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Sydney's Mosquitoes: Their role as disease vectors

William Anthony Crocker

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Sydney's Mosquitoes: Their role as disease vectors

Abstract
Mosquito-borne disease is a significant public health issue in Australia. Australia's populous southern cities, including Sydney, are home to a wide variety of mosquito-borne pathogens, many of which are known to infect humans and our animals.

The epidemiology of mosquito-borne pathogens is closely linked to the ecology of their vectors. The abundance and diversity of mosquito vector species has a huge impact on what pathogens are likely to be found in a given area.

A trapping survey of mosquitoes at a variety of sites across Sydney investigated which environmental factors influenced the diversity of mosquito fauna. Sites in the middle of the city were predicted to be dominated by a different range of species to sites further west which are mainly large, vegetated, fresh water bodies. No significant difference in species diversity or abundance was found, however brackish, estuarine sites were dominated by a very different suite of mosquito species compared with the other sites.

The trapping survey was repeated in February, March and April of 2015. High variability in mosquito assemblages was identified. Some species’ populations changed significantly over the three months, while others remained constant. Mosquito abundance changed differently at estuarine, freshwater and urban sites. Temporal and spatial factors interacted to influence mosquito vector diversity.

The mosquito samples were tested for several different species of virus, but only one was detected: the relatively benign flavivirus, Stratford. The lack of other viruses raises the possibility that Stratford could be immunising its animal reservoir (or perhaps even human) hosts against worse flaviviruses and its presence in the environment is advantageous; an idea which certainly warrants future investigation.

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Sydney’s Mosquitoes: Their Role as Disease Vectors

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This thesis is presented as part of the requirements for the award of the Degree of Bachelor of Environmental Science (Honours) of the University of Wollongong.
The information in this thesis is entirely the result of investigations conducted by the author, unless otherwise acknowledged, and has not been submitted in part, or otherwise, for any other degree or qualification.

Signed: W. Crocker

Date: 27/10/2015
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Abstract

Mosquito-borne disease is a significant public health issue in Australia. Australia’s populous southern cities, including Sydney, are home to a wide variety of mosquito-borne pathogens, many of which are known to infect humans and our animals.

The epidemiology of mosquito-borne pathogens is closely linked to the ecology of their vectors. The abundance and diversity of mosquito vector species has a huge impact on what pathogens are likely to be found in a given area.

A trapping survey of mosquitoes at a variety of sites across Sydney investigated which environmental factors influenced the diversity of mosquito fauna. Sites in the middle of the city were predicted to be dominated by a different range of species to sites further west which are mainly large, vegetated, fresh water bodies. No significant difference in species diversity or abundance was found, however brackish, estuarine sites were dominated by a very different suite of mosquito species compared with the other sites.

The trapping survey was repeated in February, March and April of 2015. High variability in mosquito assemblages was identified. Some species’ populations changed significantly over the three months, while others remained constant. Mosquito abundance changed differently at estuarine, freshwater and urban sites. Temporal and spatial factors interacted to influence mosquito vector diversity.

The mosquito samples were tested for several different species of virus, but only one was detected: the relatively benign flavivirus, Stratford. The lack of other viruses raises the possibility that Stratford could be immunising its animal reservoir (or perhaps even human) hosts against worse flaviviruses and its presence in the environment is advantageous; an idea which certainly warrants future investigation.
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Chapter 1: Background & Introduction

1.1. Background and Literature Review

1.1.1. Mosquito-Borne Disease

Mosquitoes (Diptera: Culicidae) are some of the most important organisms of concern from a public health perspective. The females of most mosquito species require a meal of fresh blood as a source of protein to develop their eggs. Male mosquitoes do not require blood. When a female mosquito finds a host, she will inject a small amount of saliva into the host’s blood vessels in order to prevent clotting. This transfer of saliva provides an opportunity for various pathogens to move between the mosquito and its hosts and from one host to the next. In this way, many different diseases, such as malaria, yellow fever and dengue, use mosquitoes as vectors.

One of the most infamous and lethal mosquito-borne diseases in humans is malaria: a disease caused by a genus of protists called Plasmodium. The protist reproduces sexually in mosquitoes of the genus Anopheles and asexually in the red blood cells of a human host, where it produces symptoms like fevers, pain and vomiting and often kills the victim (Carter, 2015). The World Health Organisation reported 198 million cases of malaria globally in 2013, including up to 855,000 deaths, mostly in impoverished tropical regions of the world, particularly sub-Saharan Africa (WHO World Malaria Report, 2014).

Many other mosquito-borne pathogens are viruses, such as Yellow fever and Dengue. Both diseases are widespread in tropical regions of the world and both are frequently fatal, producing symptoms like fever, rashes, pain, nausea and bleeding. These viruses are transmitted by infected mosquitoes of the genus Aedes, especially Aedes aegyptii (Henley, et al., 2013).

Most of the mosquito-borne viruses of public health concern are classified into two groups: Alphaviruses and Flaviviruses. Both of these genera have genomes made of single-stranded RNA. Flaviviruses are a genus of viruses in the family Flaviviridae and include viruses like Dengue, Stratford and Murray Valley Encephalitis. Alphaviruses are a genus of the family Togaviridae that include the Ross River, Barmah Forest and Sindbis viruses (Thompson et al., 2015, Russell & Dwyer, 2000). These viruses are described below in section 1.1.2.

Mosquitoes can also disperse multicellular parasites, including nematode worms (phylum: Nematoda). In humans, these are responsible for diseases like lymphatic filariasis-
a diseases which causes swelling of the limbs and genitals (Ottensen, et, al, 1997).
Heartworm in dogs is also caused by a mosquito-transmitted nematode (Merrill et al., 1980).

The distribution, abundance and diversity of mosquito vectors has a huge impact on
the occurrence of mosquito-borne disease. This is why they are a particularly common threat
in wetter tropical regions of the world. Most mosquito species require some amount of
standing water to breed in as mosquito larvae are aquatic. Larval development is also
dependant on temperature. This means that areas with warm climates and high rainfall have
abundant mosquito populations and therefore a higher risk of mosquito-borne disease
(Department of Medical Entomology, 2010). The relationships between mosquito abundance
and distribution and changing environmental conditions is discussed below.

1.1.2. Mosquito-Borne Disease in Australia

Many of the mosquito-borne diseases which are globally the most lethal are,
thankfully, absent from Australia. Malaria was once endemic in this with cases reported as
far south as the NSW central coast in the nineteenth century. In the early twentieth century
measures like improved sanitary engineering to remove mosquitoes’ breeding habitat and
the use of insecticides greatly reduced the presence of malaria in Australia (Russell & Kay,
2004). The World Health Organisation declared Australia malaria-free in 1981 (Russell, 2009),
however 700-800 cases occur annually in Australia in travellers infected elsewhere (NSW
Health, 2015).

Nonetheless, mosquito-borne disease is still a significant public health issue in
Australia. Annually, over six thousand people are diagnosed with mosquito-borne diseases
(Newman et al., 2008).

The majority of studies into mosquito-borne disease in Australia tend to focus on the
tropical north of the country. Northern Australia hosts a greater range of mosquito-borne
diseases and suffers more frequent cases of those diseases. It is no coincidence that northern
Australia also has a much higher abundance and diversity of mosquitoes: approximately 150
species exist in Queensland, as opposed to just 70 in Victoria (Dobrotworsky, 1965).
Mosquitoes, like many insects, are not good at regulating their internal homeostasis and so
are sensitive to fluctuations in temperature. Australia’s north, with its tropical climate, lacks
the cold winters that annually kill off mosquitoes in the south. The north’s warm climate,
coupled with the increased breeding habitat provided by higher rainfall, means that many
mosquito species maintain a year-round presence.
Dengue fever is confined to northern Queensland, in the vicinity of Cairns, Townsville and the Torres Strait, where annual outbreaks still occur (Queensland Health, 2015). The disease is caused by an arthropod-borne virus (or “arbovirus”), in the Flavivirus genus. In 2014 there were more than 1500 confirmed cases in the region. Dengue is generally spread only by the mosquito species *Aedes aegypti*, which is mostly restricted to northern Queensland, and *Aedes albopictus*, currently only found in the Torres Strait Islands (Ritchie, et al., 2006).

Australia has a range of mosquito-borne pathogens that are not confined to the tropical north and are widespread across the country. An example of this is Ross River Virus (RRV), an arbovirus from the genus Alphavirus, which causes rashes, fevers and joint pain—a disease referred to as epidemic polyarthritis. The disease is not fatal and the majority of sufferers may experience only mild symptoms, however symptoms can also become severe and debilitating and can last for months to years (Clafin & Webb, 2015). This is Australia’s most common arbovirus, occurring most frequently in the tropical north of the country, where warm temperatures and high rainfall allow its mosquito hosts to thrive year-round. Further south, RRV is mostly quite rare, but sudden outbreaks can occur in summer. RRV epidemics were reported in South Australia in 1993, 1997, 2000 and 2001, with numbers of cases ranging from 250 in 2001 to 800 in 1993 (Horwood, 2005). New South Wales Health’s records of RRV infection notification show that the disease appears in cycles, with cases spiking to around one hundred in summer, dropping to about 20 to 30 in winter. During the period of fieldwork for this study, in February, March and April of 2015, numbers of reported RRV cases were unusually high; peaking in March with 447 reported cases in NSW (NSW Health, 2015). RRV is a significant public health concern in NSW, including Sydney.

