represent surveyed locations from historical datasets and yellow dots represent the current known distribution in Australia (25.2744°S, 133.7751°E). Map base layer sourced from Australian ABS digital boundary data (Australian Bureau of Statistics, 2016) and licensed under Creative Commons 2.5 (2016).

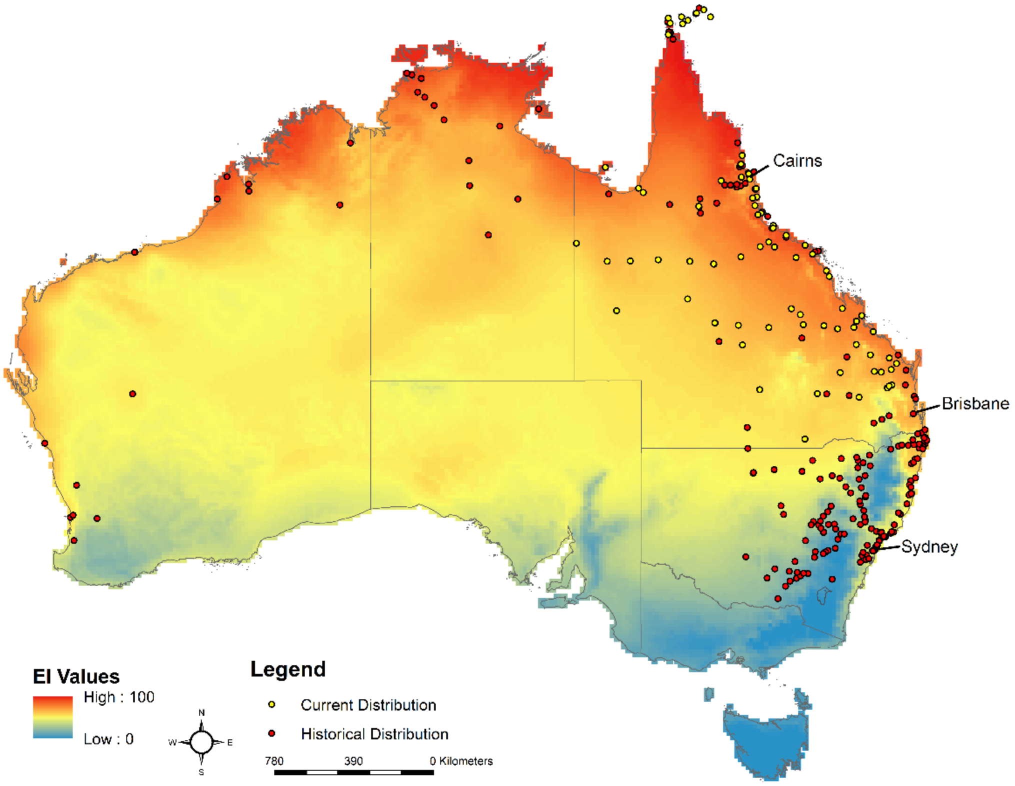


Figure 18. Australian *Aedes aegypti* distribution as predicted by CLIMEX and the Permanent Water Model (PWM). Red spots represent surveyed locations from historical datasets and yellow dots represent the current known distribution in Australia (25.2744°S, 133.7751°E). Map base layer sourced from Australian ABS digital boundary data (Australian Bureau of Statistics, 2016) and licensed under Creative Commons 2.5 (2016).

3.3.2 Potential world-wide distribution

Once the PWM was fitted to the Australian historical distribution, it was possible to observe world-wide differences in *Ae. aegypti* distribution in range margins when compared with the NDM. These include the United States (Figure 19A, B), South America (Figure 19C, D), South East Asia (Figure 19E, F) and Europe (Figure 19G, H). Areas in North America where the PWM predicted increased suitability included the Southwest and Far West in dryer states such as Texas and California (Figure 19A). There was very little difference between NDM and PWM to suitability in the south eastern states such as Georgia and where *Ae. aegypti* is currently found in Florida (Figure 19A). There was very little change in suitability in the cold margin of South America, where a modest increase in range for southern Argentina (Figure 19C, D) was observed. South East Asia had moderate increases in the core of its distribution through Thailand, Laos and Cambodia, while the cold and dry margin in the north east of India saw the largest increases in suitability over the NDM (Figure 19E, F). Without permanent water sources, rainfall in southern Europe is too low for widespread *Ae. aegypti* favourability (Figure 19H). However, with the introduction of irrigation into the model, coastal areas of the Mediterranean increase in favourability and the PWM predicts historical presence of the species (Figure 19G).

3.3.3 Favourability for growth over the past 126 years in eastern Australia.

The PWM revealed a number of candidate breakpoints that had the highest log-likelihood of a rapid switch in climate suitability, with two candidate breakpoints at 1926 and 1979 (Table 7 and Figure 20). Only Dubbo, Inverell, Muswellbrook and Tenterfield were predicted to have EI values below 20 when the PWM was fit to the survey sites (Appendix 2). When fitting the model with these two break points, results indicate that the slope prior to 1926 was significantly different to zero ($P < 0.01$, Table 8). From 1926 to 1979 the slope was significantly lower than the slope prior to 1926 (Table 8, $P = 0.02$). Finally, after 1979 the slope was not significantly lower than prior to 1926 (Table 8, $P = 0.11$). Untransformed to the original EI scale, these results indicated a 0.0005, 0.0004 and 0.0003 change in suitability per year for the pre 1926, 1926-1979 and post 1979 slopes, respectively.

Although there was a significant difference in slopes between the first break point, where EI tended to flatten between 1926 and 1979, all three slopes were positive throughout the time period examined and across all sites (Figure 21). Applying log-likelihood to estimate rapid changes in climate suitability in the NDM did not reveal any obvious breakpoints, with the most likely point at start of the climate data (1903) and a smaller point around 1972 (Appendix 3). The LME model was unable to be applied to the NDM outputs due to a number of historical locations being unsuitable for *Ae. aegypti* presence. These included Dubbo, Inverell, Muswellbrook, Tenterfield, Penrith and Toowoomba where a high number of EI values were zero (Appendix 4).

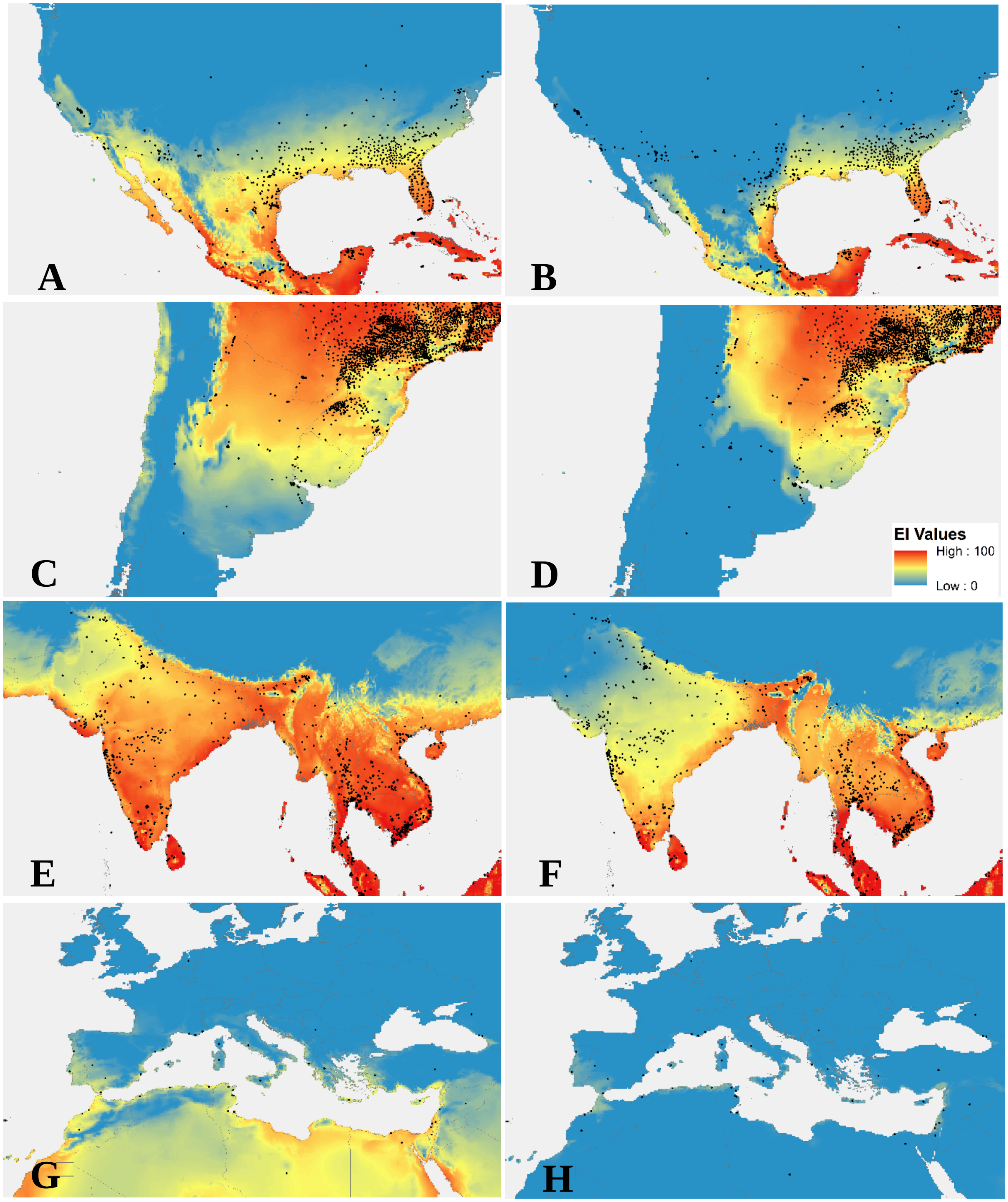


Figure 19. World cold-margin distributions of *Aedes aegypti* as predicted by the Permanent Water Model (A,C,E,G) and Native Distribution Model (B,D,F,H) developed in CLIMEX. Map base layer sourced from GADM (University of Davis, 2012) digital boundary data

Table 6. CLIMEX parameter values developed iteratively for the *Aedes aegypti* permanent water model against a previous parameter set developed by Khormi and Kumar (2014). Dashes indicate parameters that were not applied in models. Heat stress parameters were not applied in the Permanent Water Model as these had little effect on predicted distributions in cold/dry margins of geographic distribution. Parameter estimates taken from Focks (1993) indicated by a cross symbol (†) and Eisen et al. (2014) indicated by a hash (#).

Parameters	Description	Khormi and Kumar (2014)	Permanent Water Model
	Irrigation		
Summer	Volume (mm)	0	10 (PWM*)
Winter	Volume (mm)	0	10 (PWM*)
	Temperature parameter (C)		
DV0	Lower threshold of temperature for population growth	18	11.8 #
DV1	Lower optimal temperature for population growth	25	24 #
DV2	Upper optimal temperature for population growth	32	32 †
DV3	Upper threshold of temperature for population growth	38	41 †
	Moisture		
SM0	Lower threshold of soil moisture	0	0.06
SM1	Lower limit of optimal range of soil moisture	0.2	0.16
SM2	Upper limit of optimal range of soil moisture	0.5	2.5
SM3	Upper threshold of soil moisture	-	4
	Stress indices		
TTCS	Threshold of cold stress (C)	-	7
THCS	Weekly accumulation of cold stress (Week-1)	-	-0.0001
DTCS (Weekly)	Degree-day threshold of cold stress	-	15
DHCS	Weekly rate of accumulation of cold stress	-	-0.0001
TTHS	Threshold of heat stress (C)	38	-
THHS	Weekly accumulation of heat stress (Week-1)	0.9	-
SMDS	Threshold of dry stress	0.001	-
HDS	Weekly accumulation of dry stress	-0.001	-
DTCD	Cold-Dry Degree-day Threshold	-	25
MTCD	Cold-Dry Moisture Threshold	-	2
PCD	Cold-Dry Stress Accumulation Rate	-	0.0005
DVCS	Cold Stress Day-degree Temperature Threshold	-	11.8 #
DVHS	Heat Stress Day-degree Temperature Threshold	-	40
PDD	Degree-days to complete the life cycle	-	285

* irrigation added iteratively to the Native Distribution Model.

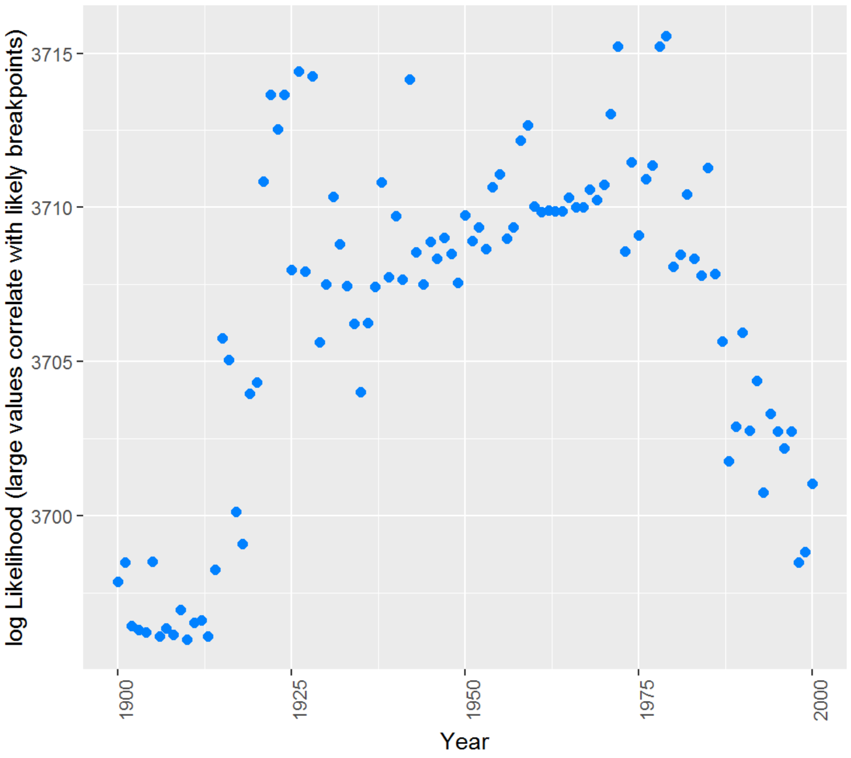


Figure 20. Estimation of rapid changes (break points) in Ecoclimatic Index (EI) from the Permanent Water Model, as determined by maximum likelihood. The years where the highest values occur represent points where trends in environmental conditions changed rapidly from suitable to unsuitable (or unsuitable to suitable) for *Aedes aegypti* presence (see text for more details).

Table 7. Highest values from Figure 20 representing the likelihood of a breakpoint (rapid change in EI) in the Permanent Water Model between 1900 and 2000.

Year	Log Likelihood
1979	3,715.57
1978	3,715.22
1972	3,715.21
1926	3,714.40
1928	3,714.25
1942	3,714.17
1924	3,713.65
1922	3,713.65
1971	3,713.04
1959	3,712.68
No Break Points	3,684.30

Table 8. Coefficients of fixed effects in Permanent Water Model analysed by a piecewise linear mixed effects statistical model. Breakpoints (where rapid changes in the Environmental Index (EI) occurred) were modelled at 1926 and 1979 and changes in EI were modelled around these points. Location data used in the model is from 20 sites where *Aedes aegypti* was historically present in Australia.

	Value	SE	DF	t-value	*P*-value
1889-1926					
(Intercept)	-1.02348	0.34	2495.00	-2.97	0.00*
1927-1979					
(Intercept)	0.96363	0.40	2495.00	2.39	0.02*
1980-2015					
(Intercept)	0.82971	0.53	2495.00	1.56	0.12
Year (1889-1926)	0.00085	0.00	2495.00	4.66	0.00*
1927-1979 * Year	-0.00051	0.00	2495.00	-2.43	0.02*
1980-2015 * Year	-0.00043	0.00	2495.00	-1.59	0.11

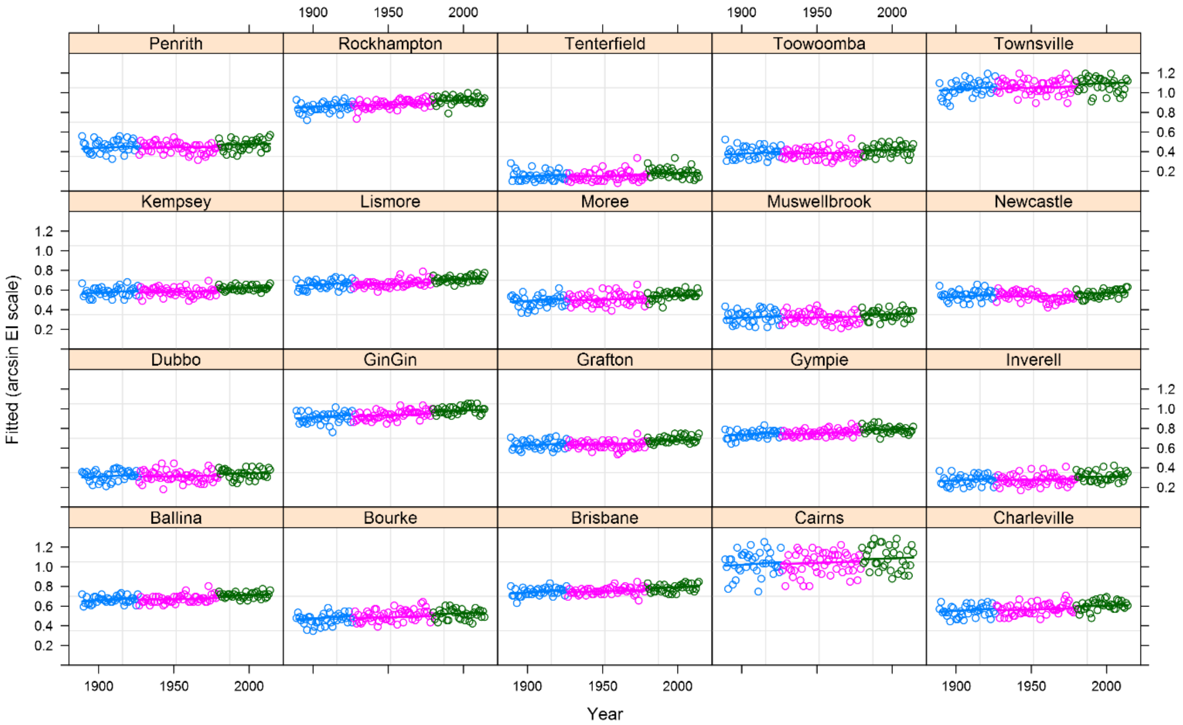


Figure 21. Fitted linear mixed effects model where slopes represent trends in Environmental Index (arcsine transformation) between identified break points and across locations where *Aedes aegypti* was historically present in Australia.

3.3.4 Ecoclimatic favourability in Brisbane over the past 100 years.

As the LME model showed no negative relationship with climate, the Brisbane EI data from the NDM and PWMs was graphed as a simple linear model as mean EI per decade (Figure 22). While the PWM predicted EI values showed a small positive trend and low variability, the slope of the NDM EI data revealed an increase in mean EI from 59.5 (95% CI 5.4) in 1889-1899 to 68.3 (95% CI 3.2) in 2010-2014. At no point did the EI of Brisbane drop below 20, the point below which a population would struggle to maintain establishment (Sutherst et al., 2007). Of note is the smaller variation in EI within the NDM after the time *Ae. aegypti* was thought to have been eliminated (after 1957) when compared to before this time (Figure 22).

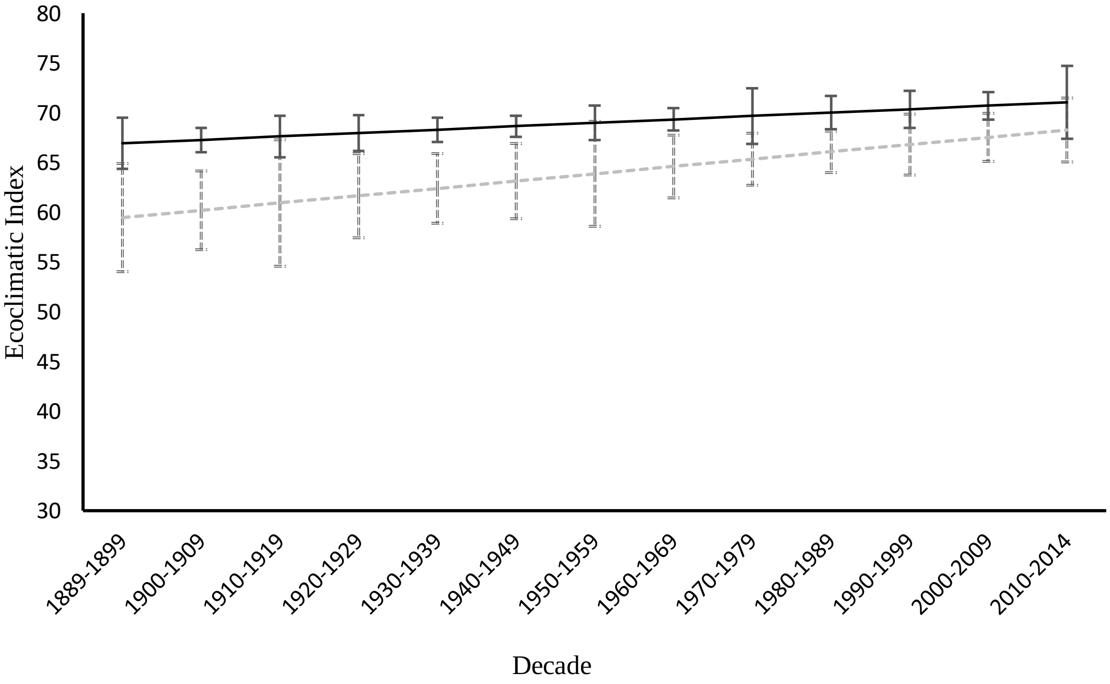


Figure 22. Untransformed Ecoclimatic Index in Brisbane predicted by CLIMEX for the past 126 years. Lines represent the models with Permanent Water Model (black) and Native Distribution Model (grey) and error bars are one standard deviation.

3.4 Discussion

The importance of *Ae. aegypti* as the primary vector of dengue has led to many attempts at predicting the distribution of the mosquito and its diseases (Khormi and Kumar, 2014, Kraemer et al., 2015a, Campbell et al., 2015). A novel approach was taken in this chapter, which incorporated a semi-mechanistic model and the presence of permanent water within the environment, to predict the presence of *Ae. aegypti* in the cold and dry range margins of its potential distribution. Results match well with historical distributions, which in the case of Australia, may have been due to the existence of large water storage containers. The model predicts areas that are suitable in California, where there is currently ongoing spread of *Ae. aegypti* through the central and southern areas of the state (California Department of Public Health, 2016). Likewise in Europe, there are established populations currently found on the eastern Black Sea coast (ECDC, 2017) and historically in Greece, where a large dengue epidemic occurred during the late 1920s (Rosen, 1986, Schaffner et al., 2013).

Predicting the distribution of *Ae. aegypti* in cold and dry range margins is fraught with the complicated nature of growth around critical thermal limits and the temporary nature of populations within these areas. It can be difficult to distinguish whether historical presence data is representative of an established population, a transient introduction or repeated transient introductions at high risk sites, such as those found on traditional trade routes. As such, attempts to model potential distributions are often inaccurate. Models tend to suffer from a number of problems relating to insufficient occurrence data and incorrect parameter selection. For instance, some authors only consider contemporary distributions (Hopp and Foley, 2001, Khormi and Kumar, 2014, Kraemer et al., 2015a) and do not include historical presence data from places such as Europe and Australia.

One of the most recent and advanced species distribution models for *Ae. aegypti,* using boosted regression trees and machine learning, does not include presence data from before 1960 (Kraemer et al., 2015a). As such, this model underestimates the presence of *Ae. aegypti* in dryer regions, such as southern Australia, the central and western United States, the Mediterranean and higher altitude areas of Central America and Asia. A prior CLIMEX model attempting to map the world-wide distribution of *Ae. aegypti* assumed that dengue case location data is a sufficient predictor of presence (Khormi and Kumar, 2014). This line of reasoning is inherently flawed due to the potential for other mosquito species to vector the disease both within and outside the distribution range of *Ae. aegypti*. For instance in 2014, *Ae. albopictus* was declared the primary vector of a dengue outbreak in Japan, in a location where *Ae. aegypti* is not currently present (Saho et al., 2015). A study by Yang et al. (2014) also demonstrated a spatial gradient between the two mosquito species in Taiwan, and implicated *Ae. albopictus* as the only vector in an epidemic of dengue in areas where *Ae. aegypti* did not occur. Thus prediction of mosquito distribution using disease presence data will inaccurately estimate the risk of transmission by *Ae. aegypti*.

Mechanistic models that link climatic data, micro-climates, incorporate rainfall and evaporation rates in a range of containers, population dynamics, disease transmission and link these to urban environments, are likely to provide more accurate estimates of vector risk (Kearney et al., 2009, Kraemer et al., 2015a, Campbell et al., 2015). These models have the capacity to measure interactions at the micro-niche where *Ae. aegypti* interacts with humans in domestic environments. Predictions using CLIMEX are semi-mechanistic and more applicable to large scale, simpler risk models, where future changes in climate can be predicted. Both the NDM and PWM make broad scale climatic predictions and do suffer from a lack of resolution when evaluating the risk of *Ae. aegypti* presence at smaller resolutions. However, CLIMEX outputs can be combined with other spatial layers such as urban environments, transport links, population density and disease data to create finer-scale “mashups” (or composite maps) of risk surfaces over larger areas (Kriticos et al., 2015b). For instance one could overlay other spatial maps, such as urban areas or evaporation data, and remove all cells which did not fall within favourable areas to create a composite risk map. Another method developed by Kriticos et al. (2015b) overlaid different maps of with-and-without diapause models for *Helicoverpa armigera* and took the highest EI value for each spatial cell, reasoning that the most adapted allele would predominate. Combining maps in this way can add an extra dimension in the ability of species distribution maps to estimate find-scale risk from semi-mechanistic and climate based predictions.

A limitation of the models developed in this chapter may be that they under predict development around the lower critical temperature, which was estimated as 11.8°C (Eisen et al., 2014). CLIMEX models development around this point with a linear relationship, however growth rates around this cut-off point are not linear and this is explored further in Chapter 4 (Colinet et al., 2015).

By incorporating a water storage effect into *Ae. aegypti* models, geographic distributions are increased (the species is no longer moisture limited) and are more likely to predict the potential range of the species. This effect is most pronounced in semi-arid areas such as central Australia and Texas, or locations where natural rainfall is primarily in the winter, such as California and the Mediterranean. Areas where distributions have increased during recent droughts include Australia and California (Queensland Health, 2012, California Department of Public Health, 2016). *Aedes aegypti* eggs are desiccation resistant and rainfall is not necessarily the primary driver of these incursions. There are a number of other reasons for the presence of the species which may include increases in larval habitat such as water storage or cryptic sites (Kay et al., 2000, Pontes et al., 2000, Montgomery et al., 2004, Trewin et al., 2013), human mediated transport (Lumley and Taylor, 1943, Guagliardo et al., 2014), little competition by other mosquitoes within the container niche (ECDC, 2017), and a warming trend during drought years (Bureau of Meteorology, 2017, NOAA, 2017). As such, this species is highly invasive, adapted to domestic environments and any changes that increase the suitability of domestic environments will be rapidly exploited if the species is present.

Applying statistical analysis to the NDM presented a number of issues for why it was not possible to model climate suitability using a LME model. First, accurate break points were unable to be determined using the changes in Ecoclimatic data during the past 126 years. In a previous study to examine the effect of biological control on distribution, Sims-Chilton et al. (2010) were able to determine their model break point when their species (Groundsel Bush) was targeted for biological control. Although an attempt to determine break points when *Ae. aegypti* went extinct in New South Wales (~1943) and Brisbane (~1957) was made, proximate years to these time points did not accurately predict changes in climate. And secondly, a number of sites, particularly in N.S.W. where *Ae. aegypti* had been identified historically were unable to be used in the statistical model. In these sites EI values were zero for the majority of years, and therefore a LME model was unable to be applied. When maximum likelihood was applied across all sites in the PWM, distinct changes in EI values were differentiated to enable two break points to be determined. Analysis indicated that when permanent water was available, climate suitability increased throughout eastern

Australia from the start of records until 1926, when EI values flattened, and again started to rise in 1979 until the present day. While conditions did not deteriorate, results suggest that if permanent water sources (such as rainwater tanks) were present, it is unlikely that climate would have played a role in the disappearance of the species from southern areas of Australia. Removing this potential habitat from the landscape likely played a major role in the disappearance of the species in southern areas. It must be noted that the historical presence of *Ae. aegypti* in southern New South Wales was heavily influenced by steam trains and their attendant water storage (Figure 4; Lumley and Taylor, 1943). Detections in areas where the PWM does not predict suitability are likely due to introductions along train lines. Populations of *Ae. aegypti* may not have persisted in these areas after winter, even if permanent water was present. This seasonal relationship is likely similar to the historical presence of *Ae. aegypti* in the north eastern United States and Europe, where the species would have been introduced by trading ships during summer periods but died out during the cold of winter (Gubler et al., 2014).

This study explores an alternative hypothesis for the historical range reduction of *Ae. aegypti* in eastern Australia by examining the effect of climate on habitat suitability. Results indicated a general trend of increased suitability for the past 126 years throughout the eastern Australia and as such, did not support the hypothesis that climate played a role in the disappearance of *Ae. aegypti*, if water storage was present. However, CLIMEX models show a clear distinction between potential suitability for *Ae. aegypti* in Australia and whether or not water is present in the environment. It is therefore likely that climate played a role in arid areas of central New South Wales and southern Queensland if permanent water was removed from the landscape. Here low rainfall patterns are likely to have restricted the ability of the species to persist and may have contributed to the disappearance of the species in these areas.

The NDM model suggests areas along the east coast including Brisbane and Sydney, have remained suitable for the persistence of the species. This is likely due to the convergence zone created between the Pacific Ocean and the Great Dividing Range, where sea breezes create fronts of unstable and humid air that can trigger thunderstorm and regular rainfall. Alternatively, it is interesting that the NDM model does not predict suitability where the species is currently found in some areas of central and southern Queensland. This suggests that populations in these areas would be highly dependent on the availability of suitable larval habitat such as cryptic sites or water storage that protect juvenile stages during extreme cold and/or dry periods. As such, populations in western areas of southern and central Queensland may be susceptible to elimination campaigns where larval habitat is targeted (O'Gower, 1956, Kay et al., 2000, Pettit and Kurucz, 2014).

Results do not support the hypothesis that climate played a major role in the disappearance of the species from Brisbane. Ecoclimatic Indexes in both the NDM and PWMs suggest Brisbane has remained suitable for the presence of *Ae. aegypti* for the past 126 years, although these conditions are not as optimal as those found in Cairns and other tropical areas (Figure 21). The suitability of south east Queensland is supported by survival and development results in Chapter 4 and suggests other factors are likely to have played a larger role in the disappearance of the species. Alternate mechanisms are addressed in Chapter 2 and by other authors (Beebe et al., 2009, Russell et al., 2009, Williams et al., 2010). Most notable is the role that larval habitat plays in the persistence of *Ae. aegypti*, particularly in areas marginal to the core geographic distribution of the species. *Aedes aegypti* disappeared from Darwin around the same time period that it did in Brisbane and both locations share similar characteristics of sub-optimal habitat, with long, dry winter periods. Medical entomologists have suggested the primary reason for the disappearance in both locations was the introduction of reticulated water and subsequent removal of rainwater tanks (O'Gower, 1956, Marks, 1957). It is likely therefore, that the removal of key larval habitat was an important driver in the extinction of the species in these marginal habitats. Trends in both models suggest climate suitability increased throughout the last century, with mean EI increasing across the region. Of particular interest is the decreasing variation in EI after *Ae. aegypti* was eliminated and until the present day, even though the region experienced an extended drought around the start of the 21st century. It is likely this increasing suitability is a function of a warming climate, typically predicted by climate change models. Future work should look at the role that climate predictions will have on the Australian distributions of *Ae. aegypti* when permanent water sources are included. It is likely that southern Australian cities, such as Melbourne, will become more suitable and the large increase in rainwater tanks observed in southern states may need to be addressed as part of vector surveillance.

Chapter 4. Rainwater tanks as a risk factor for *Aedes aegypti* establishment: survival and development under winter conditions in Brisbane, Australia.

4.1 Introduction

A key determinant of insect distribution and persistence are the micro-climate gradients in which previous generations have successfully reproduced (Kearney and Porter, 2009). These gradients vary from near optimal within the core, to borderline suitable and unsuitable at the margin of a species' geographical distribution (Eisen et al., 2014). In recent years there has been renewed interest in predicting the spread of *Ae. aegypti* into cool range margins, primarily due the increased variability in temperature and rainfall associated with climate change and the importance of the species as a disease vector (Eisen et al., 2014). In particular, rising temperatures, unpredictable rainfall and urban landscapes modified to these conditions may impact *Ae. aegypti* distribution, daily activity patterns and peak annual population abundance in cool range margins (Jetten and Focks, 1997, Beebe et al., 2009, Kearney et al., 2009, Russell et al., 2009, Bader and Williams, 2012).

The degree to which temperature affects seasonal phenology of *Ae. aegypti* in Australia will vary due to differences in climate between locations and potential adaptive changes within the mosquito as a response to climate (Kearney et al., 2009). Cold resistance in Drosophila has been linked to species distributions, where significant relationships exist between traits and climatic variables when phylogeny is controlled for (Kellerman et al., 2012). Likewise, it has been shown that the timing of diapause in *Ae. albopictus* is an adaptation to seasonal climate variation, facilitating the ongoing establishment of the species in the United States (Armbruster, 2016). It is unclear whether populations of *Ae. aegypti* exhibit adaptations to climate (such as cold resistance) in Australia. As such, it will be important to understand cold resistance within-and-between populations if we are to understand the potential for future invasions into areas south of its current distribution in Australia and elsewhere.

The distribution of *Ae. aegypti* is more complex than just climatic constraints and there are numerous factors that may play a role (Taylor, 1943, Russell, 1998). *Aedes aegypti* persists within domestic environments due to the many protected microclimates found within (Kearney et al., 2009, Jansen and Beebe, 2010). This adaptation has permitted the species to extend its distribution worldwide and into areas where it may not otherwise be able to physiologically endure. One factor affecting *Ae. aegypti* presence is the availability of suitable larval habitat (Taylor, 1943, Russell, 1998). The species readily exploits urban areas by ovipositing and developing in the multitude of man-made containers and avoiding extremes in temperature and desiccation within these environments. Of particular interest is the effect that large permanent water storage containers, called rainwater tanks, have on the geographic distribution and persistence of the species at range margins within Australia. A number of authors have estimated that the lower critical temperature for *Ae. aegypti* immature stages is approximately 11.8°C, which is the point below which no development occurs (Carrington et al., 2013, Eisen et al., 2014). This point is determined by linear regression using development measured at a range of constant temperatures, but in reality the relationship between temperature and growth rates is rarely linear (Zahiri et al., 2010, Golizadeh and Zalucki, 2012, Colinet et al., 2015). This is because temperatures fluctuate greatly throughout each day, each season, along a latitudinal gradient and within the environmental niche an organism experiences (Colinet et al., 2015).

Numerous studies have measured the survival and development of *Ae. aegypti* at and around temperatures proximate to the lower critical temperature. Richardson et al. (2011) calculated mean development time as 31-32 days with ~22% survival at a constant 16°C. Tun-Lin et al. (2000) estimated it took 39.7 days for adults to emerge, with 23.5% survival at a constant 15°C. Carrington et al. (2013) reared mosquitoes at temperatures recorded from field sites in Thailand to determine how fluctuating temperatures can alter immature development. They found that a large diurnal thermal range of 18.6°C around a mean of 16°C significantly reduced the mean development time of *Ae. aegypti* to pupation when compared to constant and small (7.6°C) temperature fluctuations. The authors found that diurnal thermal ranges significantly influenced development time but not survival when compared to a constant temperature regime (Carrington et al., 2013). Studies on Australian *Ae. aegypti* have shown 100% mortality at constant temperatures between 10°C and 12°C (Tun-Lin et al., 2000, Richardson et al., 2011).

In Australia, rainwater tank volumes can range from 500L to 100,000L. Water has a high specific heat capacity, a property that allows it to store a large amount of heat energy. The ability of large volumes of water to resist large fluctuations in temperature is called low thermal inertia (Maréchal, 2006). These two properties of large water bodies provide a mechanism by which rainwater tanks buffer potentially large fluctuations in temperature that occur in the Australian environment. It has been hypothesized that the buffering effect of a rainwater tank could protect all life cycle stages of *Ae. aegypti* in cold range margins, where climatic conditions are not favourable for persistence throughout the year (Kearney et al., 2009, Chapter 2). Thus rainwater tanks, a common feature in urban environments in Australia, may have enabled the historical range of *Ae. aegypti* and may facilitate expansion in the future.

Another Australian urban species, *Ae. notoscriptus,* is a suspected vector of Ross River and Barmah Forest viruses (Ritchie, 1994, Watson and Kay, 1998, Watson, 1999). Russell (1986) suggested that *Ae. notoscriptus* may have a competitive advantage over *Ae. aegypti* in cooler areas and this may have resulted in competition between the two species as *Ae. notoscriptus* intruded into urban environments during the last century (Russell et al., 1984, Russell et al., 2009). Both species are commonly found in rainwater tanks, and it is unknown how rainwater tanks affect the survival and development of these two container-inhabiting mosquitoes. Due to a more southerly distribution in Australia (Endersby et al., 2013), it is likely that winter temperatures in Brisbane will have less of an effect on the development and survival of *Ae. notoscriptus* than *Ae. aegypti*.

To examine whether rainwater tanks provide a suitable environment for the survival and development of two important disease vectors in Brisbane, a range of environmental characteristics of rainwater tanks and smaller containers (buckets) were observed during winter conditions. Fluctuating temperature treatments were then applied to *Ae. aegypti* and *Ae. notoscriptus* larvae in environmental chambers. It was hypothesised that 1) rainwater tanks (small temperature fluctuation treatment) would increase *Ae. aegypti* and *Ae. notoscriptus* survival to adults during winter conditions when compared with survival in buckets (high temperature fluctuation treatment); 2) that *Ae. aegypti* sourced from the southern limit of its Australian distribution would have higher survival and lower "winter" development time than *Ae. aegypti* sourced from a northern location; 3) there would be higher survival and faster development in *Ae. notoscriptus* than *Ae. aegypti* under "winter" condition in both tank and bucket treatments.

4.2 Methods

4.2.1 Environmental observations

To measure temperature fluctuations in different container types over winter in Brisbane (27.47° S, 153.03° E), rainwater tank surveys were conducted fortnightly from the start of June until September 2014. HOBO Pendant® Data Loggers were placed into ten rainwater tanks and ten buckets, and tank locations were divided into high (>66%), medium (33-65%) and low (0-32%) shade categories. Air temperatures were measured outside of tanks in full shade using the same type of data logger. Tanks were checked fortnightly to ensure they remained sealed throughout the study. Suburbs where tanks were observed in Brisbane included: Greenslopes (27.51° S, 153.05° E), Moorooka (27.54° S, 153.03° E), Salisbury (27.55° S, 153.03° E), Sunnybank (27.58° S, 153.06° E), Camira (27.63° S, 152.91° E), Indooroopilly (27.50° S, 152.97° E) and St Lucia (27.50° S, 153.00° E). A black 9L bucket, representing the most common type of container in Brisbane backyards (Darbro et al., 2017 unpubl. data), was placed in a northerly position next to each tank, with similar shade conditions. For comparison with a location where *Ae. aegypti* had never been observed previously, tanks and buckets with high and low shade regimes in Adelaide were also logged during winter. For a comparison with summer temperatures, high and low shade regimes in Brisbane from the start of December 2014 until the end of February 2015 were logged.

