MODELLING THE DEMOGRAPHY AND CONTROL OF DISEASE-CARRYING TROPICAL MOSQUITOES IN NORTHERN AUSTRALIA

By

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Abstract

Mosquito-borne pathogens constitute a major burden of disease for humans globally, and are predicted to increase in range and incidence given climate change projections based on 21st Century emissions scenarios for greenhouse gases. Understanding the contributions of environmental variation and density feedback to changes in vector population abundance is essential for designing effective control programmes and predicting disease outbreaks in humans. In my thesis I outline a population ecologist's five-step plan for mosquito control, and define the parameters needed to create spatially explicit demographic models of mosquito population dynamics using an example disease-vector system in Darwin, Northern Territory, Australia. My spatio-temporal models of larval abundance treat two important vector species in the Northern Territory: *Aedes vigilax* and *Culex annulirostris*. I show how larval habitats used by the saltwater-influenced breeder *Ae. vigilax* and the obligate freshwater breeder *Cx. annulirostris* are separated both spatially and temporally in a tidally influenced swamp. I identify adult abundance in the previous month as the most important temporal driver of larval densities in both species, providing a clear dynamical link between the two main life phases in mosquito development: the aquatic larval stage and the mobile adult stage. My field experiments show that the main vector control programme in the Northern Territory, aerial larvicide application, is effective at suppressing adult emergence of *Ae. vigilax*, whereas other possible larval control measures such as vegetation removal via burning or slashing are not as effective in this context. My experiments reveal that current larval sampling procedures alone are inadequate for quantifying larval abundance or adult emergence. Further manipulation experiments show that reducing *Ae. vigilax* larval densities results in the emergence of larger adults, and that this relationship between larval density and adult emergence size is tempered by environmental conditions such as changes in nutrient levels across different larval habitats. Mosquito body size is linked to vital rates such as fertility and adult survival that are important determinants of disease transmission probability. My measurements of body size of remotely trapped female *Ae. vigilax* adults reveal evidence for longer survival time since

emergence in larger adults, and together these findings define an important mechanism of density feedback in mosquito populations; competition for resources at the larval stage results in smaller adult emergence sizes, and therefore, lower adult survival and fertility. My block-bootstrapped generalised linear models of a widespread tropical and subtropical disease in Australia – Ross River virus – show that environmental proxies of adult vector abundance, rather than trapped vector abundance data, are the most accurate predictors for the highest priority health statistic: wet season outbreaks of Ross River virus cases. These results demonstrate that quantifying the environmental determinants of variation in larval habitat quality and the subsequent production of adults provides the means to construct models that are likely more accurate for predicting disease transmission than expensive residential vector monitoring systems. Given that low-income tropical developing countries are the hardest hit by mosquito-borne diseases, cost-effective solutions to deal with the current and future burden of mosquito-borne disease are of paramount importance to global human health.

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i. *Aedes vigilax* and *Culex annulirostris* larval abundance data, water presence data, monthly *Ae. vigilax* and *Cx. annulirostris* adult abundance data and monthly spraying hours data for the Leanyer/Holmes Jungle swamp complex spanning November 2000 to December 2006 (Chapter 2).

ii. Monthly counts of laboratory-confirmed cases of Ross River virus infections in the Darwin region notified to the Northern Territory Centre for Disease Control, and monthly *Ae. vigilax* and *Cx. annulirostris* adult abundance data from 11 locations around Darwin spanning January 1991 to December 2007 (Chapter 5).

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“The mosquito’s a clever little bastard. You can track him for days and days until you really get to know him like a friend. He knows you’re there, and you know he’s there. It’s a game of wits. You hate him, then you respect him, then you kill him.”

– Roy Spim, Mosquito Hunter.

Mosquito Hunter’s Sketch, Monty Python’s Flying Circus

Contents

CHAPTER 1

INTRODUCTION

Background

Less than 10 % of the 3500 named mosquito species are vectors of disease; however, this relatively small pool of eukaryotic species cause more human suffering than probably any other group of organisms. Over one million people die from mosquito-borne diseases such as malaria, dengue fever, and yellow fever every year; mosquito-borne diseases are responsible for 14% of all infectious and parasitic disease disability-adjusted life years (DALYs) (World Health Organization, 2004). While there are many ways to treat and prevent these diseases, pharmaceutical-based solutions ultimately become intractable during large outbreaks and in the absence of vaccines for many of these diseases, we are reliant on mosquito control to reduce pathogen prevalence and the probability of disease transmission (Walker and Lynch, 2007). Tropical developing countries are by far the hardest hit by mosquito-borne disease; in fact, 95% of the global burden of disease from mosquito-borne pathogens occurs in poverty-stricken tropical regions such as sub-Saharan Africa, Central and South America, and South-East Asia (Fig 1, World Health Organization, 2004). Cost-effective solutions to deal with the current and future burden of mosquito-borne disease are therefore of paramount importance to global human health.

Climate-change models based on 21st-Century emissions scenarios predict increases in temperature, sea level, and, depending on location, increases or decreases in rainfall and evaporation (IPCC, 2007). Changes in climate conditions can affect the transmission of vector-borne diseases in three different ways: (*i*) by influencing the reproduction of pathogens within vectors and hosts, (*ii*) by affecting human behaviour and activity, and (*iii*) by altering the population dynamics and range of vector species (Zhang et al., 2008; Paaijmans et al., 2009).

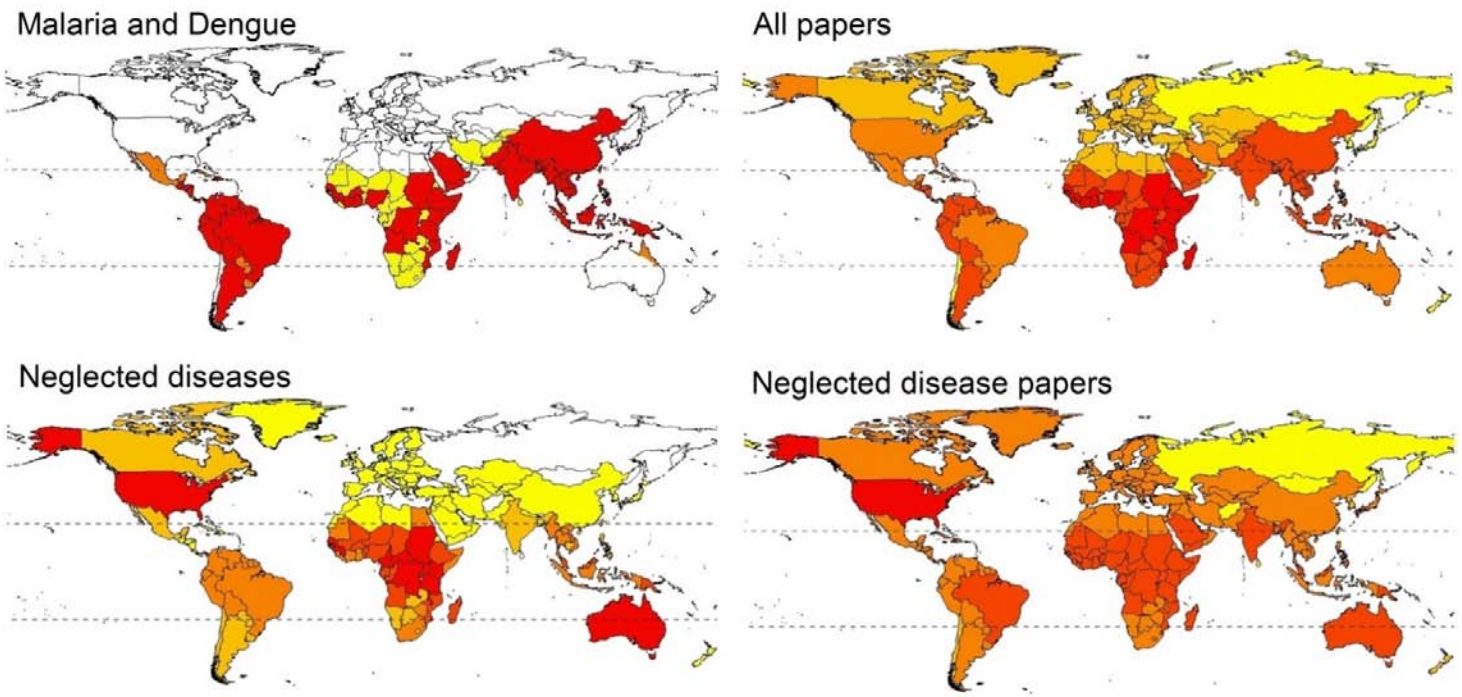

Figure 1 Distribution by country of the quantitative papers published since 2001 on mosquito control: 446 studies sourced using *ISI web of Science* [http://isiknowledge.com] and search terms 'mosquito', 'control' and 'model'. Panels show the global distribution of (*i*) malaria and dengue fever (yellow = malaria, orange = dengue, red = both), (*ii*) quantitative mosquito control research in the last 10 years (all papers) (from yellow = low to red = high), (*iii*) neglected mosquito-borne disease incidence (not malaria or dengue) (from yellow = low to red = high), and (*iv*) non-malaria or dengue research (neglected disease papers) (from yellow = low to red = high). Horizontal lines delineate tropics.

The projected increase of 1.4 to 5.8 ^{o}C in mean global temperature by 2100 is predicted to allow the expansion of vector population ranges into areas that have previously been disease-free (Campbell-Lendrum and Woodruff, 2007; Kearney et al., 2009; Lafferty, 2009; Jansen and Beebe, 2010; Tonnang et al., 2010). Increasing temperatures year-round will also increase the seasonal period when disease transmission is possible, and through the reduction of pathogen incubation time, increase the likelihood of vector infection and transmission (Lafferty, 2009; Paaijmans et al., 2009). Rising sea levels will increase the larval habitats available for saline water-breeding vectors such as *Anopheles sundaicus*, *Culex sitiens*, *Culex tarsalis* and *Aedes camptorhynchus*, and there is the possibility that freshwater-breeding species will adapt to increased salinity in the presence of larger availability of brackish and saline water habitats, along with changes in precipitation that will affect the availability of freshwater habitats (Zhang et al., 2008; Chaves and Koenraadt, 2010; Ramasamy and Surendran, 2011). These climatic effects on vector population sizes and ranges will, however, be tempered by the effects of changes in human activities and movements.

Changes in human population sizes, movements, socio-economic status and land use are all factors likely to interact with climate change, and synergies between these different variables and changes in local climatic patters will contribute to the spread and intensity of vector-borne disease (Campbell-Lendrum and Woodruff, 2007; Kearney et al., 2009). Anthropogenic influences on current distributions of vector species and parasite reservoirs include size of human settlements, housing conditions, type and size of water supply, vector control practices, and deforestation and other land-use changes (Sutherst 2004; Campbell-Lendrum and Woodruff, 2007; Yasuoka & Levis 2007; Kearney et al., 2009). In particular, poor socio-economic conditions, which are often linked to lower housing conditions and open, untreated water supplies, continue to enable disease transmission to occur (Sutherst 2004). Increasing human population density, coupled with the possiblity of environmental refugees, will lead to large poverty-ridden areas without the necessary

infrastructure for the safe storage and distribution of clean and waste water, providing more breeding sites (such as used containers and tires) for vectors within urban areas. In addition, increasing pressure on the currently under-resouced public heath infrastruces in many tropical developing countries will exacerbate the morbidity and mortality associate with vector-borne disease (Sutherst 2004; Campbell-Lendrum and Woodruff, 2007).

Synergies between land use and climate change might also affect vector populations. Deforestation has had both positive and negative affects on vector populations due to increases in surface temepratures, drier conditions, and shifts to sun-tolerant species (Sutherst 2004; Yasuoka & Levis 2007). The deadly combination of habitat fragmentation and/or destruction and climate change is also likely to increase extinction rates, and this could alter existing vector-host-parasite relationships (Sutherst 2004).

Variation in local patterns of climate change will differently affect vector species and diseases. For example, changes in temperatures affect vector development, vector survival and many other individual components of the infection transmission cycle. For temperate climates, this could mean that threshold temperatures needed for malaria might become more common; however, the extremely high temperatures that slow parasite development could also become more common, particularly in tropical environments (Pascual et al., 2006; Campbell-Lendrum and Woodruff, 2007; Paaijams et al., 2009).

Some of the other potential negative effects of climate change on vector species include: increasing temperatures will result in higher adult and larval mortality and egg desiccation, and changes in precipitation and sea levels will influence the ecology of seasonally ephemeral saltwater and freshwater swamplands, resulting in large changes in the available larval habitat (Yang et al., 2008a; Zhang et al., 2008; Kearney et al., 2009; Lafferty, 2009; Russell, 2009; Russell et al., 2009a; Ramasamy and Surendran, 2011). Predicting the risk of potential outbreaks and spread of vector-borne disease under climate change scenarios therefore depends critically on a sound knowledge of the ecology of the current climatic envelopes of vector species, the endogenous and exogenous drivers of

vector population dynamics, and the vector-disease ecology and transmission cycle.

The management of vector-borne disease can take several paths: one can treat the symptoms of disease, vaccinate against the disease, or prevent disease transmission. Only treating the symptoms of the disease results in a massive financial burden, particularly for often poverty-stricken countries, and is intractable for the mosquito-borne diseases that cause high human mortality. While some progress has been made towards vaccination against vector-borne disease (Amina et al., 2010; Appaiahgari and Vrati, 2010; Kumar et al., 2010), and in some cases, has been a highly effective method of breaking the host-parasite-disease cycle (World Health Organization, 2011). For most vector-borne diseases, the large-scale, practical applications of this method still remain some years away due to the high associated costs (Tediosi et al., 2009). Preventing disease transmission can be achieved through either preventing vectors from carrying disease, using genetic manipulation or mosquito pathogens that reduce vector fitness, or preventing host contact with the vectors of disease (Rasgon, 2008; Wilke et al., 2009; Scolari et al., 2011). Vector/host contact can be reduced either through managing host contact with adult mosquitoes, using bed nets and mosquito repellents, or through actual reduction of vector populations using control methods such as insecticides and larvicides, larval habitat reduction or introduction of predators, or the introduction of transgenic mosquitoes incapable of disease transmission into wild populations (Langeler and Snow, 1996; Mount, 1998; Patz et al., 2000; Killeen et al., 2002a; Walker and Lynch, 2007; Chandra et al., 2008; Russell and Kay, 2008; Shaalan and Canyon, 2009; Govella et al., 2010).

Current control programs targeting adult mosquitoes are compromised by the development of resistance to control agents such as pyrethroid-based insecticides (Yewhalaw et al., 2011) and the difficulty in successfully targeting highly vagile adult vectors (Killeen et al., 2002a); while a large amount of work has been done on genetic population control, the immediate practical applications of these methods are dubious (Fish, 2008; Raghavendra et al., 2011).

The larval stage of vectors are exclusively aquatic and are thus restricted to water bodies that are more amenable to control, such as the application of chemical or biological agents, the introduction of larval predators, or the physical reduction of suitable habitat (Killeen et al., 2002a; Fillinger et al., 2003; Russell et al., 2003; Fillinger and Lindsay, 2006; Walker and Lynch, 2007; Chandra et al., 2008). Recently, the focus for vector-borne disease control has turned to 'integrated vector management', which combines the suppression of larval stages of vectors with the prevention of human contact with adult vectors via indoor residual spraying and insecticide-impregnated bed nets (Ginsberg, 2001; Killeen et al., 2002a; Utzinger et al., 2002; Walker and Lynch, 2007; Russell and Kay, 2008).

Many different statistical models have been developed and applied since the debut of the classic Ross-Macdonald malaria models which predict vector-borne disease prevalence and transmission, to quantify vector population control measures and to develop more effective control regimes (Ross, 1911; Macdonald, 1952; Lord, 2007). Many of these studies have focused on developing predictive models to describe and quantify regional habitat associations and general geographic distribution of disease and disease vectors; however, relatively few studies explicitly incorporate the more intricate details of vector ecology such as the relative contributions of intrinsic and extrinsic drivers of both larval and adult vector population dynamics. The majority of the last decade's literature on vector control and vector-borne disease modelling focuses on the two diseases that cause the highest mortality and morbidity: malaria and dengue fever (n = 276 papers, Fig. 1). Other mosquito-borne disease such as lymphatic filariasis, yellow fever, Rift Valley fever, West Nile fever, Japanese encephalitis and other arboviral encephalitides still contribute to the global burden of health; however, they are often of a lesser emphasis for mosquito-borne disease and control research (n = 169 papers). Research that focuses on these 'neglected' mosquito-borne diseases tends to be done mainly in high-income countries (e.g., USA) rather than in the developing countries in Africa, Central/South America or South-East Asia that have equivalent or higher incidences of neglected mosquito-borne diseases (Fig. 1).

Statistical models of disease transmission and mosquito control making use of the current ecological knowledge available will aid the quantification and implementation of cost-effective vector control programmes for these neglected areas of research. Mosquito population dynamics are arguably the most important predictors of incidence of mosquito-borne disease, along with region-specific measures of exposure and climatic variables that affect pathogen survival and transmission. There are several excellent reviews of mosquito population dynamics and studies examining how different mosquito species react to local and global environmental conditions (e.g. Juliano, 2007; Lord, 2007; Jansen and Beebe, 2010). Rather than another exhaustive review of vector population dynamics, I propose in this chapter a population ecology-focused five-step plan for mosquito control that will incorporate the wealth of ecological knowledge already present in the literature, and identify areas to direct future funding and research.

Modelling population dynamics

The life history of mosquitoes is complex: there is an aquatic juvenile stage, incorporating egg, larval and pupal stages, and a mobile winged adult stage. These adult and juvenile stages occupy different micro-environments and so their development and survival are affected by different intrinsic compensatory mechanisms and extrinsic processes (Fig. 2). Compensatory density dependence occurs when population growth rate is dependent on population density, i.e. as density increases, the percent survival and/or reproduction of individuals decrease (Sinclair and Pech, 1996). The factors that cause the loss of reproduction or increase in mortality are the compensatory feedback mechanisms. These mechanisms act jointly with extrinsic processes, such as environmental variation and control programs, to alter population growth rates. It is important, therefore, to quantify the different drivers influencing population dynamics, and the degree of interaction between them, to have a comprehensive understanding of the reasons for variation in vector population densities and therefore the impact of any vector control program (Saether, 1997; Wang et al., 2006; de Little et al.,

2007).

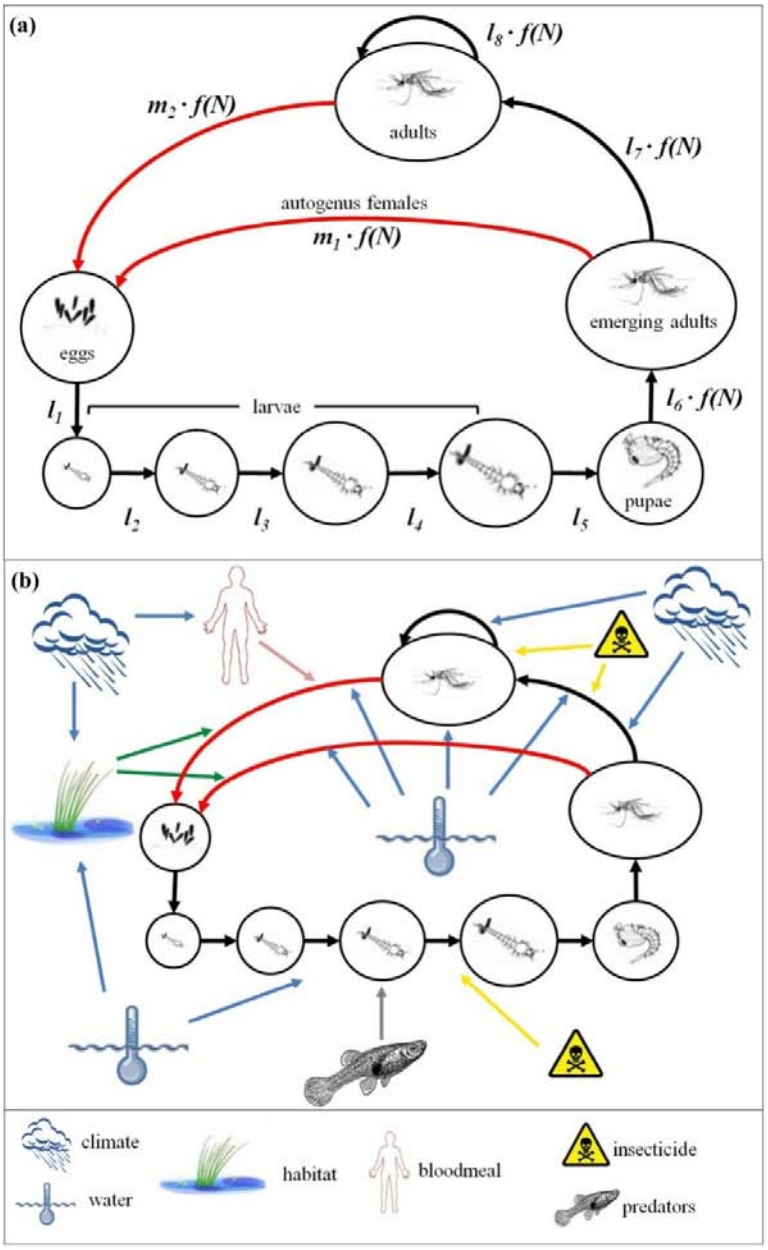

Figure 2. Mosquito life-cycle graphs. (a) Demographic stage-structured life cycle graph, where l_i = probability of survival from stage i to i +1, m_1 = fecundity of autogenous females (females capable of developing the first batch of eggs without a blood meal), and m_2 = fertility of blood-fed females. Vital rates that vary in response to changes in density (emergence survival (l_6), adult survival (l_7, l_8) and fertility (m_1, m_2)) are shown where $f(N)$ represents a function of density. (b) Demographic stage-structured life cycle graph showing the various environmental variables that affect vital rates. **climate** = season, rainfall, temperature and tides; **water** = water qualities such as pH, salinity, temperature and nutrient concentrations; **habitat** = available larval habitat (number of ephemeral pools, vegetation type); **blood meal** = probability of acquiring a blood meal (dispersal); **insecticide** = mosquito control measures (insecticides, larvicides); and **predators** = aquatic predators of larvae.