Another significant alphavirus is Barmah Forest Virus (BFV). It is the second most common arbovirus in Australia (Russell & Dwyer, 2000), with far fewer reported cases annually than RRV. There are no known fatalities and BFV is generally asymptomatic, but it can cause fevers, skin rashes and muscle pains. It has never been fatal, although cases occur in every state and territory of Australia. From 1995 to 2008 15,592 cases were reported, with the greatest number of cases occurring in northern Queensland (Naish, et al., 2011). Cases of BFV have been increasing in Australia over recent decades, possibly as a result of increasing urban expansion (Russell & Dwyer, 2000). Several common and widespread mosquito species are able to act as vectors for BFV, including *Aedes vigilax* and *Culex annulirostris* (Boyd & Kay, 2000).
Murray Valley Encephalitis (MVE) is a mosquito-borne Flavivirus found across northern Australia and New Guinea, with occasional outbreaks occurring further south into NSW, generally west of the Great Dividing Range. MVE infection is usually asymptomatic, although a small proportion do suffer mild illness and fever. An even smaller proportion of infected people will develop encephalitis. (Queensland Health, 2014) MVE has a very low fatality rate. The virus’s main vector is the extremely common mosquito, Culex annulirostris (Knox, et al., 2012).

Kunjin is a flavivirus, a milder strain of West Nile virus, first isolated from Cx. annulirostris in northern Queensland in 1960. Kunjin infections in humans rarely cause symptoms, but can cause headaches, fatigue, rashes and joint and muscle pains (Russell & Dwyer, 2000). In horses, the disease is much more serious. A 2011 outbreak of encephalitis caused by Kunjin in southeastern Australia resulted in a 10% to 15% mortality rate in infected horses. Kunjin occurs most frequently (in both humans and horses) in the tropical north of Australia, with occasional cases and outbreaks occurring further south, including inland NSW, west of the Great Dividing Range (Prow, 2013).

Kokobera virus (KOKV) is a flavivirus that has been isolated from mosquitoes throughout Australia and Papua New Guinea, originally isolated from Cx. annulirostris in northern Queensland in 1960 and has since been isolated from mosquitoes in the Northern Territory, Western Australia and NSW (Russell, 1995). Infections rarely cause symptoms, although, as with many of the viruses mentioned in this study, it can cause fevers, lethargy, headaches and rashes. The virus has also been isolated from Ae. vigilax, and Ae. camptorhynchus (Mackenzie, 2001). Kokobera and the closely related Stratford virus (STRV) were originally classified as separate species within the Japanese encephalitis virus (JEV) complex, but genetic studies have since reclassified STRV as a subtype of KOKV (Heinz et al., 2000). STRV was first isolated from Ae. vigilax specimens collected in Cairns, Queensland, in 1961 and has since been isolated from mosquitoes trapped at various localities across Queensland and NSW. Seroepidemiological studies have suggested that STRV occasionally infects humans, but there has been no reported association with human disease (Nisbet, et al., 2005, Mackenzie, 2001).

Edge Hill virus (EHV) is a mosquito-borne flavivirus that has been isolated during mosquito-surveillance programs conducted in the Northern Territory, Queensland, Western Australia and NSW. EHV is the only member of the Yellow Fever virus subgroup to be detected in Australia. Unlike its lethal cousin, EHV is not a major human health concern. The virus was only once implicated in human disease, causing joint and muscle pain and
fatigue. EHV has most commonly been isolated from *Ae. vigilax* in northern coastal NSW. EHV has mainly been associated with infection in marsupial; antibodies having been detected in wallabies, kangaroos and bandicoots (Macdonald, *et al.*, 2010, Blok, *et al.*, 1984).

Despite the focus on the tropical north, mosquito-borne disease is still a significant public health issue for Australia’s populous southern cities, including Sydney. As mentioned above, RRV remains a regular threat, as do diseases like BFV, although MVE is restricted to west of the Great Dividing Range. This study will focus on the diversity of mosquitoes and their potential public health risk to the Sydney metropolitan area.

### 1.1.3. Mosquito Species Diversity Influences Disease Risk

A major driving factor of arboviral disease risk is the variation in the assemblage of different mosquito species present. Different pathogens require different vectors and not all mosquitoes are suitable vectors for all pathogens. As a result, some mosquito species have the potential to transmit many different pathogens; others may only host one or two pathogens or may be hosts only very rarely. Many more species pose no disease threat whatsoever. This study will attempt to produce a snapshot of the diversity of mosquitoes across Sydney and how that diversity changes in time and space. Several examples of common mosquito species and their disease vector status are described below:

*Culex annulirostris* is widespread across much of the Asia-Pacific region and is a common pest species in Australia, where it was first described (Mottram & Kettle, 1997). *Cx. annulirostris* is a species whose immature, larval stages are often found in both permanent and temporary fresh and brackish water bodies, regardless of how clean or polluted, sunny or shaded, deep or shallow, the water may be. This adaptability makes them generally very common across their range. They are particularly abundant during the hottest and wettest months of the year from late summer to early autumn (Becker, *et al.*, 2010). In Australia’s tropical north, *Cx. annulirostris* is also a known vector for Japanese encephalitis virus (JEV) (Hall-Mendelin, 2012). Further south, including in the region around Sydney, *Cx. annulirostris* is a major vector for several diseases, including Ross River virus (RRV) and Murray Valley encephalitis (MVE) and dog heartworm (Doherty, 1977, Bemrick & Moorhouse, 1968). This is an example of a species found in many different habitats which is a competent vector for a variety of pathogens.

The species *Aedes vigilax* is an abundant pest species in coastal regions across large areas of Oceania, including Australia’s eastern coast. *Ae. vigilax* is commonly known as the
saltmarsh mosquito as it is limited to breeding in brackish saltmarsh and mangrove areas. *Ae. vigilax* is considered the major vector for RRV and BFV in coastal areas of Australia, including Sydney (Kay, 1982, Dale, *et al.*, 2014) as well as being a major nuisance biter. Like *Cx. annulirostris*, *Ae. vigilax* is a vector for many pathogens, however its restriction to brackish waters means it is only of public health concern in coastal areas.

The freshwater-breeding *Anopheles annulipes* is not known to readily transmit any pathogens in Australia today (Boyd & Foley, 2007), although members of the *Anopheles* genus are responsible for transmitting malaria overseas. Laboratory tests have shown *An. annulipes* has the potential to be a malaria vector (Department of Medical Entomology, 2010) and has been implicated in the small outbreaks of the disease that occurred in the Sydney region after the Second World War, when soldiers infected while fighting in tropical south-east Asia returned home (Rieckmann & Sweeney, 2012). Since malaria’s eradication from Australia, *An. annulipes* has not been shown to be an important carrier of any human disease; however it is believed to be a major vector of myxomatosis in rabbits (Foley, *et al.*, 2006). *An. annulipes* is a ubiquitous species, but as it is not a competent vector of many pathogens it is of little public health concern.

*Aedes alternans* is a large mosquito species that is common in both coastal and inland areas of south-eastern Australia. Despite being a pest that will readily bite humans, *Ae. alternans* is not a public health risk as it is not generally considered to be a disease vector (although RRV has been isolated from specimens on the NSW south coast). The larvae of *Ae. alternans* actually prey on the larvae of other mosquito species, so the species’ presence may have a net positive effect on public health in an area (Well, *et al.*, 1994, Department of Medical Entomology, 2010).

*Aedes notoscriptus* is a very common mosquito found across Australia and Melanesia with occasional occurrences in New Zealand. It has also recently been detected as an invasive species in southern California (Peterson and Campbell, 2015). *Ae. notoscriptus* is a nuisance-biting pest capable of transmitting RRV. It is also the major vector of dog heartworm. This species is so common, in part, because of its ability to breed in even very small containers of stagnant water and so can be found a long way from natural water bodies. *Ae. notoscriptus* is a dominant part of the mosquito fauna in more temperate areas of Australia as it has a greater tolerance for cooler temperatures than tropical container breeding species, like *Ae. aegypti* (Williams and Rau, 2011, van Uitregt *et al.*, 2013).

The southern house mosquito, *Culex quinquefasciatus*, is a common species in coastal NSW and is one of the most cosmopolitan mosquito species, found on every continent.
except Antarctica. This species is a major domestic pest in tropical, subtropical and temperate areas, regularly biting humans (Chaves and Kitron, 2011). *Cx. quinquefasciatus* is an example of a species shown to be a potential vector for a wide range of pathogens. Laboratory studies have shown it is capable of transmitting MVE, and Kunjin. MVE has been isolated from specimens collected in Western Australia. It also acts as a vector for dog heartworm, fowl pox and, in some areas, myxomatosis (Russell, 1996, Boyd & Kay, 2000).

The examples described above demonstrate just how much the vector competence, and therefore the public health risk, of mosquitoes can vary from species to species. Because of this, it is important to understand the species diversity of mosquitoes in an area in order to accurately assess the risk of mosquito-borne disease.

### 1.1.4. Temporal Habitat Change Influences Mosquito Species’ Diversity

This study will look at how the abundance and species assemblage of mosquitoes in Sydney changes in the short-term, over the course of three months. Sydney’s climate is temperate enough that adult mosquitoes only survive in any number from mid-Spring to mid-Autumn. Winters are too cold for mosquitoes to remain active. From late spring to early autumn, mosquito numbers in Sydney boom in response to the warmer weather. As such, rates of mosquito-borne disease infection spike during summer, especially later on in the season around February and March when higher rainfall provides even more breeding habitat (Bureau of Meteorology, 2015).

Insects like mosquitoes have very little ability to maintain thermostasis and so are very sensitive to changes in temperature. They also require specific types of water bodies to breed in: stagnant or flowing, brackish or fresh, clean or dirty, small or large. Some species specialise in a range of temperatures and a specific type of breeding water conditions, while others are more generalist. This means that, as conditions change over time, one species’ abundance and range may increase, while others may shrink, thus changing the diversity of mosquito species- and mosquito borne pathogens- present in an area.

