Mosquito (Diptera: Culicidae) communities across land uses in tropical Australia

The Ecology of Disease by Olaf Hajek

Thesis submitted by

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“Nature does nothing uselessly” (Aristotle 350BCE)

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

16th June 2016

(Signature) (Date)

Dagmar Meyer Steiger

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Chapter 2 investigated mosquito communities across an anthropogenic disturbance gradient in the wet and dry season in far north Queensland, Australia and is in press (Parasites & Vectors) as: *Mosquito communities and disease risk influenced by land use change and seasonality in the Australian tropics* by D.B. Meyer Steiger, S. A. Ritchie and S.G.W. Laurance. Dagmar Meyer Steiger conceived the idea, collected and analysed the data, and wrote the manuscript and the chapter. Susan Laurance helped develop the idea; Susan Laurance and Scott Ritchie assisted with the writing; Peter Woods produced the map of the study area (Figure 2.1); John Bosworth, Mark Jackson, Roger Steiger and Sandra Taylor assisted with field work; Paul Zborowski and John Clancy helped with species identifications.

Chapter 3 assessed which mosquito species utilised small artificial containers across an anthropogenic disturbance gradient in far north Queensland, Australia and is being prepared for submission as: *Land use change and breeding mosquitoes in tropical Australia: an experimental approach using small artificial oviposition containers* by D.B. Meyer Steiger, S. A. Ritchie and S.G.W. Laurance. Dagmar Meyer Steiger conceived the idea, collected and analysed the data, and wrote the chapter. Susan Laurance and Scott Ritchie helped with writing; Scott Ritchie and Chris Paton gave advice on the rearing of immature mosquitoes; Mick Townsend helped caring for the immatures in the laboratory. Roger Steiger and Mick Townsend assisted with field work.

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Chapter 5 evaluated a novel technique to sample mosquitoes in Torres Strait, Australia and has been published as: Meyer Steiger, D. B., S. A. Ritchie, and S. G. Laurance. 2016. *Land use influences mosquito communities and disease risk on remote tropical islands: a case study using a novel sampling technique*. American Journal of Tropical Medicine and Hygiene 94:314-321. Dagmar Meyer Steiger conceived the idea, collected and analysed the data and led the writing. Susan Laurance and Scott Ritchie helped conceive ideas and helped with the writing. Susan Laurance assisted with analysing the data. Roger Steiger helped constructing passive box traps. Bob Cooper, Paul Zborowski and John Clancy helped with species identifications. Kylie Anderson and Peter Wood helped with the map (Figure 5.1). Natalie Dillon taught me how to extract RNA from FTA® cards, Janani Jayanthan assisted with the extractions and Sonja Hall-Mendelin, Jamie McMahon, Jane Cameron, Doris Genge and Glen Hewitson from Queensland Health Forensic and Scientific Services in Brisbane carried out the PCR work. Susan Laurance, Signe Dalsgaard, Laila Herringer, Françoise Yoko Ishida, Michal Segoli and the rangers of Saibai, Boigu, Badu and Moa assisted with the field work.

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THESIS ABSTRACT

Mosquito-borne diseases cause mortality and morbidity worldwide. Diseases, such as malaria, dengue and yellow fever, have emerged or re-emerged in recent decades and are often induced by anthropogenic land use changes. Deforestation, road construction and conversions of land use (e.g. from natural environment to urbanisation or agriculture) are examples of human modified environments. These modifications alter original habitats and species compositions and, in relation to mosquito-borne diseases result in novel juxtapositions of vectors, hosts and pathogens. Hot spots for the emergence or re-emergence of mosquito-borne diseases are tropical regions due to high biodiversity, vast land clearances and human encroachment into these areas. Furthermore, remote, tropical regions are especially vulnerable to the emergence of mosquito-borne diseases as surveillance can be logistically demanding and expensive and thus prevent early disease detection.

My thesis examined if mosquito communities respond to land use changes in tropical Australia and additionally, if a newly developed technique to capture adult mosquitoes can be applied in remote localities. I explored mosquito communities across anthropogenic disturbance gradients in several ways: (1) capturing adult mosquito from three habitats (man-made grassland, forest edge and rainforest interior) during the wet and dry season in a peri-urban environment near Cairns; (2) sampling immature mosquitoes from small, artificial containers from the same habitats; and (3) capturing adult mosquitoes from urban and sylvan habitats from four remote islands (Saibai, Boigu, Badu and Moa) in the Torres Strait where I used a novel trap design.

To evaluate if mosquito communities are influenced by anthropogenic land use, I carried out adult mosquito sampling in three habitats (man-made grassland, rainforest edge and rainforest interior) in the outskirts of Cairns and in two habitats (sylvan and urban) in the Torres Strait. I found that adult mosquito communities varied in response to anthropogenic modified habitats in both locations. The Cairns sampling revealed that the mosquito community from rainforest interior was distinctly different to the grassland community and that forest edge acted as an ecotone with shared communities from both forest interior and grasslands. I also found that important vector species (*Aedes vigilax*, *Culex annulirostris*) were able to persist all year round and occurred mainly in grasslands (Chapter 2). A similar pattern was evident from the Torres Strait sampling where urban and sylvan habitats supported distinctly different mosquito communities with disease-competent species, such as *Aedes albopictus*, *Aedes aegypti and Culex quinquefasciatus* occurring more in urban areas than in sylvan habitats (Chapter 5).

I sampled immature mosquitoes from small, artificial ovitraps across the three habitats in Cairns and from two trap locations (traps were either placed on the ground or above ground) to evaluate female oviposition preferences. I found that most species chose to lay their eggs in grassland traps and that none of the species preferred to oviposit in forests traps. I also found that traps located on the ground had four times more emergents than traps located above ground. *Aedes notoscriptus*, an important disease vector, was mostly reared from grassland traps. Additionally, I observed that water temperature (ranging between 13.7°C and 43.5°C) had no influence on the number of emergents and that mosquito eggs were able to hatch in instalments (Chapter 3).

Mosquito sampling in remote areas poses unique challenges for disease surveillance and detection. Sampling female mosquitoes is heavily dependent on attractants to lure them into traps. Carbon-dioxide (CO_2), in the form of dry ice or from gas cylinders, is

commonly used. However, dry ice is unavailable in remote areas and gas cylinders are difficult or even prohibited to transport. I therefore aimed to assess the usefulness of CO_2 derived from sugar and yeast as an attractant and trialled different CO_2 concentrations in temperature controlled experiments. The concentrations which produced the most CO_2 were then compared to dry ice in field situations (in three tropical forest habitats). I found that traps baited with dry ice captured more mosquitoes than yeast-baited traps; but more importantly that there were no differences in the mosquito community composition (Chapter 4). An additional challenge is that most mosquito traps require a source of electricity which is rarely obtainable in remote field locations. I developed a novel sampling technique to capture mosquitoes by coupling a non-powered trap with CO_2 derived from sugar/yeast fermentation (Chapter 5).

Conventional disease detection methods involve sentinel animals, such as chickens or pigs, or large pools of dead mosquitoes which can be very expensive and labour-intense in remote areas. A suitable alternative are honey-soaked Flinders Technology Associates cards (FTA® cards) which preserve viruses but at the same time deactivate them. Mosquitoes taking a honey-meal from the FTA® cards expel saliva which can be used for disease detection by eluting viral RNA. I used four FTA® cards in each of the non-powered traps in the Torres Strait and even though weak infections were initially detected, they were not significant (Chapter 5).

In summary, my thesis demonstrates that mosquito communities in peri-urban environments and on remote islands in tropical Australia are strongly influenced by land use. This could have potential impacts for disease transmission to humans, domestic animals and wildlife, especially where immense anthropogenic pressures continue to change natural environments irrevocably. Strong projected growth in

human population and the subsequent demand for space will further impact already fragile environments.

My thesis may contribute to achieve a more cost-effective and logistically less demanding method to monitor mosquitoes in remote localities and thus allowing permanent and continuous disease surveillance.

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CHAPTER 1

Introduction

1.1 Anthropogenic land use changes

Anthropogenic land use changes, such as the conversion of natural landscapes into agricultural lands or urbanisations, are largely due to demands of rapidly growing human populations (Dirzo and Raven 2003). Other events which lead to anthropogenic land use changes, especially in developing nations, are government initiatives of in- and trans-migrations into sparsely populated frontier areas, like rainforests. The main purpose of these migrations is to exploit natural resources, but an accompanying feature is the construction of houses, roads and powerlines which further decimate forests (Lambin et al. 2001). The encroachment of humans into these areas and the resulting land use modifications can have additional repercussion including changes to existing pathogen-vector-host relationships which can lead to zoonotic diseases where pathogens or vectors move across the species barrier (Schrag and Wiener 1995, Daszak et al. 2001).

1.2 EIDs – Emerging infectious diseases

Zoonotic diseases comprise up to 75% of the global burden of emerging infectious diseases (Grace et al. 2013). Morse and Schluederberg (1990) defined emerging infectious diseases (EIDs) as: infections which have newly appeared in a population or are rapidly increasing in incidence, geographic range or are caused by newly-evolved pathogens. Examples of EIDs include dengue (Morse 1995), ebola (Jones et al. 2008),

avian malaria (van Riper et al. 1986), amphibian chytrid (Skerratt et al. 2007) and yellow fever (Mackenzie et al. 2004).

EIDs are often linked to anthropogenic land use changes (Table 1.1). These land use changes cause habitat transformations by disrupting existing species compositions and thus, resulting in novel interactions between pathogens, vectors and hosts (Patz et al. 2000, Wolfe et al. 2000, Wilcox and Gubler 2005). Human infections with sylvatic yellow fever are one such example of novel interactions between pathogens, vectors and hosts. The change in transmission cycle occurs because mosquitoes no longer obtain their blood-meals from monkeys in the upper layers of the rainforest, but instead move to the understorey in logged forests and feed on humans, potentially transmitting the yellow fever virus (Strode 1951, Spielman and D'Antonio 2001, Barrett and Monath 2003). Furthermore, environmental modifications due to anthropogenic land use changes often generate highly favourable conditions for mosquito vector species. Deforested areas, for example, create open habitats which receive more sunlight than forested habitats (Camargo and Kapos 1995). More sunlight and warmer conditions can increase mosquito densities owing to faster rates of immature development and higher availability of food resources (Rejmankova et al. 1996, Bayoh and Lindsay 2003, Ye-Ebiyo et al. 2003).

Table 1.1: Emerging and re-emerging infectious diseases grouped according to land use change. Bold lettering indicates diseases transmitted by mosquitoes.

Urbanisation	Deforestation	Agricultural Practices
Malaria	**Malaria**	**Malaria**
Dengue	**Eastern equine encephalitis**	**Japanese encephalitis**
Chikungunya	**Yellow fever**	**St. Louis encephalitis**
Epidemic polyarthritis	**La Crosse encephalitis**	**West Nile fever**
West Nile fever	Kyasanur Forest disease	**Western equine encephalitis**
St. Louis encephalitis	Lyme disease	**Venezuelan equine encephalitis**
Lyme disease	Loiasis	Oropuche fever
Plague	Leishmaniasis	
	Onchocerciasis	

adapted from Gubler 1998

1.2.1 Mosquito-borne diseases

Mosquito-borne pathogens cause malaria, dengue, yellow fever, encephalitis, filariasis and other medical conditions (Becker et al. 2010). Malaria was until recently the most important mosquito-borne disease. It used to account for 300 – 500 million clinical cases and over a million deaths per year. In 2015, there were 214 million reported cases and approximately 450’000 deaths worldwide (WHO 2015). It now appears that dengue fever and dengue haemorrhagic fever affect more people than malaria with an estimated 400 million infections per year (Bhatt et al. 2103). The World Health Organisation states that dengue cases are not only increasing but that the virus is expanding into new areas and that explosive outbreaks are occurring. Increasing human infections with chikungunya and Zika viruses have also been reported in recent times. Although seldom fatal, both of these infections can be debilitating. Additionally, Zika virus has been implicated as the cause of microcephaly in babies and Guillain-

Barré syndrome among other illnesses. Interestingly, dengue-, chikungunya- and Zika viruses can all be transmitted by *Aedes aegypti* and *Aedes albopictus* (WHO 2016), with reports of dengue and chikungunya not only co-circulating in some cities, e.g. Dehli, India (Singh et al. 2012) but both being transmitted by co-infected mosquitoes (Vazeille et al. 2010).

Australia is also at risk from mosquito-borne disease outbreaks. In the past 20 years, it has seen the emergence of Kunjin virus in horses in New South Wales, experienced outbreaks of Japanese encephalitis in the Torres Strait and suffered through periodic dengue epidemics in north Queensland (Hanna et al. 1996, Hanna and Ritchie 2009, Frost et al. 2012). Many mosquito-borne diseases are not acquired locally (for example chikungunya, malaria, Zika) but are detected when infected people fall ill after visiting an outbreak area overseas (Knope et al. 2014). The most commonly acquired mosquito-borne diseases in Australia are Ross River, Barmah Forest, Kunjin, Murray Valley and dengue fever. Although dengue is not endemic in north Queensland, infections occur almost every year (Knope et al. 2014). Local transmissions arise when *Ae. aegypti* takes a blood-meal from a viraemic person subsequently spreading the virus when taking further blood-meals (Hanna and Ritchie 2009).

1.2.2 Mosquitoes of medical importance in Australia

The most important mosquito vector in Australia is *Culex annulirostris* which is capable of transmitting numerous viruses (amongst them: Ross River, Murray Valley, Kunjin, Japanese encephalitis viruses) (Kay and Standfast 1987, Ritchie et al. 1997, Marshall 1998, van den Hurk et al. 2003). It was also the most dominant species captured during my fieldwork in the Cairns area and the Torres Strait for Chapter 2, 4 and 5. Other species of interest include *Aedes notoscriptus* and *Aedes vigilax;* both are known vectors for Ross River and Barmah Forest viruses (Doggett and Russell 1997, Russell and Kay 2004) and laboratory studies have demonstrated that both could act

as yellow fever vectors in Australia (van den Hurk et al. 2011). Of even more concern are *Ae. aegypti* and *Ae. albopictus*; the first is the key dengue vector in northern Australia and the latter is not yet established on the Australian mainland. However, for at least the past decade it has established populations on the majority of the Torres Strait islands (Ritchie et al. 2006a) and it is regularly intercepted at mainland ports and airports (Knope et al. 2014). *Ae. albopictus* is not only widespread in tropical but also in temperate climate zones. Both species were originally forest species (Gratz 2004) but are now closely associated with domestic environments; breeding in small artificial containers and not dispersing far from their place of emergence. My research in the Torres Strait, detected *Ae. albopictus* mostly from urban areas on the islands of Badu and Moa (Chapter 5). *Anopheles farauti* is currently of lesser concern due to the absence of endemic malaria in Australia since 1981 (WHO 1983). It used to be the primary malaria vector in Australia and still is so in Indonesia and Papua New Guinea (Beebe et al. 2000). I captured *An. farauti* mainly in grassland habitats near Cairns (Chapter 2) and on Boigu island (Chapter 5).

1.3 Mosquitoes

Currently, ~3,500 species of mosquitoes have been identified, and with the exception of Antarctica, they occur on every continent in almost any habitat (Clements 1992, Becker et al. 2010). Australia is home to ~300 species with most of them occurring in the tropical regions of Queensland, Northern Territory and Western Australia. Mosquitoes belong to the order Diptera in the family Culicidae which is divided into 3 subfamilies: *Anophelinae, Culicinae* and *Toxorhynchitinae.* Most mosquitoes (> 2900 species) belong to the subfamily *Culicinae* (MCAA 2011). Only a small fraction (~10%) of mosquito species is of health concern, but this small fraction accounts for the transmission of some of the worst diseases of humankind (Manguin and Boëte 2011).

1.3.1 Mosquito life cycle

A female mosquito usually only mates once as sperm are sufficient to fertilise all the eggs she will produce during her life. A blood meal, providing vital nutrients, is generally required for egg development (an exception is the ability to perform autogeny which allows females to produce their first batch of eggs without a blood meal). Depending on the species, 30 to 300 eggs are laid at a time on the surface of water, on aquatic vegetation, moist soil, in natural water holding bodies or in artificial containers (see Chapter 3 for more detailed descriptions on oviposition sites). If conditions are suitable, eggs hatch after 2 - 3 days into larvae and after 4 moults turn into pupae. Mating occurs soon after emerging from the pupa (Service 2008, Becker 2010).

Carbohydrates in the form of plant nectar and juices are necessary for both the female and male mosquito, whereas protein in the form of blood meals is only required by females for the production and development of eggs. The source of the blood meals is species specific; some mosquito species are generalists and others are more specialised and feed on amphibians, reptiles, birds or mammals only. Time of blood feeding is also species dependent, some are diurnal, nocturnal or crepuscular (Service 2008, Becker et al. 2010).

The life cycle of mosquitoes reveals how and why land use change enables their proliferation: exploitation of almost any aquatic habitat (Becker et al. 2010), high reproductive capacity generating large numbers of offspring (Attardo et al. 2005) and fast developing rates are further enhanced by increasing temperatures and food resources. Mosquitoes are also able to adapt to a range of environmental conditions; some species can generate a first batch of eggs without a blood meal (autogeny), produce desiccation resistant eggs and perform instalment hatching (Becker et al. 2010, Kassim et al. 2012, Ariani et al. 2015).

1.3.2 Disease transmission

Mosquitoes are infected with a pathogen (infectious agents such as bacteria, protozoa, viruses and helminths) when blood feeding on the reservoir host (a vertebrate where pathogens complete their life cycle). Subsequently, after the pathogen develops or replicates, the infected mosquito can transmit it when taking further blood-meals from other vertebrates (Becker et al. 2010, Attardo et al.2005). This type of transmission is called horizontal transmission. Vertical or transovarial transmission occurs when the female mosquito transfers the acquired pathogen to her offspring (e.g. *Aedes vigilax* infected with Ross River and Barmah Forest viruses). Recently, it has been implied that a mosquito-borne disease pathogen (Zika virus) can also infect humans through sexual (Foy et al. 2011, Enserink 2015, Musso et al. 2015) and perinatal transmission (Besnard et al.2014).

1.4 Thesis scope and structure

There have been a large number of studies on mosquito species implicated in disease transmission world-wide; however, most of these studies have primarily focussed on vector populations in urban areas (e.g. Kay 1979, Chadee 1991, Mitchell 1995, Reiter et al. 1995, Keating et al. 2004, Sattler et al. 2005, Matthys et al. 2006, Ritchie et al. 2006a, Williams et al. 2006, Garcia-Rejon et al. 2008, Kay et al. 2008, Strickman and Fonseca 2012, Chirebvu and Chimbari 2015). Investigations into mosquito communities, especially in natural habitats are rare (but refer to: Standfast and Barrow 1969, Lounibos 1981, Cooper et al. 1996, Leisnham et al. 2004, Vittor et al. 2006, Junglen et al. 2009, Thongsripong et al. 2013). My research focussed on the comparison of mosquito communities along disturbance gradients in two localities in tropical Queensland: Cairns and the Torres Strait islands. To my knowledge, no research has yet examined the mosquito community in natural habitats in the Torres

Strait and very little is known about how land use changes influence mosquito community composition in tropical Australia.

This doctoral thesis is divided broadly into three topics: sampling of adult and immature mosquitoes in the tropical lowland area north of Cairns; sampling of adults on remote, tropical islands in the Torres Strait and evaluating alternative methods and attractants to sample adult mosquitoes in remote areas. The thesis contains six chapters: this introduction which explains some of the terminology used and provides basic background information on the life cycle of mosquitoes and their role in disease transmission; four empirical chapters and a general conclusion chapter.

1.4.1 Chapter 2: Mosquito communities and disease risk influenced by land use change and seasonality in the Australian tropics

One of the most important anthropogenic land use changes in the Australian tropics is deforestation. It is estimated that up to 75% of rainforest has been cleared since colonial settlement, mainly for sugarcane, banana and livestock production (Webb and Tracey 1981, Rasiah et al. 2004, Cofinas and Creighton 2008).

This chapter (in press in Parasites & Vectors) investigated the adult mosquito community across an anthropogenic disturbance gradient of grassland, forest edge and forest interior habitats in the tropical lowlands of north Queensland, Australia. I sampled mosquitoes with CDC miniature traps (Appendix 1; Fig. 1) and applied non-metric multidimensional scaling ordinations to examine if the mosquito community varied in response to habitat type. I found distinct community differences between forest interior and grassland habitats, with the forest edges functioning as an ecotone with overlapping communities from both habitats. Furthermore, from a human health perspective I determined that important disease vectors, such as *An. farauti*, *Culex*

gelidus and *Mansonia septempunctata* occurred predominantly in these man-made tropical grasslands.

As the study region experiences a monsoonal climate, I also compared the mosquito community between the wet and the dry seasons. I observed that important disease vectors (*Ae. notoscriptus, Ae. vigilax*, *An. farauti* and *Cx. annulirostris*) were still present during the dry season which could imply that disease transmission is possible at any time of the year. I discuss what strategies mosquitoes may apply to persist and reproduce during adverse conditions such as dry seasons.

1.4.2 Chapter 3: Land use change and breeding mosquitoes in tropical Australia: an experimental approach using small artificial oviposition containers

Mosquitoes are prolific breeders and can use a large variety of habitats to oviposit. Of considerable health concern are mosquitoes which utilise artificial containers in domestic environments. For example, *Ae. aegypti* has made the transition from its native African natural tree-hole breeding habitat to the most feared domesticated mosquito species world-wide. In urban areas, *Ae. aegypti* exclusively utilises small artificial containers. This species is the major vector of yellow fever, dengue and Zika viruses.

In this chapter I explored mosquito oviposition behaviour with the use of small artificial containers across the same anthropogenic disturbance gradient of grassland, forest edge and forest interior habitats as in Chapter 2. I deployed small plastic containers (Appendix 1; Fig. 2) on the ground and at 1.5 m height in the three habitats to determine which species utilised them for oviposition. These containers were left in the field for three weeks after which they were transferred to the laboratory to rear the deposited eggs and larvae until they emerged. I found that grassland, forest edge and

forest interior traps yielded 44%, 33% and 23% of the emergents respectively, and that most emergents were from traps which were placed on the ground. I identified six species, all of them known tree-hole breeders. Terrestrial traps had higher larval records than arboreal traps, with a greater preference for grassland habitats compared to edge or forest habitats. I also observed that mosquitoes emerged for up to 60 days after having been transferred from the field to the laboratory and that water temperature did not appear to influence the number of emergents. I discuss the factors which could have contributed to the duration of emergence and how the immature mosquitoes appear tolerant of presumably adverse conditions in water temperature.

1.4.3 Chapter 4: Overcoming the challenges of mosquito sampling in remote localities: a comparison of CO_2-attractants on mosquito communities in three tropical forest habitats

Remote, tropical regions are predicted hot-spots for emerging and re-emerging mosquito-borne diseases (Jones et al. 2008). Disease surveillance and mosquito monitoring in remote regions can be challenging due to a lack of suitable attractants to lure mosquitoes into traps. The most common attractant is carbon-dioxide, either in the form of dry ice or from pressurised gas cylinders (Gillies et al. 1980; Kline 1994; Kline 2006). These CO_2 sources are expensive, difficult to transport and often unavailable in remote areas (Mboera and Takken 1997, Saitoh et al. 2004, Smallegange et al. 2010). A cheap and easily obtainable alternative source of CO_2 can be generated by combining yeast, sugar and water (Saitoh et al. 2004).

In this chapter, published in Medical Entomology, I first determined the amount and production longevity of CO_2 by yeast fermentation under different temperature scenarios in laboratory experiments. Then I conducted field experiments (4 x 4 Latin Square sampling design) in three different types of forests to compare mosquito captures with attractants derived from my yeast fermentation versus dry ice; the same

traps (CDC) as in Chapter 2 were used. I found that dry-ice baited trap generally captured more mosquitoes than yeast-baited traps. More importantly, however, I found no difference in the composition of mosquito species captured with the two different attractants when samples were compared using ordination analyses. In the discussion I explain why there were differences in the number of captures between the different treatments and how the number of captures could be increased by adding further attractants.

1.4.4 Chapter 5: Land use influences mosquito communities and disease risk on remote tropical islands: a case study using a novel sampling technique

As stated above, sampling mosquitoes in remote areas can be challenging. In addition to an easily obtainable CO_2 attractant, suitable traps are needed. Almost all commercially available traps used to collect adult mosquitoes rely on a source of electricity. The traps I used in Chapter 2 and 4 require 6 volt batteries to operate a fan; furthermore, they are fragile to transport because some components are made of glass. A powerless trap, called a passive box trap (Appendix1; Fig. 3), designed by Professor Scott Ritchie has been used in several mainland areas in Australia, although with CO_2 provided by pressurised gas cylinders or dry ice (Ritchie et al. 2013, van den Hurk et al. 2014). Building on the findings of the previous chapter, I developed a novel sampling technique by coupling non-powered traps with CO_2 derived from yeast fermentation (Appendix 1; Fig. 4).

The aim of this chapter, published in American Journal of Tropical Medicine and Hygiene, was to trial this novel sampling technique in the Torres Strait, a remote tropical island archipelago. I investigated if mosquito communities varied between sylvan (forest) and urban habitats on four islands (Saibai, Boigu, Badu and Moa); I also compared a standard trap (BG-Sentinel™ trap; Appendix 1; Fig. 5) to the non-powered

trap. This comparison was only carried out in the villages due to the need for continuous power, making this trap unreliable in sylvan habitats.

I found that my novel sampling technique was successful in remote locations as >11,000 mosquitoes were captured in this study. The comparison between this technique and the standard trap in villages revealed no differences in mean captures or in species composition. By applying non-metric mutlidimensional ordinations to the data collected from this novel trap design, I found that the mosquito communities varied significantly between habitats (forest and urban) and also between islands.

A very important outcome of this study was that human land use significantly affected presence of mosquito vectors as greater numbers of disease-competent species occurred in urban habitats. *Ae. aegypti* and *Ae. albopictus* were almost exclusively retrieved from villages. I discuss why the mosquito communities may be different between urban and sylvan habitats and between the northern low-lying islands and the southern, continental islands.