As mentioned previously, vector management programs can contribute to alleviating vector-borne disease in one of two ways: (*i*) reducing vector/host contact through integrated vector management: suppressing vector population sizes through larval control programs and preventing host contact with adult vectors via indoor residual spraying and insecticide-impregnated bed nets; or (*ii*) reducing the capacity of vectors to carry and transmit disease. It has long been accepted that effective and cost-efficient suppression of vector populations requires an understanding of how the populations vary in space and time (Killeen et al., 2004; Ferguson et al., 2010). The capacity of the a vector to acquire a pathogen through an infected blood meal, incubate the pathogen, and then infect a host through subsequent blood meals is affected by variation in vital rates such as adult survival, longevity, fertility and dispersal (Garrett-Jones, 1964; Jennings and Kay, 1999; Gimnig et al., 2002; Luz et al., 2003; Manoukis et al., 2006; Maciel de Freitas et al., 2007; Schneider et al., 2007; Bevins, 2008; Vaidyanathan et al., 2008). Therefore, accurate disease management and prediction is reliant on an understanding of how both vector population size, and also vectorial capacity specific vital rates, vary with changes in environmental conditions and intrinsic feedback mechanisms.

The population dynamics of invertebrates have traditionally been considered to be influenced predominantly by environmental variation, as evidenced by the majority of past research on vector species devoted to defining only the environmental conditions that influence changes in population size, development rates and individual survival. Only recently has there been progress in defining and evaluating the component effects of intrinsic (density-dependent) and extrinsic (environmental) processes on mosquito population dynamics (e.g. Yang et al., 2008a,b; Russell et al., 2011), and this is a field that needs much development, particularly in the areas of accurately incorporating both processes into models of mosquito population dynamics vector-borne disease, and programs for vector control.

Density feedbacks in mosquito population dynamics

Untangling the mechanism and expression of density dependence is particularly

important when trying to understand the population dynamics of organisms such as mosquitoes that have fast life histories, or recruitment-driven population dynamics. This is due to these species' capacity for exponential population growth at low densities, which declines rapidly with increases in population density (Saether et al., 2002; Sibly et al., 2005). Density dependence refers to the dependence of population growth rate (r) on population density (N), and both compensatory and depensatory density feedbacks occur within populations. While depensation refers to an positive relationship between mortality or fitness (rate of population growth) and population density – i.e., as the population size increases the % mortality decreases and vice versa; compensation occurs when a reduction in density leads to a reduction in the competitive feedbacks on various vital rates, which in turn engenders a temporary increase in population growth rate (Sinclair and Pech, 1996). Therefore, care must be taken when designing a vector control programme that the control mortality is not compensated for by an increase in some other vital rate leading to rapid population recovery (Juliano, 2007).

The expression of these different density feedback phenomena is often identified by studying the dynamics of long-term time series of population sizes (Brook and Bradshaw, 2006); however, as both compensatory and depensatory density feedback mechanisms can be operating in different vital rates within the population at any point in time, these phenomenological studies cannot necessarily reveal the true nature of the density feedback processes at play. Indeed, if depensation in one vital rate and compensation in another vital rate are equal, then the population dynamics might not display any phenomenological signal of density dependence, despite the presence of strong density feedback mechanisms. Therefore, to understand more completely the complex mechanisms of density feedback operating within a population, it is important to examine the different vital rates separately and in conjunction with each other, e.g., using density manipulation experiments (Fordham et al., 2009). Only a complete and explicit understanding of how these density feedback processes operate within the most influential and measureable vital rates, along with

quantified mortality from control measures, can the design and evaluation of vector control programmes that actively reduce adult vector abundance be done effectively (Fig. 2).

Accurate representation of density feedback is largely a neglected area in vector population and vector-borne disease modelling, despite some phenomenological evidence for compensatory density feedback signals in mosquito abundance time series (Yang et al., 2008a,b; Yang et al., 2009; Russell et al., 2011), and substantial experimental evidence for the mechanisms of compensatory density feedback in mosquito populations (Hawley, 1985; Lord, 1998; Agnew et al., 2000; Agnew et al., 2002; Reiskind et al., 2004; Gama et al., 2005; Juliano, 2007; Ellis, 2008; Legros et al., 2009; Medici et al., 2011). Even within those population dynamics or disease models that do incorporate density feedback, often the specific form or mechanism of density feedback is not provided; rather, density dependence is included merely as a ceiling abundance on the number of fertile mosquitoes (Ermert et al., 2011a,b), or as a larval mortality rate related to some (largely assumed) constant carrying capacity of larval habitats (Eisenberg et al., 1995a,b; Ritchie and Montague, 1995; Ahumada et al., 2004; Pascual et al., 2006; Otero et al., 2008; Schaeffer et al., 2008; Erickson et al., 2010a,b). In fact, where compensatory density feedback in mosquito populations has been quantified, the main mechanism is via per capita resource limitation and larval competition (Agnew et al., 2002; Gimnig et al., 2002; Barrera et al., 2006; Juliano, 2007; Gavotte et al., 2009). However, high densities do not generally elicit higher larval mortality; instead, they tend to give rise to smaller body sizes in emerging adults, with resultant decline in adult survival rates, longevity, dispersal capacity and fertility (Agnew et al., 2002; Gimnig et al., 2002; Hugo et al., 2003; Manoukis et al., 2006; Juliano, 2007; Bevins, 2008; Gavotte et al., 2009; Reiskind and Lounibos, 2009).

A notable exception in the quantification of compensatory density feedback dynamics is in container-inhabiting mosquitoes (Focks et al., 1993a,b; Focks et al., 1995; Williams et al., 2008a; Magori et al., 2009; Williams et al., 2010; Xu et al., 2010). In the container-inhabiting mosquito simulation model 'CIMSiM', compensatory density feedback is modelled as

resource limitation in the larval stages resulting in declining larval and pupal development weights, and the subsequent reduction in juvenile survival and emerging adult weight and fertility (Focks et al., 1993a). However, so far CIMSiM has been used only to model the population dynamics of the container-breeding species *Aedes aeygpti*, and does not account for the effects of compensatory density feedback on adult survival and longevity (Williams et al., 2008a). While population growth rate of fast life history organisms such as mosquitoes are not usually highly sensitive to adult survival, vectorial capacity increases exponentially as adult survival rises, making unbiased estimates of adult vector survival and longevity crucial parameters for models of vector-borne disease (Garrett-Jones, 1964).

The relationship between compensatory density feedback in vector populations and their capacity for subsequent disease transmission is complex. While reduction in density can lead to higher adult survival and an ensuing increase in vectorial capacity (Garrett-Jones, 1964), smaller adults (produced either from high-density larval conditions or reduced development rates arising from stressful environmental conditions) of some vector species are more susceptible to disease infection and dissemination (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et al., 2011), whereas other species become more susceptible to infection and dissemination as body size increases, and some species display no effect of body size on susceptibility (Nasci et al., 1994; Sumanochitrapon et al., 1998; Jennings and Kay, 1999; Alto et al., 2005; Westbrook et al., 2010; Muturi et al., 2011). A detailed understanding of species and disease-specific infection and dissemination relationships is therefore crucial for predictions of vector-borne disease prevalence and prevention.

If local population extinction is the aim of the vector control programme, it might be possible to exploit another form of density feedback (depensation, also known as 'Allee effects') manifested in some small populations. Depensation can occur when certain demographic, genetic or behavioural component effects, such as failing to locate mates, inbreeding depression or when there are too few individuals to saturate predator populations,

are manifested in small populations (Stephens et al., 1999; Williams et al., 2008b). Given extreme enough control measures, the culling of individuals could theoretically force a population density below an 'Allee threshold' where it would proceed to extinction without further intervention (Tobin et al., 2011). However, the evidence for and strength of Allee effects vary greatly among species (Gregory et al., 2010), and care needs to be taken when defining the Allee threshold, or 'population extinction point'. In some container-breeding mosquito species, control-induced mortality can elicit compensatory effects at densities of even two or three individuals per container (Agnew et al., 2002), so the existence in at least short-term depensatory mechanisms in mosquitoes is potentially dubious. Sophisticated modelling of mosquito larval and adult abundance long-term time series, along with laboratory and field experiments, are crucial to determine the sensitivity of different life stages and population densities to these various compensatory and depensatory regulatory processes. These quantified sensitivities can then be incorporated in models to simulate the complex dynamics of vector populations and therefore provide an understanding of the programs needed to control vector populations effectively despite compensatory feedbacks, or even to exploit possible depensatory feedbacks in small populations.

Environmental variation in mosquito population dynamics

Environmental variation affects the amount of resources (e.g., food availability, breeding habitat) available for any population in time and space. Mosquito population dynamics are often highly sensitive to climatic and seasonal fluctuations because juvenile life history stages (eggs, larvae and pupae) require ephemeral water habitats (Service, 1993; Chase and Knight, 2003). Most studies that seek to define the relationship between environmental resources and vector population size use estimates of adult abundance from traps to infer extrinsically forced changes in population size (Glass, 2005; Adams and Kapan, 2009; Carver et al., 2010; Sullivan, 2010). However, because the relationship between adult emergence and larval abundance is often non-linear (due to larval predation, compensatory density feedbacks and

patchy resource distribution), and each stage (adult and juvenile) occupies different microclimates, it is important to decouple the environmental conditions driving vector population dynamics into those that affect adults (e.g., humidity, ambient temperature) and those that define larval habitats (e.g., rainfall, tides, water qualities, vegetation types) (Fig 2).

Models describing population dynamics and their interaction with environmental conditions have been constructed for some mosquito species (Cai and Li, 2010; Parham and Michael, 2010; Sullivan, 2010; Moulay et al., 2011). Climatic variables commonly incorporated such as temperature, humidity, and rainfall are most often collected by meteorological monitoring stations. However, the local microclimates of larval habitats can vary in ways that cannot always be extrapolated from the data available at such scales: emergent and aquatic vegetation can reduce predator access and provide shelter for larvae from wind and waves, and water qualities such as pH, temperature, salinity and nutrient concentration affect larval development and survival at fine spatial scales (Grieco et al., 2005). The area of water defining larval habitats, while largely driven by precipitation and/or tidal flow, is also highly dependent on landscape features and type of land cover (Shaman et al., 2002; Morin and Comrie, 2010). That larval habitat quality is so highly localised renders extrapolations made from meteorological data problematic, and emphasises the need for regional definitions that are species- and disease-specific (Jansen and Beebe, 2010). Regional models are also important to predict fine-scale changes in vector population size and ranges under climate change.

Defining spatiotemporal variation in regional habitats is also useful for current vector control programmes. Mosquito larvae are the most vulnerable stage of the life cycle because they cannot easily avoid control measures. Historically, the most effective campaign against pathogen-carrying mosquitoes was the eradication of the malaria vector *Anopheles gambiae* from Brazil in the 1930s and 1940s through larval control (Killeen et al., 2002a,b). Accurately quantifying the production of emerging adults m^{-2} of larval habitat and the intrinsic and extrinsic processes that define their vital rates can assist in predicting the effectiveness of a

vector suppression programme, by helping managers to understand the interplay of compensatory and depensatory mortality within local vector populations, and therefore what resources and control regimes will be necessary to achieve targets of vector population suppression and disease transmission reduction (Kearney et al., 2009; Williams et al., 2010).

Sensitivity analyses can determine 'vulnerable' mosquito life stages

Sensitivity analysis of models of mosquito population dynamics can reveal the life stages most responsible for increases in vector density and thus, identify where control should be focussed to maximise efficiency and effectiveness. Sensitivities represent the relative importance of particular stage-specific vital rates for changing population growth rate (Caswell, 2001). Organisms such as insects, that have fast life histories (early onset of reproduction, rapid ontogenetic development, and large egg clutches), commonly exhibit highly variable population densities that are most sensitive to recruitment, i.e., fertility and survival of juvenile life stages (Saether et al., 2002). Indeed, this conclusion has thus far been supported in previous sensitivity analyses of stage-structured demographic models in some mosquito populations (Pascual et al., 2006; Williams et al., 2010).

However, population growth is only one aspect of vector ecology that affects the capacity of the vector population to transmit pathogens to other hosts. Sensitivity analyses can also be applied to coupled models of vector population dynamics and epidemiology to examine the sensitivity of disease transmission rates to various mosquito life-history traits, such as adult body size and survival, further informing integrated vector management programmes (Styer et al., 2007; Ruan et al., 2008; Stolk et al., 2008). While sensitivity analysis is a highly useful tool for models examining vector control or disease transmission, considerable care should be taken in the interpretation of results. This is because vital rates are unlikely to vary independently, and population densities are influenced by environmental variation and intrinsic compensatory and depensatory feedbacks (McCarthy et al., 1995). To make robust inference from any sensitivity analyses, it is therefore again important to

understand how these processes explicitly affect different life stages.

Tropical disease-carrying mosquitoes in Darwin, Northern Territory, Australia

The tropics are where vector-borne diseases dominate (Fig. 1). The climatic conditions, high humidity, high temperatures and frequent rainfall are ideal for vector breeding conditions because there is no winter senescence of populations, and year-round transmission of disease is possible. Developing tropical countries also carry the majority of the global burden of vector-borne disease, indicating a need for the development of cost-effective control programmes. In the tropical city of Darwin, Northern Territory, Australia, mosquito population monitoring and control constitutes one of the largest recurrent land management programmes launched in the 1980s and continuing to this day (Yang et al., 2008b). The program consists of adult mosquito monitoring using CO_2-baited traps positioned around urban areas, larval surveys in the swamp complexes surrounding the city, and larval control measures, including both larval habitat engineering and regular application of larvicides (Whelan, 1989; Brogan et al., 2002; Whelan, 2007a; Yang et al., 2008a; Yang et al., 2009). Relatively uniquely, therefore, Darwin vector management presents an excellent example system to model, because unlike most tropical countries, it has extensive data and funded control.

The program focuses on the two highest abundance vector species in the region: the salt marsh mosquito *Aedes vigilax* (Skuse) and the freshwater-breeding common banded mosquito *Culex annulirostris* (Skuse). Both *Cx. annulirostris* and *Ae. vigilax* are important vectors of Ross River virus and Barmah Forest virus, arboviruses that have no vaccination, and result in substantial human morbidity and economic losses nationally (Merianos et al., 1992; Russell and Dwyer, 2000; Jacups et al., 2008a). Ross River virus is Australia's most epidemiologically important vector-borne disease, with annual notifications per capita in the Northern Territory more than four times the national average (Carver et al., 2010; Australian Government Department of Health and Aging, 2011). Both species breed prolifically in the

large swamp complexes that lie to the northeast of Darwin, and with large populations of *Ae. vigilax* during the cycles of high tides that flood the swamps in the late dry season, and high populations of the freshwater breeding *Cx. annulirostris* during the wet season once inundation from heavy rains and freshwater stream runoff transforms the tidally influenced swamps into suitable habitats (Russell and Whelan, 1986; Whelan, 1987; Whelan, 1989, Whelan, 2007a,b; Yang et al., 2008a).

This is a highly complex system to monitor and manage, although the past and current control programs are based on an understanding of the ecology of the system, the effectiveness of the current management regime at suppressing adult vector population size, and how this suppression translates to reductions in future disease incidence, have not yet been quantified. In this thesis I will examine the current management efforts to control vector population dynamics in Darwin from a population ecologist's perspective. Specifically, my aims are to:

1. Define the spatiotemporal larval habitats of the most common vectors in Darwin, *Ae. vigilax* and *Cx. annulirostris*, using a long-term (7-year) time series of larval abundances, and various spatial (vegetation, water presence, elevation) and temporal (measures of high tide, monthly rainfall, control effort, abundance of adult vectors) variables. These models will also examine the relative contribution of intrinsic (vector population density) and extrinsic (environmental, larval control) processes to change in larval population abundance trends over time. I hypothesise that because of strong compensatory density feedback mechanisms present in vector population dynamics, adult abundance will be most important temporal driver of larval density in both species, and that there will be species specific spatial and temporal environmental drivers of larval abundance (vegetation type, elevation, and tidal range) that are related to the saline breeding habitats of *Ae. vigilax*, and the freshwater breeding habitats of *Cx. annulirostris* **(Chapter 2)**.
2. Quantify the current larval control mortality (via larvicide application) for both larval

populations and emerging adults of *Ae. vigilax* across different habitat types and microclimatic conditions (pH, water temperature, salinity etc) using large-scale field experiments. These experiments will also examine the effectiveness of other, possibly less expensive, control methods (vegetation removal), and will quantify the production of *Ae. vigilax* adults per unit area in the absence of control across different larval habitat types and microclimate conditions. To make further inference from larval sampling measurements and datasets, these experiments will also assess the effectiveness of current larval sampling procedures. I hypothesise that (1) adult *Ae. vigilax* emergence rates will vary according to different larval abundance, vegetation types, and water quality, and that lower emergence is expected in areas of lower larval abundance, and in less brackish water; (2) aerial application of *Bti* effectively decreases *Ae. vigilax* larval abundance and therefore, adult emergence across larval habitats in the swamp; (3) environmental manipulation is an economically effective vector control surrogate for aerial *Bti* application; and (4) the combined effects of aerial *Bti* application and environmental manipulation will be the most effective method of reducing *Ae. vigilax* larval abundance and adult emergence **(Chapter 3).**

3. Quantify the effects of variation in larval densities on emerging adult size (wing length) in *Ae. vigilax* via field density manipulation experiments. These experiments will also examine how the effects of different larval densities on adult emergence vary across habitats, and provide information on *Ae. vigilax* life history – sex ratio of emerging adults, and rates of autogeny and average fertility of *Ae. vigilax* females via wing length relationships defined in previous studies (Hugo et al., 2003). I will also examine the effect of body size (wing length) on adult *Ae. vigilax* survival and dispersal via measuring the frequency of body sizes at different times since emergence and different distances from the emergence site. I hypothesise that adult *Ae. vigilax* female longevity increases with body size, and therefore predict that as the distance from emergence sites increases, there will be a higher proportion of larger female

adult *Ae. vigilax* mosquitoes caught, and as time progresses, smaller mosquitoes will die faster, leading to an increase in the *mean* body size of the female adult *Ae. vigilax*. Also, I predict that as larval densities increase, female *Ae. vigilax* body size at emergence will decline, and that emergent *Ae. vigilax* body size will vary with habitat type and environmental conditions regardless of larval density **(Chapter 4)**.

4. Compare the effectiveness of models of biologically plausible environmental proxies for adult abundance to models of relative adult abundance measures (from the local residential CO_2 mosquito trapping programme) for predicting human Ross River virus cases in Darwin. I hypothesise that mosquito trapping programs do not accurately measure the adult female mosquito population size or vectorial capacity; as such, I predict that environmental proxies for mosquito population dynamics constructed from quantified ecological relationships defined in the previous chapters will more accurately predict RRv incidence than measures of vector abundance from trapping programs **(Chapter 5)**.
5. Define how the insights into *Ae. vigilax* and *Cx. annulirostris* ecology I provide would be used to create a spatially explicit, stage-structured demographic model of vector population dynamics that incorporates intrinsic (density-dependent) and extrinsic (environmental) processes on different life stages and vital rates. Also, develop proposals for future research areas for modelling vector population dynamics in these and other species, and examine the possibility of coupling fully parameterised spatially explicit demographic models to epidemiological models of disease transmission **(Chapter 6, Conclusions)**.

CHAPTER 2

QUANTIFYING THE DRIVERS OF LARVAL DENSITY PATTERNS IN TWO TROPICAL MOSQUITO SPECIES TO MAXIMIZE CONTROL EFFICIENCY

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CHAPTER 3

EXPERIMENTAL COMPARISON OF AERIAL LARVICIDES AND HABITAT MODIFICATION FOR CONTROLLING DISEASE-CARRYING *AEDES VIGILAX* MOSQUITOES.

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Abstract

BACKGROUND: Microbial and insect-growth-regulator larvicides dominate current vector control programmes because they reduce larval abundance and are relatively environmentally benign. However, their short persistence makes them expensive, and environmental manipulation of larval habitat might be an alternative control measure. *Aedes vigilax* is a major vector species in northern Australia. A field experiment was implemented in Darwin, Australia, to test the hypotheses that (1) aerial microbial larvicide application effectively decreases *Ae. vigilax* larval presence, and therefore adult emergence, and (2) environmental manipulation is an effective alternative control measure. Generalised linear and mixed-effects modelling and information theoretic comparisons were used to test these hypotheses.

RESULTS: It is shown that the current aerial larvicide application campaign is effective at suppressing the emergence of *Ae. vigilax*, whereas vegetation removal is not as effective in this context. In addition, the results indicate that current larval sampling procedures are inadequate for quantifying larval abundance or adult emergence.

CONCLUSIONS: This field-based comparison has shown that the existing larviciding campaign is more effective than a simple environmental management strategy for mosquito control. It has also identified an important knowledge gap in the use of larval sampling to evaluate the effectiveness of vector control strategies.