Data loggers were placed under a floatation device in each tank, to a depth of 30 cm below water surface, where water temperature remained relatively stable when compared with surface temperatures. Floatation devices were attached to a tape measure suspended from the roof of the tank and measurements of water evaporation were taken from tanks and buckets for calculations of volumes lost. A number of rainwater tank characteristics were measured: temperature inside and out of each tank, volume of water, evaporation rate, humidity, dew point, pH, salinity, conductivity, total dissolved solids, shade, and presence of associated infrastructure, including first flush devices (a separate pipe for collecting sediment before water enters a tank). The five-sweep netting technique was used for sampling larvae (Knox, 2007). The presence/absence of adults was visually estimated from sieves as tanks were opened. After the first survey, two tanks were disconnected from input water sources to estimate evaporation rate and to control for mosquito larval presence. First flush devices were monitored for mosquito presence and abundance, along with total volume of water and whether or not they had sediment or leaves. Archerfield (-27.57°

S, 153.01º E) and Adelaide airport (-34.95º S, 138.52º E) climate data were selected as these represented the closest station to the rainwater tanks surveyed (Bureau of Meteorology, 2015).

4.2.2 Survival and development rate trial

Aedes aegypti colonies were established from eggs sourced from field sites in Cairns (-16.92º S, 145.78º E) and Gin Gin (-24.99º S, 151.95º E), Queensland, in January 2015. The Gin Gin colony originated from 30 eggs collected from nine ovitraps at three houses within the town, while the Cairns colony was made from hundreds of eggs collected in five ovitraps from a number of properties. North Queensland is considered the core range of the species in Australia and is considered to have the highest genetic diversity of Australian populations (Rasic et al., 2014). A PCR test (Ulrich et al., 2016) for the presence of *Wolbachia* revealed zero prevalence from sixty mosquitoes. Colonies were maintained in a climate controlled insectary at 26 ± 1°C, a 70% (± 10%) relative humidity and a 12:12 hour light cycle with twilight period. Colonies were maintained at >500 individuals per generation and adults were blood fed on an adult volunteer for 15 minutes, 2 days after emergence for 3 days (QIMR Berghofer Medical Research Institute human ethics form P2273). Eggs were collected from both colonies after generation two for use in experiments. *Aedes notoscriptus* eggs were taken directly from three ovitraps sourced from a single backyard in Brisbane. Eggs were hatched synchronously using vacuum immersion in water at room temperature (24°C) for one hour.

Fifty first-instar larvae from each mosquito strain were randomly chosen and transferred into containers one hour after hatching, for a total of 400 larvae per strain per treatment. A total of eight white plastic containers (183 x 152 x 65mm) were used per mosquito strain, per temperature treatment, in a randomized block design and 500mL of pre chilled tap water (aged 2 days) was placed into each container. Larvae were fed with TetraMin© ground fish food (Tetra, Germany) standardized to the high treatment of Hugo et al. (2010), with food concentrations estimated per larvae per volume each day. Containers were covered and topped up daily with pre-chilled, aged water to replace evaporation. Deaths were recorded and any excess food was removed before feeding. Larval counts per instar stage were made daily and confirmed with photographs taken of each container. Trays were rotated within environmental chambers and insectary shelves daily to prevent bias based on location within the chamber. Survival was recorded if adult mosquitos had completely emerged from pupal cases.

A fluctuating temperature regime (mean maximum, mean minimum) was established from tank and bucket measurements during the coldest week experienced in Brisbane during July (Appendix 5). Temperature regimes were applied for tank and bucket treatments in environmental chambers at two hourly time steps. A control treatment was set up for a constant 26°C (± 1°C) regime in a temperature controlled insectary. Light regimes for all treatments were set at a 10:14h cycle, typical of Brisbane in July, and humidity was set at 75% which is comparable to those observed in rainwater tanks during winter (Appendix 6).

4.2.3 Development models

To estimate the lower critical threshold using fluctuating temperatures, we applied the model of Dallwitz et al. (1992) who present a simple development rate function:

$$\sum_{T_{start}}^{T_{end}} r = \begin{cases} b_1(T - h_1) & when\ T \geq h_1 \\ 0 & when\ T < h_1 \end{cases} \tag{5}$$

where r is the development rate expressed as a percentage per day; b_1 is the percentage development per day per degree above the lower critical temperature; h_1 is the threshold temperature; and T is the temperature. The daily development rate for each treatment regime $r(d)$ as:

$$r(d) = \frac{1}{\tilde{\pi}(dT)} \tag{6}$$

Here $\tilde{\pi}(dT)$ is the median development time to adult emergence for each treatment regime. The median time to development was chosen based on the tail associated with the distribution of emergence times for both treatment regimes. As temperatures were recorded every half hour, the daily rate of development was equal to the sum of each 30 minute interval (eq 5). From equation (5), h_1 was iteratively adjusted until the daily linear rates of development was optimized and had the same slope for both temperature regimes (high and low fluctuation).

The estimated lower critical temperature was then applied to a degree day model to estimate the total number of heating degree days (HDD) for each treatment regime with the model;

$$\sum_{T_{start}}^{T_{end}} HDD = (T - h_1) \times \left(\frac{1}{T_t}\right) \tag{7}$$

Where T_t is the total number of time units in a day and h_1 is the lower critical temperature threshold.

Additionally, the lower critical threshold was estimated using a non-linear modelling approach. To do this a number of published datasets were compiled from multiple continents, including data from this chapter, for median and mean time to pupation across the range of *Ae. aegypti* developmental temperatures (Gilpin and McClelland, 1979, Rueda, 1990, Tun-Lin et al., 2000, Richardson et al., 2011, Carrington et al., 2013). Datasets were chosen to demonstrate the large variation in potential growth rates, and not as a comparison between continents. Each dataset was converted to a daily development rate and means of both constant and fluctuating temperature regimes were included. Seven of the inbuilt non-linear equations were applied using the R package 'devRate', which estimates model parameters using non-linear least squares (Kontodimas et al., 2004, Shi et al., 2011, Briere et al., 1999, Briere et al., 1999, Hilbert and Logan 1983, Logan et al., 1976, Sharpe and DeMichele, 1977). The model with the highest correlation between observed and fitted residuals was chosen. Confidence bands were estimated using a linear approximation approach with the non-linear regression models, applying the methods of Bates & Watts (2007) in the R package 'investr'. All estimations were made in R version 3.3.2 (R Core Team, 2017).

4.2.4 Statistical analysis

To assess the effect of temperature on survival to adult in all treatments, Kaplan Meier (log-rank) survival analysis was used (Pepe and Fleming, 1989). Student's t-tests and ANOVA were used to compare mean survival, development times and degree days for *Ae. aegypti* and *Ae. notoscriptus* strains in simulated tank, bucket and control treatments. Student t-tests were used to compare air temperature, humidity and dew point from fortnightly measurements taken internally and externally of ten tanks across Brisbane. HDD models at 30 minute intervals and a lower critical temperature of 11.8°C were constructed for the constant, tank and bucket temperature treatments and results were compared with t-tests. Cooling degree day (CDD) models for bucket treatments were also calculated to estimate cold stress. All analyses were done using R version 3.2.2 (R Core Team, 2017) with the 'nlme', 'survival' libraries and 'survminer' used for plotting survival curves.

4.3 Results

During winter 2014 Brisbane experienced a mean rainfall of 36.3mm/month, a mean air temperature of 15.6°C (SD = 2.02) with a mean maximum of 22.8°C (SD = 2.1), a mean minimum of 8.3°C (SD = 3.4; Table 9) and an absolute minimum of 0.5°C (Bureau of Meteorology, 2015). Adelaide experienced a mean air temperature of 11.7°C (SD = 2.04), a mean maximum of 16.0°C (SD = 2.3) and minimum of 7.4°C (SD = 3.0; Table 9), and an absolute minimum of 0.5°C (Bureau of Meteorology, 2015).

4.3.1 Field container temperatures relative to critical thresholds

Rainwater tanks surveyed during winter 2014 had mean temperatures of 16.8°C (range = 11.3°C, SD = 1.9), with mean maximum of 20.8°C and mean minimum of 13.2°C (Table 9). Buckets were recorded having a mean temperature of 16.3°C (range = 29.9°C, SD = 4.1), with a mean maximum of 27.3°C and mean minimum of 13.0°C (Table 9). The relative difference between the mean weekly temperature in tanks consistently stayed above the mean weekly temperatures in air throughout the winter (mean relative difference tank/air = 1.3°C, SD = 0.14), while the relative difference in mean bucket temperatures to weekly mean air temperatures was 0.55°C (SD = 0.14; Figure 23).

Mean hourly temperatures of tanks in high and low shade never dropped below the lower critical temperature during a 24 hour cycle in July (Figure 24), and the minimum daily temperature of any tank dropped below the lower critical temperature on 5.4% (5 of 92 days) of the total period (Figure 25). Temperatures below the lower critical temperature coincided with tanks in high shade or containing under 500L of water at the time (Figure 25, Appendix 6). In buckets the mean hourly temperature (high and low shade regimes) and mean daily minimum (all shade regimes) dropped below the lower critical temperature throughout July (Figure 24) and winter (Figure 26), respectively. Daily temperatures in buckets were estimated to drop below the lower critical temperature 66.3% (61/92) and 93.3% (28/30) of the time in winter and July, respectively. The lowest temperature observed in buckets was 5.4°C, tanks 11.24°C and air 0.5°C during July. Differences between mean air temperature measured internally (mean = 21.4°C, SD = 0.99) and externally (mean = 21.74°C, SD = 0.94) of individual tanks fortnightly, were not significant (t(12) = 0.26, *P* = 0.80). Adelaide conditions were generally unfavourable for *Ae. aegypti* larval development, with the high shade tank and bucket mean temperatures consistently below the lower critical temperature (Figure 27A). Conditions in Brisbane during summer were within the optimal temperature range for *Ae. aegypti* in both rainwater tanks and buckets (Figure 27B).

4.3.2 Non-biological measurements field containers

The mean volume of water in all tanks was 4,098L (minimum = 222L, maximum = 10,758L, Appendix 6) with changes in tank volume decreasing by a maximum of 1,625L and increasing by 4,306L over a two week period. Tanks that were not connected to inlet pipes had a mean evaporation rate of 0.01L/day (range = 0.27L, SD = 6.2). Mean evaporation rate for buckets in low shade was 1.47L (SD = 1.06), medium shade 0.98L (SD = 0.60) and high shade 1.01L (SD = 0.71) per fortnight, with a maximum of 4.3L and minimum of 0.002L. Evaporation rates were not significantly different between bucket shade treatments per fortnight (t(18) = 1.34, *P* = 0.29). At evaporation rates similar to the low shade treatment and assuming a linear relationship, the water in 9L buckets used in experiments would take approximately 105 days to evaporate completely.

First flush devices were observed on 50% of tanks, with one tank having two on separate inputs. The mean volume recorded inside first flush devices was 12.9L (SD = 19.7) with a maximum volume of 81L. Humidity was tested internally and externally to all tanks fortnightly. Humidity was significantly higher inside tanks (mean = 78.1, SD = 11.0) than measured externally (mean = 48.8, SD = 10.7; t(8) = -9.9, $P < 0.001$). Likewise, differences between the mean dew point internally (mean = 17.2°C, SD = 4.2) and externally (mean = 9.9°C, SD = 3.7) per fortnight indicated a significantly higher internal dewpoint (t(12) = -4.47, $P < 0.001$). All measurements of water quality, humidity, dewpoint and temperatures for tanks were recorded for reference (Appendix 6).

4.3.3 Mosquito presence in field containers during winter

Mosquito life cycle stages were observed in tanks throughout the entire survey period (Figure 28). *Aedes notoscriptus* was the primary species observed, with a total of 1,820 (mean = 26, SD = 71.64) immature stages being surveyed from tanks. One *Aedes tremulus* (Theobald) fourth instar was also sampled. The tanks that were disconnected from rainfall had adults and/or larvae present the first fortnight of surveys but were never observed again. The total abundance of immature mosquitoes in first flush devices was 200 (mean = 4.8, SD = 18.7) and larval presence ranged from 12.5% to 62.5% of first flush devices surveyed fortnightly (Appendix 7).

Table 9. Temperatures recorded in air at Archerfield Airport, (-27.57º S, 153.01º E), tanks and buckets from Brisbane and Adelaide Airport (-34.95º S, 138.52º E) during winter (1st June until 31st August), 2014.

Location	Mean °C (SD)	Maximum Daily Range (°C)	Mean Daily Maximum °C (SD)	Mean Daily Minimum °C (SD)
Brisbane Air	15.6 (2.02)	23.0	22.8 (2.1)	8.4 (3.4)
Brisbane Tanks	16.9 (1.89)	11.3	19.7 (1.4)	14.5 (1.7)
Brisbane Buckets	16.2 (4.10)	29.9	28.5 (3.4)	10.7 (2.7)
Adelaide Air	11.7 (2.04)	9.5	16.0 (2.3)	7.4 (3.0)
Adelaide Tanks	11.2 (1.57)	11.7	12.9 (1.4)	9.5 (1.0)
Adelaide Buckets	10.3 (1.73)	9.3	13.5 (1.2)	6.7 (1.5)

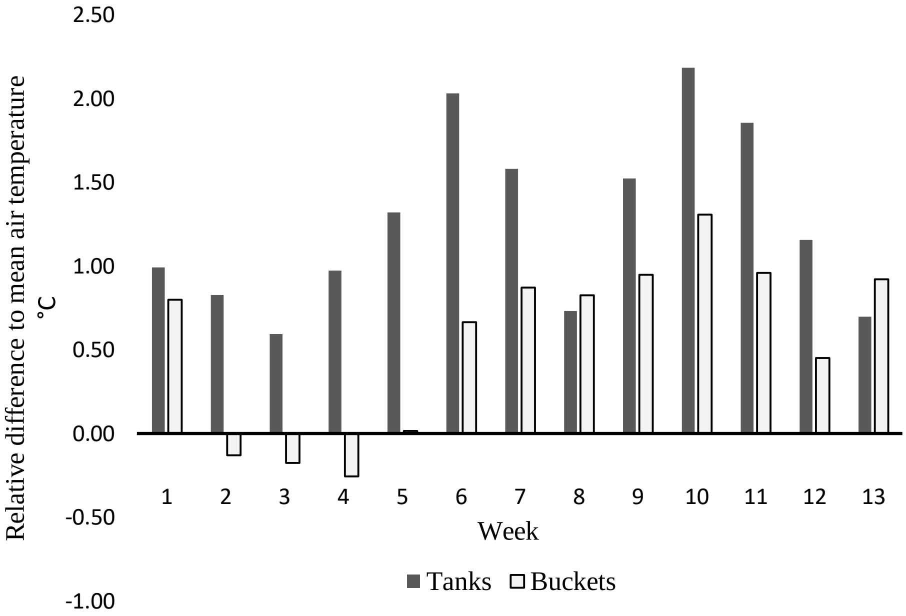


Figure 23. Relative difference in mean weekly temperature for rainwater tanks and buckets to air temperature in 100% shade during winter in Brisbane, 2014.

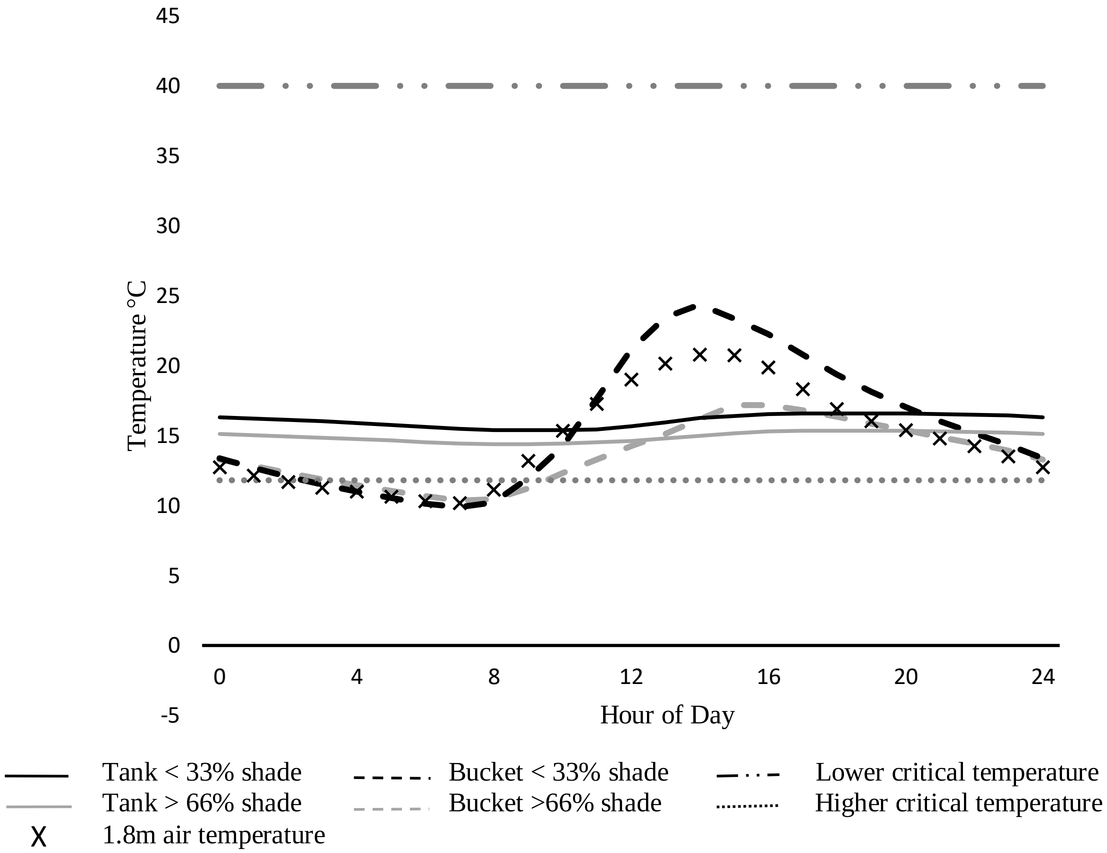


Figure 24. Mean hourly temperatures from buckets and rainwater tanks during July in Brisbane, 2014. Air temperatures are recorded from 100% shade (crosses) and critical thresholds of *Aedes aegypti* are displayed.

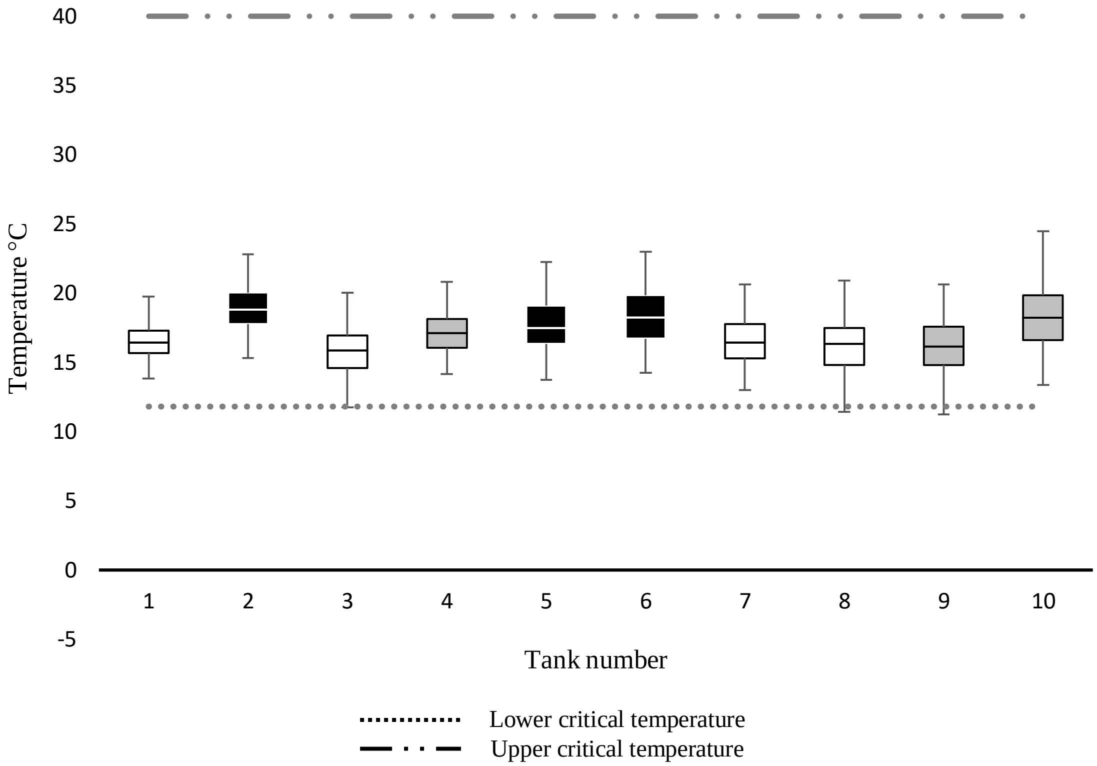


Figure 25. Interquartile ranges of temperatures of individual rainwater tanks in Brisbane during winter 2014. Lower critical temperature threshold (11.8°C) taken from Eisen et al. (2014) and upper threshold (40.0°C) from Richardson et al. (2013).

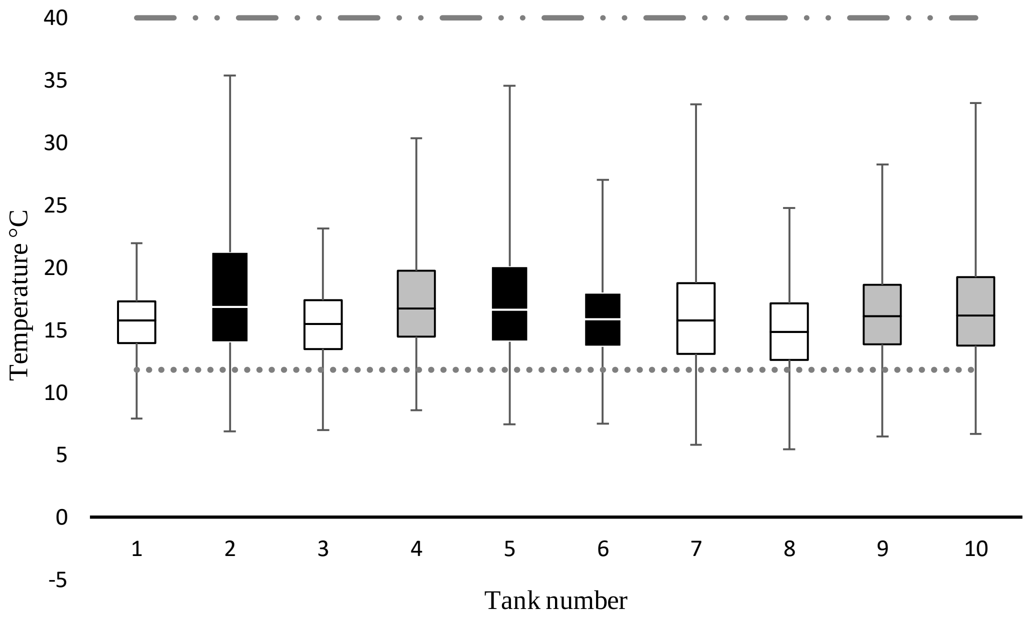


Figure 26. Interquartile range of temperatures of individual buckets in Brisbane during winter 2014. Lower critical temperature threshold (11.8°C) taken from Eisen et al. (2014) and upper threshold (40.0°C) from Richardson et al. (2013).

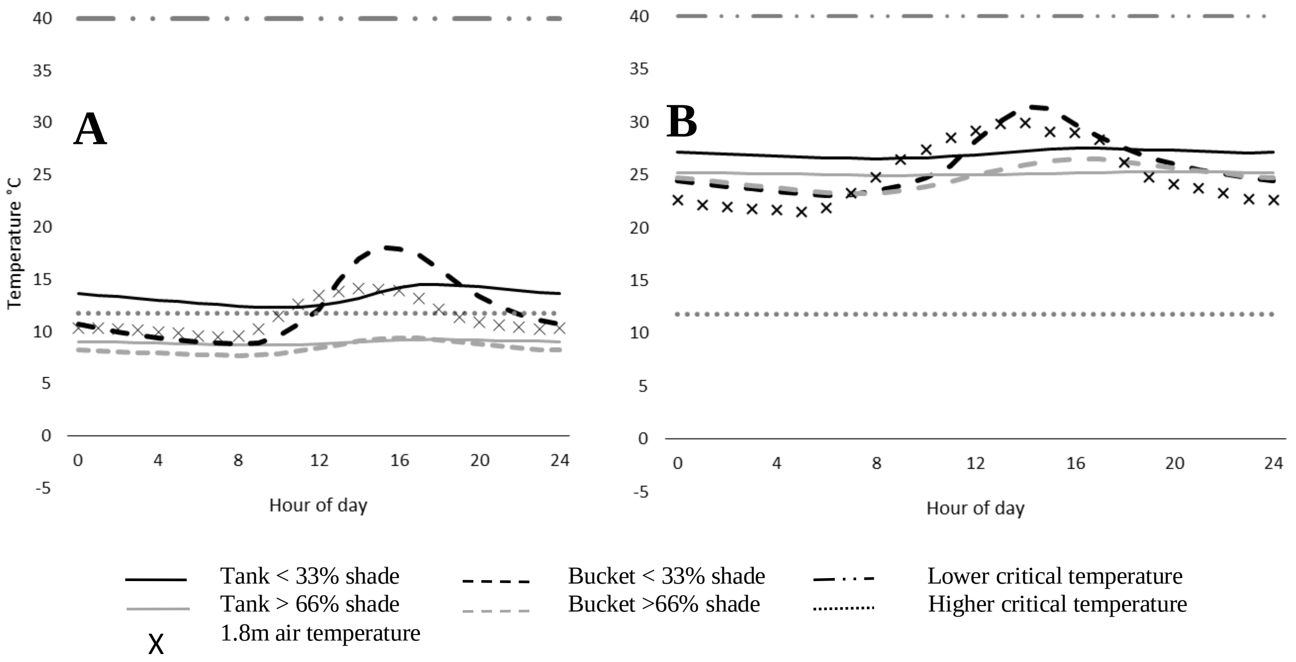
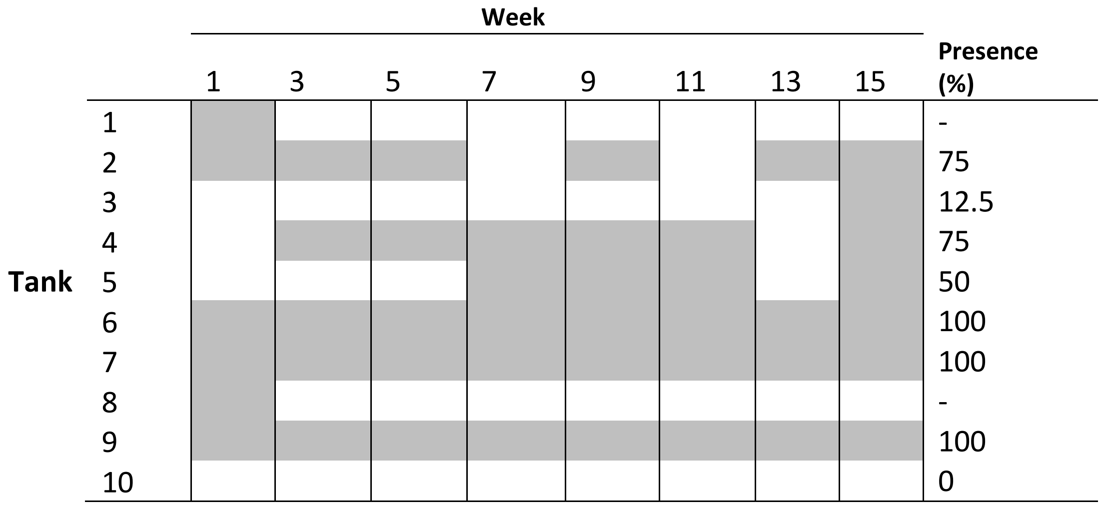


Figure 27. (A) Mean hourly temperatures from buckets and rainwater tanks during July in Adelaide, 2014. (B) Mean hourly temperatures from buckets and rainwater tanks during January in Brisbane, 2015. Air temperatures are recorded from 100% shade (crosses) and critical thermal limits of *Aedes aegypti* are displayed. Lower critical temperature threshold (11.8°C) taken from Eisen et al. (2014) and upper threshold (40.0°C) from Richardson et al. (2013).



		Week								
		1	3	5	7	9	11	13	15	**Presence (%)**
	1	▨								-
	2	▨	▨	▨		▨		▨	▨	75
	3								▨	12.5
	4		▨	▨	▨	▨	▨		▨	75
Tank	5				▨	▨	▨		▨	50
	6	▨	▨	▨	▨	▨	▨	▨	▨	100
	7	▨	▨	▨	▨	▨	▨	▨	▨	100
	8	▨								-
	9	▨	▨	▨	▨	▨	▨	▨	▨	100
	10									0

Figure 28. Presence of *Aedes notoscriptus* life cycle stages in sealed rainwater tanks during winter in Brisbane, 2014. Dashes indicate tanks which contained mosquitoes when surveyed initially, however these tanks were subsequently disconnected from inlet pipes to prevent immatures being washed into tanks during rainfall events.

4.3.4 *Aedes aegypti* development and survival under fluctuating temperatures in simulated containers.

In environmental chambers, temperatures within containers were 1°C (SD = 0.98 tanks, SD = 0.2 buckets) less than programmed temperatures, likely due to the thermal capacity of water stored within chambers. Log-rank tests indicated that the rainwater tank temperature regime (low fluctuation) increased *Ae. aegypti* survival to adult when compared to the bucket temperature regime (high fluctuation; $\chi^2 = 59.7$, df = 1, $P < 0.001$). There was higher survival in the rainwater tank treatments for both Cairns (Figure 29; $\chi^2 = 18.3$, df = 1, $P < 0.001$) and Gin Gin strains (Figure 30; $\chi^2 = 47.8$, df = 1, $P < 0.001$) when compared with their respective bucket treatment (Table 10).

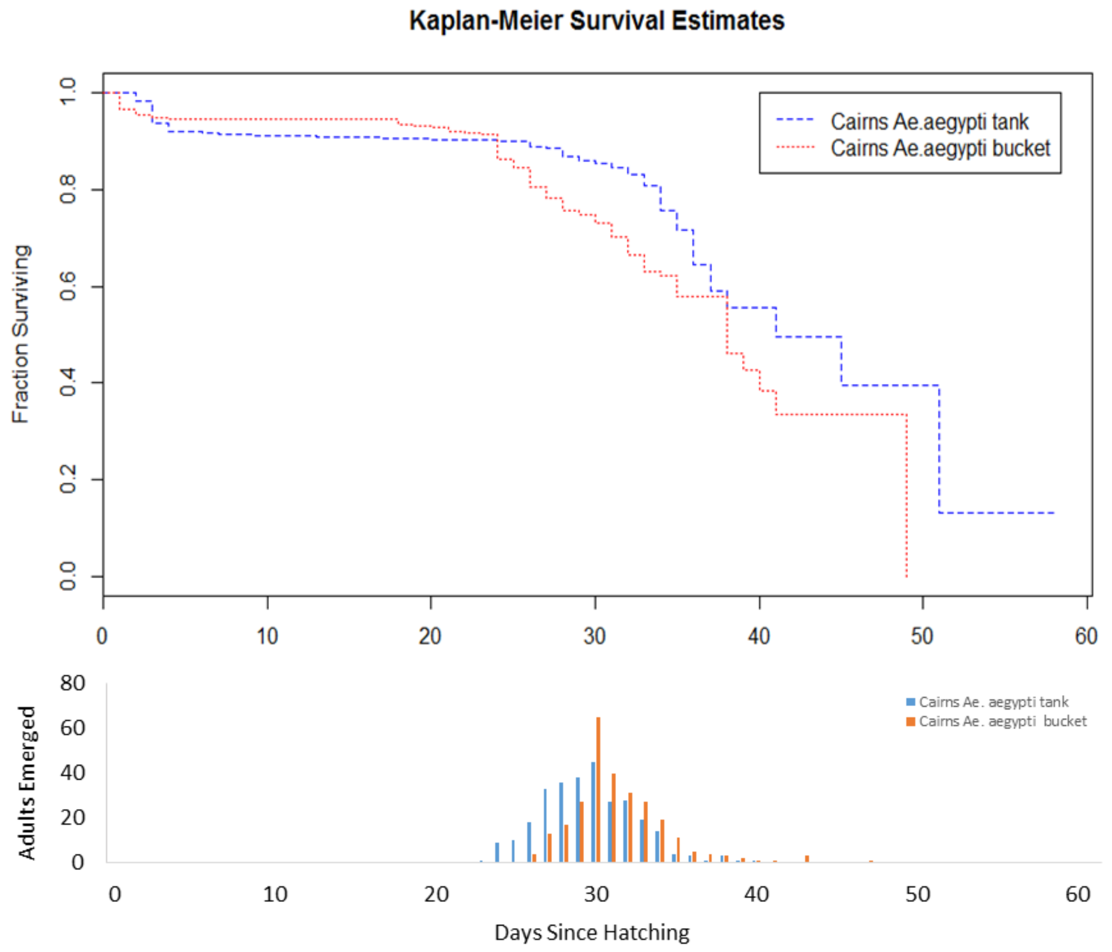


Figure 29. Survival curves and histogram of emergence comparing Cairns *Aedes aegypti* strain in tank and bucket temperature treatments.

Aedes aegypti from the Cairns strain had higher survival in both tank (χ^2 =5.2, df = 1, *P* = 0.022) and bucket temperature regimes (χ^2 = 24.7, df = 1, $P < 0.001$) when compared to the Gin Gin strain in each regime. Regarding development time, no differences in mean development time between any of the tank or bucket treatments were detected (F(1,29) = 1.48, *P* = 0.234; see Table 11). Likewise, strain had no effect on mean development time (F(1,29) = 0.646, *P* = 0.428). The interaction effect was not significant (F(1,28) = 0.242, *P* = 0.627).

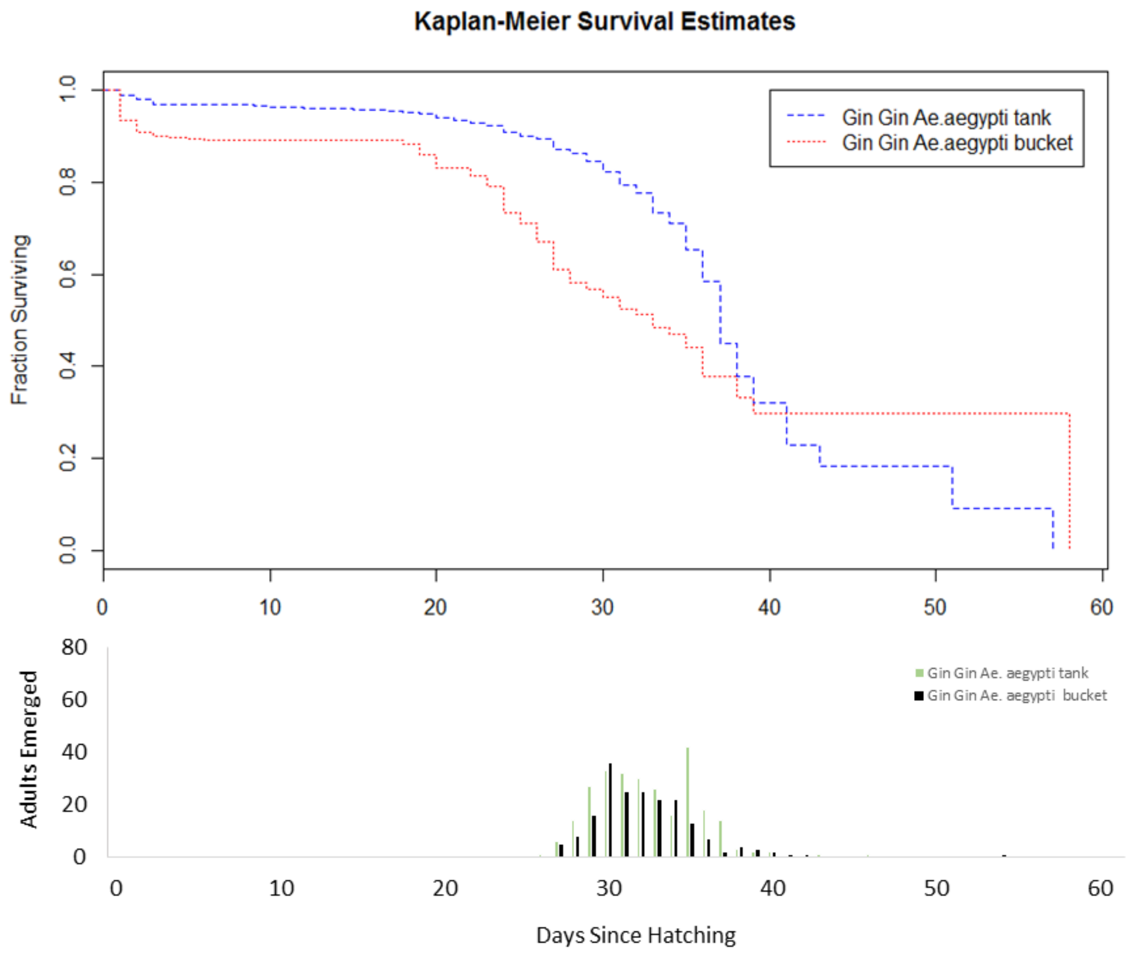


Figure 30. Survival curves and histogram of emergence comparing Gin Gin strain of *Aedes aegypti* in tank and bucket temperature treatments.

4.3.5 *Aedes notoscriptus* development and survival under fluctuating temperatures

There was no difference in mean developmental times between *Ae. notoscriptus* tank and bucket treatments ($t(15) = -0.14$, $P = 0.9$), however the bucket treatment had a higher survival rate than the tank treatment ($\chi^2 = 44.5$, df = 1, $P < 0.001$; Figure 31). There was significantly higher survival in Cairns ($\chi^2 = 326$, df = 1, $P < 0.001$; Figure 32) and Gin Gin ($\chi^2 = 371$, df = 1, $P < 0.001$) *Ae. aegypti* strains when compared to *Ae. notoscriptus* tank treatment (Table 10). Likewise, *Ae. notoscriptus* had lower survival in the bucket treatment when compared to *Ae. aegypti* strains from Cairns ($\chi^2 = 131$, df = 1, $P < 0.001$; Figure 33) and Gin Gin ($\chi^2 = 50.3$, df = 1, $P < 0.001$).