In the longer term, the distribution of mosquito species and the diseases they carry can be influenced by changes in climate. Mosquitoes are known to move out of areas where the climate is becoming less suitable (e.g colder and/or drier) and into areas with more hospitable conditions.

Modelling by Hongoh, *et al.* (2012) using current presence/ absence data for the species *Culex pipiens* (an important vector for West Nile and other arboviruses) in southern
Canada suggested that a few degrees of warming of the global climate increases the area of suitable habitat much further north and further inland. The study also suggested that rising temperatures would increase the survival rate of *Cx. pipiens* larvae, leading to an increase in the mosquito’s abundance as well.

Schaffner, *et al.*, in a 2013 study, described the distribution and abundance of five invasive mosquito species of the genus *Aedes*, which are established in Europe and the various viruses they are known to transmit in laboratory and field settings. They suggested the introduction of these invasive mosquitoes was the source of outbreaks of chikungunya and dengue fever in Europe in 2007 and 2010. Both of these years were unusually warm with heatwaves occurring in Europe in the summer. Schaffner, *et al.* suggested that warming temperatures lead to an increase in the abundance of invasive *Aedes* species and warned that as the climate warms, these outbreaks may become more common. This is a view supported by Beckers (2008) who investigated how mosquito abundance had changed in past decades in Germany’s Rhine Valley. Warming average temperatures were associated with a lengthening of the mosquito breeding season and an increased risk of arboviral disease transmission.

Gould and Higgs (2008) described changing global patterns of several arboviral diseases, including the mosquito-borne chikungunya, west Nile virus and Rift Valley fever virus. Chikungunya, a rarely fatal but often chronic disease that causes rashes, fever and joint pain, is spread by the species *Aedes aegypti* across much of Africa and Asia. In urban settings, particularly in Asia, the abundance of chikungunya is most closely linked to availability of container breeding habitat. Seasonal changes in rainfall and temperature tend to influence its incidence only in rural areas, suggesting that changes in climate will affect chikungunya incidence differently between urban and rural areas. Gould and Higgs also referred to the 2007 chikungunya outbreak in Europe mentioned by Schaffner *et al.* (2013) they suggested that the outbreak was due mainly to a mutant chikungunya strain adapting to infect *Aedes albopictus*- an invasive species present in southern Europe- rather than climate change expanding vector distribution.

Gould and Higgs (2008) cited Rift Valley fever as a much clearer example of a mosquito-borne arbovirus whose incidence is strongly affected by climate: The disease’s outbreaks in eastern Africa correlated strongly with changes in rainfall due to the El Niño/ Southern Oscillation phenomenon, suggesting that future climate change could greatly increase either its range or its incidence or both. Even just looking at the examples of
chikungunya and rift valley, it can be seen that any effect of climate change on rates of arboviral disease will vary between types of pathogen as well as by vector species.

In an Australian context, Kearney, et al. (2009) used weather data and information about known physiological parameters to produce a model to predict how the range and abundance of the dengue-carrying species *Aedes aegypti* (currently restricted to northern Queensland) is likely to change with a warming climate. They suggest that an increase in average temperature could extend *Ae. aegypti*'s current range southward, towards New South Wales. An increase in rainfall in the tropics could allow the species to spread westward into the Northern Territory; in both cases, potentially bringing dengue fever with it.

Many of the studies that investigate the role of climate change in changing rates of mosquito-borne diseases are ones that produce predictive models based on available mosquito presence and absence data. The exact nature of how mosquito-borne disease risks will change with climate is not as clear or predictable as these models may imply. Even if an area becomes more suitable as habitat for particular mosquitoes, it is still not guaranteed that a species will disperse into that area, or that it will bring any pathogenic organisms with it. In a 2009 review paper, Russell describes some of the limitations many predictive-model-based studies have in being able to realistically anticipate climate change’s effect on mosquito and pathogen distribution. He emphasises the complexity of the ecology of mosquito-borne disease and points out that, while some diseases may become more common in some areas, this may be offset by their decline elsewhere. Russell cites malaria as an example of a disease whose increase is more often a result of failing local control programs than any change in climate or weather conditions: Incidence of malaria infection worldwide correlates much more strongly with insufficient public health measures than with climate.

Beebe et al. (2009) suggested mosquito distribution may not be directly affected by climate change. Instead, the effect may be indirect; a result of changing human activity, rather than changes driven by mosquito species’ physical tolerance of temperature and moisture change. The proliferation of rainwater storage tanks as a means of reducing demand on mains water supplies during droughts lead to an increase in the amount of breeding water available for the dengue-transmitting species *Aedes aegypti*. If the installation and maintenance of these tanks is not properly controlled, their use could lead to an increase in the range and abundance of *Ae. aegypti* and thus increase the public health risk.
Shorter-term changes in mosquito abundance and distribution can be influenced by
many factors besides climate. Changes in land use also impact the abundance and
distribution of mosquito species. Changes in shade and nutrient run-off are often a result of
deforestation and changing agricultural practices. Leisnham et al. 2004 demonstrated the
flow-on effect this can have on mosquito populations in New Zealand. Manipulating the
amount of available sunlight and nutrients present in the mosquitoes’ breeding water
significantly influenced both how many mosquito larvae matured to adulthood and how
fast they did so.

Townroe and Callaghan (2014) described how the expansion of urban areas has been
changing that Britain’s mosquito fauna. Mosquitoes in highly urbanised areas were
generally less diverse but occurred in greater densities, due to factors such as the increased
presence of small water containers, such as plant pots and water butts, as well as the urban
heat island effect which keeps air and water temperatures higher in urban areas than rural
ones. Urban areas had a much larger mosquito population than rural areas. As many of
the world’s cities continue to grow and spread, this change in land use will likely change the
array of mosquito and pathogen species people are exposed to.

In an Australian context, Steiger, et al. (2011) sampled mosquito populations along
anthropogenic disturbance gradients from untouched rainforest to cleared grassland in
northern Queensland. The species assemblage in cleared grasslands was identical to that
found around the edges of the forest. Only in the interior of the forest was the species
assemblage any different. Seven mosquito species were only found in the disturbed habitat
outside of the forest interior, suggesting that the change in habitat brought about by
changing human land-use introduced new mosquito species and so changed the range of
insect vectors present in the area.

Shorter-term seasonal weather changes can alter the abundance and diversity of
mosquitoes. Mosquitoes’ sensitivity to changing temperature, moisture and breeding water
quality means their numbers can fluctuate over weeks and months as well as years and
decades.

Many species’ life-cycles speed up or slow down in response to changing
temperature and habitat availability. During warmer and wetter periods, eggs can hatch into
larvae and then emerge as adults over the course of a single week. When conditions are less
favourable, particularly as temperatures drop in autumn and winter, the rate of
reproduction slows. Once temperatures drop below a particular threshold (which varies
between species), some mosquitoes enter a state of hibernation, or diapause to wait until
conditions warm again, while others species just die off and leave their eggs or larvae to survive until the next season (Denlinger, et al., 2014, Webb, 2013).

Although mosquito numbers in general peak in summer and decline in winter, not all species’ number rise and fall at the same time or by the same amount. Each species follows its own pattern of boom and bust in a given year and between different years. Studies from the US (Chuang, et al., 2011, Walsh, et al. 2008), Malaysia (Rozilawati, et al., 2007), the UK (Medlock, et al., 2006), Argentina (Miceli & Campos, 2003) and many others show examples of different mosquito species responding to seasonal changes in different ways. In Australia, two common estuarine species Ae. vigilax and Cx. sitiens, have been shown to peak in abundance in Sydney two months apart from each other in February and April, respectively (Webb & Russell, 1999). Therefore, it is important to consider how seasonal changes affect species diversity as well as simple abundance.

Seasonal change can influence mosquito-borne disease risk, not only by changing the abundance and diversity of mosquitoes, but also by changing their behaviour. Oliveira et al. (2011) tested blood-fed Culex erraticus mosquitoes in Alabama in order to determine from which species the blood meals had come. The host preference of Cx. erraticus was shown to shift between avian and mammalian hosts from March to September of each year (Mendenhall et al., 2012).

Seasonal changes in temperature can also affect the behaviour of the pathogens themselves, not just of their mosquito vectors. Ruiz, et al. (2010) found that increasing air temperature was the strongest temporal predictor of increased infection of Culex mosquito species with West Nile virus in Illinois, USA. This study will focus on how seasons change mosquito species’ abundance, however it is important to note that seasonal changes can also affect disease risk by changing vector behaviour.

1.1.5. Spatial Habitat Change Influences Mosquito Species’ Diversity

The abundance and distribution of mosquito species changes according to environmental variation across space as well as in time. At the largest scales, a mosquito species can have a very wide distribution. In Australia, there is a much greater diversity of species present in the tropical north of the country than in the temperate south (Dobrotworsky, 1965), however, many of the species that feature in this paper, including Cx annulirostris, Ae. vigilax and Cx. sitiens are found widely across Australia and the Asia-Pacific (Department of Medical Entomology, 2010).
A given species may be found over a wide area, but its abundance can vary hugely over smaller, local distances. In most cases, adult mosquitoes do not venture far from the water body in which they hatched (Blaustein, et al., 2004), which means that the types of water bodies present in an area are the main factor in determining what adult mosquito species are present. Different species specialise in taking advantage of different breeding water habitat.

Breeding-water salinity is a major factor influencing the abundance and distribution of mosquito species. Certain species specialise in breeding in brackish water in environments like mangroves and saltmarshes. Different species’ eggs and larvae can survive in water with different levels of salinity. The brackish-water species, Culex sitiens, has been shown to have the highest larval survival rate in water that is 66% as salty as seawater, whilst the freshwater species Culex quinquefasciatus will die in water that brackish (Roberts, 1996). Because of this great range in salt tolerance, brackish, estuarine areas are likely to have a different assemblage of mosquito vector species than areas with more freshwater.