Introduction

The control of vector populations is the first line of defence against outbreaks of vector-borne disease and their associated public health and economic impacts. Each year, millions of people die from vector-borne diseases such as malaria, dengue and yellow fever or suffer from chronic illness as a result (Gubler 1997; World Health Organization and UNICEF 2005). While there are many ways to treat and prevent these diseases, pharmaceutical-based solutions, where they exist, ultimately become intractable during large outbreaks. Recently, the focus for vector-borne disease control has turned to 'integrated pest management', which combines the suppression of larval stages of vectors with the prevention of human contact with adult vectors via indoor residual spraying and insecticide-impregnated bed nets (Killeen, et al. 2002aa; Utzinger, et al. 2002; Walker and Lynch 2007; Russell and Kay 2008).

The efficacy of chemical insecticide-based vector-control tools will be potentially compromised by the evolution of resistant vector populations (Hemingway and Ranson 2000); however, non-chemical insecticide-based methods that target vector larvae have had some success in reducing vector populations and the concomitant pathogens they transmit (Gatton, et al. 2004). Mosquito larvae are a vulnerable part of the life cycle because they cannot easily avoid control measures given that they are confined to their aquatic breeding sites until emergence as adults. As a consequence, microbial larvicides such as *Bacillus thuringiensis* var. *israelensis* (*Bti*), which dominates current broad-scale field larval control programs, can more effectively reduce target vector populations. Microbial larvicides are highly effective at suppressing vector numbers, are environmentally benign for non-target organisms, and due to the complex of insecticidal proteins present, are less likely to result in resistance than chemical insecticides (Russell, et al. 2003; Brown, et al. 2004; Walker and Lynch 2007; Fillinger, et al. 2008; Ostman, et al. 2008; Wirth, et al. 2010).

The short-activity persistence of larvicides such as *Bti* (2 to 3 days) means that while this method is useful for immediate control of high larval densities, repeated and costly applications are typically required to suppress vector densities over the long term (Walker and

Lynch 2007; Russell and Kay 2008). Source reduction, or environmental management, is another potentially cost-effective method for vector control that refers to the modification of vector habitat to discourage larval development (Turner and Streever 1999; Thullen, et al. 2002; Walker and Lynch 2007). While broad-scale, long-term, engineered changes to wetland systems can be effective at eliminating larval habitats (Turner and Streever 1999), temporary manipulation of habitat such as seasonal vegetation removal can also reduce vector populations and is not as ecologically disruptive as permanent modification (culvert removal, increased drainage, filling operations) (Whelan 1989; Turner and Streever 1999; Brogan, et al. 2002; Walker and Lynch 2007; Russell and Kay 2008; Yang, et al. 2008a).

Mosquitoes are sensitive to environmental changes brought about by vegetation removal because their survival, density and distribution are influenced by small changes in microclimatic conditions (Milby and Meyer 1986; Jeffery, et al. 2005; Yasuoka and Levins 2007). Emergent and semi-aquatic vegetation removal can create a hostile aquatic microclimate for vector larvae by allowing greater predator access, less shelter from wind and wave actions, and reduced protection from extreme temperatures and evaporation (Grieco, et al. 2005). There are many cases where vegetation removal has been successful in reducing larval abundance and oviposition (Wallace, et al. 1990; Whittle, et al. 1993; Thullen, et al. 2002; Grieco, et al. 2005; Lawler and Dritz 2005). However, vegetation removal over broader scales has also been correlated with increases in vector numbers (Yasuoka and Levins 2007), clearly showing that this form of vector control is ecosystem- and species-specific, and again highlighting the need for a detailed understanding of local vector ecology to implement effective control measures.

The effectiveness of vector control methods is realized as a reduction in adult vector numbers. However, in the case of larval vector control, linking the relative size of the adult population to the effects of control at the larval stage is challenging. In most situations, larval abundance or density estimates are used as a proxy for emerging adult numbers (Getis, et al. 2003; Gu and Novak 2005); however, larval sampling often underestimates true larval

densities, and it does not account for pre-emergence mortality (Workman and Walton 2000; Thullen, et al. 2002). Although monitoring adult numbers can track local vector population dynamics and quantify population trends resulting from control, monitoring only the adult population cannot detail the absolute effect of larval control on vector numbers due to the confounding influences of density feedback on larval survival, larval habitat availability, adult dispersion, and the alternating activities of blood-meal seeking and oviposition (Smith, et al. 2004). It is therefore important to quantify the relationship between larval abundance and adult emergence before any conclusions about the effectiveness of a control measure can be drawn.

The government of the Northern Territory of Australia currently spends approximately AU$400,000 annually on aerial applied larvicide for mosquito population control around the city of Darwin (Whelan, et al. 2009). The main aim of the control program is to suppress emergence of the salt-marsh mosquito *Aedes vigilax* in the swamp complexes adjacent to the northern residential suburbs of Darwin. *Ae. vigilax* is recognised as a vicious biter and is also a major vector of Ross River and Barmah Forest viruses in coastal and sub-coastal areas (Russell and Dwyer 2000; Jacups, et al. 2008a). Therefore, effective control of a major nuisance species that is one of the primary vectors of these pathogens is a high priority for public health management in the Northern Territory (Jacups, et al. 2008a). The major method of control currently employed is ground and aerial application of the microbial insecticide *Bti* (Whelan 1989; Whelan 2007a; Yang, et al. 2008a), although there have been previous successful attempts to remove larval habitats permanently in some areas of Darwin through environmental modification (Whelan 1989; Brogan, et al. 2002; Yang, et al. 2008a).

Ae. vigilax eggs are mainly oviposited on damp mud at the base of vegetation, and can withstand long periods of desiccation until favourable hatching conditions occur (Sinclair 1976). Tides and rainfall that flood the swamp complexes create ephemeral pools suitable for larvae, and high numbers of adults often emerge following extremely high spring tides and/or high rainfall after the habitats have been dry for a variable period (Chapter 2; Yang, et al.

2008a; Yang, et al. 2009). *Ae. vigilax* oviposition and egg density are strongly correlated with the presence of vegetation (Turner and Streever 1997; Dale, et al. 2002), and previously, large-scale engineering environmental modification methods, such as drain infilling, filling and culvert removal, that aim to increase tidal flushing in coastal swamps have been used to control this species, with some success at reduction of larval habitats (Whelan 1989; Turner and Streever 1999; Brogan, et al. 2002). The current aerial larval control measures for *Ae. vigilax* populations in Darwin only affect the immediate rate at which the generations fill available larval habitat, and do not appear to have a long-term impact on potential population size (Chapter 2; Yang, et al. 2008a).

The principal aim of the *Bti* spraying program is to reduce or dampen the emergence of adults following a breeding initiation event, such as a high tide or rainfall. Although several studies have found evidence for reductions in surveyed larvae numbers and indoor resting adult populations in conjunction with local larvicide application (Fillinger, et al. 2009), this outcome has never been quantified experimentally. To this end, I designed and implemented a field experiment to (i) evaluate current aerial larval control procedures across the swamp complex to the northeast of Darwin, and (ii) examine the relative effectiveness of alternative mosquito control measures such as environmental modification. I hypothesized that:

(1) adult *Ae. vigilax* emergence rates will vary according to different larval abundance, vegetation types, and water quality; lower emergence is expected in areas of lower larval abundance, and in less-brackish water;

(2) aerial application of *Bti* effectively decreases *Ae. vigilax* larval abundance and therefore, adult emergence across larval habitats in the swamp;

(3) environmental manipulation (vegetation removal via shears or localized burning) is an effective vector control surrogate for aerial *Bti* application; and

(4) the combined effects of aerial *Bti* application and environmental manipulation (vegetation removal via shears or localized burning) will be the most effective method of reducing *Ae. vigilax* larval abundance and adult emergence.

Methods

Study site

I chose the Leanyer/Holmes Jungle swamp complex (LHJ swamp) that lies approximately 2 km to the northeast of Darwin, Northern Territory, Australia, as the study site because this area is close to dense human settlement, contains a complex of different vegetation types, and is regularly surveyed and sprayed for mosquito larvae by Medical Entomology of the Northern Territory Department of Health (Whelan 1989). I collected emergent mosquitoes from four vegetation types in the LHJ swamp: (1) closed canopy mangrove (*Avicennia marina)* forest, (2) an area where the brackish water reed *Schoenoplectus litoralis* fringes the edge of the mangrove forest, (3) an area dominated by *S. litoralis*, and (4) an area dominated by the freshwater water chestnut, *Eleocharis dulcis* (Fig. 1).

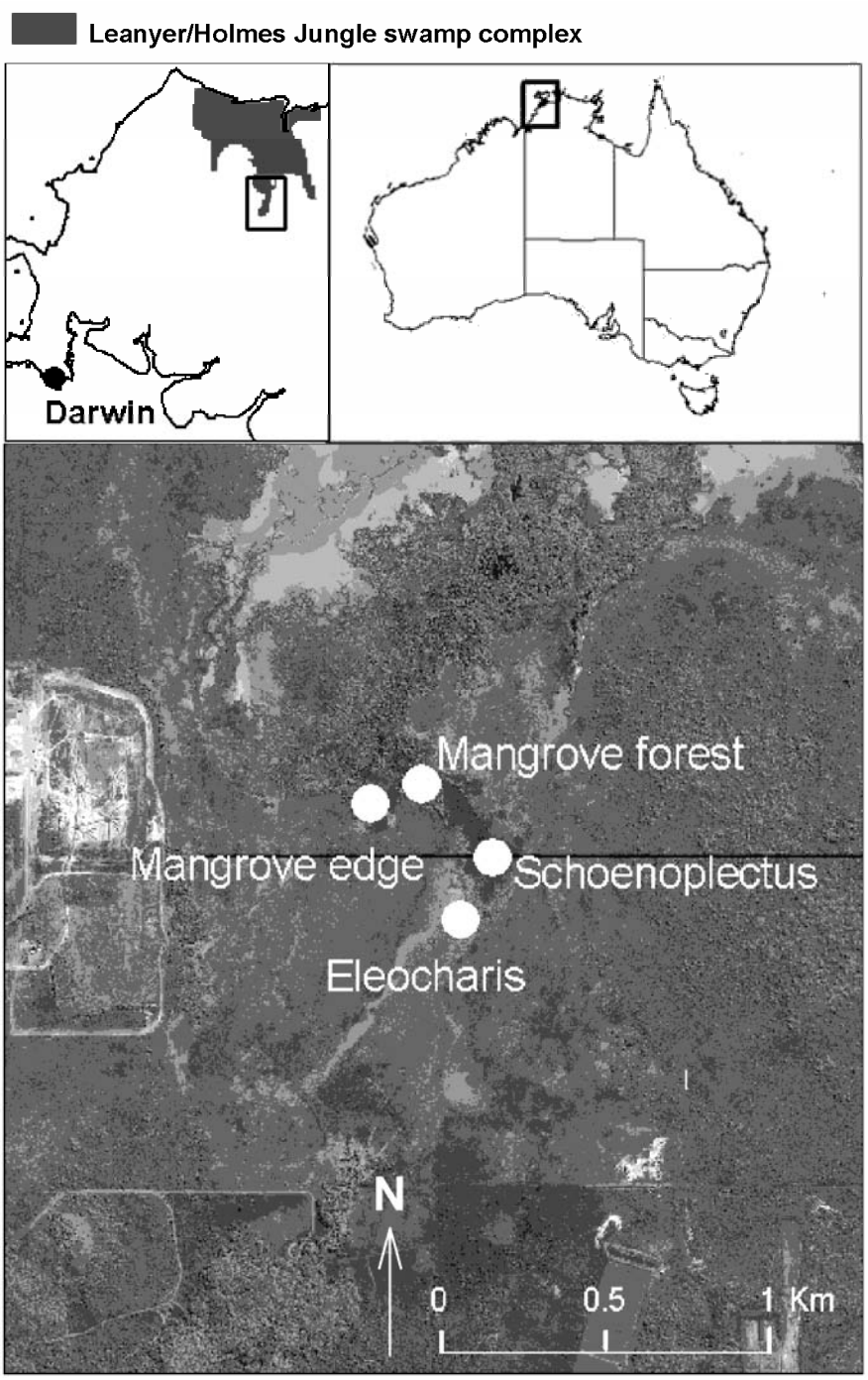

Figure 1 Study site: The Leanyer/ Holmes Jungle swamp complex near Darwin, Northern Territory, Australia, showing the four different vegetation types in which 60 emergence traps were distributed: Mangrove forest – closed canopy mangrove (*Avicennia marina)* forest, Mangrove edge – an area where *Schoenoplectus litoralis* reeds fringe the edge of the mangrove forest, Schoenoplectus – an area dominated by the brackish water reeds, *Schoenoplectus litoralis*, and Eleocharis – an area dominated by the freshwater reeds, *Eleocharis dulcis*.

Experimental design: Larval Traps

I established larval traps before the highest monthly high tide events in October 2007 and November 2008 when all the sites were dry. The larval traps consisted of 1 m^2 galvanized metal frames, 20 cm in height, which had vertical rectangular holes on two sides that were covered with fine mesh that allowed water to flood the trap, but prevented the movement of mosquito larvae into or out of the trap (Fig. 2). I dug larval traps 0.05 m into the muddy substratum and attached a pyramid-shaped mosquito net to the top of the traps to prevent oviposition of non-target species in the plots, and also to capture emerging adult *Ae. vigilax*. During the aerial spraying event, traps that were not exposed to the *Bti* 'spraying treatment' were covered with plastic sheets and had the holes on the side of the traps blocked to prevent *Bti*-contaminated water from moving inside of the non-sprayed traps (Fig. 2).

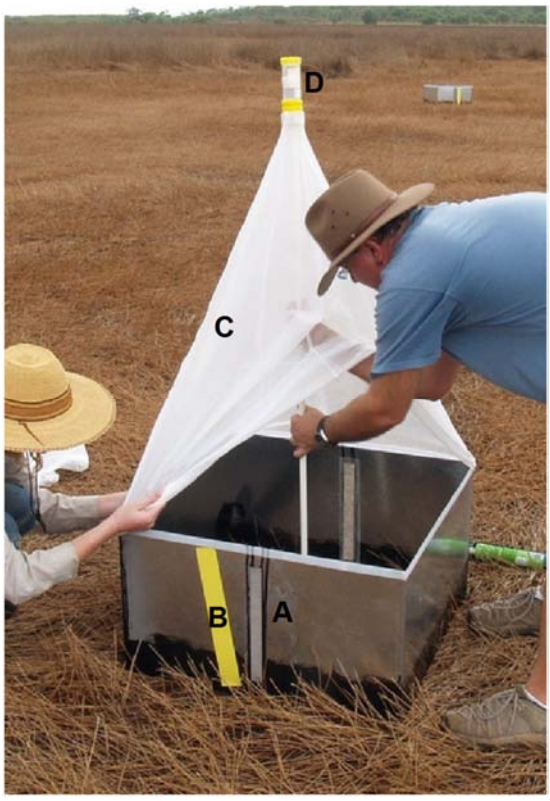

Figure 2 Emergence trap design: mesh-covered holes allow tidal flooding without larval movement (A); yellow magnets applied over holes prevent unwanted larvicide entering trap (B); mesh emergence tent prevents unwanted species' oviposition inside trap (C) and also catches emerging adults (D).

Experimental design: Quantifying mosquito emergence and larval sampling.

To measure uncontrolled mosquito emergence, I placed five traps in October 2007 in each of the four vegetation types (Mangrove forest, Mangrove edge, Schoenoplectus Eleocharis). To examine the effectiveness of the current larval sampling procedure in relation to uncontrolled mosquito emergence, I placed 10 traps in the Schoenoplectus habitat during the November 2008 high tide event.

Experimental design: Spraying, Slashing and Burning as vector control.

I only applied the different mosquito control treatments to traps within the vegetation types known to produce the highest numbers of emerging Ae. vigilax at that time of year, namely Schoenoplectus litoralis and Eleocharis dulcis (Russell and Whelan, 1986). I placed five trap replicates each within the Schoenoplectus and Eleocharis habitats in October 2007 for each of the three different treatments of vegetation removal (via shears or localized burning) and spraying:

(i) Spraying: I exposed traps to Vectobac (Bti larvicide) sprayed from a Jetranger helicopter at a concentration of 1.5 L ha^{-1} at a height of approximately 2 metres on 29 and 30 October 2007

(ii) Burning: I removed vegetation within the frames via localized burning prior to the October 2007 high tide event. I achieved this within the traps by igniting the vegetation using a hand-held blow torch. I burned vegetation to ground level where possible but did not remove charred vegetation remains from the trap.

(iii) Slashing: I removed vegetation within the larval traps using pruning shears prior to the October 2007 high tide event to determine the effects of vegetation removal only. I trimmed the vegetation as close to ground level as possible and removed it from the frames.

To test whether Bti application had an interactive or additive effect with either of the vegetation removal treatments (via shears or localized burning) within the Schoenoplectus

habitat, I also placed five traps in October 2007 for each of the treatment interactions: vegetation removal via localized burning and spraying, and vegetation removal via shears and spraying.

Larval sampling and adult emergence

The LHJ swamp was flooded by a series of high tides during October 2007 and November 2008. In 2007, the highest tide that occurred was 7.9 m on Saturday, 27 October; however, a tide capable of inundating a large area of the swamp (7.7 m in height) occurred the previous evening, and the following two days (7.9 and 7.6 m). In 2008, the highest tide (7.8 m) occurred at on Friday, 14 November, and tides capable of inundating the swamp (7.2 and 7.6 m) also occurred during the previous few days. I monitored the traps each day leading up to these high tide events, and once the traps were flooded, larval sampling commenced. Each day following initial flooding, I sampled *Ae. vigilax* larval abundance in the traps using a prescribed dipping procedure: I did five dips (one in each corner of the trap and one in the centre) using a standard dipper (190 ml volume). I counted live larvae and then returned them to the traps and calculated the final estimate of larval abundance by summing the counts of larvae across the five dips $trap^{-1}$ day^{-1}. To quantify the ability of the above sampling procedure to estimate true larval density reliably, I removed and counted all larvae within each of 5 traps according to the methods outlined in Service (1993) once the larvae in the traps reached the 3^{rd} instar in November 2008. I removed the water by bailing with buckets and sieved through fine mesh. I carefully removed larvae to smaller storage containers for counting in the lab. Bailing continued until I sieved two or more buckets that contained no larvae, and observed no larvae rising to breathe at the surface of the remaining water in the traps.

After observing emerging adults in emergence traps, I ceased larval sampling in October 2007. I manually caught emerging adults by sucking them into sampling containers using a small aspirator. This was repeated daily until emergence within the traps had ceased.

During November 2008, the five control traps to test for numbers of emerging adults failed due to an unexpected spraying event and unfortunately, I collected no adults that year.

Larval sampling procedure

To assess whether the larval sampling procedure used accurately measures true larval density, I compared two Gaussian distributed (identity link function) linear mixed effects models. The response variable was final trap density (Table 1), and the explanatory variable was average larvae per dip. Trap ID was included as a random effect.

Water chemical and physical properties

I measured water pH, conductivity, and dissolved oxygen in each trap daily using a Horiba U10 water meter (HORIBA Ltd., Kyoto, Japan). I measured temperature 5 cm above the water surface using Dallas DS1923 Hygrochron iButtons (Maxim Integrated Products, Sunnyvale, CA, USA). Temperature readings were taken every 5 minutes, and from these I calculated the mean for the daylight hours of each day.

Larval presence and adult emergence across habitat types

To examine whether local environmental conditions affected larval presence or adult emergence, I developed statistical model sets where we included pH, conductivity (mS cm^{-1}), dissolved oxygen (mg l^{-1}) and mean daily temperature (^{o}C) as variables in binomial error-distribution (logit link function) generalised linear mixed-effects models (GLMM) for larval presence, and Poisson (logit link function) GLMM for adult emergence. Random effects included in the larval presence GLMM were trap and vegetation type (trap was nested within vegetation) and vegetation was also included as a random effect in the adult emergence models. From these model comparisons, I identified the water qualities that were the most important drivers of larval presence and adult emergence, and then combined these via a principal components analysis (PCA). I included the first principal component, representing

the key environmental conditions affecting larval presence or adult emergence, in further analyses.

To examine the differences in larval presence and adult emergence across different habitat types, I developed binomial (logit link function) GLMM of larval presence and Poisson (log link function) generalised linear models (GLM) of adult emergence. Explanatory variables I included in the models were vegetation type (mangrove forest, mangrove edge, *Schoenoplectus* and *Eleocharis*), local environmental conditions (first principal component from the PCA of pH, conductivity, dissolved oxygen and mean daily temperature), and trap water depth (mm) to control for the confounding effects of variable water depths between traps. I also included median larval presence in all the adult emergence models to control for the confounding effects of varied larval numbers among traps.

Spraying and vegetation removal (via shears or localised burning) as control methods

For the two habitat types that had traps exposed to spraying and vegetation removal treatments (*Schoenoplectus* and *Eleocharis*), I used a before-after/control-impact (BACI) experimental design to examine the effectiveness of these different methods at reducing larval presence (Stewart-Oaten, et al. 1986). I sampled larval in (*i*) control traps, (*ii*) traps that had vegetation removed (either via shears or localized burning), and (*iii*) traps exposed to *Bti* for 2 days before and 4 days after the 'impact' (the spraying event). Larval presence was the response variable for sets of binomial (logit link function) GLMM, and the variables I included in the models were vegetation treatment (removal via localized burning, removal via shears, or control), spray treatment (*Bti* application or shielded), time period (before or after spray event), local environmental conditions (first principal component representing environmental conditions) and water depth (mm). Random effects included in the GLMM were trap and vegetation type (*Schoenoplectus* or *Eleocharis*); I nested trap within vegetation. Statistical evidence for an interaction between time period (before or after) and spray treatment (*Bti* application or shielded) indicates an effect of spraying on larval presence.

I also constructed model sets to examine the effects of spraying, vegetation removal treatment and environmental conditions on adult emergence. Total adult emergence per trap was the response variable of sets of Poisson (log link function) GLMM, and explanatory variables were: vegetation treatment (removal via localized burning, removal via shears, or control), spray treatment (*Bti* application or shielded), median water depth (mm) and median larval abundance. I included vegetation type (*Schoenoplectus* or *Eleocharis*) as a random effect.