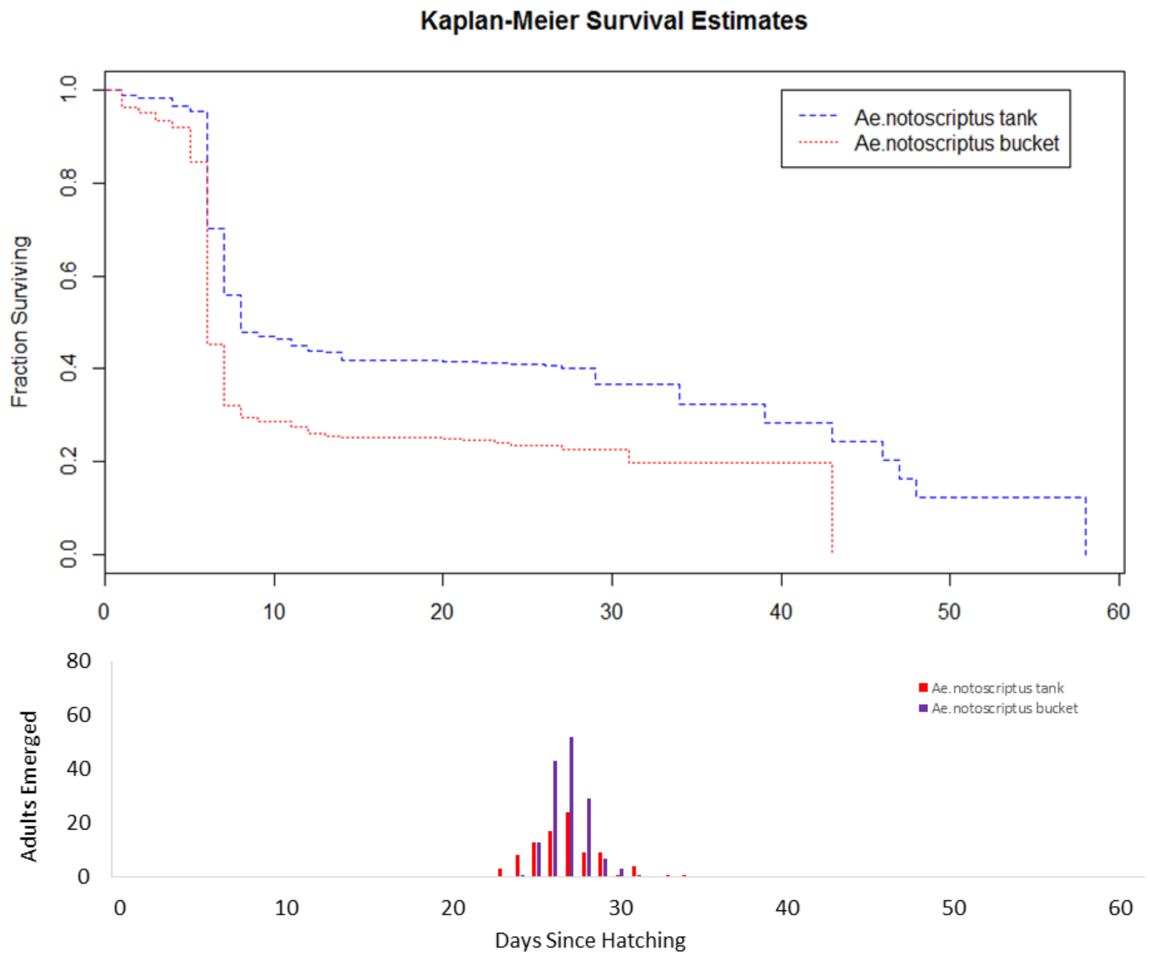


Figure 31. Survival curves and histogram of emergence comparing *Aedes notoscriptus* in tank and bucket temperature treatments.

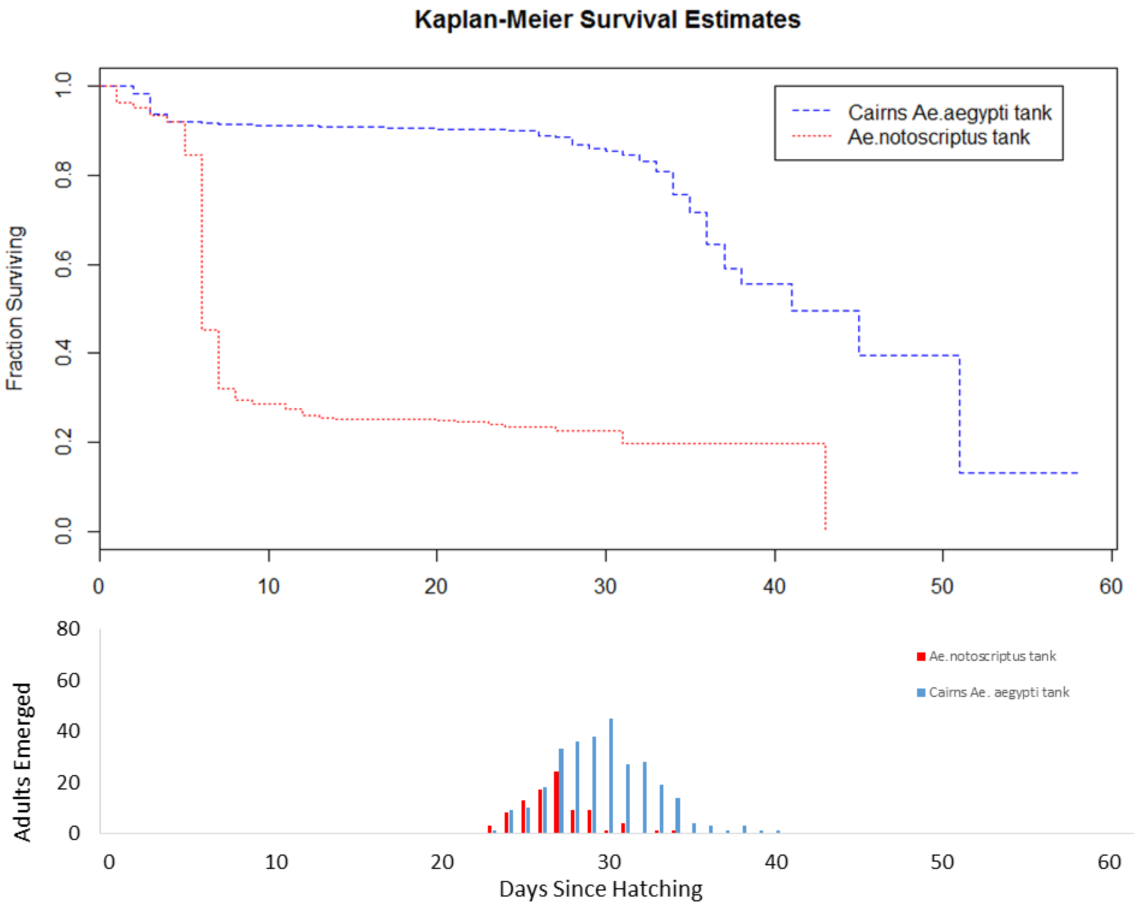


Figure 32. Survival curves and histogram of emergence comparing Cairns *Aedes aegypti* strain with *Aedes notoscriptus* in the tank temperature treatment.

There were significant differences in development time between *Ae. notoscriptus* and both strains of *Ae. aegypti* within tank treatments (F(2, 21) = 19.57, $P < 0.001$; Table 11). A post hoc test revealed significant differences in development time between *Ae. notoscriptus* and Gin Gin ($P < 0.001$) and *Ae. notoscriptus* and Cairns strains ($P < 0.001$) in the rainwater tank treatment. There were significant differences in mean development time between *Ae. notoscriptus* and *Ae. aegypti* strains in the bucket treatment (F(2, 21) = 19.57, $P < 0.001$). A post hoc test revealed differences in mean development time between *Ae. notoscriptus* and the Gin Gin strain ($P < 0.001$) and *Ae. notoscriptus* and the Cairns strain ($P < 0.001$) in bucket treatments.

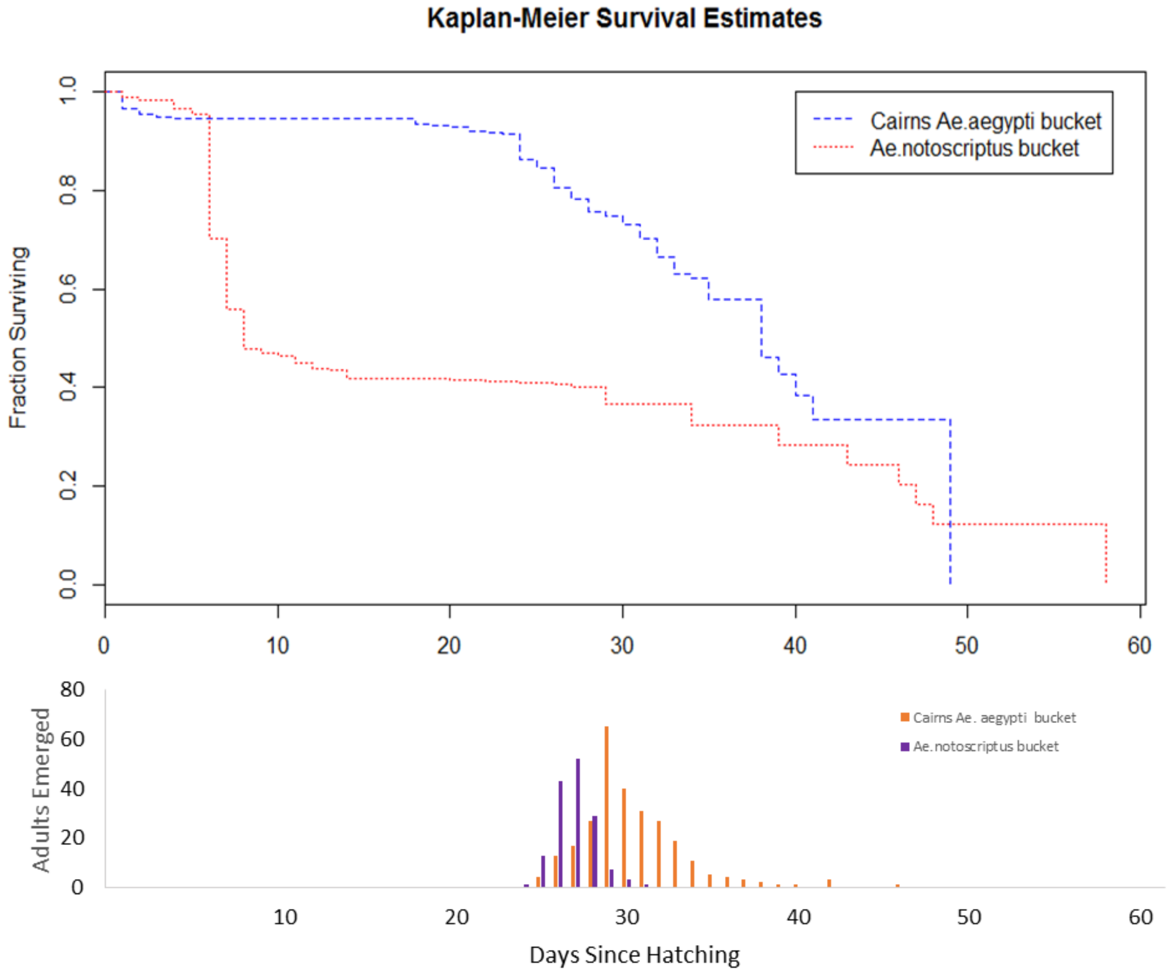


Figure 33. Survival curves and histogram of emergence comparing Cairns *Ae. aegypti* strain with *Ae. notoscriptus* in the bucket temperature treatment.

The Cairns *Ae. aegypti* strain had higher survival at constant 26°C than the Gin Gin strain (χ^2 = 4.3, df = 1, P = 0.038) and *Ae. notoscriptus* (Table 10; χ^2 = 25, df = 1, P < 0.001). A comparison between Gin Gin and *Ae. notoscriptus* controls indicated that the Gin Gin control strain had a significantly higher survival (χ^2 = 9.2, df = 1, P = 0.002). There were no significant differences in development times between the Cairns, Gin Gin or *Ae. notoscriptus* control strains (Table 11; F(2, 21) = 3.276, P = 0.058).

Table 10. Survival of Cairns and Gin Gin *Aedes aegypti* strains and *Aedes notoscriptus* in rainwater tank (small fluctuation), buckets (large fluctuation) and 26°C control (constant) treatments.

Treatment	Strain/Species	Adults Surviving	Deaths	Total	%
Tanks	Gin Gin *Ae. aegypti*	268	134	402	66.7
	Cairns *Ae. aegypti*	291	94	385	75.6
	Ae. notoscriptus	90	309	399	22.6
Bucket	Gin Gin *Ae. aegypti*	193	207	400	48.3
	Cairns *Ae. aegypti*	274	141	415	66.0
	Ae. notoscriptus	149	248	397	37.5
Control	Gin Gin *Ae. aegypti*	391	11	402	97.3
	Cairns *Ae. aegypti*	389	4	393	99.0
	Ae. notoscriptus	354	29	383	92.4

Table 11. Mean, standard error, minimum and maximum development time for Cairns and Gin Gin *Aedes aegypti* strains and *Aedes notoscriptus* in rainwater tank (small fluctuation), buckets (large fluctuation) and 26°C control (constant) treatments.

Treatment	Strain/Species	Mean Days to Adult	SE	Min	Max
Tanks	Gin Gin *Ae. aegypti*	32.48	0.19	26	46
	Cairns *Ae. aegypti*	32.70	0.20	23	58
	Ae. notoscriptus	26.78	0.22	23	34
Bucket	Gin Gin *Ae. aegypti*	32.22	0.23	27	54
	Cairns *Ae. aegypti*	31.37	0.18	26	47
	Ae. notoscriptus	26.89	0.07	24	31
Control	Gin Gin *Ae. aegypti*	8.88	0.03	7	11
	Cairns *Ae. aegypti*	9.05	0.04	7	15
	Ae. notoscriptus	8.86	0.03	8	11

Based on the lower critical temperature of 11.8°C (Carrington et al., 2013, Eisen et al., 2014), the Cairns strain *Ae. aegypti* required 125.7 (SE ± 0.57) HDDs and Gin Gin 123.4 (SE ± 0.45) HDDs to develop into adults at a constant 26°C temperature. There was a small but significant difference between the HDDs required (t(14) = 2.28, $P = 0.046$). There was no significant difference in HDDs between Cairns (mean = 80.6, SE ± 2.04) and Gin Gin strain (mean = 81.1, SE ± 2.16) tank treatments (t(14) = -0.71, *P* = 0.401), or Gin Gin (mean = 107.2, SE ± 1.72) and Cairns strain (mean = 104.1, SE ± 1.64) bucket treatments (t(14) = -1.26, *P* = 0.23). The Cairns *Ae. aegypti* strain in the bucket treatment experienced a mean of 47.2 (SE ± 0.27) and Gin Gin 48.5 (SE ± 0.35) CDDs. As the lower critical temperature for *Ae. notoscriptus* is unknown, I did not calculate degree days for development.

4.3.6 Linear estimate of *Aedes aegypti* lower critical threshold and degree day estimates

The median developmental time for the tank regime was 32 days and bucket regime 31, resulting in a total development rate of 0.03125 and 0.03226 per day for the tank regime bucket regime, respectively. Iterating through different lower critical thresholds, the linear development equation (eq. 5) was optimized for each of the daily development rates above until the slope of each development equation was equal. The lower critical threshold was estimated as 10.004°C for both treatments (h_2). Setting the lower critical threshold to this temperature in the heating degree day development model (eq. 7), it was estimated that *Ae. aegypti* undertook 4.24 and 4.38 daily HDDs for the tank and bucket regimes, respectively. To estimate the total number of degree days taken per regime, the median development time was multiplied with daily HDD to give a total of 138.18 (SD ± 13.7) and 138.83 (SD ± 13.8) HDDs for the tank and bucket regimes, respectively.

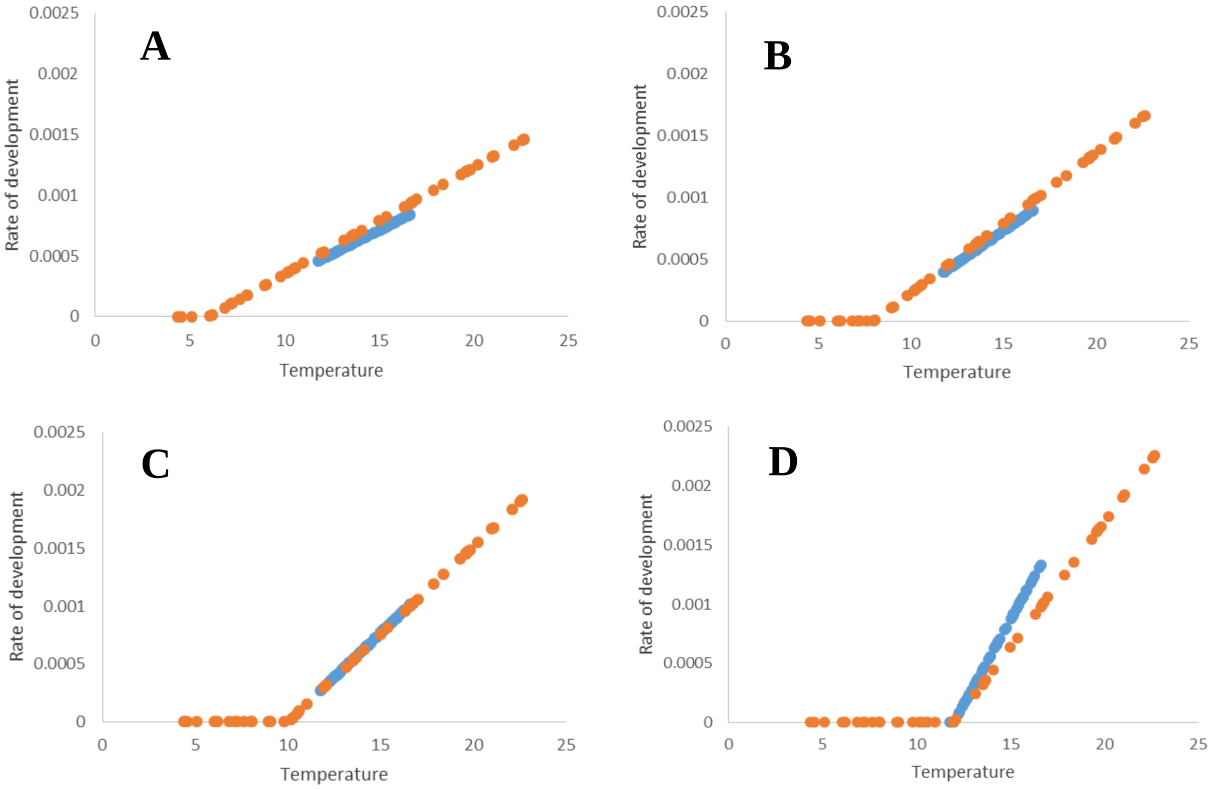


Figure 34. Demonstration of the iterative process for fitting of the zero development threshold (A = 6°C, B = 8°C, C = 10°C and D = 12°C) based on a linear rate of development (eq. 5) from two fluctuating temperature regimes. Blue is the low fluctuation and orange the high fluctuation.

4.3.7 Non-linear estimate of *Aedes aegypti* lower critical temperature

A number of non-linear curves were fit to published *Ae. aegypti* development rates (Table 12). Correlations between observed and fitted values were similar across for most equations, however the model with the highest correlation, and allowed us to estimate a zero development threshold, was the Logan et al. (1976) model, which had a correlation of 0.899 (Figure 35). This model does not have a parameter for the lower critical threshold, so the equation was solved for the point where the development line crosses the x axis, which was 9.21°C (Table 12). Other estimates for the lower development threshold suggest the range is between 6.55°C and 12.38°C and confidence intervals around these estimates vary considerably (Table 12). Parameter estimates were included for the Sharpe and DeMichele (1977) non-linear model used in CIMSiM (Focks et al., 1995), however, it is impossible to estimate an accurate zero development threshold with this model. All non-linear models and their fit can be found in Appendix 8.

Table 12. Parameter estimates, confidence intervals and observed versus expected correlations (cor) for non-linear models of *Aedes aegypti* development rates under constant and fluctuating temperatures sourced from scientific literature.

Model	devRate Model	cor	Lower Threshold	95% CI	Upper Threshold	95% CI	Parameter 1	Par 2	Par 3	Par 4	Par 5	Par 6
Kontodimas et al. (2004)	kontodimas_04	0.894	10.84	1.90	41.34	0.95	0.000052					
Shi et al. (2011)	perf2_11	0.891	12.38	1.95	40.67	0.81	0.013491	0.1931				
Briere et al. (1999)	briere1_99	0.883	10.003	3.08	40.13	0.26	0.000106					
Briere et al. (1999)	briere2_99	0.893	12.15	2.55	40.44	0.87	0.000077	1.439				
Hilbert and Logan (1983)	hilbertLogan_83	0.898	6.55	31.3	45.38	197.8	3.154	62.28	7.622			
Logan et al. (1976)	logan10_76	0.899	9.2091		43.16	11.24	1.1276	0.1347	47.22	12.644		
Sharpe and DeMichele (1977)	sharpeDeMichele_77	0.900	N/A		N/A		32.2	14068.5	-264.96	-76397	282.52	87234

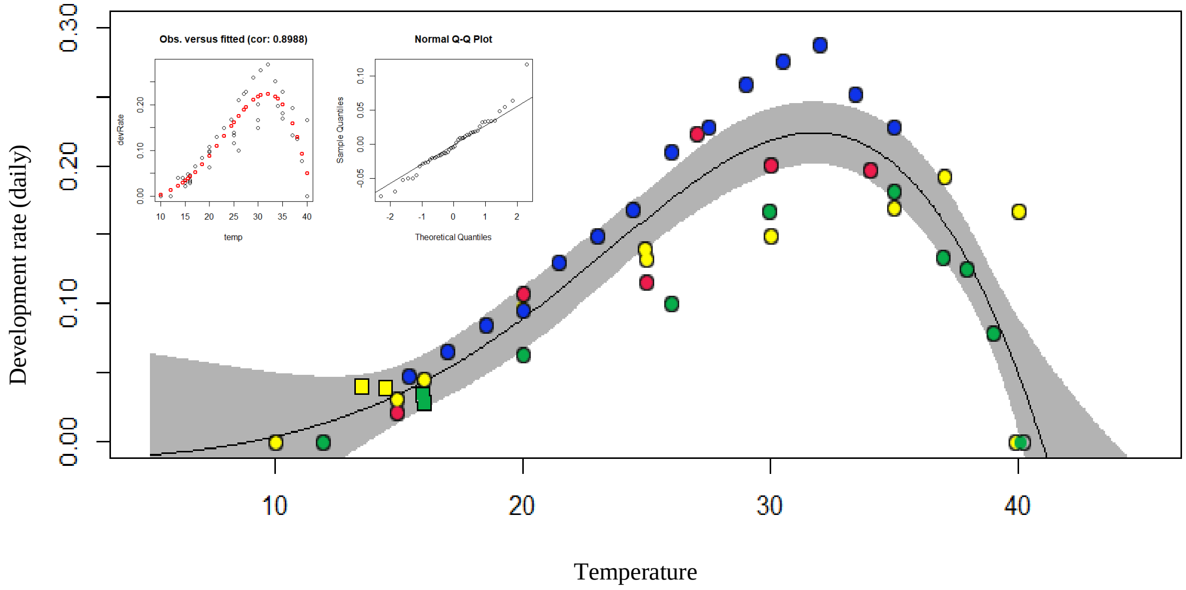


Figure 35. Non-linear development model for *Aedes aegypti* using mean and median time to pupation using equation developed by Logan et al (1976) and development data from Chapter 4, Gilpin and McClelland, 1979, Rueda, 1990, Tun-Lin et al., 2000, Richardson et al., 2011 and Carrington et al., 2013. Colours represent different continents; Africa (blue), Australia (yellow), South East Asia (green) and North America (red); and spots representing constant and squares fluctuating temperatures.

4.4 Discussion

Historically, *Ae. aegypti* was present in Brisbane during the early twentieth century (Cooling, 1923, Hamlyn-Harris, 1931, Trewin et al., 2017 accepted PLoS NTD, Chapter 2). However, this distribution was at a time when unsealed rainwater tanks were common. Recent modelling has suggested that conditions in Brisbane may be inhospitable for the species during winter particularly if rainwater tanks were not present (Kearney et al., 2009, Williams et al., 2010). In this context I discuss my findings on whether conditions found in two common backyard container types are favourable for the persistence of *Ae. aegypti* immatures in Brisbane during winter.

In Brisbane air, tank and bucket mean temperatures were comparable, with weekly differences not exceeding 2.2°C throughout winter. The largest relative difference in mean weekly temperature occurred between tank and air temperatures, with tanks consistently retaining a higher mean temperature than air throughout the winter. This reflected the high thermal mass of water in rainwater tanks and an ability to retain heat when the surrounding air temperatures are dropping. Not only do large volumes retain heat, they buffer or resist rapid fluctuations in air temperature through low thermal inertia. The low thermal inertia observed in rainwater tanks resulted in mean weekly minimum and maximum temperature fluctuation rarely varying by more than ±5°C and rarely crossing the lower critical threshold for development (Eisen et al., 2014).

Shade regimes in tanks were generally uninformative due to the differences in water volume between tanks and fluctuations observed. However, the two tanks that dropped below the lower critical temperature were at full volume (~3000L) and in ~100% shade (south facing or covered in trees). Changes in container temperature are largely driven by ambient air temperature and direct solar radiation, and results suggests direct solar radiation influences tank temperatures above lower critical temperature in Brisbane (Kearney et al., 2009). In buckets, dark colouring would have minimized solar reflectance and enhanced solar radiation absorption. Buckets in the low shade regime tended to increase to a larger maximum temperature and maintained a raised temperature for longer than buckets in high shade. Thus, it is likely that low shade buckets would hasten development during winter. At night in Brisbane, buckets of all shade regimes stabilized with air temperature around 12am. This resulted in minimum temperatures in buckets dropping below the lower critical threshold throughout July. Humidity levels of ~70% and high dewpoints over the surface of the water in rainwater tanks indicated that the air cavity may protect mosquito lifecycle stages when conditions outside are unfavourable. It is likely the observed conditions would protect eggs and adults from desiccation stress during periods of low humidity that occur during Australian winters (Russell et al., 2001, Faull and Williams, 2015).

Aedes notoscriptus larvae were consistently present in ‘sealed’ tanks throughout the winter. Frequent rainfall throughout the period likely resulted in eggs or larvae being flushed into tanks. Tanks with no larvae or adults present had their inflows disconnected early in the survey. This suggests that roof guttering and other rainwater tank infrastructure may play an important role in seeding tanks with larval mosquitoes during rainfall events. In this way rainwater tanks may be classified as population sinks if they remained sealed to the egress of mosquitoes throughout the lifecycle of the product. Although the rainwater tanks in this study were all sealed and mosquitoes were unable to escape, any small gap in seals, meshes or plastic would likely allow mosquitoes developing inside tanks to escape. Determining where eggs were being laid was not an objective of this study, however photos of infrastructure associated with the most productive tanks suggest that eggs or larvae are washed in directly from roof guttering with high levels of organic matter or sitting water (Appendix 9). The role that gutters play as a source to container inhabiting mosquitoes has been identified previously in north Queensland (Montgomery and Ritchie, 2002). The presence of *Ae. notoscriptus* larvae was observed in first flush devices throughout winter, and this suggests that these containers could act as a source to both local adult mosquito populations and larvae in tanks.

No evidence was found to support the original hypothesis that *Ae. aegypti* would not survive in simulated winter temperatures from Brisbane. Instead, 48-67% of larval were observed to survive in bucket treatments representing the coldest week observed, where the mean temperature (13.5°C) was close to the lower critical temperature of Eisen et al. (2014). Fluctuating temperatures can have varying effects on life span and not necessarily in an advantageous direction (Colinet et al., 2015). This suggests that the higher survival observed in the tank treatments was the result of lower cold stress (CDD) when compared to the bucket treatment. Large fluctuations exert high energy demands on exposed insects and when temperature ranges extend far over or under critical thresholds, somatic damage can result (Colinet et al., 2015). While the large fluctuation experienced in the bucket regime received the benefit of temperatures approaching the optimal temperature range of *Ae. aegypti,* it also experienced potentially damaging temperatures ~7°C below its lower critical threshold. It is likely that this damage contributed to the differences between survival results and the previous work by Carrington et al. (2013), who observed higher survival in their large fluctuation when compared to the lower fluctuation. Temperature fluctuations in the current Brisbane study had lower maximum and minimum mean temperatures (22.8°C and 4.5°C respectively) which may have resulted in larvae experiencing longer periods below the lower critical threshold when compared to the Carrington et al. (2013) range of 25.3°C and 6.7°C. This chapter did not compare results to a similar mean as previous authors have (Tun-Lin et al., 2000, Richardson et al., 2011, Carrington et al., 2013) due to the nature of the containers that were observed. The difference in mean temperature observed in the current Brisbane study may have contributed to the differences in survival between treatments, however these differences serve to support the hypothesis that rainwater tanks provide a thermally buffered habitat during periods of stress.

There were differences in survival between *Ae. aegypti* strains, with the Cairns strain having higher survival in all treatments, when compared with the Gin Gin strain. Although these were different within the experiment, survival results for both treatments were above or equal to those observed in previous studies studying survival at constant temperatures (Tun-Lin et al., 2000, Richardson et al., 2011, Carrington et al., 2013). As the Cairns strain was sourced from a population studied previously from North Queensland (Tun-Lin et al., 2000, Richardson et al., 2011), the increased survival in *Ae. aegypti* treatments in the current study may have been due to water husbandry or feeding regime applied. Here, excess food was removed and water was topped up from a pre-chilled source daily. Finally, higher survival in the Cairns strain suggested results do not support the original hypothesis that southern populations would be adapted to colder temperatures.

Estimates of development time around the lower critical temperature were consistent with other studies of Australian *Ae. aegypti* populations (Tun-Lin et al., 2000, Richardson et al., 2011). These studies estimated mean emergence times of 30-39 days for constant temperatures regimes of 15°C and 16°C (Tun-Lin et al., 2000, Richardson et al., 2011). Interestingly, no difference in mean development time was observed between simulated temperature regimes in the current study. However, differences in the number of HDDs required for development were observed, with mosquitoes within the tank regime requiring a lower number of degree days to develop than the bucket regime. Assuming 11.8°C as the lower critical temperature, the HDDs estimated in this chapter using a degree day model are likely to be inaccurate due to difficulties associated with estimating the lower critical temperature. In the mosquito literature, the lower critical temperature is typically calculated using regression to estimate the point where the linear function crosses the temperature-axis (Tun-Lin et al., 2000, Carrington et al., 2013, Eisen et al., 2014). In reality, development rates at this lower threshold tend to decay in an exponential fashion and thermal minimums estimated using linear functions will have a high margin of error compared to estimations using non-linear methods (Allsopp et al., 1991). For example, the number of HDDs calculated for a small fluctuation around an inaccurate lower critical temperature, will tend to underestimate the total HDDs required for development compared to rates entering the linear part of the developmental curve (Allsopp et al., 1991). Thus interpreting development rates around thermal minimums must be done with caution.

The linear and non-linear estimates show the relative inaccuracy associated with developing estimates of the lower critical temperature in insects (Allsopp et al., 1991). The lower critical temperature for *Ae. aegypti* is clearly lower than what is currently accepted as the value for this limit (11.78 ~11.8°C), estimated by linear regression (Carrington et al., 2013, Eisen et al., 2014). Estimating the lower critical temperature via constant temperatures represents an artificial condition for most insects (Allsopp et al., 1991) and thus fluctuating temperatures are likely to give more accurate estimates of this threshold value. At cold temperatures, development decelerates due to the slowing of molecules and a decrease in enzyme activity (Lee Jr, 1991). This effect means that the development curve flattens almost asymptotically as in approaches the point of zero development (Allsopp et al., 1991). Thus, these findings indicate that the lower critical threshold is likely lower than previously estimated and therefore estimates of *Ae. aegypti* survival and development (particularly those using degree days) may have underestimated the ability of the species to withstand cold temperatures.

Aedes notoscriptus had higher survival in the simulated bucket treatment when compared to the rainwater tank treatment. This reversal of what was observed in *Ae. aegypti* survival may be the result of a lower critical temperature in *Ae. notoscriptus,* although this threshold is currently unknown. If it were considerably lower, *Ae. notoscriptus* results may reflect a larger time spent within the ideal temperature range, similar to what was observed in *Ae. aegypti* by the Carrington et al. (2013) study. As so little is known about these ranges, it is hard to draw firm conclusions. Likewise, conclusions may be influenced by the different handling of eggs and this may have been reflected in the lower survival rate in the *Ae. notoscriptus* controls. However, *Ae. notoscriptus* is not readily colonized and our method of egg handling ensured we removed the inbreeding effects of colonizing this species. Development of *Ae. notoscriptus* was faster in both container treatments when compared with *Ae. aegypti* and this was expected based on the distribution of *Ae. notoscriptus* in colder regions of Australia. Survival was considerably lower, however, and results did not validate the hypothesis that *Ae. notoscriptus* would have higher survival than *Ae. aegypti* strains under each temperature regime. There is not a lot known about the biology of *Ae. notoscriptus* and recent evidence suggests cryptic lineages within Australian populations (Endersby et al., 2013). The work by Endersby et al. (2013) shows a clear divide between northern and southern populations, and as such there is the potential for certain lineages to be more cold adapted than others.

Another explanation for the observed results is that *Ae. notoscriptus* has lower survival in earlier instars if conditions are not within the ideal range for development. Results from the control treatment indicated a high survival of >90%, however, in both container treatments survival dropped rapidly during early instar stages. Typically entomologists allow *Ae. notoscriptus* eggs to sit for many hours or days before healthy larvae are selected for experimentation (Russell, 1986, van Uitregt et al., 2012, Nicholson et al., 2015). First instar larvae were selected immediately after one hour of vacuum pumping. Although the size of larvae was not measured, size differences were observed between *Ae. aegypti* and *Ae. notoscriptus* first instars. A low survival rate of *Ae. notoscriptus* in late winter (6 adults from 1200 eggs) was observed in another study where eggs were taken from an insectary colony and raised in rainwater tanks (Trewin unpubl. data). Personal observations suggest that first instar *Ae. aegypti* emerge from the egg at a greater size, and this may allow them to overcome environmental stresses around the time of emergence.

To conclude, we observed that *Ae. aegypti* was able to survive and emerge as adults when subjected to the coldest temperatures recorded in both buckets and rainwater tanks from Brisbane during winter, 2014. Our results do not support the hypothesis that southern *Ae. aegypti* populations would be adapted to colder temperatures, however a limitation to our conclusion includes a lack of replication at the population level. The low thermal inertia of rainwater tanks meant that mean weekly temperature fluctuations rarely varied by more than ±5°C and mean temperatures remained above the Eisen et al. (2014) lower critical threshold for the species. However, it is unlikely this lower threshold is accurate, as our results show it is possible to set lower threshold using a non-linear approach. Although we did not simulate containers smaller than 9L, it can be inferred that containers where water does not remain after 32 days in winter would likely result in the death of larvae. Our results support the hypothesis that rainwater tanks provide a thermally buffered habitat during periods of environmental stress. Thus, any containers with large volumes of water in southern areas of Australia are likely to increase the risk of establishment of *Ae. aegypti* and other urban vectors in the future.

Chapter 5. Movement and distribution of disease vectors in an urban landscape: the influence of domestic rainwater tanks.

5.1 Introduction

Understanding how rainwater tanks influence the movement and distribution of disease vectors is essential to managing the risk of establishment of *Ae. aegypti* in Brisbane. In two experiments, one surveying mosquitoes around different tank spatial configurations and the second using mark-release-recapture (MRR) methodologies, this chapter describes how an urban landscape with rainwater tanks influences the population, distribution and movement of *Aedes* mosquitoes over time. For the full literature review for this chapter see Introduction Section 1.5.1. It was hypothesised that a) rainwater tanks would act as attractants to container-inhabiting mosquitoes, increasing adult catches and eggs laid in traps situated in close proximity to these large water storage containers, and b) barriers to movement, such as roads, would limit the movement of marked mosquitoes and inhibit dispersal into the surrounding urban environment.

5.2 Methods

5.2.1 Study area

Gin Gin (-24.99ºS, 151.95ºE) is a small rural town situated on a main highway 300km north of Brisbane, in the eastern foothills of the Great Dividing Range which experiences hot, humid summers and dry winters (Bureau of Meteorology, 2015). The urban landscape of Gin Gin is generally open; properties are around 800m^2 each in size and little to no fencing or hedge-like greenery on boundaries. Dwellings in the town are typically raised off the ground by 1-1.5m and made primarily of wood, with tin rooves. Some are the typical Queenslander style, raised to 2m, and the space underneath utilized for storage and laundry. Roads are generally 30m wide fence to fence, and a two lane highway passes through the middle of the town. A population of *Ae. aegypti* was discovered in Gin Gin by health authorities in 2011 (Queensland Health, 2012). A comprehensive survey to describe larval habitats and the distribution of *Ae. aegypti* in 2012 revealed 73% (347/473) of dwellings had a rainwater tank (Queensland Health, 2012), with a large number of these tanks present for over 20 years, and in various states of disrepair and in various states of disrepair (rusted rooves and entrances, warped sieves, signs of UV degradation).

The research protocol received ethical approval by the University of Queensland, CSIRO and QIMR Berghofer Medical Research Institute (human ethics form P2273) and endorsement by state health authorities and the local council. As part of the ethics approval process, a suppression plan was developed that entailed physical larval habitat source removal or treatment with S-Methoprene pellets, inspection of tanks within the trapping area for compliance with regulations and larval presence, remedy of non-compliant tanks, and indoor residual spraying offered to households within 200m of the release sites. Extensive public engagement with town residents preceded experiment 2, including the formation of a community reference group that met throughout the study period (dates attended 13/1/16, 27/01/16, 26/04/16, Chair: Councillor Wayne Honor) to discuss research goals and enhance communication between the researcher and the residents. A town-hall meeting (date 27/01/16) was undertaken for residents to hear about the research and give feedback or voice concerns. Residents who volunteered to have traps placed in their yards provided written approval, and were offered free insect repellent and a can of insecticide surface spray during the experiment. At the completion of the experiment, volunteers were also provided a gift voucher as compensation for electricity use. All news media associated with this study can be found in Appendix 11.

5.2.2 Experiment 1: The effect of rainwater tanks on vector abundance

Nine properties were selected to investigate the effect of rainwater tank presence on mosquito abundance. Three properties had unsealed/non-compliant rainwater tanks, three had sealed/compliant rainwater tanks and three did not have a rainwater tank. Houses were selected with similar levels of shade condition, house condition and yard condition as defined by Tun-Lin et al. (1995a), to attempt equivalent suitability for mosquitoes. Tank condition (open, sealed, no tank), total number of potential water holding containers present, number of containers containing water and containers with mosquito larvae present were described and recorded at each property. Using Google Earth ® (2017), the area (m^2) of tree shade and shade attributable to buildings was estimated at each property. To increase attractiveness of traps, all larval habitats were emptied of water, removed from yards or treated with S-Methoprene sand before and during the experiment. Four ovitraps were placed in each yard containing aged rainwater and lucerne with the pellet to volume ratio described by Ritchie (2001) and water-proof sandpaper (80 grit) used as an oviposition substrate. Traps were collected fortnightly for 10 weeks from the second of February until the thirteenth of April, 2015. Eggs were counted and larvae reared to fourth instar before being identified to species at the fourth instar via microscopy using the taxonomic keys of Marks (1967). To examine the influence of rainfall on oviposition, linear regression was used to compare the total number of eggs laid in ovitraps with rainfall, with a lag of two, three and four weeks. The response variable (number of eggs laid) was $\log_{10}$ transformed to normalize residuals and satisfy the assumptions of linear regression. To test the hypothesis that a higher abundance of container inhabiting mosquitoes would be observed in premises with unsealed tanks, an analysis of variance (ANOVA) was applied to egg counts for each species. As egg numbers were highly variable, the mean number of eggs for the four traps in each property was used. This removed all zero values from the data, which would have reduced the sum of squares and falsely increased significance. A $\log_{10}$ transformation was used to make the variance independent of the mean. All statistical analysis was done in R version 3.3.2 (R Core Team, 2017).

5.2.3 Experiment 2: Mark, release and recapture of disease vectors

An initial compliance survey was undertaken to find rainwater tanks with *Ae. aegypti* present for marking. As part of this survey, the condition of the rainwater tanks were recorded and included construction type, compliance status, fault type, age estimate (old/new) and species of mosquitoes if present. Inspections for larval mosquitoes in rainwater tanks used a five sweep methodology (Knox et al., 2007) and samples were stored in ethanol for identification.

An array of traps was deployed to investigate the effect of rainwater tanks on mosquito movement and distribution. The trap array was set amongst a diverse range of buildings within 250m of two release sites. The trapping area included 26 residential dwellings, three churches, a public pool, a lawn bowls club, a kindergarten, six commercial buildings, five community centres and a primary school. Environmental conditions were taken from the nearest measurement devices to the study sites as possible; Bundaberg Airport (wind direction, speed, humidity) which is 40km from the release site; rainfall from Gin Gin Post Office 680m from the release site; and data loggers at each release site recorded temperature in a shaded location every 30 minutes. The study area was surrounded by large barriers to movement which minimized unwanted population gains from outside the area (such as parklands, a school and the highway; Figure 36).