Another aim of this chapter was to establish whether the following viruses were present at the time of the sampling in the Torres Strait: dengue, chikungunya, Ross River, Japanese encephalitis, Kunjin, West Nile and Murray Valley (the evaluation of disease presence was not included in the publication). Conventional disease detection methods involve sentinel animals, such as chickens or pigs, or large pools of dead mosquitoes which can be very expensive and labour-intense in remote areas. A new alternative is the use of honey-soaked nucleic acid preservation cards (FTA® cards) which preserve viruses but at the same time deactivate them. Mosquitoes taking a honey-meal from the FTA® cards expel saliva which can be used for disease detection by eluting viral RNA. I used four honey-soaked FTA® cards in each of the non-powered traps in the

Torres Strait study. Although weak virus detections were initially detected, upon reanalyses they were considered not significant.

1.4.5 Chapter 6: Conclusion

In the final chapter I summarise the main findings from the data chapters and discuss the implications of this research and suggest future research avenues.

1.5 Notes on publications and species

Thesis chapters which have been published or are in press have been modified only to minimise unnecessary repetition and to ensure a consistent style throughout the thesis. The pronouns “we” and “our” in the publications have been replaced with “I” and “my” respectively.

Mosquito genera names were abbreviated with two letters as using only one letter can create confusion (i.e. *Aedes* and *Anopheles*; *Coquillettidia* and *Culex*). Reinert (2009) provides an updated list of abbreviations for generic-level taxa (2-letters for genera and 3-letters for subgenera) of the family Culicidae.

CHAPTER 2

Mosquito communities and disease risk influenced by land use change and seasonality in the Australian tropics

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2.1 Abstract

Anthropogenic land use changes have contributed considerably to the rise of emerging and re-emerging mosquito-borne diseases. These diseases appear to be increasing as a result of the novel juxtapositions of habitats and species which can result in new interchanges of vectors, diseases and hosts. In tropical Australia, mosquito populations were sampled across an anthropogenic disturbance gradient of grassland, rainforest edge and rainforest interior habitats, in both the wet and dry seasons. I captured ~13,000 mosquitoes from 288 trap nights across four study sites. A community analysis identified 29 species from 7 genera. Even though mosquito abundance and richness were similar between the three habitats, the community composition varied significantly in response to habitat type. The mosquito community in rainforest interiors was distinctly different to the community in grasslands, whereas forest edges acted as an ecotone with shared communities from both forest interiors and grasslands. I found two community patterns that will influence disease risk at my study sites; first, that disease vectoring mosquito species occurred all year round. Second, that anthropogenic grasslands adjacent to rainforests may increase the probability of novel disease transmission through changes to the vector community on rainforest edges, as most disease transmitting species predominantly occurred in grasslands.

2.2 Introduction

The emergence and re-emergence of mosquito-borne diseases can often be linked to human land use changes such as deforestation, agriculture and urbanisation (Morse 1995, Gubler 1998, Patz et al. 2000, Vittor et al. 2009, Foley et al. 2007). These land use changes may influence disease prevalence and distribution by increasing breeding habitats, food resources, and changing vector-host relationships (Bayoh and Lindsay 2003, Norris 2004, Tuno et al. 2005, Vittor et al. 2009). Tropical deforested habitats are open, well lit and warmer compared to secondary and primary forests (Camargo and Kapos 1995). These characteristics may increase the survival and growth rates of mosquito larvae (Ye-Ebiyo et al. 2003, Bond 2005, Tuno et al. 2005, Vittor et al. 2009). Newly available habitats for mosquitoes, such as irrigation systems, dams and other water-holding bodies, have also enabled mosquitoes to spread into previously uninhabitable areas (Amerasinghe and Ariyasena 1990, Harb et al. 1993).

A principle risk factor in the emergence of zoonotic diseases (diseases that transfer from other animals to humans) is the alteration of the vector-host relationship due to land use modification (Schrag and Wiener 1995, Daszak et al. 2001). This change in relationship occurs when a vector is introduced to a new habitat or exposed to a new host. Human infection with sylvatic yellow fever is one such example (Spielman and D'Antonio 2001, Barrett and Monath 2003). Within their natural environment, the yellow fever virus (*Flavivirus* spp.) is mainly transmitted by *Hemagogus, Sabethes* and *Aedes* mosquitoes to monkeys in the rainforest canopy. After logging and land clearing, mosquitoes followed the canopy edge to the ground where they fed upon and infected humans (Strode 1951, Spielman and D'Antonio 2001, Barrett and Monath 2003).

Seasonality in the tropics can influence mosquito populations, as the duration of wet and dry seasons affects larval development and adult abundance. Wet season rains create more breeding habitats, and elevated humidity levels extend the lifespan of

adults, thus prolonging disease transmission rates (Patz et al. 2000). For example, dengue outbreaks regularly coincide with wet seasons in Brazil, Thailand and Australia (Hanna et al. 1998, Luz et al. 2008, Wongkoon et al. 2013).

Mosquito-borne diseases such as malaria, yellow fever and chikungunya are thriving worldwide, especially in the tropics. The tropical regions of Australia could also be vulnerable to these diseases as potential vectors are present and disease transmission could arise due to infected people entering the country (Longbottom 1996, Bryan et al. 1998). For example, potential vectors which occur in tropical Australia are: *Anopheles farauti* and *Anopheles annulipes* for spreading malaria (Lee et al. 1989), and *Aedes aegypti* for the transmission of yellow fever and chikungunya (Johnson et al. 2008, van den Hurk et al. 2010). Human populations in Australia's tropical regions can also be at risk from mosquito-borne infections as attested by outbreaks of dengue, Ross River fever, Barmah Forest fever, Japanese encephalitis and Murray Valley encephalitis (Mackenzie et al. 1998). There is very little known about the ecology of these diseases or their vectors in the Australian tropics and if environmental change has influenced their prevalence.

My study investigated the mosquito community structure and composition across an anthropogenic disturbance gradient of grassland, forest edge and forest interior habitats in the tropical lowlands of north Queensland, Australia. The main objectives were to evaluate how mosquito abundance, number of species and species composition differed between the three habitat types and across seasons. This study presents a template to assess how landscape disturbances are able to influence mosquito species composition and distribution in the tropics and how those changes may influence mosquito borne diseases.

2.3 Methods

2.3.1 Study area

This study was conducted in the Wet Tropics bioregion of north eastern Australia. Study sites were located approximately 10 to 15 km north of Cairns city, (16° 50'S, 145° 41'E; Fig. 2.1), which provides an ideal setting for the analysis of mosquito response to land use changes as Cairns' human population is growing and urban areas are expanding into agricultural and forest habitats. The population has more than doubled within 25 years (1981 – 2006) from 70,762 to 147,538; and it is expected to be 1.4 to 1.7 times larger by 2031 (DIP 2008), resulting in further land use changes (Bohnet and Pert 2010). Cairns is also an important tourist destination with an international airport and seaport for cruise liners and container ships; all of which have the potential to introduce exotic infectious agents into the country (Reiter and Sprenger 1987, Mitchell 1995, Daly et al. 1996, Knope et al. 2013).

Annual rainfall is ca. 2000 mm yr^{1} and strongly seasonal with a wet season from December to May and a dry season from June to November. Temperature reaches an annual mean maximum and minimum of 29°C yr^{1} and 20.8°C yr^{1} respectively. The lowland evergreen rainforests within the study area are classified locally as Notophyll Vine Forests (Type 7) (Tracey 1982). These forests contain trees that are 15 – 24 m in height with emergents such as *Acacia polystachya*, *Eucalytpus pellita* and *Eucalyptus tessellaris* on the ridges (Tracey 1982). The forests have experienced some anthropogenic disturbances such as the removal of timber species (Keto and Scott 1986), and fires that have escaped from cane farms (Griggs 2007). The grasslands adjacent to the forests were man-made and dominated by 2 - 4 m high non-native grasses, shrub species, and some pioneer rainforest trees.

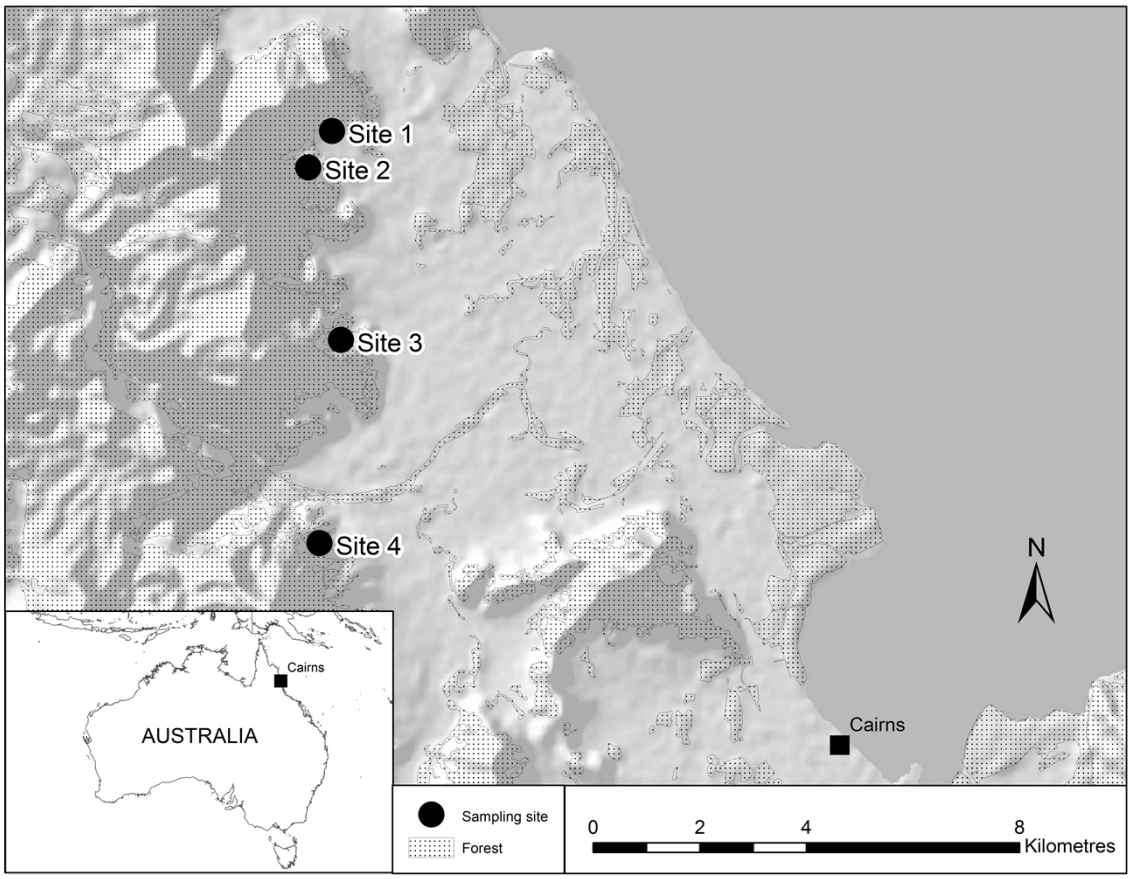


Figure 2.1: The study area, north of Cairns, Australia showing the four sampling sites which feature similar ecological habitats and environmental gradients.

2.3.2 Sampling methods for mosquitoes

Field work was conducted between October 2011 and August 2012 at four sites with similar ecological habitats and environmental gradients. Every site was sampled four times; twice in the wet season and twice in the dry season. I captured adult mosquitoes from three different habitats: forest interior, forest edge and adjacent grassland. The distance between each of these habitats was at least 100 meters. Mosquitoes were collected using Center for Disease Control and Prevention (CDC) light traps (model 512, John W. Hock Company, Gainesville, Florida; Appendix 1; Fig. 1). The traps were modified by removing the light bulbs to avoid sample bias – as some mosquito species

may be more attracted to light than others; to reduce attracting non-target insects that can damage mosquitoes; and to increase battery life (Kline 2006). All traps were run with 6 volt batteries. Six traps were set up in each habitat at each site resulting in 18 trap catches per site per sampling period. Traps were established along a transect at least 20 meters apart and were placed at a height of approximately 1.5 meters above ground level. The traps were baited with CO_2 (2 kg dry ice per trap) in insulated containers, which were placed directly above the traps. Vaseline petroleum jelly was applied to suspension ropes to deter green ants (*Oecophylla smaragdina*) from reaching the caught mosquitoes. Traps were in operation for 24 hours to ensure that both diurnal and nocturnal mosquito species were captured. After collecting the traps, species were stored in the insulated containers on the remaining dry ice and taken back to the laboratory where they were placed into freezers (-21°C) for further storage.

2.3.3 Mosquito identification and subsampling procedure

Mosquitoes were identified to species level using taxonomic keys (Lee et al. 1980 - 1989, van den Hurk et al. 1999, MCAA 2011) and with the assistance of taxonomic experts. The identification of mosquitoes is time consuming as it generally requires the keying out of each individual. Due to the large numbers of mosquitoes captured I applied a subsampling procedure that required the random selection of individuals for identification, this method was previously tested and was found to accurately predict species diversity and maintain species proportions within the community (Meyer Steiger et al. 2012). The subsampling procedure involved randomly choosing 30 individuals from each trap. If traps had < 30 captures, all individuals were identified. This entailed that traps were emptied onto graph paper where 30 randomly generated points were marked. The mosquitoes which landed on or closest to each point were then identified, resulting in a total of 4,504 individuals which represents 35% of the total capture. To measure the appropriateness of the subsampling procedure I used

Coquillettidia near crassipes, a bright orange species, as a surrogate to compare actual abundance within a sample with those predicted by subsampling.

2.3.4 Data analysis

I assessed whether the applied subsampling technique accurately described species composition by comparing the relative abundance of one species (*Cq.* nr. *crassipes*) in the subset with its actual abundance in the whole sample, by using Spearman's rank-order correlation. Only traps which had captured abundant captures (≥ 80 mosquitoes) were used which resulted in 30 traps analysed. The reason I only included traps with ≥ 80 mosquito captures was due the results of my pilot study which showed that cumulative species richness begins to plateau at 80 individuals.

I estimated the differences in mean abundance for each habitat type (forest interior, forest edge and grassland) and each season (wet and dry season) using a two-way ANOVA (independent factorial design with fixed factors). Variables were log-transformed to satisfy the assumptions of the residuals conforming to a normal distribution and in homogeneity of variances. A two-way ANOVA was also used to assess whether there was a difference in mean number of species between habitats and seasons. Data were not transformed prior to the analysis as statistical assumptions were met. The data for the early and late dry seasons, and the early and late wet season were pooled to derive the dry season and wet season data respectively. Rank-abundance diagrams distinguished changes in species dominance between habitats and seasons. To evaluate if mosquitoes were sampled adequately under my sampling design, I constructed species accumulation curves to display the cumulative number of species collected against the measure of sampling effort. The sampling effort is all data across the three habitats. Chi-square tests were applied to investigate whether the different mosquito tribes and subfamilies had habitat preferences. To examine major gradients in the composition of mosquito communities

and vector communities (vector communities consist of species which are able to transmit alpha-, flaviviruses and protozoan parasites) between habitat types and seasons, I performed nonmetric multidimensional scaling (NMS) ordinations. NMS is a robust and commonly used method for ecological community data as it does not assume data to be normally distributed (Minchin 1987, Clarke 1993). The ordination was performed by using the recommend default options (McCune and Mefford 1999). Data were log (x + 1) transformed prior to analysis. Monte Carlo randomization tests (250 runs) were used to determine whether the ordination axes explained significantly more variation than expected by chance. A Bonferroni correction was used to reduce the likelihood of type II errors, where $P = 0.15/x$ (x represents the number of mosquito species multiplied by two or three axes and 0.15 is the experiment wise error rate) (Chandler 1995). Permutation-based nonparametric MANOVAs (PerMANOVAs) (Anderson 2001), followed by pairwise comparisons for significant results, were employed to distinguish differences in mosquito communities for the three habitat types. Finally, I assessed whether there were differences between commonly captured mosquitoes (> 40 individuals) with similar ecological and biological characteristics by applying two-way ANOVA tests. Species were grouped according to the following three ecological and biological characteristics: geographical range in Australia, breeding environments and time of blood-feeding with the use of published life history and distributional data (Lee et al. 1989, MCCA 2011). The geographical range of species occurrence was divided into four groups: *very restricted* (species restricted to north Queensland only); *restricted* (species restricted to either north Queensland and Northern Territories or to Queensland and New South Wales); *medium* (northern Australia: Queensland, Northern Territories and Western Australia) and *wide* (all of Australia, except Tasmania). Mosquito species were further classified as using the following known breeding environments: *ground water* and *containers* (natural and artificial). Preferred time of blood-feeding of species was divided into *diurnal*, *nocturnal*

and *crepuscular* (dawn and dusk). Geographical range data were square-root transformed to fulfill the assumptions of two-way ANOVAs.

I used SPSS statistical package (SPSS Statistics for Windows 22.0, Armonk, New York, USA) for most analyses, except for the ordination analyses and the PerMANOVAs for which I used PC-ORD (PC-ORD 6.0, MjM Software, Gleneden Beach, Oregon, USA). Where required, data was tested for normality by using Kolmogorov-Smirnov tests and Levene's tests for homogeneity of variances.

2.4 Results

2.4.1 Subsampling

At the outset, I verified the accuracy of my subsampling method for estimating the relative abundance (proportion) of mosquito species. I found a strong positive correlation between the estimated and actual abundance in my samples (Spearman rank-order correlation $r_s = 0.833$, $P < 0.001$) which infers that this subsampling technique adequately estimated species composition.

2.4.2 Mosquito captures

In total, 12,854 mosquitoes were captured in 288 trap-nights across 16 grassland-edge-rainforest gradients. On average 93 ± 86 (mean ± SD) mosquitoes were captured per trap. Mosquito captures were quite evenly distributed across the three habitats as there was no significant difference between the mean captures per habitat (2-way ANOVA $F_{2,18} = 0.109$, $P = 0.897$). However, seasonality influenced mosquito abundance strongly. Thirteen times more mosquitoes were captured during the wet season than during the dry season sampling period (2-way ANOVA $F_{1,18} = 65.555$,

$P < 0.0001$) (Fig. 2.2). No effect of interaction occurred between habitat and season (2-way ANOVA $F_{2,18} = 0.150$, $P = 0.861$).

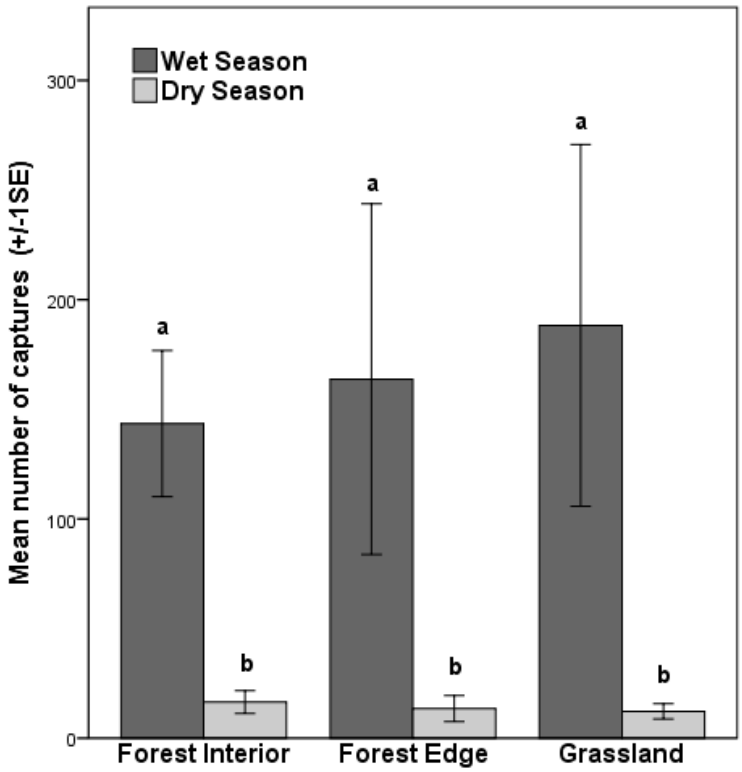


Figure 2.2: The mean number of mosquitoes caught in forest interior, forest edge and grassland habitats during the wet and dry season. Mean values are similar in the three habitats but are significantly lower in the dry season than those in the wet season. Error bars denote the standard error. Different letters denote significant differences.

Using the subsampled data set, I identified four tribes and two subfamilies of mosquitoes (Table 2.1) in the community. Members of the mosquito tribes Aedini and Mansoniini were found mostly in forest interior habitats ($\chi^2_1 = 186.39$, $P < 0.001$ and $\chi^2_1 = 30.26$, $P < 0.001$), whereas members of the tribe Culicini were predominantly captured in grassland habitats ($\chi^2_1 = 92.57$, $P < 0.001$). No habitat preference was found for the Sabethini tribe ($\chi^2_1 = 2.46$, $P > 0.05$). Most (95%) individuals were in the

subfamily Culicinae, with only 5% in Anophelinae. Mosquitoes in the subfamily Culicinae were mostly captured inside forests ($\chi^2_1 = 21.84$, $P < 0.001$) compared to mosquitoes in the subfamily Anophelinae which appeared to prefer open habitats, as they were significantly more abundant in grasslands compared to forest edges and forest interiors ($\chi^2_1 = 75.20$, $P < 0.001$).

Table 2.1: Mosquito abundance by tribe and subfamily from the three habitats in tropical Australia (*df = 2; **df = 1).

	Forest Interior		Forest Edge		Grassland		Total		Chi-Square test	
	n	%	n	%	n	%	N	%	χ^2	*P* <
Tribe										
Aedini	953	60.66	689	47.03	444	31.11	2086	46.74	186.39	0.001*
Culicini	479	30.49	633	43.20	824	57.75	1936	43.38	92.57	0.001*
Mansoniini	106	6.75	63	4.30	42	2.94	211	4.73	30.26	0.001*
Sabethini	17	1.08	9	0.62	0	0.00	26	0.58	2.46	N.S.**
Subfamily										
Anophelinae	16	1.02	71	4.85	117	8.20	204	4.57	75.20	0.001*
Culicinae	1555	98.98	1394	95.15	1310	91.80	4259	95.43	21.84	0.001*

2.4.3 Mosquito species

The mosquito community was very diverse with 29 species (27 species in the wet season and 24 species in the dry season) identified from 7 genera (Table 2.2). The genera *Aedes* recorded the highest number of species (10 spp.), whereas *Culex* spp. were captured most frequently (1936 individuals). Four species dominated the total samples, together contributing to 74% of the captures; *Culex annulirostris* (36%), *Verrallina lineata* (16%), *Aedes notoscriptus* (11%) and *Aedes vigilax* (11%). The most dominant species in the wet season were *Cx. annulirostris* and *Ve. lineata*. During the

dry season*, Cx. annulirostris* was again the most abundant species, followed by *Ae. notoscriptus*. Some species, such as *Ae. alternans*, were only captured during the wet season and other species, such as *Ae. lineatopennis* were predominantly trapped during the dry season (Table 2.2).

Table 2.2: Mosquito species identified from each habitat (FI = forest interior, FE = forest edge, GR = grassland) in tropical Australia and the pathogens they may transmit.

	Wet Season				Dry Season			
	FI	**FE**	**GR**	**Total**	**FI**	**FE**	**GR**	**Total**
Species								
Aedes alboscutellatus [?]	10	7	14	31	24	12	0	36
Aedes alternans [a]	1	5	4	10	0	0	0	0
Aedes kochi [a,d,e]	2	30	26	58	3	3	8	14
Aedes lineatopennis [a,g]	1	4	55	60	0	1	0	1
Aedes notoscriptus [a,b,d]	144	119	32	295	97	62	34	193
Aedes palmarum [?]	15	2	0	17	24	7	1	32
Aedes quasirubithorax [?]	0	0	3	3	2	0	0	2
Aedes quinquelineatus [?]	16	14	0	30	66	17	3	86
Aedes tremulus [a,b,]	10	2	3	15	3	1	0	4
Aedes vigilax [a,b,d,e]	86	106	108	300	45	57	71	173
Anopheles annulipes s.l. [a,c,d,e]	1	0	4	5	3	3	5	11
Anopheles bancroftii [c,e]	0	0	0	0	1	0	0	1
Anopheles farauti [b,c,e]	8	58	99	165	3	10	9	22
Coquillettidia nr.*crassipes* [c,f]	95	49	7	151	6	7	4	17
Culex annulirostris [a,b,d,e,g]	390	456	544	1390	38	77	125	240
Culex bitaeniorhynchus [b,e]	0	1	0	1	0	0	0	0
Culex cubiculi [?]	0	0	0	0	0	1	0	1
Culex gelidus [a,b]	2	36	115	153	0	1	6	7
Culex hilli [?]	12	18	2	32	6	5	0	11
Culex pullus [b]	16	26	12	54	0	1	1	2
Culex sitiens [e]	5	6	12	23	10	5	7	22
Mansonia septempunctata [a]	5	4	27	36	0	1	3	4
Mansonia uniformis [a,b,e,f]	0	2	1	3	0	0	0	0
Tripteroides atripes [?]	0	1	0	1	0	0	0	0
Tripteroides magnesianus [?]	4	2	0	6	6	0	0	6
Tripteroides sp.	4	6	0	10	3	0	0	3
Verrallina carmenti [a]	20	4	0	24	13	27	3	43
Verrallina funerea [a,b,g]	0	1	0	1	0	0	0	0
Verrallina lineata [a]	353	193	71	617	31	41	10	82
Total	1200	1152	1139	3491	384	339	290	1013

a = alphaviruses (may cause Barmah Forest, chikungunya, Ross River, Sindbis)
b = flaviviruses (may cause dengue, Murray Valley-, Australian-, Kunjin- & Japanese encephalitis; yellow fever, Edge Hill, West Nile)
c = *Plasmodium spp.* (may cause human and/or avian malaria)
d = *Dirofilaria immitus* (may cause dog heartworms)
e = *Wuchereria bancrofti* (may cause filariasis in humans)
f = *Brugia malayi* (may cause filariasis in humans)
g = *Orbivirus spp*.(may cause epizootic hemorrhagic disease in ruminants & macropods)
? = unknown

Mean mosquito species richness was similar in all habitats (2-way ANOVA $F_{2,18}$ = 0.692, P = 0.514) but differed significantly between the seasons (Fig. 2.3). More species were captured in the wet season than in the dry season (2-way ANOVA $F_{1,18}$ = 15.720, P = 0.001). There was no significant interaction between habitat and season (2-way ANOVA $F_{2,18}$ = 0.692, P = 0.514). Species accumulation curves from my sampling design show the difference in species capture rate between the three habitats. The asymptotes of the curves suggest that my traps captured all the attracted species present in the habitats. However, it is possible that I did not trap either rare species or those not attracted to the traps or bait used (Fig. 2.4).