Combined effects of control methods

To examine whether the combined effects of vegetation removal via localized burning and spraying or vegetation removal via shears and spraying, are the most effective methods of reducing larval presence and adult emergence, I analysed data from the *Schoenoplectus* traps, as this was the only habitat to receive the combined treatments.

Again I used a BACI design, where I sampled larval in control traps, traps that had vegetation removed (either via shears or localized burning), and traps exposed to *Bti* for 2 days before and 4 days after the spraying event. Larval presence was the response variable for sets of binomial (logit link function) GLMMs, and the variables included in the models were vegetation treatment (removal via localized burning, removal via shears, or control), spray treatment (*Bti* application or shielded), time period (before spray event or after spray event), local environmental conditions (first principal component from the PCA of pH, conductivity, dissolved oxygen and mean daily temperature) and trap water depth (mm). I coded trap as a random effect.

To examine the effects of combined vector control methods on adult emergence, I constructed sets of Poisson (log link function) GLM, with total adult emergence per trap as the response variable and explanatory variables were vegetation treatment vegetation treatment (removal via localized burning, removal via shears, or control), spray treatment (*Bti* application or shielded), median water depth (mm) and median larval presence.

Model comparison

To rank and weight models, I used Akaike's information criterion, corrected for small samples (AIC_c), as an estimate of Kullback Leibler (K-L) information loss (Burnham and Anderson 2002) (i.e., statistical likelihoods that have been bias-corrected to account for number of parameters fitted, somewhat akin to a measure of model parsimony). I calculated the difference between the model's AIC_c and the top-ranked model's (ΔAIC_c), and the relative model weights ($wAIC_c$) (Burnham and Anderson 2002). Thus, the strength of evidence ($wAIC_c$) for any particular model varies from 0 (no support) to 1 (complete support) relative to the entire model set, and evidence is not assessed based on some arbitrary probability of making a Type I error (i.e., rejecting a null hypothesis when in fact, it is true). I assessed the amount of variance in the response variable captured by each model (i.e., structural goodness-of-fit) as the percent deviance explained (% DE) relative to the null deviance.

I used an individual variable-ranking method to determine the relative importance of different predictor variables in separately and jointly explaining deviance in adult emergence (Wanger, et al. 2009). Firstly, I dropped each predictor variable individually from the saturated model (model containing all possible predictor variables), and assessed the change in %DE. Secondly, I added each predictor variable individually to the null model, and again measured %DE. I calculated the changes in %DE relative to the saturated and null model and then summed them as total variable deviance. I rescaled total variable deviance to sum up to one (relative deviance) and ranked variables according to the relative deviance explained. I did all analyses using the R Package V2.9.0 (R Development Core Team 2011).

Results

Larval sampling, larval presence and adult emergence across habitat types

There was no statistical evidence for a relationship between average number of larvae per dip and final larval density, indicating that this form of larval sampling is not an accurate measure

of mosquito production from a given habitat; rather, it simply provides an approximate measure of larval presence or absence (Table 1).

Further, taken across all habitats, my analyses did not reveal statistically meaningful differences in the effects of local environmental conditions for either larval presence or adult emergence; even though there was some variation across habitat types. The ranges of the environmental conditions the developing larvae experienced were: pH 6.69 - 4.39, dissolved oxygen 5.91 – 5.35 mg l^{-1}, salinity 71.48 – 67.4 mS cm^{-1}, and mean daily temperature 35.49 – 32.34 ^{o}C. The most parsimonious model explaining the variation in *Ae. vigilax* adult emergence included median larval presence and trap water volume, although there was also support for models including local environmental conditions and habitat type (*Schoenoplectus*, *Eleocharis*, mangrove forest, mangrove edge) (see Appendix 3a). The individual variable ranking revealed that median larval presence and habitat type (*Schoenoplectus*, *Eleocharis*, mangrove forest, mangrove edge) explained 81.9 and 14.3% relative deviance in adult emergence, respectively (Table 2a).

Both water depth and habitat type were the best predictors of differences in larval presence (see Appendix 3a). Larval presence was highest in *Schoenoplectus,* then mangrove forest and mangrove edge, and lowest in *Eleocharis* (Fig 3).

(a) Average larval density

Trap ID	15/11/08	16/11/08	17/11/08	18/11/08	Final density
12	0	1.8	0.8	1.2	73
14	0.2	9.8	1.6	2.2	71
16	0	1.8	1.6	1	45
18	0	2.4	0	0.6	111
20	0	3.4	1	-	91

(b) Model comparison

Model	AIC_c	ΔAIC_c	$wAIC_c$	%DE
density ~ 1	-165.87	0.00	<0.999	0
density ~ dip	-130.44	35.44	>0.001	8.2

Table 1 (a) Numbers of larvae averaged over 5 sampling dips taken each day for 3 days, and (b) Comparison of two models used to assess the ability of larval sampling dips to predict true larval density per trap, using information-theoretic model selection. Final densities were estimated by removing all the water in the traps and counting total larval numbers. This occurred on 18/11/08 for traps 12-18 and on 17/11/08 for trap 20. Explanatory variable is average larvae per dip, and statistics shown Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight ($wAIC_c$) scaled relative to a total sum of 1, and percent deviance explained by the model (%DE).

Predictor variables	%DE deletion	%DE addition	Rel. deviance
(a) Habitat Type Models			
median larval presence	21.6	26.8	81.9
habitat type	0.9	7.6	14.3
trap water volume	0.1	1.0	1.8
local environmental conditions	0.1	1.2	2.1
(b) Spraying or Vegetation Removal Models			
exposure to aerial *Bti* application	22.6	27.2	46.9
median larval presence	14.4	21.6	33.9
vegetation removal (via shears or burning)	6.5	1.4	7.4
local environmental conditions	4.8	3.0	7.3
trap water volume	4.3	0.5	4.6
(c) Combined Control Methods Models			
exposure to aerial *Bti* application	25.0	52.6	60.0
median larval presence	4.7	34.4	30.2
trap water volume	7.0	0.3	5.6
vegetation removal (via shears or burning)	2.2	1.8	3.0
local environmental conditions	0.4	0.6	0.8
combined *Bti* and vegetation removal	0.2	0.2	0.3

Table 2 Individual explanatory strength of predictor variables for different model sets of *Aedes vigilax* adult emergence. Habitat type = mangrove forest, mangrove edge, *Schoenoplectus, Eleocharis*

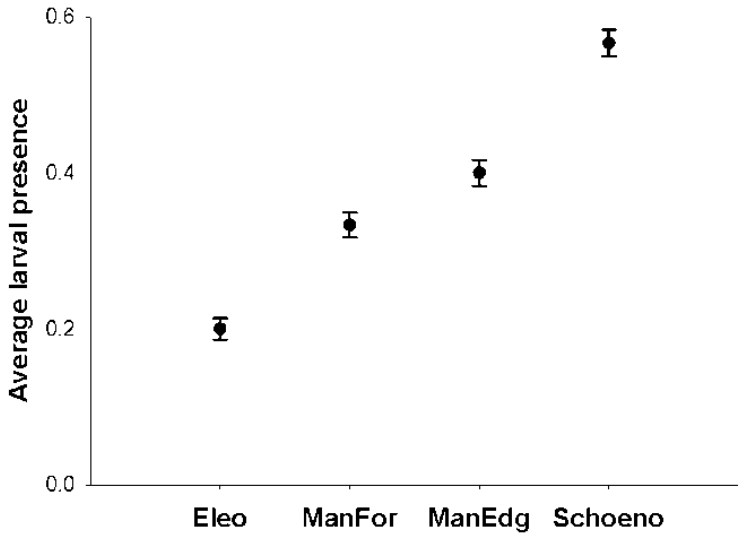

Figure 3 Average *Aedes vigilax* larval presence sampled across different habitat types (where 0 = no larvae and 1 = larvae present) with standard error bars. Habitat types are: Eleo – an area dominated by the freshwater reeds, *Eleocharis dulcis*; ManFor – closed canopy mangrove (*Avicennia marina)* forest; ManEdg – an area where *Schoenoplectus litoralis* reeds fringe the edge of the mangrove forest; and Schoeno – an area dominated by the brackish water reeds, *Schoenoplectus litoralis*.

Spraying and vegetation removal (via shears or localised burning) as control methods

In the traps that were exposed to aerially applied *Bti*, 97% fewer adults emerged than in unexposed traps (Fig 4a). I also found statistical evidence for an effect of vegetation removal (via shears or localized burning) on adult emergence numbers (see Appendix 3b). For this model set, the individual variable ranking revealed that exposure to aerial *Bti* (spray), median larval presence and vegetation removal explained 46.9, 33.9 and 7.4% relative deviance in adult emergence, respectively (Table 2b). My models predict that, with all other variables held equal, vegetation removal via localized burning will reduce adult emergence by 41% (95% confidence interval: 22 – 55%) and vegetation removal via shears will reduce adult emergence by 57% (95% confidence interval: 44 - 66%).

The larvae models showed no evidence for any effect of vegetation removal (via shears or localized burning) on larval presence, and hardly any evidence for the interaction term between time and impact (spraying) (see Appendix 3b).

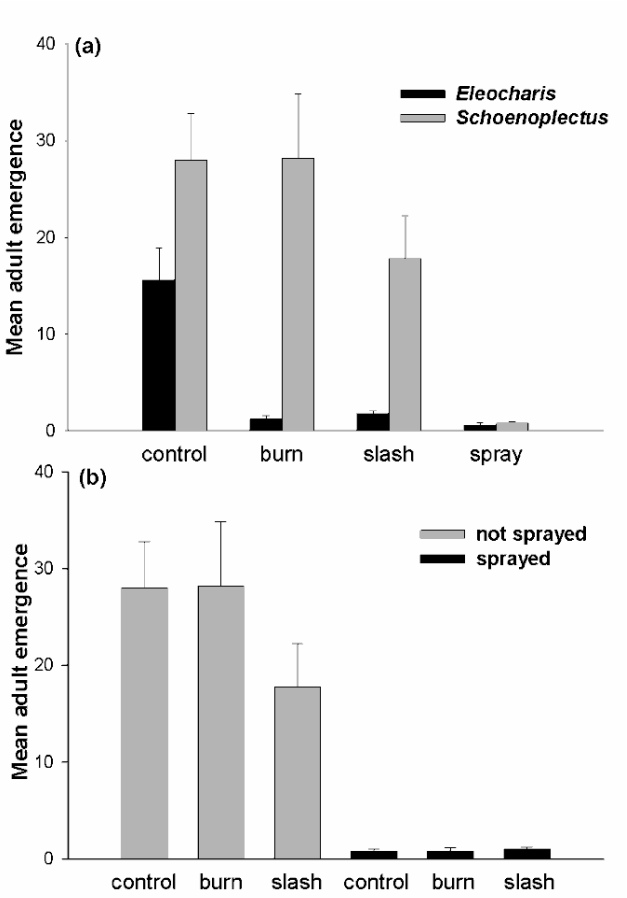

Figure 4 (a) Mean number of adult *Aedes vigilax* that emerged from traps in *Schoenoplectus litoralis* and *Eleocharis dulcis* habitats with standard error bars. Treatments applied to traps include: control – no manipulation, burn – vegetation burnt, slash – vegetation mowed, and spray – trap exposed to aerial larvicide application. (b) Mean number of adult *Aedes vigilax* that emerged from traps in *Schoenoplectus litoralis* habitat with standard error bars. Treatments applied to traps include: control, burn, slash. Black columns indicate traps that were exposed to aerial larvicide application, and grey columns indicate traps that were shielded from aerial larvicide application.

Combined effects of control methods

In the model set examining the effects of combined vector control methods on adult emergence, although there was strong evidence for the separate effects of aerial *Bti* application and vegetation removal (via shears or localized burning), there was no statistical evidence that combining these different control measures will further reduce adult emergence numbers (see Appendix 3c). Individual variable ranking revealed that exposure to aerial *Bti* (spray) explained 60.0%, median larval presence explained 33.9%, trap water volume explained 5.6%, and vegetation removal explained 3.0% of the relative deviance in adult emergence (Table 2c). Adult emergence was lowest in the traps that had the treatments of vegetation removal via localized burning and *Bti* exposure, vegetation removal via shears and *Bti* exposure, and just *Bti* exposure, than the traps that had vegetation removal via shears but no *Bti* exposure. Emergence was highest in the traps that had vegetation removal via localized burning and the control traps (Fig 4b).

In the model sets examining the effects of combined vector control methods on larval presence, there was no evidence that combining vegetation removal via shears and *Bti* exposure or vegetation removal via localized burning and *Bti* exposure was more effective at reducing larval presence than just *Bti* exposure alone (see Appendix 3c).

Discussion

I found clear evidence that larval presence and numbers of emerging adult Ae. vigilax were reduced – by an average of 95% – after the aerial application of Bti larvicide, across a range of different vegetation types and water qualities. Applications of Bti-based products can be highly effective at reducing larval densities over a range of mosquito vectors including Ae. vigilax (Fillinger, et al. 2008; Ostman, et al. 2008; Russell and Kay 2008; Geissbuhler, et al. 2009). I have also shown that other control methods based on vegetation removal, via either shears or localized burning, can reduce adult emergence in Schoenoplectus and Eleocharis habitats by around 50%. Uniquely, I compared several different forms of control for Ae.

vigilax, separately and in combination – aerial larvicide application and environmental manipulation – and in so doing I provide direct confirmation that larvicide application is the most effective control method (of those considered at these experimental scales) for reducing adult emergence of this species from a range of larval habitats. I have also shown that the experimental larval sampling methods do not accurately measure larval abundance or adult emergence numbers. This is important for vector control, as larval surveys are commonly used to determine areas of high or low density for control procedures (Getis, et al. 2003; Gu and Novak 2005).

Larvicide control of vectors

Monthly tidal inundation of breeding habitats during the dry season stimulates desiccation-resistant *Ae. vigilax* eggs to hatch, resulting in the new generation hatching, growing and emerging at the same time. Therefore, *Bti* application during the larval stage will ensure suppression of the entire generation, making the timely application of this method relatively cost effective for this species. By contrast, species which breed in semi-permanent or standing water, and slowly build population density over longer periods rather than exhibiting monthly peaks in emergence, will require repeated applications of *Bti* over at least fortnightly intervals to control each generation, and will therefore be more expensive. *Culex annulirostris*, the mosquito species that dominates the LHJ swamp once it is inundated with fresh water during the wet season, exhibits this pattern of breeding (Chapter 2; Russell 1986; Yang, et al. 2008a). This might be why studies of *Cx. annulirostris* population dynamics have revealed little to no long-term effects of opportunistic *Bti* larvicide application on this species (Chapter 2; Yang, et al. 2008a).

Environmental manipulation for vector control

Vegetation removal can lead to a decrease in larval numbers, presumably by increasing exposure to higher temperatures and removing protection from predators, wind and wave

action. Removal can also facilitate control in areas where larvicide application or diffusion is impeded by thick, emergent vegetation (Janousek and Olson 1994; Thullen, et al. 2002; Grieco, et al. 2005; Lawler and Dritz 2005; Leisnham, et al. 2005). I found that the removal of *Eleocharis* or *Schoenoplectus* reeds (either via shears or localized burning) reduced emergence of *Ae. vigilax*, but was not as effective as aerial *Bti* application. One possible reason relates to the traps that were designed to exclude predators and thereby reduce the confounding effects of predation on larvicide-induced mortality. The traps might have provided some shade, wind and wave protection, thus negating the effects of vegetation removal. Another factor influencing my results might be the timing of vegetation removal; I removed the vegetation in my experiments by just prior to inundation and hence, after egg deposition had occurred following a previous inundation. It is possible that oviposition, larval hatching and adult emergence would have been appreciably less in the burned or sheared plots if the vegetation removal had occurred prior to egg laying. This would have provided a less attractive egg-laying environment with more direct sunlight, and by allowing the ground surface to dry much sooner, it might have more quickly become unsuitable for continued egg laying (Sinclair 1976). Further study involving traps that allow predator access to the larvae, or broad-scale (i.e., over 100s of metres) treatment plots without treatment frames, would allow me to explore more fully the benefits of vegetation removal as a larval control measure for this species. Broad-scale plots with unburnt areas and areas burnt prior to any egg deposition episodes would also allow aspects such as the time of burning, open water areas and wave action, and predator effects to be examined in more detail. Larger-scale vegetation manipulation would likely restrict the distribution of larvae to marginal areas, such that the total area of breeding would be smaller and hence, easier to survey and control.

Ultimately, the efficacy and long-term cost effectiveness of any control measure depends on how well the intervention is matched to the ecology of the species targeted. Environmental manipulation might be an effective vector control method in some cases, but this strategy can also negatively affect non-target species (Grieco, et al. 2005). Also, incomplete control

procedures that reduce larval densities rather than exterminating all larvae allow the survivors greater access to resources, with the corollary that they will emerge as larger adult mosquitoes potentially capable of surviving longer, dispersing farther and infecting more people (Jirakanjanakit, et al. 2007; Juliano 2007; Gavotte, et al. 2009).

Larval sampling, larval presence and adult emergence across habitat types

The end goal of any vector control programme is the reduction, or if possible, elimination of the adult vector population and therefore, by logical extension, the reduction in incidence of vector-transmitted pathogens and disease. An essential step that is often missed in examining the effectiveness of larval control methods, however, is quantifying the direct effects of the larval control on adult emergence (Fillinger, et al. 2009). However, adult emergence trap results are not necessarily indicative of adult numbers caught in CO_2 traps because emergence cages remove the problems associated with partial or incomplete spraying in landscape control operations, and adult dispersal from outside control areas.

My experiment aimed at quantifying larval abundance by counting all larvae captured in single traps, and showed that the experimental larval sampling (dipping) procedures do not adequately quantify larval abundance or adult emergence. At best, the sampling method used only measures larval presence or absence, and there was little evidence for a correlation between larval abundances and adult emergence numbers. This is an important result because it suggests that the current practice of using larval surveys to determine where and when larval control should be applied might not quantify larval abundance or density (only presence or absence). The implication for mosquito control is that managers may instead opt to apply larvicide in all likely habitats and forego larval sampling altogether. Otherwise, habitat-specific models predicting larval vector population abundances would need to be developed for a wider suite of species and habitats.

It is important to have a thorough understanding of the ecological features of vector breeding sites to implement effective and efficient control within resources available, and certain larval

habitat environmental conditions have been previously identified as important in determining larval habitat suitability. These include water salinity, pH, and dissolved oxygen, ambient temperature, and vegetation presence, habitat and type (Milby and Meyer 1986; Hearnden and Kay 1997; Depinay, et al. 2004; Jeffery, et al. 2005; Barrera, et al. 2006; Yasuoka and Levins 2007). For *Ae. vigilax*, I found that although there was some influence of different vegetation types and water qualities, no single environmental attribute was identified as the principal correlate of larval development or adult emergence. *Ae. vigilax* are able to breed effectively in a relatively wide range of temperatures and salinities (Sinclair 1976; Lee, et al. 1984), and the maxima and minima of the microclimate environmental parameters I measured fell well within these ranges. During the spring tides, the LHJ swamp acts as an ideal *Ae. vigilax* larvae incubating environment; none of the water qualities affect larval survival, so generation times will be as rapid as developmental instar progression allows.

It is imperative that effective control is implemented in environments such as these, where human occupation is so close to large populations of insect vectors. My results showed that although there were some combined effects of local environmental conditions and vegetation removal (via shears or localized burning), aerial larvicide application remained the most effective control method of reducing adult vector emergence across all habitats. Previous studies have not identified an effect of different larval habitat environmental conditions on the effectiveness of larvicide at suppressing larval densities (Russell, et al. 2003; Brown, et al. 2004). I did find that vegetation type added some statistically useful explanatory capacity to models of larval presence, however: *Ae. vigilax* larval presence was highest in mixed mangrove forest edge and *Schoenoplectus* reed and pure *Schoenoplectus* reed habitats, and lowest in the *Eleocharis* reed habitat. This is further evidence that the tidally flooded *Schoenoplectus litoralis* reed beds of the coastal swamps, which are highly productive habitats for *Ae. vigilax*, are indeed an important target for control (Russell and Whelan 1986).

Conclusions

The current control method of aerial larvicide application is effective at suppressing adult emergence of *Ae. vigilax* across a range of habitat types, and vegetation removal can also be an effective control alternative for this species in some habitats, but this conclusion should be investigated with larger-scale experiments. The trade-off in terms of cost, negative environmental consequences of environmental manipulation versus the risk of eventual resistance (Paris, et al. 2011) and other longer-term negative effects of larvicide application remain unclear. However, this area warrants more investment, as comparative approaches such as this study are rarely done. I have also identified an important knowledge gap in evaluating the effectiveness of larval vector control strategies.

Quantification of the relationship between larval sampling measures, larval abundance and/or density, and adult vector emergence is essential to determine whether control methods are indeed reducing the productivity of larval habitats. Larval densities measured during sampling surveys are routinely used to direct control efforts; therefore, extreme care needs to be taken when interpreting the findings of such surveys. Using larval sampling surveys as an indication of larval presence or absence only, or better still, supplementing larval sampling surveys with models of the climatic, environmental and intrinsic factors that drive vector population dynamics to determine optimum control strategies can assist, with the corollary that good predictive models based on the ecology of local vector populations might eventually supplant expensive and time-consuming larval surveys.

CHAPTER 4

THE RISE OF THE SUPER MOSQUITO: HOW DENSITY SUPPRESION INCREASES MOSQUITO BODY SIZE AND INFECTION POTENTIAL

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Text in manuscript

NOTE:
Statements of authorship appear in the print copy of the thesis held in the University of Adelaide Library.