Thirty-eight trap groups, each comprising one Biogents-Sentinel (BGS; Biogents GmbH, Regensburg, Germany) and two Gravid *Aedes* Traps (GAT; Ritchie et al., 2014), were set at roughly concentric distances (25m, 75m and 125m) from each release site prior to releases. Overlap between sites allowed for trapping up to 265m for release sites. Traps were classified into two categories according to our hypotheses: one would expect to observe mosquitoes, relative to the proximity to a tank (ie. <25m of a tank) and where one would not expect to observe mosquitoes (>25m from a tank) in each concentric distance. Traps were set at a minimum distance of 25m from release sites to allow released mosquitoes to mix with the background population (those mosquitoes occurring independently of the study). The release block is defined as the continuous group of houses surrounding the release sites that are together surrounded by a road (Figure 36). GATs were treated with residual chemical (Mortein Barrier Spray – imiprothrin 0.3g/Kg, deltamethrin 0.6g/Kg) and the same number of lucerne pellets per unit volume in ovitraps (Ritchie, 2001). BG traps were run on mains electricity or 12V battery.

Two unsealed rainwater tanks that were non-compliant with state regulations were selected as sources for adult mosquitoes. Accordingly, the properties at which these tanks were located were chosen as the two release sites. The tanks were surveyed and found to contain both *Ae. aegypti* and *Ae. notoscriptus* immatures during Experiment 1. Tanks were disconnected from downpipes from September until February 2016 to allow mosquito eggs to accumulate whilst preventing hatching induced by immersion. Tanks were flooded with filtered tap water at the end of summer (February) to simulate a large rain event after a dry period. The degree day model developed in Chapter 4 was used to estimate time to adult emergence. To collect adults for marking, emergence traps were designed and constructed, and placed over tank entrances. Emergence traps were operated for four days before releases (for trap design see Appendix 10). Adults were maintained on a 10% sucrose solution inside the collection cup, before transfer to large cages twice daily. Adults were stored in cages at room temperature and maintained using a 10% sucrose solution. Brilliant General Purpose Fluorescent Pigments (Brilliant Group San Francisco, USA) were used to mark mosquitoes and different colours (Green, Yellow, Pink, Purple and Blue) indicated date of release and release site. This marking methodology was used due to a number of previous experiments which demonstrated that dusts have no negative influence on life span (Muir and Kay, 1998, Watson et al., 2000b, Kluiters et al., 2015). Dusting was performed using a similar technique developed for use with midges (Kluiters et al., 2015), where beakers were pre-dusted and bulb-dusters circulated dusts in wind currents. Mosquitoes were released on 5 different days over a 7 day period next to each non-compliant collection rainwater tank (16th, 17th, 20th, 22nd and 23rd of February 2016). Mosquitoes were collected from each trap group daily over the 13 day period after initial release. Background and recaptured mosquitoes were stored at -20°C after collection and marked status identified using microscopy under ultraviolet (365nm) and white lights. Mosquitoes were considered marked if they had greater than three dust particles.

Figure 36. Trap layout including trap groups (1 BG, 2 GATs) set at <25m to tanks (red circles) and >25m from tanks (blue circles). Annuli represent 25, 75 and 125m from each release site (purple triangle).

5.2.4 Data collection and statistical analysis

The probability of daily survival (PDS) was estimated using the methods of Gillies (1961), where regression coefficients were calculated from $\log_{10}(n+1)$ transformed count data over day of recapture and the PDS as the antilog$_{10}$ of the slope. The average life expectancy (ALE) was calculated from the PDS using the methods of Niebylski and Craig Jr (1994) and a geometric distribution as 1/-log$_e$PDS. To compare survival between sites, a one-way ANOVA was used to compare for significant differences in the log(recaptures) per day. Movement, including MDT and FR$_{50}$ and FR$_{90}$ of all species of released mosquitoes was calculated as per Lillie et al. (1981) and White and Morris (1985). These methods compensate for unequal trap densities within each annulus, as was the case in our trapping design of the current study due the heterogeneous nature of the urban environment. To assess the difference in catch rates between BG traps and GATs, we conducted two binomial logistic regressions on recaptured and background counts of *Ae. notoscriptus* females. Population estimates were based on closed population methods (Southwood and Henderson, 2009) where all five assumptions are addressed by the current study. These include: 1) marked insects should not be affected by being marked, 2) marked insects should become completely mixed with the local population, 3) random sampling with respect to marked insects, 4) trap samples are measured at discrete time intervals in relation to total time, and 5) population gains or losses do not change over the period of study. A Lincoln-Peterson Index was applied and modified with a Bailey correction where low capture rates were observed (Southwood and Henderson, 2009).

The daily and total number of mosquitoes captured, mean angle of dispersion (a) and length of each vector (r) was calculated for *Ae. notoscriptus* females using circular descriptive statistics (Zar, 1999). To test if observed dispersion deviated from non-directional dispersion from each release site, a Watson one sample U^2 test (Zar, 1999) was used. A Watson's large-sample nonparametric test was applied to determine whether all movement and movement external to the release block had a common mean direction with wind direction during the experiment. R code and functions for this test were from 'circular statistics' in R (Pewsey et al., 2013). Mean wind direction and vector and scalar wind speeds were calculated from 3pm and 9am readings using the R package '*Openair*' (Carslaw and Ropkins, 2012, Grange, 2014). All calculations and analyses were done using R version 3.2.2 (R Core Team, 2017).

Two hypotheses were tested regarding the urban landscape using contingency table analysis using the χ^2 statistic. These hypotheses included: 1) Female mosquitoes would be more likely to be caught in association with rainwater tanks (<25m) than away from rainwater tanks (>25m), and 2) more *Ae. notoscriptus* and *Ae. aegypti* were likely to be caught in traps located on the release block than in traps located in blocks separated by roads from the release block.

Using the method applied in Watson et al. (2000b), the percentage of mosquitoes that could potentially transmit disease during the study was estimated. Their method assumes constant mortality and probability of daily survival within the population. Transmission rates (Ross River – mouse infection rate, Barmah Forest – head and thorax; Watson and Kay, 1998, Watson, 1999) and extrinsic incubation period in *Ae. notoscriptus* were 9 and 5 days for Ross River (Watson and Kay, 1998) and Barmah Forest virus (Watson, 1999) respectively. A lag of 2 days from emergence was included to allow for development of flight muscles and time to find a blood meal (Watson et al., 2000a).

5.2.5 Isotropic Gaussian kernel: model framework

To compare traditional methods of measuring movement, dispersal kernels were parameterized and the mean distance travelled for female *Ae. notoscriptus* was estimated using an isotropic Gaussian diffusion model. In this case, the dispersal kernels were defined as a temporally-evolving probability density function (PDF) over two-dimensional space, where the probability of a mosquito being trapped per unit area is a function of the distance from the release location and time.

The PDF for an isotropic Gaussian diffusion can be written as a function of *x* and *y* coordinates in space and time *t*:

$$f(x, y|\, t) = (2\pi\sigma^2 t)^{-1/2} e^{\frac{-(x^2+y^2)}{\sigma^2 t}}, \tag{8}$$

where $\sigma\sqrt{t}$ can be thought of as a standard deviation (in both x and y directions) that grows with time. If the mosquitoes disperse in space and time according to such a model, then one might expect the rate at which they are caught at a given location to be related to their predicted density. To model the counts of mosquitoes caught in traps, a Poisson model was applied, but where the mean of the Poisson distribution over some trapping period $[t_1, t_2]$ at location (*x*, *y*) is modelled as $\theta(x, y, t_1, t_2) = K \int_{t_1}^{t^2} f(x, y, t)dt$, where K is an empirical scaling parameter used to relate the trap counts to the integral of the diffusing Gaussian PDF over the trapping period. K may be considered as a measure of how effective a trap is at catching mosquitoes given the density function for the diffusion at the location of the trap. The likelihood function for data under this model can be written as:

$$L(\sigma^2, K) = \prod_{i=1}^{N} \frac{\theta(x_i,y_i,t_{i,1},t_{i,2})^{c_i} e^{-\theta(x_i,y_i,t_{i,1},t_{i,2})}}{c_i!}, \tag{9}$$

where c_i is the number of mosquitoes caught in the time interval $[t_{i,1}, t_{i,2}]$ for a trap located at coordinate (x_i, y_i) and there are N such data collected in the study. It is often more convenient to work with the log-likelihood, for reasons of numerical stability and ease of differentiation. The log-likelihood follows simply as:

$$l(\sigma^2, K) = \sum_{i=1}^{N} \left(c_i \log \theta(x_i, y_i, t_{i,1}, t_{i,2}) - \theta\left(x_i, y_i, t_{i,1}, t_{i,2}\right) - \log(c_i\,!) \right), \tag{10}$$

where *log* denotes the natural logarithm. The parameters σ and K were estimated by the method of maximum likelihood with optimisation carried out via a Nelder-Mead simplex method as per the optimisation *optim*() routine in R. Confidence intervals on these two parameters were also computed using a profile likelihood() function (Cox, 2006).

Once the maximum likelihood estimates $\hat{\sigma}$ and $\hat{K}$ had been obtained, the probability distribution was obtained for the absolute distance travelled from the origin as a function of time, from the original isotropic Gaussian diffusion model (eq. 8). Under this model, the absolute distance travelled from the origin, d, by time t follows a scaled χ-distribution (closely related to the well-known χ^2 distribution) with two degrees of freedom having PDF:

$$f(d|t) = \frac{d}{t\sigma^2} e^{\frac{-d^2}{2t\sigma^2}}. \tag{11}$$

Since the distance travelled by an individual over its lifetime depends upon longevity under normal circumstances, rather than the distance travelled before it is trapped, a probability distribution for the lifetime of *Ae. notoscriptus* females was required. For simplicity, an exponential lifetime model was used with the PDF:

$$f(t) = \frac{1}{\lambda} e^{-t/\lambda} \tag{12}$$

where λ is the ALE and t denotes lifetime. λ was taken to equal 7.11 days (see results). In practice, other lifetime distributions such as the Weibull distribution could be employed in place of the exponential distribution where sufficient data were available. MDT, FR_{50} and FR_{90} were finally estimated by Monte Carlo simulation. Ten thousand lifetimes $(t_1, \ldots, t_{10,000})$ were randomly sampled from the female *Ae. notoscriptus* lifetime distribution. Next, for each sampled lifetime (t_i) a distance travelled, d_i, was sampled from the χ-distribution above, but where t was set equal to t_i. The resulting values for the distances travelled in a lifetime, $d_1, \ldots, d_{10,000}$, were then used to compute the sample mean, 50th percentile and 90th percentile to estimate the MDT, FR_{50} and FR_{90} respectively.

5.3 Results

5.3.1 Experiment 1: Ovitrapping around rainwater tanks

A total of 27,556 eggs were collected over the 10 week experimental period, with 2,831 hatching before collection and 3,820 killed due to desiccation or fungal contamination. Of the remaining eggs, 17,470 (83.6%) survived until 4th instar for identification. Most eggs were laid by *Ae. notoscriptus* (98.1%), followed by *Ae. aegypti* (1.7%) and *Ae. tremulus* (0.2%). There were also 1,999 *Culex quinquefasciatus* and eighteen *Toxorhynchites speciosus* larvae found inside ovitraps. Low collections of *Ae. aegypti* eggs meant it was impossible to perform statistical analysis for this species. *Aedes aegypti* was, however, predominantly found around unsealed rainwater tanks throughout the experiment; 300 eggs were laid in six ovitraps from three premises (272 from a single premises) with non-compliant rainwater tanks, a single egg was collected from two premises with sealed rainwater tanks, and no eggs were collected from premises without rainwater tanks.

For the ANOVA comparing the mean number of *Ae. notoscriptus* eggs (Table 13), the proportion of tree shade, building shade and building area per premises did not explain any of the variation in oviposition and were removed from the anlalysis. As containers with mosquito larvae present were too intermittent to be used in the analysis as predictors, they were removed from the analysis. Tank treatments were not significantly different when tested against premises (F(2,6) = 0.47, NS, unsealed = 1.57, compliant = 1.78, no tank = 1.86). However, there was a significant difference between dwellings (F(6,24) = 5.56, $P < 0.001$) and weeks (F(4,24) = 6.69, $P < 0.001$, wk2 = 20.1, wk4 = 1.92, wk6 = 1.93, wk8 = 1.24, wk10 = 1.48, LSD = 0.44). There was no interaction between week and tank treatments (F(8,24)=0.90, NS), hence the two factors were independent. Rainfall in the three weeks prior to emptying traps explained 72% of the variation between weeks (b = 0.0041, SE = 0.009; Figure 37).

Table 13. The mean number of *Aedes notoscriptus* eggs laid per week ($\log_{10}$ transformed and untransformed means in parenthesis) over a 10 week period. Treatments were three replicate premises with unsealed tanks (1) three premises with sealed tanks (2) and three premises with no tank (3).

		Tank Condition		
	Replicate	Unsealed	Compliant	No Tank
Premises	1	1.40 (39.9)	1.35 (27.4)	1.42 (45.5)
	2	1.43 (45.7)	1.80 (94.1)	1.93 (94.2)
	3	1.89 (81.6)	2.17 (172.3)	2.23 (285.4)

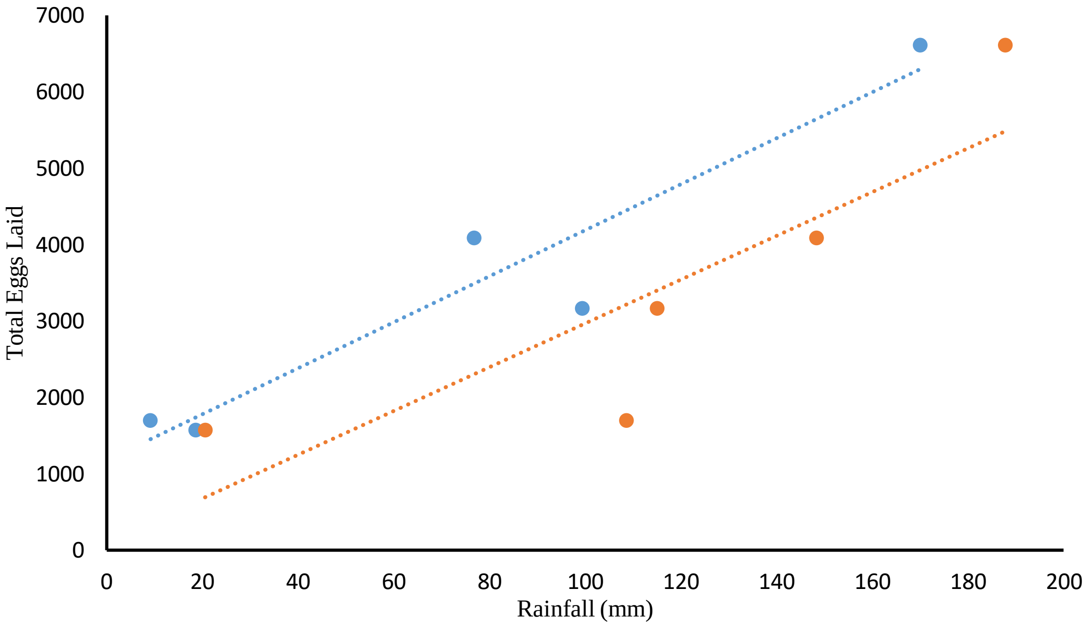


Figure 37. Eggs laid fortnightly by *Aedes notoscriptus* in ovitraps as predicted by cumulative rainfall in Gin Gin, Australia, at a three week delay (orange) and 4 week delay (blue).

5.3.2 Experiment 2: Mark, release and recapture

A survey of rainwater tanks within 200m of the release sites revealed 58 tanks, of which 53.5% (31/58) were compliant with state legislation. Tank construction was primarily polyethylene (37.9%), followed by galvanized tin (31%), fibreglass (15.5%) and galvanized concrete (10.3%; Table 14). Reasons for not complying with regulations included; damaged or unsealed overflow (exit sieve; 44%), holes in roof material (26%), entrance sieve degradation (15%), holes in side wall (8%) and warped design (exposing holes, 7%). Twenty-four tanks were surveyed for the presence of mosquitoes, and of those sampled, 41.7% (10/24) contained larvae of at least one of four species. Forty percent (4/10) of tanks containing mosquito larvae had *Ae. aegypti* present.

Table 14. Tank construction, and frequency of non-compliance and presence of mosquitoes (including percent present) in non-compliant tanks from Gin Gin, Australia.

Tank Construction	Age	Tank Count	% Tank Construction	Non-compliant Tank Count	% Non-compliant	Mosquitoes Present	% Mosquitoes Present
Polyethylene	new	22	37.9	6	27.3	2	33.3
Galvanized	old	18	31.0	11	61.1	3	27.3
Fibre Glass	old	9	15.5	4	44.4	2	50.0
Galvanized Concrete	old	6	10.3	4	66.7	1	25.0
Pressed Metal	new	2	3.4	1	50.0	1	100
Concrete	old	1	1.7	1	100	1	100
Total		58		27	46.6	10	37.0

5.3.2.1 Emergence, release and recapture data

Mosquitoes were released over a period of seven days, with the largest releases occurring on the first two days. A total of 1,714 mosquitoes emerged from the rainwater tank at site one; A*e. notoscriptus* being the most prevalent species with 722 males and 702 females released, followed by *Ae. aegypti* with 49 males and 16 females released and *Culex quinquefasciatus* (Say) with 14 males and 37 females (Table 15). Two female *Toxorhynchites* speciosus (Skuse) and 1,163 non-biting chironomid adults (*Chironomus cloacalis* (Skuse)) were trapped emerging from the same rainwater tank. Deaths during the marking process were minimal, with three male (6.1%) and no female *Ae. aegypti*, and 18 female (2.6%) and 44 male (6.1%) *Ae. notoscriptus* failing to fly away. Site two failed to produce any viable adult mosquitoes, likely due to contamination by large numbers of *Toxorhynchites speciosus* larvae. A total of 71 (10.1%) marked *Ae. notoscriptus* females and no males were collected in trap arrays (Figure 38, Table 15). Of the marked *Ae. aegypti* released, two females (12.5%) and seven males (14.3%) were collected (Figure 38, Table 15). A total of 1,401 unmarked mosquitoes (background) from thirteen species were collected throughout the experiment, of which 27 were female and six male *Ae. aegypti*, 382 female and 13 male *Ae. notoscriptus* and 80 female and 94 male *Cx. quinquefasctiatus*.

Mean daily minimum and maximum temperatures during the experiment were 22.4°C (SD ±1.36) to 34.1°C (SD ±2.30) and 26.5°C (SD ±2.02) to 31.3°C (SD ±4.66) for air and tank temperatures respectively, and the mean relative humidity at 09:00 hours was 69.1% (SD ±8.46) and at 15:00 hours, 57.9% (SD ±7.70). Wind direction was predominantly from the south east. Total rainfall for the two and three weeks leading up-to-and during the release was 119.4 and 233.0mm, respectively.

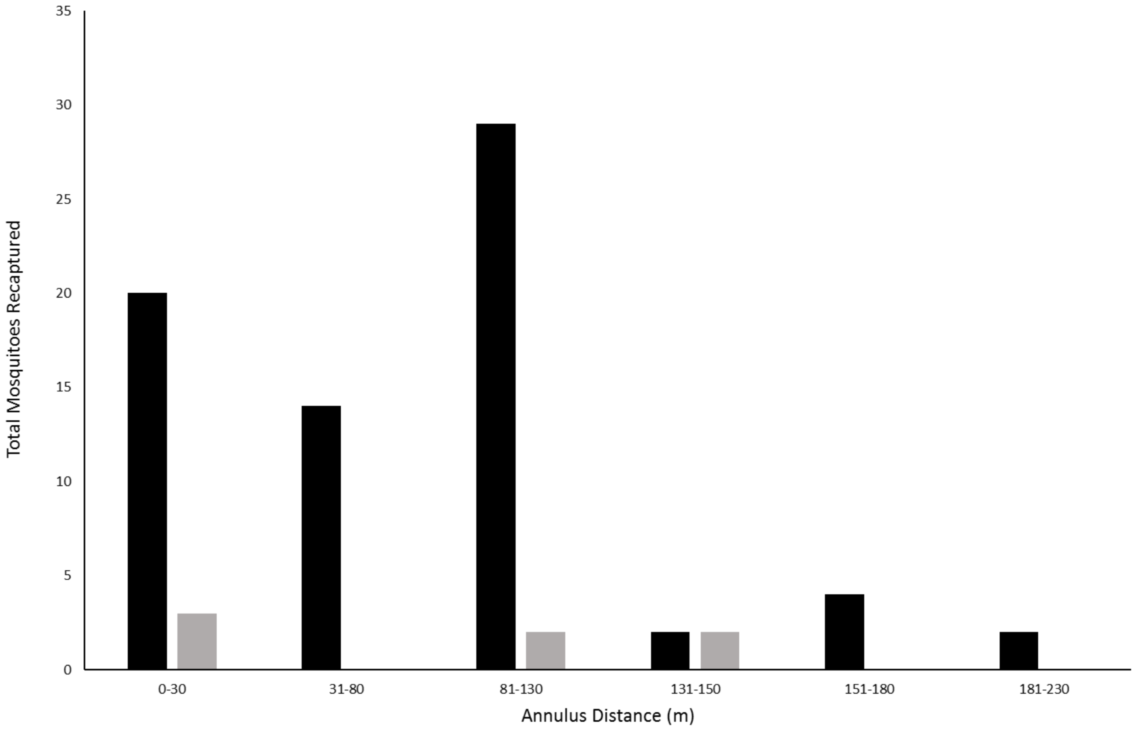


Figure 38. Frequency of marked *Aedes notoscriptus* females (black) and *Aedes aegypti* males (grey) collected in traps to distance travelled based on annuli from release site used for mean distance travelled (MDT) calculations.

Table 15. Total male and female *Aedes aegypti* and *Aedes notoscriptus* released by date and number recaptured from each site.

Release Date	Release Location	Species	Sex	# Released	# Died	# Escaped	Total Emerged	# Recaptured	% Recaptured
16/02/2016	Site 1	***Ae. notoscriptus***	F	71	1	0	72	7	9.9
			M	90	2	3	95	0	0
		Ae. aegypti	F	2	0	0	2	0	0
			M	2	0	0	2	0	0
	Site 2	***Ae. notoscriptus***	F	160	1	10	171	20	12.5
			M	246	9	14	269	0	0
		Ae. aegypti	F	1	0	0	1	0	0
			M	-	-	-	-	-	-
17/02/2016	Site 1	***Ae. notoscriptus***	F	305	7	11	323	25	8.2
			M	224	6	9	239	0	0.00
		Ae. aegypti	F	-	-	-	-	-	-
			M	1	0	0	1	1	100
20/02/2016	Site 1	***Ae. notoscriptus***	F	70	5	0	75	14	20
			M	73	9	0	82	0	0
		Ae. aegypti	F	6	0	0	6	0	0
			M	31	3	0	34	4	12.9
22/02/2016	Site 2	***Ae. notoscriptus***	F	65	0	2	67	4	6.2
			M	54	12	4	70	0	0
		Ae. aegypti	F	7	0	0	7	2	28.6
			M	14	0	0	14	2	14.3
23/02/2016	Site 2	***Ae. notoscriptus***	F	31	4	0	35	1	3.2
			M	35	6	2	43	0	0
		Ae. aegypti	F	-	-	-	-	-	-
			M	1	0	0	1	0	0
	Totals	***Ae. notoscriptus***	F	702	18	23	743	71	10.1
		Ae. notoscriptus	M	722	44	32	798	0	0
		Ae. aegypti	F	16	0	0	16	2	12.5
		Ae. aegypti	M	49	3	0	52	7	14.3
			Total	1489	65	55	1609	80	5.4

5.3.2.2 Differences in trap efficiency for target species

Over the period of the experiment, BG traps (494 trap days) collected a total of 49 marked and 196 of the background population of *Ae. notoscriptus* females, while GATs (988 trap days) collected a total of 22 marked and 185 background females, respectively. BG traps caught more female *Ae. notoscriptus* per day per trap (mean = 0.50, SE ± 0.05) than GATs (mean = 0.21, SE ± 0.02). A comparison of trap efficiency suggested BG traps were more likely to capture marked ($P < 0.001$, Odds Ratio 2.28, 95% CI 1.74-2.99) and background *Ae. notoscriptus* females ($P < 0.001$, Odds Ratio 2.83, 95% CI 1.63-5.03) than GATs.

5.3.2.3 Population size, survival, and dispersal

One-way ANOVA revealed no significant differences in survival between release sites for female *Ae. notoscriptus* (F(1,20) = 2.12, $P = 0.145$), so results were combined. Likewise, male *Ae. aegypti* counts were combined by site and dust marking. For survival analysis, total recaptured female *Ae. notoscriptus* and male *Ae. aegypti* were plotted against days since release, with numbers declining over the study period (Figure 39). Regression coefficients were -0.061 (N = 71, $r^2 = 0.62$, $P < 0.001$) and -0.060 (Figure 39; N = 7, $r^2 = 0.5$, $P = 0.181$), which correspond to a PDS of 0.87 and 0.88 for female *Ae. notoscriptus* and male *Ae. aegypti*, respectively. The ALE for each release was calculated from the slope of the PDS as 7.11 and 7.21 days for female *Ae. notoscriptus* and male *Ae. aegypti* respectively. Using the PDS and assuming constant mortality, it was estimated that 43.0% (95% CI ± 3.7) and 24.5% (95% CI ± 3.2) of the female *Ae. notoscriptus* released would survive the extrinsic incubation periods for Barmah Forest and Ross River viruses, respectively. Based on the number of *Ae. aegypti* adults emerging from the tank and recaptures, it was estimated that the total population was 160 females (95% CI ± 149) and 159 males (95% CI ± 87). Assuming two people per house in Gin Gin, this equates to between 0.2 and 5.9 adult *Ae. aegypti* females per person within the study area. Finally, the population of female *Ae. notoscriptus* was estimated as 3,776 (95% CI ± 957) adults, or between 54 and 91 *Ae. notoscriptus* females per person.

A comparison of the isotropic Gaussian model to the traditional methods of calculating MDT, FR_{50} and FR_{90} for *Ae. notoscriptus* females showed notable differences (Table 16). For the isotropic model, Sigma was estimated to be 61.90 (95% CI = 44.0 – 100.0) and 47.02 (95% CI = 32.1– 88.1) and dispersion parameter estimates 57.2 and 28.1 for site one and site two, respectively. When sites were combined over the entire study, Sigma was 57.6 (95% CI = 43.8–83.1) and the dispersion parameter 42.3. The isotropic Gaussian model centred on Site 1 indicates that over 13 days, female *Ae. notoscriptus* have the potential to disperse throughout the northern half of Gin Gin (Figure 40). The maximum distances travelled by female *Ae. notoscriptus* and male *Ae. aegypti* were 219m and 147m, respectively. Daily mean distance travelled by female *Ae. notoscriptus* tended to increase up to day ten, before decreasing when the number of females recaptured declined (Figure 41).

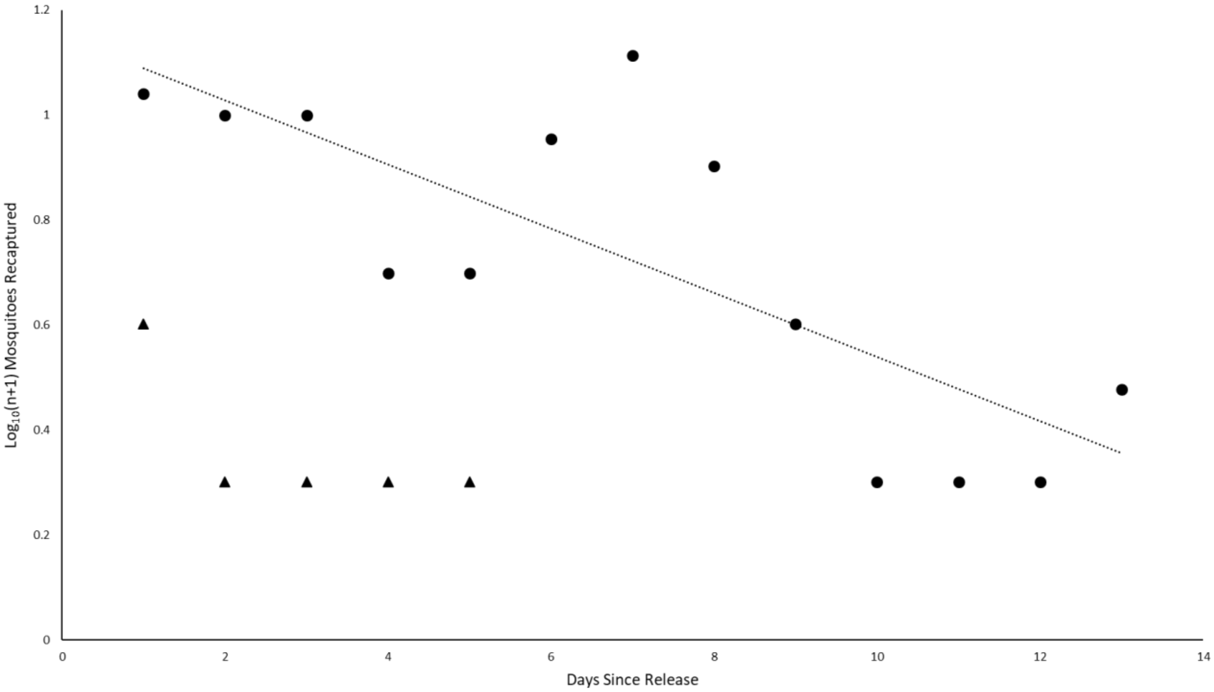


Figure 39. Log$_{10}$(n+1) transformed recapture rates for *Aedes notoscriptus* females (circles, linear equation: y = -0.0611x + 1.15, R² = 0.62) and *Aedes aegypti* males (triangles) over the study period.

Table 16. Comparison of mean distance travelled (MDT) estimates by *Aedes notoscriptus* females using the traditional Lillie et al. (1981) method and the isotropic Gaussian model over a 13 day period. FR_{50} and FR_{90} represent the flight range over which 50% and 90% of the population have travelled during the period. † There were not enough recaptures to estimate the dispersal of male *Aedes aegypti* using the isotropic Gaussian model.

Species	Method	Site	MDT (m)	FR_{50} (m)	FR_{90} (m)
Female *Aedes notoscriptus*	Traditional	1	91.2	60.3	144.7
		2	78.6	83.7	204.7
		Combined	85.0	65.0	163.1
	Isotropic	1	190.4	151.8	387.1
		2	180.3	144.9	368.2
		Combined	177.4	148.5	341.6
Male *Aedes aegypti*†	Traditional	Combined	88.7	63.2	147.8

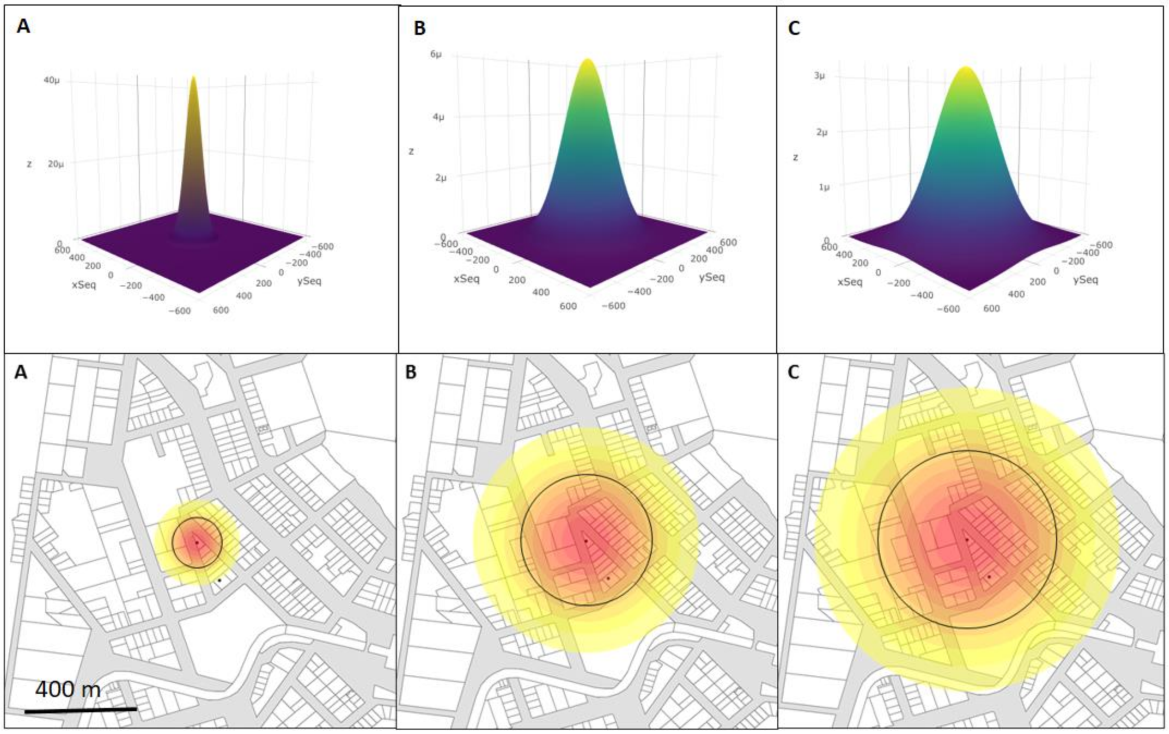


Figure 40. Isotropic Gaussian dispersal kernel of female *Aedes notoscriptus* movement from Site 1 in 3D (top) and 2D (bottom) after (A) one day, (B) seven days and (C) 13 days. Black line represents the mean distance travelled over the time period.

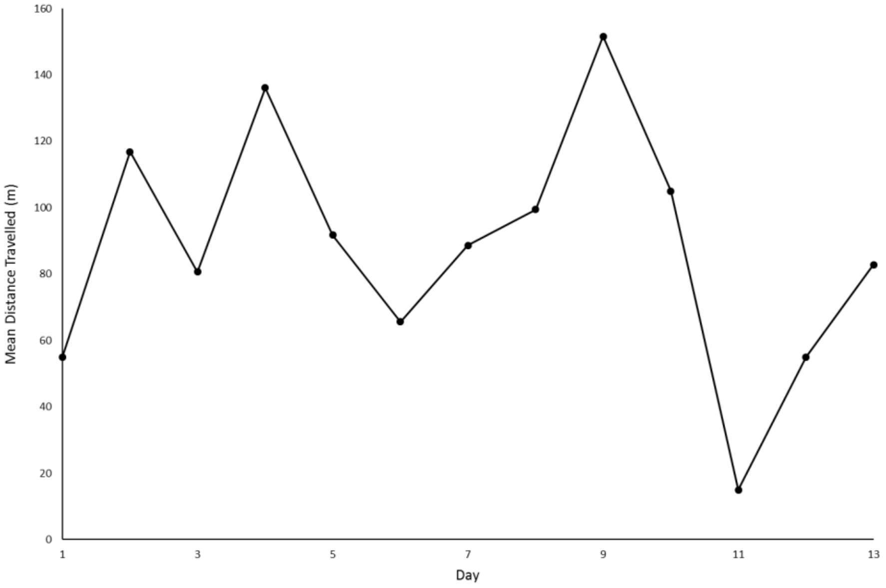


Figure 41. Daily mean distance travelled (metres) per day by female *Aedes notoscriptus* using the methods of Lillie et al. (1981). Data is combined from both release sites.

The mean angle of movement for *Ae. notoscriptus* females tended to be towards the northeast throughout the experiment (Figure 42A). The mean wind direction for the monitoring period was from 127.2 degrees (between the southeast and east-south east; Figure 42B.) and were significantly different to the daily mean angle of movement for all female *Ae. notoscriptus* recaptures (n = 71, $P < 0.001$), or those that had moved across roads (n = 29, $P < 0.001$). The Watson U^2 test for trends in dispersion showed significant differences to a uniform distribution from both release sites (Site 1, n = 46, $P < 0.001$; Site 2, n = 25, $P < 0.001$). The mean vector and scalar wind speeds were 16.3m/s and 20.5m/s^{-1}, respectively.

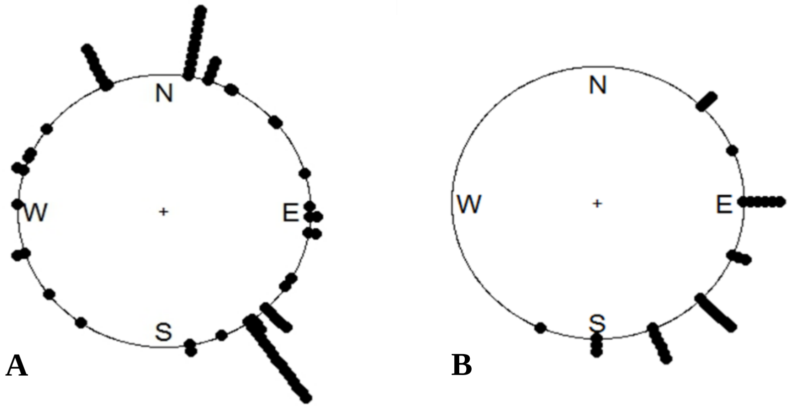


Figure 42. Circular descriptive statistics showing; A. direction of travel by recaptured female *Aedes notoscriptus* females with relation to release sites and; B. daily wind direction recorded at 9am and 3pm at Bundaberg Airport.

5.3.2.4 The role of urban landscape features

Roads did not prevent the movement of released female *Ae. notoscriptus* into surrounding blocks. However, when trapping effort was taken into consideration, significantly more females were likely to be caught within the release block than outside ($\chi^2 = 19.12$, N = 494, OR = 3.77, 95% CI = 2.01-7.065, $P < 0.001$). All recaptured male and female *Ae. aegypti* were caught within the release block. The presence of tanks within the landscape had no effect on recaptured *Ae. notoscriptus* females within the release block ($\chi^2 = 2.72$, N = 91, $P = 0.12$), outside the release block ($\chi^2 = 1.19$, N = 403, $P = 0.32$) and throughout the entire study site ($\chi^2 = 2.27$, N = 494, $P = 0.13$). Recaptures of released *Ae. aegypti* males and females were too low to perform statistical analysis, however, the background population of *Ae. aegypti* females were four times more likely to be trapped around rainwater tanks ($\chi^2 = 9.86$, N = 494, OR = 4.47, 95% CI = 1.62-12.3, $P < 0.001$) than elsewhere. The background population of *Ae. notoscriptus* females were more likely to be captured in traps next to tanks than those traps not by tanks ($\chi^2 = 5.09$, N = 494, OR = 1.53, 95% CI = 1.05-2.16, $P = 0.03$).

5.4 Discussion

5.4.1 Recapture rates

The distribution of a newly established *Ae. aegypti* population is influenced by access to larval habitat with >99% of eggs laid in dwellings with unsealed rainwater tanks. In Gin Gin, unsealed tanks may act as a source to the *Ae. aegypti* population throughout the year by permitting persistence during the colder and dryer winter months. It was hypothesised that large water storage containers, such as rainwater tanks, are important for overcoming hurdles to establishment and the ongoing persistence of new populations, especially in areas that experience sub-optimal temperatures and rainfall. Large domestic water storage containers, like rainwater tanks, are potential drivers of *Ae. aegypti* populations in Australia and elsewhere (Tun-Lin et al., 1995b, Mariappan et al., 2008). Some 70% of dwellings in Gin Gin contained rainwater tanks and almost half could potentially provide habitat for mosquitoes. In a previous suppression attempt, rainwater tanks were not targeted as a key source of recruitment to the population within the town (Queensland Health, 2012). As such, any attempt to manage the population in the future should seek approval to seal or remove all non-compliant rainwater tanks (Chapter 2).