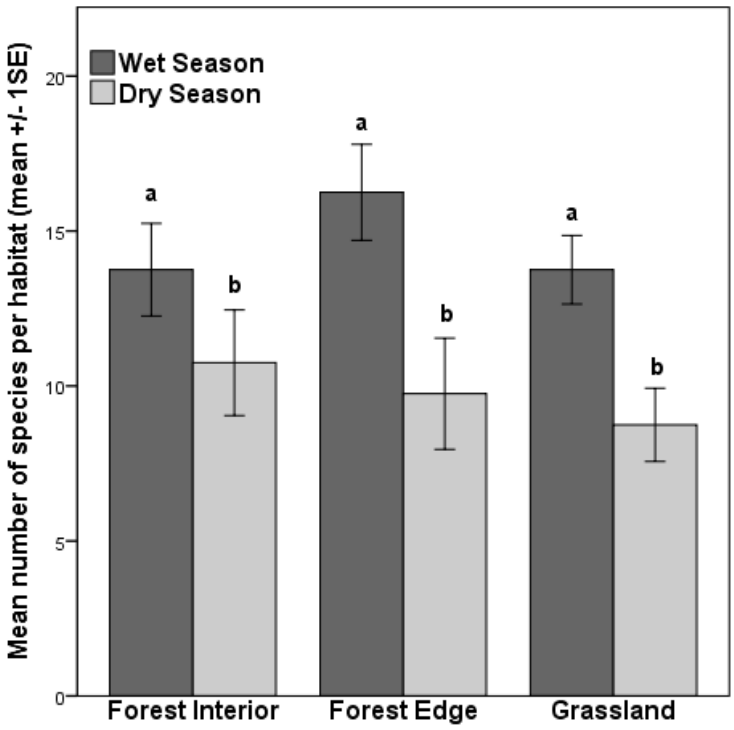


Figure 2.3: Mean number of mosquito species captured in forest interior, forest edge and grassland habitats during the wet and dry season in each habitat. Mean species richness was similar between the three habitats but significantly fewer species were captured in the dry season compared to the wet season, especially along forest edges and grassland sites. Error bars denote the standard error. Different letters denote significant differences.

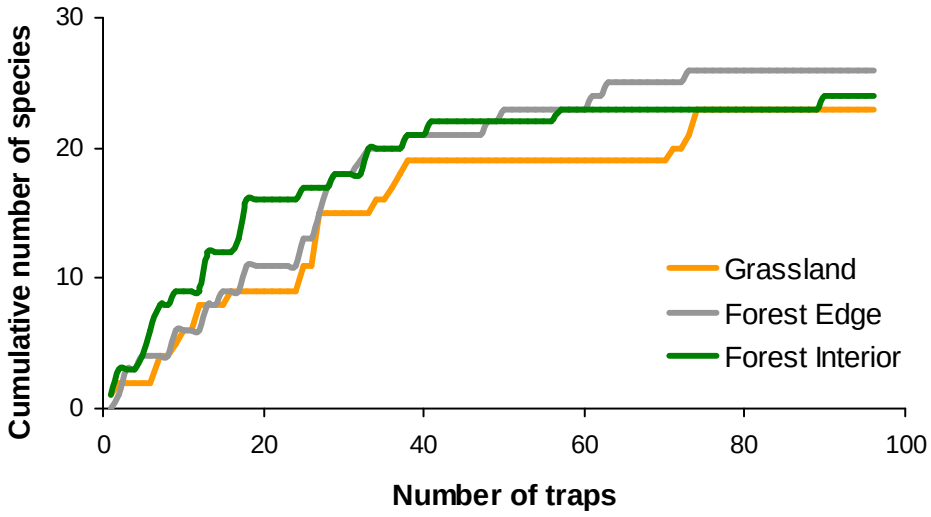
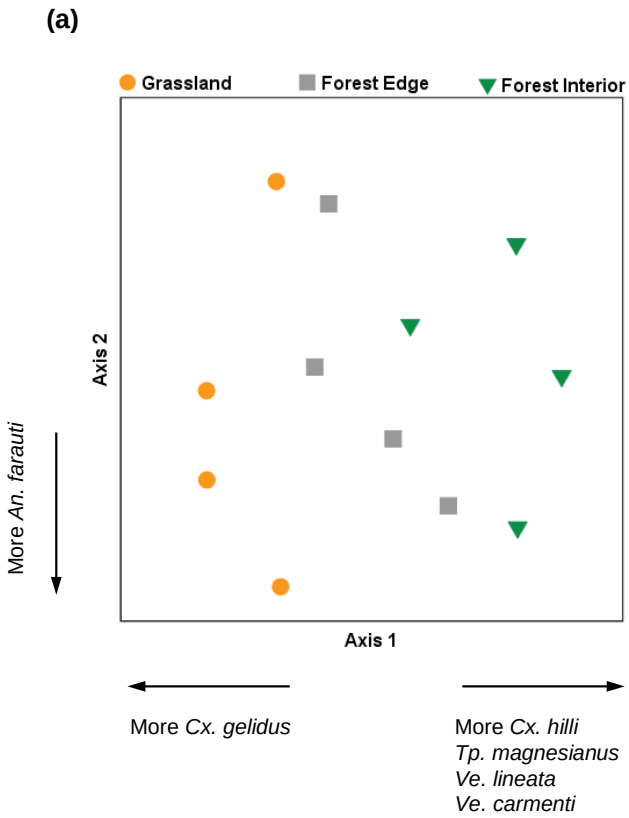


Figure 2.4: Species accumulation curves for mosquitoes sampled from forest interior, forest edge and grassland habitats suggest that most common species were captured. The curves display adequate sampling effort for all habitats and indicate that further sampling would not have produced the discovery of more species, except for very rare ones.

2.4.4 Community composition.

Using ordination analyses, I examined the mosquito community composition in three ways. First, I combined data for the wet and dry season (Fig. 2.5 a) and determined that the mosquito community varied in response to habitat type, especially between forest interior and grassland sites (PerMANOVA: *pseudo F* = 2.164, *P* = 0.028). The NMS Axis 1 (which captured 45% of the total variation) and Axis 2 (capturing 42% of the variation) both discriminated mosquito communities in forest interiors that differed from those in grasslands. Of the 22 species examined, 6 were significantly correlated with these axes (Table 2.3). Secondly, when I explored the influence of seasonality on the community structure and found an identical and significant pattern of rainforest and

grassland separation: (wet season PerMANOVA: *pseudo F* = 2.274, *P* = 0.028) and (dry season PerMANOVA: *pseudo F* = 1.608, *P* = 0.026). The wet season ordination (Fig. 2.5 b) explained 91% of the variation in the data (Axis 1: 55%, Axis 2: 36%), with 21 species examined and 8 significantly correlated with these axes (Table 2.3). The dry season analysis (Fig. 2.5 c) explained 93% of the total variation in the data set with 6 out of 19 species significantly correlated with these axes (Table 2.3). In summary, all three ordination analyses revealed that the mosquito communities were different between forest interior and grassland sites and that an overlap in species composition existed between forest interior and forest edge sites and between grassland and forest edge sites.



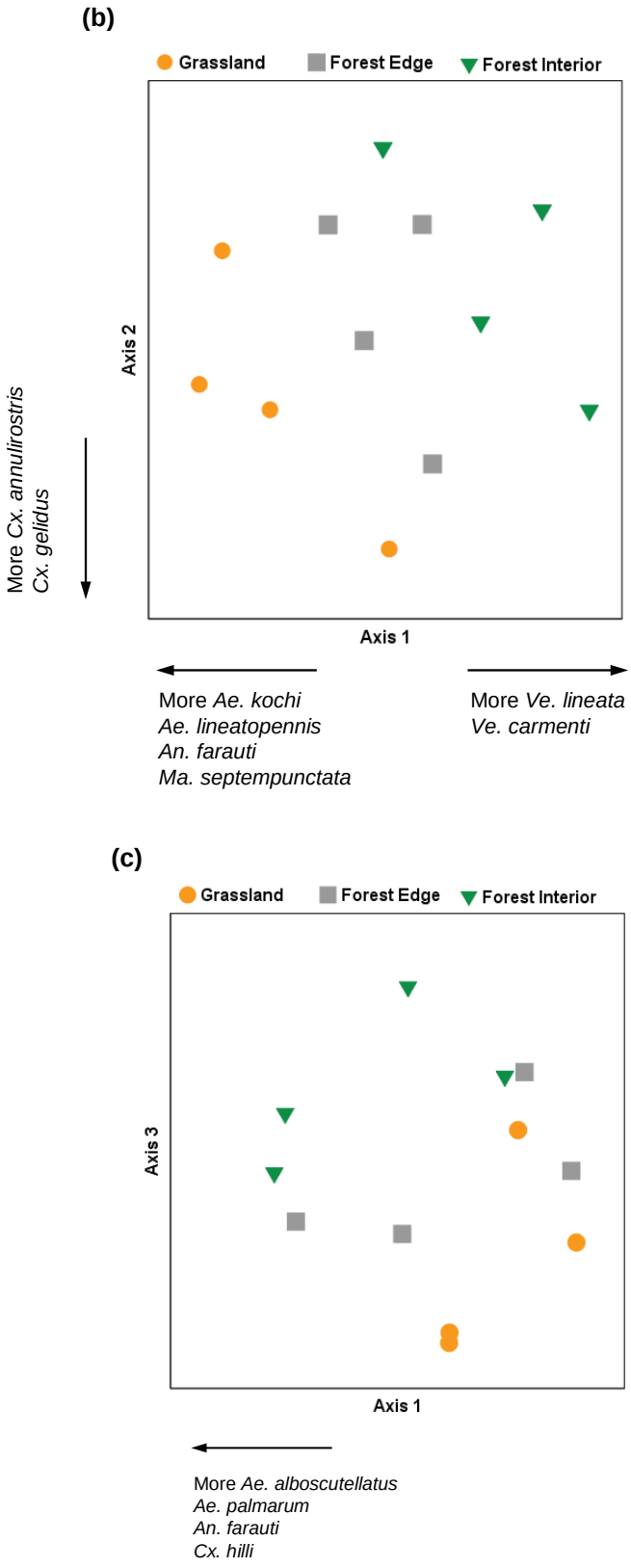


Figure 2.5: Ordination analyses (NMS) show that the mosquito community varied strongly in response to habitat type. Forest interior sites are distinctly different from grassland sites when (**a**) the data for the wet season and dry season were combined,

the data for the wet season (**b**) and the dry season (**c**) (only two of the three dimensions obtained in the analysis are displayed) were analysed separately.

Table 2.3: Pearson correlation values for mosquito species with two or three ordination axes produced by non-metric multidimensional scaling (NMS). Correlation values in boldface were significant ($P < 0.005$) using a Bonferroni-corrected alpha value.

	All Seasons *		**Wet Season**		**Dry Season**		
	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2	Axis 3
Species							
Aedes alboscutellatus					**-0.871**	0.319	0.246
Aedes kochi			**-0.784**	0.096			
Aedes lineatopennis			**-0.762**	0.281			
Aedes palmarum					**-0.866**	-0.088	-0.103
Aedes quinquelineatus					-0.796	0.387	0.087
Aedes vigilax					0.150	**0.787**	0.023
Anopheles farauti	-0.370	**-0.854**	**-0.769**	0.470			
Culex annulirostris			0.039	**-0.793**			
Culex gelidus	**-0.845**	0.441	-0.393	**-0.791**			
Culex hilli	**0.799**	0.235			**-0.839**	0.005	0.243
Mansonia septempunctata			**-0.837**	0.131			
Tripteroides magnesianus	**0.792**	-0.278					
Verrallina carmenti	**0.775**	-0.508	**0.770**	0.257			
Verrallina lineata	**0.815**	0.357	**0.777**	0.140	-0.194	**-0.789**	0.529

* All Seasons (wet and dry seasons combined).

2.4.5 Mosquito vector community

I further examined the habitat preference of known disease vectors - mosquito species capable of transmitting alpha-, flaviviruses and protozoan parasites and found a significant difference between rainforest interior and grassland sites (Fig. 2.6). The two ordination axes collectively explained 87.1% of the total variation with 8 species

significantly correlated with these axes (PerMANOVA: *pseudo F* = 2.502, *P* = 0.029) (Table 2.4). Notably, I observed more known disease vectors in grasslands than in forest habitats.

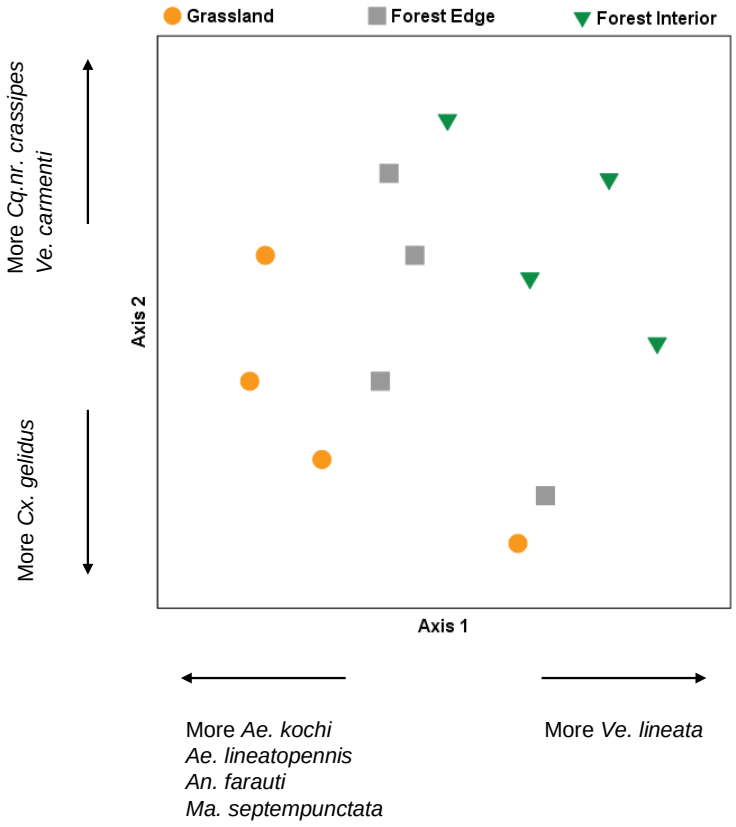


Figure 2.6: Ordination analysis (NMS) of the vector community displays a distinctly different species composition for forest interior and grassland sites.

Table 2.4: Pearson correlation values for the vector community (disease transmitting mosquito species) with two ordination axes produced by non-metric multidimensional scaling (NMS). Correlation values in boldface were significant ($P < 0.005$) using a Bonferroni-corrected alpha value.

	Axis 1	Axis 2
Species		
Aedes kochi	**-0.811**	-0.062
Aedes lineatopennis	**-0.767**	0.248
Aedes vigilax		
Anopheles farauti	**-0.879**	0.325
Coquillettidia.nr. *crassipes*	0.081	**0.786**
Culex annulirostris		
Culex gelidus	-0.289	**-0.902**
Mansonia septempunctata	**-0.865**	0.013
Verrallina carmenti	0.183	**0.921**
Verrallina lineata	**0.820**	0.301

I further investigated the mosquito community structure of each habitat type by examining species dominance using rank-abundance diagrams (Fig. 2.7 a, b and c). Overall, *Cx. annulirostris* was the most dominant species. During the wet season, it dominated grasslands and forest edges, and shared dominance of rainforest interiors with *Ve. lineata*. In the dry season, *Cx. annulirostris* continued to dominate the grasslands but shared dominance of forest edges with the rainforest species *Ae. notoscriptus*. Only one species dominated rainforest interior (*Ae. notoscriptus*).

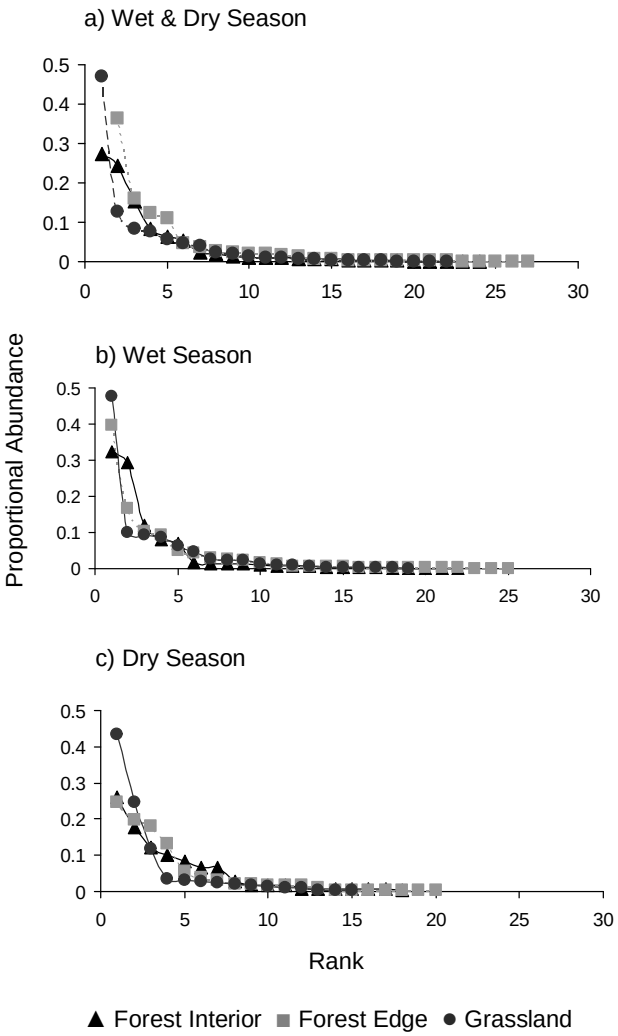
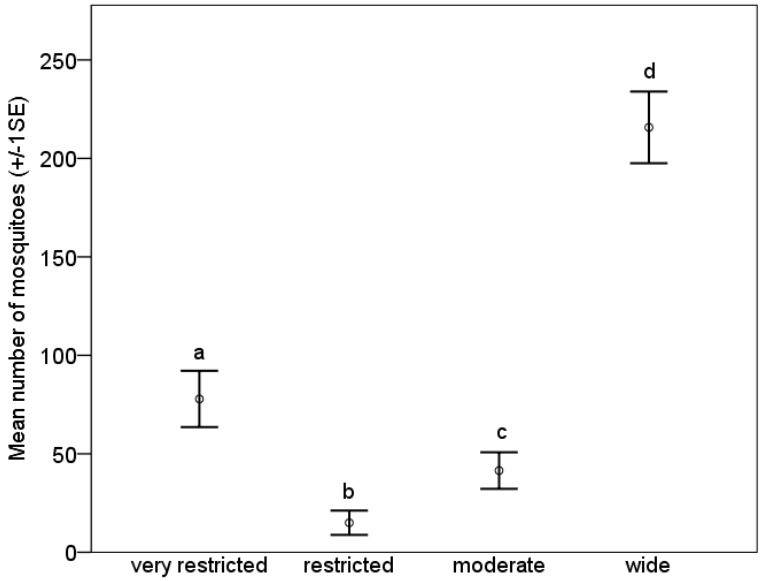


Figure 2.7: Rank-abundance diagrams displaying the diversity of mosquito species in the three habitats; taking into account not just the number of species (richness) but also the distribution of individuals among species (evenness). Overall (a) (wet and dry season combined) forest interior had 2 dominant species; forest edge and grassland had one dominant species. In the wet season (b) two dominant species were discovered in the forest interior but only one dominant species in both grassland and forest edge. During the dry season sampling (c) one dominant species was captured in the grassland and forest edge and two species dominated the forest interior.

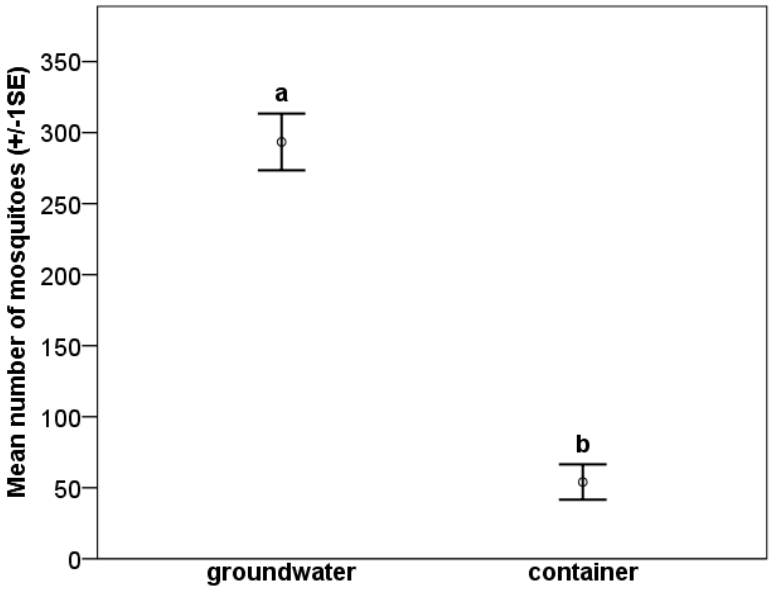
2.4.6 Mosquito characteristics

I found that ecological and biological characteristics strongly influenced mosquito captures in the study area. Commonly captured mosquitoes (>40 individuals) had a wide geographical range (Fig. 2.8 a), were ground water breeders (Fig. 2.8 b) and nocturnal blood-feeders (Fig. 2.8 c). The mosquito group which has a wide geographical range in Australia was captured nearly three times more than the second most common group (the very restricted range group) (2-way ANOVA $F_{3,36}$ = 75.045, P < 0.0001). All groups were significantly different from each other (Tukey HSD post hoc tests P < 0.018). No differences in mean captures occurred in the three habitats (2-way ANOVA $F_{2,36}$ = 0.163, P = 0.85); however, there was an interaction effect between groups and habitat (2-way ANOVA $F_{6,36}$ = 5.286, P = 0.001), with rainforest habitats supporting more species with a very restricted range than the other habitats. Mosquitoes which belong to the group of groundwater breeders were captured six times more often than container breeders (2-way ANOVA $F_{1,18}$ = 106.086, P < 0.0001). Habitat type had no influence on mean captures (2-way ANOVA $F_{2,18}$ = 0.173, P = 0.842) and there was no interaction effect between groups and habitat type (2-way ANOVA $F_{2,18}$ = 2.070, P = 0.155). I found that there was a significant difference of time of feeding amongst the collected mosquitoes (2-way ANOVA $F_{2,27}$ = 12.459, P < 0.0001). Mosquitoes captured in this study, blood-feed predominantly at night (54%), followed by the crepuscular group (28%) and the diurnal group (18%). Nocturnal and diurnal feeders differed significantly (Tukey HSD post hoc test P < 0.0001) as did nocturnal and crepuscular feeders (Tukey HSD post hoc test P = 0.004). There was no significant difference between the diurnal and crepuscular group (Tukey HSD post hoc test P = 0.431). Habitat had no influence on the mean captures (2-way ANOVA $F_{2,27}$ = 2.522, P = 0.099) and no interaction effect was detected (2-way ANOVA $F_{4,27}$ = 1.613, P = 0.200).

(a) range



(b) breeding



(c) feeding

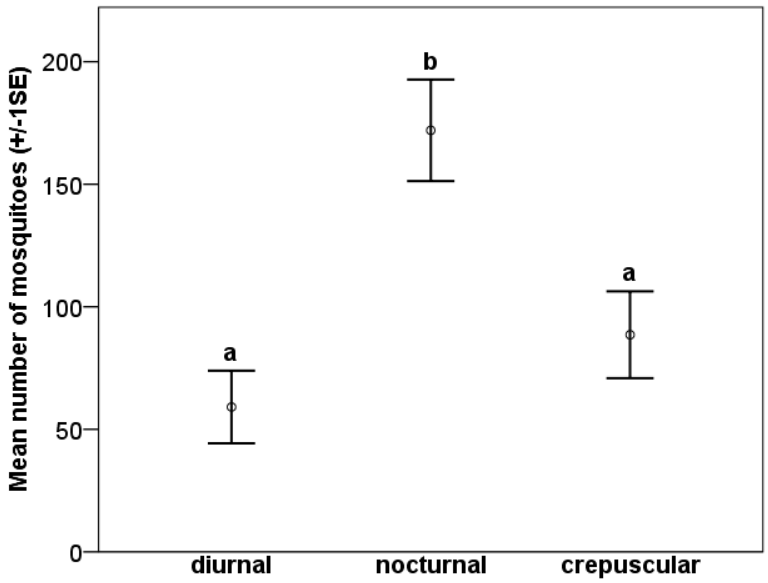


Figure 2.8: Mean number of captured mosquitoes in regards to geographical range, breeding habitat and time of feeding in tropical Australia. Most captures were from mosquitoes which have a wide distribution (**a**), use groundwater environments for depositing eggs (**b**) and blood-feed mainly during the night (**c**). Different letters denote significant differences.

2.5 Discussion

Communities in naturally-occurring ecotones are often an integration of species from adjacent habitats (Fortin et al. 2000). I observed a similar pattern in mosquito communities although across an anthropogenic disturbance gradient from tropical grassland to rainforest, where forest edges supported mosquito species from both habitats. This landscape pattern continued throughout the year, despite a seasonal influence on mosquito abundances. The majority of the mosquitoes at my study area showed significant traits such as groundwater breeders, nocturnal blood-feeders and a wide geographic distribution across Australia. My analysis of disease risk found a

significant difference between grassland and forest habitat, with a greater abundance of known disease vectors in grasslands.

Forest edges acting as ecotones, may produce a novel juxtaposition of mosquito communities which could have wide-reaching consequences for mosquito-borne disease transmission (Despommier et al. 2006) as an increase of endemic viruses is more likely to occur in disturbed habitats than in pristine primary forests. For instance, Junglen et al. (2009) found that the mosquito genera *Aedes*, *Anopheles* and *Culex* were more commonly encountered in disturbed habitats and contained more virus isolates than forest mosquitoes.