Permanent natural and constructed freshwater bodies, such as ponds, lakes and wetlands, provide habitat for freshwater-breeding mosquito species. In particular, they are favoured by species whose larvae need deeper fresh water with dense vegetation to live in. The larvae of some species, including those in the genera Coquillettidia and Mansonia, have modified siphons that enable them to attach to the submerged parts of aquatic plants. These species require more permanent vegetated water bodies, both to provide cover for their larvae to hide in and to over-winter when temperatures drop. Other species, like Anopheles annulipes prefer to breed in permanent water-bodies with floating mats of algae for their larvae to hide in (Webb, 2013). This requirement for water permanence and vegetation means that areas with freshwater lakes and wetlands are likely to have an array of species not found in other areas.

Not all freshwater species depend on permanent vegetated water to breed in. Some, like the extremely common Aedes notoscriptus, have adapted to breed in ephemeral pools and puddles (Montgomery & Ritchie, 2002). These species can develop fast and can adapt to small, shallow and stagnant water. These are the species that commonly take advantage of artificial containers, including water tanks, flower pots, blocked gutters and so on. Although container-breeding species can take advantage of water bodies in many different habitats, they are most abundant in highly urbanised areas, where small water containers are the only water available. In these environments, they do not need to compete with freshwater or
estuarine species and so make up a much larger percentage of the mosquito fauna (Armistead, 2012, Webb, 2013).

The geography of the Sydney metropolitan area contributes to its abundant and diverse mosquito population. The eastern part of the city is built around several river estuaries with abundant mangrove and saltmarsh habitat used by brackish-water species (Mitchel & Adam, 1989). Further west is both the upper, freshwater portion of the rivers as well as many freshwater lakes and wetlands, both natural and man-made. Newer housing developments in Sydney’s expanding western suburbs are often built around constructed lakes and wetlands designed to provide aesthetically pleasing green space as well as to improve drainage and provide habitat for wildlife. All of this provides abundant habitat for freshwater-breeding mosquito species (Russell, 1999).

The more heavily urbanised parts of the city provide an opportunity for container-breeding mosquitoes. By taking advantage of a city’s drains, flower pots, water tanks and puddles, container-breeding species particularly thrive in Sydney’s most urbanised central and eastern suburbs where there is less competition from freshwater and brackish-water breeding species.

This study will seek to build up a picture of how the species assemblage of mosquitoes changes across the Sydney area, taking into account how it changes between areas with estuarine, freshwater and urban container habitats and which species most influence these changes.

1.1.6. Zoonotic Disease and Mosquitoes

When a mosquito that has recently had a blood meal is trapped during a survey, genetic assays can be used to determine the identity of the host from whence the meal came. Many mosquito species have been shown to be able to take advantage of any blood-meal host available and will come into contact, both with humans and animals on a regular basis. A survey of blood meals from common mosquitoes in Western Australia (Johansen, et al., 2009) showed that, although some species showed preferences for birds or marsupials or large mammals, all regularly fed on a wide range of vertebrate species. The most common species in the study, Culex annulirostris and Aedes camptorhynchus are widespread across Australia and are well known to bite humans and transmit a variety of arboviruses.

A more extreme example of mosquitoes adapting to multiple hosts comes from a study of mosquitoes sampled in and around two different zoos in South Carolina, USA.
Nine common mosquito species were found to have fed on humans, local native wildlife and the exotic captive animals in the zoos, including mammalian, avian, reptilian and amphibian hosts. This included many examples of one mosquito containing a mixed blood-meal from two or more species. This study demonstrated mosquitoes’ ability to adapt to novel hosts as well as the potential variety of inter-species contact they can provide to pathogens.

The presence of a particular mosquito-borne disease in an area is often dependant, not only on the presence of its insect vectors, but also the presence of its major animal reservoir host. An example of this is the outbreak of Murray Valley Encephalitis in the Murray-Darling Basin in western NSW that occurred in the February of 2011: the virus was detected in sentinel chickens along the Murray River and several reported and suspected cases of MVE infection occurred in humans, including three deaths, that year. MVE, as mentioned above, is spread by the freshwater-breeding mosquito Culex annulirostris. 2010 - 2011 saw high rainfall and flooding across much of Australia, including the Murray-Darling Basin, as a result of a La Niña weather event. The rising floodwaters contributed to the outbreak of MVE in two ways: Firstly, the flooding increased the amount of breeding habitat available for Cx. annulirostris, leading to an increase in abundance of MVE’s primary vector, however the most influential factor that lead to the outbreak is believed to have been an influx of migratory waterbirds into flood-affected areas. Waterfowl are the main reservoir hosts for MVE, which is enzootic in the tropical north of Australia and tends to appear further south only during times when its reservoir hosts fly south to take advantage of new, flood-affected habitat (Knox, et al., 2012).

An example relevant to the Sydney region is the ecology of RRV: Outbreaks of RRV occur in the presence of macropods; their primary animal reservoirs. Common macropod species like swamp wallabies (Wallabia bicolor) and grey kangaroos (Macropus, subgenus: Macropus) are commonly infected with RRV in coastal regions of Australia (Russell, 2002). Even where the local mosquito fauna includes competent RRV vectors (Webb & Russell, 1999), if these reservoir hosts are not present, RRV will not be in circulation in the environment. A comparison of the frequency of RRV appearing in mosquito specimens from two different river estuaries in Sydney reveals this. The Georges River, in southern Sydney has areas of native vegetation on either side of the river, including national parks and a large military reserve. This abundance of habitat means the river supports a large population of native macropods. Mosquitoes trapped in the vicinity of the Georges River regularly test positive for Ross River RNA. The nearby Parramatta River is a similar estuarine system with
an identical mosquito species assemblage, also including competent RRV vectors. However, the river runs through the middle of Sydney and is much more heavily urbanised. The patches of vegetation along its shores are isolated by urban development on all sides and so are devoid of macropods. RRV has almost never been detected in the Parramatta River estuary as a result of the lack of reservoir hosts in the area (McLean, et al., 2015, Department of Medical Entomology, 2010).

In the case of both MVE and RRV, as with many mosquito-borne diseases, changing vector abundance and distribution is not the only factor that determines whether an outbreak of disease will occur or not. Many mosquito-borne diseases are zoonotic in origin. Even if their main vector species are abundant in an area, it is the presence or absence of their reservoir species that has the final say in whether or not they will present a risk to the human population.

The Australian white ibis (*Threskiornis molucca*) is another example of a native species that exist in great numbers in the Sydney area. In the last 30 years, ibis populations have increased both their abundance and distribution, expanding into urban areas to take advantage of the city’s parks, garbage dumps and rubbish bins. Their abundance in public space and recreation areas and lack of shyness around humans leads to frequent contact between ibis and humans (Martine, et al., 2012). Many wild avian species are known to harbour zoonotic disease, including mosquito-borne flaviviruses (Dickerman et al., 1976). Although it is still unclear if ibis are a reservoir species for any arboviral disease, there is evidence both that they can be infected by at least one arboviral disease of public health concern and that they are fed on by the same mosquito species that also target humans: In a study of ibis in Queensland, which tested blood samples from 88 ibis for viral RNA (Epstein et al., 2007), one tested positive for Kunjin, a mosquito-borne flavivirus similar to MVE in both taxonomy and the symptoms it causes. This was the only mosquito-borne arbovirus found in the ibis. Genetic analysis of mosquito blood meals from several Australian coastal cities, including Sydney, shows that common mosquito species frequently feed on different birds, including ibis (Jansen, et al., 2009).

This study was originally intended to include testing blood samples from urban ibis in Sydney to determine what vector-borne pathogens they may be carrying, but due to time restrictions this component of the study could ultimately not be included.

It is important to determine the species identity of the major reservoirs of arboviral diseases, as a given virus will only be hosted by certain species. The common brushtail possum (*Trichosurus vulpecula*) is another example of a species that has adapted really well to
William Crocker (4051087)

life in Australia’s cities. The possums thrive in woodlands and parklands in urban and peri-urban environments in much the same way that wallabies and gray kangaroos do. However, Hill et al.’s 2008 study into RRV exposure in Sydney’s urban possum populations found no RRV antibodies in any of the 82 possums tested. From this, it can be seen that the presence of macropods in a peri-urban area suggests that RRV might be present, but the presence of brushtail possums does not.

In order to better assess the potential risk of an outbreak of mosquito-borne disease in a given area, it is important to consider not only which vector species, but also which potential animal reservoirs may be present.

1.2. Introduction of Study

The Sydney region has a wide variety of mosquito species. Some of these are known to be efficient vectors for a variety of pathogens, while others pose no known health risk at all. As such, the assemblage of mosquito species present has a huge influence on what mosquito-borne pathogens are present. This study will look at how these species change in abundance and distribution across the metropolitan area.

A lot of the variation in mosquito species assemblage can be explained based on what breeding water habitat is present in an area. Different mosquito species specialise in breeding in permanent freshwater bodies, ephemeral pools and containers and brackish estuarine areas. Sydney has all three of these water types in abundance, so this study will compare how the mosquito species assemblage varies between areas with different available mosquito-breeding habitat.

Mosquito populations also change in response to variations in weather over time. Different species’ abundances respond differently to changes in temperature and rainfall. This means that the public health significance of mosquitoes is not static. Mosquito vector diversity and abundance varies over time as well as spatially. This study will account for these changes by surveying the changing diversity of mosquitoes over three months, starting in February, typically the peak of Sydney mosquito season.