The second experiment describes the first mark, release, recapture experiment in Australia to use a natural, emerging population of mosquitoes, and to test the effect of urban landscape features on mosquito movement. Recapture rates for *Ae. notoscriptus* (10.2% females, 0% males) were similar to previous studies of the species (Muir and Kay, 1998, Russell et al., 2005), as were recapture rates for *Ae. aegypti* (12.5% females, 14.3% males). The trap layout was designed to minimize trapping out effects by avoiding trap placement close to release sites, and traps were only placed in outdoor locations. Thus, it is likely that recapture rates were potentially lower than could have been expected if trapping had occurred inside dwellings and at release sites which was a common practice in previous Australian studies (Muir and Kay, 1998, Russell et al., 2005). Although mosquitoes did not travel with the wind, results suggest flight directions may have been appetitive, with females predominantly moving upwind, potentially sensing odour plumes (Dekker and Cardé, 2011). Interestingly, only thirteen male *Ae. notoscriptus* were captured during the entire trapping period, and none were recaptured from the 722 males released. No males were recaptured in a previous study (Watson et al., 2000b) and it is possible that mate selection in this species (Trewin unpubl. data) is different from *Ae. aegypti* (where males search for females and thus remain in close proximity to females), that BGS traps and GATs do not attract large numbers of male *Ae. notoscriptus*, or that survival of male *Ae. notoscriptus* is extremely low.

5.4.2 Trap sensitivity

BG Sentinel traps are considered one of the most sensitive trap types for collecting *Aedes* adults (Williams et al., 2006) and results of the current study concur; recapture of a naturally emerging female *Ae. notoscriptus* was at an average rate greater than that observed with CDC light traps baited with carbon dioxide in a previous study in Brisbane (Watson et al., 2000b), and at percentages similar to what has been observed in other *Aedes* MRR studies (Maciel-de-Freitas et al., 2006, Gouagna et al., 2015, Li et al., 2016). Male *Ae. aegypti* were recaptured at a higher percentages in BGS traps than females which is similar to what has been observed in other studies on this species and trap type (Maciel-de-Freitas et al., 2006, Williams et al., 2006, Ball and Ritchie, 2010). Collections of the background population of mosquitoes suggests BGS traps to be three times more likely to catch *Ae. notoscriptus* females than GATs. This result is similar to comparisons done by Ritchie et al. (2014) and Johnson et al. (2016) where BGS were more efficient at collecting adult *Aedes* mosquitoes. As is the case with female *Ae. aegypti*, it is likely the higher catch rate in BGS traps was partially due to mosquitoes exhibiting a variety of behaviours and not solely oviposition (the primary target of GAT traps). This is due to the tendency for BGS traps to capture mosquitoes exhibiting a range of physiological stages (Ball and Ritchie, 2010).

5.4.3 Population statistics

Estimations of male *Ae. aegypti* ALE and PDS were considerably higher than those estimated in previous MRR experiments. This was likely due to a combination of factors including the high rainfall and humidity during the study, and the large error value associated with the low number of recaptures. The slope of the regression curve was likely influenced by recaptures at four and five days post release. However this is not unusual, as recaptures of male *Ae. aegypti* have been observed from 6 and 12 days in previous studies (Maciel-De-Freitas et al., 2007a, Lacroix et al., 2012). Estimations of female *Ae. notoscriptus* ALE and PDS were considerably higher than those estimated by Watson et al. (2000b) who observed a daily survival of 0.77 and 0.79 for two cohorts, respectively. The number of adult females calculated that would survive the extrinsic incubation periods of Ross River and Barmah Forest viruses were also considerably larger than previous estimates. Both estimations assumed constant mortality and therefore does not take into consideration changes in this parameter over time. A more accurate assessment would include an independent study to estimate field mortality. The large abundance of *Ae. notoscriptus* within the study site (145 adult females per house) suggests this container inhabiting mosquito is highly domesticated, and would present a major biting nuisance. Moreover, the species has the ability to contribute to the urban transmission cycle of Ross River and was implicated as a vector during the 1994 epidemic in Brisbane (Ritchie, 1994). Estimation of the number of released female *Ae. notoscriptus* surviving the extrinsic incubation periods for both viruses and the large distances calculated in the isotropic model, suggest that *Ae. notoscriptus* can survive, disperse widely and potentially contribute to the spread of disease in an urban setting.

5.4.4 Isotropic Gaussian movement

The success of disease elimination strategies such IIT and SIT are reliant upon the degree of population mixing. A better understanding of the small-scale movement of mosquitoes within and between urban landscapes is therefore essential, typically at the block level. Results from the majority of previous mark release recapture studies that use MDT assume movement is a discrete, linear distance (the distance travelled to the trap). Likewise, current kernel methods attempting to describe insect movement are based on patterns of seed dispersal where distances are also discrete (Cousens et al., 2008, Clobert, 2012). Recent applications to male mosquito movement have also applied this technique (Winskill et al., 2015). These methods only measure discrete linear distances over an entire study period and fail to capture the fine scale temporal component of movement and evolution of the dispersal kernel with time. Therefore, they potentially underestimate dispersal because measurements do not consider further movement once an individual is removed from the landscape (as would be the case when a seed lands or a mosquito is caught in a trap).

The process by which insects search for hosts and oviposition sites is considered highly stochastic and random walk models are often applied (Freeman, 1977, Kareiva and Shigesada, 1983, Cain, 1985, Levin, 1986, Dufourd and Dumont, 2012, Zalucki et al., 2015). By contextualizing behaviour in this way, movement is analogous to a diffusion process where insects disperse into the surrounding landscape in continuous space and time (as seen in Figure 40). Directionless or isotropic movement has been observed in female *Ae. aegypti* dispersal. Maciel-de-Freitas and Lourenço-de-Oliveira (2009) found mosquitoes exhibited no preference for a particular direction and travelled up to 690m from the release site. The unique design of the Maciel-de-Freitas and Lourenço-de-Oliveira (2009) study was that it tested dispersal in an area where container frequencies were not manipulated and geographic barriers did not constrain movement. Likewise, the direction of female *Ae. notoscriptus* movement in the current study was random, so the method for assuming isotropic dispersal can be justified. Estimates of distance moved using a diffusion process tended to be larger than those observed by traditional methods where individuals are removed and no longer contribute to movement. Interestingly, these results may suggest that studies using marked eggs to measure adult dispersal may offer a more accurate method for measuring maximum dispersal distances and the probability of movement (Reiter et al., 1995, Liew and Curtis, 2004, Maciel-de-Freitas et al., 2004). In studies such as these, adults are not removed from the population and thus continue exhibiting normal behaviours (such as host seeking and skip oviposition) as they continue their 'random walk' through the landscape. Movement of male and female *Ae. aegypti* are likely to be very different processes. Further refining of the isotropic dispersal method will be necessary by investigating the characteristics of the landscape that would inhibit or facilitate movement in certain directions.

5.4.5 Landscape effects

There are a number of factors which predict the increased likelihood of container inhabiting mosquito presence such as; tree and building shade, oviposition sites and access to blood meals (Hemme et al., 2010, Heersink et al., 2015). There was no influence of tree or building shade on released or the background population of female *Ae. aegypti* and *Ae. notoscriptus* when measured using absolute values calculated using GIS methods. This could be due to a number of reasons including an un-stratified landscape within the study site or not considering the different 'types' of shade caused by vegetation and building structures. However, there was a predictive relationship between rainwater tanks and the presence of *Ae. aegypti*. In both experiments, a higher prevalence of *Ae. aegypti* was observed around sealed and unsealed rainwater tanks. Results from experiment two showed that *Ae. aegypti* females were four times more likely to occur around rainwater tanks than in locations without them. In experiment one, the best predictor of *Ae. notoscriptus* eggs laid in ovitraps was the total rainfall in the three weeks before traps were collected. Interestingly, the presence of rainwater tanks was not a significant predictor for *Ae. notoscriptus* oviposition, with females laying equal numbers of eggs in dwellings with no rainwater tank, a sealed rainwater tank and an unsealed rainwater tank.

In experiment two it was hypothesised that more *Ae. notoscriptus* females would be caught around rainwater tanks than away from them, but the null hypothesis was accepted. These results could be explained by a number of factors. First, the dispersal of *Ae. notoscriptus* was observed to be large for a container inhabiting species, with barriers such as roads having little influence on movement. As such, the species is capable of travelling larger distances in search of an oviposition site, than the scale of a dwelling or block over which experiments were designed (Figure 40C.) and is likely one reason why no differences between treatments were observed in the ovitrapping data. Second, it is possible that the effect of trap arrays were more sensitive to *Ae. notoscriptus* females than the effect of a rainwater tank presence. Grouping of three traps in an environment where the majority of other containers were removed is likely to be an attractive oviposition site for a species that can disperse widely. It is possible that the presence of a rainwater tank does not provide a level of attraction greater than that offered by these trap groups for female *Ae. notoscriptus*. Although rainwater tanks are still utilized as larval habitat, the ability of *Ae. notoscriptus* to oviposit over a large area suggests that this container category unlikely represents a key oviposition site for the species.

For sterile and incompatible insect technologies to be used effectively, it will be important to understand how urban landscapes affect male movement. It is likely that landscape features, such as roads and access to oviposition sites would influence movement and how mixed an *Ae. aegypti* population is. Typically, it is thought that male *Ae. aegypti* exhibit a relatively short flight range, with a MDT of between 30-80m for females and 30m for males (Muir and Kay, 1998). Maciel-de-Freitas et al. (2007b) explored distances travelled by two body size cohorts, observing a MDT of smaller males as 32m, larger males as 42m and a maximum distance of 131.7m for both. Tsuda et al. (2001) consistently observed males moving further than females from both release sites in their study, and daily MDT were consistent with those observed by the majority of studies. There have been exceptions, however, as Harrington et al. (2005) observed two individuals travelling a maximum distance of between 400 and 456m and Lacroix et al. (2012) caught over 10% of sterile males between 200 and 225m. As such, *Ae. aegypti* male movement studies show considerable variation in results and have the added limitation of reduced lifetime relative to females. The MDT for males in the current study (traditional method - 88.7m) was larger than previously recorded in Australia and is likely due to a number of factors. Firstly, conditions during the experiment were ideal and the open nature of the urban landscape in Gin Gin enhanced movement. Next, a lack of favourable habitat at release site 1 meant males may have continued to move through the landscape. Another possibility is that released males moved towards locations where the natural population of female *Ae. aegypti* were caught (around rainwater tanks). It is possible that males, fuelled by a sucrose meal, had the energy to move greater distances through the landscape in search of females, than if they had not been fed.

Here I show the effect of a moderate barrier (>30m) on male *Ae. aegypti* movement, where all recaptured males failed to cross roads around the release block. The inability to cross roads suggest that optimized sterile male release strategies will need to take into consideration the effect of barriers for population mixing and ideal coverage. For eradication campaigns to achieve suppression, sterile male releases may need to be done on every block of a city or town. However, results also suggest that once released, males are capable of moving through the landscape and seeking out females within each block. As such a single release site on each block might be all that is required. Although male *Ae. aegypti* did not move across the 30m barriers in this experiment, the roads in Gin Gin have not prevented the spread of the species throughout the town. Rainwater tanks continue to present ideal larval habitat and play a role in the population dynamics of disease vectors in urban environments. The large number of rainwater tanks reinstalled in Australian cities over the past decade represent a significant risk for the establishment of disease vectors such as *Ae. aegypti*. If communities are to remain vector-safe, attention must be given to minimising the risk of invasive mosquitoes through the appropriate surveillance and management of rainwater tanks.

Chapter 6. Network simulation of an invasion of *Aedes aegypti* through rainwater tanks in Brisbane, Australia.

6.1 Introduction

Large epidemics of dengue once plagued Brisbane, with 70-90% of the human population infected, and it was suggested rainwater tanks contributed to *Ae. aegypti* populations responsible for these epidemics (Hamlyn-Harris, 1931, Lumley and Taylor, 1943). Following the removal of the majority of rainwater tanks during the mid-1900s, *Ae. aegypti* disappeared from the city (Chapter 2). Following the re-introduction of rainwater tanks in the early 21st century due to drought, health authorities have been concerned with *Ae. aegypti* re-establishing in the city through these large permanent water storage containers. The goal of designing a spatially explicit model was to use movement data from individual mosquitos to simulate the spread of a population between rainwater tanks. In doing so, I sought to understand how far an invasion would spread and which areas of Brisbane represented the highest risk of establishment and spread. Simulations were based on different non-compliance scenarios to give an indication of what one could expect to observe in the future, based on the current and previous compliance status of rainwater tanks.

Mosquito simulation models typically focus on disease spread or population dynamics (Focks et al., 1993a, Magori et al., 2009, Reiner et al., 2013, Reiner et al., 2014), with very little attention on fine-scale population movement. Renewed interest in population elimination through new technologies such as IIT and SIT has increased focus on modelling the spread and dispersal of mosquito populations (Dufourd and Dumont, 2012, Lees et al., 2014, Winskill et al., 2015). Spatially explicit network models are a novel method by which the connectivity of mosquito populations can be explored. In these models, mosquito movement and population dynamics are simulated across representative landscapes in order to identify pathways for movement and connectivity between key resources (Ferrari et al., 2014). Little focus has been applied to modelling fine scale mosquito movement primarily due to the computational cost and paucity of data available for host seeking behaviour over smaller distances (Manore et al., 2015). With advances in computing capacity, increases in the availability of open source spatial mapping and applicable software libraries, it has become possible to simulate the movement of a large mosquito population within realistic urban landscapes.

This chapter focuses on the characteristics of a newly established mosquito population as it moves through the landscape of Brisbane, Australia, utilizing large water storage containers called rainwater tanks. When unsealed or 'non-compliant' with regulations, rainwater tanks are ideal habitat for all life-cycle stages of *Ae. aegypti* and have the potential to act as a major population source (Tun-Lin et al., 1995b). In the Brisbane City Council it is estimated that there are ~100,000 rainwater tanks which represents approximately 40% of premises (Figure 9, Chapter 1). The modelling approach used in the current study applied the Repast Simphony 2.4.0 (North et al., 2013) Java libraries to create a network based on known rainwater tank locations. I then simulate the movement of an *Ae. aegypti* population based on mark, release, recapture data published by Russell et al. (2005). A number of network metrics were measured from simulated population spread to help understand which suburbs represent the highest risk of spread with varying scenarios of rainwater tank non-compliance. This chapter is laid out as follows; a description of the network simulation and model development is found in section 6.2.1-8. This is followed by a description of model outputs and statistical analysis in section 6.2.9-10. Results are used to define risk metrics for statistical local areas (SLA: areas similar to suburbs) within the Brisbane City Council Area in section 6.3. I conclude with a discussion of results and implications for managing risk and the spread of an *Ae. aegypti* population through rainwater tanks.

6.2 Methods

6.2.1 Background

The model description follows the ODD (Overview, Design concepts, Details) protocol (Grimm et al., 2010, Grimm et al., 2006). The network model was written in Java and the Repast Simphony 2.4.0 toolkit for agent-based modelling (North et al., 2013).

6.2.2 Model purpose

The spatially explicit network model was designed to simulate an invading population of female *Ae. aegypti* in a landscape of rainwater tanks of known locations, and under differing scenarios of regulatory compliance. Based on what is known about *Ae. aegypti* movement and behaviours, the model was designed to predict which suburbs and rainwater tank configurations represent the highest risk of mosquitoes within the urban context of Brisbane. In the model, female *Ae. aegypti* move in response to the landscape configuration based on published estimates of population growth, survival and movement parameters (Table 17). The model was applied to explore the spread of mosquitoes (measuring total numbers of tanks where populations establish) how connected these populations are within the landscape, and to predict emergent risks at the suburb level.

6.2.3 Entities, state variables and scales

The model consists of rainwater tank agents within a spatially explicit landscape, consisting of various urban configurations defined primarily by roads. Datasets consist of publically available locations of rainwater tanks from rebates collected by local council (Figure 43; Brisbane City Council, 2012) and road locations from a state geospatial database (Figure 44; Queensland Government, 2017). Rainwater tanks represent ideal habitat for mosquitoes, as they provide all the requirements for development of the species; reliable larval food resources, protected and ideal temperature range and humidity requirements (Chapter 4), a surface for oviposition and resting, a permanent or semi-permanent source of water where larval density is unlikely to be restrictive and a location close to adult food resources. The spatial landscape within the model is called a 'Repast context' and the 'Repast geography' context class was used to display and interpret GIS data. The model runs on a two week time step, within which mosquito populations and movement probabilities are calculated and populations reset after every 6 months to conservatively simulate the effect of the winter season. The landscape is made up of statistical local area (SLA) boundaries, the spatial measure used by the Australian government as political and statistical boundaries when the rainwater tank data was collated in 2011. Each model replicate was run for selected SLAs within the area of Brisbane, Australia over five years (ArcGIS shapefile, Figure 43). The spatial locations of all roads were incorporated as line strings within the boundary of Brisbane (Figure 44).

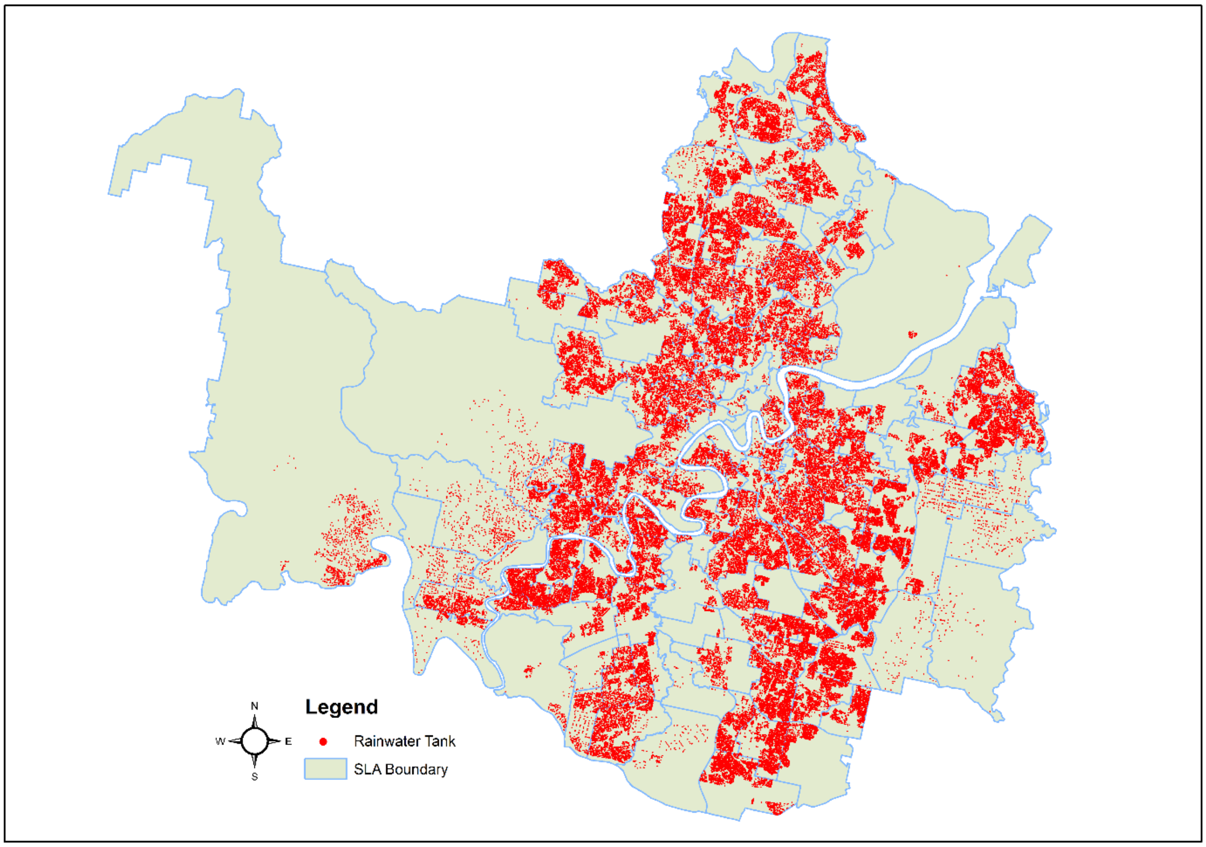


Figure 43. The location of rainwater tanks (Brisbane City Council, 2012) and statistical local areas within Brisbane, Queensland, used in modelling mosquito spread. Map base layer sourced from Australian ABS digital boundary data (Australian Bureau of Statistics, 2016) and licensed under Creative Commons 2.5 (2016).

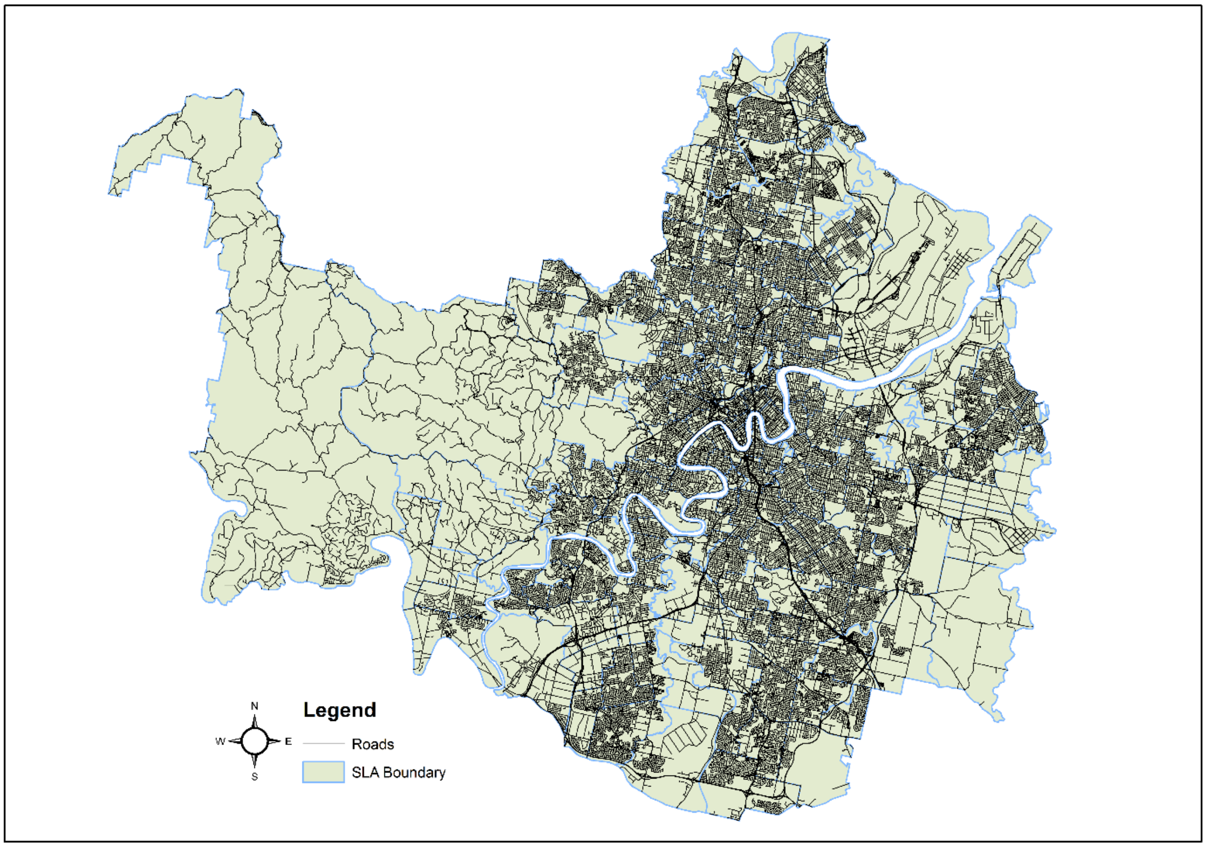


Figure 44. The location of the road network and statistical local areas within Brisbane, Queensland, used as a barrier to mosquito spread in modelling. Map base layer sourced from Australian ABS digital boundary data (Australian Bureau of Statistics, 2016), Queensland Government (2017) and licensed under Creative Commons 2.5 (2016).

I compared the effect of different levels of rainwater tank compliance on mosquito spread at the SLA level. As such, landscapes represent with four scenarios of non-compliance; high (90%) represents non-compliance rates when regulations were first introduced into Brisbane 100 years ago (Elkington, 1913); the half non-compliance scenario (50%) represents the condition of rainwater tanks when the last large epidemic of dengue occurred in Brisbane during 1925/26 (Hamlyn-Harris, 1927a), a contemporary ‘worst-case scenario’ was selected at 30% non-compliance, and a low scenario (10%) was chosen to represent the condition of tanks during the last known entomological surveys (Darbro et al., 2017 unpubl. data). A compliance state (yes/no) was randomly assigned to each tank before running the model for each iteration. The selection on the initial tank infested with mosquitoes was chosen for each SLA; the centroid was defined, and the closest tank to the centroid selected. However, if this initial point was isolated (>500m from another point) or surrounded by main roads (double roads), the next closest tank was subsequently selected. A SLA would not be chosen for simulation if: the only tanks available were close to the SLA boundary, tanks within the SLA were separated by a large geographic distance, central tanks were isolated without chance of forming a link, or the total number of tanks in the SLA was less than 100 (such as the central business district).

The model takes the isotrophic Gaussian dispersal kernel developed in Chapter 5 and derives movement probabilities from the *Ae. aegypti* movement dataset of Russell et al. (2005) from Cairns, Australia. As *Ae. aegypti* tend to avoid crossing roads, this same dataset was used to estimate the probability of a mosquito crossing a road over a two week period (Russell et al., 2005). The probability by which mosquitoes would remain at the original tank was estimated as half the length of the release site premises in Cairns (20m) from the same study (Russell et al., 2005). The probability of mosquitoes moving from one rainwater tank to another was derived from a cumulative distribution function that considers all available rainwater tanks within the maximum distance of travel (500m).

6.2.4 Processes overview and scheduling (network topology and movement)

The processes occurring within the model are split into two parts; a ‘population model’ within each node (tank) and a ‘movement behaviour’ model representing links between nodes (Figure 45). The mosquito population in each node is a simple logistic growth population model:

$$\frac{dN}{dt} = rN\left(\frac{K-N}{K}\right) \tag{13}$$

Where *N* is the number of individuals in a population, *t* is time, *r* is the maximum per capita growth rate and *K* is the carrying capacity (Table 17, Figure 46). This carrying capacity was estimated to be less than the maximum *Ae. aegypti* adults that would likely emerge from the maximum larvae (6,601 larvae) observed in a rainwater tank by Tun-Lin et al., 1995b. The rate of growth is based on a published *Ae. aegypti* population model where the simulation starts with ten egg laying females and reaches equilibrium at approximately 200 days (Dye, 1984). As this model is continuous and in reality mosquitoes are discrete individuals, tanks were only allowed to become infested once a population was greater than 1 mosquito.

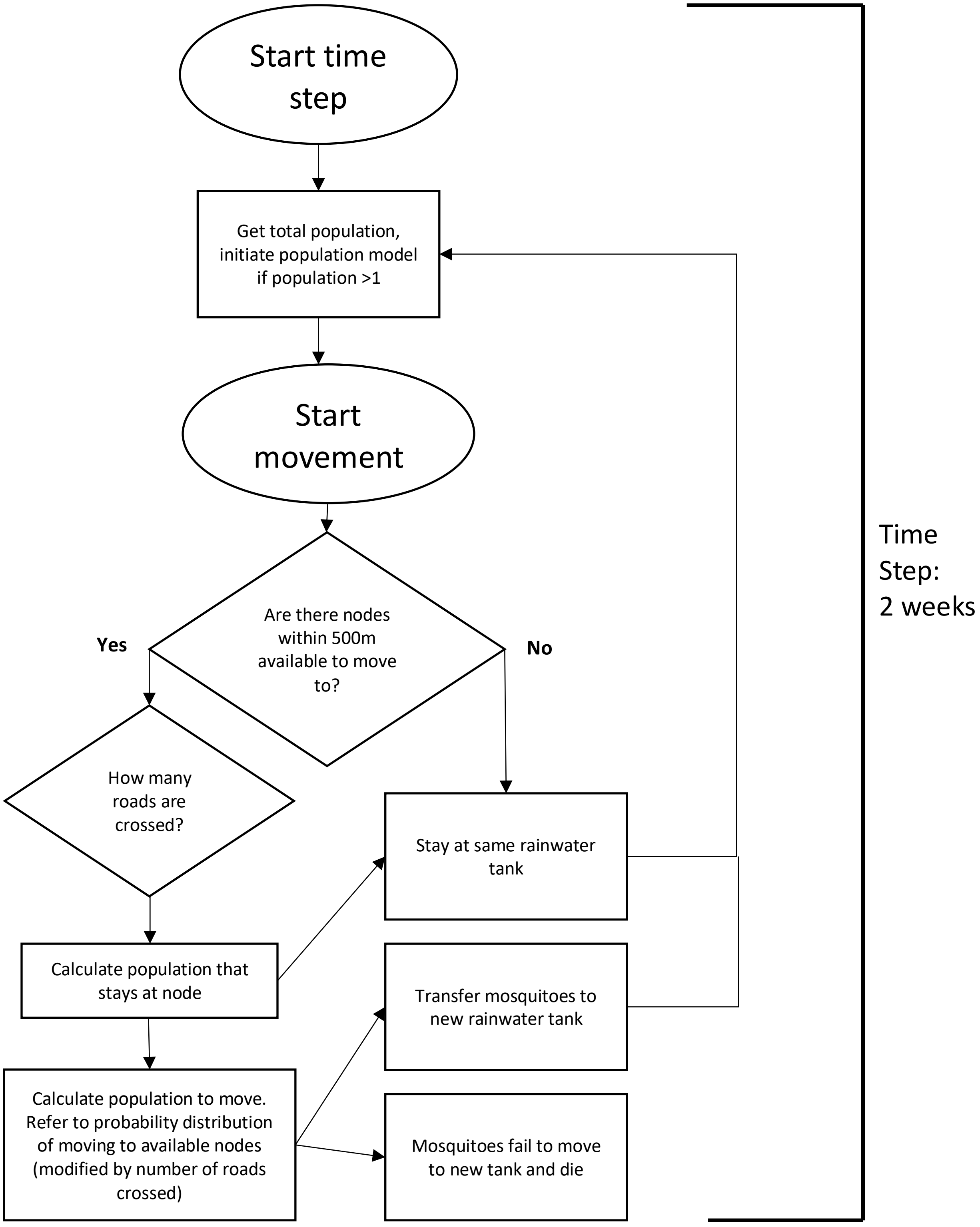


Figure 45. Model overview showing the different processes taken by the mosquito population through the network over the two week period of a time step.

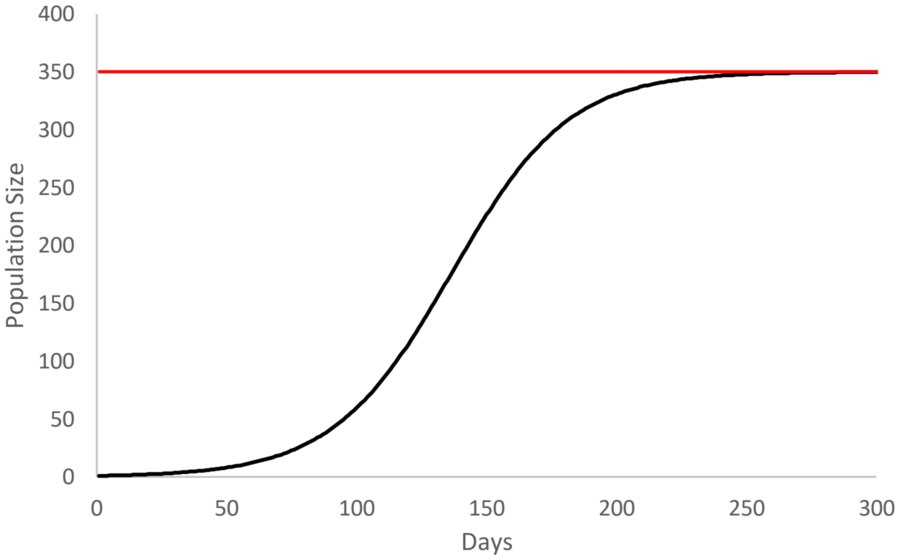


Figure 46. *Aedes aegypti* population model for each infested rainwater tank (node). Red line represents the applied carrying capacity for each node based on the maximum adults that could emerge from larvae observed in a rainwater tank by Tun Lin et al., (1995b)(6,061 larvae).

Aedes aegypti movement data, including probability of average life expectancy (λ), isotropic Gaussian kernel, and probability of crossing a road was developed from mark recapture data in Cairns, Australia (Russell et al., 2005). First, the isotropic Gaussian diffusion model was developed, where movement is based on Brownian motion or in insect terms, an infinitesimal random walk through the landscape (Chapter 4). The isotropic Gaussian probability density function (iGPDF) is implemented as:

$$f(x, y|\, t) = (2\pi\sigma^2 t)^{-1/2} e^{\frac{-(x^2+y^2)}{\sigma^2 t}}. \tag{14}$$

The diffusion model is linked to the mark-recapture data by modelling a trap count, C_i, from a trap at location (x_i, y_i) over some interval [a,b] as the outcomes of a Poisson process with intensity function proportional to $\Lambda_i = p^{r_i} \int_a^b f(x_i, y_i|\, t)\, dt$. Here, p is the thinning that is applied to the Poisson process because a road is present between the trap and the release location and r_i is the minimum number of roads that must be crossed to reach the trap, so that

$$C_i \sim \text{Poisson}(k\Lambda_i). \tag{15}$$

The parameter p_R provides a proportionate reduction in the numbers of mosquitoes that cross a road and is akin to the probability of a mosquito crossing a road which was calculated as p_R = 0.184 (95% Confidence Interval Upper 0.187, Lower 0.181). Using this model for the observed data, the scaling parameter, *k*, the standard deviation, σ, and the road thinning parameter, *p*, can be estimated through maximum likelihood with optimization carried out via a Nelder-Mead simplex method via the *optim*() routine in R (Chapter 5).

As the iGPDF model is temporally-evolving, the lifetime of a mosquito can be modelled and a random estimate for how far an individual will fly in this lifetime derived. This component of the model starts with estimating the average lifetime for *Ae. aegypti* (λ) from probability of daily survival (PDS) in the Cairns MRR data using the methods of Niebylski and Craig Jr (1994) here λ is 1/-$\log_e$PDS (Table 17). The estimated average lifetime (7.1 days) is within the range of those observed in other papers estimating *Ae. aegypti* PDS (Muir and Kay, 1998, Maciel-de-Freitas et al., 2004). As the distance travelled relies on how long an individual lives, this was randomly sampled as *m* = 1,000,000 lifetimes, $t_1, \ldots, t_\mathrm{m}$ for female *Ae. aegypti* lifetimes using an exponential lifetime PDF:

$$f(t) = \frac{1}{\lambda} e^{-t/\lambda}. \tag{16}$$

These samples were used to randomly generate a set of movement coordinates (x_1, y_1), …, $(x_\mathrm{m}, y_\mathrm{m})$ using the probability density function in equation (eq. 14), conditional on each of the sampled lifetimes. These coordinates were in turn used to generate the absolute travel distances for each sampled lifetime, so that for the *i*th coordinate, this was $d_\mathrm{i} = ({x_i}^2 + {y_i}^2)^{1/2}$.

The iGPDF was then integrated over an estimate of the maximum distance *Ae. aegypti* have been observed dispersing without barriers to movement (Maciel-de-Freitas and Lourenço-de-Oliveira, 2009). A probability mass function (PMF) was created by returning the absolute distance travelled at 5m intervals for ease of reference within the network model (Figure 47). For estimating the probability of movement an empirical cumulative distribution function (CDF) was integrated from the static PMF (Figure 48). For each link to form between nodes, the number of roads is counted along the link and the probability of moving the distance is modified by the number of roads. The proportion of the population staying and leaving the current node was based on the equation:

$$\pi\,(i,j) = \frac{(1-p_{stay})p(i,j)\times {p_R}^{R(i,j)}}{\sum_{k=1}^{n} p(i,N_k)\times {p_R}^{R(i,N_k)}}, \tag{17}$$

and

$$\pi\,(i,i) = p_{stay},$$

where i is node that is a source of infestation; $N = \{N_1, \dots, N_n\}$ is a set of neighbouring nodes connected to i that represent non-compliant tanks, of which j is a member ($j \in N$); $\pi\,(i,j)$ is the proportion of the population that move from node i to node j; p_{stay} is the proportion of the population that stays at node i; $p(i,N_k)$ is the proportion moving the distance between node i and N_k; p_R is a parameter that represents the probability that a mosquito crosses a road; and $R(i,N_k)$, the number of roads that must be crossed in order to move between node i and node N_k, becomes a function of all possible probabilities of movement along the CDF for each distance to a surrounding susceptible node. Once all potential links have formed to surrounding nodes, a proportion of the population of mosquitoes is moved along each link that has formed to each targeted node. The distance mosquitoes move before returning to the original node was defined as approximately half the length of a typical residential block in Cairns (20m) and the proportion of the population staying within this distance is defined by the CDF. A uniform random number is drawn between 0 and 1 against the CDF for the probability that some proportion of the mosquito population will move the distance along the link to the targeted node. If the random chance of movement is unsuccessful, the mosquitoes within that proportion die, but if successful they populate the targeting tank. At the end of the step in the model, the original population is updated with the mosquitoes that returned, those that died and those that moved to the targeting tank. Within the display network of the model, a link is formed between the two nodes where movement was successful. This process happens for each tank populated with mosquitoes at each time step.

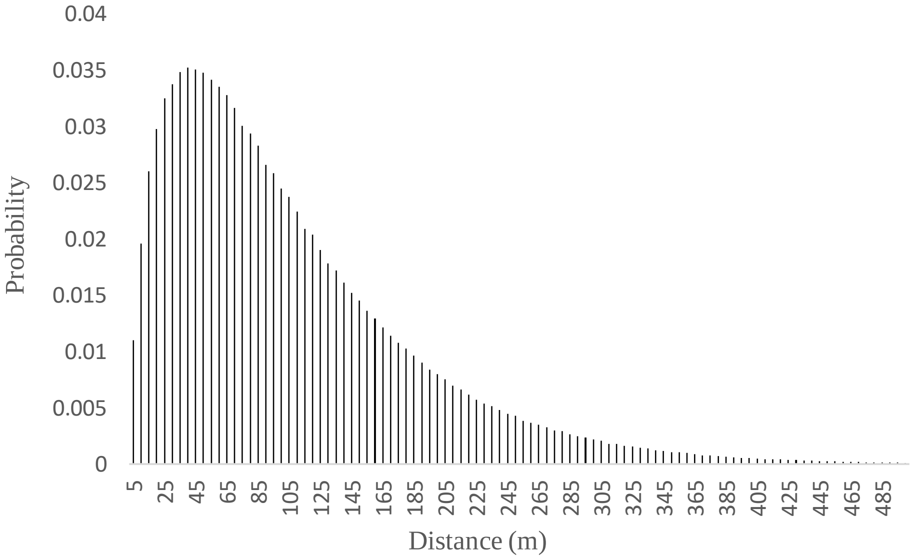


Figure 47. iGPMF: The probability mass function (PMF) used to estimate *Aedes aegypti* movement by sampling from an isotropic Gaussian dispersal model which assumes unconstrained dispersal over a two week period. Data used to estimate distance travelled and average life expectancy from Russell et al. (2005).