I found edges are hot beds of potentially disease vectoring species and that they have an important role in facilitating disease transmission across the landscape. Disturbed and degraded habitats are avoided by numerous forest species (Laurance 2004, Ernst and Roedel 2008, Hillers et al. 2008). However some, especially invasive and generalist species seem to prefer these habitats (Brown and Hutchings 1997, Dranzoa 1998) which may explain why the mosquito community composition was significantly different between man-made grasslands and forest interior sites in this study. A previous, short-term study I conducted in the same area (pilot study; Meyer Steiger et al. 2012) also showed that grasslands supported a markedly different community to inside forests. These distinct differences in mosquito community composition between grassland and forest interior sites may be because certain mosquito species (e.g. *An. farauti* and *Cx. gelidus*) find open habitats such as grasslands more attractive than closed habitats.

The question that then arises is “why do some mosquitoes prefer open habitats to closed habitats”? The answer might be that open and disturbed habitats feature environmental characteristics such as higher temperatures and light levels, higher pH-

and lower salt levels, which can accelerate larval growth and increase larval survivorship. These changing environmental conditions also contribute to faster growth of algae – an important food source for mosquito larvae (Camargo and Kapos 1995, Patz et al. 2000, Ye-Ebiyo et al. 2003, Bond 2005, Tuno et al. 2005, Vittor et al. 2009).

It was not unexpected that more mosquitoes and species were captured in the wet season compared to the dry season. Mosquitoes are commonly associated with rainfall (Russell 1998a, Patz et al. 2000). However, it was surprising to find that some important disease vectors such as *Ae. notoscriptus*, *Ae. vigilax, An. farauti* and *Cx. annulirostris* in my study area were able to persist in the dry season. This suggests that disease transmission could potentially occur at any time of the year.

Previous studies have found that refugia with favourable microclimatic conditions may help a mosquito population to persist year round. For example, Hightower et al. (1998) found that malaria vectors (*Anopheles* spp.) in Kenya retreat to vegetation around permanent water bodies during the dry season, allowing for year round reproduction. Water-filled tree holes and abandoned snail shells can also be used for oviposition (Lounibos 1981). Additionally, anthropogenic changes to the environment like the construction of irrigation areas and dams allow mosquitoes to breed regardless of seasons (Mulla et al. 1987, Ghebreyesus et al. 1999, Singh et al.1999, McMichael 2001). Jardine et al. (2004) demonstrated that the Ord River Irrigation Area in Western Australia is responsible for mosquitoes breeding even during the driest month of the year. For example, *Cx. annulirostris*, the most abundant species in this study and responsible for transmitting numerous viruses (Ross River, Kunjin, Murray Valley and Japanese encephalitis) (Marshall 1998, van den Hurk et al. 2003) was found to be very active during the dry season (Jardine et al. 2004).

There may be other mechanisms which contribute to the year-round persistence of mosquitoes. Biological, physiological and ecological attributes such as desiccation-resistant eggs, egg dormancy, diapause and larval development in moist soil, leaf litter or plant axils and adult and larvae hibernation, aestivation, quiescence and diapause could be mechanisms for survival in environments with seasonal periods of droughts (Louinbos 1981, Beier et al. 1990, Minakawa et al. 2001, Yang et al. 2008, Denlinger and Armbruster 2014). Additionally, some adult mosquitoes are able to aestivate by gonotrophic dissociation (the pausing of egg production despite acquiring several blood meals) or by performing diapause (Swellengrebel 1929, Sanburg and Larsen 1973, Jupp 1975, Washino 1977, Benoit 2010). Lastly, man-made and natural shelters may provide refugia for inactive parous (having laid eggs at least once) female mosquitoes (Russell and Whelan 1986). Captured mosquitoes from my study most likely used one or a combination of the strategies outlined above to persist year-round. For example, *Ae. notoscriptus* has both desiccation-resistant eggs and the ability to perform hibernation and diapause during larval and adult life stages (Cooling 1924, Graham 1939). However, the eggs of *Cx. annulirostris*, the most dominant species in this study, are prone to desiccation (Lee et al. 1989); but adults can perform diapause (Jupp 1975) and are known to lay eggs in shallow, grassy pools only hours after a rainfall event (McDonald and Buchanan 1981). This is in accordance with the findings of this study as most *Cx. annulirostris* were captured in grasslands.

I expected that mosquito species richness would be highest in forest interior habitats as tropical forests often support greater diversity within forest interiors and decline along forest edges (Laurance 2004, Ernst and Roedel 2008, Hillers et al. 2008). However, this pattern was not observed in this study for mosquito diversity. In contrast, the number of species was quite evenly distributed across the three habitat types. Even though the sampling effort was sufficient, rare species, most notably canopy specialists, could be absent from my samples as a consequence of trap height

(~1.5 m). More extensive sampling at different vertical strata would most likely increase the probability of capturing such rare species in future studies (Jansen et al. 2009a).

I believe that it is crucial to comprehend the impacts of landscape disturbance, especially the impacts of deforestation and forest fragmentation, on mosquito communities. Commonly-captured mosquito species in my study were found to be widespread in geographic distribution, ground breeders and nocturnal feeders. I consider the current distribution of these species to be the result of land clearing and agriculture (predominantly sugar cane). Prior to European arrival, tropical rainforests, open eucalypt forests and estuarine vegetation blanketed the region (Kemp et al. 2007), and the mosquito communities would have been dominated by species that were more restricted in distribution to these different habitats.

My observations of changing mosquito communities in response to land use, support the concept that new or re-emerging mosquito-borne diseases could arise in areas where land use changes occur. Disease transmission will almost certainly emerge via common mosquito species captured across all habitats and seasons (e.g. *Cx. annulirostris*) or through species more prolific within open habitats, such as man-made grasslands (e.g. *An. farauti, Cx. gelidus, Ma. septempunctata*) (Meyer Steiger et al. 2012). Previous studies from tropical areas have already demonstrated that human-induced land use changes, such as deforestation, are responsible for a rise in important disease vectors such as Anopheline and Aedine (Patz et al. 2000, Matthys et al. 2006, Yasuoka and Levins 2007, Olson et al. 2010). However, my study is the first to suggest that common species and species which are potential disease vectors can maintain populations across a land use gradient throughout the year.

In summary, I demonstrated that the mosquito community in north Queensland strongly responded to anthropogenic land use changes. My results displayed that there

is a diverse mosquito community in tropical Australia, but more importantly that the community composition varies considerably between forests and disturbed habitats. Additionally, most disease transmitting species predominantly occur in grasslands created by humans. This strong influence of anthropogenic land use change on mosquito communities could have potential implications for pathogen transmission to humans and wildlife. I also found that vectors of mosquito-borne diseases, such as *Cx. annulirostris*, can persist all year round, further increasing disease risk. Considering that human-induced land use changes and human population growth are advancing rapidly in tropical regions, it is of the utmost importance to predict future disease risk. Historically, mosquito studies have mainly focused on single species life cycles in association with urban environments and I suggest further ecological studies are necessary to understand how land use changes will influence disease dynamics of the whole community in order to predict and prevent future health threats.

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CHAPTER 3

Land use change and breeding mosquitoes in tropical Australia: an experimental approach using small artificial oviposition containers

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3.1 Abstract

Anthropogenic land use changes have been implicated in the emergence and resurgence of mosquito-borne diseases, especially in the tropics. In tropical Australia, immature mosquitoes were sampled from artificial oviposition traps across an anthropogenic disturbance gradient of grassland, rainforest edge and rainforest interior habitats. I reared 3,347 mosquitoes from 72 traps that were placed either on the ground or above ground. I found that habitat type had no influence on the number of mosquitoes reared, but trap location was important. Traps placed on the ground yielded four times more emerging mosquitoes than traps above ground. Six species from three genera (*Aedes*, *Tripteroides* and *Toxorhinchites*) were identified, with the majority (93%) of the individuals comprising two species: *Aedes palmarum* and *Aedes notoscriptus*. Most species were reared from disturbed habitats (forest edges and grasslands). An important disease vector, *Ae. notoscriptus*, was predominantly raised from grassland site traps. My findings suggest that artificial containers on the ground, in combination with disturbed environments, provide ideal mosquito larval habitat thus increasing the risks of disease transmission.

3.2 Introduction

The emergence and resurgence of mosquito-borne diseases in tropical regions are often strongly correlated with anthropogenic land use changes. One of the most destructive land use change in the tropics is the clearing of rainforests. Consequently deforested areas either become weed-infested open grasslands or are transformed into human settlements and agricultural landscapes (Patz et al. 2000). Such modified environments can be beneficial for some mosquito species due to the creation and expansion of suitable breeding sites. Mosquito breeding sites encompass a wide range of natural and artificial habitats. Natural habitats range from large water bodies (swamps, marshes, rivers and lake edges) to small collections of water (tree-holes, plant axils, snail shells and fallen leaves). Similarly, artificial habitats also range from large water bodies (rice fields, irrigation channels, roadside ditches, latrines) to small collections of water (artificial water-holding containers such as domestic utensils, car tyres, roof gutters, etc.) (Rejmankova et al. 1991, Silver 2008, Nikookar et al. 2016).

Habitat selection for oviposition (the act of depositing eggs) by the gravid mosquito is species dependent. Some species have the ability to utilise a great variety of oviposition sites whereas others are more selective (Silver 2008). For example, *Culex annulirostris*, the most important arbovirus vector in Australia (Doherty 1977, van den Hurk et al. 2003), can exploit almost any available water source (permanent, temporary, fresh, saline, turbid, stagnant) for oviposition, including larger artificial containers such as water- and sewage tanks (Belkin 1962, Kay 1979). Females of the closely-related genera *Coquillettidia* and *Mansonia* oviposit only in the presence of aquatic or semi-aquatic plants as their immobile and constantly submerged larvae need to attach to vegetation to obtain oxygen (Laurence 1960, Russell 1999). In contrast, no aquatic vegetation is necessary for *Anopheles arabiensis* of Western Kenya, who oviposit in small, temporary and highly turbid habitats containing algae (Gimnig et al. 2001).

Mosquito larval sampling is a valuable surveillance tool for vector control (Ndenga et al. 2011) whereby the management of larval habitats acts as source reduction (Slooff 1987, Samson et al. 2015). For example, an extensive malaria control program in Zambia determined anopheline larval habitats in copper mining communities. The findings were subsequently used to implement management strategies to eliminate or modify these larval habitats (in Utzinger et al. 2001). Anopheline larvae (*An. arabiensis*) were also the target of a larval survey conducted from breeding sites associated with the creation of a dam in Ethiopia. It was discovered that larvae were present all year round (and therefore extending the length of the malaria transmission season) due to leakage areas around the dam and its irrigation canals. Draining, filling and shading of these leakages reduced larval sources and resulted in a decrease of adults by nearly 50% (Yohannes et al. 2005). Vector control also includes the source reduction of artificial containers suitable for oviposition. Some of the most debilitating mosquito-borne diseases are transmitted by vectors that exploit artificial containers for oviposition, e.g. *Aedes aegypti* (Christophers 1960), *Aedes albopictus* (Robertson and Hu 1935), *Aedes notoscriptus* (Russell 1986), *Culex gelidus* (Sirivanakarn 1976) and *Culex quinquefasciatus* (Marks 1965).

Mosquito larvae and pupae from natural oviposition sites are typically sampled by net-sweeping, dipping, siphoning and pipetting (see Lounibos 1981, Rejmankova et al. 1991, Brown et al. 1992, Sattler et al. 2005, Ritchie et al. 2006a, Le Goff et al. 2014, Chirebvu and Chimbari 2015, Samson et al. 2015, Sulesco et al. 2015, Nikookar 2016). Studies which use artificial containers are often supplied with laboratory mosquito eggs or larvae of a particular species. For instance, Gimnig et al. (2002) employed plastic wash basins (35 cm diameter x 13 cm deep), prepared to resemble natural habitats, which were stocked with freshly emerged laboratory-reared anopheles larvae. This experiment was performed to evaluate if larval development was density-dependent under manipulated conditions. Tun-Lin et al. (2000) placed a variety of

artificial containers of different sizes in shaded and unshaded urban settings in north Queensland. Laboratory-raised *Aedes aegypti* larvae were added and ovipostition by wild mosquitoes was prevented. This research examined how temperature influenced larval development, survival rate and size of emergent adults.

Studies with the main aim of attracting a target species to oviposit in artificial containers are frequently employed for vector surveillance. For example, dengue vector research relies heavily on monitoring and controlling *Ae. aegypti* larvae in domestic settings. Lethal ovitraps, often containing pyrethroids, are deployed to kill gravid mosquitoes (Zeichner and Perich 1999, Perich et al. 2003, Williams et al. 2007, Ritchie et al. 2008). The evaluation of different sized-containers is also an integral part of vector control. Richardson et al. (2013) used a variety of containers (pot plant bases, 420 ml drinking cups, car tyres, 35 L and 100 L waste bins) under sun and shade conditions in temperate and tropical locations to record and compare development and survival rates of *Ae. aegypti*.

Fewer studies are performed to attract females of different species to use small, artificial containers for oviposition. Derraik and Slaney (2005) tested if container aperture size and nutrient preferences influenced oviposition in native and exotic species in New Zealand. In Florida, Obenauer et al. (2009) used tin cans (11 cm in height and 7.5 cm in diameter) to evaluate if infusions attracted container breeding mosquitoes at different elevations and habitats.

In this study, I investigated mosquito breeding behaviour by employing small artificial containers across an anthropogenic disturbance gradient of grassland, forest edge and forest interior habitats in the tropical lowlands of north Queensland, Australia. My main objectives were to determine which species used these artificial containers for

oviposition and whether numbers of emerging mosquitoes were influenced by habitat type, trap location and water temperature.

3.3 Methods

3.3.1 Study area

I conducted this study in the Wet Tropics bioregion of north eastern Australia, at the same four sites as the sampling for Chapter 2 (Fig. 2.1).

3.3.2 Sampling methods for mosquito larvae

Field work was conducted from January to May 2013. At each of the four study sites, I established three parallel transects, with one transect in the forest interior (>100 m from the nearest edge), one along the forest-edge treeline, and one in the adjoining grassland (>100 m from the nearest forest). I used artificial oviposition traps (Appendix 1; Figure 2) which were black plastic containers (12 cm high and 14 cm in diameter) with 4 mm holes drilled 3 cm below the rim to allow rainwater to discharge. Black containers were selected as this colour seems to be preferred by gravid mosquitoes to oviposit (Yanoviak 2001). Strips of sandpaper, partially immersed in water, were added to the containers to provide a surface for egg-attachment (Leisnham et al. 2004). Each trap received one (0.5 mg) lucerne (alfalfa) pellet as an attractant for ovipostion and as a food source for the larvae. Traps contained 0.7 L of water; if needed, water was replenished during the trial. Data loggers were added to record water temperature. Containers were covered with 0.5 cm galvanised wire mesh to obstruct animals from drinking the water (Leisnham et al. 2004). Organic matter (e.g. leaves) was not provided, but was not prevented if occurrence was natural. At each site, three traps per habitat were placed on the ground and three traps were placed ~ 1.50 m above ground resulting in 72 total trap catches. The oviposition traps were left at the sites for 21 days

and I assumed that no mosquitoes had emerged from the traps while out in the field as no pupal cases were detected, although it is possible that the pupal cases had disintegrated or were ingested by the larvae.

3.3.3 Rearing of larvae in laboratory

Ovipostion traps were moved to a temperature controlled room (26°C; 75% relative humidity) at James Cook University Cairns. The contents of each trap were transferred into clear food storage containers which were the same size as the ovitraps. Containers were covered with fine mesh to prevent hatched mosquitoes from escaping. Larvae were fed yeast (Tandaco®, Australia, dry yeast) and fish food (Tetra Min® Germany, tropical tablets) once per week and fresh water was added when needed. Emerging mosquitoes were removed every second day for species identification.

3.3.4 Data analysis

I used a two-way ANOVA (independent factorial design with fixed factors) to compare whether habitat type and trap location had an effect on the number of mosquitoes reared from the artificial ovitraps. Variables were square-root transformed to satisfy the assumptions of the residuals conforming to a normal distribution and in homogeneity of variances. I applied Chi-square tests (Yate's correction for continuity was used if there were only two categories tested and only species which had cells higher than the expected frequency of 5 were examined) to determine which habitat mosquito tribes and species preferred for depositing their eggs. Spearman's rank-order correlation analysis was used to establish whether there was a correlation between the number of emergent mosquitoes and water temperature (I tested minimum, mean and maximum water temperature). The failure of 6 data loggers resulted in 66 of 72 oviposition traps being analysed.

SPSS 22.0 statistical package was used for all analyses. Where required, data were tested for normality by using the Kolmogorov-Smirnov tests and Levene's tests for homogeneity of variances.

3.4 Results

3.4.1 Mosquito emergents

From the 72 artificial oviposition traps across the grassland-edge-rainforest gradients 49 produced mosquitoes. In total, I reared 3,347 mosquitoes with an average of 68 ± 12 (mean±SE) mosquitoes per trap. In each of the three habitats, 51% of the emerging mosquitoes were female and 49% were male. Although more mosquitoes in total were reared from grassland (44%) and forest edge (33%) traps than forest interior traps (23%), this difference was not significant because of high among-trap variability in captures within each habitat type ($F_{2,18}$ = 1.517, P = 0.246). However, trap location had a significant effect on the numbers of mosquitoes reared; four times more mosquitoes emerged from traps located on the ground compared to traps which were placed above ground ($F_{1,18}$ = 19.306, P < 0.0001 (Fig. 3.1). No interaction effect was observed between habitat and the location of the traps ($F_{2,18}$ = 0.134, P = 0.875).

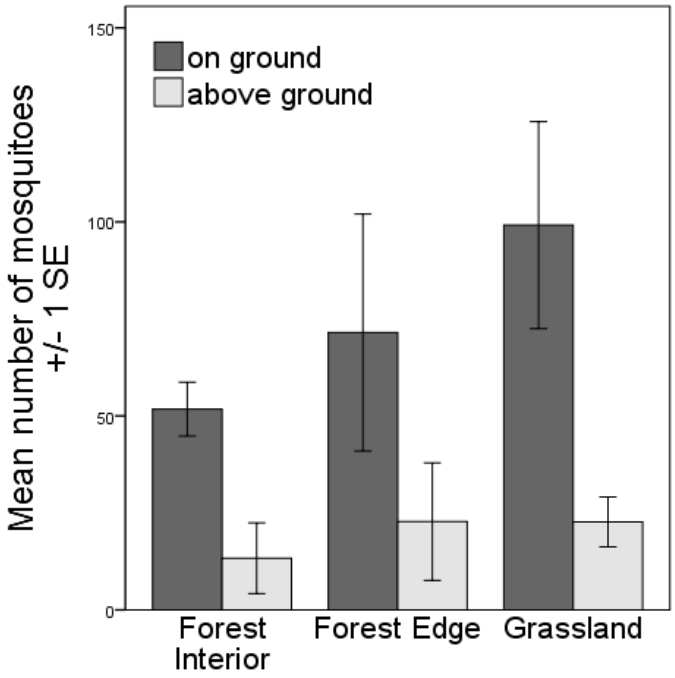

Figure 3.1: Mean number of mosquitoes reared from artificial ovitraps from three different habitats and two different trap locations. Habitat had no effects on the mean number of mosquitoes reared, but significantly more mosquitoes were reared from traps located on the ground.

Mosquitoes reared from traps consisted of three tribes and one subfamily of mosquitoes. All mosquitoes were in the subfamily Culicinae and most (97%) individuals were in the Aedini tribe, with only 3% in Sabethini. Members of the Aedini appeared to prefer more open habitats to lay their eggs as significantly more mosquitoes emerged from grassland and forest edge traps compared to forest interior traps ($\chi^2_2 = 166.03$, $P < 0.001$; Table 3.1). Individuals of the Sabethini seemed to avoid forest locations altogether as most mosquitoes were reared from traps which were placed in grassland habitats ($\chi^2_2 = 143.64$, $P < 0.001$).

Table 3.1: Mosquito emergents by tribe from three habitats in tropical Australia (FI = Forest Interior; FE = Forest Edge; GR = Grassland).

Tribe	FI	FE	GR	Total		Chi-Square test (df=2)	
				N	%	χ^2	*P* <
Aedini	771	1112	1369	3252	97.16	166.03	0.001
Sabethini	7	1	86	94	2.81	143.64	0.001
Toxorhynchitini	0	0	1	1	0.03	n.a	n.a

3.4.2 Influence of water temperature

Even though water temperature in the traps ranged from 13.7 °C to 43.5°C, I found that this had no effect on the number of emerging mosquitoes (Spearman's rank-order correlation tests: minimum temperature r_s = - 0.121, P = 0.334; mean temperature r_s = 0.021, P = 0.865; maximum temperature r_s = - 0.137, P = 0.274) (Figure 3.2). Water temperature had also no effect on the number of emergents from the two different trap locations (Table 3.2).

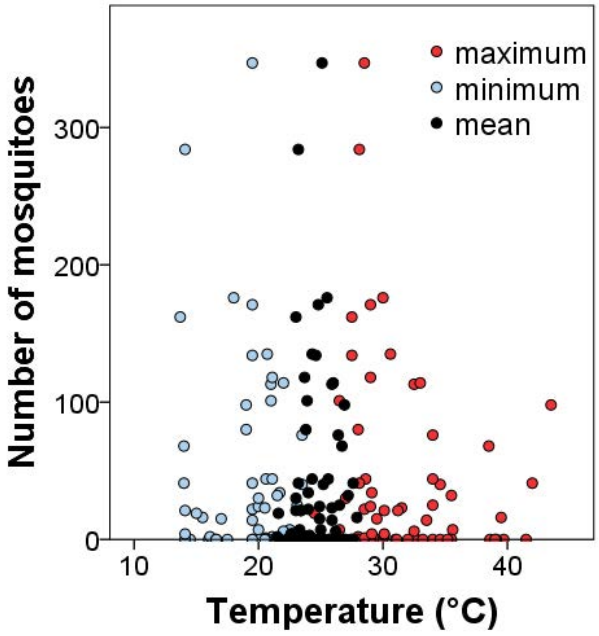

Figure 3.2: Spearman's rank-order correlations show that water temperature (maximum, minimum and mean temperature) had no influence on the number of emerging mosquitoes.

Table 3.2: Spearman's rank-order correlation values (r_s) indicate that water temperature did not influence the number of emerging mosquitoes from the two trap locations.

Trap Location	Temperature	r_s	*P* value
on ground	minimum	-0.167	0.379
on ground	maximum	-0.139	0.464
on ground	mean	-0.065	0.735
above ground	minimum	0.066	0.701
above ground	maximum	0.056	0.744
above ground	mean	0.112	0.516

3.4.3 Mosquito species

A total of six mosquito species from three genera utilized the artificial ovitrap for reproduction (Table 3.3). The genus *Aedes* was not only the most species rich (4 species) but was also numerically the most dominant genus (97% of individuals compared to only 2.8% for *Tripteroides* and 0.2% for *Toxorhynchites*). Two species constituted 93% of identified mosquitoes: *Aedes palmarum* (49%) and *Aedes notoscriptus* (44%). *Ae notoscriptus* was present in 73 % of the 49 positive ovitraps, *Ae. palmarum* was recorded in 50% of the traps while *Ae. tremulus* and *Tp. magnesianunus* occurred in 10% of the traps. 57% of the traps contained a sole species and two or more species co-occurred in 43% of the traps. I investigated the mosquito larval community composition of each habitat type by examining species dominance with rank-abundance diagrams. *Ae. palmarum* was the most dominant species both in forest interior and forest edge sites and *Ae. notoscriptus* was the most dominant species in the grasslands (Fig. 3.3).

Table 3.3: Total number of mosquito species reared from each habitat in north Queensland and the pathogens they may transmit.

	Forest Interior		Forest Edge		Grassland		
	on ground	above ground	on ground	above ground	on ground	above ground	Total
Species							
Aedes kochi [a,d,e]	0	1	0	0	0	0	**1**
Aedes notoscriptus [a,b,d]	223	159	211	212	444	234	**1483**
Aedes palmarum [?]	388	0	645	43	529	36	**1641**
Aedes tremulus [a,b]	0	0	1	0	124	2	**127**
Tripteroides magnesianus [?]	7	0	1	0	86	0	**94**
Toxorhynchites speciousus [*]	0	0	0	0	1	0	**1**
Total	**618**	**160**	**858**	**255**	**1184**	**272**	**3347**

a = alphaviruses (may cause Barmah Forest, chikungunya, Ross River, Sindbis)
b = flaviviruses (may cause dengue, Murray Valley-, Australian-, Kunjin- & Japanese encephalitis; yellow fever, Edge Hill, West Nile)
c = *Dirofilaria immitus* (can cause dog heartworms)
d = *Wuchereria bancrofti* (can cause filariasis in humans)
? = unknown
* = can not transmit diseases as it does not feed on blood

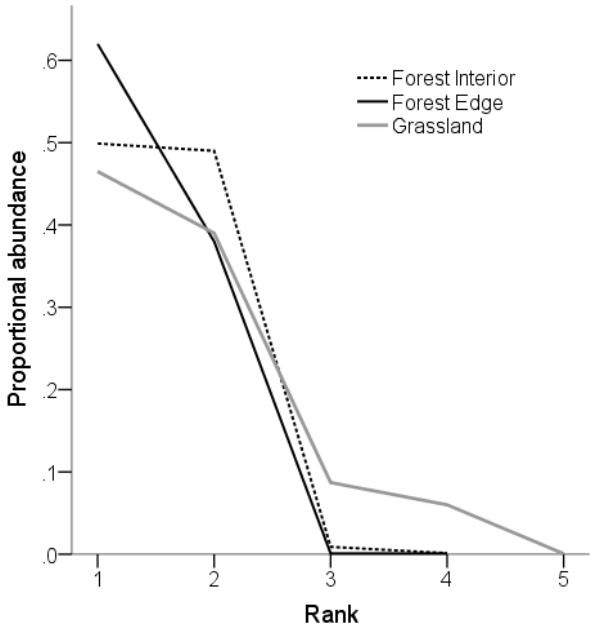

Figure 3.3: Rank-abundance diagram displaying the diversity of mosquito species reared from each of the three habitats.