Although Sydney no longer experiences outbreaks of mosquito-borne diseases like malaria that are lethal in humans, outbreaks of diseases like Ross River fever and Barmah Forest still affect the population. Diseases that are lethal to animals, such as Kunjin in horses and heartworm in dogs are still present. The many mosquito species in the Sydney region spread a variety of pathogenic organisms. Most common of all are the alphaviruses and
flaviviruses. These viruses vary greatly in rates of infection and the severity of the diseases they cause. The alphavirus, Ross River can have outbreaks of several hundred cases and produces debilitating fever and pain. By contrast the flavivirus, Edge Hill, was implicated only once in causing disease symptoms in a human host. This diversity of viruses, some dangerous, others benign, means it is important to keep track of the variety of which viruses are present where so as to better assess the public health risk. This study will include assays to detect what alphaviruses and flaviviruses that mosquitoes from the surveyed areas might be carrying and how that changes between habitat types and over the three-month study period.

1.3. Study Aims

1. To investigate the differences in mosquito assemblages across different water bodies in Sydney.
2. To investigate how variation in mosquito species assemblages varied from February to April at these sites.
3. To identify the presence of flaviviruses in different mosquito species at different sites.
Chapter 2: Methods

2.1. Study Sites

A total of 11 study sites were chosen from across the Sydney metropolitan region on the basis that they represented important roost or foraging sites for Australian White Ibis and that there were known potential mosquito habitats (i.e. natural or constructed wetlands) in the nearby area. These selection criteria were applied to candidate study sites with reference to published literature and discussion with experienced bird, mosquito and wetland researchers. A summary of these sites in the Sydney region is provided in Table 2.1 and Figure 2.1.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Location (Lat/Long)</th>
<th>Water type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deepwater Park, Milperra</td>
<td>33°56'57.5&quot;S 150°58'36.8&quot;E</td>
<td>Estuarine</td>
</tr>
<tr>
<td>Landing Lights Wetlands, Arncliffe</td>
<td>33°56'35.6&quot;S 151°09'10.2&quot;E</td>
<td>Estuarine</td>
</tr>
<tr>
<td>Sydney Olympic Park, Homebush</td>
<td>33°50'34.2&quot;S 151°04'48.5&quot;E</td>
<td>Estuarine</td>
</tr>
<tr>
<td>Driftway Reserve, Pemulwuy</td>
<td>33°48'38.4&quot;S 150°55'53.1&quot;E</td>
<td>Freshwater</td>
</tr>
<tr>
<td>Lake Annan, Mount Annan</td>
<td>34°03'17.4&quot;S 150°45'37.0&quot;E</td>
<td>Freshwater</td>
</tr>
<tr>
<td>Lake Gillawarna, Lansdowne</td>
<td>33°54'25.0&quot;S 150°58'52.6&quot;E</td>
<td>Freshwater</td>
</tr>
<tr>
<td>Mundurama Reserve, Ambarvale</td>
<td>34°05'50.6&quot;S 150°47'09.3&quot;E</td>
<td>Freshwater</td>
</tr>
<tr>
<td>Nurragingy Reserve, Doonside</td>
<td>33°45'38.3&quot;S 150°51'29.3&quot;E</td>
<td>Freshwater</td>
</tr>
<tr>
<td>Centennial Park, Sydney</td>
<td>33°54'03.1&quot;S 151°14'01.8&quot;E</td>
<td>Urban</td>
</tr>
<tr>
<td>Rockdale Bicentennial Park</td>
<td>33°57'47.8&quot;S 151°08'45.2&quot;E</td>
<td>Urban</td>
</tr>
<tr>
<td>Royal Botanic Gardens, Sydney</td>
<td>33°51'51.5&quot;S 151°13'00.9&quot;E</td>
<td>Urban</td>
</tr>
</tbody>
</table>

Table 2.1: Table showing the names, locations and water types of the sites where mosquito trapping in this study was carried out.
2.2. Mosquito Collection

Adult mosquitoes were collected at each of the 11 sites on three separate occasions; 10-11 February, 11-12 March and 8-9 April 2015. These sampling periods were selected to coincide with the warmer periods of the year when mosquitoes were most likely to be active while also sampling approximately 10 days or more following environmental triggers for increased mosquito abundance (e.g. rainfall or tidal flooding of estuarine wetlands). Mosquitoes were sampled using carbon dioxide baited encephalitic virus surveillance (EVS) traps (Rohe and Fall 1979). At each of the 11 study sites, four replicate trap sites were established. The location of individual trap sites was determined based on areas where mosquitoes were likely to seek refuge that were humid, away from wind and direct sunlight and there was approximately 200 to 300m spacing between each trap site. The EVS traps consisted of a metal bucket insulated with Styrofoam and half-filled with dry ice (approximately 500g). The traps were hung, 0.5 to 1m above the ground, on chains from poles, tree branches, fences and similar structures already present at sites. Small holes
drilled in the bottom of the cans allowed gaseous CO\textsubscript{2} from the sublimating dry ice to slowly escape. Hooked to the underside of each can was a small section of plastic pipe, containing a small battery-powered fan. The bottom half of this pipe was inserted into a tube, or ‘sock’ of fine plastic netting, at the bottom of which lead into a plastic container. Host-seeking mosquitoes, looking for an animal to feed on, were attracted to the CO\textsubscript{2}. When they approached the trap, they were sucked into the tube by the fan, through the sock and into the plastic container beneath (see figure 2.2). On each collection occasion, the traps were set out in the afternoons, left overnight and collected again in the morning.

**Figure 2.2**: Diagram describing the type of trap used to catch mosquitoes. Mosquitoes were attracted to CO\textsubscript{2} emanating from the dry ice in the trap. They were then sucked by the fan into the container below. Four of these traps were hung from trees/ fences, etc. at each site by the chain at the top of the bucket.

CO\textsubscript{2}-based mosquito traps, such as those used in this study, are commonly used by studies into mosquito ecology as they capture mosquitoes intact and attract very little bycatch. Female mosquitoes use the scent of CO\textsubscript{2} from exhaling animals as a way of finding

Each round of trapping took three days: On the first day, four traps each were set out at Nurragingy, Driftway, Lake Gillawarna, Deepwater Park and Lake Annan. On the second day the traps were retrieved, emptied and their dry ice replenished. They were then set out again at Olympic Park, the Royal Botanic Gardens, Centennial Park, Landing Lights, Rockdale Bicentennial Park and Mundurama Reserve. On day three, the traps were collected from the last sites and emptied again. This three-day procedure was carried out three times: the 10\textsuperscript{th} and 11\textsuperscript{th} of February, 11\textsuperscript{th} and 12\textsuperscript{th} of March and 8\textsuperscript{th} and 9\textsuperscript{th} of April 2015.

Once trapped, the mosquitoes were brought back to the lab and killed by placing them in the freezer. They were then identified by species, using the taxonomic keys of Russell 1990, and sorted into tubes of up to 100 individuals per tube. Mosquitoes were pooled together from all four traps at each site from each trapping date.

2.3. Arbovirus Testing

To determine the key mosquitoes species most likely involved in local arbovirus transmission cycles, a review of literature reporting isolation of arboviruses from field collected specimens was conducted. In addition, the results of previous years’ arbovirus surveillance work by Medical Entomology (Pathology West – ICPMR Westmead) were used to determine the study sites and mosquito species, most likely involved in local arbovirus transmission cycles. In order to efficiently use available time and resources, samples from these sites were tested first, with the intention of testing the remaining sites if these first ones tested positive. The sites chosen were Deepwater Park, Sydney Olympic Park (both saltmarsh), Pemulwuy (freshwater), Centennial Park (urban) and Lake Gillawarna (freshwater). The species chosen were those known to be competent arboviral vectors as well as those abundant at most sites. They were: Cx. annulirostris, Ae. vigilax, Ae. notoscriptus, Cx. sitiens and Ae. procax. A maximum of 100 mosquitoes were tested per pool. The number of species and number of sites chosen lead to 79 pools being tested (see appendix 1).

Each selected tube of mosquito specimens had four plastic beads added to it along with 2mL of Phosphate-Buffered Saline (PBS). The tubes were shaken for forty minutes until the mosquitoes were thoroughly pulverised into a liquid. The tubes were then centrifuged to settle the remaining solids so that the liquid supernatant could be extracted. Neat samples of supernatant and samples diluted with distilled water to a 1:10 concentration were used to test for virus RNA.
RNA was extracted from the samples using a QIAGEN EZ1 Mini Virus Kit v 2.0 according to manufacturer’s instructions. Viral RNA was detected using two different 1-step real-time TaqMan qRT-PCR assays: one with probes for three different alphaviruses: Ross River (RRV), Barmah Forest (BFV) and Sindbis (SINV), the other for the flaviviruses Murray Valley Encephalitis (MVE), Kunjin (KUN), Kokobera (KOKV), Edgehill (EHV) and Stratford (STRV).

2.4. Weather Data
The Bureau of Meteorology records for average maximum and minimum temperature and total rainfall were obtained for January, February, March and April of 2015- the three months of trapping as well as the month before. This data was obtained from the Sydney Airport, Bankstown Airport, Campbelltown, Prospect Dam, Olympic Park and Observatory hill weather stations as these are the stations closest to the study sites. Tidal data was also obtained for Sydney (Fort Denison). The mean maximum and minimum temperatures and total rainfall for each month were obtained from each station. The data from all the stations were then averaged together to show the average minimum and maximum temperatures and average total rainfall for each month.