Abstract

Reducing the risk of human vector-borne disease is the primary role of mosquito control. Mosquito body size, an important aspect of vector fitness, is affected by larval density and environmental conditions. Through routine monitoring, I measured body size of female adult *Aedes vigilax* at various distances from their emergence sites, and at two different times since emergence. I also measured body size in female adult *Ae. vigilax* emerging from field experiments to examine the effects of environmental variation and manipulated larval densities on adult body size. I found some evidence for longer survival since emergence in larger adult *Ae. vigilax* females, and a statistically supported negative relationship between larval density and emerging adult female size, the strength of which depended on environmental conditions. Low larval densities result in larger adults with higher survival rates, increasing the probability that vectors will survive the extrinsic disease incubation period and successfully transmit disease. Therefore, mosquito population demography, in particular estimates of larval density, should be incorporated into vector-borne disease models used to inform control efforts. Two applied implication of the work arise: (1) mosquito-control should not ignore low-density larval sites because of their potential to produce more effective vectors, and (2) to benefit fully from the effects of high-density larval competition for resources on adult vector body size larval control is best applied towards the latter stages of larval development.

Introduction

Mosquito-borne pathogens constitute a major burden of disease for humans globally: mosquito-borne disease is responsible for 11% of all infectious and parasitic disease Disability-Adjusted Life Years (DALYs) (Russell and Dwyer, 2000, World Health Organization, 2004). In many parts of the world, climate-change models based on 21st century emissions scenarios predict increasing vector population size and ranges, and an associated increase in many vector-borne diseases (Hay et al., 2002; Chase and Knight, 2003; Campbell-Lendrum and Woodruff, 2007); therefore, improving the effectiveness of disease prevention and vector population control is a paramount concern for improving human health. Given the relative lack of pharmaceutical-based solutions, mosquito control programmes are recognised globally as the most economically viable tools to reduce pathogen prevalence and disease expression (Walker and Lynch, 2007). To develop effective vector population control measures, many studies have focused on developing models to describe and quantify habitat associations and general geographic distribution of disease and disease vectors (Grieco et al., 2006; Lindsay et al., 2007; Yang et al., 2008a; Yang et al., 2009). However, due to time, data or financial constraints, the more intricate details of vector ecology – such as the relative importance of intrinsic and extrinsic vector population dynamics and the interactions between the pathogen, vector and host – are rarely considered in these models (Reiskind and Wilson, 2004; Tong et al., 2008; Bellan, 2010; but see Yang et al 2009).

'Vectorial capacity' is the ability of a vector to acquire a pathogen though an infected blood meal, develop a disseminated infection and infect a host during subsequent contact (Garrett-Jones, 1964). Vectorial capacity is affected by variation in life-history traits, demography, physiological status and environmental conditions, such as vector survival rate, host-seeking behaviour, biting persistence, susceptibility of the vector to infection, blood-meal sizes, a vector's energetic reserves, dispersal potential, temperature, and humidity (Jennings and Kay, 1999; Gimnig et al., 2002; Luz et al., 2003; Manoukis et al., 2006; Maciel de Freitas et al., 2007; Schneider et al., 2007; Bevins, 2008; Vaidyanathan et al., 2008). Some

traits are also linked to vector body size: susceptibility to infection and dissemination are higher in smaller females of some vector species (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et al., 2011), and higher in larger females of other species (Nasci et al., 1994; Sumanochitrapon et al., 1998; Westbrook et al., 2010), and survival and dispersal are higher in larger females of many vector species (Agnew et al., 2002; Gimnig et al., 2002; Manoukis et al., 2006; Bevins, 2008; Gavotte et al., 2009; Reiskind and Lounibos, 2009). These particular population-demographic traits are important aspects of vectorial capacity, especially for pathogens with long extrinsic incubation periods such as malaria (Nasci, 1991; Sumanochitrapon et al., 1998; Gimnig et al., 2002; Bevins, 2008).

Density-dependent reduction in larval growth, and consequent smaller adult emergence sizes, have been observed for a wide range of vector species (Agnew et al., 2002; Gimnig et al., 2002; Hugo et al., 2003; Barrera et al., 2006; Reiskind and Lounibos, 2009). Competition for nutrients at the larval stage has been proposed as the main mechanism by which increased mosquito density inhibits growth and survival (Agnew et al., 2002; Gimnig et al., 2002; Barrera et al., 2006; Gavotte et al., 2009). To maximise cost effectiveness, mosquito-control strategies often aim to target only prolific, or high-density larval habitats (Gu et al., 2008). Low-density larval habitats are more difficult to detect and target; therefore, these habitats are either avoided or missed during control. The question then arises: will the emergent adults in low-density populations be larger and have higher vectorial capacities than their high-density counterparts, and what is the effect of larval density control in high density habitats? Possible mechanisms for this increased fitness in low density habitats or following reduction of high densities include longer survival times due to higher availability of resources to the larvae, per individual, and longer-distant dispersal following emergence (thereby exposing more people) due to increased vigour of the adults. Effective and sustainable vector control therefore requires knowledge of whether the control mortality is additive to natural mortality or if it is compensated by increased vector survival or reproduction (Chapter 2; Juliano, 2007; Yang et

al., 2008a,b; Yang et al., 2009), and also a detailed understanding of specific vector/disease relationships to determine if infection and dissemiation is higher in smaller or larger females (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Nasci et al., 1994; Sumanochitrapon et al., 1998; Jennings and Kay, 1999; Alto et al., 2005; Alto et al., 2008a,b; Westbrook et al., 2010; Muturi et al., 2011).

The salt marsh mosquito *Aedes vigilax* is a major vector of Ross River virus in Australia (Russell and Dwyer, 2000; Jacups et al., 2008a), and thousands of dollars are spent each year on *Ae. vigilax* larval control (Chapter 3; Yang et al., 2008a). *Ae. vigilax* oviposit on mudflats and along pool edges that are regularly inundated by tides and rainwater (Sinclair, 1976). Due to the ephemeral nature of these shallow pools, high densities of larvae can develop, creating both density-dependent and nutritional stresses (Jennings and Kay, 1999). Indeed, various fitness-related life-history traits of *Ae. vigilax* are affected by larval density: wing size, autogeny (capability of developing the first batch of eggs without a bloodmeal) and fertility (Hugo et al., 2003). *Ae. vigilax* adults have a high dispersal capacity (up to 9 km) (Chapman et al., 1999) which is aided by increased wing size, and transmission of Ross River virus occurs 4-5 days after vector infection, with maximum transmission reached at 10-13 days post emergence (Vale et al., 1992). Therefore, identifying the relationship between ecological processes, such as larval competition and adult survival and dispersal, would assist in making explicit the connection between intrinsic and extrinsic pressures on individuals and related epidemiological models of population size and disease-transmission potential.

Many studies examining the effect of density on life-history and vector-capacity traits are laboratory based, and while relevant for species that breed in natural or artificial containers (e.g. tree holes, pitcher plants, discarded tyres), patterns arising from laboratory manipulation can break down in field situations because the outcomes of density-forced competitive interactions depend mainly on resource quantity and availability during development (Bevins, 2007; Bevins, 2008). Field studies that examine variability in adult body size often only report environment-induced, rather than density-induced, variation in

body size (Baqar et al., 1980; Grimstad and Haramis, 1984; Klowden et al., 1988; Grimstad and Walker, 1991; Nasci and Mitchell, 1994; Sumanochitrapon et al., 1998), so we are often unable to decouple the effects of environmental conditions and larval density on body size and other linked traits (Schneider et al., 2007).

Here I explore the links between larval density, environmental conditions, vector body size and related vector-capacity traits, by examining female body size of both emerging and feeding adult populations of *Ae. vigilax*. I use demographic data collected from ongoing monitoring of the adult population at different times since emergence and different distances from the larval emergence sites. I also implemented two field experiments: one examining the effects of environmental variation on adult body size, and the other a manipulation experiment examining the effects of larval density on adult body size. I hypothesized that (1) adult *Ae. vigilax* female longevity, and consequent dispersal capacity, increases with body size, so larger adult female *Ae. vigilax* mosquitoes will be caught farther from emergence sites; over time, smaller mosquitoes will die faster giving rise to an increase in the *mean* body size of adult females; (2) higher densities of larvae will result in smaller female *Ae. vigilax* body size at emergence; and (3) emergent *Ae. vigilax* body size will vary with habitat type and environmental conditions regardless of larval density.

Methods

Study site

The Leanyer/Holmes Jungle swamp complex (LHJ swamp), which lies to the northeast of Darwin, Northern Territory, Australia, was chosen as the study site because it is the largest source of pest mosquitoes for the major urban centre of Darwin. It also contains a complex of different vegetation types, and is regularly surveyed and sprayed for mosquito larvae by Medical Entomology of the Northern Territory Department of Health and Families (Brogan et al., 2002). Emergence traps were established before the highest monthly high tide events in October 2007 and November 2008, when the site was dry (i.e., prior to the commencement of

the wet season). For details on emergence trap design see Chapter 3. The traps were covered with plastic sheets during monthly aerial spraying operations, and the holes on the side of the traps covered to prevent the spray-contaminated water from moving inside.

Experimental design and treatments

In October 2007, emergence traps were established within the swamp in four different vegetation types: (1) closed canopy mangrove (*Avicennia marina*) forest, (2) an area where *Schoenoplectus litoralis* reeds fringe the edge of the mangrove forest, (3) an area dominated by the brackish water reeds *Schoenoplectus litoralis* and (4) an area dominated by the reed *Eleocharis dulcis*. Five traps were placed in each of the four vegetation-habitat types.

To establish the background emergence size of *Ae. vigilax* females, five 'control' emergence traps were established in November 2008 in an area dominated by *Schoenoplectus litoralis*. Ten other emergence traps were also established in this vegetation type, and once the larvae hatched, the larval densities of five of these traps were reduced and the collected larvae used to supplement the density of the five remaining traps. The average density of *Ae. vigilax* in the *Schoenoplectus* habitat of the LHJ swamp was 78 (± 25) larvae 1 m^{-2} larval trap, and average emergence was 30 (± 26) adults per trap (Chapter 3). It is often difficult to achieve exact replication of manipulation in the field (Pedersen et al., 2004; Fordham et al., 2009), and it is especially difficult to determine larval density from larval sampling procedures (Chapter 3; Workman and Walton, 2000; Thullen et al., 2002); therefore, I reduced the larval density (removed larvae) in the traps where a low count of larvae was observed during sampling. On average, trap larval density was reduced by 15-20 larvae, or increased (supplemented) by 30 larvae per trap.

Larval sampling and adult collection

The LHJ swamp was flooded by a series of monthly high tides during October 2007 and November 2008. In 2007, the highest tide that occurred was 7.89 m on Saturday 27 October;

however, a tide capable of inundating a large area of the swamp also occurred the previous evening, and the following two days. In 2008, the highest tide (7.76 m) on Friday 14 November, and a tide capable of inundating a large area of the swamp, also occurred on the previous day and the following two days. The traps were monitored daily leading up to these high tide events, and once the traps were flooded, larval sampling commenced. Each day following initial flooding, *Ae. vigilax* larval abundance was sampled in the traps using a prescribed dipping procedure: five dips (one in each corner of the trap and one in the centre) were done per trap using a standard dipper (190 ml volume). Larvae were counted and then returned to the habitats and the final estimate of larval abundance was obtained by summing the counts of larvae across the five dips/trap/day. Once the first emerging adults were observed in traps, larval sampling ceased. Emerging adults were caught by manually sucking them into sampling containers using a small aspirator. This was repeated daily until emergence within the traps had ceased. During November 2008, the five control traps, three supplemented traps and four reduced-density traps failed due to an unexpected spraying event and unfortunately no adults were collected from these traps.

Water temperature in each trap was measured daily at mid-day using a Horiba U10 water meter (HORIBA Ltd., Kyoto, Japan). Water volume of each trap was also recorded by measuring water height (mm) at each corner of the trap, and multiplying the mean of these values by trap area.

Adult trap collection

Adult mosquito population density is measured weekly using CO_2-baited mosquito traps by the Medical Entomology Branch of the Northern Territory Department of Health and Community Services at various locations around Darwin for routine surveillance (Russell and Whelan, 1986; Yang et al., 2008b). Four of these trap locations were selected as study sites (Karama, Palm Creek, Holmes Jungle and Marrara Round Swamp) (Fig. 1). These sites were chosen to sample variability in sizes of the female *Ae. vigilax* adults at different distances

from their probable emergence site (LHJ swamp). The LHJ swamp is the nearest *Ae. vigilax* breeding site to the four CO_2 traps selected, so I assumed that most of the mosquitoes caught in these traps emerged there. Distance to emergence site was measured as the shortest distance between the CO_2 trap and the edge of the tide-inundated breeding area of LHJ swamp (Karama = 1.58 km, Palm Creek = 2.33 km, Holmes Jungle = 0.99 km, Marrara Round Swamp = 2.75 km). On two occasions, a sample of 50 adult female *Ae. vigilax* were selected randomly from the total trapped adults at the four sites. The first sample was taken 11 November 2008, two weeks after a series of high tides on 15-16 October (7.5 and 7.7 m, respectively) gave rise to a mass emergence event of *Ae. vigilax.* The second sample was taken on 25 November; one week after several high tides on 14-15 November (7.56 and 7.76 m, respectively) triggered another mass emergence event. Despite these samples being taken in different months, I believe that the surrounding environmental conditions were similar enough to be able to compare the differences in adult size between these two samples as a product of time since emergence or distance from emergence site, rather than as a result of other unmeasured conditions. Due to consistently low densities of trapped adults in at Marrara Round Swamp, samples of only 6 and 25 were taken at this site on 11th November and 25th November respectively.

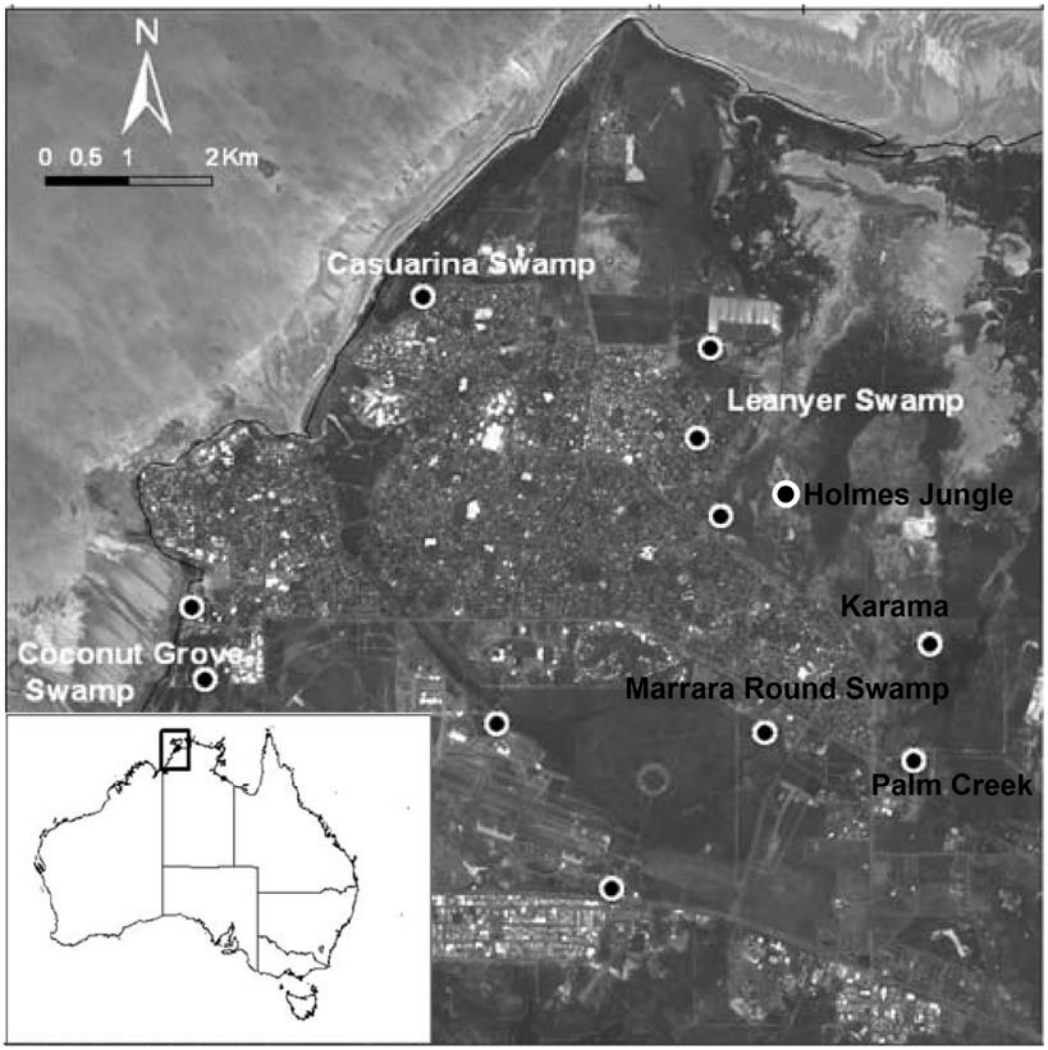

Figure 1 Adult mosquito monitoring trap sites (black dots with white outlines) around Darwin, Northern Territory, Australia. Traps used in this study were Marrara Round Swamp, Karama, Holmes Jungle and Palm Creek.

Analyses

Adult mosquito body size was measured as the distance from the arculus to the wing tip on the dissected left wing of each mosquito, as per Hugo et al. (2003). The three different datasets of wing lengths (adult traps, manipulation experiment and habitat experiment) were used to test three different hypotheses:

(1) *Adult traps* - To examine whether larger female adult *Ae. vigilax* mosquitoes were caught farther from emergence sites, and as time progresses, whether smaller mosquitoes die faster and therefore the mean body size of the female adult *Ae. vigilax* population increases. Distance and time were included as variables in linear mixed-effects models. The response variable was wing length (mm); trap ID (Karama, Palm Creek, Holmes Jungle or Marrara Round Swamp) was included as a random factor to account for within-trap non-independence of measurements (e.g., unmeasured site-specific effects).

(2) *Manipulation experiment* - To determine whether higher densities of larvae result in smaller female *Ae. vigilax* body size at emergence, I fitted linear regression models that included various predictors of variation in emerging female wing length. Predictors considered included larval density (supplemented or reduced), temperature (mean daily temperature measured at mid-day), and water (volume of water in emergence trap). I fit a linear regression model set consisting of only single-term models and ignoring trap ID as a random effect, due to insufficient replication because of trap failure (see explanation above).

(3) *Habitat experiment* – To examine whether emergent *Ae. vigilax* body size varies with habitat type and environmental conditions, regardless of larval density, I constructed general linear models where the response variable was emergent female wing length, and explanatory variables were vegetation type (mangrove forest, mangrove edge, *Schoenoplectus* and *Eleocharis*) and trap density (number of adults that emerged per trap) and an interaction term between vegetation type and trap density.

To rank and weight models according to their statistical strength of evidence given the data, I

used Akaike's information criterion corrected for small samples (AIC_c) as an estimate of Kullback-Leibler (K-L) information loss (Burnham and Anderson, 2002). The difference between the model's AIC_c value and the top-ranked model (ΔAIC_c), and the relative model weights (w_i), were calculated (Burnham and Anderson, 2002, R Development Core Team, 2011). Thus, the strength of evidence (w_i) for any particular model varies from 0 (no support) to 1 (complete support) relative to the entire model set.

Models were compared using the information-theoretic evidence ratio (*ER*) (Burnham and Anderson, 2002). The evidence ratio is calculated as the w_i of any one model divided by a simpler comparison model w_i. The *ER* therefore estimates how many more times likely the model in question is over the model(s) to which it is being compared (Burnham and Anderson, 2002). All analyses were done using the R Package V2.9.0 (R Development Core Team, 2011).

Results

Adult traps

I found evidence for time since emergence having an effect on wing length despite the top-ranked model being the null (i.e. same average wing length at all distances and times) (information-theoretic evidence ratio [*ER*] = 1.43, Table 1, Fig 2). In terms of effect size, my model-averaged prediction of mean wing length of the adult female population two weeks after emergence was 1.02 % (0.2 -1.8 %; 95 % confidence intervals) larger than the mean female population wing length one week after emergence. I found no evidence for any statistically meaningful effect of distance from emergence site affecting wing length (*ER* = 38.20, Table 1).

Models	k	AIC_c	ΔAIC_c	$wAIC_c$	%DE
wing length ~ 1 + (1|trap)	4	69.970	0	0.573	0
wing length ~ time + (1|trap)	5	70.680	0.710	0.402	2.50
wing length ~ distance + (1|trap)	5	77.272	7.302	0.015	0.87
wing length ~ time + distance + (1|trap)	6	77.889	7.919	0.011	3.00

Table 1 Outcome of the linear mixed-effects models and information-theoretic model inference metrics examining variation in body size of *Aedes vigilax* females caught in CO_2 traps, where wing length (mm), a proxy for body size, is the response, and possible predictor variables are time (either 2 weeks or 3 weeks since emergence (binary)), distance (the distance from the adult trap to the nearest larval presence site in the swamps (km)), and the random effect: 1|trap (trap location, i.e., each trap is a unique random effect). Shown are the number of fitted model parameters (k; includes intercept), Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE). %DE is a measure of the structural goodness-of-fit of the model.

Manipulation experiment

In the traps where emergence occurred (two larvae-supplemented traps and one larvae-reduced trap), the adult emergence density of the supplemented traps (72) was almost twice that of the reduced trap (34). The top-ranked model for adult female wing length included negative effects of emergence density (Table 2, Fig 2). Model averaged predictions show a reducing density by half results in a 5.75 % (7.70 % - 3.74 % CI) increase in wing length.