I assessed the patterns of abundance for the four most common species along the forest interior to grassland gradient. Two general patterns were detected: none of the mosquito species preferred to oviposit inside forests and most species chose grassland traps to lay their eggs (Fig. 3.4). These patterns were further supported by a highly significant association between certain mosquito species and particular habitats: *Ae. notoscriptus* (χ^2_2 = 104.061, P < 0.001) and *Tp. magnesianus* (χ^2_2 = 146.638, P < 0.001) were found to have a preference for ovipositing in grasslands and *Ae. palmarum* mainly utilised traps along forest edges (χ^2_2 = 83.458, P < 0.001).

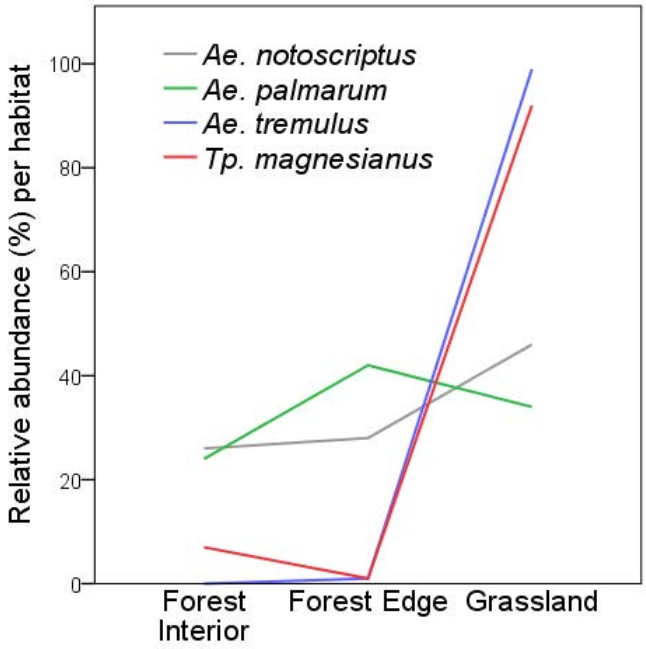

Figure 3.4: Trends in relative abundances of mosquito species reared from artificial containers from forest interior, forest edge and grassland habitats in tropical Australia.

Species accumulation curves suggest that I have adequately sampled the larval community from all three habitats as approaching asymptotes were observed before the sampling process was terminated. However, increased sampling effort could result in the discovery of more species, especially rarer ones (Fig. 3.5).

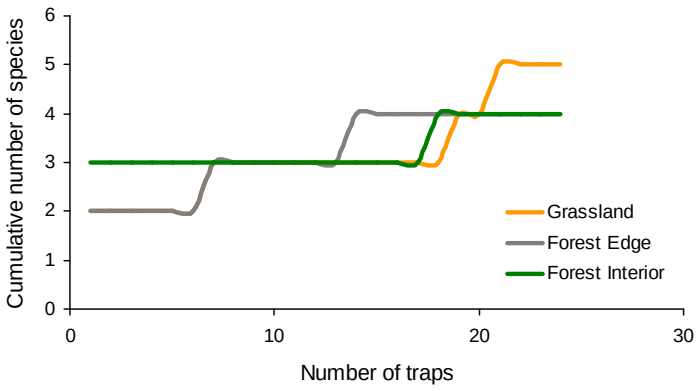

Figure 3.5: The species accumulation curves display adequate sampling effort for all habitats.

Mosquitoes emerged for up to 60 days from being placed in the temperature controlled laboratory. Generally, on ground traps produced mosquitoes longer than above ground traps in all habitats (Fig. 3.6). Forest interior and forest edge traps had later emergents than grassland traps. The last emergent was *Ae. notoscriptus* from an above ground trap from forest interior (60 days) and the second last emergents were *Ae. palmarum* and *Tp. magnesianus* who emerged after 55 days from on ground traps from forest interior.

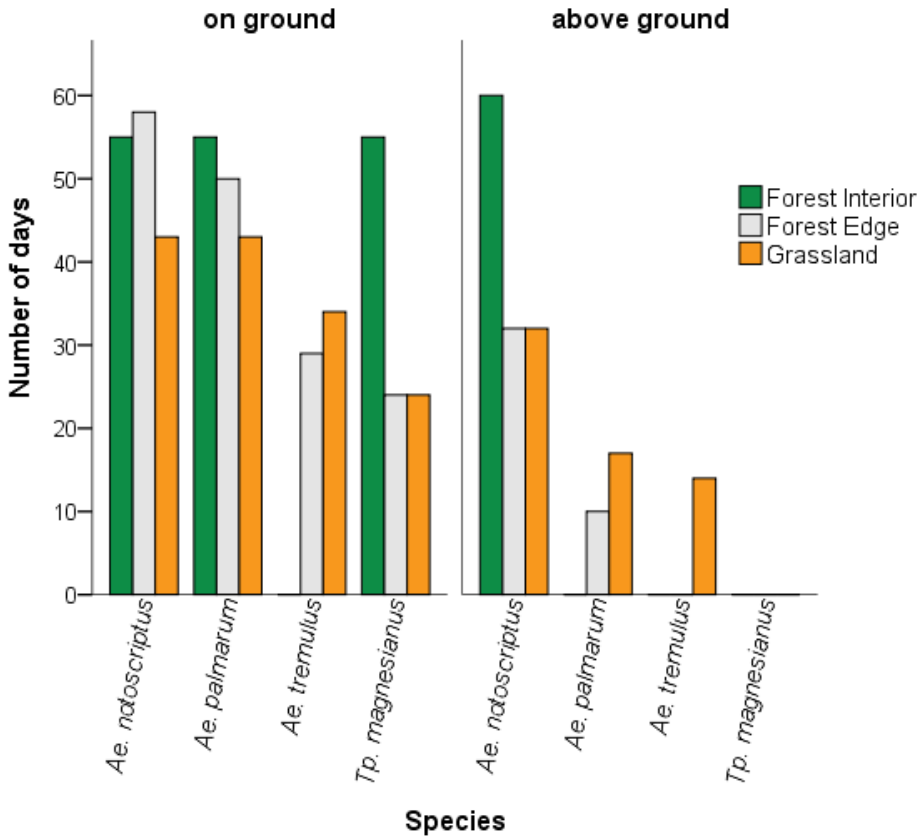

Figure 3.6: The time (number of days) the four most common species produced emergents from on ground and above ground traps.

3.5 Discussion

The gravid mosquito's choice of the oviposition site invariably affects the survival and growth of the immature aquatic stages and thus the number of emerging adults. In this study I employed small artificial oviposition containers at two height levels across an anthropogenic disturbance gradient of three habitats. The results revealed that habitat type had no influence on the number of emergents and that traps placed at ground level produced more emergents than traps located above ground.

Although, statistically (due to high trap variability affecting the mean) I failed to demonstrate that habitat type had an effect on the number of emergents. However, numerically grassland traps yielded nearly twice as many emergents than forest interior traps. Higher immature mosquito abundance from artificial containers has also been observed in open areas compared to forested areas in New Zealand (Leisnham et al. 2004); and in Kenya, Tuno et al.(2005) reported that larval survival was 56% in open habitats compared to 1.5% survival along forest edges and inside forests.

I obtained more mosquitoes emerging from traps placed at ground level compared to traps located above ground (1.5 m). Previous studies have shown oviposition preferences for ground level traps. Ae*. albopictus* and *Ae. subalbatus* deposited more eggs in bamboo traps on ground level compared to traps at 3.5 m and 7.0 m in height (Amerasinghe and Alagoda 1984). Williams et al. (2006) reported more captures of *Ae. aegypti* from sticky ovitraps located on the ground than from traps located 1.75 m above ground. The authors explain that strong winds could have made trap access more arduous at a height of 1.75 m due to this elevation being above the flight boundary layer for *Ae. aegypti*. The flight boundary layer (within this layer insects can fly freely, above it insects are exposed to the wind) varies between species, wind velocity, season and topography (Clements 1999). There is consensus, however, that oviposition preferences related to height are not only species dependent but also

affected by geographical location (Obenauer et al. 2009). For example, Chadee (1991) observed that *Ae. aegypti* in Trinidad as opposed to *Ae. aegypti* in Cairns (Williams et al. 2006), laid twice as many eggs in above ground traps (1.2 m) than in ground level traps. *Ae. triseriatus* in Indiana (Novak et al. 1981) mainly used oviposition traps in the canopy whereas in Wisconsin ground level traps were preferred (Scholl and de Foliart 1977). It is also known that some species show no preference or are opportunistic in regards to trap heights. For instance, Amerasinghe and Alagoda (1984) found that *Ae. novalbopictus* in Sri Lanka deposited similar amounts of eggs into traps located at 0 m, 3.5 m and 7.0 m above ground.

I observed and was surprised that mosquitoes emerged for up to 60 days after the traps were removed from the field and maintained in the temperature controlled laboratory. Several factors could account for the long period of emerging mosquitoes: autogeny, instalment hatching, egg diapause or density dependency. Whether species are autogenous (no blood meal is necessary for at least the first production of eggs) or anautogenous (need to blood feed in order to reproduce) (Roubaud 1929, Clements 1992) is influenced by geographical location, environmental conditions, genetics, nutrition, population dynamics and host availability (Spielman 1957, Clements 1992, Ariani et al. 2015). Autogeny has, for example, been recorded for *Ae. aegypti* (only true for certain genotypes) (Ariani et al. 2015), *Ae. caspius* (Ahmed 2013), *Ae. atropalpus* (Telang et al. 2006), *Ae. togoi* (Junkum et al. 2003), *Cx. pipiens* complex (Californian population) (Strickman and Fonseca 2012), *Cx. molestus* (Kassim et al. 2012) and *Cx. annulirostris* (Kay et al. 1986). Successful autogeny typically depends on a nutrient rich diet for larvae and the availability of sugar sources for adults (Ariani et al. 2015). I did not provide nectar or any other sugar sources to the emergents and I am therefore doubtful that autogeny was responsible for the extended emerging period as it seems highly unlikely that the acts of emergence, mating, egg maturation and oviposition could have been completed within the time frame of two days (as emergents were

collected every 2nd day). In this case the extended period of emergents can most likely be explained by instalment hatching due to high larval density.

Instalment or asynchronous hatching in mosquito eggs, even if laid at the same time or from the same egg batch, is quite common and is a reproductive strategy to ensure species survival, especially in temporary larval habitats. Temporary larval habitats, such as salt marshes or treeholes, receive irregular inundations from tides or rainfall (Gillett 1955, Wilson and Horsefall 1970, Becker 1989, Andreadis 1990). Following inundations, elevated microbial activities cause a reduction in oxygen concentration which appears to activate egg hatching, especially in *Aedes* species (Gjullin et al.1941). It is therefore possible that I induced egg hatching each time I added water to the field traps and later to the containers in the laboratory. Additionally, instalment hatching in this experiment was probably also due to larval density in two ways: high larval densities can decrease egg hatching (Livdahl et al. 1984, Livdahl and Edgerly 1987) and larval development time is decelerated, predominantly owing to food availability (Bar-Zeev 1957, Reisen et al. 1984, Agnew et al. 2000, Tuno et al. 2005, Hancock et al. 2016). Many studies on density-dependency in the immature stages of mosquitoes have been published, although most of them were performed under laboratory conditions (Fish and Carpenter 1982, Hard et al. 1989, Fisher et al. 1990, Smith et al. 1995) where response variables are easier to control than in field-based studies. Field-based studies have mainly been conducted on phytotelmata breeding species (Lounibos 1981, Bradshaw and Holzapfel 1989, Lounibos et al. 2003).

Another well documented factor affecting immature mosquito development is water temperature (Mohammed and Chadee 2011, Kassim et al. 2012, Hancock et al. 2016). Large variations exist in optimal and lethal water temperatures for the development of different species, with geographical location playing an important role in the lower and upper lethal thresholds of water temperature (Becker et al. 2010). Within these

thresholds, immature mosquito development is faster as temperature rises (Brust 1967, Hagstrum and Workman 1971). For example, Bayoh and Lindsay (2003) demonstrated that the optimum temperature range for *An. gambiae* s.s. is between 28°C and 32°C where development time from egg to adult lasted 10 days; whereas at 18°C the development time increased to 23 days; water temperatures below 18°C and above 34°C yielded no emergents. At the other end of the temperature spectrum is the subarctic *Ae. impiger* which can develop at 6°C but it lasts 40 days; at 9°C it takes 24 days and at 13°C 15 days (Haufe and Burgess 1956). Some species, such as the second most abundant species (*Ae. notoscriptus*) in my study, display plasticity in regards to water temperatures. Kay et al. (2008) observed that *Ae. notoscriptus* can tolerate temperature ranges from 9°C to 37°C in field situations. Conversely, Williams and Rau (2011) observed that larvae did not survive to second instar in a 35°C temperature controlled study. The most likely explanation for this small discrepancy of the upper threshold could be that water temperatures fluctuate in the field whereas in laboratory experiments they stay constant, which could be the reason as to why there was no association of water temperature in the field and the number of emergents in my study. Accelerated larval development time, which could intensify with climate change, can have considerable implications for disease transmission. The faster and increased production of female mosquitoes also increases biting rates and therefore the probability of infection (Garrett-Jones and Ferreira 1964, Kovats et al. 2001).

The six species which produced emergents from the traps are known tree-hole breeders and often co-occur (Williams et al. 2006 and 2013, Kay et al. 2008, Ritchie et al. 2008). Most tree-hole breeders also exploit other oviposition sites such as rock-pools, pitcher-plants, bromeliads, cavities created by buttress roots, fallen palm-fronds, hollow bamboo stems and artificial containers (Russell 1986, Lee et al. 1989, Clements 1999). Tree-hole breeders generally have a preference to oviposit in shady areas, but interestingly I reared the most emergents from grassland traps. This is in stark contrast

to previous findings, especially for *Ae. notoscriptus* which was the second most abundant species in this study. Cooling (1924) noted that its breeding habitats were deeply shaded and located within dense vegetation and other studies have made similar observations (Hamlyn-Harris 1928, Foot 1970, Laird 1990, Leisnham et al. 2004). For example, Leisnham et al. (2004) detected immature *Ae. notoscriptus* nearly exclusively in containers inside forests. When I compared habitat preferences for the four most commonly emerged species (*Ae. notoscriptus, Ae. palmarum, Ae. tremulus and Tp. magnesianus*) in this larval study with my research on the adult community at the same sites (Chapter 2), I find that these four species were most frequently captured in forest interior habitat (Meyer Steiger et al. in press).

This poses the question as to why my findings do not reflect the results of past studies. Geographical location and climatic variations are unlikely to be the causes as studies from other areas and temperate regions also state *Ae. notoscriptus*' preference for forested or at least shady sites (Foot 1970, Leisnham et al. 2004). The number of emergents could have been influenced by the presence of predacious mosquito larvae; however, this is doubtful as I only raised a single individual of the predatory *Toxorhynchites speciousus* and it was from a grassland trap which yielded 41 *Ae. palmarum* emergents. Competition for oviposition sites due to the presence of other species can probably also be excluded as *Ae. notoscriptus* immatures are often found in association with other tree-hole species (Lee et al. 1982, Williams et al. 2006, Ritchie et al. 2008).

One of the following scenarios could be accountable for the preference of grassland oviposition by *Ae. notoscriptus* and the other species from this study. First, container-breeding females often perform skip-oviposition (distributing eggs singly among multiple oviposition sites) (Mogi and Mokry 1980, Edgerly et al. 1998, Watson et al. 2000). This strategy has been observed for *Ae. aegypti*, *Ae. albopictus*, *Ae.*

polynesiensis and *Ae. notoscriptus* (Rozeboom et al. 1973, Reiter et al. 1995, Watson et al. 2000). Second, host-seeking females may disperse from forests to blood-feed on animals in the adjacent grasslands. Native mammals, such as wallabies, are often observed feeding in the grasslands of the study area (pers. observation). *Ae. notoscriptus* is mammalophilic (Johansen et al. 2009) and is known to feed on marsupials (Lee et al. 1957). Once female mosquitoes have obtained their blood-meal, they need to rest for 2 - 4 days (in tropical climates) (Reiter et al. 1995); and they tend to do so nearby (Le Menach et al. 2005, Becker at al. 2010) as their body weight can triple after a blood-meal (Nayar and Sauerman 1975). The mosquitoes in this study could have rested in the grassland vegetation and when ready to lay eggs utilised the closest oviposition options. Mosquito dispersal is dictated by searching for blood-meal hosts and oviposition sites and hence a shorter distance between these two is probably advantageous (Reiter et al. 1995, Le Menach et al. 2005). Third, some females may just simply be opportunistic ovipositors and return to their original habitat once they have oviposited (Edgerly et al. 1998, Edman et al. 1998). Finally, immmature mosquitoes in forest traps could have been subjected to parasitism by nematodes, fungi or bacteria and thus produced less emergents. *Coelomomyces* species are widespread fungal parasites infecting and killing larvae of many mosquito species, including *Ae. notoscriptus* (Laird 1959).

I realise that this short-term study only offers limited insight into mosquito breeding behaviour in the three habitats. Undoubtedly further sampling, especially during the dry season, could provide a more detailed representation of mosquito breeding dynamics across the chosen anthropogenic disturbance gradient.

I believe that future health threats from mosquito-borne diseases are most likely to eventuate from species which leave their traditional breeding habitats. *Ae. notoscriptus* has already successfully made this transition from sylvan habitat to become the main

native peri-domestic artificial container breeding mosquito in Australia (Russell 1986). It is a vector of Ross River and Barmah Forest virus (Doggett and Russell 1997) and dog heartworm (Russell and Geary 1996); and laboratory studies disclosed that it has the competence to vector yellow fever (van den Hurk et al. 2011) and Rift Valley fever (Turell and Kay 1998). *Ae. palmarum* and *Tp. magnesianus* have to date not been implied in disease transmission. *Ae. tremulus* seems to be severely understudied even though isolates of Ross River, Sindbis, Kunjin and Murray Valley encephalitis viruses have been retrieved (Russell 1998b). *Ae. notoscriptus*, *Ae. palmarum*, *Ae. tremulus* and *Tp. magnesianus* commonly co-occur in artificial containers (Williams et al. 2006, Kay et al. 2008, Ritchie et al. 2008, Williams et al. 2013). Using small artificial containers as a surveillance tool at the periphery of urban areas could detect which species utilise them and would allow more precise predictions on the future spread of potential disease vectors. I would also recommend, unless height preferences for targeted species are already known, to place ovitraps at various distances from the ground. It also needs be taken into account that mosquito immature development is not only species specific, but also varies with geographical location and environmental conditions.

In summary, this study demonstrated that small artificial containers in grasslands, especially at ground level, are particularly productive mosquito larval habitats in tropical Australia. It is also quite evident, that immature mosquitoes are able to employ a variety of strategies for surviving adverse conditions which has further implications for vector control and therefore disease monitoring.

CHAPTER 4

Overcoming the challenges of mosquito sampling in remote localities: a comparison of CO_2-attractants on mosquito communities in three tropical forest habitats

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4.1 Abstract

Emerging infectious diseases are on the rise with future outbreaks predicted to occur in frontier regions of tropical countries. Disease surveillance in these hotspots is challenging because sampling techniques often rely on vector-attractants that are either unavailable in remote localities or difficult to transport. I examined if a novel method for producing CO_2 from yeast and sugar produces similar mosquito species captures compared to a standard attractant such as dry ice. Across three different vegetation communities, I found traps baited with dry ice frequently captured more mosquitoes than yeast-baited traps; however there was little effect on mosquito community composition. Based on my preliminary experiments, I find that this method of producing CO_2 is a realistic alternative to dry ice and would be highly suitable for remote field work.

4.2 Introduction

Emerging infectious diseases are a growing global concern because of their risk to human and wildlife health. In remote tropical regions, zoonotic diseases are predicted to surface where humans and wildlife live in close proximity (Taylor et al. 2001, Jones et al. 2008). Disease surveillance in these areas is rare; partly because vector sampling in these remote regions is limited by a lack of access to standard trap attractants. Baits or trap attractants are a standard feature of vector surveillance and mosquito community studies (Kline 2006, Silver 2008). Different live baits, such as humans, other mammals and birds have been used (Sasa et al. 1951, Buescher et al. 1959, Meyer and Bennett 1976, Emord and Morris 1982, Costantini et al. 1993), but mostly live baits are replaced by odour attractants which stimulate mosquitoes to approach and enter traps (Costantini et al. 1993). The most common attractant to lure female host-seeking mosquitoes is carbon dioxide (CO_2) (Gillies et al. 1980, Kline 1994, Kline et al. 2006, Silver 2008).

Convenient forms of CO_2 sources are pressurised gas cylinders and dry ice in the form of solid blocks or dry ice pellets. Pressurised gas cylinders have the advantage that the release of CO_2 can be controlled using a calibrated regulator and can provide CO_2 over prolonged periods (Ritchie et al. 2007). However, gas cylinders are heavy, expensive and can not be transported on aircrafts due to safety regulations. Dry ice is lighter and relatively less expensive than pressurised gas cylinders, but needs to be stored at approximately -80°C and also cannot be transported on aircrafts. Neither dry ice nor pressurised gas cylinders are readily available in remote areas (Mboera and Takken 1997, Saitoh et al. 2004, Smallegange et al. 2010). Therefore alternative sources of CO_2 need to be identified. Saitoh et al. (2004) demonstrated that a substitute method to generate CO_2 can be achieved by combining yeast, sugar and water. These ingredients are cheap, easy to obtain and light enough to carry into remote field locations.

In this study, I investigated the effectiveness of yeast-generated CO_2 as a method for sampling mosquitoes in remote localities in two ways. First, I explored how yeast-sugar combinations influenced CO_2 production in laboratory trials at different temperatures. Second, through a series of replicated field trials in three different vegetation types (rainforest, mangrove and dry forest) I compared mosquito capture rates and community composition between standard dry ice- and yeast-baited traps.

4.3 Methods

4.3.1 Laboratory experiment for quantifying CO_2 production from yeast

I determined the amount and production longevity of CO_2 generated by yeast fermentation in laboratory experiments at James Cook University in Cairns. The fermentation of yeast and sugar is temperature-dependent (Saitoh et al. 2004); hence I conducted 24 hour trials at 20°C, 25°C and 30°C. Six different treatment combinations of baker's yeast and white sugar were examined to identify the highest CO_2 yield. Each treatment consisted of a 4 litre volume water bottle which had 1 litre of tap water added (Table 4.1). The production of CO_2 was measured by the water displacement method. The initial reading was taken two hours after mixing the solutions for one hour; successive readings were taken every three hours for one hour for the duration of 24 hours. As a baseline, I also measured the CO_2 produced from 1.5 kg of dry ice.

4.3.2 Study sites for field experiments

The comparison of the effectiveness of yeast-produced CO_2 versus dry ice in capturing mosquitoes was undertaken as a series of field experiments in the wet tropics region of northern Australia. Mosquitoes were sampled along the edges of three different habitats: lowland evergreen rainforest (16° 48' S, 145° 40' E), mangrove forest (16° 49' S, 145° 42' E) and dry forest (16° 46' S, 145° 39' E) north of Cairns. In this region, annual rainfall is ca. 2000 mm yr^1 and strongly seasonal with a wet season from

December to May. Temperature ranges from an annual mean maximum of 29˚C yr^{1} to a mean minimum of 20.8˚C yr^{1}.

4.3.3 Field experiments for evaluating the efficacy of yeast-produced CO_2

Field work was performed from 26th December 2011 to 30th March 2012. Adult mosquitoes were collected using CDC (model 512) traps (Appendix 1; Fig. 1), with light bulbs removed to reduce by-catch. A 4 x 4 Latin Square sampling design (Cochran and Cox 1957) was conducted along the edges of three different vegetation types: lowland rainforest, mangrove forest and dry forest; each vegetation type was sampled twice. Four traps were set-up at least 50 m apart and were placed at a height of ~ 1.5 meters above ground level and rotated for four days.

At each vegetation type, four experimental treatments were tested: (1) control trap without bait; (2) trap baited with 1.5 kg dry ice pellets in an insulated container; (3) trap baited with three yeast/sugar/water solutions and with exhaust tubes attached to the trap lid and a single tube above the lid (referred to as Yeast 1); (4) trap baited with three yeast/sugar/water solutions (as in 3) and with an exhaust tube attached to trap lid and two tubes above the lid (referred to as Yeast 2) (Fig. 4.1). I used two different set-ups for the yeast-baited traps to evaluate if controlling where CO_2 was released influenced mosquito capture. Yeast-baited traps consisted of 3 bottles (4 litres each) with different sugar and yeast combinations (see Table 4.1). The yeast/sugar/water solutions and the dry ice pellets were replaced each day. The insulated containers holding the dry ice and the bottles containing the yeast/sugar/water solutions were placed above ground to prevent animals (i.e. feral pigs) from destroying or displacing them.