2.5. Statistical Analysis
Analysis was done using JMP Pro 11 and PRIMER 7 software.

A PERMANOVA analysis was used to determine if the differences in trapping months and site water types had a significant influence on the species assemblage of mosquitoes. SIMPER analysis was used to show which species were most characteristic of water types or months. The differences in species assemblages from the three site water types were then visualised using a non-metric multi-dimensional scaling (MDS) graph.

Mosquito abundance data was log transformed to meet assumptions of normality. The effect of trapping month and site water type on mosquito abundance was tested using repeated-measures MANOVA. The sphericity chi-squared test was not significant, therefore unadjusted univariate F-test statistics are reported. This same MANOVA design was also used to test the effect of month and water type on the abundances of the six influential species found in the SIMPER analysis. Species richness did not vary as extremely as mosquito abundance, so those data did not need to be log-transformed.

Testing for virus RNA produced very few positive results so these were simply listed. For the full data set for all 84 pools, see Appendix 1.
Chapter 3: Results

3.1. Weather & Tide Data

Average monthly temperatures declined from January to April (Figure 3.1). Rainfall was highest in April, lower in January and lowest in February and March (Figure 3.2). The maximum tide height during the February trapping round was 1.4m, in March it was 1.5m and in April 1.65m (Figure 3.3).

![Monthly Average Temperatures](image1)

*Figure 3.1 (left):* Average maximum and minimum temperatures for the three months of trapping and the month before. Mean temperatures were averaged out from the Bureau of Meteorology station nearest the study sites.

![Monthly Total Rainfall](image2)

*Figure 3.2 (left):* Total monthly rainfall for the three months of trapping and the month before. Rainfall was averaged out from the Bureau of Meteorology station nearest the study sites.
Figure 3.3 (above): Predicted maximum tide heights for Sydney (Fort Denison). Dates of trapping are shown with dotted lines. Data from the Bureau of Meteorology:
3.2. Mosquito Abundance

A total of 13,982 mosquitoes were trapped. Mosquito abundance changed over the three months which varied depending on the type of water body at the site. Trapping month and water type both had a significant effect on mosquito abundance (see Table 3.1). Urban and freshwater sites behaved very similarly, changing only slightly over the three months. Estuarine sites behaved somewhat differently with a much greater abundance of mosquitoes in February, dropping rapidly in March and then falling in a similar way to the other site types in April (see figure 3.4).

Figure 3.4 (left): Graph showing how mosquito abundance changed over the three rounds of trapping from February to April at Freshwater, Urban and Estuarine sites. Error bars are standard error.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>2</td>
<td>6.4048</td>
<td>0.0018*</td>
</tr>
<tr>
<td>Water type</td>
<td>2</td>
<td>8.6798</td>
<td>0.0123*</td>
</tr>
<tr>
<td>Month × Water type</td>
<td>4</td>
<td>5.3246</td>
<td>0.0003*</td>
</tr>
<tr>
<td>Error</td>
<td>602</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 (Above): Results of a repeated-measures MANOVA analysis of the effect of trapping month and site water type on the average abundance of mosquitoes (log transformed). Asterisks indicate a significant result.
3.3. Mosquito Species Richness

A total of twenty mosquito species were counted. Estuarine sites were more species-rich than freshwater or urban ones across the entire study period (Figure 3.5). Trapping month had the most significant effect on species richness, which declined at freshwater and estuarine sites, but remained constant at urban sites. Freshwater sites were more species-rich than urban ones in February and March but had fewer species on average in April. Despite both water type and month of trapping being significant, there was no significant interaction between the two (see table 3.2).

![Figure 3.5](graph.png)

*Figure 3.5 (left):* Graph showing how mosquito species richness changed over the three rounds of trapping from February to April at Freshwater, Urban and Estuarine sites. Error bars are standard error.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>2</td>
<td>9.0902</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Water type</td>
<td>2</td>
<td>4.6212</td>
<td>0.0463*</td>
</tr>
<tr>
<td>Month × Water type</td>
<td>4</td>
<td>2.0710</td>
<td>0.0891</td>
</tr>
<tr>
<td>Error</td>
<td>602</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 3.2 (Above):* Results of a repeated-measures MANOVA analysis of the effect of trapping month and site water type on the species richness of mosquitoes. Asterisks indicate a significant result.
3.4. Mosquito Community Composition

There was a significant change in species assemblage between the three months and the three water body types, but there was no significant interaction between the two factors (see table 3.3).

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>Pseudo-F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>2</td>
<td>2.2677</td>
<td>0.011*</td>
</tr>
<tr>
<td>Water type</td>
<td>2</td>
<td>3.9714</td>
<td>0.010*</td>
</tr>
<tr>
<td>Site (water type)</td>
<td>8</td>
<td>7.1208</td>
<td>0.001*</td>
</tr>
<tr>
<td>Month x Water type</td>
<td>4</td>
<td>1.1894</td>
<td>0.251</td>
</tr>
<tr>
<td>Month x Site (Water)</td>
<td>16</td>
<td>3.3895</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Table 3.3 (Above):* Analysis of the effect of water type (saltmarsh, freshwater or container) on mosquito community composition using PERMANOVA. Asterisks indicate significant results.

**Figure 3.6 (Above):** A non-metric multi-dimensional scaling (MDS) graph showing the relative similarity of mosquito species assemblage in each sample to that of every other sample, separated by the type of water bodies present at the site. Based on the PERMANOVA results described in Table 3.4. All three trapping sessions’ data are pooled.
The range of species trapped in February was significantly different to those in March but not in April. March and April were not significantly different. Freshwater and urban sites were not significantly different. Estuarine sites were significantly different from both freshwater and urban sites (Table 3.4).

<table>
<thead>
<tr>
<th>Months</th>
<th>t</th>
<th>p</th>
<th>Water Types</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>February and March</td>
<td>2.14</td>
<td>0.008*</td>
<td>Freshwater and Urban</td>
<td>1.19</td>
<td>0.171</td>
</tr>
<tr>
<td>February and April</td>
<td>1.33</td>
<td>0.112</td>
<td>Freshwater and Estuarine</td>
<td>2.11</td>
<td>0.037*</td>
</tr>
<tr>
<td>March and April</td>
<td>1.22</td>
<td>0.195</td>
<td>Urban and Estuarine</td>
<td>2.97</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

Table 3.4 (above): Table showing the results of PERMANOVA pair-wise tests comparing how different the species assemblages of mosquitoes are between the three different months of trapping and between the three different site water types. Asterisks indicate a significant result.

When the assemblage of species at freshwater, urban and estuarine sites were compared with each other via SIMPER analysis, six species were found to have made a significant contribution to the differences in species assemblage between all three water types. These were *Culex annulirostris*, *Aedes notoscriptis*, *Cx. quinquefasciatus*, *Cx. sitiens*, *Coquillettidia linealis* and *Ae. vigilax* (Table 3.5).

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>% Contribution to difference between water types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freshwater &amp; Urban</td>
</tr>
<tr>
<td><em>C. annulirostris</em></td>
<td>22.17</td>
</tr>
<tr>
<td><em>Ae. notoscriptus</em></td>
<td>24.27</td>
</tr>
<tr>
<td><em>Ae. vigilax</em></td>
<td>5.06</td>
</tr>
<tr>
<td><em>Cq. linealis</em></td>
<td>7.29</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>11.28</td>
</tr>
<tr>
<td><em>Cx. sitiens</em></td>
<td>8.75</td>
</tr>
</tbody>
</table>

Table 3.5 (above): Table showing what contribution each of the most influential species made to the differences in species assemblage between freshwater and urban sites, Freshwater and estuarine sites and estuarine and urban sites.
Cx. annulirostris, Ae. Vigilax, Cq. linealis and Cx. sitiens were most abundant at estuarine sites. Cx. quinquefasciatus was most abundant at freshwater sites and Ae. notoscriptus was most abundant at urban sites (Table 3.6). For the full results of the SIMPER analysis, see Appendix 2.

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Average Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban</td>
</tr>
<tr>
<td>Cx. annulirostris</td>
<td>1.65</td>
</tr>
<tr>
<td>Ae. notoscriptus</td>
<td>2.48</td>
</tr>
<tr>
<td>Ae. vigilax</td>
<td>0.31</td>
</tr>
<tr>
<td>Cq. linealis</td>
<td>0.52</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>0.61</td>
</tr>
<tr>
<td>Cx. sitiens</td>
<td>0.63</td>
</tr>
</tbody>
</table>

*Table 3.6 (above):* Table comparing the relative average abundances at the site water types of the six species that contributed to the differences in species assemblage between all three water types.
3.5. Influential Species’ Abundance

Each of the six influential species behaved differently between water types and over the course of the three months (see Figure 3.7). Trapping month had a significant effect on the abundance of *Ae. notoscriptus*, *Ae. vigilax* and *Cx. annulirostris*. Site water type significantly influenced *Ae. notoscriptus*, *Ae. vigilax* and *Cx. sitiens*. *Coquillettidia linealis* was the only one of the six most influential species that was not significantly influenced by either water type or trapping month (see Table 3.7).

*Figure 3.7 (above)*: Graphs depicting the change in abundance of the six mosquito species that influenced the dissimilarity of all three site water types to each other. The changes in abundance are shown over the three trapping dates and for each water type. Error bars are standard error.
### Table 3.7 (above): Results of a repeated-measures MANOVA analysis of the effect of trapping month and site water type on the average abundance of the six mosquito species that were influential in producing differences in species assemblage between the three water types. F-ratios are G-G adjusted. Asterisks indicate a significant result.
3.6. Viral RNA

None of the 79 samples tested positive for the alphaviruses, Ross River, Barmah Forest or Sindbis. Seven of the pools of samples tested positive for flavivirus RNA. Sequencing then showed this belonged to Stratford Virus (STRV) (see table 3.8). See Appendix 2 for a complete list of the PCR test results, including the sites, trapping month and mosquito species.