Models	**k**	**AIC_c**	**ΔAIC_c**	***w*AIC_c**	**%DE**
wing length ~ density	2	-65.147	0	0.989	56.57
wing length ~ temp	2	-56.063	9.083	0.011	39.00
wing length ~ 1	1	-44.967	20.180	<0.001	0
wing length ~ water	2	-42.854	22.293	<0.001	0.84

Table 2 Outcome of the linear regression models and information-theoretic model inference metrics from the larval density manipulation experiment, where wing length (mm), a proxy for body size of emerging adult female *Aedes vigilax*, is the response, and possible predictor variables are density (total number of adult *Aedes vigilax* that emerge per trap), temperature (mean daily water temperature (^{o}C) measured at mid-day), and water (median trap water volume of trap). Shown are the number of fitted model parameters (k; includes intercept), Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 (*w*AIC_c) and percent deviance explained (%DE). %DE is a measure of the structural goodness-of-fit of the model.

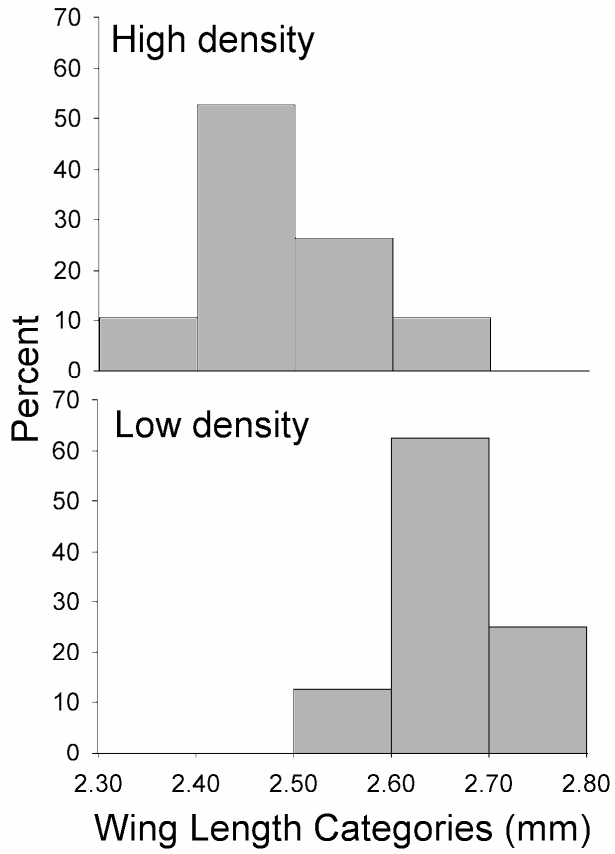

Figure 2 Frequency distribution of female *Aedes vigilax* body size (indicated by wing length) from emerging mosquitoes collected from low density (n=34) and high density (n=72) traps during the November 2008 density manipulation field experiment.

Habitat experiment

The sex ratio of emerged adults across all habitats was 1.2:1 (female to male), and the mean wing length of females was 2.83mm (±0.17). I found evidence for vegetation type, density and the interaction between vegetation and density as predictors of female adult wing length (*ER* > 999, Table 3); evidence for the interactive term between density and vegetation shows that the effect of density on adult emergence size varies among habitats. I found a negative relationship between emergence density and adult body size in the mangrove forest, mangrove edge and *Schoenoplectus* habitats, however there was a positive relationship between emergence density and adult body size in the *Eleocharis* habitat (Fig 3).

Models	k	AIC_c	ΔAIC_c	$wAIC_c$	%DE
wing length ~ density*vegetation	8	-267.92	0	>0.999	47.1
wing length ~ veg	4	-205.38	62.54	<0.001	25.0
wing length ~ density + veg	5	-203.99	63.93	<0.001	25.3
wing length ~ density	2	-159.09	108.83	<0.001	4.0
wing length ~ 1	1	-152.77	115.15	<0.001	0

Table 3 Outcome of the linear regression models and information-theoretic model inference metrics from the larval habitat experiment, where wing length (mm), a proxy for body size of emerging adult female *Aedes vigilax*, is the response, and possible predictor variables are density (total number of adult *Aedes vigilax* that emerge per trap), and vegetation (mangrove forest, mangrove edge, *Schoenoplectus* reeds, *Eleocharis* reeds). Shown are the number of fitted model parameters (k; includes intercept), Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE). %DE is a measure of the structural goodness-of-fit of the model.

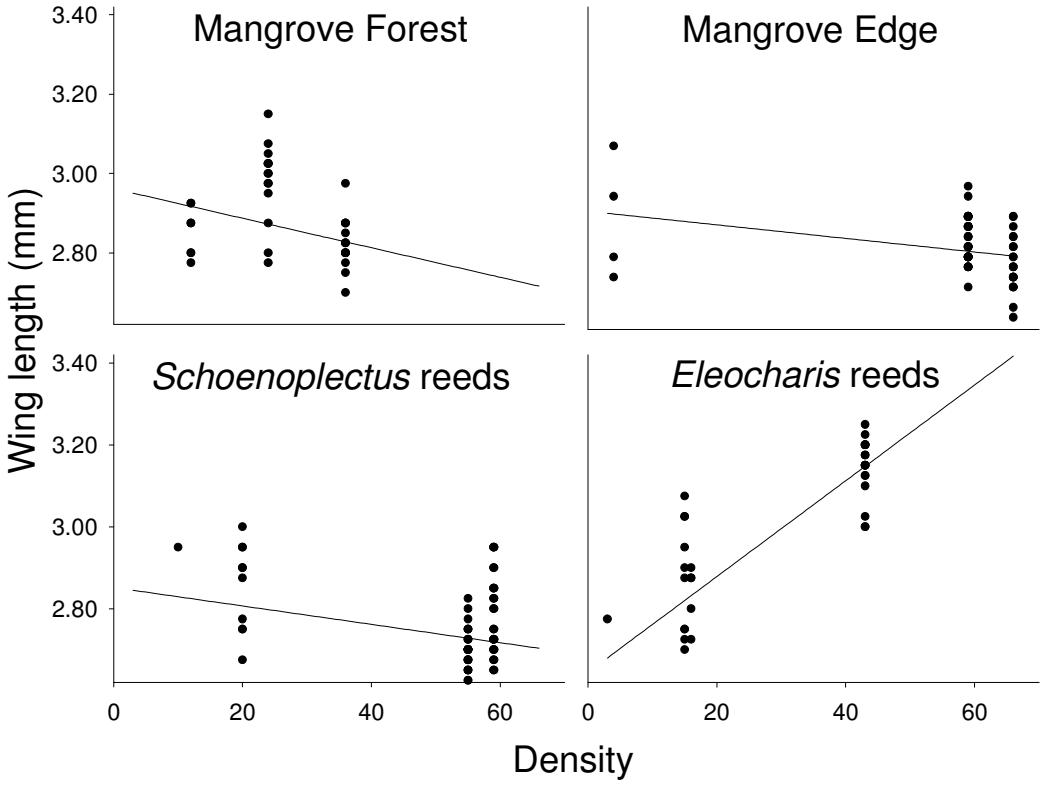

Figure 3 Relationship between body size (indicated by wing length) and density (number of emerged adults per trap) of *Aedes vigilax* females collected from different habitat types (mangrove forest, mangrove edge, *Schoenoplectus* reeds, and *Eleocharis* reeds) during the October 2007 habitat field experiment.

Discussion

I examined larval competition as a mechanism driving density feedback in *Ae. vigilax* body size and thus, its vectorial capacity; reducing larval density had the effect of increasing the body size of emergent mosquitoes, but this relationship depended on the vegetation type in which the larvae were reared. Nutritional competition among larvae is well documented as the principal mechanism for density feedback in insect vectors; higher larval densities mean more competition for resources, and therefore result in smaller emerging adults (Agnew et al., 2002; Gimnig et al., 2002; Hugo et al., 2003; Barrera et al., 2006; Juliano, 2007; Reiskind and Lounibos, 2009). This relationship has been quantified previously for container-breeding species (Agnew et al., 2002; Barrera et al., 2006), and in laboratory experiments (Hugo et al.,

2003; Reiskind et al., 2004; Reiskind and Lounibos, 2009), but mine is the first broad-scale evidence for a negative density effect in the field and across a gradient of natural habitats. Unfortunately, the loss of five background emergence (control) replicates, three supplemented replicates and four reduced replicates (because no females survived to emergence), limited my ability to define clearly the relationship between larval density and adult emergence size for this species.

There is a large body of work examining the effects of adult vector size on vectorial capacity traits. In some cases, larger individuals are more susceptible to viral infection from a bloodmeal (Nasci et al., 1994; Sumanochitrapon et al., 1998; Westbrook et al., 2010), although in others, smaller individuals are more susceptible (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et al., 2011). This relationship appears to be species- and disease-specific, and previous research has shown no correlation between Ross River virus infection rates and *Ae. vigilax* female body size variation from larval nutrition changes (Jennings and Kay, 1999). Larger individuals from most mosquito species live longer, have greater energetic reserves, and have larger dispersal ranges and therefore, a longer potential time to infect hosts and so transmit pathogens (Hawley, 1985; Agnew et al., 2002; Gimnig et al., 2002; Manoukis et al., 2006; Bevins, 2008; Gavotte et al., 2009; Muturi et al., 2010). Taking my adult trap data as representative of the adult *Ae. vigilax* population, despite sampling issues, I can corroborate these conclusions at a broad spatial scale. Measurements taken from weekly adult monitoring stations around Darwin at different times since emergence demonstrate that larger female adults do indeed live longer (Fig. 4). However, the effect size of time on frequency of body size is small (time since emergence explains 2.5% of the deviance in body size, Table 1), and more experimental evidence is required to test the body size/density trade-off further.

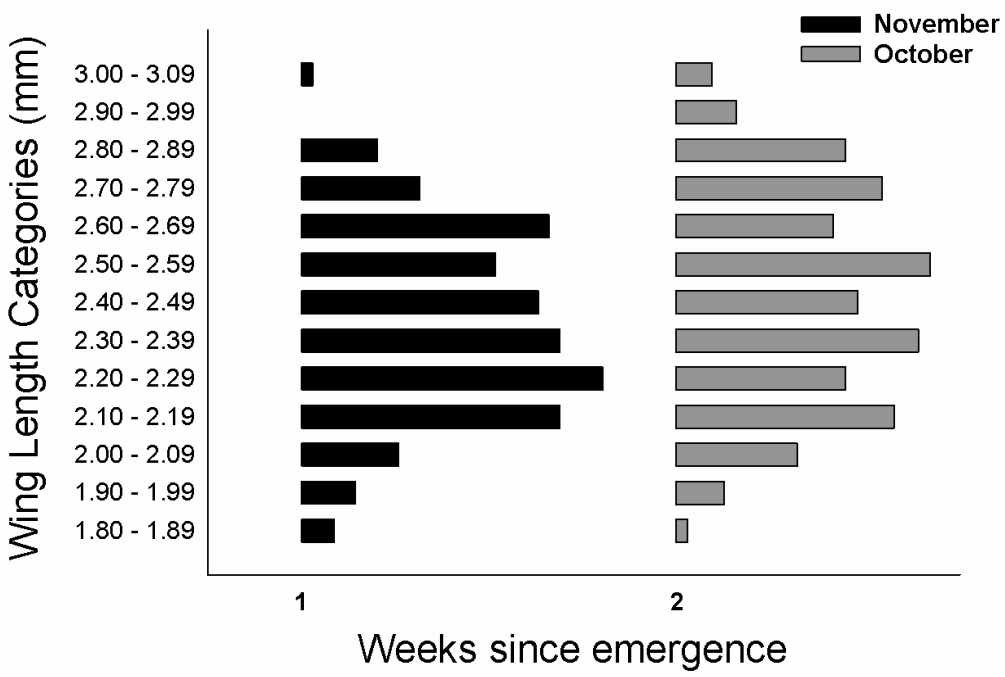

Figure 4 Frequency histograms of female *Aedes vigilax* body size (indicated by wing length) collected from four adult monitoring traps at various distances from the emergence site in November 2008 (one week after emergence) and October 2008 (two weeks after emergence).

While vectorial capacity increases linearly with some traits such as vector competence (ability to transmit disease), it increases exponentially as mosquito survival rises (Garrett-Jones, 1964). This is because vector-borne pathogens must undergo a within-vector incubation period before they can be transmitted to a new host. The disease/vector incubation period can vary from just one week to up to a month (Brownstein et al., 2003), so a mosquito must at least survive longer than the initial non-feeding and disease-incubation periods combined to become a successfully transmitting host. This strongly non-linear timing response has important implications for control measures, as explained below.

Aedes vigilax are major vectors of Ross River virus, so density effects on body size are essential considerations any vector control models or management interventions. The maximum transmission of Ross River virus is reached 10 to 13 days after vector infection (Vale et al., 1992), thus *Ae. vigilax* adults must survive at least two weeks to reach this state. Consequently, only the proportion of the mosquito population that survives this period

actually contributes to disease transmission (Brownstein et al., 2003; Cook et al., 2008). As such, the longer vectors survive, the higher the density of infected mosquitoes in the population and the greater the likelihood of disease transmission. Therefore, interventions which only reduce, but do not eliminate, larvae in targeted habitats may lead to the perverse effect of actually elevating the disease-transmitting potential of the females who survive to adulthood (Juliano, 2007). The corollary is that high-density larval environments should produce smaller, shorter-lived adults with lower disease-transmission potential – offset to a degree by the fact that there are more potential vectors available. While this theory holds for species where vector competence (susceptibility to viral infection and dissemination) is positively correlated with, or unaffected by, vector body size (Nasci et al., 1994; Sumanochitrapon et al., 1998; Jennings and Kay, 1999; Alto et al., 2005; Westbrook et al., 2010; Muturi et al., 2011), in cases where vector body size is inversely related to vector competence (i.e., smaller females are more susceptible to infection and dissemination; Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et al., 2011), this relationship will also need to be taken into account, further emphasing the importance of defining the relationship between vector life history and disease infection.

I found a negative relationship between larval density and adult body size in three of the four different larval habitats that dominate the LHJ swamp. In the fourth habitat, *Eleocharis* reeds, I found a positive relationship between larval density and body size (Fig 3). While larval competition is one mechanism driving density feedback in body size, there are also a suite of environmental factors known to affect adult mosquito body size, such as nutrient levels, ambient temperature, and predation level (Baqar et al., 1980; Rae, 1990; Hugo et al., 2003; Costanzo et al., 2011). The larval traps I used were designed to exclude predators, and previous work has shown no significant differences in water temperature, pH, salinity, and dissolved oxygen across the four different larval habitats; and *Ae. vigilax* larval survival and development is not affected by the gradients of these microclimate environmental parameters

across the habitats sampled (Chapter 3). It is possible that the nutrient levels present in some traps in the *Eleocharis* reed habitat were higher than in the other traps and habitats, allowing larvae in those traps greater access resources, and therefore a larger emergent size. Further study investigating nutrient levels across the different habitat types would allow us to more fully explore the effects of different larval habitats on emergent body size.

Most predictive models of vector-borne disease transmission do not include the vector's larval stages explicitly, and so they cannot determine realistically the sensitivity of disease transmission to larval control measures (Tong et al., 2008; Bellan, 2010). When using prescriptive vector control models, it is therefore crucial to consider not only environmental conditions and mosquito abundance, but also the skew of body size frequency in the adult population. While some models of disease transmission incorporate metrics of vector survival (Garrett-Jones, 1964), adult survival is often difficult to gauge in wild populations (Hugo et al., 2010). A metric that combines body size (as a proxy for adult survival) and adult abundance could therefore represent a better predictor of disease transmission potential. However, depending on species, this would mean that any changes to vector size might increase vector competence. A detailed knowledge of the species-disease relationship is therefore crucial to understand these complex relationships.

In conclusion, my experiments have revealed a crucial connection between larval density and vectorial capacity. This is also one of the first field studies that allows us to decouple the effects of different larval habitats from the effects of larval density on adult mosquito body size and the subsequent linked life-history traits. Along with informing current models of disease transmission, these findings are also directly applicable to current vector management programs. I propose two methods of elevating current vector-control effectiveness: (1) mosquito-control methods should not ignore low-density larval sites as they have the potential to produce more effective vectors, and (2) to benefit fully from the effects of high-density larval competition for resources on adult vector body size larval control is best applied towards the latter stages of larval development.

CHAPTER 5

EFFICIENT PATHWAYS TO MODELLING VECTOR-BORNE DISEASE OUTBREAKS: USING ENVIRONMENTAL DATA IN PLACE OF VECTOR ABUNDANCE.

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Text in manuscript

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Abstract

Vector population dynamics are important predictors of vector-borne disease outbreaks in humans that act synergistically with climatic variables that affect pathogen survival and transmission. Adult vector abundance is most often estimated by trapping to infer demographic patterns and consequently, to predict disease risk, yet such sampling programs are expensive and difficult to run. Vector population dynamics are highly complex and intricately modified by stochastic environmental conditions. I determine the utility of using estimates of vector abundance to predict accurately the incidence of a widespread mosquito-borne disease in Australia – Ross River virus (arbovirus). I ask whether incidence of the disease can be more accurately predicted using environmental proxies for vector population dynamics versus vector abundance data *per se*. Using block-bootstrapped generalised linear modelling, I developed a multi-model prediction of monthly human Ross River virus cases based on combinations of times series of trapped vector abundance and environmental proxies of vector population dynamics. I found that wet-season specific environmental proxy models were the most accurate at predicting the highest priority health statistic: wet season outbreaks of virus cases. I conclude that adult vector trapping abundances are not necessarily the most effective predictors of vector-borne disease outbreaks in this case, and that ecologically accurate environmental proxies are a cheaper and effective alternative to adult vector monitoring for predicting outbreaks of vector-borne disease.

Introduction

Vector-borne diseases are a major cause of morbidity and mortality for humans globally, representing 14% of all infectious and parasitic disease Disability-Adjusted Life Years (DALY) (Russell and Dwyer, 2000; World Health Organization, 2004). Climate change models predict an increase in many vector-borne diseases, mainly indicated through predicted increases in vector population size and range (Hay et al., 2002; Chase and Knight, 2003; Campbell-Lendrum and Woodruff, 2007). The adequate provision of health services to deal with the current and future burden of vector-borne disease depends on accurate disease surveillance and outbreak prediction. Mosquito population dynamics are arguably the most important predictors of incidence of mosquito-borne disease, along with measures of exposure and climatic variables that affect pathogen survival and transmission.

Estimates of adult abundance from trap programs are commonly used phenomenologically to infer demographic patterns in vector populations (Glass, 2005; Adams and Kapan, 2009; Carver et al., 2010; Sullivan, 2010). However, accurate measurements of the highly vagile adult vector populations are economically and energetically costly, and not always obtainable (McIver et al., 2010). Also, the relationship between vector population dynamics and vector-borne disease epidemiology is complex, depending on many more elements than simple adult vector abundance, of which vector behaviour, longevity and dispersal ability, disease incubation period, and host population density and distribution are a few (Glass, 2005; Adams and Kapan, 2009; Hu et al., 2010a; Stresman, 2010, Sullivan, 2010). A basic first step for models seeking to make inference about the relationship between vector abundance and disease incidence is defining the ecological factors affecting vector population dynamics (Fish, 2008; Adams and Kapan, 2009). Such models might identify environmental variables that better predict disease occurrence than data from trap programs. This is because mosquito population dynamics are highly complex and intricately modified by stochastic environmental conditions (Chapter 2; Yang et al., 2008b; Yang et al., 2009; Russell et al., 2011). Because mosquitoes require ephemeral aquatic habitats for the development of their

larvae and pupae, the relative amount of standing water in an environment due to rainfall or tidal patterns is often used as a predictor of vector population size or carrying capacity (Chapter 2; Chase and Knight, 2003; Yang et al., 2008a; Russell et al., 2011). Some models of vector-borne disease already use these environmental predictors of vector population size or 'environmental proxies' in place of unavailable measures of adult vector abundance; for example, incidences of both malaria and dengue are commonly modelled using rainfall as a proxy for adult vector abundance (Carver et al., 2010; Hu et al., 2010b; Sullivan, 2010). However it is important to remember these cases are species- and disease-specific, and that as much as possible models should be tailored to encompass the complex relationships between exogenous and endogenous process that influence vector population dynamics and disease transmission (Yang et al., 2008a).

Clearly, mathematical modelling has been identified as a key area of investment in the ongoing war against vector-borne disease (Fish, 2008; Ferguson et al., 2010; Hu et al., 2010a; Sullivan, 2010). The importance of creating statistically robust models is paramount, as one of the major sources of error in scientific predictions and subsequent decision making is choosing the 'wrong' model (Taper et al., 2008). The use of information criteria to evaluate multiple models representing competing hypotheses allows any inference to be based on all models and their relative distance from reality, and therefore reduces the possibility of model selection error (Burnham and Anderson, 2002; Brook and Bradshaw, 2006). Most models of vector populations and vector-borne disease are the results of correlational studies using time-series analyses (Jacups et al., 2008a; Tong et al., 2008; Carver et al., 2010; Hu et al., 2010b; Stresman, 2010), and they suffer from confounding variables, in particular, climatic variables, and hence are prey to the risk of statistical over-fitting (Krebs and Berteaux, 2006; Knape and de Valpine, 2011). In the face of climate change, the key issue is not how well the models of vector-borne disease fit the data, but how good they are at predicting future disease outbreaks. Measures of model fit do not necessarily equate with the model's predictive ability; therefore, validating the models' ability to predict disease outbreaks is essential.