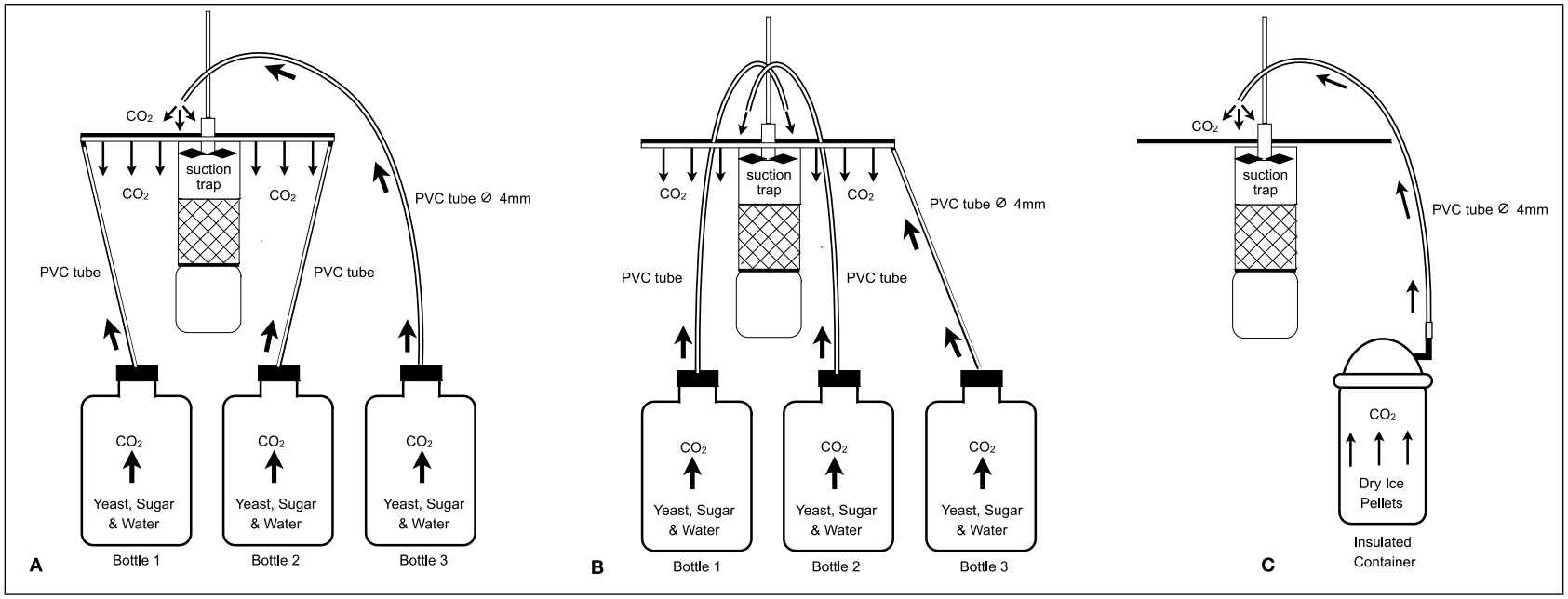

Figure 4.1: The set-up of the traps baited with yeast (A & B) and dry ice pellets (C). Bottle 1 contained 30 g yeast and 250 g sugar, bottle 2 contained 20 g yeast and 250 g sugar and bottle 3 contained 30 g yeast and 500 g sugar; all bottles had 1 liter of water added. The insulated container held 1.5 kg of dry ice.

Traps were operated for a 24 hr period starting in the afternoon (from 13:00 – 15:00hrs). The 24 hr period ensured the capture of both diurnal and nocturnal mosquito species. After collecting the traps, species were stored in insulated containers and taken back to the laboratory. Mosquitoes were identified to species level using identification keys (Lee et al. 1980 - 1989, van den Hurk et al. 1999) and with the assistance of taxonomic experts.

4.3.4 Data analysis

I evaluated whether yeast-generated CO_2 outputs varied between treatments and temperatures by using a two-way ANOVA with Tukey's HSD post hoc tests. Two yeast/sugar solutions: (1) containing 20 g yeast and 500 g sugar and (2) containing 15 g yeast and 250 g sugar were discarded prior to statistical analysis due to very low and limited time of CO_2 production at all three temperatures (Table 4.1).

I compared the effectiveness of dry ice- to yeast-baited traps in two ways. First, I examined differences in abundance and species richness at each vegetation type using one-way ANOVAs (data were square-root-transformed) with Tukey's HSD post hoc tests (SPSS 17.0). Second, I examined the structure and composition of the mosquito communities using non-metric multidimensional scaling (NMS) ordination analyses with the PC-ORD package (McCune and Mefford 1999). Data were log-transformed prior to analysis. Monte Carlo randomization tests (100 runs) were used to determine whether the ordination axes explained significantly more variation than expected by chance. A Bonferroni correction was used to reduce the likelihood of type II errors, where $P = 0.15/46$ (46 represents the number of mosquito species multiplied by two axis and 0.15 is the experiment wise error rate).

4.4 Results

4.4.1 CO_2 production from yeast and dry ice

The mean amount of yeast-generated CO_2 was significantly different between all treatments ($F_{3,312}$ = 53.154, P < 0.001; Tukey's HSD P < 0.05) and all temperatures ($F_{2,312}$ = 78.107, P < 0.001; Tukey's HSD P < 0.001). No effect of interaction occurred between treatments and temperatures which could have influenced the mean CO_2 production ($F_{6,312}$ = 1.644, P = 0.135). I found three different ratios of yeast to sugar (30 g yeast : 250 g sugar; 20 g yeast : 250 sugar and 30g yeast : 500 g sugar) were the most productive in terms of volume and continuity. Together these three treatments produced approximately 140 ml CO_2/minute at 30°C; 110 ml CO_2/ minute at 25°C and 60 ml CO_2/ minute at 20°C (Table 4.1). In order to ensure both high CO_2 production and continuity, I decided to use all three treatments together with each trap in our field experiments. The mean amount of CO_2 produced from 1.5 kg of dry ice was 351 ml/minute.

Table 4.1: The effects of six different yeast and sugar combinations on the average and peak production of CO_2 at temperatures of (a) 30°C, (b) 25°C and (c) 20°C.

Treatment (g/1litre water)	Mean CO_2 (ml/min) ± 1SDM	Peak CO_2 (time) (ml/min) ± 1SDM
a) 30°C		
30 g yeast + 250 g sugar*	54.2 ± 24.3	17 hrs (99.2 ± 6.9)
30 g yeast + 500 g sugar*	44.2 ± 12.0	8 hrs (59.2 ± 4.5)
20 g yeast + 250 g sugar*	40.2 ± 8.7	8 hrs (49.2 ± 4.6)
20 g yeast + 500 g sugar^	7.0 ± 11.0	5 hrs (26.7 ± 3.1)
15 g yeast + 250 g sugar^	10.6 ± 12.4	8 hrs (33.2 ± 5.5)
15 g yeast + 150 g sugar	24.5 ± 16.0	11 hrs (50.5 ± 10.2)
b) 25°C		
30 g yeast + 250 g sugar*	47.1 ± 21.6	17 hrs (81.7 ± 7.1)
30 g yeast + 500 g sugar*	36.2 ± 9.7	17 hrs (47.5 ± 5.2)
20 g yeast + 250 g sugar*	26.5 ± 10.4	8 hrs (37.5 ± 4.5)
20 g yeast + 500 g sugar^	3.2 ± 7.2	5 hrs (21.6 ± 3.3)
15 g yeast + 250 g sugar^	9.6 ± 14.3	14 hrs (36.0 ± 2.0)
15 g yeast + 150 g sugar	17.5 ± 12.0	11 hrs (37.5 ± 3.7)
c) 20°C		
30 g yeast + 250 g sugar*	30.5 ± 13.5	11 hrs (52.6 ± 5.7)
30 g yeast + 500 g sugar*	17.2 ± 9.1	8 hrs (36.4 ± 3.5)
20 g yeast + 250 g sugar*	12.4 ± 8.9	8 hrs (29.6 ± 6.1)
20 g yeast + 500 g sugar^	2.3 ± 3.8	5 hrs (9.8 ± 2.4)
15 g yeast + 250 g sugar^	1.9 ± 2.9	11 hrs (6.5 ± 1.8)
15 g yeast + 150 g sugar	9.8 ± 7.8	8 hrs (19.2 ± 2.9)

Significant treatments with high average and consistent CO_2 productions are highlighted with *. Treatments which produced low amounts of CO_2 for a limited time only were discarded prior to statistical analysis and are denoted with ^.

4.4.2 Field experiments

In total, I captured 4,417 mosquitoes of 31 species across 3 vegetation types (Table 4.2); with most individuals caught in the mangroves (83.4%). Rainforest captures (9.2%) were similar to dry forest captures (7.4%). At all sites, baited traps captured significantly more mosquitoes than unbaited control traps (rainforest: $F_{3,28} = 9.009$, $P < 0.01$; mangroves: $F_{3,28} = 19.760$, $P < 0.01$; dry forest: $F_{3,28} = 25.391$, $P < 0.01$; all tests one-way ANOVA with Tukey's HSD). A comparison of mosquito abundance between yeast-baits and dry ice found that there was no difference in rainforest ($P > 0.05$) but there were significant differences in mangroves ($P < 0.01$) and dry forest ($P < 0.01$). There was no difference in mosquito abundance between the yeast-baited traps in all vegetation types ($P > 0.05$) (Fig. 4.2).

Table 4.2. Mosquito species captured with the four treatments in the three sampled vegetation types in north Queensland (Symbols denote C = control; DI = dry ice; Y1 = yeast/sugar solution 1 and Y2 = yeast/sugar solution 2).

	Rainforest					Mangroves					Dry Forest				
Species	**C**	**DI**	**Y1**	**Y2**	**Total**	**C**	**DI**	**Y1**	**Y2**	**Total**	**C**	**DI**	**Y1**	**Y2**	**Total**
Aedes alboscutellatus	1	10	3	2	16	0	3	0	2	5	0	3	0	1	4
Aedes kochi	0	8	4	4	16	1	259	77	99	436	0	39	8	7	54
Aedes notoscriptus	0	53	9	12	74	0	50	24	22	96	0	46	18	5	69
Aedes palmarum	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
Aedes quinquelineatus	0	0	1	2	3	0	0	0	0	0	0	1	2	2	5
Aedes tremulus	0	0	0	0	0	0	1	1	0	2	0	6	0	0	6
Aedes alternans	0	0	1	0	1	0	3	0	0	3	0	0	0	0	0
Aedes lineatopennis	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Aedes vigilax	0	41	29	85	155	1	124	24	31	180	0	5	0	2	7
Anopheles bancroftii	0	0	0	2	2	0	0	0	0	0	0	1	0	0	1
Anopheles annulipes s.l.	1	1	3	4	9	0	0	0	0	0	0	0	1	0	1
Anopheles farauti	0	2	3	3	8	2	167	57	54	280	0	7	2	0	9
Coquillettidia nr *crassipes*	1	6	2	3	12	0	34	6	6	46	0	0	0	0	0
Culex annulirostris	1	10	5	4	20	4	1002	213	223	1442	0	74	9	7	90
Culex bitaeniorhynchus	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Culex edwardsi	0	1	0	1	2	0	0	0	0	0	0	0	0	0	0
Culex gelidus	0	0	0	0	0	0	4	1	0	5	0	1	0	0	1
Culex sitiens	1	4	4	3	12	1	97	35	47	180	0	0	0	0	0
Culex squamosus	0	0	0	0	0	0	1	1	1	3	0	0	0	0	0
Culex pullus	0	4	3	5	12	0	70	24	25	119	0	5	5	10	20
Culex hilli	0	0	0	0	0	3	177	26	9	215	0	3	1	0	4
Culex halifaxii	0	0	0	0	0	0	2	0	0	2	0	0	0	0	0
Mansonia septempunctata	0	0	1	0	1	0	58	3	3	64	0	2	0	0	2
Mansonia uniformis	0	2	2	3	7	0	15	5	4	24	0	0	0	0	0

Mimomyia sp.	0	0	0	0	0	0	7	9	2	18	0	0	0	0	0
Tripteroides magnesianus	0	0	0	0	0	0	4	15	3	22	0	0	0	0	0
Tripteroides sp.	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0
Uranotaenia sp.	0	0	0	0	0	1	2	28	13	44	1	0	1	0	2
Verrallina carmenti	0	0	0	0	0	0	80	5	9	94	0	3	0	0	3
Verrallina funerea	0	0	0	0	0	0	140	73	77	290	0	0	0	0	0
Verrallina lineata	0	11	2	5	18	0	8	0	0	8	0	14	0	0	14
males	0	5	11	9	25	1	23	14	6	44	1	4	11	14	30
damaged	1	6	1	3	11	4	33	10	12	59	0	3	0	1	4
Total	6	168	84	150	408	18	2365	652	648	3683	2	217	58	49	326

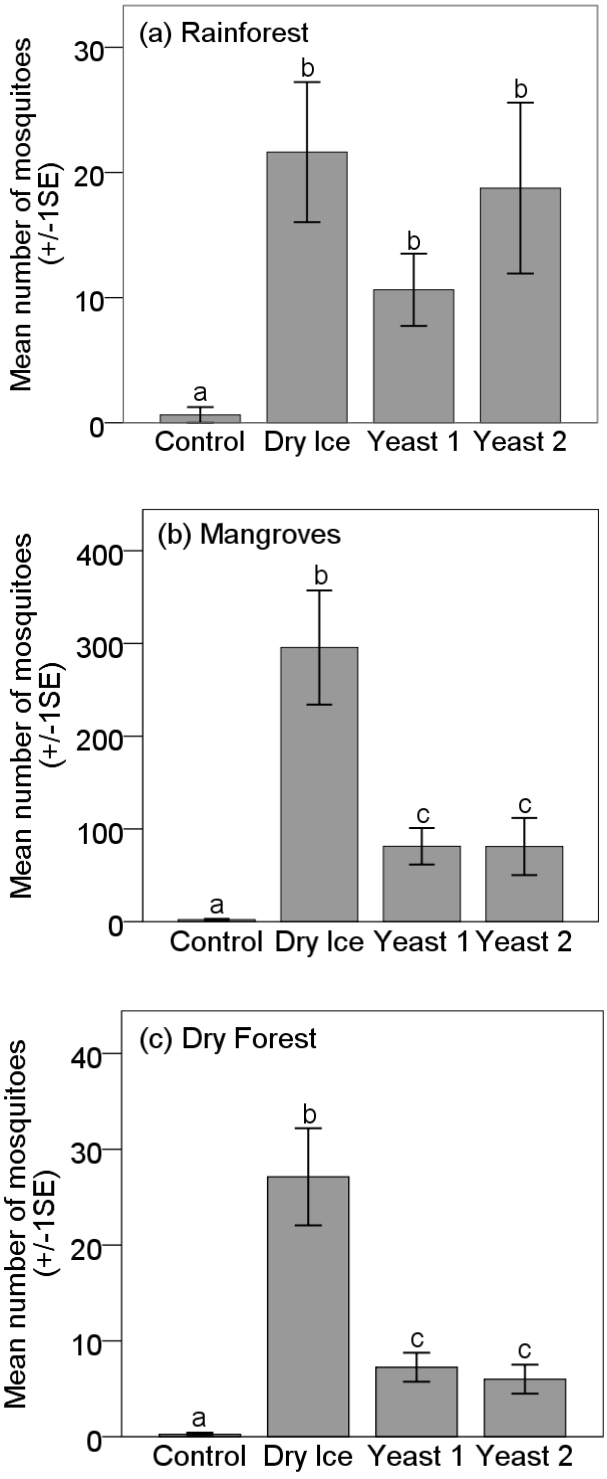

Figure 4.2: The mean number of mosquitoes caught with control, dry ice, yeast 1 and yeast 2 treatments in (a) rainforest, (b) mangroves and (c) dry forest - (y-axes vary). Different letters denote significant differences.

Baited and unbaited traps influenced mosquito species richness, with the unbaited traps having the least species richness in all three vegetation types (rainforest: one-way ANOVA; $F_{3,28} = 5.65$, $P < 0.01$; mangroves: one-way ANOVA; $F_{3,28} = 17.97$ $P < 0.01$; dry forest: $F_{3,28} = 11.12$, $P < 0.01$). The type of CO_2 generating bait did not affect the mean number of species captured in the rainforest and in the mangroves (Tukey's HSD, $P > 0.05$) however, species richness was higher in dry ice baited traps in the dry forest (Tukey's HSD, $P < 0.01$) (Fig. 4.3). Where CO_2 was released on yeast-baited traps had no effect on species richness in any of the three vegetation types (Tukey's HSD, $P > 0.05$).

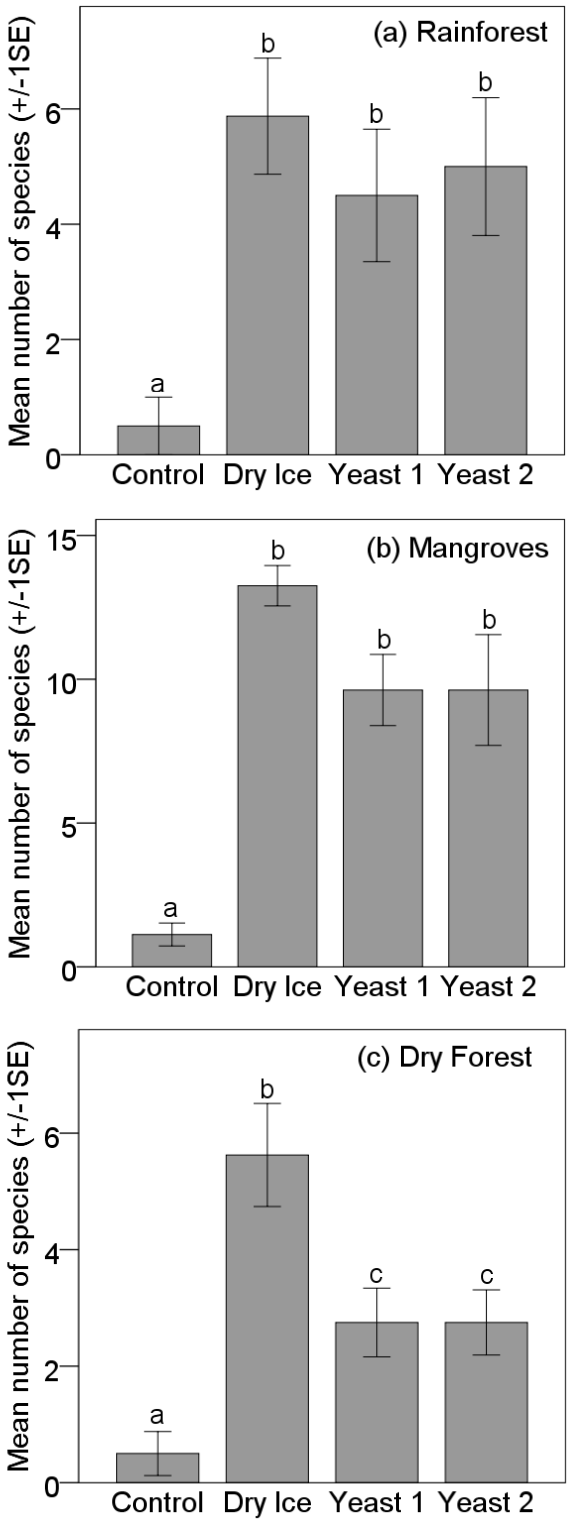

Figure 4.3: Mean number of species captured with control, dry ice, yeast 1 and yeast 2 treatments in (a) rainforest, (b) mangroves and (c) dry forest - (y-axes vary). Different letters denote significant differences.

The mosquito community varied strongly in response to vegetation type but not to bait type. The ordination analysis (NMS) identified two axes that explained 88.9% of the data variation and was significantly correlated with 17 mosquito species (Table 4.3). Axis 1 distinguished between dry forest sites, which correlated with the higher abundances of *Aedes quinquelineatus*, on the left hand side of the figure (4.4), and mangrove and rain forest sites that were correlated with *Aedes vigilax*, *Coquillettidia* near *crassipes*, *Culex sitiens* and *Mansonia uniformis* to the right. The second axis highlighted the dissimilarity between mangroves and rainforest sites due to the presence of species such as *Aedes kochi*, *Anopheles farauti*, *Culex hilli*, *Culex annulirostris* and *Cx. sitiens* (Fig. 4.4). At mangrove and rainforest sites there was no effect of bait type, in the dry forest where mosquito captures were lowest there was some effect.

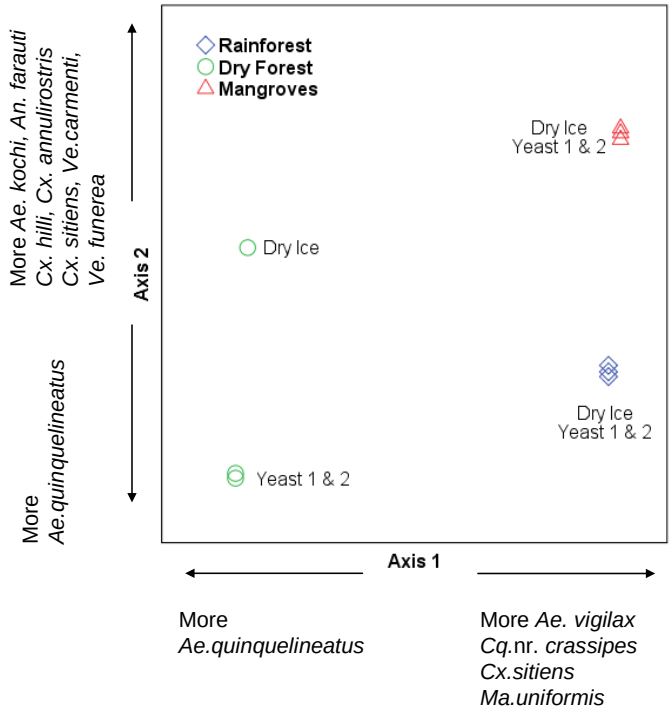

Figure 4.4: Ordination analysis of mosquito communities displaying that the mosquito community composition varied strongly in response to vegetation type, but not to treatment type. All three vegetation types have distinctly different communities. Baited traps captured similar species, especially in the rainforest and mangroves.

Table 4.3: Pearson correlation values of 17 mosquito species from baited traps with two ordination axes produced by non-metric multidimensional scaling.

Species	Axis 1	Axis 2
Aedes kochi	0.256	**0.894^**
Aedes quinquelineatus	-0.659	**-0.797#**
Aedes vigiliax	**0.908^**	0.576
Anopheles farauti	0.542	**0.944^**
Coquillettidia nr.*crassipes*	**0.837+**	**0.692***
Culex annulirostris	0.289	**0.906^**
Culex sitiens	**0.798#**	**0.839+**
Culex pullus	0.326	**0.740***
Culex hilli	0.326	**0.834#**
Culex squamosus	0.523	**0.876+**
Mansonia septempunctata	0.361	**0.770***
Mansonia uniformis	0.865	**0.727***
Mimomyia spp.	0.494	**0.826#**
Tripteroides spp.	0.597	**0.741***
Uranotaenia spp.	0.391	**0.722***
Verrallina carmenti	0.336	**0.850+**
Verrallina funerea	0.522	**0.873+**

Note: Correlation values in boldface were significant at *$P < 0.05$; #$P < 0.01$; +$P < 0.005$; ^$P < 0.001$.

The mosquito community from all three vegetation types constituted 31 mosquito species in 9 genera (Table 4.2). *Culex* and *Aedes* were the most species rich genera (n = 9 species) but *Culex* was numerically dominant (> 48% of individuals compared to 26% for *Aedes*). Four species constituted 59% of captures: *Cx. annulirostris* (35%), *Ae. kochi* (11%), *Ae. vigilax* (7%), and *An. farauti* (6%) with all of them more frequently captured in traps baited with dry ice than traps baited with yeast. All species were present in all CO_2 traps except for those that were very rare (< 4 individuals), with dry ice catching *Aedes lineatopennis*, *Culex halifaxii*, *Tripteroides spp.* and yeast traps catching *Culex bitaeniorhynchus*. In the mangroves and dry forest, *Verrallina lineata*

were only captured in traps augmented with dry ice. *Aedes tremulus*, in the dry forest, was also only caught in dry ice baited traps.

4.5 Discussion

Despite some exceptions, there is little difference in the composition of mosquito species trapped with the two types of CO_2 attractants. Dry ice does generate more CO_2 (own measurements & Saitoh et al. 2004) and would be the preferred attractant if funds and logistics were not limiting. CO_2 derived from yeast and sugar has demonstrated to be a suitable alternative attractant to capture mosquitoes in remote localities.

Not surprisingly the CDC miniature light traps without attractants were not very successful in capturing mosquitoes. However once CO_2 attractants, be it in the form of dry ice pellets or yeast produced, were added captures increased. Whether traps baited with yeast/sugar solutions had one or two plumes did not make a difference. Dry-ice augmented traps generally, but not always, caught more mosquitoes than yeast-augmented traps; these results are in agreement with previous studies from temperate urban areas in Japan (Saitoh et al. 2004) and from residential areas in Malaysia (Oli et al. 2005). For example, Saitoh et al. (2004) not only captured the dominant mosquito species (*Aedes albopictus* and *Culex pipiens*) in both the yeast and dry-ice baited traps; but the captures also displayed similar species composition for other dipterans and for coleopterans, hemipterans, hymenopterans and psocopterans. Oli et al. (2005) caught five species of mosquitoes in their comparative study. The same numbers of *Culex vishnui* and *Aedes aegypti* were captured with CO_2 produced from dry ice and yeast; the other three remaining species (*Culex quinqufasciatus*, *Ae. albopictus* and *Armigeres subalbulatus*) were more attracted to the CO_2 produced by dry ice. In a Kenyan highland village (> 1100 m above sea level) Smallegange et al.

(2010) demonstrated that mosquito captures were further enhanced by adding worn socks (releasing human foot odour) to the traps, which resulted in comparable mosquito captures for CO_2 produced from yeast and from pressurised gas cylinders.

The fermentation of yeast and sugar not only produces CO_2 but also ethyl alcohol (Buchner and Rapp 1897) and other compounds (Hazelwood et al. 2008) which may be a deterrent or attractant to certain mosquito species. Other species possibly have host preferences and could be predominantly anthropophilic in which case our yeast CO_2 production (140 ml/min, 110 ml/min or 60 ml/min respectively) may not be sufficient in attracting these species, as humans produce approximately 275 ml/min (Schmidt-Nielsen 1997). These explanations could account for the absence of *Ve. lineata* in the mangroves (n= 8) and dry forest (n = 14); *Ae. tremulus* (n = 6) in the dry forest and *Aedes alternans* (n=3) in mangroves in yeast augmented traps. For example, *Ve. lineata* readily takes blood meals from humans (Ritchie et al. 2006b) and the addition of a further attractant such as 1-octen-3-ol (octenol) (Takken and Kline 1989, Ritchie and Kline 1995, van den Hurk et al. 2006), ammonia (Braks et al. 2001) or L-lactic acid (Dekker et al. 2002) could be used to improve trap success. *Anopheles gambiae*, the major malaria vector in Africa, displays high anthropophilic behaviour and the addition of a combination of ammonia (NH_3) (Braks et al. 2001), lactic acid and carboxylic acids (especially tetradecanoic acid) seems to be highly successful in attracting this target species (Smallegange et al. 2005); however, these attractants could prove to be expensive and too difficult to obtain in remote locations. Further studies need to be carried out to estimate whether additional attractants in combination with yeast produced CO_2 could be used for disease or vector surveillance to target specific mosquito species. More studies may also be required to determine if seasonality has an influence on the capture of particular species. Furthermore, the trap design could be expanded with more bottles of yeast and sugar solutions attached in order to gain more CO_2. Yeast generated CO_2 may also be useful for the collection of

other blood-feeding invertebrates. For instance, *Triatoma infestans*, the vector responsible for the transmission of Chaga's disease, has also been successfully trapped using yeast-generated CO_2 (Guerenstein et al. 1995), and Saitoh et al. (2004) caught species belonging to the families Ceratopogonidae and Psychodidae.