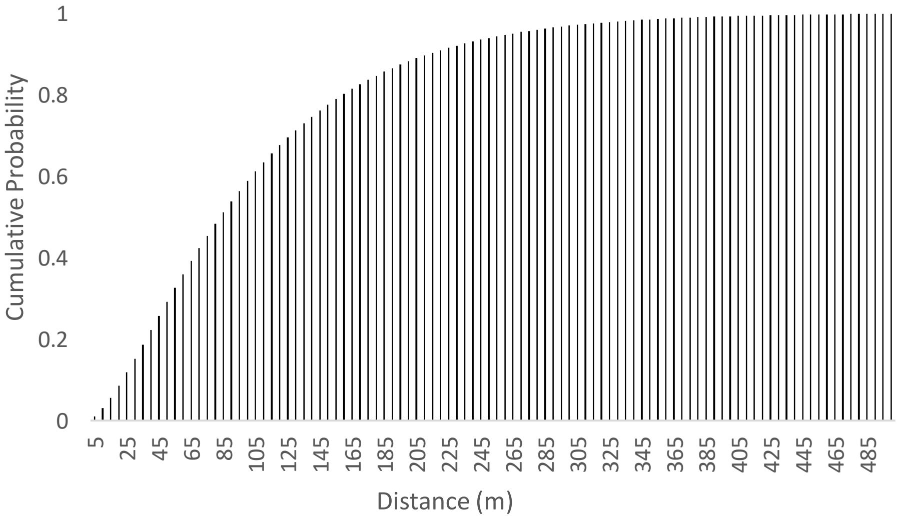


Figure 48. CDF: Cumulative density function developed from the PMF (Figure 47) and used to estimate the probability of *Aedes aegypti* population moving out of a source tank to its destination, over a 2 week period.

Table 17. Parameter attributes of agents within the network simulation (parameters, values, source).

Parameter	Values	Source
Movement Parameters		
Distance	iGPDF	Russell et al. (2005)
Average life expectancy (λ)	7.1 days	Russell et al. (2005)
Isotropic Gaussian PDF	see equation 14	Chapter 5
Field of View	360°	Maciel-de-Freitas and Lourenço-de-Oliveira (2009)
Directionality	based on random walk	
Time Step	2 weeks	
Time Step Distance Max	500 m	Maciel-de-Freitas and Lourenço-de-Oliveira (2009)
Probability of staying at original node	8.60%	
Agent Parameters (rainwater tanks)		
Tank agents	object ID	
Infected tank	True or False	
Compliance with regulations	True or False	
r (maximum per capita growth rate)	0.4	Dye (1984)
Carrying capacity (*K*)	350	Dye (1984)
Begin movement	tank population >1	
Tank spatial location	x,y coordinate in space	Brisbane City Council (2012)
Out-link	polyline	
Landscape Parameters		
Roads	polyline	
Probability of crossing a road (p_R)	0.184	Developed from Russell et al. (2005)
SLA	polygon	Australian Bureau of Statistics (2016)

6.2.5 Design concepts

The model was designed to explore the influence of urban spatial structure on the movement behaviour of an invasive *Ae. aegypti* population. In designing the model, the formulation was intentionally kept simple to balance general applicability across the landscape with the need to incorporate mechanistic realism at smaller perception ranges (Lima, 1998). The network simulated:

- Habitat selection
- Movement and population redistribution

6.2.6 Basic principles

The model is primarily concerned with population spread and distribution through urban landscapes. Here movement is modified by roads which are thought to act as a barrier to *Ae. aegypti* movement (Russell et al., 2005, Hemme et al., 2010, Chapter 4). The decisions by which mosquitoes move through the landscape or stay is based on published movement data of *Ae. aegypti* (Russell et al., 2005). The pattern that emerges is the result of the interaction among the number of rainwater tanks with mosquito populations through time, the connectivity of rainwater tanks, and the movement of mosquitoes across large urban landscapes (SLAs).

6.2.7 Stochasticity

The non-compliance state of each rainwater tank was randomly generated in each replication of the model, thus creating a stochastic landscape for each SLA. Movement pathways (out-links) formed within the model based on probabilities along a PMF of *Ae. aegypti* movement. The final stochastic feature of the model was the randomized probability that mosquito movement would occur. This entailed the random probability of moving along the CDF, and determined whether mosquitoes would move, stay in the same location, or death occurs.

6.2.8 Initialization

The starting population for each initial infested rainwater tank was set to 10 mosquitoes, while the remaining tanks were randomly assigned a compliance status (compliant or non-compliant). The model was initialized using the batch run capabilities of the Repast toolkit and simulated over the entire parameter space of rainwater tanks, with four scenarios of compliance (mentioned above) over each selected SLA. Each iteration of the model was run for 26 weeks (13 model ticks) representing the likely active season in Brisbane. To simulate winter, each infested tank was reset to 10 mosquitoes before the model was allowed to continue run up to a fixed period of 5 years.

6.2.9 Output

The three main outputs of the model at each time step were; 1) whether a node has mosquitoes, 2) the number of mosquitoes within each rainwater tank, and 3) the total number of links from a source rainwater tank to recipient tanks (out-links). For each SLA and scenario of non-compliance the model was run 30 times. Real-time movement is displayed as a network using the Repast Edge and Network classes as out-links and are recorded in the model as connections are formed. Mosquitoes infest non-compliant rainwater tanks (as nodes) as they move through the network. The complete network topology for each SLA is added to the Repast context for display at the end of each simulation step. All statistics were output to .csv files for analysis.

6.2.10 Statistical analysis

6.2.10.1 Model covariates. To mitigate the effect of the modifiable areal unit problem, results were reported at the largest areal unit available, in this case the SLA. Covariates were extracted using ArcGIS (ESRI, 2017) at the SLA level and included total rainwater tanks, tanks per square km, total area of SLA (km^2), total properties (lot and plan from cadastre), mean property size (m^2), properties per square km, total cadastral plans, mean plan size (m^2), total roads, total road length (km), roads per square km, mean road length (km^2), total population, population density (km^2) and socio-economic decile (Table 18). Multicollinearity between predictors was estimated through variance inflation factors (VIF) and any with values over 10 were removed in order to prevent correlation between predictors. Datasets were provided by the Brisbane City Council (Brisbane City Council, 2012), Australian Bureau of Statistics (Australian Bureau of Statistics, 2011) and the Queensland government (Queensland Government, 2017).

Table 18. Predictors used in negative binomial and regression statistical models to estimate the risk of mosquito spread in SLAs.

Statistical Predictors	Value	Unit	Reference
Human population	Number, density	Total individuals	Australian Bureau of Statistics (2011)
Cadastre data (lot)	Total number, area	m²	Queensland Government (2017)
Cadastre data (plan)	Total number, area	m²	Queensland Government (2017)
Cadastre data (lot/plan)	Total number, area	m²	Queensland Government (2017)
Roads	Total number, total length, mean length	km	Queensland Government (2017)
SLA Area	Area	km²/m²	Queensland Government (2017)
Rainwater tanks	Number, density	Total rainwater tanks	Brisbane City Council (2012)
Socioeconomic decile	1-10	Decile	Australian Bureau of Statistics (2011)

6.2.10.3 Out-degree distribution. The degree of a node is the number of connections that a focal node has with the remainder of the network, and the degree distribution is a measure of the frequency by which nodes connect across the entire network (Proulx et al., 2005). In a directed network, out-degrees (out-links) are the number of outgoing connections to other nodes and this measure was used to describe the connectivity within the network. One way to describe the distribution of out-links within a network is the Zeta distribution (Walck, 2007), where out-links are positive integers. The Zeta distribution has the PMF:

$$p(k) = \frac{k^{-\alpha}}{\zeta(\alpha)} \ , \tag{18}$$

where *p(k)* is the probability that a node has k out-links, $\alpha \geq 1$ is the exponent parameter of the distribution and $\zeta(\alpha)$ is the Riemann zeta function. This is the normalising constant for the PMF to ensure that all probabilities sum to one. A commonly employed approach to estimating the parameter, α, is to observe that:

$$\log\big(p(k)\big) = \log\big(\zeta(\alpha)\big) - \alpha \log(k). \tag{19}$$

This means that if the logarithm of the observed *p(k)* was plotted against the corresponding values of $\log(k)$, under this power-law model, the data should roughly follow a straight line with gradient equal to $-\alpha$. The resulting function was fit to the linear form by least-squares linear regression and the r^2 was estimated using R. Then, 10,000 random values were simulated from a power distribution with the parameters of α and $x_{min} = 1$ for each non-compliance scenario, and the CDFs of observed and expected distributions were compared (see below).

A more complex model for describing the power law function of the network out-link distribution is to use a discrete two parameter model (Clauset et al., 2009). This function is the traditional way of describing a scale-free network topology where:

$$P(k) = Ck^{-\alpha}. \tag{20}$$

Here the observed fraction of nodes in the network with exactly k out-links formed with other nodes in the network (for each non-compliance scenario) was defined as *P(k)*. As this distribution converges at zero, a lower bound $x_{min} > 0$ must be determined (Clauset et al., 2009) and the normalizing constant, *C,* is defined as:

$$C = \frac{\alpha - 1}{x_{min}}$$

To estimate parameters via maximum likelihood and model the power law distribution from the data, the R package 'poweRlaw' was used. Once model parameters were estimated, 10,000 random samples were generated from a power distribution to compare with the observed distribution.

A simpler, one parameter model to describe the out-link distribution of the entire network is the geometric distribution (Walck, 2007). This discrete version of the exponential distribution is used for demonstrating the number of trials (failures) up to and including the first "success", where the probability of success, p, is assumed to be the same for each trial. As such, p is the probability that the first success requires k independent trials, and each subsequent probability of success is modified by the kth trial. Two different discrete probability distributions were compared over the four non-compliance scenarios where the PMF:

$$P(k) = p \times (1-p)^{(k-1)}. \tag{21}$$

Here p is a parameter of the shifted geometric distribution, that must be estimated and k = 1, 2, 3, … i. In this case, the probability of a success occurs when an out-link does not form and this probability is modified by the number of out-links in the distribution. The parameter (p) for each geometric model was estimated using maximum likelihood and the mean number of trials (or out-links) was estimated by:

$$E(k) = \frac{1}{p}$$

To test the hypothesis that either of the observed distributions were the same as expected distributions (best fit to both geometric and power law distributions), a two sample Kolmogorov-Smirnov test was used. The null distribution in this case was that the observed distribution was similar to the expected distribution. To do this, distributions were converted to empirical CDFs, ‘ks.test’ was applied with a two sided hypothesis test from the base R package and statistical significance was determined as $P < 0.05$.

6.2.10.2 Total infested tanks. To determine which SLAs represented the highest risk of mosquito spread through non-compliant rainwater tanks, a number of analyses were performed on model outputs. To determine which SLAs were the highest risk of mosquito spread, model outputs were chosen that represented the 90% rainwater tank non-compliance scenario. This scenario represented the highest potential nodes for mosquitoes to spread between. As the size of a mosquito population within each SLA was highly correlated with the number of rainwater tanks (Appendix 12), infested tanks were chosen for analysis. The total and mean number of infested rainwater tanks per SLA (over 30 replicates, rounded to the nearest integer) were calculated. Due to over dispersion in rainwater tank count data where the variance was considerably larger than the mean, a generalized linear model with negative binomial errors for prediction was used. Here the PMF for the negative binomial:

$$f(y; \theta) = \binom{y+r-1}{y} \times \theta^{y}(1-\theta)^{r}, \tag{22}$$

where r is the number of failures, y the number of successes and θ the probability of success. This distribution gives the probability of $r - 1$ successes and y failures in $y + r - 1$ trials, and success on the $(y + r)^{\text{th}}$ trial. The glm.nb() with a log link function ‘MASS’ library was applied for analysis in R v3.3.2 (R Core Team, 2017). Residuals were normally distributed and a forward selection methodology was applied to choose optimal predictors, improve model fit and minimize

AIC. Model predictions were back-transformed to identify which SLAs were at highest risk for infested rainwater tanks.

To estimate rates of spread across different compliance scenarios (in this case total infected tanks over 5 years), ten high (> 90th percentile), medium (50th percentile) and low risk (< 10th percentile) SLAs were selected as predicted by the negative binomial model. Replicates were extracted from each SLA for the 10%, 30% and 90% non-compliance scenarios. The risk profiles of each SLA, as predicted by the model, were ranked into quartiles, displayed using ArcGIS and the selected ten high, medium and lowest risk suburbs were added for effect in displays/ figures.

6.2.10.4 Maximum out-links per SLA. To predict which SLAs consistently had the highest connectivity or "hubs" within the tank network, maximum out-links for each SLA per compliance scenario and replicate of the model were derived. Multiple regression was then applied to maximum out-links for the 90% rainwater tank non-compliance scenario, as this provided the greatest potential for nodes with high connectivity. No transformation of the dependant variable was necessary as residuals were normally distributed and the lm() function was applied from the base R package for analysis. The linear estimator has the form:

$$y = a + b_1 \times X_1 + \ b_2 \ \times \ X_2 \ldots + \ b_p \times X_p \tag{23}$$

Here y is the dependent variable, a is the intercept, $b_{1 \ldots p}$ are the partial coefficients of the slope and $X_{1 \ldots p}$ are the independent variables. Again, a forward selection methodology was applied to choose predictors and improve model fit using the multiple R^2 value. Independent variables were significant at the 0.05 level for all models. To visualize connectivity within selected areas from high, medium and low risk SLAs (as predicted by the infested tanks risk statistical model) heat maps were created in ArcGIS using the kernel density tool within Spatial Analyst. This tool was applied to visualize groups of nodes within the landscape with high mean out-links after five years. Output cell size was set to 1 metre, search radius 75 metres, with output values as densities and the planar method was applied.

6.2.10.5 Maximum distance of mosquito spread. To understand the area a mosquito infestation may spread through the urban landscape, suburbs from a high and moderate risk category were selected. Fifteen replicate invasions were allowed to proceed for 5 years under each scenario of non-compliance. The greatest width and height in metres, passing through the initial infestation of each network replicate(i), was recorded for each compliance scenario. As widths and heights of infestations were never constant, the mean area $(\bar{A})$ was calculated for *n* iterations of the model in km^2 as:

$$(\bar{A}) = \sum_{i=1}^{n} \left(\pi \times (Height_i) + (Width_i)\right) \div n \tag{24}$$

6.3 Results

6.3.1 Data cleaning

Modelling focused on SLAs that had a large total number and consistent distribution of rainwater tanks across urban areas. This resulted in the removal of 15 SLAs that were not suitable for modelling spread. These SLAs were primarily inner city areas where tanks were not present or larger peri-urban areas with residential developments on the periphery of the SLA boundary.

6.3.2 Out-link degree distribution

At the highest non-compliance scenario (90%), nodes formed on average 16,387 total links (SD ± 317.5) to surrounding nodes after 5 years, per model run (Figure 49). This was followed by 9,376 (SD ± 198.8), 2,434 (SD ± 53.1), and 757 (SD ± 53.7) links within the 50%, 30% and 10% non-compliance scenarios, respectively. Mean out-degrees for the 90%, 50%, 30% and 10% scenarios were 2.49 (SD ± 1.77), 2.20 (SD ± 1.44), 1.98 (SD ± 1.22) and 1.58 (SD ± 0.85), respectively. Modelling the cumulative out-degree distribution as a Riemann zeta function, the values of α were -3.91 ($R^2 = 80.9\%$), -3.86 ($R^2 = 82.9\%$), -3.88 ($R^2 = 85.76\%$) and -4.15 ($R^2 = 87.8\%$) for the 90%, 50%, 30% and 10% scenarios respectively (Table 19, Figure 50). CDFs for each scenario were not significantly different to fitted power-law distributions with $x_{min} = 1$ (Table 19, Figure 50). Estimating x_{min} via maximum likelihood indicated that out-link distributions from 10% (k.s. test, $P = 0.112$), 50% (k.s. test, $P = 0.234$) and 90% (k.s. test, $P = 0.495$) scenarios all followed a power-law distribution (Table 19, Figure 50 A/E/G). However, the Kolmogorov-Smirnov test revealed that the 30% scenario was significantly different from what was expected under a power-law distribution fitted to model output (k.s. test $P = 0.019$; Table 19, Figure 50 C/D). When assuming a geometric distribution as a description of the frequency of out-links, the 90% (k.s. test, $P = 0.056$), 50% (k.s. test, $P = 0.059$) and 10% scenarios (k.s. test, $P = 0.087$; Table 19) did not differ from the expected best-fitting distributions. The 30% scenario was significantly different from what would be expected from a geometric distribution (k.s. test, *P* = 0.031; Table 19).

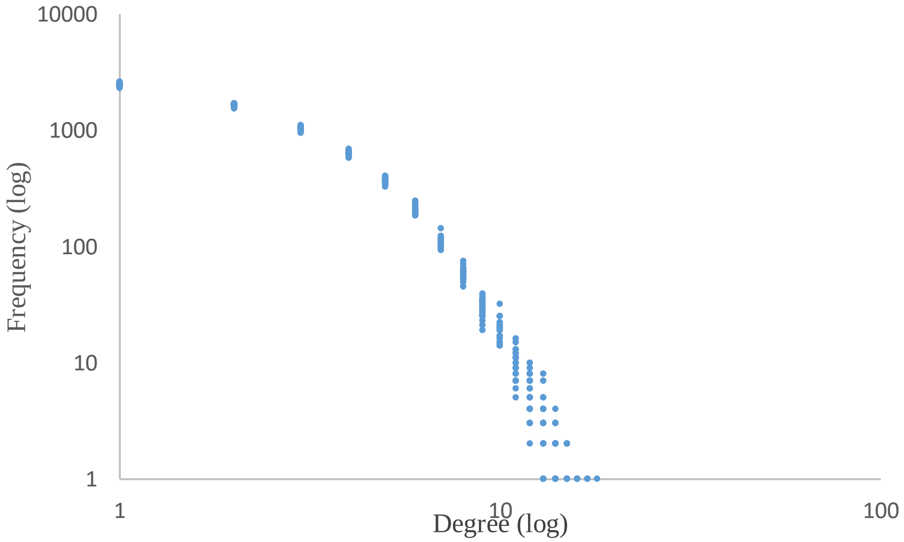


Figure 49. Log-log plot of the out-link degree distribution for the 90% rainwater tank non-compliance scenario after 30 simulations.

Table 19. Parameters estimated for geometric and power-law distributions (one and two parameter) of out-link degree distributions for each rainwater tank non-compliance scenario in the model. *P* values represent results of hypothesis tests by Kolmogorov-Smirnov hypothesis tests of observed and expected CDFs for each distribution.

Non-compliance		**Parameters estimated by MLE**			**(Obs vs Exp)**
Scenario	**Distribution**	***p***	α	x_{min}	***P* value**
10	Power law		4.15	1	0.980
30	(x_{min} = 1)		3.88	1	0.869
50			3.86	1	0.636
90			3.91	1	0.275
10	Power law		9.45	5	0.112
30	(MLE x_{min})		9.46	8	0.019 *
50			8.42	8	0.234
90			6.57	8	0.495
10	Geometric	0.39			0.087
30		0.34			0.031 *
50		0.31			0.059
90		0.29			0.056

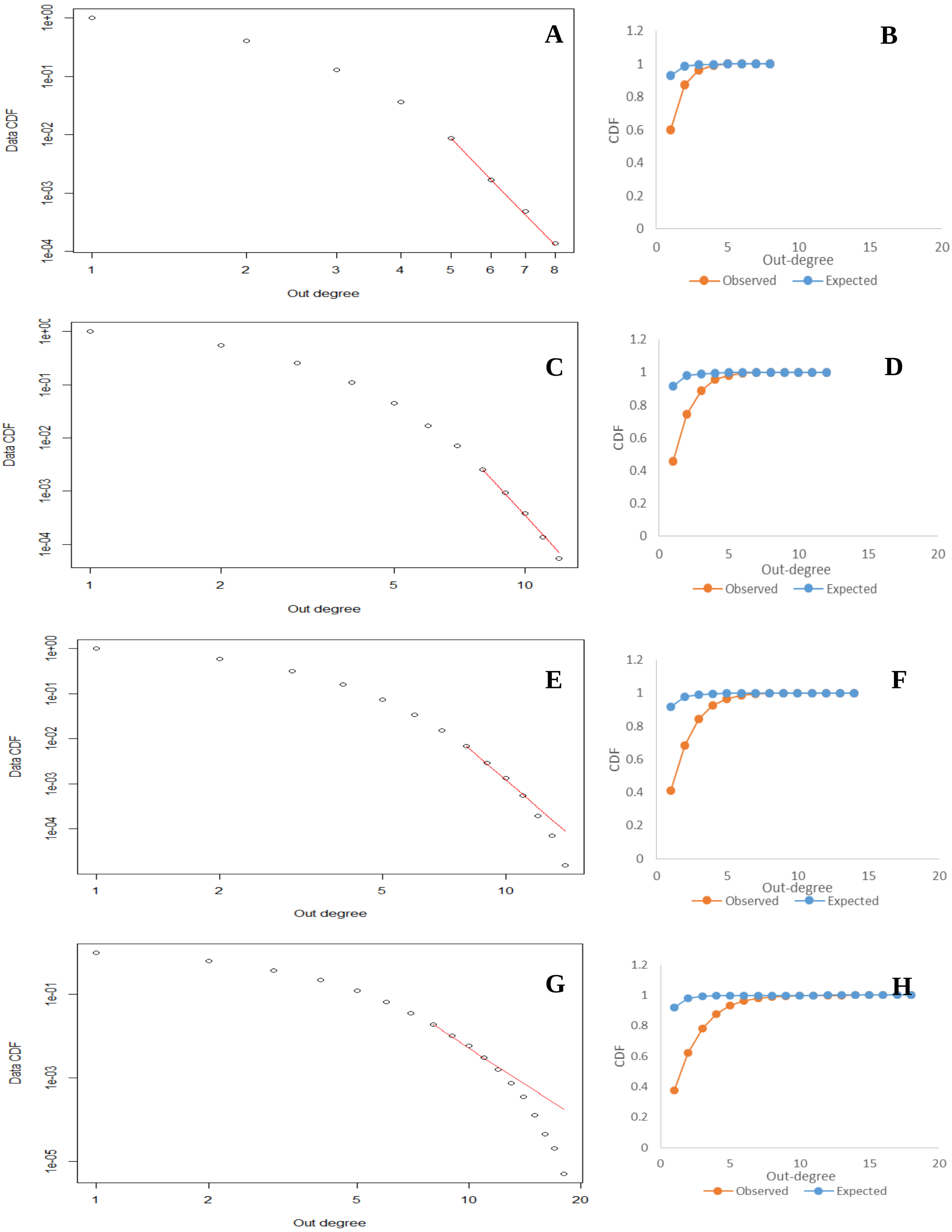


Figure 50. Observed CDF of node out-degrees (log scale) attempting to fit a power distribution from maximum likelihood estimation of x_{min} and α (red line)(A,C,E,G). Cumulative distribution functions of observed (orange) versus expected (blue) modelled under power distributions from linear regression estimation of (B,D,F,H). Different rainwater tank non-compliance scenarios are represented as; 10% (A/B), 30% (C,D), 50% (E,F) and 90% (G,H).

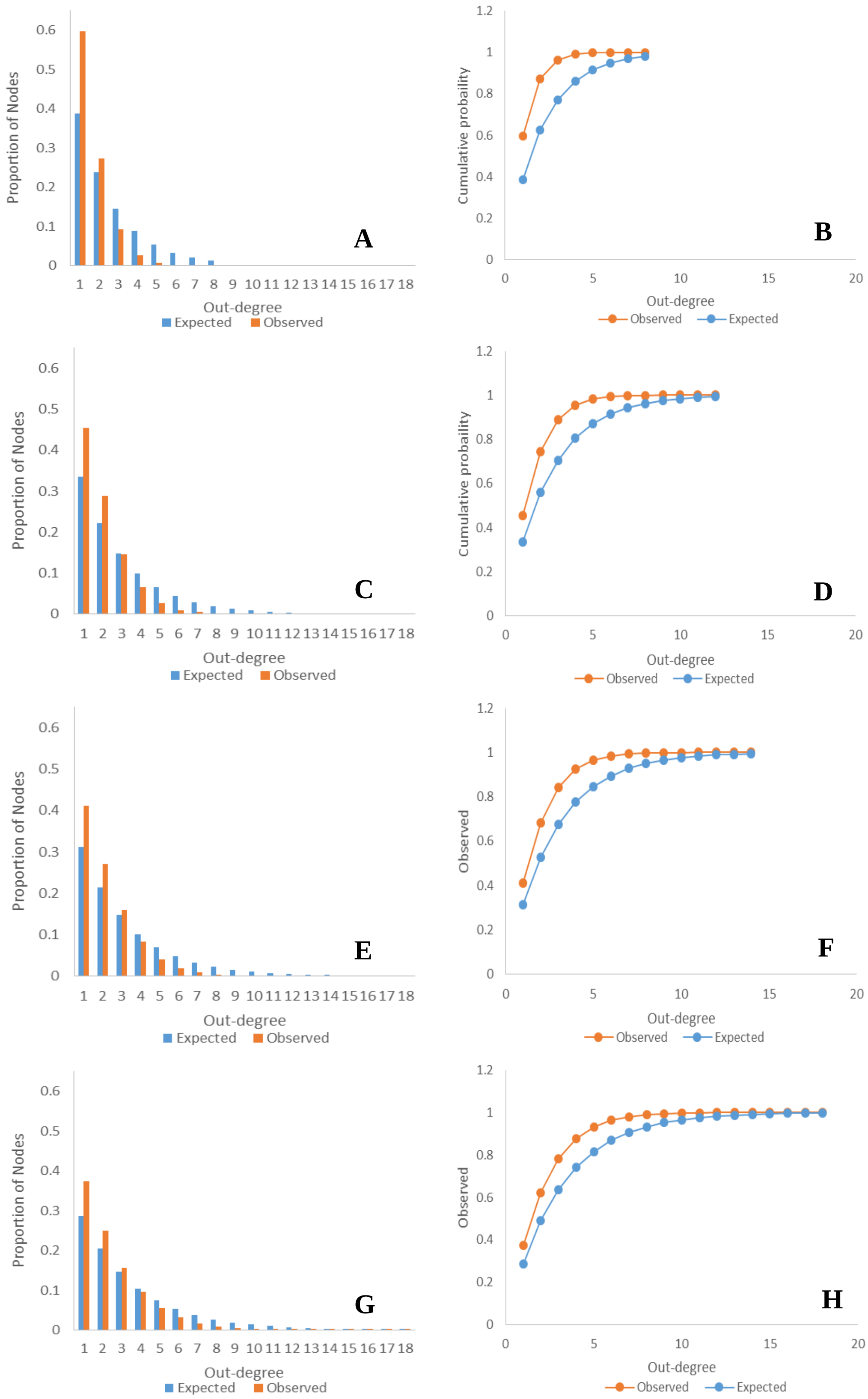


Figure 51. Observed proportion of out-degrees per node and proportion expected if observed followed a shifted geometric distribution (A,C,E,G) and cumulative distribution functions of observed (orange) versus expected (blue) distributions (B,D,F,H). Different non-compliance scenarios are represented as; 10% (A/B), 30% (C,D), 50% (E,F) and 90% (G,H).

6.3.3 Infested rainwater tanks

Descriptive statistics for the mean number of, and percentage of total infested rainwater tanks after 5 years for each non-compliance scenario were calculated (Table 20). Results show the highest mean infestation was for the 90% scenario, although this represented a smaller percentage of total non-compliant tanks than the 50% scenario and a similar percentage as the 30% scenario (Table 20). As expected, the 10% non-compliance scenario represented a lower percentage and mean number of infested tanks than all other scenarios (Table 20). Results of the negative binomial model predicting total infested rainwater tanks showed a three way interaction between the covariates; tanks per km^2, the mean size of a property and the mean road length of SLAs (Table 21).

Table 20. Descriptive data of model outputs including the mean number of infested tanks, mean number of non-compliant tanks and the maximum number of infested tanks over 5 years under different rainwater tank non-compliance scenarios.

Non-compliant rainwater tank scenarios	Mean infested tanks (% of non-compliant)	SE	Non-compliant tanks (mean)	Max # infested tanks
10%	6.1 (12.2)	0.4	63.6	22
30%	30.0 (18.9)	1.9	190.6	93
50%	54.1 (21.0)	2.9	317.9	160
90%	84.8 (19.1)	3.9	571.7	210

Table 21. Results of negative binomial analysis comparing the number of infested tanks (90% non-compliance scenario) predicted over 5 years from 140 SLAs within Brisbane.

Coefficients	**Estimate**	**Std Error**	**z value**	***P* value**
Intercept	4.191	0.344	12.179	<0.001
Tanks/km^2	-0.002	0.003	-0.863	0.388
Mean property size (km^2)	-78.198	2.830	-0.713	0.476
Mean road length (km)	-2.019	26.584	-2.942	0.003*
Tanks/km^2 : Mean road length	0.029	0.024	1.226	0.220
Mean road length : Mean property size	167.331	67.766	3.744	<0.001*
Tanks/km^2 : Mean property size	4.637	1.239	2.469	0.014*
Tanks/km^2 : Road Length : Property size	-21.841	9.664	-2.26	0.024*

Null deviance: 274.99 on 139 degrees of freedom
Residual deviance: 132.50 on 132 degrees of freedom
AIC: 681.65

Number of Fisher Scoring iterations: 1
Theta: 12.99
Std. Err. 4.85
2 x log-likelihood: -663.65

6.3.4 Maximum out-links and connectivity

To understand which landscapes promoted the highest connectivity, nodes that were most frequently represented in the top 1% of nodes with high out-links (within the 90% non-compliance scenario) were examined. Of these "hubs" (those with total out-links between 9 and 18), 15% (64/431) were primarily identified as nodes where the original infestation began. After thirty replications these nodes represented 47.8% (944/1,975) of all nodes with high out-degrees in the top 1% of hubs. Heat maps revealed groups of nodes within the landscape with high connectivity (Figure 52). Low risk SLAs (Figure 52 A/B) had considerably lower connectivity and area of spread than medium (Figure 52 C/D) and high risk SLAS (Figure 52 E/F). Multiple regression suggested SLAs with high node connectivity were predicted by an interaction between the number of tanks per km^2 (tank density)and the mean size of a property, and a model which explained 49.9% of the variation (Table 22).

Table 22. Results of regression analysis comparing the maximum number out-links in each SLA (90% non-compliance scenario) over 5 years within Brisbane.

Coefficients	Estimate	Std. Error	z value	*P* value
Intercept	2.067	5.63E-02	36.722	<0.001
Tanks/km^2	1.53E-03	3.27E-04	4.691	<0.001
Mean Property Size/km^2	-1.42E+01	2.55E+00	-5.577	<0.001
Property size : Tanks/ km^2	6.08E-01	2.34E-01	2.596	0.011

Residual standard error: 0.2553 on 136 degrees of freedom
Multiple R-squared: **0.499**, Adjusted R-squared: **0.489**
F-statistic: 45.28 on 3 and 136 DF, *P* value: < 0.001

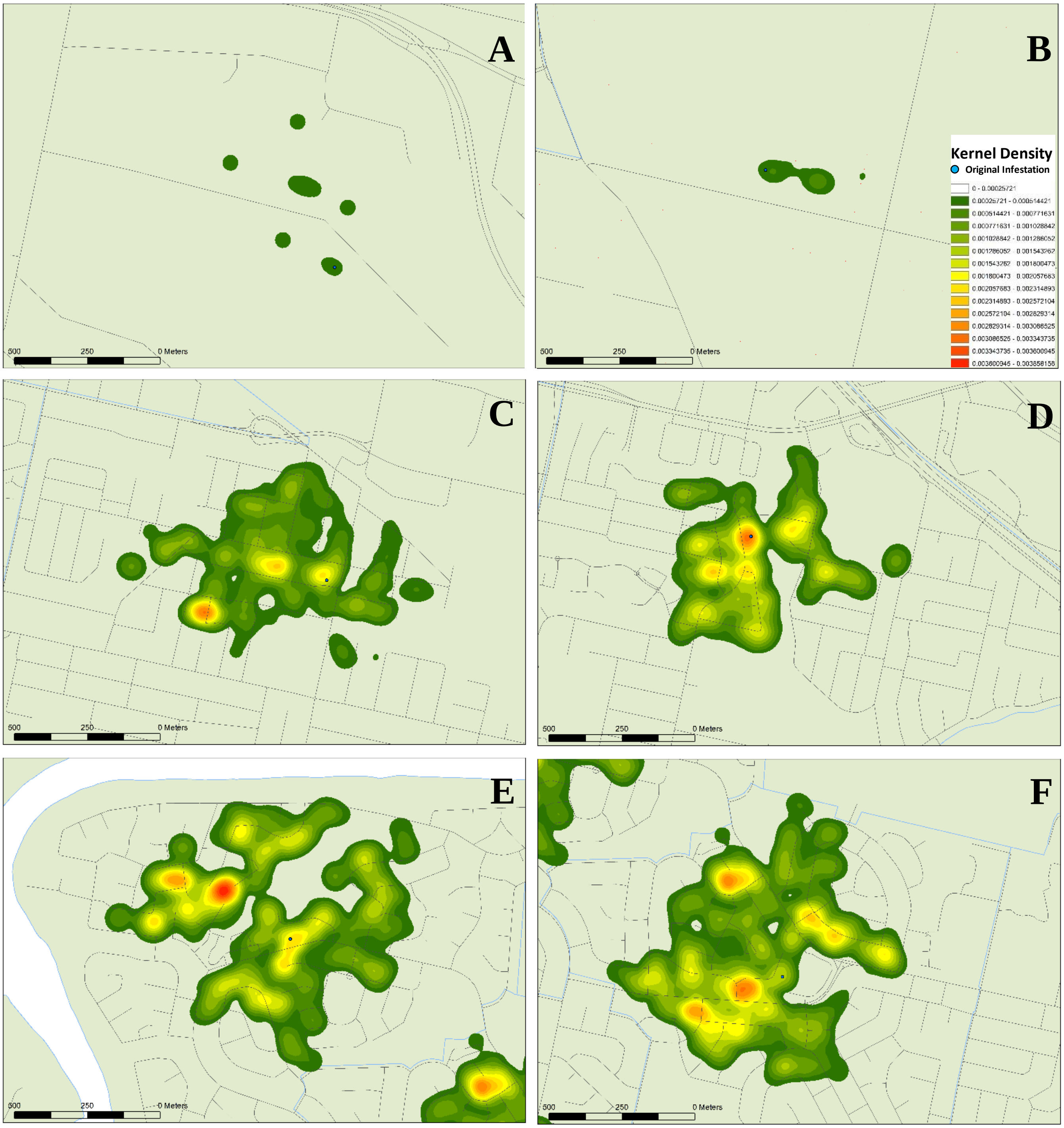


Figure 52. Heat maps representing connectivity (mean out-links) of nodes within low (A,B) medium (C,D) and high (E,F) risk SLAs under a 90% non-compliance scenario. Blue circle represents node of initial infestation.

When predicting risk across statistical models, both the regression model and the negative binomial model predicted similar SLAs as high risk. As such, outputs of the negative binomial model (total infested tanks) were used to estimate and display risk of population spread. In the 90% non-compliance scenario, the high risk SLAs tended to be predicted by a large densities of rainwater tanks, followed by the mean area of residential properties, and these SLAs tended to be aggregated spatially within Brisbane (Figure 53).

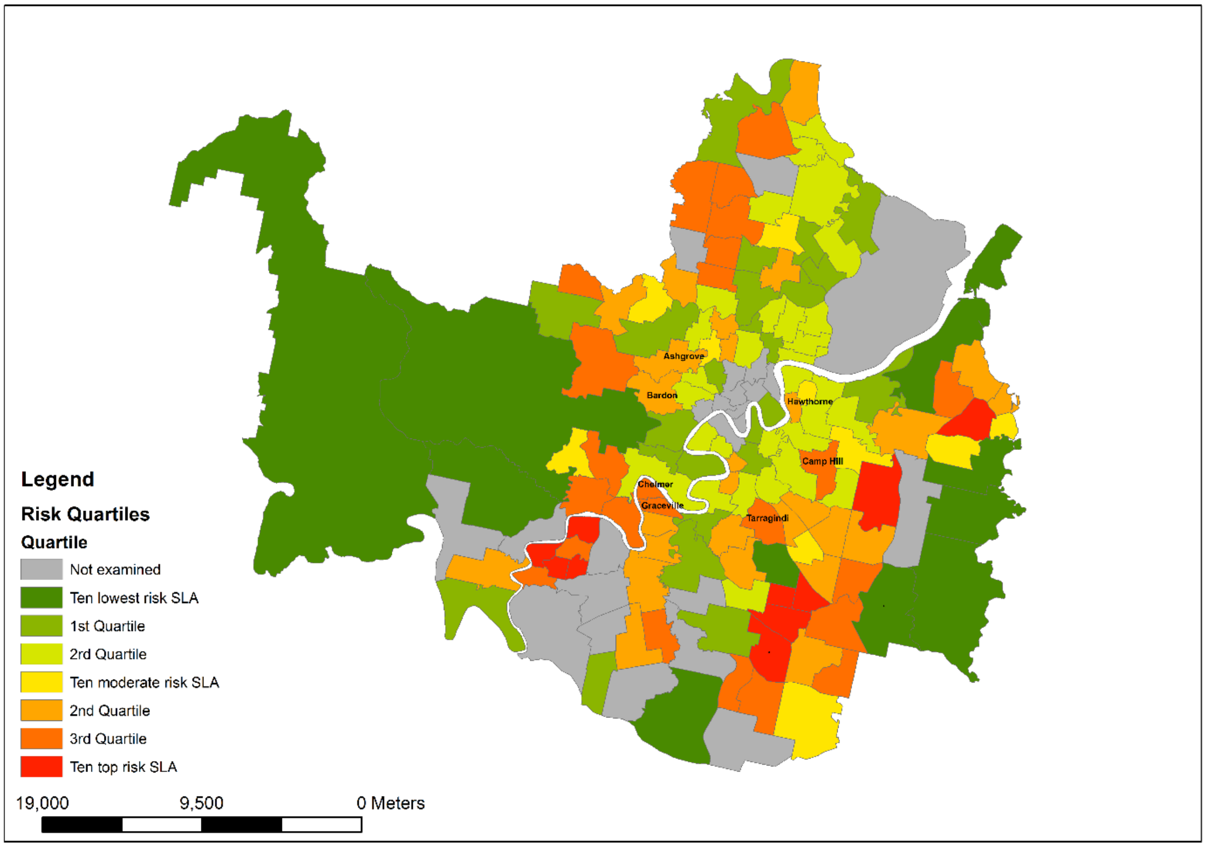


Figure 53. Risk map of Statistical Local Areas, indicating where the highest (red), moderate (yellow) and low (dark green) numbers of tanks infested with mosquitoes occur during the 90% non-compliance scenario. Suburbs with higher tank density and older house design (likely unscreened) are included for visualization.

6.3.5 Population spread

Once suburbs that represented the greatest risk of spread (total infested tanks) were selected, we ran the model to understand the area of an invasion under each non-compliance scenario after five years. The greatest spread was observed in the 50% non-compliance scenario, which had a mean area of 0.842km^2 (SD ± 0.096), followed by the 30% (mean = 0.722km^2, SD ± 0.152), 90% (0.703km^2, SD ± 0.088) and 10% (0.216km^2, SD ± 0.031) scenarios, respectively (Figure 54).