Ross River virus is Australia's most epidemiologically important vector-borne disease and annual notifications per capita in the Northern Territory are more than four times the national average (Carver et al., 2010; Australian Government Department of Health and Aging, 2011). Previous research on Ross River virus has linked outbreaks of the disease to environmental conditions such as precipitation, tidal regimes and temperatures that are intricately linked to the ecology of the main Ross River virus vectors and marsupial reservoir hosts (Hu et al., 2004; Gatton et al., 2005; Tong et al., 2005; Hu et al., 2006a,b; Woodruff et al., 2006; Jacups et al., 2008a,b; Tong et al., 2008; Williams et al., 2009; Carver et al., 2010; McIver et al., 2010; Pelecanos et al., 2010). While temperate studies of the disease have found patterns of seasonality in outbreaks associated with climatic conditions and vector populations, in tropical areas the outbreak occurrences reflect the year-round vector breeding conditions (Carver et al., 2010). In the absence of an effective vaccine and/or treatment, efforts to reduce Ross River virus transmission are primarily based on vector control or avoidance of vector exposure strategies (Harley et al., 2005). In the Northern Territory in particular, mosquito population monitoring and control constitutes one of the larger recurrent land management programs launched in the 1980s and continues today (Yang et al., 2008b). The mosquito monitoring programme, which consists of CO_2-baited traps positioned around urban areas, is intended to act as an indicator of adult mosquito abundance around Darwin as well as surveying mosquito species assemblages for exotic mosquito presence (Jacups et al., 2008b). The ecology of the two main vectors of Ross River virus in Darwin, *Aedes vigilax* and *Culex annulirostris*, has been well studied and the main exogenous drivers of each species are accumulated ephemeral water in swap complex around Darwin due to spring high tides (for *Ae. vigilax*) and wet season rainfall (for *Cx. annulirostris*) (Chapter 2; Russell and Whelan, 1986; Whelan et al., 1997; Whelan, 1989; Whelan et al., 2003; Whelan, 2007a; Yang et al., 2008a,b; Kurucz et al., 2009; Yang et al., 2009).

Here I examine the utility of multi-model inference and model averaging to create accurate predictions of disease outbreaks. Using Ross River virus incidence in Darwin as a

case study, I examine the ability of measures of vector abundance from the adult monitoring programme compared to environmental proxies of vector abundance (tidal and rainfall data) to accurately predict outbreaks of Ross River virus. I hypothesise that the current mosquito trapping programs do not accurately measure the adult female mosquito population size or vectorial capacity, so combining environmental proxies for mosquito population dynamics will represent a more cost-effective and accurate method of predicting disease incidence than measures of vector abundance from trapping programs..

Methods

Data Collection

I used laboratory-confirmed cases of Ross River virus infections in the Darwin region notified to the Northern Territory Centre for Disease Control. I calculated case counts per calendar month using date of diagnosis, and the completed dataset spans 204 months (17 years), from January 1991 to December 2007.

The Medical Entomology Branch of the Northern Territory Department of Health and Community Services have measured weekly adult mosquito abundance using CO_2-baited mosquito traps continuously since 1991 at 11 locations around Darwin, Northern Territory of Australia (Russell and Whelan, 1986; Yang et al., 2008a,b). I considered average monthly adult female mosquito abundance (all species), and monthly adult female mosquito abundance of the two major vectors of Ross River virus, *Cx. annulirostris* and *Ae. vigilax*. The abundance of these two vectors makes up an average of 59% of the monthly abundance in all mosquito traps, with other local vectors *Aedes notoscriptus* and *Aedes phasceatus* contributing only a negligible amount to monthly mosquito trap abundances (2 and 0.2%, respectively).

The Australian Bureau of Meteorology provided monthly data for climatic variables covering the same interval as the Ross River virus dataset (www.bom.gov.au). These included summed rainfall (mm), number of rain days (where a rain day is defined as ≥ 1 mm rain),

humidity measured as average vapour pressure (hPa), evaporation (mm), average daily maximum temperature (^{o}C), maximum temperature (^{o}C), average temperature (^{o}C), average daily minimum temperature (^{o}C), minimum temperature (^{o}C), maximum tide height (m), mean tide height (m), and frequency of tides higher than or equal to 7.4 m (tides > 7.4 m generate temporary saltwater habitats ideal for the oviposition and larval development of *Ae. vigilax* in the swamps around Darwin (Whelan 1987, Yang et al., 2008b)).

Principal components analyses

In a highly seasonal environment such as the wet-dry tropics, environmental attributes used as model covariates tend to be highly correlated. This is particularly true of variables based on similar environmental phenomena such as frequency of high tides and maximum tide height, or humidity and rainfall. One way of dealing with the complexities of multicollinearity is to use principal components analysis (PCA) (Jolliffe, 2004). I completed PCAs for four groups of related environmental predictors: 1) Temperature - average daily maximum temperature (^{o}C), maximum temperature (^{o}C), average temperature (^{o}C), average daily minimum temperature (^{o}C) and minimum temperature (^{o}C); 2) Wetness – humidity measured as average vapour pressure (hPa), evaporation (mm), summed rainfall (mm), and number of rain days; 3) Tides – maximum tide height (m), mean tide height (m), and frequency of tides higher than or equal to 7.4 m; and finally 4) Rain – summed rainfall (mm), and number of rain days. PCAs were completed for the whole dataset and for subsets representing wet season and dry season (see Appendices 3a to 3d). I included the principal components of these analyses that explained > 75% of the variation in the each of the four groups in further analyses in place of the equivalent environmental predictor variables because the principal components are uncorrelated (Jolliffe, 2004).

Mosquito trap abundance models

I developed negative binomial (log link function) generalised linear models to examine the

relationship between mosquito trapping abundances and Ross River virus infection. The response variable was monthly Ross River virus case counts, and explanatory variables included in the models were mean monthly mosquito abundance (all species), mean monthly *Ae. vigilax* abundance and mean monthly *Cx. annulirostris* abundance. Transmission of Ross River virus occurs 4 – 5 days after vector infection, with maximum transmission reached at 10 – 13 days, and patients classically present with symptoms following an incubation period of 7 – 9 days (Fraser and Cunningham, 1980; Vale et al., 1992). Thus, the likely time from vector incubation and infection to host infection, incubation and presentation is roughly 11 – 22 days. Therefore, average monthly abundance and also a lag of 1 month for mosquito numbers were included in models. I also included season (wet/dry), human exposure (number of public holidays per month) and Ross River virus immunity (peak Ross River virus case count from the previous year) in the models. I included the number of public holidays per month as measure of change in risk of human exposure to vectors of Ross River virus (Lincoln et al., 2006).

Environmental proxy abundance models

Monthly abundance of the two major Ross River virus vectors, *Ae. vigilax* and *Cx. annulirostris*, can be approximated by the main environmental variables that drive their population dynamics: tides and rainfall, respectively (Chapter 2; Yang et al., 2008b; Yang et al., 2009). I developed negative binomial (log link function) generalised linear models to examine the relationship between environmental proxies of mosquito abundance and Ross River virus cases. The response variable was monthly Ross River virus case counts, and explanatory variables included were the first *tidal* and *rain* principal components. In the Northern Territory, the average time from inundation of *Ae. vigilax* and *Cx. annulirostris* larval habitats to adult emergence is 9 – 13 days (Sinclair, 1976; Lee et al., 1989). Therefore, I included lags of 1 and 2 months of the environmental proxies in the models. These lagged variables also account for zoonotic amplification cycles that can precede human cases. Season

(wet/dry), human exposure (number of public holidays per month) and Ross River virus immunity (peak Ross River virus case count from the previous year) were also included in the models.

Model averaging

To rank and weight models, I used Akaike's information criterion corrected for small samples (AIC_c) as an estimate of Kullback Leibler (K-L) information loss (Burnham and Anderson, 2002). I also calculated the difference between the model's AIC_c value and the top-ranked model (ΔAIC_c), and the relative model weights ($wAIC_c$) (Burnham and Anderson, 2002). Thus, the strength of evidence ($wAIC_c$) for any particular model varies from 0 (no support) to 1 (complete support) relative to the entire model set.

Time series data are usually autocorrelated, thereby violating the assumption of independence in linear models. I use block bootstrapping to resample my time series data and return bootstrapped datasets. Blocks of data were resampled randomly with replacement from the original time series, and then joined together in a random order to create the uncorrelated 'bootstrapped sample (Carlstein et al., 1998; Politis and White, 2004; Patton et al., 2009). I then applied the model fitting process to 100 bootstrapped samples, and the median and 95% bootstrapped confidence intervals (2.5 and 97.5 percentiles) of the test statistics: AIC_c, ΔAIC_c, $wAIC_c$ and percent deviance explained were used to rank and weight models. Models were compared using the information-theoretic evidence ratio (*ER*) (Burnham and Anderson, 2002). The evidence ratio is calculated as the $wAIC_c$ of any one model divided by a simpler comparison model $wAIC_c$. The *ER* therefore estimates how many more times likely the model in question is over the model(s) to which it is being compared (Burnham and Anderson, 2002). I used the R Package V2.12.1 for all analyses (R Development Core Team, 2011).

Combined models

To determine the partial contribution of trap abundance and environmental proxies to

explaining variance in Ross River virus cases, I created a model set that included all models with a substantial empirical support (>2/3 $w\text{AIC}_c$) from the vector trap abundance and vector environmental proxy model sets. I also included other explanatory variables known to affect vector survival and virus transmission: first temperature and wetness principal components.

I also developed mosquito trap abundance, environmental proxy abundance and combined model sets for the two separate monsoonal seasons. I classified data from November to April as 'wet season' and data from May to October as 'dry season'. I also included season-specific principal components of environmental variables in these models.

Validation

To validate model predictions, I used two years of new data collected from January 2006 to December 2007 (i.e., data not use to construct models). The mean and variance of the environmental variables in the new (validation) dataset (2006-2008) are equivalent to the mean and variance of the variables in the original dataset (1991-2005); therefore, I was able to use the corresponding coefficients for the environmental principal components to predict from these variables. Using model-averaging, I predicted monthly Ross River virus cases for the validation period for all three model sets (full dataset, wet season and dry season), and calculated the mean squared error between predicted and measured number of cases per month.

Woodruff et al. (2002) defined a Ross River virus outbreak year as any financial year in which the number of cases exceeded 1 standard deviation above the mean number of cases during the study period. I define a Ross River virus outbreak therefore as any month in which the number of cases exceeded 1 standard deviation beyond the mean number of cases for that month given my choice of a monthly interval. Using the model-averaged predictions of Ross River virus cases for the validation data, I calculated various sensitivity parameters of the models for predicting monthly Ross River virus outbreaks during the wet season. These included accuracy (percentage of outbreaks and non-outbreaks correctly predicted), sensitivity

(proportion of outbreaks that are correctly identified as outbreaks), specificity (proportion of non-outbreaks correctly identified as non-outbreaks), positive predictive value (probability that a predicted outbreak really is a measured outbreak), and negative predictive value (probability that a predicted non-outbreak really is a measured non-outbreak).

I also used these new data to examine the accuracy and Ross River virus outbreak predictive ability of previous models of Ross River virus in Darwin (Jacups et al., 2008b).

Results

Principal components analyses

Temperature PCA: The PC1 was used for the full and dry data sets, and all the measurements of temperature increased with increasing PC1. Both the PC1 and PC2 were used for the wet season dataset: maximum and average temperature measurements increased with increasing PC1, while minimum measurements increased with decreasing PC2.

Wetness PCA: The PC1 was used for the full dataset, and evaporation increased with increasing PC1, while all other *wetness* PCA variables decreased. Both PC1 and PC2 were used for the wet and dry data sets. For the wet dataset, evaporation increased with increasing PC2, and all other variables increased with PC1, whereas for the dry, evaporation decreased with increasing PC2, and all the other variables decreased with increasing PC1.

Rain PCA: The PC1 was used for all data sets, and for the full and wet data sets, both variables increased with increasing PC1, whereas for the dry dataset, they decreased.

Tidal PCA: The PC1 was used for all data sets, and all the measurements of tide height increased with increasing PC1.

(For a full description of results, see Appendix 4a.)

Mosquito trap abundance models

The top-ranked mosquito trap abundance models for the full, wet and dry season datasets all included mean monthly *Ae. vigilax* abundance, mean monthly *Cx. annulirostris* abundance

and 1 month lags of mean *Ae. vigilax* and *Cx. annulirostris* abundances (Table 1a,b,&c). As expected, there was a positive correlation between Ross River virus cases and mean monthly *Cx. annulirostris* abundance, and 1 month lags of mean *Ae. vigilax* and *Cx. annulirostris* abundances for the full dataset and wet season models (Fig. 1). Paradoxically, I also found evidence for a negative relationship between mean monthly *Ae. vigilax* abundance and Ross River virus cases (Fig. 1). The relationship between dry season Ross River virus cases and lags of mean *Ae. vigilax* and *Cx. annulirostris* abundances were opposite to those found for the other datasets (Fig.1). This is probably related to the large difference in the deviance explained by the vector trap abundance models for the dry season compared to the full and wet season datasets: 23% compared to 45% (Table 1b&c).

Table 1 Comparison of the top-ranked vector trap abundance models and the null models used to assess change in monthly Ross River virus cases for (a) the full time series (January 1991 to December 2005) (b) the wet season time series (November to April, 1991 to 2005), and (c) the dry season time series (May to October 1991 to 2005). Explanatory variables considered are: CxA_{lag1} = 1 month lag of mean monthly *Culex annulirostris* trap abundance, CxA = mean monthly *Culex annulirostris* trap abundance, AeV_{lag1} = 1 month lag of mean monthly *Aedes vigilax* trap abundance, AeV = mean monthly *Aedes vigilax* trap abundance, season = wet or dry, holidays = number of public holidays per month and RRv_{lag} = peak Ross River virus cases from the previous wet season. Shown are the number of fitted model parameters (*k*; includes intercept), and the median score (range of score in parentheses) from 100 block bootstrap iterations for Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE). See Appendix 4b for full model sets.

Models	k	Median AIC_c	Median ΔAIC_c	Median $wAIC_c$	Median %DE
(a) Full dataset					
CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag}	8	824.82	0.000	0.378 (0.276-0.435)	45.18 (41.45-50.23)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag}	7	825.10	0.224	0.29 (0.223-0.38)	45.12 (41.04-50.12)
CxA_{lag1} + CxA + AeV + season + holidays + RRv_{lag}	7	828.82	2.269	0.105 (0.053-0.18)	44 (40.36-49.5)
CxA_{lag1} + CxA + AeV + season + RRv_{lag}	6	829.85	3.065	0.077 (0.028-0.124)	43.97 (39.88-49.42)
null	1	929.95	102.925	0 (0-0)	0 (0-0)
(b) Wet season					
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}	7	467.41	0.000	0.259 (0.205-0.343)	44.98 (38.52-55.54)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag}	6	467.58	0.303	0.183 (0.128-0.238)	44.17 (38.15-53.73)
CxA_{lag1} + AeV_{lag1} + AeV + holidays + RRv_{lag}	6	467.66	0.693	0.142 (0.084-0.201)	44.18 (36.93-52.40)
CxA_{lag1} + AeV_{lag1} + AeV + RRv_{lag}	5	467.92	1.257	0.116 (0.058-0.156)	43.93 (36.71-50.68)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays	6	469.94	1.565	0.106 (0.037-0.136)	42.24 (36.56-52.14)
CxA_{lag1} + AeV_{lag1} + CxA + AeV	5	469.98	3.052	0.057 (0.017-0.112)	41.77 (36.22-51.17)
CxA_{lag1} + AeV_{lag1} + AeV + holidays	5	470.89	4.025	0.040 (0.010-0.094)	41.65 (35.44-50.89)
CxA_{lag1} + AeV_{lag1} + AeV	4	470.95	4.894	0.027 (0.006-0.065)	41.29 (35.14-49.57)
Null	1	514.32	50.004	0 (0-0)	0 (0-0)
(c) Dry Season					
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}	7	311.92	0.000	0.492 (0.404-0.555)	23.2 (18.72-27.51)
AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}	6	313.68	1.488	0.231 (0.165-0.291)	21.55 (16.91-25.97)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays	6	322.26	5.104	0.036 (0.003-0.139)	16 (13.23-20.53)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag}	6	321.07	5.881	0.027 (0.007-0.1)	16.93 (12.96-22.1)
holidays + RRv_{lag}	3	320.27	6.198	0.021 (0.003-0.068)	16.08 (13.94-19.86)
Null	1	338.04	23.439	0 (0-0)	0 (0-0)

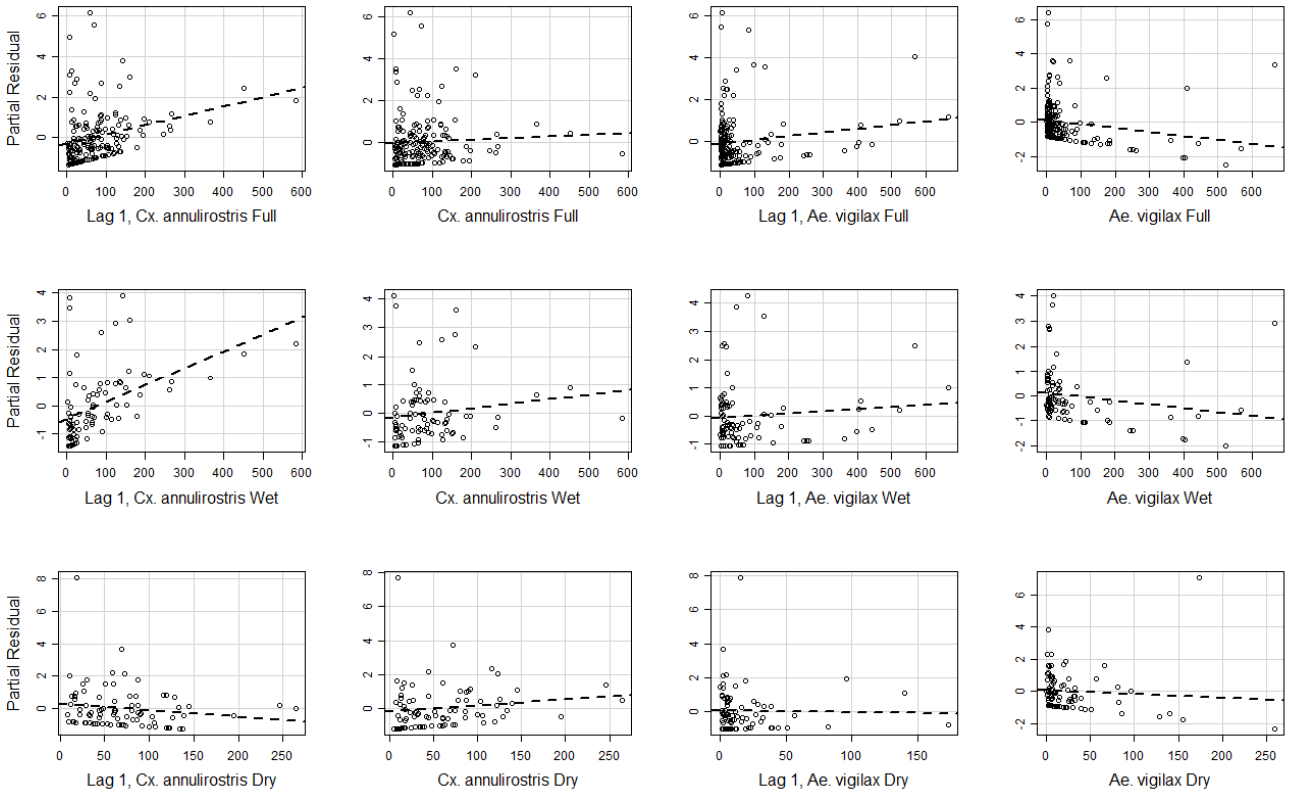

Figure 1 Partial residual plots derived from the highest-ranked vector trap abundance models of monthly Ross River virus cases for the full time series (Full) and the wet seasons only (Wet) and dry seasons only (Dry). Variables examined are: mean *Aedes vigilax* monthly abundance, 1 month lagged mean *Aedes vigilax* monthly abundance, mean *Culex annulirostris* monthly abundance, 1 month lagged mean *Culex annulirostris* monthly abundance. Dashed lines indicate the least-squares relationship.

Environmental proxy models

Top-ranked proxy abundance models included 2 and 1 month lags of both environmental proxies: *tidal* and *rain* principal components, for all three datasets (Table 2a,b,&c). I found evidence for positive relationships between Ross River virus and both lags (1 and 2 months) of the *rain* PC1 and the 2 month lag of the *tidal* PC1, and a negative relationship between Ross River virus and the 1 month lagged *tidal* PC1 for all three model sets (full dataset, wet season and dry season) (Fig. 2). Extrapolating these results through the *tidal* PCA for all three model sets, Ross River virus increases with mean monthly tide height, maximum tide height and frequency of high tides 2 months previously, and decreases with a 1 month lag of all the tidal variables. These relationships are as expected given that the tidal variables are an environmental proxy for adult *Ae. vigilax* abundance (Yang et al., 2008a; Yang et al., 2009).

For the full dataset and wet season models, the relationship between Ross River virus and rainfall and number of rain days were the same as expected given that these rain variables are an environmental proxy for adult *Cx. annulirostris* abundance (Yang et al., 2008a,b): Ross River virus increases with rainfall and rain days at lags of 1 and 2 months. However, extrapolating through the PCA I found evidence that in the dry season, Ross River virus decreases with increasing rainfall and number of rain days.

Table 2 Comparison of the top-ranked environmental proxy models and the null models used to assess change in monthly Ross River virus cases for (a) the full time series (January 1991 to December 2005) (b) the wet season time series (November to April, 1991 to 2005), and (c) the dry season time series (May to October 1991 to 2005). Explanatory variables considered are: $tide_{lag2}$ = 2 month lag of tidal first principal component, $tide_{lag1}$ = 1 month lag of tidal first principal component, $rain_{lag2}$ = 2 month lag of rain first principal component, $rain_{lag1}$ = 2 month lag of rain first principal component, season = wet or dry, holidays = number of public holidays per month and RRv_{lag} = peak Ross River virus cases from the previous wet season. Shown are the number of fitted model parameters (*k*; includes intercept), and the median score (range of score in parentheses) from 100 block bootstrap iterations for Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE). See Appendix 4b for full model sets.