In summary, I can conclude that although the production of CO_2 from sugar and yeast fermentation is lower than from dry ice, it is an adequate alternative to capture mosquitoes in remote tropical vegetations where commercial forms of CO_2 are unavailable.

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CHAPTER 5

Land use influences mosquito communities and disease risk on remote tropical islands: a case study using a novel sampling technique

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5.1 Abstract

Land use changes, such as deforestation and urbanisation can influence interactions between vectors, hosts and pathogens. The consequences may result in the appearance and rise of mosquito-borne diseases, especially in remote tropical regions. Tropical regions can be hotspots for the emergence of diseases due to the high biological diversity and complex species interactions. Furthermore, frontier areas are often haphazardly surveyed as a result of inadequate or expensive sampling techniques, which limit early detection and medical intervention. I trialled a novel sampling technique of non-powered traps and a CO_2 attractant derived from yeast and sugar to explore how land use influences mosquito communities on four remote, tropical islands in the Australian Torres Strait. Using this technique I collected > 11,000 mosquitoes from urban and sylvan habitats. I found that human land use significantly affected mosquito communities. Mosquito abundances and diversity were higher in sylvan habitats compared to urban areas, resulting in significantly different community compositions between the two habitats. An important outcome of my study was determining that there were greater numbers of disease-vectoring species associated with human habitations. Based on these findings I believe my novel sampling technique is a realistic tool for assessing mosquito communities in remote regions.

5.2 Introduction

Anthropogenic land use changes, such as deforestation, agricultural practices, road construction, hydrological transformations and urbanisation create immense impacts on biological processes and ecosystem functions. The consequences of these environmental changes are not only the loss of biodiversity and ecosystem services but also the change in vector ecology patterns. Modifications of vector-host-pathogen interactions can result in an increase of emerging infectious diseases, especially mosquito-borne diseases (Patz et al. 2000, Williams 2012). For example, the rise and emergence of malaria and yellow fever in South America and Africa, and dengue in South-East Asia can all be linked to anthropogenic land use (Walsh et al. 1993, Morse 1995, Patz et al 2000, Vasconcelos et al. 2001, Reisen 2010).

Pathogens transmitted by mosquitoes inflict significant health hazards to humans, wildlife, livestock and pets worldwide. For example dengue, the most important viral mosquito-borne disease is believed to infect an estimated 400 million people yearly (Bhatt et al. 2013). Avian malaria is almost certainly responsible for the extinction of nearly 50% of native bird species in Hawaii (van Riper et al. 1986). Rift Valley fever causes high miscarriage- and death-rates in cattle, sheep and other domestic ruminants resulting in major economic losses (Bird et al. 2009). Many of these mosquito-borne diseases are emerging in new geographical locations or are re-emerging in areas where they had previously been declared absent (Morse and Schluederberg 1990, Gubler 2002).

Remote tropical areas are the predicted hotspots for emerging and re-emerging diseases because these are the regions, where diseases from zoonotic and vector-borne pathogens are often most concentrated, human population growth and density may be high, and surveillance often inadequate (Wolfe et al. 2007, Jones et al. 2008). Surveillance of mosquito communities and the arboviruses they vector yields vital data

on their distribution and prevalence that is crucial for biosecurity measures and disease management. Yet, in remote regions, mosquito surveillance can be logistically demanding and expensive. Most mosquito traps require a source of electricity to operate a fan or a suction device and suitable attractants. Carbon dioxide (CO_2) derived from dry ice or from pressurised gas cylinders is a commonly employed attractant, but in remote locations sources of CO_2 or electricity are often not available. Hence, there is a desperate need to develop and trial new vector sampling methods that can be built and monitored within remote communities. Powerless traps, like the passive box trap (PBT) (see full description in Ritchie et al. 2013) have been employed successfully in several cities in Australia with CO_2 obtained from pressurised gas cylinders or dry ice (Ritchie et al. 2013, van den Hurk et al. 2014). As these CO_2 attractants are difficult to transport to remote localities, I have also successfully trialled a novel method of producing CO_2 from sugar, yeast and water (Saitoh et al 2004, Oli et al. 2005, Smallegange et al. 2010). In Chapter 4, I compared mosquito captures from standard traps (Center for Disease Control (CDC) light trap model 512, John W. Hock Co., Gainesville, Florida) baited with dry ice versus my sugar, yeast and water method and found that while dry ice resulted in greater overall captures, mosquito species compositions were similar between the two methods (Meyer Steiger et al. 2014).

Disease detection from mosquitoes requires the storage of individuals at -70°C to preserve viruses. Keeping and transporting mosquito specimens at such low temperatures in and from remote areas is not only challenging but expensive (Ritchie et al. 2003). Fungal contamination of mosquitoes can also inactivate viral RNA which complicates disease detection even further (Ritchie et al. 2007). An alternative option is the use of FTA® cards (Flinders Technology Associates; Whatman International Ltd., Maidstone, UK) which can be stored at ambient temperature. These cards contain chemicals to preserve and deactivate viral RNA. They can be soaked in a honey solution as a potential food source and when infected mosquitoes feed they expel

saliva containing viral RNA which can be detected by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) (Hall-Mendelin et al. 2010, Ritchie et al. 2013, van den Hurk et al. 2007 and 2014). This approach also addresses a second issue in disease detection which is discerning between mosquitoes that have fed upon an infected host (and hold infected blood in the gut upon capture) versus mosquitoes that can transmit an infection because the parasite has developed in the mosquito to an infective stage. Identifying mosquito species that are competent vectors (i.e. susceptible to infections), is important for surveillance and this method offers a significant advantage in the field. The current limitation however is that mosquitoes expel only a small quantity of saliva (~ 4.7 nl) making false negatives a possibility in the genetic testing (van den Hurk et al. 2014).

In the study reported here, I used the unique combination of PBT and sugar-yeast produced CO_2 to study mosquito communities in the Torres Strait, a remote tropical island archipelago, situated between Papua New Guinea and Australia. Using this method I investigated if the mosquito community composition, abundance and species richness varied between urban and sylvan (forest vegetation) habitats. In the Torres Strait, mosquitoes involved in disease transmission, have been studied in urban communities since the 1980s; however, no research has yet examined the mosquito community in natural habitat. Additionally, I used FTA® cards for screening the captured mosquitoes for dengue (subtypes 1 - 4), Ross River, Japanese encephalitis, chikungunya, West Nile, Kunjin (subtype of West Nile) and Murray Valley viruses.

5.3 Methods

5.3.1 Study area

This study was conducted during the wet season (January to April) in natural vegetation and in villages on the remote islands of Saibai (9° 23’S, 142° 37’E), Boigu

(9° 14’S, 142° 13’E), Badu (10° 09’S, 142° 10’E) and Moa (10° 09’S, 142° 10’E) in the Torres Strait (Fig. 5.1). These islands were selected because of their location, their similarity in size, replication potential and that we were granted permission to carry out field work by indigenous elders and island authorities. Saibai and Boigu are low-lying, swampy alluvial mud islands, dominated by mangroves, saltpans and grasslands and are situated approximately 5 km south of the Western Province of Papua New Guinea. Badu and Moa are continental islands with eucalypt forests and grasslands and are located approximately 90 km south of Papua New Guinea (Stanton et al. 2009). The climate of the islands is tropical monsoonal with most of the annual rainfall (~ 1,600 mm yr^{1}) occurring between December and April. Temperature reaches an annual mean maximum of 30.4 $^{o}C\ yr^{1}$ and a mean minimum of 24.6 $^{o}C\ yr^{1}$ (Bureau of Meteorology 2014).

Early human land use in the Torres Strait included the burning and clearing of vegetation for plant cultivation (Haddon 1912, Harris 1977). All of the islands where my fieldwork was performed have been subjected to annual burning for thousands of years (Harris 1977). In more recent times (late 18^{th} and early 19^{th} century), many islands were heavily deforested to provide fuel for the bêche-de-mer (sea-cucumber, trepang) industry and for missionary steamers; and also for the construction of boats and slipways (Shnukal 2004). Landscape alterations to the already fragile island environments were also caused by the introduction of exotic plants (e.g. yam, taro, banana, cassava, sugarcane, sweet potato, tobacco, bamboo, and sisal) and animals (e.g. dogs, pigs, goats, Rusa deer, horses, chickens, wallabies) (Haddon 1912, Harris 1977).

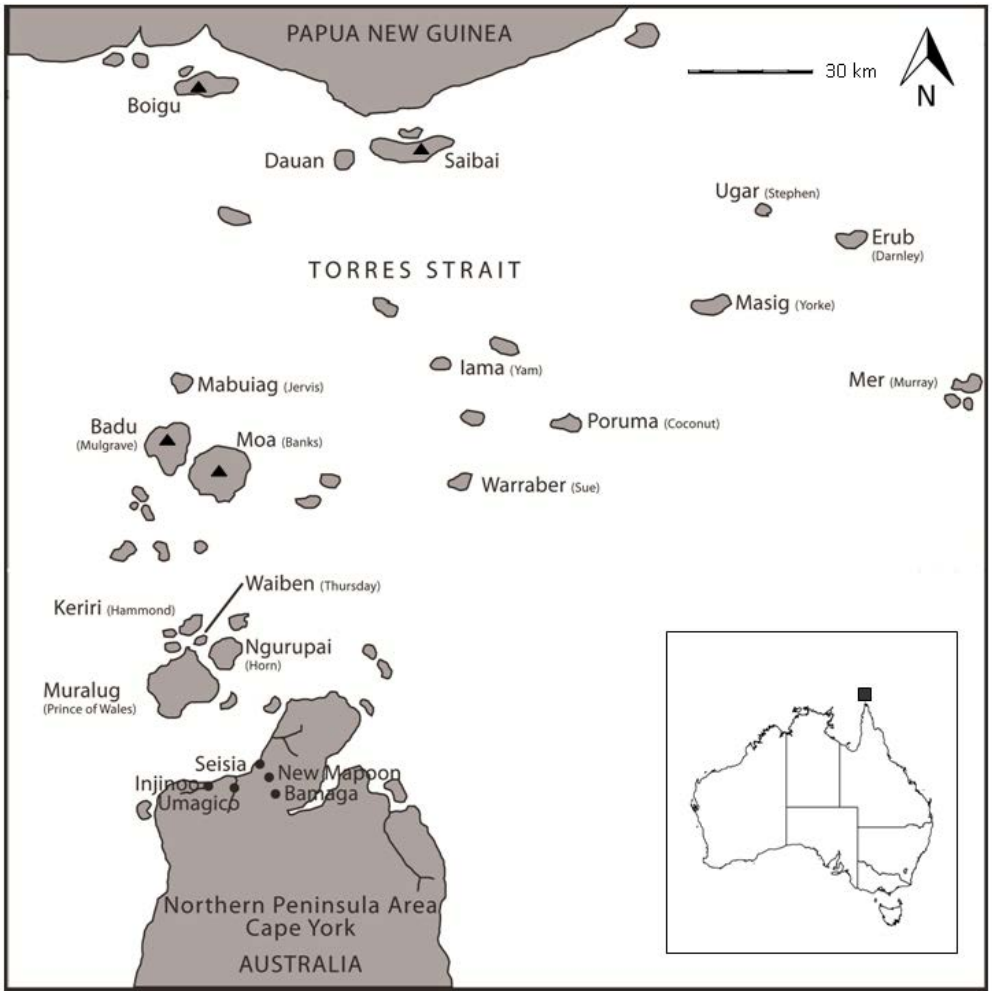

Figure 5.1: Map of the study area in the Australian Torres Strait. Mosquitoes were collected from the islands of Saibai, Boigu, Badu and Moa (▲).

5.3.2 Sampling methods for mosquitoes

Field work was conducted during the wet seasons 2013 and 2014. Adult mosquitoes were collected using non-powered passive box traps (PBT; Appendix1; Fig, 3 and Fig. 4) (Ritchie et al. 2013) with the following modification: the entry bowl was painted black which is attractive to many mosquito species (Browne and Bennett 1981, Hoel et al. 2011). The traps were baited with CO_2 (derived from sugar/yeast fermentation) from 15 L volume water bags (containing 4 L of water, 1 kg of sugar and 40 g of yeast)

which produced ~ 120 ml CO_2 /minute; this solution was replaced after two nights. Traps were placed at a height of approximately 0.5 m above ground level and spaced at least 20 m apart. On each island, I placed five traps in villages (close to residential houses and communal areas such as health centres, rangers sheds and council offices) and five traps in natural vegetation (along the first tree line of mangrove forests; the surrounding matrix were grasslands supporting mainly native grasses and *Pandanus* spp.). All traps were operating for four nights on each island in two consecutive years, resulting in a total of 320 trap nights. Mosquitoes were removed from traps at the end of the four night sampling period.

I had initially intended to include a comparison between the BG-Sentinel™ traps (Biogents AG, Regensburg, Germany. Appendix 1; Fig. 5) and passive box traps, both in natural vegetation and villages. However, due to battery failures in the field samples, I present only data for the villages (where electricity powered the traps). I placed five BG traps in each village on each island where they were in use for four consecutive nights in the 2013 wet season, which resulted in a total of 80 trap nights.

5.3.3 Mosquito identification

Mosquitoes were identified to species level using taxonomic keys (Lee et al. 1980-1989) and with the assistance of taxonomic experts (Richard Russell, John Clancy, Paul Zborowski and Bob Cooper). *Culex annulirostris* and *Culex sitiens* were pooled as they belong to the morphologically indistinguishable members of the *Cx. sitiens* subgroup in this study area (Chapman 2000).

5.3.4 Disease detection using FTA® cards

I placed four honey-coated FTA® cards into each passive box trap to collect mosquito saliva. Each card was also treated with blue food-dye (to stain the individuals that have taken a honey-meal) and liquid fipronil (poison to kill mosquitoes). Mosquitoes taking a

honey-meal from these cards expel saliva which can be used for disease detection by eluting and extracting viral RNA (Ritchie et al. 2013). I closely adhered to the protocol suggested by van den Hurk et al. (2014). Briefly, the FTA® cards were cut into thin strips and together with 1 mL of sterile H_2O were placed into 5 mL sample tubes. Sample tubes were vortexed for 15 seconds every 5 minutes for a total of 20 minutes. A pipette was used to extort 140 µl of the resulting liquid from each tube. Viral RNA was extracted from these eluates by using QIAamp Viral RNA Mini Kits (QIAGEN, Clifton Hill, Australia) following the instructions provided by the manufacturer. I performed RNA elution and extraction from FTA® cards at the molecular laboratory at James Cook University in Cairns.

Real-time RT-PCR (TaqMan) assays with synthetic probe and primer controls (Appendix 2; Table 1) as well as no-template controls were used to evaluate if dengue (subtypes 1 – 4), Ross River, Japanese encephalitis, chikungunya, West Nile, Kunjin (subtype of West Nile) and Murray Valley viruses were present. Assays were performed at Queensland Health Forensic and Scientific Services in Brisbane.
The reaction mix consisted of 0.4 µL of Superscript™ III RT/Platinum® Taq mix (Invitrogen, Carlsbad, U.S.A), 10 µL of 2 x master mix provided by manufacturer, primers and probes to required final concentration, 50nM ROX Reference Dye, 5 µL of extracted viral RNA or synthetic control, and H_2O to produce a final volume of 20 µL. The TaqMan RT-PCR cycling conditions were as follows: one cycle at 50°C for 5 min, one cycle at 95°C for 2 min, 50 cycles at 95°C for 3 sec and at 60°C for 30 sec. A threshold cycle number (Ct) of ≥ 45 cycles indicated that no RNA was detected. Samples with a Ct value <45 cycles were further investigated. A second extraction and TaqMan assay was performed to confirm initial result. If sample was positive again, PCR and sequencing were undertaken.

5.3.5 Data analysis

I evaluated if mosquitoes were sampled adequately in both habitat types under my sampling design by constructing species accumulation curves to display the cumulative number of species collected against the measure of the sampling effort. I used linear mixed effects models (with Restricted Maximum Likelihood) to examine relationships between the response variables: mosquito captures and diversity (Fisher's alpha) as a function of land use (village versus natural habitat). Habitat was a fixed factor in the model and island was a random factor. Variables for the capture data were log (x + 1) transformed. I examined major gradients in the composition of mosquito communities across study sites by undertaking non-metric multidimensional ordination analysis (NMS). I used NMS in two ways: 1) to evaluate if the mosquito community varies in response to habitat type and island and 2) to evaluate if the vector community (species transmitting alpha-, flaviviruses and protozoan parasites) varies in response to habitat type and island. Data were log (x + 1) transformed prior to analysis. Monte Carlo randomization tests (250 runs) were used to determine whether the ordination axes explained significantly more variation than expected by chance. A Bonferroni correction was used to reduce the likelihood of type II errors, where $P = 0.15/x$ (x represents the number of mosquito species multiplied by the number of axes and 0.15 is the experiment wise error rate) (Chandler 1995). Permutation-based nonparametric MANOVAs (PerMANOVAs) (Anderson 2001) are a method that allows for examining within and between categories examined in the ordination, followed by pairwise comparisons to distinguish differences among mosquito communities on the four islands. I used an independent samples t-test with log-transformed data to compare mean captures of mosquitoes and NMS ordination analysis to compare the mosquito community composition between BG and passive box traps.

All modeling was performed in R (R Development Core Team 2009), using the package nlme (Pinheiro and Bates 2000) and following a standard protocol for data

exploration and model validation (Zuur et al. 2009). For the ordination analyses and the PerMANOVAs I used PC-ORD 6.0 package (McCune and Mefford 2011) and SPSS 22.0 (IBM 2013) statistical package was used for the t-test. Where required, data was tested for normality and for homogeneity of variances by using Kolmogorov-Smirnov and Levene's tests.

5.4 Results

5.4.1 Mosquitoes in urban and sylvan habitats

My novel sampling method resulted in the capture of 11,109 mosquitoes. I found that habitat type influenced mosquito captures significantly with generally higher captures in sylvan habitat compared to village locations (linear mixed effects model; Table 5.1). I identified 27 species from 7 genera (Table 5.2). The genus *Aedes* was the most dominant genus (n = 4385 captures) and was represented by 13 species. Two species were most frequently captured, together contributing up to 70% of the captures; *Cx. sitiens* subgroup (37%), and *Ae. kochi* (33%). The most dominant species on the northern low-lying islands, Saibai and Boigu, was *Cx. sitiens* subgroup*;* whereas on the southern continental islands, Badu and Moa, *Ae. kochi* was the most dominant species. Some species, such as *Anopheles* spp*.* and *Coquillettidia* spp. were captured only on Saibai and Boigu and *Aedes albopictus* was trapped only on Badu and Moa*. Culex quinquefasciatus* and *Mansonia uniformis* were captured only on Saibai. Mosquito diversity was also higher in natural vegetation compared to villages (Fisher's alpha diversity index; Table 5.1).

Table 5.1: Results of linear mixed models fitted to mosquito abundance and diversity estimates across four tropical islands. Significance levels: $P < 0.01$*; $P < 0.001$**; $P < 0.0001$***.

Covariate	df	Abundance (SE)	Fishers-Alpha (SE)
(Intercept)	35	1.418 (0.209)***	1.072 (0.278)**
Habitat	35	0.506 (0.125)**	0.448 (0.158)*

Table 5.2: Mosquito species captured with the passive box trap in the two habitats on the four islands in the Torres Strait and the pathogens they may transmit.

	Saibai		Boigu		Badu		Moa	
	Village	Natural Vegetation	Village	Natural Vegetation	Village	Natural Vegetation	Village	Natural Vegetation
Species								
Aedeomyia catasticta [g]	0	2	0	22	0	0	8	288
Aedes aegypti [a,b]	0	0	1	0	0	0	0	0
Aedes albopictus [a,b]	0	0	0	0	5	0	19	1
Aedes alternans [a]	0	1	0	0	0	0	0	0
Aedes aurantius	0	3	0	6	0	0	0	0
Aedes culiciformis	0	0	0	4	22	3	3	0
Aedes kochi [a,d,e]	3	721	0	6	1416	1038	337	117
Aedes littlechildi	0	0	0	0	44	5	2	2
Aedes notoscriptus [a,b,d]	0	1	0	45	15	13	25	3
Aedes scutellaris [b]	4	9	2	28	1	11	25	4
Aedes tremulus [a,b]	2	47	0	14	5	9	6	5
Aedes vigilax [a,b,d,e]	4	0	0	20	0	5	0	2
Aedes spp.	0	0	0	1	0	0	0	0
Anopheles farauti [b,c,e]	16	51	181	677	0	0	0	0
Anopheles hilli [a,c]	0	0	0	1	0	0	0	0
Anopheles meraukensis [a]	0	0	21	1	0	0	0	0
Coquillettidia nr. *crassipes* [c,f]	6	786	0	88	0	0	0	0
Coquillettidia xanthogaster [a,b]	0	8	0	0	0	0	0	0
Culex sitiens * [a,b,d,e,g]	291	1935	53	1746	10	5	4	18
Culex hilli [?]	0	0	0	0	1	0	0	0
Culex quinquefasciatus [b,d,e]	65	0	0	0	0	0	0	0
Mansonia uniformis [a,b,e,f]	3	109	0	0	0	0	0	0
Tripteroides magnesianus[?]	0	3	0	12	0	7	0	55
Tripteroides spp.	0	0	0	0	0	0	0	1
Verrallina carmenti [a]	0	40	0	6	20	8	0	12
Verrallina funerea [a,b,g]	36	111	6	192	3	1	0	29
Verrallina lineata [a]	0	1	0	0	0	0	1	0
damaged	3	48	5	45	0	3	0	0
Total	**433**	**3876**	**269**	**2914**	**1542**	**1108**	**430**	**537**

*Consists of the morphologically similar species *Cx. annulirostris* and *Cx. sitiens.*

a = alphaviruses
b = flaviviruses
c = *Plasmodium* spp.
d = *Dirofilaria immitus*
e = *Wuchereria bancrofti*
f = *Brugia malayi*
g = *Orbivirus* spp.
? = unknown/not suspected to transmit diseases

Species accumulation curves from the passive box trap design show two patterns. First, the difference in species capture rate between the two habitats and second, the gradual asymptoting of the curves which suggests the traps are catching all the attracted species present in the habitats (Fig. 5.2). Hence, it is possible that I have missed to capture either rare species or those not attracted to this trap design.

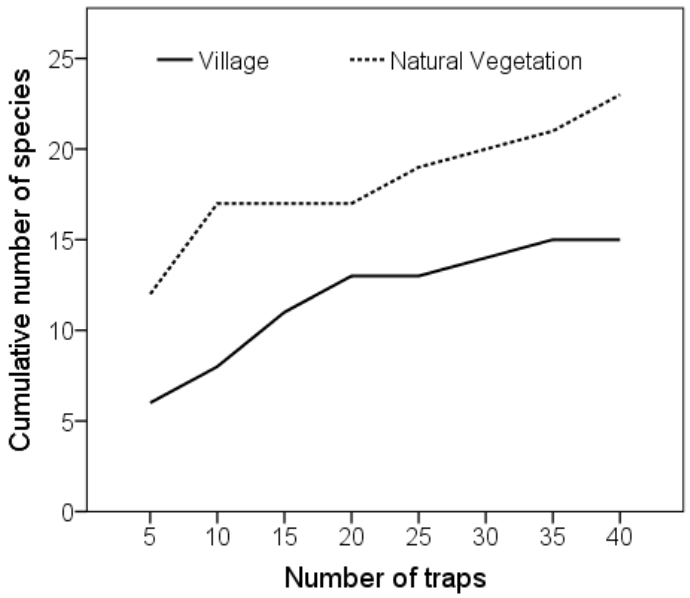

Figure 5.2: Species accumulation curves suggest that most common mosquito species attracted to our trap design in villages were sampled, whereas further sampling in natural vegetation could have resulted in capturing more species, especially rare ones.

5.4.2 Community composition

Using ordination analysis (NMS), I examined the mosquito community composition in two habitats on the four islands. Both sampling years were used for the analysis, as

years had no effect on the community composition (PerMANOVA: *pseudo* $F_{1,15}$ = 1.03, P = 0.375). I found that the mosquito community strongly varied in response to island (PerMANOVA: *pseudo* $F_{3,15}$ = 6.86, P = 0.0002) and pairwise comparisons showed that Saibai and Boigu supported a similar community composition, but different to Badu and Moa (Table 5.3, Fig. 5.3). Natural vegetation and villages (PerMANOVA: *pseudo* $F_{1,15}$ = 6.32, P = 0.0008) also supported different mosquito communities as seen between the continental islands on Axis 1 and the low-lying islands on Axis 2. The interaction effect of distinctly different island and habitat effects was also significant (PerMANOVA; *pseudo* $F_{3,15}$ = 2.99, P = 0.005), with a greater relative similarity seen between villages and natural vegetation in the continental islands of Badu and Moa from the Axis 2 perspective. The two NMS ordination axes together explained 91% of the total variation (Axis 1: 48%, Axis 2: 43%). Of the 27 species examined, 6 were significantly correlated with these axes (Table 5.4): *An. farauti*, *Cx. sitiens* subgroup and *Verrallina funerea* were more frequently associated with Saibai and Boigu than with Badu and Moa and *Ae. kochi*, *Aedes tremulus* and *Verrallina carmenti* were more frequently associated with natural vegetation.

Table 5.3: Pairwise comparisons testing whether mosquito communities differ between the four islands (values in boldface were significant).