<table>
<thead>
<tr>
<th>Site</th>
<th>Trapping Month</th>
<th>Mosquito Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deepwater</td>
<td>February</td>
<td>Ae. procax</td>
</tr>
<tr>
<td>Deepwater</td>
<td>February</td>
<td>Ae. vigilax</td>
</tr>
<tr>
<td>Deepwater</td>
<td>February</td>
<td>Ae. vigilax</td>
</tr>
<tr>
<td>Deepwater</td>
<td>April</td>
<td>Cx. annulirostris</td>
</tr>
<tr>
<td>Sydney Olympic Park</td>
<td>March</td>
<td>Ae. vigilax</td>
</tr>
<tr>
<td>Lake Gillawarna</td>
<td>February</td>
<td>Ae. vigilax</td>
</tr>
<tr>
<td>Lake Gillawarna</td>
<td>March</td>
<td>Ae. vigilax</td>
</tr>
</tbody>
</table>

*Table 3.8 (above):* Listing of the site, month of collection and species of the pooled mosquito samples that tested positive for STRV, the only virus to test positive in this study
Chapter 4: Discussion & Conclusions

4.1. Urban & Freshwater Sites not Distinctly Different

Urban and freshwater sites were expected to have different abundance and diversity of mosquitoes. Instead, the two water types’ mosquito fauna was not significantly different in terms of abundance (Figure 3.4), species richness (Figure 3.5) or species assemblage (Table 3.4).

The sites chosen as “urban” and “freshwater” sites were quite similar but all urban sites were closer to the CBD of Sydney while the freshwater sites were more westerly. The “urban” sites (The Royal Botanic Gardens, Centennial Park and Rockdale Bicentennial Park) all have vegetated ornamental ponds and other nearby water bodies. Some of the traps set out in Centennial Park were hung up in the paperbark swamps in the area, whilst the Rockdale Bicentennial Park traps were hung up in the immediate proximity to a large, well-shaded stagnant pond. Given the sheer abundance of freshwater mosquito habitat at these sites, it is not surprising that they should be virtually identical to the sites designated “freshwater”. Future studies should consider trapping mosquitoes away from such green spaces in order to determine if highly urbanised areas have a distinct suite of mosquito species or not. It also is important to note that this study trapped only adult mosquitoes. Even if the larvae have specific habitat requirements, a matrix of different larval microhabitats in close proximity is likely to produce a more diverse adult mosquito fauna in a given area.

The nature of the mosquito species themselves may also contribute to the similarity between “urban” and “freshwater” sites. The distinction between a “freshwater” and “container-breeding” species is not as clear as that between brackish and freshwater species. In general, still freshwater provides sufficient breeding habitat for most mosquito species. Whilst some might prefer deeper water to hide in, or vegetation to attach to, or smaller ponds with less competition, many mosquitoes can breed in various freshwater conditions. As can be seen in table 3.7, influential species like Coquillettidia linealis and Culex annulirostris numbers were not significantly influenced by water type and they were not distinctly more or less abundant in any one water type than any of the others.

One implication to be taken from these findings is that even the most urbanised areas of central Sydney have the same range of mosquito disease vectors present as areas on the outskirts with natural water bodies and remnant bushland. Sydney’s abundant green spaces, remnant vegetation and natural and artificial water bodies means that even the very middle
of the city has a range of freshwater-breeding mosquito species present. Any differences in the arboviruses present in the environment will therefore not be due to the available vectors, but instead caused by factors like the presence of reservoir host animals.

More broadly, these findings highlight the fact that mosquito habitat is rarely homogenous, especially in cities like Sydney with plentiful green space. Urban and peri-urban areas contain a matrix of mosquito habitats. It is important to use trapping surveys like the one in this study to see what mosquitoes are present whereas this is difficult to predict based on environmental factors alone. Although it may be intuitive that vegetated lakes and swampy bushland have a greater range and abundance of mosquitoes than the inner city, the actual situation as regards mosquito-borne disease vectors may be quite different.

4.2. Estuarine Sites Differed from the Others

Mosquitoes at the estuarine sites were more abundant (Figure 3.4) and speciose (Figure 3.5) and had a significantly different assemblage of species (Table 3.4) than those at the other sites. Mosquito abundance overall did change with time, being much higher in February than March or April, however most of this difference occurred only in the estuarine sites. As discussed in section 1.1.5, some species’ larvae grow best in waters that are salty enough to kill other species (Roberts, 1996). The vast majority of Australia’s population live on or near the coast and almost all of its major cities are built around river estuaries. As such, it is important to understand how much the risk of mosquito-borne disease is likely to differ in estuarine areas from elsewhere.

The two common estuarine species *Aedes vigilax* and *Culex sitiens* were two of the species that had the greatest influence on differing species assemblage. *Ae. vigilax* and *Cx. sitiens* were by far the most common species in estuarine sites but were much less abundant elsewhere (Table 3.6). Despite both being abundant, these two species are very different in terms of the public health risks they pose. *Ae. vigilax* is a nuisance-biting pest and a major vector for disease-causing pathogens, most notably the alphaviruses Ross River (RRV) and Barmah Forest (BFV) (Kay, 1982, Dale et al., 2014). *Cx. sitiens*, on the other hand, is not widely considered to be any sort of threat or nuisance. Although it has been shown to be a potential vector for Japanese encephalitis in Asia (Vythilingham, et al., 2002) and in Australia has been able to transmit RRV in a laboratory setting (Fanning et al., 1992) the public health risk it poses is believed to be negligible. As the two most common estuarine mosquitoes are so different- one, a significant disease vector and nuisance biter, the other essentially
harmless- this finding emphasises the importance of understanding the diversity and ecology of each mosquito species rather than just the abundance of mosquitoes as a whole.

4.3. Change in Mosquito Diversity over Time

The results of this study emphasise that the way a mosquito population changes over time is highly variable, even between sites that are close together in the one city. Figure 3.4 shows that the average mosquito abundance did not change significantly from February to March to April at the urban and freshwater sites. By contrast, the estuarine sites saw high mosquito numbers in February that then dropped sharply by March to be the same as the other two sites from March to April. Other studies have shown that peaks in the number of estuarine mosquito species tend peak in summer when warmer temperatures and lower tides occur together and leave plenty of warm, brackish pools that remain out of reach for predatory fish due to low water levels (Kokkinn, et al., 2009, Carlson & Vigliano, 1985). The effect of predation on mosquitoes is an important factor in changing mosquito diversity; one which future studies should consider.

The six species examined in figure 3.7 are each examples of how changing environmental conditions affect each mosquito species in its own way. *Aedes notoscriptus* particularly stood out as behaving differently to the other mosquitoes: Its numbers dropped then rose again from February to April at estuarine and freshwater sites, but at urban sites its numbers remained constant. Despite the lack of difference in mosquito abundance and diversity overall between urban and freshwater sites, it would seem that the distinction between the two habitats does hold true for this particular species. Studies into the habitat preferences of *Ae. notoscriptus* show that this species can remain abundant despite changing temperatures so long as it has suitable breeding habitat. *Ae. notoscriptus* is known to be able to breed in almost any fresh water but thrives in small, shaded, stagnant containers with no competition or predators, such as plant pots, buckets and bromeliad axils (Kay, et al., 2008, Williams & Rau, 2011). The urban sites in this study; Centennial Park, the Royal Botanic Gardens and Rockdale Bicentennial Park, all had plenty of this type of habitat. The Royal Botanic Gardens is especially suitable for *Ae. notoscriptus* with its large, regularly watered bromeliad collection under the shade of many trees. The example of *Ae. notoscriptus* shows that, given the right conditions, at least some mosquitoes have the capacity to overcome the effects of seasonal weather changes and remain abundant where they otherwise would not. It is worth noting that, while the species richness at estuarine and freshwater sites showed a declining trend from February to April, urban sites did not do this; they were as species-rich in April as they were in February (see figure 3.5). It is entirely possible that human
modification of the environment, providing well-watered gardens with plenty of breeding habitat is serving to buffer certain species against the effect of seasonal weather change.

Ultimately, seasonal change in mosquito abundance and diversity is driven by a variety of factors that interact with and confuse the influence of changing temperature and rainfall. Particular habitat characteristics and the biology of a given mosquito species can alter which factors are important in regulating their numbers. The data from this study represent only three, brief snapshots of Sydney’s mosquito fauna. Higher-resolution data taken over multiple seasons would provide a clearer picture of any patterns in mosquito species’ abundance. Even so, it can be seen how complex the task of predicting mosquito vector diversity can be, with tremendous potential for variation across short distances in space as well as short intervals of time.

4.4. Why was only Stratford Virus Detected?

The only virus RNA detected in this study belonged to Stratford virus (Table 3.8). These results raise two questions: Firstly, why was only Stratford detected? Secondly, what does Stratford’s presence in the environment mean in terms of mosquito-borne disease risk in the Sydney region?

Public health surveillance regularly detects a variety of viruses in the Georges River and Homebush areas—which include the Deepwater Park, Lake Gillawarna and Sydney Olympic Park sites in this study. The most common virus isolates detected belong to RRV, with Stratford only appearing occasionally (Department of Medical Entomology, 2015). The lack of RRV detected in this study is doubly surprising given that during the three months when these specimens were collected, New South Wales saw its largest outbreak of RRV infections in years. The previous four years’ seasons peaked at about 100 notifications, but March of 2015 saw 447 RRV cases (NSW Health, 2015). There are several potential explanations for why this study detected so few viruses. The relatively small sample size and limited trapping occasions in this study may have reduced its ability to detect RRV.