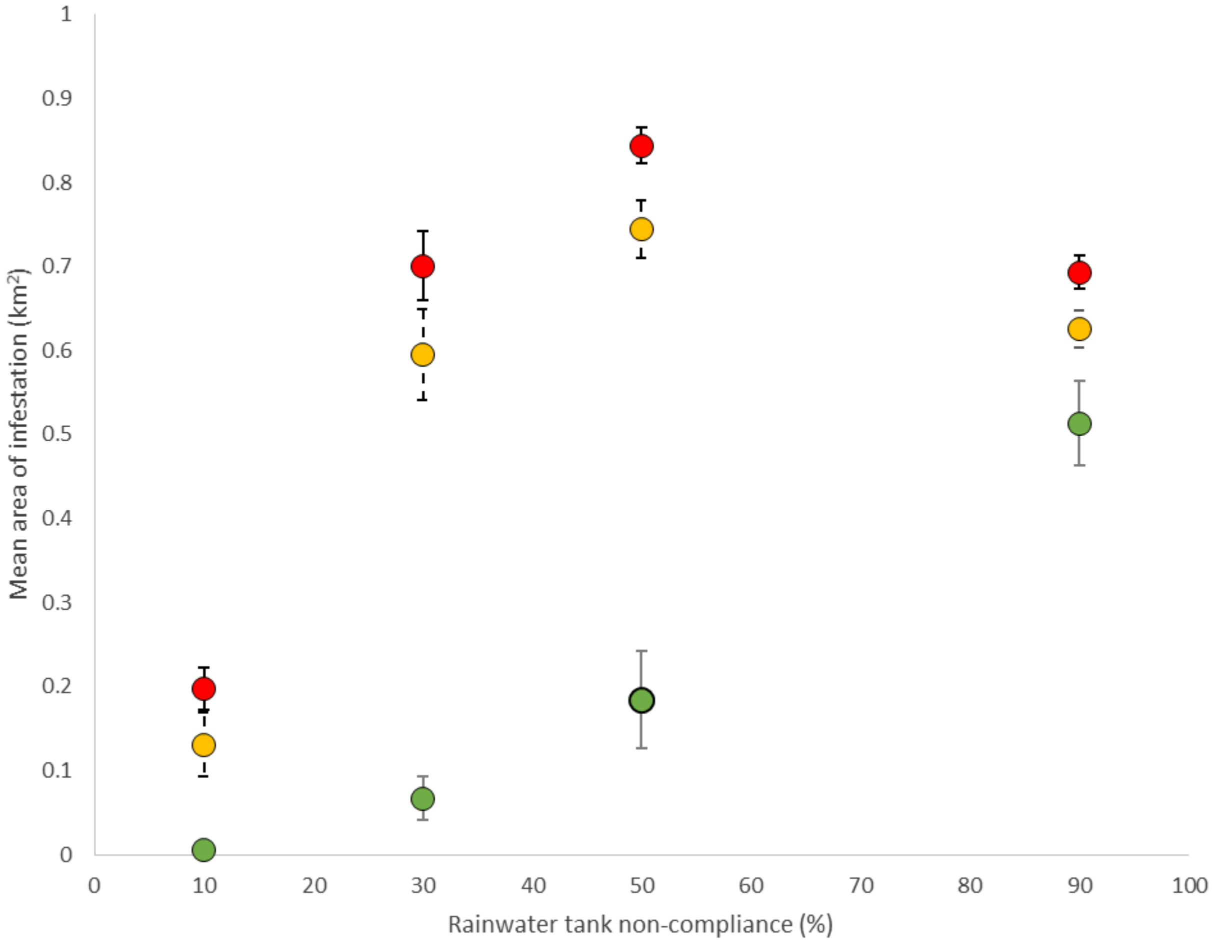


Figure 54. Plot of mean infestation area (km^2) for each scenario of non-compliance (10%, 30%, 50% and 90%) in SLAs with a high (red), medium (orange) and low (green) risk of spread (total infested tanks) over a period of 5 years. Error bars represent standard error of the mean.

6.4 Discussion

6.4.1 Model outcomes and implications for management

As climate instability continues to influence patterns of water storage, there is an increasing need to understand how mosquito vectors spread through urban landscapes, particularly those connected by key larval habitat. The primary goal of this model was to understand the implications of mosquito spread between rainwater tanks, and identify areas of risk within Brisbane. The prevalence of rainwater tanks is currently greater than 40% of households in high risk SLAs. As such, results suggest that risk is primarily driven by the number of rainwater tanks in the landscape, followed by property size and length of road. Total infested tanks (within each SLA) were used to understand how spatial covariates can predict the spread of mosquitoes between tanks, and allow for a comparison of SLAs with different risk profiles. Simulations show that for each level of non-compliance that was chosen, high tank density, moderate-to-large property size and small road lengths resulted in the largest number of infested tanks. Property sizes with a high tank density are likely to represent areas of residential housing further than 7km from the centre of the city, have the space to contain a rainwater tank and require greater amounts of water for garden use. These areas contain residential neighbourhoods with a mean property size of ~800 to 1,000m^2, were settled primarily during the 1970s and 80s and are dominated by cul-de-sac designs. Ideally, surveillance of rainwater tanks and mosquito vectors should target these high risk SLAs, and examples include Middle Park, Robertson, and Sunnybank (Figure 53). The design of these areas provides more continuous space for the spread of a population before a population needs to cross a road and establish another infestation (Figure 52). Interestingly, socio economic status did not influence spread, as SLAs with a higher decile are more likely to contain rainwater tanks than lower ranks (Darbro et al., 2017 unpubl. data). We did not include areas where historically, dengue was once a major issue (such as South Brisbane, Fortitude Valley and Spring Hill)(Aust Med Gaz, 1905) as they no longer contain high numbers of rainwater tanks. However, this does not necessarily mean they are low risk to *Ae. aegypti* establishment (there would be many potential cryptic habitats) and therefore should still be part of any surveillance activity.

Although rainwater tanks represent ideal larval habitat, non-compliant tanks are also a key driver of disease and mosquito presence (Chapter 2). Connectivity within the landscape is enhanced once tank density and non-compliance rates start to increase. With current non-compliance levels likely to be between 10 and 30% (Brian Montgomery, Queensland Health, pers. comm.), rainwater tanks in Brisbane may be approaching ideal conditions for establishment and rapid spread. Network connectivity metrics suggest there is something different about the degree distribution for a network where 30% of rainwater tanks are non-compliant, when compared to other scenarios. This finding is also supported by the increased area of spread observed in the same non-compliance scenario, which suggests there is something about the rate of spread that occurs between 10% and 30% non-compliance within the network. When non-compliance levels were set to 10%, spread was limited and did not enter the rapid phase of population growth observed in the other scenarios after 5 years. It is interesting to note that non-compliance levels of ~10% were recorded in Brisbane around the time *Ae. aegypti* was driven to extinction in the mid-1900s (Chapter 2) and non-compliance levels of <10% did not predict *Ae. notoscriptus* distribution or larval abundance (Heersink et al., 2015). It is possible that non-compliance rates under 10% have a protective effect, much like the epidemiological theory called the "mass-action principle" (Fine, 1993). This principle states that incidence (infestation frequency) is related to the product of prevalence (proportion infested) multiplied by susceptibles (non-compliant tanks) and is the theory underlying herd immunity (Paul, 1979). As such, it might be prudent for health authorities and councils to limit non-compliance levels to less than 10%, particularly in suburbs which are at high risk for establishment and spread.

When planning for an intervention involving a potential *Ae. aegypti* incursion, rainwater tanks acting as "hubs" or "key containers" are likely to be those targeted by management (Tun-Lin et al., 1995b). The multiple regression model (number of out-links) supported the findings above, and suggested that high risk SLAs were more likely to contain increased connectivity between rainwater tanks. Predictors again included high tank density and moderate-to-large property area as risk predictors. Nodes that acted as highly connected hubs within the relatively unconstrained scenario (90% non-compliance) tended to be those where the original infestation began. Half of all nodes within the top 1% of the degree distribution were from the original node. Being inherently spatially explicit meant nodes with high out-links were biased by the location of the initial infestation. However, when heat maps of mean out-links were displayed across the landscape it was clear that the original site of infestation, although connected with a great number of other nodes, was not necessarily the site where most connectivity occurred. Most highly connected areas tended to be observed at the corners of blocks or at the end of a cul-de-sacs (Figure 52). Interestingly, results from Chapter 5 and other work being done on *Ae. aegypti* currently (Beebe pers. comm.) show aggregations of re-captured males at the end of residential blocks. If a key container such as a rainwater tank were to be found in these corner properties, it may enhance spread between multiple blocks, due to the smaller distance mosquitoes would need to move between each of the corner properties on each block.

Currently little is known about the movement and spread of disease vectors through permanent water storage, however, modelling approaches are beginning to explore the role that urban landscapes play in population movements (Otero et al., 2008, Almeida et al., 2010). Comparatively, both the 30% and 50% non-compliance scenarios contained larger areas of spread. There are likely two reasons for why this may have occurred. First, distances between non-compliant tanks would have been greater in the 30% and the 50% scenarios, but not so far that mosquitoes consistently die when attempting movement (such as the 10% scenario). Second, it is likely the 30% and 50% scenarios had a greater number of mosquitoes moving between a smaller number of potential nodes. Thus, initial populations in destination rainwater tanks would have been larger after mosquitoes had successfully ‘jumped’ between nodes. In this way, spread would have progressed more rapidly as initial population sizes would have been initiated with greater individuals. When conditions are not ideal, mosquitoes may need to move further, to seek out favourable habitat and this has been supported by previous modelling by Otero et al. (2008) and observed in previous field experiments (Wolfinsohn and Galun, 1953, Bugher and Taylor, 1949, Honório et al., 2009). Otero et al. (2008) suggest that in areas of sub-optimal habitat (such as temperate areas), populations of *Ae. aegypti* may prevent extinction by dispersing over larger distances to new habitat. Thus, when population sources are sparsely distributed through a landscape, it may act to increase the distance of mosquito dispersal and movement.

Low dispersal is a tenet of *Ae. aegypti* movement and some authors suggest the species does not move large distances if resources are optimal where adults emerge (Edman et al., 1998, Harrington et al., 2005). It was expected that spread would occur most rapidly in high risk SLAs at the 90% non-compliance level. Although spread was relatively high, this scenario did not result in the largest spread. Instead, area of spread occurred at a slower rate than other scenarios because conditions were highly suitable at smaller distances. As such, it may be useful to consider *Ae.*

aegypti movement based on the suitability of the habitat; movement may be reduced in geographic distributions where climate and resources are optimal, while the opposite may occur where climate is sub-optimal and potential habitat is sparsely distributed.

6.4.2 Future work

Simulating the spread of a mosquito population using a network model framework in Repast and Java provided a novel foundation on which to explore the dynamics of movement through key larval habitat. The model could be improved in a number of ways; firstly, the current iteration of the model assumes temperature to be constant, and population growth similar throughout 6 months of the year. Future models could simulate time of year, taking into consideration temperature and water effects like evaporation, homeowner water use and rainfall on population growth rates in each individual tank. Next, the movement of mosquitoes with individual lifetimes and movement probabilities would likely increase stochasticity in population size, rate of spread, and the Repast environment is ideally suited to an agent based system like this (Almeida et al., 2010). Finally, the network model developed was semi-dynamic, where nodes within each scenario and iteration were randomly assigned a non-compliance status, however, the site of original infestation was always static. This minimised the number of landscape parameters and allowed for a simple interpretation of model outputs, but also ensured the emergent network was still random. Ferrari et al. (2014) found that the site of initial establishment can significantly affect the temporal dynamics of an invasion. Future work with the current network model should enhance dynamic processes by randomly selecting the initial infestation site, and running the model for longer periods to remove the bias associated with landscape configuration at the initial infestation point.

Another form of dynamic process that could be applied to the model would involve simulating mosquito control within the network to understand how manipulating node status (susceptible/infested/eliminated) may alter mosquito spread. The literature behind optimizing management processes across a network suggests multiple ways in which the spread of an organism can be controlled (Chades et al., 2011). For instance, hypotheses could be developed around whether efficient management actions should focus on highly connected nodes, surround the infestation, or move in a linear fashion through the landscape (Chades et al., 2011). Currently little is known about the connectivity of populations at the city scale and understanding the relative connectedness between blocks will be essential in the successful implementation of disease and vector suppression tools such as population replacement technologies, SIT and IIT.

Chapter 7. Discussion

Throughout Australian history, rainwater tanks have played an important role in providing reliable potable and non-potable water to households in both urban and rural regions. The switch to large-scale reticulated water across much of the country in the 1950s led to the removal of rainwater tanks, particularly in major urban centres. However, the human response to climate instability has led to the further mass installation of rainwater tanks throughout Australia during the early 2000s. A culture of water hording was encouraged and promoted, largely through government-run rebate schemes, regulated water restrictions and mandated installations in new houses. The rapid re-expansion in the number of these large water storage containers throughout major urban centres, has resulted in an estimated 300,000 rainwater tanks in the south east Queensland region. This has increased the risk of re-establishment by *Ae. aegypti* into areas south of its current distribution.

This thesis evaluated the potential for *Ae. aegypti* to re-colonize Brisbane through rainwater tanks, and assessed the potential consequences for those managing mosquito-borne disease risks in the region. This thesis explores how the removal of rainwater tanks contributed to the elimination of both *Ae. aegypti* and dengue transmission in Brisbane, and the invasion and disease risks that their re-establishment poses (Chapter 2). Applying predictive CLIMEX models to potential *Ae. aegypti* distributions in Australia enabled us to explore the role that water storage has played in the historical and current distribution of the species at range margins. Models suggested that although Brisbane provides marginal habitat, the region remains suitable for the establishment of the species (Chapter 3). These findings were supported by Chapter 4, where I assessed the ability of two populations of *Ae. aegypti* (and a population of *Ae. notoscriptus*) to develop and survive in rainwater tanks and buckets under winter temperatures in Brisbane. Chapter 5 assessed the distribution and movement of a newly established *Ae. aegypti* population in an urban environment saturated with rainwater tanks, and those results were used to develop a dispersal kernel based on a diffusion process that can estimate distance travelled over time. The final chapter (Chapter 6) modelled mosquito spread through Brisbane in a spatially explicit network approach that simulated *Ae. aegypti* population movement between rainwater tanks under different scenarios of compliance to legislative standards. The final sections of this thesis address the significance of the findings and place the work in a broader context of future risk of re-establishment. It will explore the consequences and implications of the risks posed by the mass-installation of rainwater tanks and offer mitigation strategies for implementation by those authorities responsible for the management of water resources, urban design and mosquito-borne disease.

7.1 The elimination of *Aedes aegypti* from Brisbane: lessons from history

It has been suggested that predictions of future risk in Australia should incorporate knowledge from the past (Russell et al., 2009). The absence of locally-acquired dengue cases in Brisbane since 1948, or collections of *Ae. aegypti* since 1957, suggests the species has been eliminated or is no longer in sufficient abundance or distribution for detection of the species or for it to vector arboviruses. However, the species was once highly abundant in Brisbane and large epidemics of dengue fever have been recorded. A high prevalence of unsealed rainwater tanks throughout the city may have been crucial in sustaining *Ae. aegypti* populations and, therefore, facilitating disease outbreaks. Anti-mosquito regulations were created to ensure that these permanent water sources could not harbour *Ae. aegypti*. The combination of increased surveillance by health officials targeting larval habitat (including unsealed rainwater tanks), the introduction of wide-spread reticulated water within the city, community support and regulatory enforcement all played a major role in the elimination of *Ae. aegypti* from Brisbane.

The elimination strategy incorporated elements of a top-down approach based on the success of the Gorgas (1915) work during the construction of the Panama Canal. As such, the approach in Brisbane primarily targeted the larval habitat of mosquitoes and health officials intensively surveyed residential areas and enforced regulations. Although it is likely that other factors, such as the introduction of anti-mosquito regulations, improvements to living standards and use of residual chemicals contributed to the elimination process, it was the removal of key larval habitat (such as unscreened rainwater tanks and water-holding rubbish) that acted as the primary driver of elimination. From historical records, it is clear that an educated, cooperative and sympathetic public was essential to the elimination campaign and strategy employed in Brisbane. Regulations were imposed gently, and residents were given every opportunity to resolve issues before an official process was forced upon them. The intensive campaign orchestrated by scientists, health officials and local council workers, was demonstrably successful at removing non-compliant rainwater tanks from the landscape, effectively eliminating a productive, permanent habitat from the urban landscape. The elimination of these water sources from the landscape ensured that the remaining larval habitat was too transient or rare to maintain a mosquito population throughout the year. This strategy was aided by the fact that the climate of Brisbane is sub-optimal for *Ae. aegypti* survival.

Applying this top-down strategy to Brisbane today would be expensive, time consuming and unlikely to be successful. For instance, to survey all of the ~93,000 tanks within the BCC in one year, it would thus take six teams of two people surveying a total of 360 tanks a day. If 20% of properties needed to be re-visited to enforce regulations, it would add another ~19,000 surveys, and a team of two people roughly a year to complete. With an estimated salary of $70,000AU per field officer (Darbro et al., 2017) that would cost almost $1Million AUD for the inspection team. If a wide-spread *Ae. aegypti* incursion were to be discovered in the BCC, then some of the remaining ~330,000 private dwellings without rainwater tanks would need to be included in surveillance (Australian Bureau of Statistics, 2011).

A contemporary application of the historical strategy used in Brisbane would be in the targeting *Ae. aegypti* populations in small, regional towns like Gin Gin or Charleville. There, *Ae. aegypti* populations are small, climate is suboptimal and the number of private dwellings and rainwater tanks could be easily managed. Community engagement and subsequent motivation to support collective effort to eliminate larval habitats may also be more achievable. Targeting larval habitat for elimination has been successfully applied in the Northern Territory during recent times (Whelan et al., 2005, Whelan et al., 2012) and it is likely to have a similar effect in semi-arid areas of Queensland. From my work in Gin Gin, it is clear that *Ae. aegypti* has partly established through the large number of non-compliant and unsealed rainwater tanks found in the town. Gin Gin has recently upgraded the quality of the reticulated water system but residents value the quality and taste of rainwater, so tanks are likely to remain. My surveys in the town indicated that almost 50% of those tanks are non-compliant. The Gin Gin climate is not optimal for *Ae. aegypti* and the town is small enough (473 premises with 347 rainwater tanks) for a management approach that targets larval habitat and sealed tanks. These factors increase the probability of an elimination strategy focused on large permanent habitats. Moreover, as part of the community engagement process, I ensured that ~20% of rainwater tanks in the town were no longer contributing to mosquito populations. The community in Gin Gin was supportive of those actions and it is therefore unlikely that anti-mosquito regulations would need to be implemented harshly. Although there is still the risk of large distance jumps in distribution or incursions of invasive mosquitoes through ports of entry, there is still considerable advantage in eliminating *Ae. aegypti* from small towns like Gin Gin. Currently, these towns and the major traffic corridors that link them to the rest of the country, form part of an uncontrolled pathway that threatens to bring *Ae. aegypti* to Brisbane.

7.2 Climate suitability within the Brisbane region

It is clear from the historical literature that *Ae. aegypti* was once established in Brisbane and that it caused large epidemics of dengue fever (Lumley and Taylor, 1943). Recent publications, however, have suggested that the climate may no longer be suitable for the species in the absence of rainwater tanks (Kearney et al., 2009, Williams et al., 2010, Richardson et al., 2011).. Desiccation tolerance and a capacity to exploit urban aquatic habitats that are independent of rainfall means that *Ae. aegypti* is able to exploit a range of sub-optimal environments (Christophers, 1960, Juliano et al., 2002, Faull and Williams, 2015). This is evident by current distribution maps that show the species in arid and semi-arid areas of Queensland, California and India, where cryptic sites and other artificial containers play a role in establishment and persistence (Figure 17, 19). The climate of Brisbane, however, is humid-subtropical by the Koppen scale (Köppen and Geiger, 1930), and has annual rainfall and temperatures similar to Jacksonville, Florida and Buenos Aires, Argentina where *Ae. aegypti* is currently established (Eisen et al., 2014). Thus, it is likely that climate in Brisbane would support *Ae. aegypti* independent of rainwater tanks if other containers are present.

While Brisbane represents sub-optimal habitat when compared to the rainforest and monsoonal climate zones in northern Queensland, CLIMEX modelling suggests the region sits well within the margins of what might be considered suitable habitat for persistence. Both the Native Distribution Model (NDM) and Permanent Water Model (PWM) show climate suitability along the east coast of Australia, including Brisbane. CLIMEX determines habitat suitability for organisms whose distribution is dictated by the environment. Given the restrictions of the CLIMEX model in terms of its ability to simulate water-filled containers, I used irrigation as a proxy. Adding irrigation in CLIMEX clearly increases water availability and humidity in the modelled environment, and this feature has been used to predict the suitability of habitat for fruit and other products (Sutherst and Yonow, 1998). Rainwater tanks provide a favourable micro-climate for adult *Ae. aegypti*, one that has higher humidity and lower fluctuations in temperature (Chapter 3). Although the species is capable of surviving in semi-arid and arid locations, removing desiccation stress by incorporating irrigation allowed for a greater understanding of how temperature affects the distribution. Adult reproduction and movement generally occur in temperatures above 15°C (Eisen et al., 2014). However, the larvae and eggs of *Ae. aegypti* are capable of surviving far lower temperatures (Eisen et al., 2014, Chapter 3) and ameliorate the impacts of low seasonal temperatures on mosquito establishment, dispersal and disease transmission. By adding irrigation and creating a less stressful environment in the PWM, it was possible to focus on the effect that temperature has on the potential distribution of *Ae. aegypti* in Australia.

The PWM analysis shows an increasing trend in suitability over the past 126 years, resulting from the gradual temperature increases observed globally. Even during the drought period of the early 2000s, the milder temperatures observed in Brisbane (Bureau of Meteorology, 2015) decreased fluctuations in the Ecoclimatic Index (EI). This reduced variation is unlike earlier in the past century, when greater fluctuations in EI values were observed and *Ae. aegypti* was present in Brisbane. I conclude that Brisbane climate has remained suitable for the establishment of the species throughout the past 100 years. As such, climate has not played a major role in the disappearance of the species from the city. It is the presence and frequency of containers in urban areas that strongly correlate with *Ae. aegypti* persistence and abundance (WHO, 2003). The findings of this chapter support the assertion made in Chapter 2 that the removal of specific larval habitat from urban environments that was a primary driver of the elimination process in Brisbane.

7.3 Suitability of rainwater tanks and other containers for persistence in Brisbane

Results from this thesis suggest *Ae. aegypti* is capable of developing and surviving throughout the year in Brisbane in both rainwater tanks and buckets. Hourly summer temperatures in Brisbane for tanks and buckets (under all shade regimes tested herein) were consistently within the ideal temperature range for *Ae. aegypti* as defined by Tun-Lin et al. (2000). Previous modelling has suggested that *Ae. aegypti* overwinters in Brisbane as eggs (Williams et al., 2010) and that climate is unsuitable for persistence in containers other than rainwater tanks (Kearney et al., 2009). When *Ae. aegypti* larvae were reared under fluctuating temperature regimes derived from direct observations during the coldest winter month, approximately 50% and 70% survived until adults in buckets and rainwater tanks, respectively. Temperature fluctuations in rainwater tanks rarely dropped below what has traditionally been considered the lower critical threshold of *Ae. aegypti* development. The volume of water in rainwater tanks provide a buffered environment with less thermal stress than other smaller larval habitats like buckets. Although buckets had a lower thermal stability than rainwater tanks, larvae were capable of surviving temperatures down to 4.5°C for short periods of time during this study. Hot and cold temperatures have physiological impacts on insects, would likely have influenced the reproductive ability of surviving adults in experiments (Rinehart et al., 2000), although this is something that was not tested.

The original hypothesis that southern populations of *Ae. aegypti* would be adapted to colder temperatures was not supported in the temperature tolerance study. However, results from this study and field work in Gin Gin suggested that this population may be newly established and represents an isolated population at the edge of its geographic distribution. The population may be a small remnant population or have recently established around 2011 and has undergone further selection and suppression with insecticides (Queensland Health, 2012, Chapter 4). A report to the Bundaberg council by Endersby-Harshman (2014) examined microsatellite markers from the *Ae. aegypti* population in Gin Gin and compared them to samples from northern and central Queensland (Rasic et al., 2014). They concluded that the Gin Gin population was small, had a low number of microsatellite alleles (adjusted for sample size), a moderate amount of inbreeding, and the lowest gene diversity of the samples tested (Endersby-Harshman, 2014). The central-marginal hypothesis predicts that population size, genetic diversity and genetic connectivity are highest in the core of an organism's geographic distribution, and decrease near the margins (Carson, 1959, Brown, 1984, Eckert et al., 2008). Therefore, isolated populations established by limited founding events, such as the *Ae. aegypti* found in Gin Gin, are likely to exhibit reduced genetic variability that might affect their ability to deal with thermal stress at critical temperature thresholds. It is possible that alleles related to heat/cold shock proteins (Feder and Hofmann, 1999, Colinet et al., 2010) might be identified that correlate with cold tolerance in *Ae. aegypti*.

The findings of Chapters 3 and 4 have important implications for the potential distribution of *Ae. aegypti* and the risk of establishment in southern Queensland. It is clear that larval habitat is available if *Ae. aegypti* were to return and rainwater tanks are ideal (Trewin et al., 2013). Considering the presence of historical populations of *Ae. aegypti* as far south as the Victorian boarder, it is no surprise that larvae were capable of surviving Brisbane winter temperatures in rainwater tanks. Kearney et al. (2009) and Richardson et al. (2011) postulated that rainfall was not high enough in Brisbane for buckets to represent suitable larval habitat for *Ae. aegypti* but evaporation estimates showed that any container that could hold > 3L or which retain water for greater than 32 days during the winter could be considered potentially productive. Field observations indicated that, in winter, evaporation rates in buckets are not high enough to prevent *Ae. aegypti* from completing development. Like rainwater tanks, buckets used to store water are common water storage containers in Brisbane backyards (Trewin et al., 2013). This has been observed in studies where buckets represented one of the most common and productive containers for urban mosquitoes in Brisbane (Trewin et al., 2013, Darbro et al. 2017 unpubl. data). It is clear that daily winter temperatures in the south east Queensland region are above the lower critical temperature for *Ae. aegypti* development and that larger containers like rainwater tanks provide a permanent source of thermally buffered habitat for all life cycle stages.

We used Adelaide as an example of a colder, more southerly city. Although summer temperatures were not tested, most container types here in this city are unlikely to support *Ae. aegypti* during the winter, as they fall below the lower critical temperature throughout most the day. Richardson et al. (2013) examined a range of container types in a Melbourne winter and found similar results. However, the permanent water found in rainwater tanks may still present a risk during winter in southern states. In Adelaide, rainwater tank temperatures in sunlight never dropped below the lower critical threshold. We conclude that *Ae. aegypti* may be able to establish in areas of coastal Victoria and South Australia if permanent water sources are available (Chapter 3). Indeed, populations of *Ae. aegypti* established in the San Francisco Bay Area (California Department of Public Health, 2016) and the eastern Black Sea Region (Georgia, Russia, Turkey; ECDC, 2017) demonstrates the capacity for the species to establish seasonally cold areas.

7.4 Movement and distribution of disease vectors in urban landscapes

Understanding the mechanisms that enable invasive and resident species to partition resources in a common niche is important in predicting and managing the ecological, economic, or health impacts of disease vectors (Leisnham, 2009). Competitive interactions between species at the niche determine the distribution and abundance of an invading species and eventually result in coexistence, segregation or local extinction (Schoener, 1974). There is evidence to show that temperature affects the relative competitiveness of *Ae. aegypti* and *Ae. notoscriptus* larvae (Russell, 1986), while spatial aggregations of larval habitat have been shown to occur in tropical areas (Tun-Lin et al., 1999). *Aedes aegypti* is a specialist that prefers larval habitat in artificial containers, close to domestic dwellings (Hamlyn-Harris, 1927b, Christophers, 1960) while *Ae. notoscriptus* is a generalist and shows little preference for the type of container in which it oviposits (Darbro et al., unpubl. data). Experiments in this thesis support these assertions, showing *Ae. aegypti* adults exhibit higher spatial aggregation within 25m of rainwater tanks, while *Ae. notoscriptus* adults showed no relationship to the presence of a rainwater tank (Chapter 5). Likewise, oviposition of *Ae. aegypti* was only recorded in dwellings with a non-compliant rainwater tank, while *Ae. notoscriptus* was observed ovipositing in large numbers of eggs around all dwellings whether or not they contained a rainwater tank. It seems that habitat segregation occurs between the two species and this is supported by the historical literature (Hamlyn-Harris, 1927b). Results suggest that the higher dispersal capacity of *Ae. notoscriptus* permits a broader distribution and potential for locating oviposition sites other than those around domestic dwellings. Although *Ae. aegypti* seem to prefer dwellings with rainwater tanks, it is unlikely that these large volume containers would present a niche that both species would compete for. Although nutrition levels were not tested directly, 20+ year old tanks, like those in Gin Gin, are capable of supporting a large abundance of immature mosquitoes because they build-up of organic matter over time. This was evidenced by the >1,500 adults of both *Ae. aegypti* and *Ae. notoscriptus* emerging from a single tank during the current research. *Aedes notoscriptus* is unlikely to exclude *Ae. albopictus* through larval competition alone (Nicholson et al., 2015), and neither does it outcompete *Ae. aegypti* in North Queensland (Montgomery and Ritchie, 2002), nor has it prevented *Ae. aegypti* from establishing in Gin Gin (Chapter 5). Although in high abundance throughout Brisbane, *Ae. notoscriptus* is unlikely to prevent *Ae. aegypti* establishing, particularly if rainwater tanks are present.

For sterile (SIT) and incompatible insect technology (IIT) to be effective, it will be important to understand how urban landscapes affect male movement. It is likely that landscape features, such as roads and access to oviposition sites will influence movement and the spatial heterogeneity of an *Ae. aegypti* population. This chapter showed that *Ae. aegypti* males tended to move towards areas where females were present (such as areas with rainwater tanks) and that barriers to movement (such as the >30m wide roads in Gin Gin) affect dispersal. The failure of male *Ae. aegypti* to cross roads in our experiment suggests that IIT and SIT strategies will need to consider the effect of urban barriers for maximizing population mixing and permeation of males within a landscape. For eradication campaigns to have an impact, sterile male releases may be needed on every block of a city or town. Alternatively, results suggest that released males are capable of moving through the landscape and seeking out females. A single release site on each block might be sufficient for complete coverage.

7.5 Modelling an invasion of an *Ae. aegypti* population through rainwater tanks in Brisbane

Numerous studies have simulated the population dynamics and disease transmission of *Ae. aegypti* within theoretical landscapes (Reiner et al., 2013). However, little work has modelled movement of *Ae. aegypti* within spatially explicit landscapes, likely due to difficulties with computational capacity and complexity (Almeida et al., 2010). Researchers have incorporated mosquitos as individuals or agents, but movement is often considered random and not related to published dispersal patterns (Almeida et al., 2010, Chao et al., 2013, Reiner et al., 2014). Otero et al. (2008) provides a useful example of a spatial model that simulates the movement of an *Ae. aegypti* population through a landscape. The authors use a random walk process similar to the one developed in the isotropic Gaussian dispersal kernel (Chapter 5) and could alter the diffusion coefficients to simulate different rates of dispersal (Otero et al., 2008). By parameterizing movement on a diffusion-like process, the underlying assumption of the current model was that dispersal through the landscape occurred as a random walk process with specific distance parameters. Alternatively, a Cauchy or *t* distribution could have been applied to simulate spread. Movement using these distributions would see mosquito populations diffuse and “jump” through the landscape between source nodes and a destination. Although these types of distribution were not used in the density function, we did create a “pseudo-jump” based on the probability that mosquitoes would travel the distance to the next node at each time step.

In future models, stochasticity of movement and spread could be increased by simulating individual mosquitoes as agents, instead of a proportion of the population. In this way, each individual within the population would undergo two probabilities of movement; one of the distance travelled (the PMF) and then the direction of movement (the CDF). Currently my model outputs show a high correlation between the number of infested tanks and total population size. By incorporating increased stochasticity in movement one would start to increase the variation within the landscape, start to more accurately simulate an accurate population size and further emphasise differences between SLAs. While this would be computationally more intensive, the result would be a more realistic simulation of population movement through the landscape and improve predictive ability for management decision making.

7.6 The implications for rainwater tank compliance on mosquito spread

It is widely accepted that *Ae. aegypti* movement tends to be localized and confined to relatively small distances, however some evidence suggests that dispersal can occur over greater areas if there are no barriers to movement (Maciel-de-Freitas and Lourenço-de-Oliveira, 2009). As such, it is likely that population dispersal occurs at different rates, depending on the spatial structure of the landscape in which mosquitoes are travelling. Just because *Ae. aegypti* has the potential to travel long distances, doesn't mean it will necessarily 'choose' to move, and instead may be more likely to remain within favourable habitat. The network model suggests that there was a point between 10% and 30% rainwater tank non-compliance where spread rapidly increased and then plateaued between 30-90%. There was a protective effect from rapid spread in the 10% non-compliance scenario, and this may be related to the "mass-action principle" (Fine, 1993) where incidence is related to prevalence and the "susceptibility" of the rainwater tank stock. Finally, it seems that mosquitoes in the 90% non-compliance scenario are more likely to move within a group of high density tanks than making large jumps, thus lowering the speed of spread across the landscape. This may be a limitation to our model in its current form, as it is possible that when high densities of mosquitoes are present, individuals may cross unfavourable areas and colonize areas more quickly through mechanisms such as human mediated transport (Benedict et al., 2007). Finally, results support the assertion that dispersal is driven by the availability (or density) of oviposition sites (Wolfinsohn and Galun, 1953, Reiter et al., 1995).

7.5 The effect of road length on landscape connectivity and population spread

Barriers are another landscape feature that influence the movement and connectivity of *Ae. aegypti* populations. Roads are a barrier to *Ae. aegypti* movement in urban environments, with smaller roads acting as less of an obstacle when compared to major roads (Russell et al., 2005, Hemme et al., 2010). In the model, the probability of crossing a road was set at 18% over a two week period based on observational data from mark release recapture experiments (Russell et al., 2005). As such, connectivity was higher within blocks and movement between blocks restricted, reflecting what one would expect to see in urban environments. Landscapes with high tank densities, small property sizes and small road lengths are more likely experience higher numbers of infestations if a population of mosquitoes was introduced. These landscapes tend to be represented by modern neighbourhood designs that incorporate large numbers of cul-de-sacs. In these suburbs, blocks tend to be shaped with the landscape, thus increasing the number of houses that can fit into oddly shaped tracts (eg. to the edge of rivers) such as the suburb of Westlake in western Brisbane. A non-uniform block shape enabled higher connectivity within the landscape and increased spread without forcing populations to cross roads to invade new habitat. Although these landscapes represent the highest rates of spread, they may not increase risk of establishment as modern house designs in these SLAs incorporate window screening and ducted air-conditioning which have been shown to influence disease transmission and vector abundance (Reiter et al., 2003, Ramos et al., 2008). It has been suggested that housing characteristics influence the ability of mosquitoes to find a blood meals and harbourage sites (Ritchie, 2014). The network model assumed equal probability of finding a blood meal when determining establishment. Thus, a full risk assessment of likelihood of spread through an urban landscape would need to take housing characteristics into consideration.

7.7 Surveillance for *Aedes aegypti*

Modelling suggested that all scenarios with a non-compliance rate over 30% had a similar influence on the rate of mosquito spread in SLAs. Over a 5 year period, infestations extended to a radius of approximately 500m and area of 0.7-0.9km^2. These findings may have implications for the ability to detect an incursion of *Ae. aegypti* within Brisbane and may require surveillance with a fine resolution if authorities are to detect an invasion early. If Brisbane were divided into square kilometre units (roughly the area estimated by the model that an *Ae. aegypti* population would spread after 5 years) then one would need to monitor 16,000 ovitraps a year to be certain of no establishment across the city. Ovitrapping represents one of the most sensitive methods for detection, and larvae would need to be raised and identified at 4th instar levels for each trap if morphological identification is used. Unfortunately, surveillance at this magnitude is not always an efficient use of resources, both in terms of the time taken to deploy traps and manually identify specimens, so detecting an early invasion may be almost impossible.

There are a number of emerging solutions that may increase the efficacy and optimize surveillance activities. Targeted surveillance around international First Ports of Entry is an efficient way of targeting import pathways from overseas. In south east Queensland, local and state authorities have established ovitrapping programs, preferentially targeting areas surrounding First Ports of Entry (including domestic ports and surrounding residential suburbs) which represent the next level of risk. *Aedes aegypti* and *Ae. albopictus* are regularly intercepted at these locations and, to date, targeted responses have been successful in preventing establishment. The Rapid Surveillance for Vector Presence (RSVP) system uses real-time PCR to detect a single first instar larvae in a sample containing up to 4,999 non-target larvae combined from multiple but geo-referenced ovitraps (Montgomery et al., 2017). This is a significant improvement in our ability to detect incursions of invasive species. Using this more efficient methodology, authorities can further optimize surveillance by focusing efforts in areas of high risk such as older, established suburbs with high tank densities and older housing characteristics (Ritchie, 2014). Examples of Brisbane areas with these characteristics are Graceville, Tarragindi, Camp Hill, Chelmer, Hawthorne, Ashgrove and Bardon (Figure 53). Houses in these SLAs are less likely to have adequate fly-screening than modern houses, thus allowing for greater access for adults to blood meals (Ritchie, 2014).

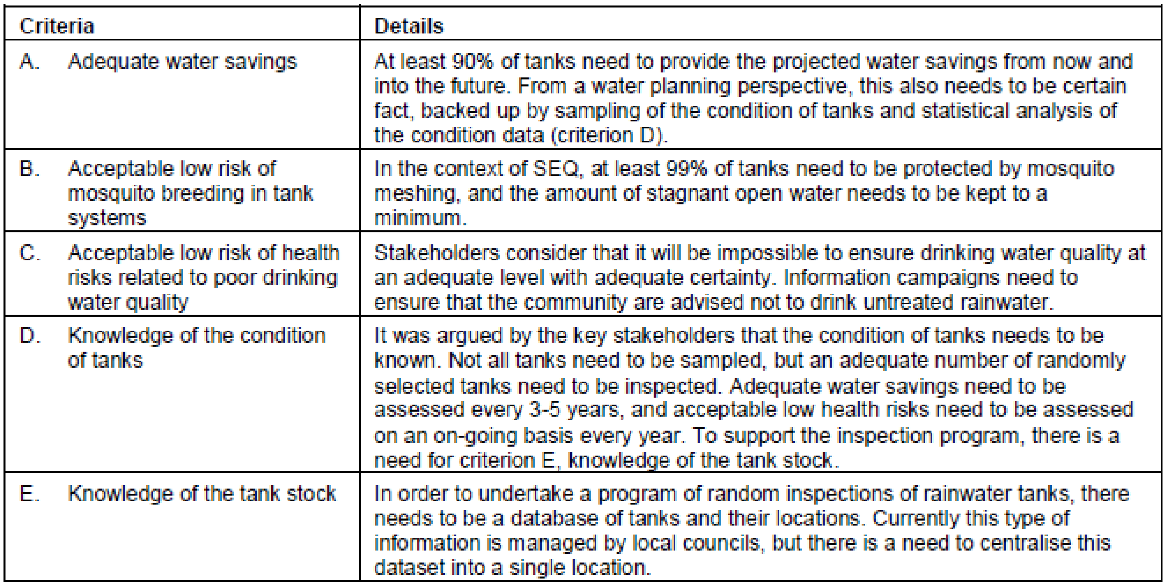
Medical entomologists have always understood the importance of engaging communities in the implementation of disease vector control initiatives (Hamlyn-Harris, 1931). In California, community engagement has been crucial in detecting populations of *Ae. aegypti* and *Ae. albopictus* invading California (Cornel et al., 2016, Metzger et al., 2017). Authorities report that education and public involvement was critical to detecting early infestations in new areas through community reporting of 'day-biting' mosquitoes (Metzger et al., 2017). Citizen science initiatives are another way to educate and increase public awareness. An innovative citizen science project is currently in development in Brisbane, applying ovitrapping and genetic methods to detect *Ae. aegypti* and other invasive mosquito species (Montgomery et al., 2017). The RSVP project relies on interested citizens to set up a mosquito oviposition trap on their property, collect the resulting eggs and send these to health authorities for processing. Similar methodologies are being used to monitor invasive mosquitoes in Europe (Kampen et al., 2015) and North America (Cohnstaedt et al., 2016). The evolution of these surveillance strategies have the potential to cover large areas over a number of years and reduce surveillance costs.