Islands	**t**	***P***
Saibai versus Boigu	1.0104	0.3594
Saibai versus Badu	2.4332	**0.0260**
Saibai versus Moa	2.2071	**0.0304**
Boigu versus Badu	2.2182	**0.0294**
Boigu versus Moa	1.8691	**0.0304**
Badu versus Moa	1.2051	0.3072

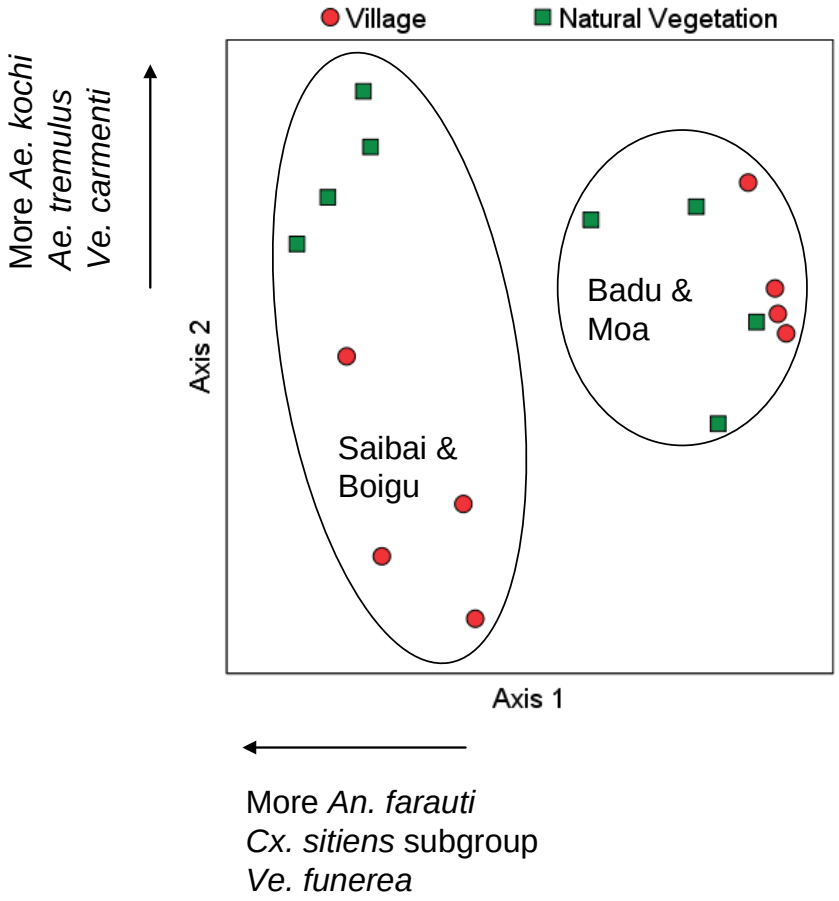

Figure 5.3: Ordination analysis of mosquito communities displaying that mosquito community composition varied strongly in response to island and habitat type. Saibai and Boigu shared a similar community composition, as did Badu and Moa.

Finally, I found that a subset of the mosquito community (16 spp.), which can transmit alpha-, flaviviruses and protozoan parasites varied significantly between the islands (PerMANOVA: *pseudo* $F_{3,4}$ = 4.87, P = 0.030). A single significant ordination axis explained 68% of the variance. Again, Saibai and Boigu supported a similar community composition, as did Badu and Moa. Of the 16 species examined, 4 were significantly correlated with this single axis (Table 5.4). *An. farauti*, *Cx. sitiens* subgroup and *Ve.*

funerea were more frequently associated with Saibai and Boigu whereas *Ae. albopictus* was mostly associated with Badu and Moa.

Table 5.4: * Mosquito community: Pearson correlation values of six mosquito species with two ordination axes produced by non-metric multidimensional scaling. Correlation values in boldface were significant ($P < 0.005$) using a Bonferroni-corrected alpha value ($P = 0.003$). **Vector community (only species vectoring alpha-, flaviviruses and protozoans were considered for the analysis): Pearson correlation values of four mosquito species with one ordination axis produced by non-metric multidimensional scaling. Correlation values were significant ($P < 0.005$) using a Bonferroni-corrected alpha value ($P = 0.009$).

	Mosquito Community*		**Vector Community****
	Axis 1	Axis 2	Axis 1
Species			
Aedes albopictus			**0.908**
Aedes kochi	0.611	**0.697**	
Aedes tremulus	-0.196	**0.790**	
Anopheles farauti	**-0.699**	-0.066	**-0.840**
Culex annulirostris	**-0.897**	0.388	**-0.980**
Verralina carmenti	-0.151	**0.748**	
Verralina funerea	**-0.776**	0.538	**-0.847**

A comparison between my novel sampling technique and the standard BG-Sentinel trap in 2013 found no differences in mean captures (t-test; $t_{38} = -1.28$, $P = 0.206$) and that both traps designs captured a similar mosquito community composition (PerMANOVA: *pseudo* $F_{1,6} = 1.14$, $P = 0.374$) with a single significant ordination axis

explaining 84% of the variance. However, there were interesting differences in the capture success of specific disease vectors. *Ae. aegypti*, for example, were captured more frequently in the BG-Sentinel trap compared to the passive box trap (which is not that surprising as the BG-Sentinel™ trap was initially designed with *Ae. aegypti* in mind). Alternatively, *An. farauti* were captured 10 times more frequently in the PBT compared to the BGT, and both trap designs were equally successful in capturing *Ae. albopictus*.

5.4.3 Disease detection

A total of 320 FTA® cards were analysed with 60% of the trapped mosquitoes having taken a honey meal. In village traps 70% and in natural vegetation traps 57% of the mosquitoes had fed on the FTA cards. Three samples tested weakly positive for chikungunya and one sample tested weakly positive for dengue (Table 5.5). However, these results could not be confirmed which suggests that there were only small amounts of RNA in the samples.

Table 5.5: Types of detected viruses in the Torres Strait. All positives were retested due to high Ct values (weak amplification) and all samples returned negative results.

Virus	Island	Habitat	Year
Chikungunya	Saibai	Natural Vegetation	2013
Chikungunya	Saibai	Village	2013
Chikungunya	Badu	Village	2013
Dengue	Badu	Village	2013

5.5 Discussion

One of the greatest hindrances to understanding the impacts of anthropogenic activities on emerging diseases is a lack of reliable, cheap and efficient equipment for the surveillance of tropical frontiers. On four remote tropical islands of northern Australia I used a novel home-made, non-powered trap baited with CO_2 derived from yeast and sugar to capture mosquitoes. Using this simple system I successfully collected sufficient samples of mosquitoes (n > 11,000) to describe important mosquito community differences between urban and natural habitats on these remote islands. Specifically, I discovered that urban mosquito communities supported a higher proportion of disease-competent species compared to natural vegetation, despite having lower total captures and lower species richness. In addition, I found that low-lying islands harboured populations of major disease vectors, most notably *An. farauti*, the primary malaria vector in Indonesia, Papua New Guinea and Australia (Beebe 2000).

I was able to recognise unique species associations allowing for the identification of sylvan and urban mosquito communities. Important disease vectors such as *Aedes aegypti, Cx. quinquefasciatus* and the recently arrived *Ae. albopictus* (Lee et al. 1989, Ritchie et al. 2006a) were almost exclusively trapped in villages. Similarly in Thailand, the same three species were also least abundant in undisturbed compared to disturbed areas (Thongsripong et al.2013). These three species originate from Africa or Southeast Asia and are now described as “domestic” mosquitoes (species successfully living in close association with humans in anthropogenically modified landscapes) in many parts of the world. Human settlements provide these mosquitoes with suitable larval habitats (artificial containers), blood meal resources and resting sites (around houses and often indoors) (Jansen and Beebe 2010). Comparative adult mosquito community studies in natural vegetations in the tropics are rare (although see Standfast and Barrow 1969, Thongsripong et al. 2013) as most studies concentrate on

single vector species, such as dengue vectors (Hanna et al. 1998, Ritchtie et al. 2006a, Garcia-Rejon et al. 2008) or peri-urban areas (Jardine et al. 2004, Keating et al. 2004, Kay et al. 2007, Jansen et al. 2009b).

Mosquito community composition not only differed between habitats but also varied across the studied Torres Strait islands. The northern low-lying islands situated close to Papua New Guinea (Saibai and Boigu) shared a very similar mosquito community that was distinct to that from the southern, continental islands (Badu and Moa). Disease vectors for malaria (*Anopheles* spp.) were only captured on the northern islands, whereas a disease vector for dengue and chikungunya (*Ae. albopictus*) was more frequently captured in the villages on southern islands. I have no explanation for not capturing *Ae. albopictus* on the northern islands and I suspect that the scarcity of swamplands in the southern islands could limit *Anopheles* spp. distributions. For example, Cooper et al. (1996) found that *An. farauti* and *Anopheles hilli* often use brackish and saline waters to oviposit and that *An. meraukensis* larvae were absent from drier (< 1,000 mm rainfall per year) areas in the Gulf region of northern Australia.

The higher capture rates observed in natural vegetation habitats may be partly due to a greater availability of species-appropriate larval habitat, particularly permanent or temporary (grassland) swamps. For example, the larvae of *Coquillettidia* and *Mansonia* are unique amongst mosquitoes; they are immobile and attach themselves to aquatic plants to obtain oxygen (Russell 1999). Another driving factor influencing capture rates may be differential attractiveness to low CO_2 levels. Mosquito species occurring in natural vegetation, such as *Aedeomyia catasticta,* are probably more zoophilic where as mosquitoes occurring in villages tend to be primarily anthropophilic and these feeding preferences may influence their attraction to different outputs of CO_2 production. For example, mosquitoes which display a high degree of anthropophily, such as *Ae. aegypti* (Ponlawat and Harrington 2005, Takken and Verhulst 2013), may

be less attracted to my traps as the CO_2 output of ~ 120ml/min is less than half that of an average human (~ 275 ml/min; Schmidt-Nielsen 1997). In contrast, zoophilic species, such as *Ad. catasticta* exploit smaller hosts (e.g. birds) (Standfast and Barrow 1969) who emit less CO_2. Furthermore, lower captures in villages could also be attributed to wind intensity in the coastal human settlements compared to the wind protected natural vegetation. The average wind speed during mosquito trapping was 13 km/hr – however maximum wind speeds were frequently over 50 km/hr (Willy Weather Australia 2014) which would almost certainly have had an influence on trap catches.

The most dominant mosquitoes captured in this study belong to the *Culex sitiens* subgroup. Among them is *Cx. annulirostris* which is the most important arbovirus vector in Australia (Kay and Standfast 1987). It is capable of transmitting Ross River, Barmah Forest, Murray Valley, Kunjin and Japanese encephalitis viruses to humans (Ritchie et al. 1997, van den Hurk et al. 2003). *Cx. annulirostris* was responsible for the outbreaks of Japanese encephalitis in the Torres Strait in 1995 and 1998 (Hanna et al. 1996 and 1999) and it was also the most dominant species captured in three different tropical habitat types (rainforest, rainforest edge and grassland) in far north Queensland (Meyer Steiger et al. 2012, see also Chapter 2) and from urban, peri-urban and melaleuca swamps (Harley et al. 2000, Jansen et al. 2009b) in the same region. *Cx. annulirostris* is regarded as a generalist species with opportunistic feeding patterns defined by host availability and it can utilize a wide variety of larval habitats including fresh water swamps, shallow grassland pools and large artificial containers such as livestock watering tanks (Lee et al. 1989). This species can also persist during dry periods (Jardine et al. 2004, see also Chapter 2). Its ability to utilise a range of hosts, habitat types and oviposition sites may account for the dominance of *Cx. sitiens* subgroup mosquitoes in my study sites.

I sampled over two consecutive wet seasons which may not reflect the community composition at other times, although the monsoonal climate in this region has distinct dry seasons with low mosquito densities. The main purpose of this study was not to capture as many mosquitoes as possible but to evaluate if my inexpensive method could be a valuable tool for mosquito surveillance in remote areas. I believe my sampling has been adequate for this purpose but acknowledge that more extensive sampling could detect other patterns. I recognize that CO_2 produced from the yeast and sugar mixture is less concentrated than CO_2 from dry ice. My prior study (Chapter 4) found that while this may affect capture rates, it did not affect the ability to describe the community composition, although, very rare species may not be captured. I think the efficacy of the passive trap utilised in this study could be further enhanced by the addition of secondary attractants, such as octenol, ammonia or lactic acid because they are effective for some species and are easily transported (Braks et al. 2001, Dekker et al. 2002, Ritchie et al. 2013). These secondary attractants may yield higher collection totals and could be used to target more species-specific captures. Additionally, I concede that there is always mosquito sampling bias depending on the type of traps used. Nevertheless, the simple CO_2 attractant and passive trap combination described in the current study is a cheap, effective and reliable surveillance device for use in remote areas without adequate access to electricity and conventional CO_2 sources (i.e. dry ice and CO_2 gas cylinders).

This study demonstrated that human settlements significantly altered the mosquito community composition in tropical landscapes, increasing the presence and abundance of anthropophilic species. Surveillance in these remote landscapes has an important role in detecting and managing disease outbreaks and I believe that the development and testing of home-made trap designs and attractants that are affordable and reliable will be crucial to this task.

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CHAPTER 6

Conclusion

The research performed for this thesis has involved the exploration of a range of topics in regards to mosquitoes in tropical Australia. As each data chapter already contains an extensive discussion, the aim of this final chapter is to concisely synthesise and emphasise the main findings and explain why these findings are significant. The last part of this conclusion outlines possible scope for future research.

6.1 Synthesis and significance of findings

The overall aim of this thesis was to determine whether anthropogenic modified environments influence mosquito communities in tropical Australia. A further aim was to evaluate if a newly developed sampling technique could be applied to trap mosquitoes in remote areas.

The sampling of adult mosquitoes across an anthropogenic disturbance gradient (man-made grassland, forest edge and forest interior) in a peri-urban environment near Cairns revealed a very diverse mosquito community consisting of 29 species (Chapter 2). This research demonstrated that forest interior and grassland habitats not only supported significantly different communities but that forest edges acted as ecotones, sharing mosquito communities from adjacent habitats. This pattern was observed both in the wet and dry seasons. I also found that disease transmitting species primarily occurred in grasslands and that *Cx. annulirostris*, an important disease vector in Australia, is able to persist all year round. Findings from this chapter provide new information about the influences of land use and seasonality on mosquito communities including the distribution of potential disease vectors in the study area. My research suggests that disease transmission could arise via common mosquito species captured across all habitats and seasons or through species more prolific within disturbed habitats as forests often have fewer disease vectors compared to anthropogenic disturbed habitats (Vittor et al. 2006, Junglen et al. 2009, Thongsripong et al. 2013).

Because some of the most debilitating mosquito borne diseases are transmitted by small container breeding mosquitoes, I examined the oviposition behaviour of mosquitoes (Chapter 3) by placing small artificial containers (traps) along the same disturbance gradient where I trapped adult mosquitoes. Although species diversity was much lower for the immature mosquito sampling from artificial ovitraps compared to the

adult sampling from Chapter 2, I observed similarities in habitat preferences. Gravid mosquitoes displayed a preference to oviposit in disturbed habitats (grassland and forest edges) rather than inside forests. In addition, oviposition behaviour was influenced by the location of traps as I reared more mosquitoes from terrestrial traps compared to above ground traps. The most important disease vector (*Ae. notoscriptus*) from this study was mostly reared from grassland containers. These findings suggest that artificial containers on the ground in combination with disturbed environments provide very productive mosquito larval habitats; thus increasing the risks of disease transmission. Mosquito species which exchange their traditional breeding habitats with more productive habitats could further increase disease transmission. This overspill has already occurred with *Ae.aegypti* and *Ae. albopictus*, two of the most important disease vectors in urban areas. Both species have made the important evolutionary jumps in behaviour, adapting from forests breeders to highly successful domestic breeders (Crosby 2006).

One of the obstacles to sampling adult mosquitoes in remote areas is the unavailability of attractants to entice mosquitoes into traps. In Chapter 4, I explored the option of using CO_2 produced by yeast and sugar as such an attractant. This CO_2 method was compared with dry ice (a standard attractant) in three field trials with both types of attractants yielding similar species captures. Findings of this chapter led to the application of yeast and sugar fermented CO_2 to capture mosquitoes in the Torres Strait (Chapter 5).

As outlined in Chapter 5, selecting suitable traps to sample mosquitoes in remote locations can be an additional obstacle. Most traps require a power source and therefore, I used the unique combination of a powerless trap and CO_2 produced by yeast and sugar. The collection of over 11,000 mosquitoes from four Torres Strait islands demonstrated that this novel sampling technique is a reliable, non-labour

intensive and inexpensive tool for sampling mosquitoes in remote areas. The results presented in regards to human land use influencing mosquito communities on the islands clearly indicated that natural habitats supported distinctly different communities than urban habitats with medically important species (*Ae. albopictus* and *Ae. aegypti*) almost exclusively trapped in villages. A further interesting result was the division of mosquito communities between the northern low-lying (Saibai and Boigu) and southern continental islands (Badu and Moa). Malaria vectors (*Anopheles* spp.) were solely captured on Saibai and Boigu, whereas vectors for dengue and chikungunya (*Ae. albopictus* and *Ae. aegypti*) were mainly trapped on Badu and Moa. The significant findings of this study first imply that vector species are more likely to be encountered in villages than in natural vegetation and second, that different vectors occur on the northern and southern islands. The northern islands are only separated from Papua New Guinea by a few kilometres which could enable an exchange of mosquito species and diseases.

The overall results of this thesis demonstrate that human disturbed environments support distinctly different mosquito communities than undisturbed environments; that disease transmitting mosquito species are predominantly found in such disturbed environments and in close proximity to human habitations.

Mosquito borne diseases seem to be proliferating in disturbed landscapes and outcomes from this research could be valid for other geographical locations and habitats where anthropogenic land uses are threatening natural environments.

6.2 Future research

Globally more than 700 million of people are affected by mosquito borne diseases (Caraballo and King 2014) and it would be an illusion to believe that these diseases could ever be eradicated; however the knowledge of why, how and where these

diseases and their vectors occur are key aspects in the assistance of reducing health impacts for human and non-human animals. Although the research into mosquito communities performed for this doctoral thesis has involved extensive sampling, it can only provide a “snapshot in time” observation and may not reflect the communities at other times. Therefore, long-term observations of mosquito communities across different disturbance gradients are needed in detecting any such changes.

Considering that anthropogenic land use changes play such an important role in the transmission of mosquito-borne diseases it is essential to understand where mosquitoes are distributed in the landscape as it can help to understand the complex dynamics of vector-host- pathogen relationships. Information gaps exist in regards to biological, ecological and behavioural characteristics for many mosquito species which could be the focus for further research. These species-specific characteristics are crucial in understanding how anthropogenic land use changes influence the vector community and thus disease risk.

Surveillance of mosquito communities is an essential part of disease monitoring and management, especially in remote areas. I demonstrated that the use of yeast-generated CO_2 in combination with a powerless trap can be a viable method to attract and capture adult mosquitoes. However, there are circumstances (e.g. disease outbreaks) where it is vital to accumulate large quantities of mosquitoes in a short period of time. Further research could be carried out to achieve an increase in CO_2 production and to evaluate additional affordable mosquito attractants for emergency situations.

Apart from anthropogenic land use changes, global warming unquestionably has an impact on the emergence and re-emergence of mosquito-borne diseases. Warmer temperatures not only increase the range extension and breeding season of vectors,

but also decrease immature development rates and extrinsic incubation periods of pathogens (Patz et al. 1996, Epstein et al. 1998, Githeko et al. 2000). For example, warmer temperatures have allowed the distribution of malaria vectors to be present where they had been previously absent. Malaria incidences have been reported from various high elevation areas in Asia, Central Africa and Latin America (Epstein et al. 1998) and *An. farauti* has been captured outside the malaria-receptive zone in Queensland, Australia (van den Hurk et al. 1998). The time taken for pathogens to develop within mosquitoes to be infectious (extrinsic incubation periods) is shorter with rising temperatures; for example dengue type 2 virus in *Ae. aegypti* takes 12 days at 30°C and 7 days at 32°C (Watts et al. 1987). The expected influence of climate change on disease epidemiology could further be addressed with different modelling approaches and laboratory experiments.

Finally, reasons contributing to the emergence of infectious diseases are complex and often interact with each other and include environmental, ecological and socio economic factors which warrants the necessity for interdisciplinary research (Patz et al. 1996, Morens et al. 2004, Wilcox and Gubler 2005, Lambin et al. 2010).

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APPENDICES

Appendix 1: Traps used to sample adult and immature mosquitoes.

Figure 1: CDC (Center for Disease Control and Prevention) Miniature Trap Model 512 (John W. Hock Company, Gainesville, U.S.A.) used in Chapter 2 and 4.

Figure 2: Oviposition trap used in Chapter 3.

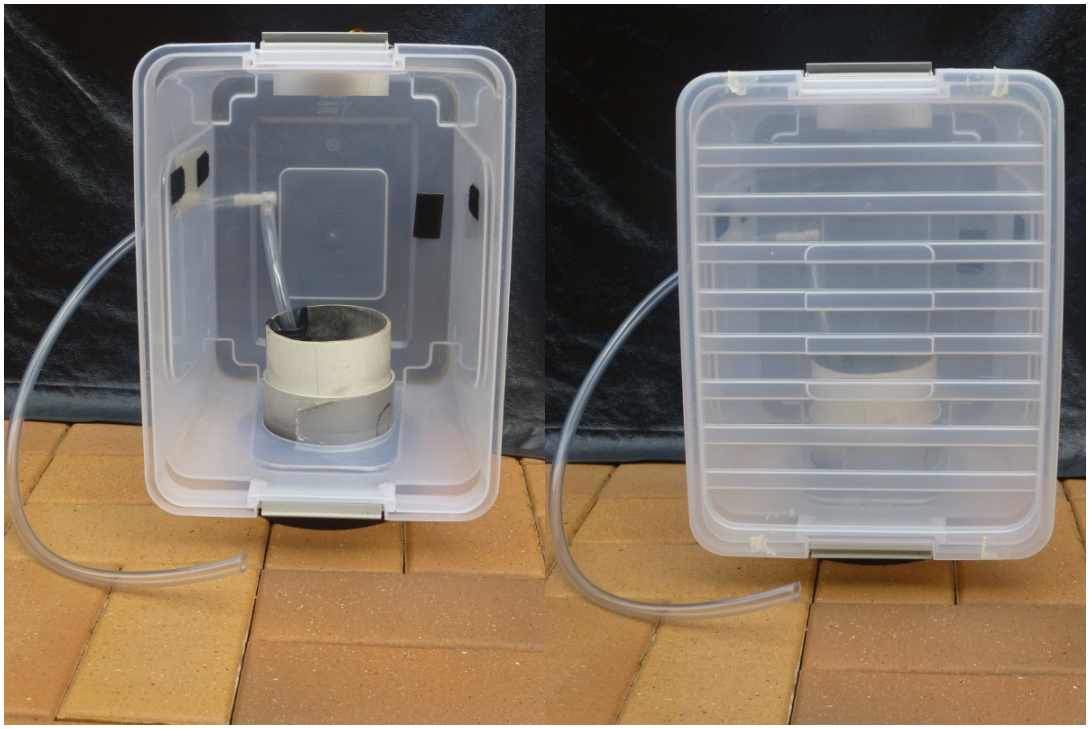

Figure 3: Non-powered passive box trap used in Chapter 5.

Figure 4: Non-powered passive box trap in combination with CO_2 produced by yeast and sugar fermentation used in Chapter 5 on the islands of Saibai, Boigu, Badu and Moa.

Figure 5: BG-Sentinel™ (Biogents AG, Regensburg, Germany) trap used in Chapter 5.

Appendix 2: Table of primers and probes used for TAQMAN assays.

Virus	Name	Targeted gene	5' - 3' sequence	Concentration per reaction	References
JEV	JEV MGB TAQ For 10486	3'UTR	GTGCTGYCTGCGTCTCAGT	600 nM	In house*
	JEV MGB TAQ REV 10566	3'UTR	GAGACGGTTRTGAGGGCTTTC	600 nM	In house*
	JEV MGB PROBE 10514	3'UTR	6FAM-ACTGGGTTAACAAATCTGACA-MGB	400 nM	In house*
MVEV	MVE-FOR	NS5	ATCTGGTGYGGAAGYCTCA	900 nM	Pyke et a.l 2004
	MVE-REV	NS5	CGCGTAGATGTTCTCAGCCC	900 nM	Pyke et a.l 2004
	MVE-FAM	NS5	6FAM-ATGTTGCCCTGGTCCTGGTCCCT-MGB	200 nM	Pyke et a.l 2004
RRV	Primer RRV E2 F	E2	ACGGAAGAAGGGATTGAGTACCA	390 nM	Hall et al. 2010
	Primer RRV E2 R	E2	TCGTCAGTTGCGCCCATA	390 nM	Hall et al. 2010
	Probe RRV E2 FAM	E2	6FAM-CAACAACCCGCCGGTCCGC-TAMRA	244 nM	Hall et al. 2010
WNVKUNV	Kunjin-F	NS5	AACCCCAGTGGAGAAGTGGA	400 nM	Pyke et al. 2004
	Kunjin-R	NS5	TCAGGCTGCCACACCAAA	400 nM	Pyke et al. 2004
	Kunjin MGB Probe	NS5	6FAM-CGATGTTCCATACTCTGG-MGB	135 nM	In house*
DEN-UNIV	DEN UNIV-F	3'UTR	AAGGACTAGAGGTTA(G/T)AGGAGACCC	30 pmol	Warrilow et al. 2002
	DEN UNIV-R	3'UTR	CG(ATTCTGTGCCTGGA	30 pmol	Warrilow et al. 2002
	DEN 2+4 UTR Probe	3'UTR	TCTGGTCTTTCCCAGCGTCAATATGCTGTT	100 pmol	Warrilow et al. 2002
CHIKV	CHIK-MA-F	E1	CCCGGTAAGAGCGGTGAA	370 nM	van den Hurk et al. 2010
	CHIK-MA-R	E1	CTTCCGGTATGTCGATGGAGAT	370 nM	van den Hurk et al. 2010
	CHIK-MA Probe	E1	TGCGCCGTAGGGAACATGCC	180 nM	van den Hurk et al. 2010
WNV	3'NC-F	ENV	CAGACCACGCTACGGCG	50 pmol	Lanciotti et al. 2000
	3'NC-R	ENV	CTAGGGCCGCGTGGG	50 pmol	Lanciotti et al. 2000
	3'NC Probe	ENV	TCTGCGGAGAGTGCAGTCTGCGAT	10 pmol	Lanciotti et al. 2000

*In house: unpublished; developed at Queensland Health Forensic and Scientific Services Public Health Virology, Brisbane.

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