There are also procedural differences between the regular public health surveillance testing and this study. This study used quantitative polymerase chain reaction (qPCR) testing on ground-up whole mosquito specimens. This method is time-consuming and requires a great deal of work identifying, sorting, pooling and processing mosquito specimens, all the while keeping specimens cold enough at every stage to prevent RNA degrading. Instead of testing of ground whole mosquitoes, public health monitoring of arboviruses in Australia mostly uses sugar-baited nucleic acid preservation cards (FTA
cards). Adult mosquitoes land on the FTA cards and feed off the sugar in them, expelling their saliva onto the card in the process. This saliva is then tested for common Australian arboviruses using qPCR. This method differs from testing whole ground mosquitoes at it is less time-consuming and because it is a more accurate way of assessing the public health risk mosquitoes pose: If an arbovirus is present in a mosquito’s body but is not in the salivary glands the mosquito will not be able to pass the virus on to a host. Testing ground whole mosquitoes can determine if the mosquitoes are infected but not if they are infectious. By testing expelled saliva instead of the whole mosquito, the FTA card method will determine if a mosquito is actually infectious rather than just infected. There is also a relative lack of evidence as to whether the FTA card method is more or less sensitive than testing ground whole mosquitoes (van den Hurk, 2012, Flies, 2015). Ultimately, the results of ground mosquito samples from this study are difficult to compare with public health records accumulated mainly via the FTA card method. The two methods are different enough that disagreeing results is inevitable. The FTA card method is better, however for assessing public health risk than the methods used in this study because it detects only infectious mosquitoes, rather than just infected ones.

Assuming only Stratford virus was present at the sites studied, the next question to ask is why. This study has repeatedly mentioned the most important factors that control the presence of a mosquito-borne virus in an area: the availability of mosquito vectors and the presence of its primary reservoir hosts. This study’s results clearly show that the necessary mosquito vectors were present: Stratford was found mostly in Ae. vigilax but also Ae. procax and Cx. annulirostris (see table 3.8), so the next thing to consider is reservoir hosts.

Relatively little is known about Stratford’s specific epidemiology in comparison to more well-studied (and dangerous) arboviruses. Flaviviruses are often associated with avian reservoir hosts. Waterbirds are known to be the primary hosts for flaviviruses like Murray Valley Encephalitis (MVE), Kunjin (KUN) and Japanese Encephalitis Virus (JEV) (Russell & Dwyer, 2000). However, Stratford’s closest relative, Kokobera virus (KOK), is most frequently associated with macropods, and to a lesser extent horses, rather than birds (Poidinger, et al., 2000, Nisbet, et al. 2005). It therefore seems likely that the presence of Stratford indicates the presence of reservoir hosts suitable for a variety of other arboviral diseases. Specifically which hosts may be harder to determine and will depend on whether Stratford behaves more like KOK (mammalian hosts) or like one of the other flaviviruses (avian hosts). One clue to this question is the detection of the one Stratford isolate at Sydney Olympic Park (SOPA). The rest of the isolates were found at Lake Gillawarna and
Both of these are by the Georges River, which has a population of macropods, particularly swamp wallabies. SOPA, however, is in the middle of Sydney’s urban area and lacks the habitat corridors that provide access to macropods. Native fauna surveys over fourteen years at SOPA have found many species of birds, but not one species of macropod (Sydney Olympic Park Authority, 2014). However, all the sites in this study do have abundant bird populations. This suggests that Stratford’s main reservoir hosts may be avian rather than mammalian, although it does not rule out the possibility of a domestic mammal host like dogs or horses.

The presence of Stratford and apparent absence of other arboviruses, in the local bird population could potentially be related. The phenomenon of cross-protective immunity has been observed in Australian flaviviruses: That is, the presence of one flavivirus in a host impedes the progress of another (Lobigs, et al. 2009). This can happen through the two viruses competing for nonimmune vertebrate hosts and because a successful host immune response to a milder virus can provide some immunity to its more virulent relatives. Examples of this phenomenon include the immunity to JEV observed in pigs previously exposed to KUN or MVE (Williams, et al., 2001) and the immunity of macaques to West Nile Virus when previously exposed to JEV (Goverdhan, et al., 1992). This raises the possibility that Stratford may be immunising Sydney’s bird population to more severe related flaviviruses.

Stratford virus may not be a direct threat to human health, but its presence in the environment does have a variety of implications. The presence of Stratford may serve as a warning that conditions are suitable for its other, more severe, flavivirus relatives. On the other hand, Stratford could be immunising its avian reservoir (or perhaps even human) hosts against worse flaviviruses and its presence in the environment is a blessing in disguise. Either way, these are possibilities that deserve investigation in future studies.

4.5. Conclusions
Mosquitoes and mosquito-borne pathogens exist in a complex relationship. Being able to understand and predict where and when mosquito-borne pathogens are likely to occur is vital in order to safeguard against outbreaks of disease.

Mosquito species differ so dramatically in their vector competence that a change in the assemblage of species in an area can be a great help or hindrance to the spread of any would-be epidemic. The assemblage of mosquito species in an area can vary greatly depending on environmental conditions, although exactly which conditions may not always be obvious. This study showed that mosquitoes in the very middle of Sydney were not
significantly different from those in peri-urban areas with nearby bushland. Brackish, estuarine areas had a significantly different mosquito fauna, in terms of abundance and diversity, than the other sites. This highlights the importance of considering estuarine areas separately when assessing the risk of mosquito-borne disease. This is of great importance in Australia, where the biggest population centres are on the coast.

The lack of arboviral RNA from any species other than Stratford may indicate one of three issues future studies should consider. Firstly, the different methods used to survey mosquito-borne viruses differ greatly in their sensitivity and the ability to detect infected versus infectious mosquitoes. Future studies might better assess whether the ground mosquito PCR or FTA card method is best for determining which viruses are actually present. Secondly, the presence of Stratford at Sydney Olympic Park suggests its primary reservoir host may be avian rather than mammalian. A future study should determine if Stratford is present in the local avian fauna in the Sydney area. Thirdly, the presence of Stratford and absence of other viruses raises the possibility that it may be providing some sort of cross-protective immunity in its reservoir hosts to other, nastier flaviviruses. This is an idea that certainly warrants investigation in future studies, as the presence of Stratford virus in an area may be an important indicator of disease risk, not because Stratford itself is a public health risk but because it may actually be protecting us from arboviral diseases.

Ultimately, this study is simply an exploration of the huge variety of factors that can influence mosquito-borne disease risk. It has shown just how much this can vary over even very small distances at local scales, even within the one metropolitan area. If any, one, solid conclusion is to be drawn it is that there is no guaranteed, one-size fits-all way of assessing the risk of mosquito-borne disease. Some mosquitoes can be a threat to us while others are harmless. Some environmental factors matter hugely in determining vector abundance and diversity, while others have no apparent effect whatsoever. Some viruses can infect and harm hundreds of people in a season, while others may actually be protecting us. This study has shown that, only through continuous and thorough monitoring and research can we learn to tell the difference.
4.6. Further Research Questions

This study has emphasised the need for further study to answer the following questions:

- Do heavily urbanised sites- without ponds, lakes, green spaces, etc.- harbour the same range of mosquito species as urban parks or bushland?
- What role do various bird species play as potential reservoir hosts of mosquito-borne pathogens?
- Is the presence of particular bird species related to the range of mosquito-borne pathogens in an area?
- What is the primary reservoir host for Stratford virus (avian, mammalian, etc.)?
- Does Stratford virus provide some cross-protective immunity to other flaviviruses in its hosts?
Chapter 5: References


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William Crocker (4051087)


Appendix 1: Details of Samples Tested for Arbovirus RNA

A total of 79 pools of mosquitoes were tested for the presence of arbovirus RNA.
None of the samples tested positive for the alphaviruses, Ross River, Barmah Forest or Sindbis. Seven of the pools of samples tested positive for flavivirus RNA. Sequencing then showed this belonged to Stratford Virus (STRV).

<table>
<thead>
<tr>
<th>Site</th>
<th>Trapping Month</th>
<th>Mosquito Species</th>
<th>No. Mosquitoes in Pool</th>
<th>Result for Flavivirus Primers</th>
<th>Result for Alphavirus Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centennial Park</td>
<td>April</td>
<td>Cx. annulirostris</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Centennial Park</td>
<td>February</td>
<td>Ae. notoscriptus</td>
<td>48</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Centennial Park</td>
<td>February</td>
<td>Cx. annulirostris</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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## Appendix 2: Complete SIMPER Analysis Results

### SIMPER

**Similarity Percentages - species contributions**

**Two-Way Analysis**

### Parameters

- Resemblance: S17 Bray Curtis similarity
- Cut off for low contributions: 90.00%

### Examines Month groups

(\textit{across all Water groups})

#### Group Feb

**Average similarity:** 51.34

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<th>Sim/SD</th>
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<th>Cum.%</th>
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#### Group Mar

**Average similarity:** 47.08

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#### Group Apr

**Average similarity:** 32.79

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#### Groups Feb & Mar

**Average dissimilarity:** 56.39

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#### Groups Feb & Apr

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<td>58.45</td>
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<td>Sim/SD</td>
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<td>Cum.%</td>
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Groups Mar & Apr
Average dissimilarity = 64.76

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<th>Cum.%</th>
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Examines Water groups (across all Month groups)

Group f
Average similarity: 36.98

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Group c
Average similarity: 52.52

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Group s
Average similarity: 57.69

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Groups f & c
Average dissimilarity = 64.27

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<th>Diss/SD</th>
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Groups f & s
Average dissimilarity = 70.50

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Page 58 of 59
William Crocker (4051087)
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Groups c & s
Average dissimilarity = 67.72

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