Finally, the development of environmental DNA (eDNA) techniques have promise for detecting invasive species in large, cryptic or hard-to-sample volumes of water such as rainwater tanks and avoids the challenge of identifying mosquito samples (Schneider et al., 2016). Mosquitoes leave traces of their DNA in the environment when they shed their cuticle or defecate (Schneider et al., 2016). Real Time PCR can be used to amplify minute amounts of DNA from water. Although challenging, the sensitivity of techniques to detect eDNA is improving. This methodology represents a major step forward in surveying rainwater tanks where, traditionally, surveyors used ladders and larval sampling nets to collect mosquito immatures. The ability to detect species by just turning on a tap at the base of a rainwater tank is sure to improve the ability to take samples for analysis. Ultimately however, the most efficient and cost effective form of protection against *Ae. aegypti* establishment and spread will be to ensure large permanent water storage containers, such as rainwater tanks, are managed appropriately.

7.8 Future management of asset stock and surveillance of rainwater tanks

Moglia et al. (2012) propose a strategy for the management of rainwater tanks in south east Queensland as a result of meetings with key stakeholders in the Department of Natural Resources and Mines, Queensland Health and the Queensland Water Commission. Criteria for the successful management of this resource are listed in Table 23. An important outcome of the Moglia et al. (2012) work identified that 99% of tanks should remain structurally intact as to prevent opportunities for mosquito breeding (Table 23).

Table 23. Criteria for the successful management of rainwater tank infrastructure from Moglia et al. (2012)



Criteria	Details
A. Adequate water savings	At least 90% of tanks need to provide the projected water savings from now and into the future. From a water planning perspective, this also needs to be certain fact, backed up by sampling of the condition of tanks and statistical analysis of the condition data (criterion D).
B. Acceptable low risk of mosquito breeding in tank systems	In the context of SEQ, at least 99% of tanks need to be protected by mosquito meshing, and the amount of stagnant open water needs to be kept to a minimum.
C. Acceptable low risk of health risks related to poor drinking water quality	Stakeholders consider that it will be impossible to ensure drinking water quality at an adequate level with adequate certainty. Information campaigns need to ensure that the community are advised not to drink untreated rainwater.
D. Knowledge of the condition of tanks	It was argued by the key stakeholders that the condition of tanks needs to be known. Not all tanks need to be sampled, but an adequate number of randomly selected tanks need to be inspected. Adequate water savings need to be assessed every 3-5 years, and acceptable low health risks need to be assessed on an on-going basis every year. To support the inspection program, there is a need for criterion E, knowledge of the tank stock.
E. Knowledge of the tank stock	In order to undertake a program of random inspections of rainwater tanks, there needs to be a database of tanks and their locations. Currently this type of information is managed by local councils, but there is a need to centralise this dataset into a single location.

Moglia et al. (2013a) draw attention to the lack of knowledge on the rainwater tank ‘asset stock’ in south east Queensland. The authors state that there are gaps in policy regarding the management of these systems. Currently in Brisbane there is “no mechanism in place for making sure that household rainwater collection systems are maintained and in a good condition” and “no provision, guidance or requirements for transfer of ownership of a rainwater tank” (Moglia et al., 2013a). Moglia et al. (2013a) undertook a survey of professionals to gauge their expectations regarding problems related to the failure of installed tanks. Issues of concern included failure of pumps/parts, integrity of mosquito meshing, structural condition, and water quality. The causes of potential problems included lack of maintenance, design failure, incorrect maintenance and poor installation (Moglia et al., 2013a). Results indicate that plumbers and water professionals estimated 46% of tanks had problems with pumps and 30% of tanks had broken or removed meshing (Moglia et al., 2013a). Most did not believe that tanks were adequately maintained by householders (Moglia et al., 2013b). These observations are informative when considering design, maintenance and surveillance strategies for rainwater tanks.

The survey results of Moglia et al. (2013b) are unfortunately not indicative of the true non-compliance rates of the tank stock in south east Queensland (Darbro et al., unpubl. data). Biermann et al. (2012) completed a survey of 223 mandated rainwater tanks in the region. Results indicated that mosquito meshing was absent in 4% of tanks, pump condition was less than good in 15% of cases (includes poor condition), gutters were in less than good condition in 6% of cases, and tank structural condition was in less than good condition in 5% of cases (including poor condition). While these results are informative in terms of non-compliance rates in mandated tanks, they only observed a small number of newly installed tanks (~5 years), which had not had time to decay or fail, so conclusions on decay rate cannot be drawn from this dataset.

7.9 Current condition of the rainwater tank stock in south east Queensland and Brisbane

There are currently ~93,000 rainwater tanks in the Brisbane City Council area with known locations and potentially many more at unknown locations. The most comprehensive survey of the rainwater tank stock in south east Queensland was undertaken by CSIRO, QIMR Berghofer and Queensland Health during the period 2010 until 2012 (Darbro et al., unpubl. data). In this survey, 2,684 rainwater tanks in 4,980 properties were examined for compliance with state regulations (Darbro et al., unpubl. data). Some 4% were non-compliant with regulations and of those that were unsealed, 65% had mosquitoes present (Darbro et al., unpubl. data). From this data it was estimated that there are potentially another 30% more unregistered rainwater tanks in the city (Darbro et al., unpubl. data). Rainwater tank compliance data from other councils in the region suggest non-compliance rates are increasing. In one council area, survey data indicates an increase in defective and unsealed tanks from 6.5% in 2010 to 25.3% in 2014 and 17.4% in 2015 from a total sample size of 2,877 tanks (Brian Montgomery, Queensland Health, pers. comm.). These results are worrying if they reflect what is currently occurring in Brisbane, particularly because they are reaching the 30% non-compliance scenario modelled as having a high potential for spread. At this non-compliance rate, there could potentially be 30,000 rainwater tanks providing potential habitat for an incursion of *Ae. aegypti*.

Because of their age, the condition of rainwater tanks in Gin Gin could potentially indicate the state of Brisbane rainwater tanks in 10-20 years. House-to-house surveys in Gin Gin revealed a compliance rate of ~50% from 58 rainwater tanks. The primary reason for unsealed rainwater tanks was exposed overflows (44%), followed by holes rusted in roofs (26%) and holes in sieves (15%). Surveys of Brisbane and other councils revealed the most common reason for non-compliance of newer rainwater tanks was owner mismanagement (Darbro et al. unpubl., data). Reasons included removal of overflows (35%) and primary sieves fitted incorrectly (27%) or removal of sieve (7%). Sieves are key to maintaining the integrity of rainwater tanks and preventing both the ingress and egress of mosquitoes but there is some evidence to suggest that these steel meshes are beginning to fail via rust or homeowner negligence (see Appendix 13). Surveys revealed that rainwater tanks could potentially harbour large mosquito populations, and any small hole in a sieve or crack could allow these mosquitoes to escape. Closer inspection determined that eggs or larvae can be washed in from roof guttering during rainfall events, even if rainwater tanks are sealed. Roof gutters increase the surface area for oviposition and potentially increase the productivity of rainwater tanks. Problematic gutters were collecting leaf litter and pooling water (Appendix 9), which stresses the importance of removing trees that are overhanging rooves, as required by Brisbane City Council Ordinances (Brisbane City Council, 1933).

Other rainwater tank condition characteristics can determine their ability to support mosquito production. Although not tested explicitly, observations of sediment and nutrient levels in newly installed rainwater tanks suggest they are unlikely to support large numbers of mosquitoes due to lack of nutrients. If devices are used to exclude sediment, vegetative debris and animal droppings then nutrients can be managed appropriately. However, the range of devices preventing nutrients in rainwater tanks can cause problems themselves if they are not maintained appropriately. For instance, homeowners may be unaware that first flush devices need to be emptied regularly: mosquito larvae have been observed in these devices after they have become blocked and begin to collected water. Flaps can be used to prevent mosquito emergence from underground pipes. However, if these are not regularly maintained they can get stuck to 'open' and no longer service their intended purpose. Despite the presence of devices to exclude sediment and nutrients in some cases, all tanks observed during surveys in Brisbane and Gin Gin contained sediment that covered the entire floor of rainwater tanks. Thus, it is unlikely that nutrients are a limiting factor for mosquito production in most tanks currently in the urban landscape.

7.10 Attitudes to maintenance and public education

Surveys indicate a lack of awareness in the community regarding the maintenance of rainwater tank infrastructure (Moglia et al., 2011). The rapid degradation of rainwater tanks in councils around Brisbane indicates that ongoing monitoring and maintenance will be essential in the near future. Compliance with those recommendations will be governed by community interactions and perceptions and by the willingness of government to promote and impose (Walton and Gardner, 2012). Collaborating for a common good versus the freedom of action regarding one's own property and management of resources within it is a modern day version of the tragedy of the commons (Hardin, 1968). Developing policy around the concerns of the community will be required if authorities seek the levels of support and participation that helped eliminate *Ae. aegypti* in the past (Chapter 2).

Queensland Health have developed guidelines regarding the ongoing maintenance of rainwater tanks which, under Queensland law, are the responsibility of the homeowner (Queensland Health, 2011). Walton and Gardner (2012) indicate that there is a low level of awareness regarding tank maintenance but that the community is willing to support measures that encourage it. Of those who were aware of their rainwater tanks, their preference was for autonomy, the freedom to control and exercise their own options for maintenance, and to be independent of government interference (Walton and Gardner, 2012). Gardiner (2010) surveyed 1,051 homeowners for attitudes to rainwater tank storage and use in south east Queensland. An interesting outcome of this study was that 31% of tank owners indicated that they had no interest in the tank on their property, likely due to compulsory installation or a subsequent purchase of the house after tank installation. This group of rainwater tank owners was not confident in managing their tank and rarely cleaned or inspected (Gardiner, 2010). Fifty percent of respondents never conducted routine maintenance (eg. cleaning leaves out of gutters or screens) unless a problem was detected (Gardiner, 2010). These outcomes suggest the first and most effective step in tank maintenance appears to be community engagement and awareness.

7.11 The future of rainwater tanks

Whether the installation of tanks has been a cultural change or just a temporary response to drought is an interesting topic for future consideration. Dam levels have increased significantly since the time of the documented tank installations, and rebates for tank installation were withdrawn in February 2013 (Moglia et al., 2013). There is now the potential for rainwater tanks to no longer hold value to the homeowner, and they may continue to degrade due to lack of owner motivation or any awareness of the risks they pose. Degradation of the tank stock has occurred at regular intervals in the past, resulting in increases in populations of *Ae. aegypti* and subsequent dengue fever outbreaks (Cooling, 1923, Hamlyn-Harris, 1931). There are now a considerable number of rainwater tanks installed (and potentially unsealed) in the urban landscape of Brisbane. These permanent water storage containers may play an important part in any future disease and vector establishment, and Brisbane authorities will need to consider their presence when planning for the future.

Final Conclusion

Rainwater tanks represent ideal larval habitat for the establishment and persistence of invasive mosquito species in areas where low rainfall and temperatures would otherwise make establishment difficult. In the past, water stored in urban landscapes has consistently been shown to be high risk for *Ae. aegypti* establishment and persistence, particularly in areas where temperatures are suitable for year–round development (Kearney et al., 2009). In recent years, the large-scale re-installation of rainwater tanks into Australian urban landscapes as a response to drought and climate instability, has increased the risk of invasion and establishment in areas south of its current distribution (Beebe et al., 2009, Kearney et al., 2009, Williams et al., 2010). These tanks may have facilitated the expansion of *Ae. aegypti* populations into areas where conditions are sub-optimal for species survival. Results show that rainwater tanks assist *Ae. aegypti* in surviving fluctuating temperatures, particularly if the upper part of the daily range approaches the optimal range for the species, and historical distributions of *Ae. aegypti* in Australia support this assertion.

Network simulations based on historical non-compliance rates of rainwater tanks showed differences in the spread of *Ae. aegypti* through the landscape and the pattern of potential Brisbane incursions. Even low levels of non-compliance (~30%) may rapidly increase the spread of the species in suburbs with high densities of rainwater tanks. This may be exacerbated by enhanced connectivity in areas with connecting yards and many cul-de-sacs (Chapter 6). In the absence of any appropriately scaled surveillance program and given the dispersal parameters of the vector, we warn that many incursions will not be detected. With the installation of over 100,000 rainwater tanks into Brisbane in the past 15 years, results are essential in informing health authorities about the risks that they pose.

Health authorities must be aware that the presence of rainwater tanks is enhancing the capacity of the landscape to support the establishment of invasive disease vectors across large urban areas. Given the public health impact and rapid global spread of dengue, chikungunya and now Zika viruses, understanding the current and future distribution of the primary vector, *Ae. aegypti* should help plan for vector surveillance, invasion and control. We have shown how the successes of past health campaigns can inform future risk assessments and incursion preparedness, particularly in areas of coastal Queensland and New South Wales where water storage once contributed to *Ae. aegypti* presence and to dengue transmission. It is no longer a question of ‘if’ they will establish, but a matter of ‘when’ will this occur. Authorities must plan for this eventuality and for the management of those landscapes that facilitate the invasion.

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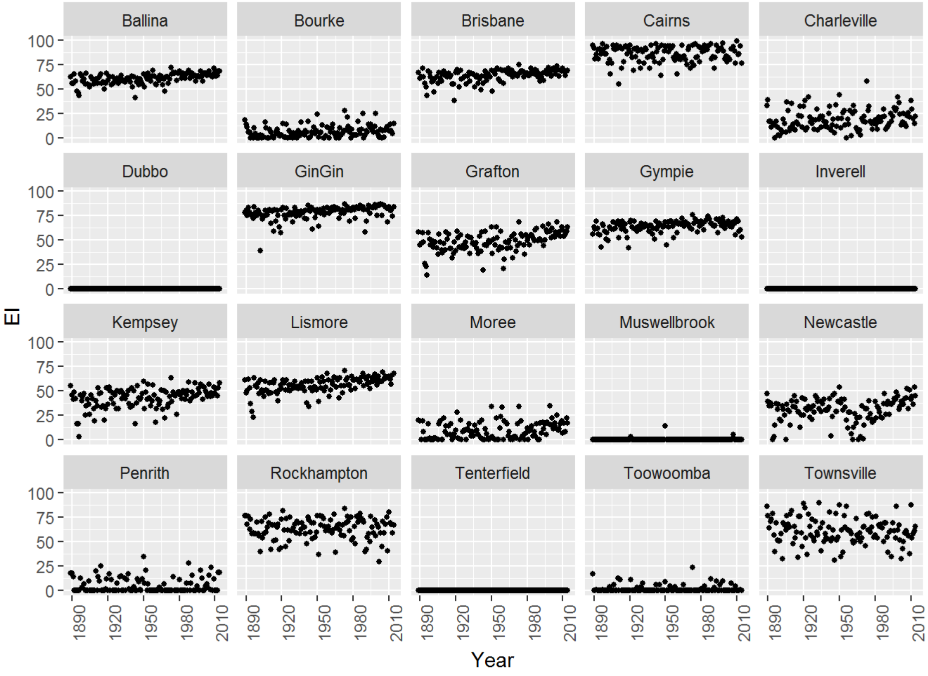
Appendix

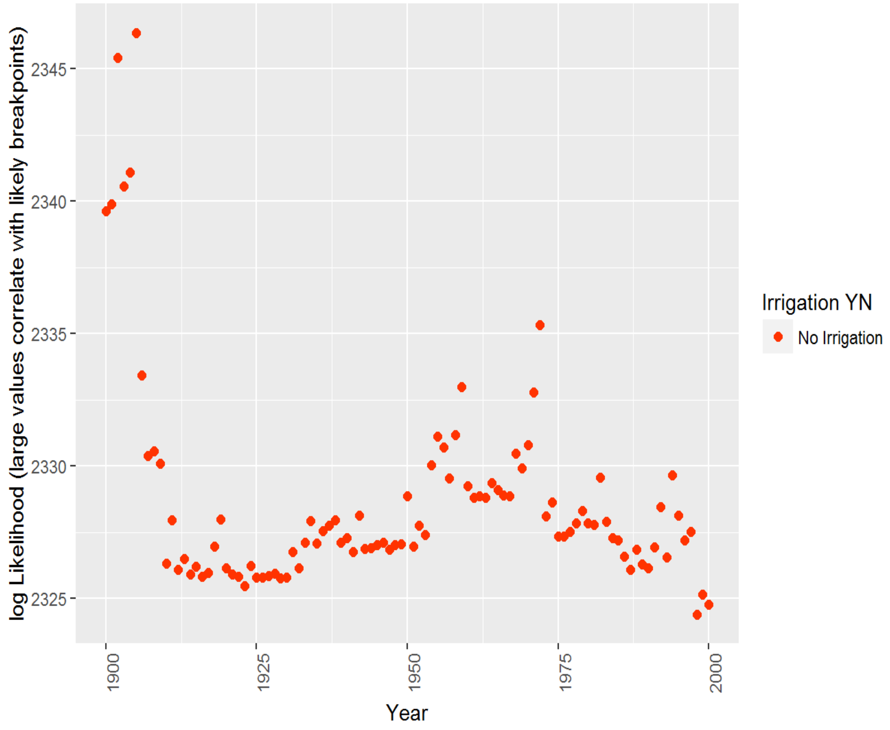
A1. Database of historical *Aedes aegypti* survey locations at cold range margins. FigShare data depository:

https://doi.org/10.6084/m9.figshare.5902945.v1

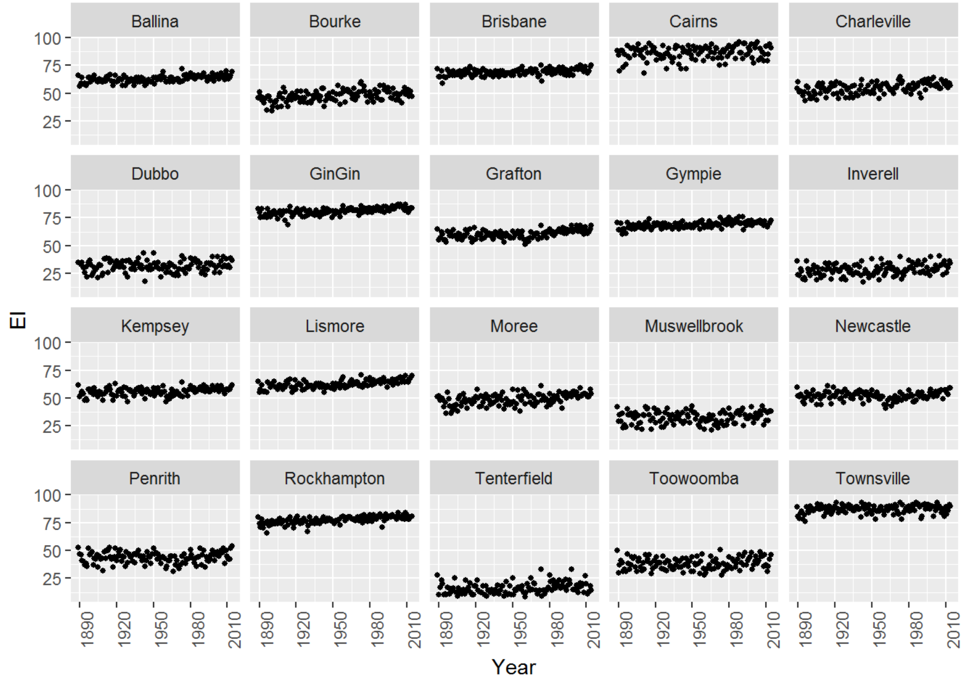
A2. Ecoclimatic Index (EI) values predicted by CLIMEX for the Native Distribution Model for a selection of sites where *Aedes aegypti* has been historically surveyed in Australia. Plots show variations in EI when compared with the Permanent Water Model (A4).



A3. Log-likelihood estimates of rapid change in Ecoclimatic Index (break points) as predicted by CLIMEX in the Native Distribution Model (no irrigation included in the model).



A4. Ecoclimatic Index values predicted by CLIMEX for the Permanent Water Model (PWM) for a selection of sites where *Aedes aegypti* has been historically surveyed in Australia. Plots show variations in EI when compared with the Permanent Water Model (A2).



A5. Temperature regimes for environmental chambers to determine survival of *Aedes aegypti* in different container categories from Brisbane, Australia.

Hour	**18:00**	**20:00**	**22:00**	**24:00**	**02:00**	**04:00**	**06:00**	**08:00**	**10:00**	**12:00**	**14:00**	**16:00**
Tank °C	17.08	16.26	15.44	14.62	13.8	12.98	13.8	14.62	15.44	16.26	17.08	17.9
Bucket °C	20.63	17.66	14.70	11.73	8.77	5.80	8.77	11.73	14.70	17.66	20.63	23.59

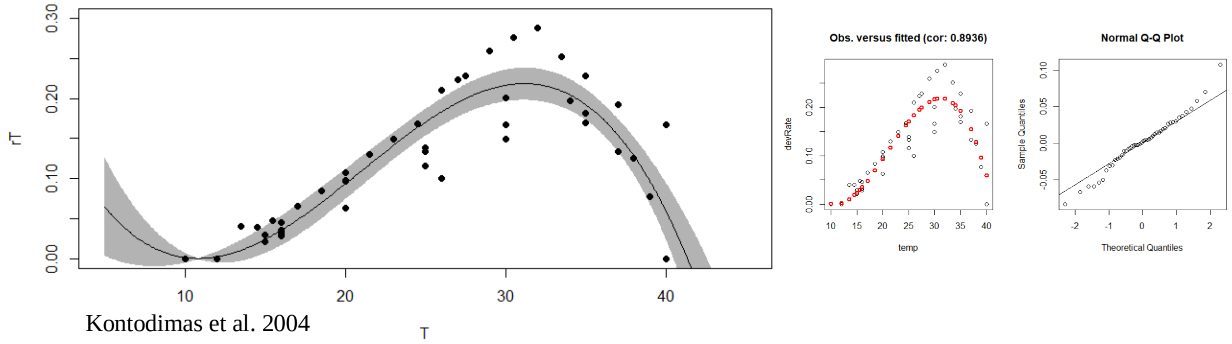
A6. Abiotic conditions within rainwater tanks and buckets in Brisbane during winter (July) 2014. Levels represent 0-32% shade cover (1), 33-65% (2) and 66-100% (3).

	Mean Bucket	**Mean Tank Air Conditions**			**Mean Tank Water Conditions**					
Shade Level	**Evaporation (L)(SD)**	**Temperature 9-11am (°C)(SD)**	**Dewpoint (SD)**	**Humidity (%)(SD)**	**Volume (L)**	**Salinity (SD)**	**pH (SD)**	**Conductivity (SD)**	**TDS (SD)**	**Larvae (SD)**
3	0.33 (0.2)	18.5 (2.2)	12.2 (4.2)	83.3 (3.6)	4618.9	16.6 (1.7)	5.2 (0.3)	21.5 (4.5)	16.0 (3.0)	0.0 (-)
1	0.92 (0.3)	21.8 (4.8)	14.5 (6.2)	85.7 (5.2)	5018.7	19.2 (3.1)	5.1 (0.4)	25.8 (7.6)	17.8 (5.2)	4.6 (3.5)
3	0.55 (0.2)	21.4 (2.0)	12.6 (5.5)	69.2 (12.6)	2094.4	16.9 (1.8)	5.0 (0.1)	22.2 (6.5)	16.0 (4.5)	2.0 (1.4)
2	0.56 (0.4)	22.1 (3.1)	14.6 (6.3)	80.0 (4.9)	9913.1	19.8 (5.8)	5.1 (0.4)	28.9 (15.8)	20.6 (11.1)	5.4 (2.4)
1	0.94 (0.7)	21.4 (2.8)	14.4 (5.3)	85.2 (9.0)	1807.4	30.8 (22.0)	6.2 (0.5)	53.2 (51.0)	38.1 (36.0)	1.8 (1.5)
1	0.36 (0.4)	24.8 (1.8)	16.2 (6.9)	85.3 (6.0)	1958.0	19.8 (4.3)	5.6 (0.2)	26.7 (10.2)	19.6 (8.1)	167.4 (144.0)
3	0.77 (0.5)	22.2 (3.3)	14.2 (6.1)	79.6 (6.4)	8993.9	57.4 (13.2)	6.6 (0.2)	115.0 (28.5)	81.4 (21.0)	10.5 (8.9)
3	0.37 (0.3)	21.5 (4.0)	12.6 (5.1)	74.1 (5.4)	2755.9	189.8 (0.5)	7.6 (0.2)	305.2 (177.0)	281.8 (1.7)	4.0 (-)
2	0.56 (0.1)	23.7 (3.8)	11.8 (4.1)	57.7 (6.8)	807.9	22.7 (3.7)	6.5 (0.6)	37.3 (9.4)	26.5 (7.0)	44.3 (46.9)
2	0.34 (0.3)	20.1 (4.5)	12.8 (5.7)	81.1 (10.1)	3015.2	16.6 (0.9)	4.8 (0.3)	22.5 (3.2)	14.9 (2.1)	0.0 (-)

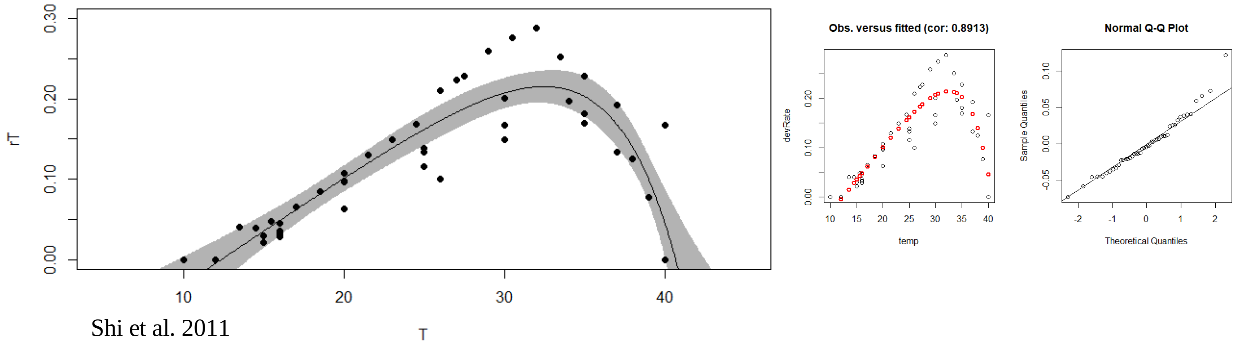
A7. Presence of *Aedes notoscriptus* immatures (grey shading) in first flush devices during winter in Brisbane, 2014. Numbers correspond to the tank which contained devices. For example, tank 10 had two separate first flush devices (10a,10b) on downpipes entering tank. Volume measures the mean volume found in each device throughout the field survey. Presence represents the percentage of surveys where at least one *Ae. notoscriptus* immature was sampled from the device.

		Week									
		1	3	5	7	9	11	13	15	**Presence (%)**	**Mean Volume (L)**
Tank	1			■						12.5	4.2
	3									0	8.6
	4									0	7.9
	8		■		■		■	■	■	62.5	42.6
	10a	■			■					25	6.4
	10b			■						12.5	7.6

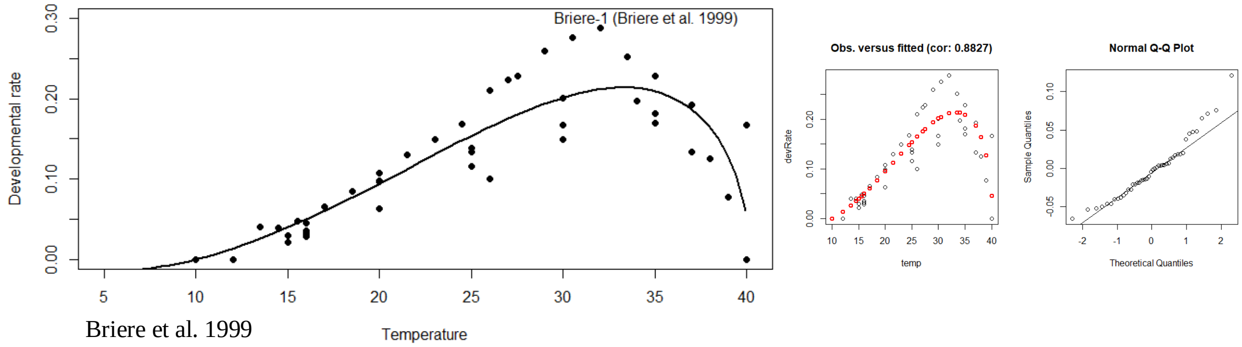
A8. Non-linear models and their fit for *Aedes aegypti* development rates (median/mean time to pupal development, black circles) and dark bands represent confidence bands where possible. Data sourced from Chapter 4, Gilpin and McClelland, 1979, Rueda, 1990, Tun-Lin et al., 2000, Richardson et al., 2011 and Carrington et al., 2013. Linear models are referenced below each plot. Smaller plots show observed values (grey circles) versus fitted model values (red circles) and Q-Q plots comparing quantiles for each distribution.



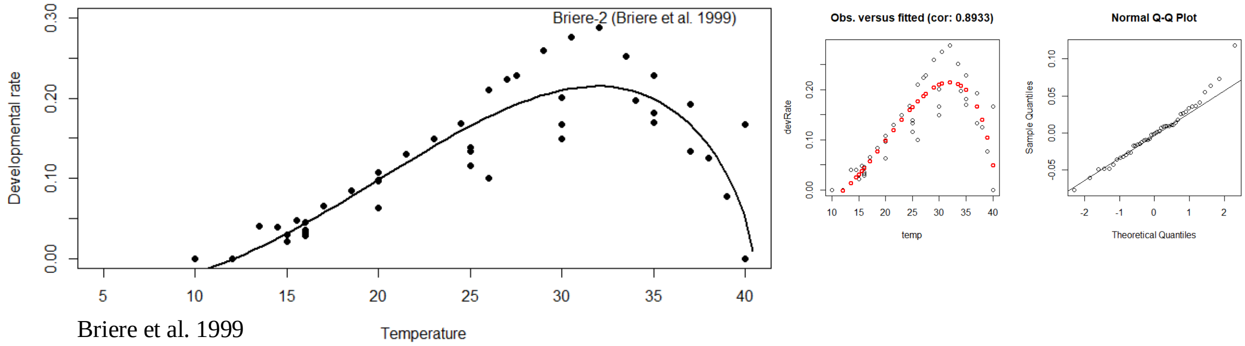
Kontodimas et al. 2004



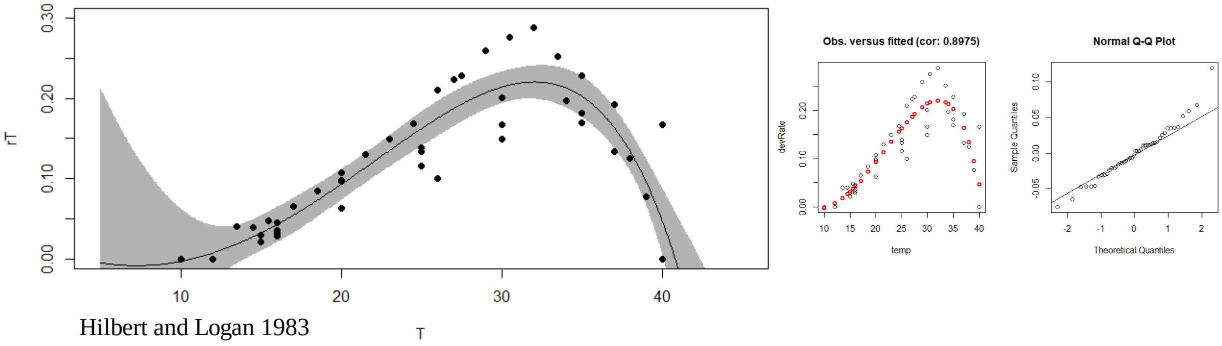
Shi et al. 2011



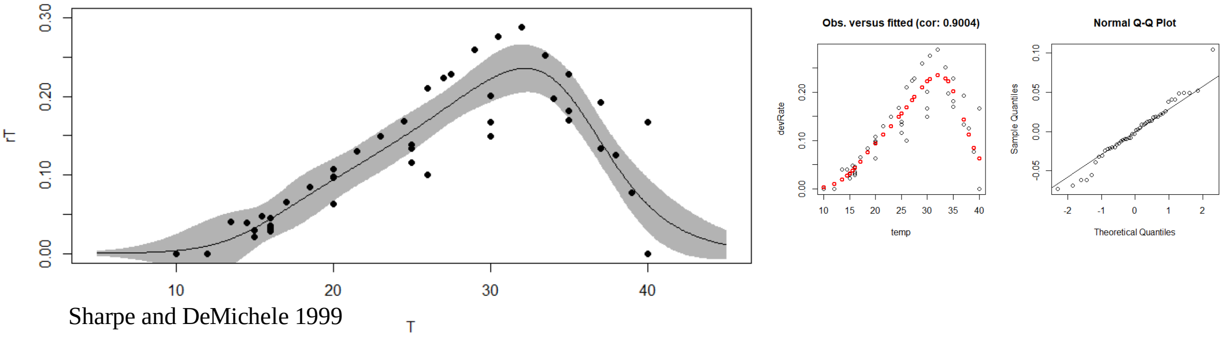
Briere et al. 1999



Briere et al. 1999



Hilbert and Logan 1983



Sharpe and DeMichele 1999

A9. Gutters observed that likely increased productivity of rainwater tanks in Brisbane and Gin Gin, 2014.

A10. Design of emergence trap including fan, battery and collection cup.

A11. News Media:

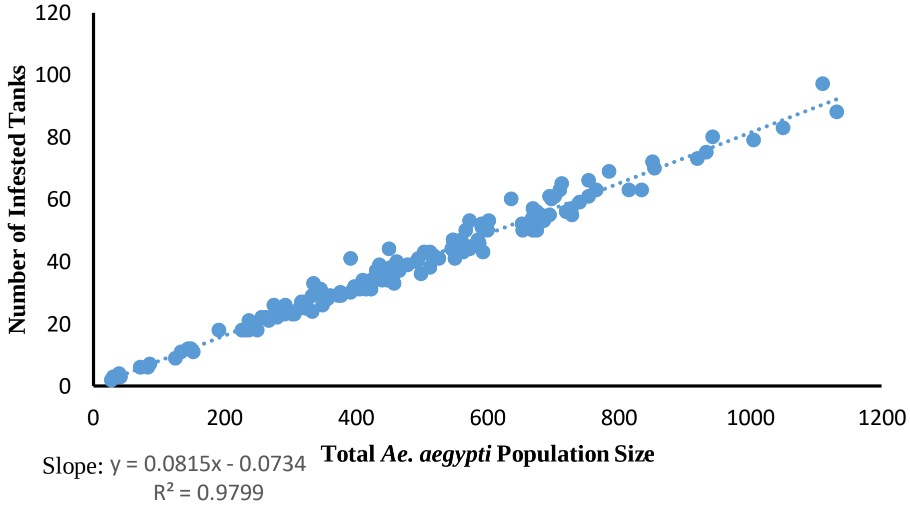
Channel 7 News

https://www.youtube.com/watch?v=B1hNEPVBfhA

ABC Wide Bay

https://soundcloud.com/abcwidebay/breakfast-dengue-fever-research-project-to-start-in-gin-gin

A12. Correlation between total *Ae. aegypti* population and the number of infested tanks in each statistical local area after five years of network simulation suggesting linear relationship.



Slope: $y = 0.0815x - 0.0734$
$R^2 = 0.9799$

A13. Sieve degradation through rusting, manufacturing failure and intentional disruption (bottom two images).

A14. Conference paper from Formal Methods in Macro-Biology: First International Conference, FMMB 2014, Nouméa, New Caledonia, September 22-24, 2014.

The challenges of developing spatially explicit network models for the management of disease vectors in ecological systems.

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Challenges of modelling vector-borne disease systems result from complexities and uncertainties inherent in the vector's behavioural ecology and its interactions in a landscape context. Network models provide a number of approaches and measures to quantify spatially-explicit systems that are consistent with the ecological process of vector dispersal, with implications for disease transmission and spread [1,2]. Here we discuss two spatially explicit vector systems as network models; (1) the movement of the invasive mosquito Aedes aegypti, which vectors a number of diseases including dengue fever, through rainwater tanks in a major urban area, (2) the movement of bats (flying-foxes), which vector Hendra virus, through urban and rural landscapes [3]. We contrast the design and applicability of these networks, comparing features and challenges inherent in modelling these systems, and discuss the use of network models as disease vector management tools with implications for disease spread.

In an ecological context, nodes often represent metapopulations and compartmentalize important demographic characteristics such as growth rate, disease transmission rate and spatial location within landscapes. In our mosquito model, rainwater tanks are nodes that are fixed in both space and time, with accurate location data available from government rebate schemes. Depending on whether nodes are exposed to the environment (non-compliant) or not, tanks are nodes that may act as sources or sinks for mosquito vectors respectively. Characteristics that govern population growth within each source node are simple to collect and model as there is a vast literature on simulating population growth within containers [4]. Within the bat-Hendra model nodes are likely to be bat camps (roosts), containing populations of vectors. The highly seasonal nature of camps and their susceptibility to variations in environment and climate result in uncertainties in spatial location of the camps. Bats have high dispersal abilities with complex movement and social behaviours. This leads to large fluctuations in the formation and removal of nodes through space and time. Foraging sites, could be additional nodes within this system, but are difficult to model explicitly due to their inherent stochasticity and have so far been ignored. Important simplifying assumptions are made in characterizing bat camps as nodes in a network model compared to rainwater tanks, as the tanks better reflect our compartmentalized concept of 'nodes' in a network. These assumptions introduce uncertainty into any conclusions that are drawn about the bat disease vector system, but this uncertainty is not made explicit.

Within a network model, edges are characterized as the flow of information between nodes and it is important that these connections reflect the scale at which nodes interact. In an ecological context this is represented by a dispersal mechanism, typically either a binary variable or a continuous function that decays with distance. In our mosquito model, edges represent the movement of mosquito vectors between rainwater tanks and are characterized through a dispersal kernel algorithm that decays with increasing distance. In this way, we connect nodes by considering topology and a distance threshold based on known dispersal rates and population size. Initially, tanks may become breeding sites and put neighbouring tanks at risk of hosting vectors when deemed non-compliant. Edges within the bat-Hendra model are similarly characterized by a distance weighted 'connectivity' function between camp nodes. In reality, the magnitude and direction of bat movement between camps and foraging sites could be represented by a large number of links and are not necessary driven solely by distance. However, there are difficulties in collecting movement data with telemetry equipment and in accessing and monitoring bat camps, foraging sites and the seasonal nature of bat movement between sites. Attempting to simulate a bat vector system with a network model is therefore fraught with difficulties in obtaining accurate data to quantify the scales at which vector movement and interactions occur.

The importance of node connectivity can be explored in an ecological context by calculating measures of diffusion and node centrality within the system. The connectivity within the mosquito system allows for a higher probability of colonization if populations are large, but is also constrained by the limited dispersal ability of the species. Measures of the number of node links and node influence on a network can indicate the risk of individual nodes as disproportionate sources of infection. The ability to effectively identify and target high risk nodes or collections of nodes is considered an important goal for vector reduction (therefore reducing disease risk) by mosquito control authorities. Connectivity within the model bat-Hendra system is based on a distance weighted probability of infected individuals moving between camps. The result of the model was that the highly connected urban camps are predicted to experience small, high frequency epidemics, occasionally sprouting travelling waves of infection linearly through rural populations [3]. How well this represents disease spread through this system is very uncertain due to the bat's high dispersal ability and complex nature of movement. Until a better understanding of the dynamics within the bat system is developed, this network model is best used alongside empirical studies as a hypothesis generating tool [5].

The large contrasts between these two disease vector networks relate to how well each model represents reality. Thus when considering whether to adopt a network modelling approach, one should consider how well studied the disease vector's behavioural ecology is, as well as its interactions with the disease and the environment in space and time. Ideally, when developing network models for biosecurity or public health authorities as management tools, suitable ecological systems to simulate are those that minimize temporal/spatial stochasticity in network design, have access to accurate spatial data and give realistic insights into vector dispersal. If assumptions generate open-ended hypotheses, there may be more value to authorities to reframe questions or consider other modelling approaches.

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