Models	k	Median AIC_c	Median ΔAIC_c	Median $wAIC_c$	Median %DE
(a) Full dataset					
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + season + holidays + RRv_{lag}$	8	812.19	0.000	0.256 (0.198-0.354)	46.06 (39.95-49.9)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + season + holidays + RRv_{lag}$	7	813.35	0.619	0.167 (0.121-0.222)	45.77 (39.58-49.76)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays + RRv_{lag}$	7	814.51	0.742	0.154 (0.094-0.213)	45.14 (39.81-49.48)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays + RRv_{lag}$	6	815.77	1.782	0.098 (0.049-0.135)	44.97 (38.85-49.23)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + season + RRv_{lag}$	7	818.14	4.993	0.017 (0.004-0.045)	44.3 (37.09-48.38)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + season + RRv_{lag}$	6	819.06	6.011	0.014 (0.003-0.042)	44.16 (37-48.16)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$	6	821.24	7.249	0.008 (0.002-0.028)	43.74 (36.48-47.41)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$	5	822.43	7.635	0.006 (0.001-0.023)	43.59 (36.01-47.05)
null	1	913.85	106.209	0 (0-0)	0 (0-0)
(b) Wet season					
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag} + holidays$	7	444.46	0.000	0.352 (0.261-0.422)	51.45 (45.98-55.68)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag} + holidays$	6	446.47	0.301	0.239 (0.182-0.316)	51.23 (45.76-55.19)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays$	6	449.07	1.918	0.121 (0.06-0.195)	49.27 (44.45-53.50)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays$	5	450.38	2.638	0.094 (0.041-0.138)	48.82 (44.35-53.21)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$	6	449.14	3.784	0.053 (0.023-0.093)	48.62 (42.72-52.56)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$	5	450.35	4.814	0.041 (0.016-0.073)	47.96 (42.53-52.03)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1}$	5	452.21	6.164	0.015 (0.005-0.039)	46.63 (41.19-51.46)
null	1	505.58	60.029	0 (0-0)	0 (0-0)

(c) Dry season	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$ + holidays	7	308.10	0.000	0.253 (0.192-0.362)	26.61 (21.9-31.43)
$tide_{lag2} + rain_{lag2} + rain_{lag1} + RRv_{lag}$ + holidays	6	308.95	0.471	0.17 (0.119-0.225)	25.67 (20.83-30.78)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$ + holidays	6	309.60	0.750	0.139 (0.082-0.19)	24.47 (19.96-29.72)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + RRv_{lag}$ + holidays	6	311.44	2.425	0.073 (0.024-0.13)	24.22 (20.04-27.8)
$rain_{lag2} + rain_{lag1} + RRv_{lag}$ + holidays	5	309.88	2.416	0.072 (0.028-0.132)	23.07 (19.19-28.54)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$	6	313.52	5.351	0.019 (0.006-0.053)	21.44 (16.74-25.94)
$rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$	5	313.73	6.481	0.01 (0.002-0.042)	20.4 (15.34-24.52)
$tide_{lag2} + rain_{lag2} + rain_{lag1} + RRv_{lag}$	5	313.94	6.503	0.009 (0.002-0.032)	20.15 (14.73-24.73)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + RRv_{lag}$	5	316.04	7.390	0.006 (0.001-0.018)	19.79 (14.89-23.89)
$rain_{lag2} + rain_{lag1} + RRv_{lag}$	4	314.31	8.822	0.003 (0.001-0.016)	18.46 (13.53-22.83)
holidays + RRv_{lag}	3	316.04	9.230	0.003 (0.001-0.008)	17.76 (13.78-22.51)
$tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1}$ + holidays	6	316.98	9.637	0.003 (0-0.05)	17.44 (13.49-22.48)
null	1	336.08	28.503	0 (0-0)	0 (0-0)

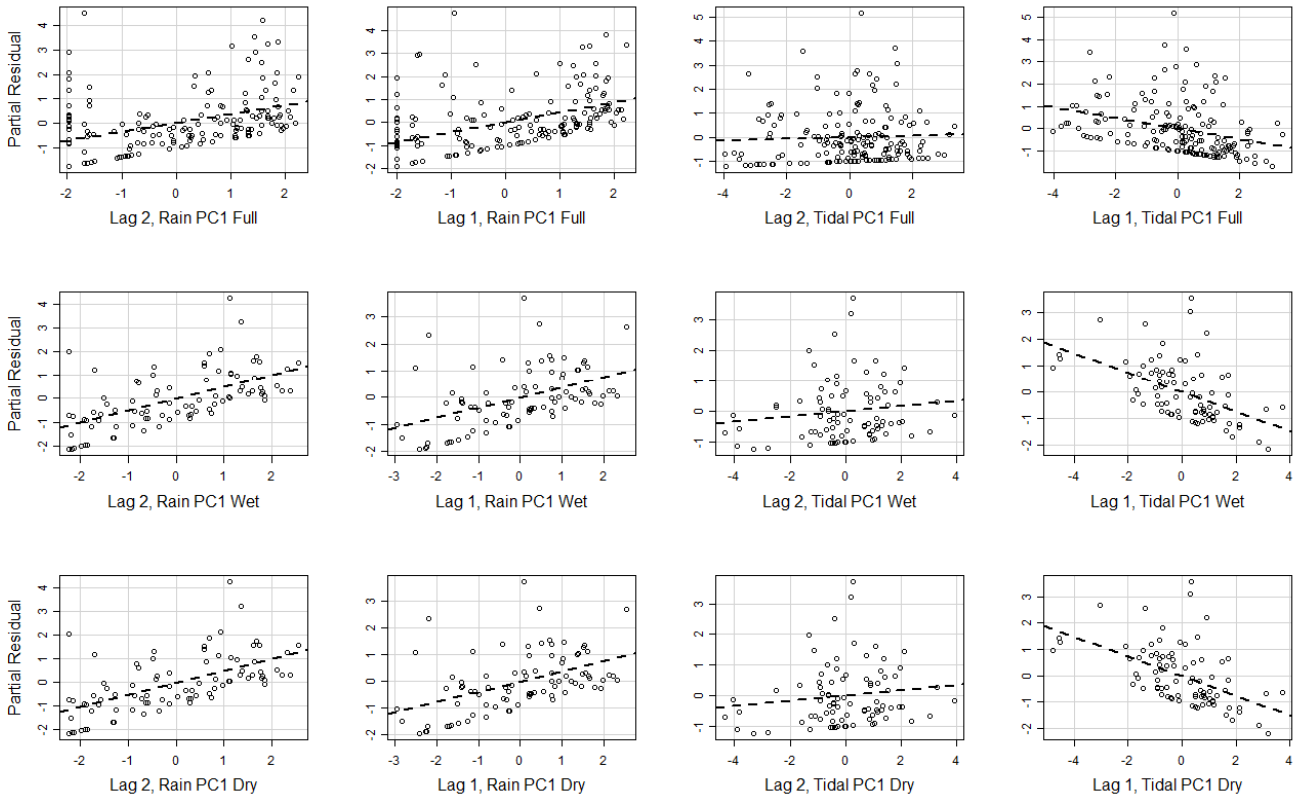

Figure 2 Partial residual plots derived from the highest-ranked environmental proxy models of monthly Ross River virus cases for the full time series (Full) and the wet seasons only (Wet) and dry seasons only (Dry). Variables examined are: first principal component of tidal data at monthly lags of 1 and 2, and first principal component of rain data at monthly lags of 1 and 2. Dashed lines indicate the least-squares relationship.

Combined models

Environmental proxy models ranked higher than the vector abundance models for the full and wet season datasets (Table 3a, b). For the dry season dataset, vector abundance models ranked higher than the environmental proxy models; however, there was strong evidence for the relationship between both types of vector abundance measures (environmental proxy or trap abundance) and Ross River virus (information-theoretic evidence ratio [*ER*] = 1.57, Table 3c). *Temperature* and *wetness* principal components were included in all the top-ranked models for the three datasets (Table 3a,b&c). Extrapolating the relationships through the PCAs, my models showed that Ross River virus increases with decreasing temperatures, and increases with increases in rainfall, number of rain days and vapour pressure (humidity). However, the relationship between Ross River virus and evaporation was different across model sets: for the full dataset and dry season, I found that Ross River virus decreases with increases in evaporation, whereas for the wet season model set Ross River virus increases with increases in evaporation (Fig 3). I also found evidence for relationships between residual immunity (Ross River virus lag) or human exposure (public holidays), and Ross River virus for all three model sets (Table 3a,b,&c).

Table 3. Comparison of the top-ranked environmental proxy models and vector trap models and the null models used to assess change in monthly Ross River virus cases for (a) the full time series (January 1991 to December 2005) (b) the wet season time series (November to April, 1991 to 2005), and (c) the dry season time series (May to October 1991 to 2005). Explanatory variables considered are: $tide_{lag2}$ = 2 month lag of tidal first principal component, $tide_{lag1}$ = 1 month lag of tidal first principal component, $rain_{lag2}$ = 2 month lag of rain first principal component, $rain_{lag1}$ = 2 month lag of rain first principal component, CxA_{lag1} = 1 month lag of mean monthly *Culex annulirostris* trap abundance, CxA = mean monthly *Culex annulirostris* trap abundance, AeV_{lag1} = 1 month lag of mean monthly *Aedes vigilax* trap abundance, AeV = mean monthly *Aedes vigilax* trap abundance, temp = temperature principal components, wet = wetness principal components, season = wet or dry, holidays = number of public holidays per month and RRv_{lag} = peak Ross River virus cases from the previous wet season. Shown are the number of fitted model parameters (*k*; includes intercept), and the median score (range of score in parentheses) from 100 block bootstrap iterations for Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE). See Appendix 4b for full model sets.

Models Full	k	Median AIC_c	Median ΔAIC_c	Median $wAIC_c$	Median %DE
(a) Full dataset					
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag} + temp + wet	10	822.74	0.000	0.135 (0.078-0.203)	48.89 (44.88-52.52)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag} + temp	9	822.96	0.556	0.098 (0.059-0.146)	48.75 (44.69-52.21)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag} + temp + wet	9	822.81	0.557	0.097 (0.068-0.149)	48.7 (44.7-52.42)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag} + temp	8	823.04	1.184	0.075 (0.048-0.11)	48.53 (44.52-52.17)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + RRv_{lag} + temp + wet	9	823.70	2.431	0.048 (0.012-0.09)	47.91 (44.23-51.81)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + RRv_{lag} + temp + wet	8	823.78	2.905	0.041 (0.009-0.07)	47.73 (43.69-51.61)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + RRv_{lag} + temp	8	824.58	3.462	0.035 (0.007-0.067)	47.54 (43.69-51.02)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + RRv_{lag} + temp	7	824.71	3.784	0.031 (0.004-0.051)	47.32 (43.44-50.88)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag} + wet	9	827.29	5.789	0.012 (0.002-0.044)	47.04 (43.14-50.6)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag} + wet	8	827.95	6.360	0.009 (0.001-0.039)	46.98 (42.9-50.49)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag}	8	827.88	6.335	0.008 (0.001-0.038)	46.97 (42.87-50.47)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag}	7	828.49	6.646	0.007 (0.001-0.028)	46.86 (42.71-50.24)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag} + temp + wet	10	823.29	7.183	0.006 (0-0.117)	46.74 (42.63-51.1)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag} + temp	9	823.57	7.712	0.004 (0-0.069)	45.93 (42.07-51.06)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag} + temp + wet	9	824.38	7.847	0.004 (0-0.068)	46.57 (42.39-51.03)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + RRv_{lag} + wet	8	828.26	7.468	0.004 (0-0.019)	46.07 (42.84-50.02)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag} + temp	8	824.51	8.082	0.004 (0-0.046)	45.86 (41.89-51.01)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + RRv_{lag} + wet	7	829.09	8.175	0.003 (0-0.013)	46 (42.65-49.9)
Null	1	929.95	115.419	0 (0-0)	0 (0-0)

(b) Wet season	k	Median AIC_c	Median ΔAIC_c	Median $wAIC_c$	Median %DE
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + temp + wet	11	434.99	0.000	0.212 (0.002-0.314)	58.18 (53.65-62.00)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + temp + wet	10	435.30	0.848	0.174 (0.001-0.251)	58.06 (53.26-61.64)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + temp + wet	10	436.36	3.064	0.069 (0-0.202)	57.09 (52.95-60.96)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + temp + wet	11	442.27	3.935	0.036 (0-0.298)	54.91 (49.82-61.58)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag} + temp + wet	10	443.07	5.874	0.023 (0-0.176)	54.16 (49.00-61.43)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + temp	9	439.63	7.704	0.007 (0-0.084)	56.07 (50.68-59.50)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + temp	8	440.25	7.882	0.007 (0-0.071)	55.87 (50.51-59.47)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + temp + wet	10	444.79	9.022	0.005 (0-0.062)	53.54 (48.68-59.44)
CxA_{lag1} + AeV_{lag1} + AeV + holidays + RRv_{lag} + temp + wet	10	445.06	9.693	0.003 (0-0.034)	52.91 (48.50-59.79)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + temp	8	441.07	9.367	0.002 (0-0.024)	54.38 (50.23-58.40)
Null	1	506.35	75.461	0 (0-0)	0 (0-0)
(c) Dry Season					
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + temp + wet	10	297.72	0.000	0.185 (0.069-0.335)	36.46 (30.51-41.54)
CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + wet	9	298.43	1.456	0.109 (0.028-0.23)	35.07 (30-39.82)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + temp + wet	10	300.50	1.557	0.081 (0.01-0.169)	32.95 (28.59-37.04)
AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + temp + wet	9	297.98	2.718	0.073 (0.028-0.153)	35.19 (29.38-40.26)
$tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + wet	9	301.51	2.618	0.056 (0.004-0.129)	32.16 (27.74-36.53)
$tide_{lag2}$ + $rain_{lag2}$ + $rain_{lag1}$ + RRv_{lag} + holidays + temp + wet	9	300.92	3.037	0.045 (0.005-0.093)	31.99 (28.16-36.34)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + temp + wet	9	301.20	3.560	0.043 (0.006-0.112)	31.47 (27.49-36.67)
AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + wet	8	299.37	3.575	0.039 (0.009-0.096)	33.75 (28.87-37.82)
$tide_{lag2}$ + $rain_{lag2}$ + $rain_{lag1}$ + RRv_{lag} + holidays + wet	8	302.04	4.291	0.026 (0.003-0.073)	31.21 (26.78-36.19)
$rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + wet	8	302.53	4.696	0.023 (0.003-0.067)	30.67 (26.6-36.14)
Null	1	335.23	40.319	0 (0-0)	0 (0-0)

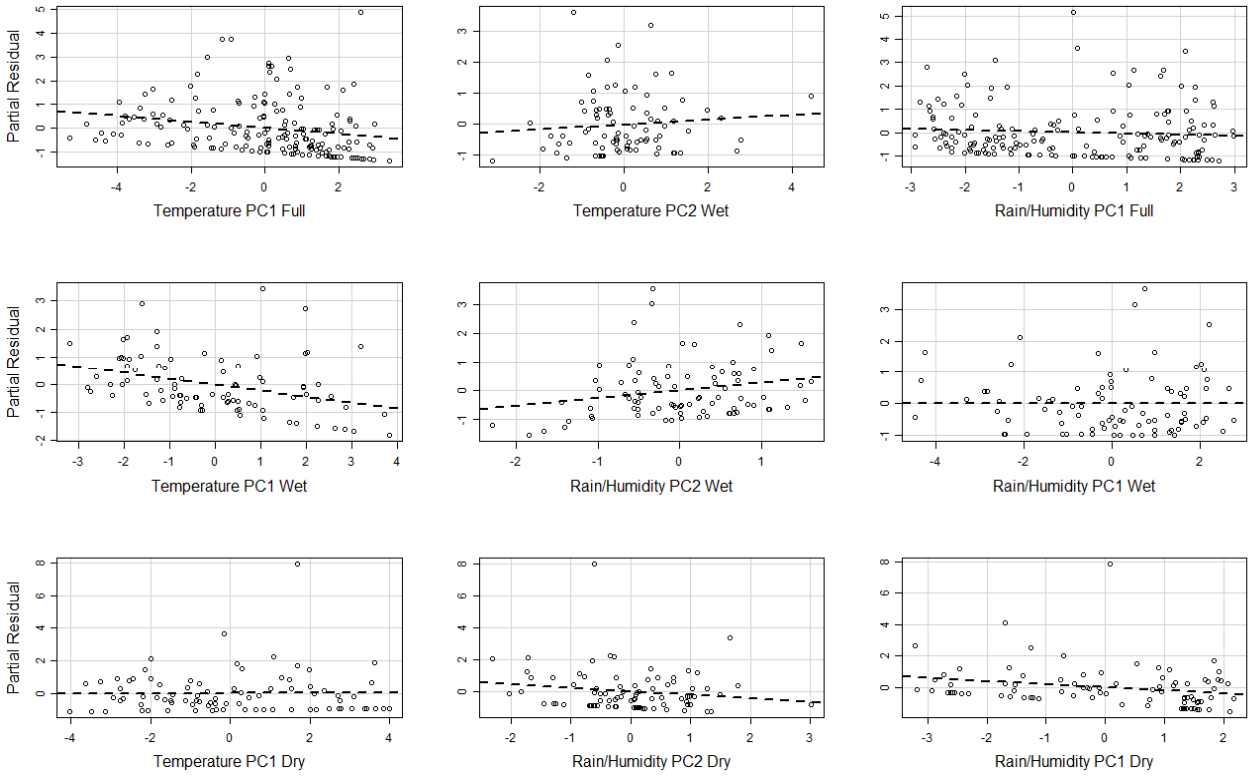

Figure 3 Partial residual plots derived from the highest-ranked vector trap abundance and environmental proxy models of monthly Ross River virus cases for the full time series (Full) and the wet seasons only (Wet) and dry seasons only (Dry). Variables examined are: first and second principal components of temperature data, and first and second principal components of wetness (Rain/Humidity) data. Dashed lines indicate the least-squares relationship.

Validation

The model-averaged predictions of Ross River virus cases overall and in the wet season using the full dataset trap abundance models were the most accurate (Table 4). Dry season cases were equally well predicted by the full dataset environmental proxy and combined model sets. While accuracy of wet season outbreak predictions was relatively high for all models except the combined wet season model set, the sensitivity of all models was low (Table 5). The wet season environmental proxy models were the best at predicting case outbreaks, and the wet season mosquito trap abundance models had the highest positive predictive value (Table 5, Figure 4). All the model sets predicted wet season non-outbreak months better than outbreak months.

Models	**Total**	**Wet**	**Dry**
Full dataset Proxy	59.88	112.83	6.92
Full dataset Trap	26.33	38.92	13.75
Full dataset Combined	57.38	107.83	6.92
Wet Season Proxy		134.58	15.67
Wet Season Trap		60.17	21.42
Wet Season Combined		237.83	16.50
Jacups *et al.* (2008b) Global	52.30	90.40	14.20

Table 4 Mean squared error between measured monthly Ross River virus cases and predicted monthly Ross River virus cases from 2006-2008. Model sets used to calculate model averaged predictions were: Proxy = only environmental proxy models, Trap = only vector trap abundance models, and Combined = both environmental proxy models and vector trap abundance models. Full dataset models were developed using a 15 year monthly dataset from 1991 to 2005, and Wet season models were developed using only the wet season months (November to April) from the 1991 to 2005 dataset. Jacups et al. 2008b Global = a global multivariate Poisson model developed by Jacups et al. (2008b).

Epidemic Wet	Accuracy	Sensitivity	Specificity	Positive pred. value	Negative pred. value
Full dataset Proxy	75 %	0	100 %	0	0.75
Full dataset Trap	75 %	33 %	89 %	0.50	0.80
Full dataset Combined	75 %	0	100 %	0	0.75
Wet Season Proxy	83 %	67 %	89 %	0.67	0.89
Wet Season Trap	83 %	33 %	100 %	1.00	0.82
Wet Season Combined	58 %	0	78 %	0	0.70
Jacups *et al.* (2008b) Global	75 %	33 %	89 %	0.50	0.80
Climate Change Model	67%	0	89%	0	0.73

Table 5 Sensitivity parameters of model-averaged predictions for wet season monthly Ross River virus outbreaks between 2006 and 2008 in Darwin. Accuracy = the percentage of outbreaks and non-outbreaks correctly predicted, Sensitivity = the proportion of outbreaks that the model correctly identifies as outbreaks, Specificity = the proportion of non-outbreaks that the model correctly identifies as non-outbreaks, Positive predictive value = the probability that an outbreak predicted by the model really is an outbreak, Negative predictive value = the probability that a non-outbreak predicted really is a non-outbreak. Model sets used to calculate model averaged predictions of outbreaks were: Proxy = only environmental proxy models, Trap = only vector trap abundance models, and Combined = both environmental proxy models and vector trap abundance models. Full dataset models were developed using a 15 year monthly dataset from 1991 to 2005, and Wet season models were developed using only the wet season months (November to April) from the 1991 to 2005 dataset. Jacups et al. 2008b Global = a global multivariate Poisson model developed by Jacups et al. (2008b). Climate Change Model = model averaged predictions from an environmental proxy model set developed for the wet season dataset using only variables for which there are projected climate change predictions for northern Australia.

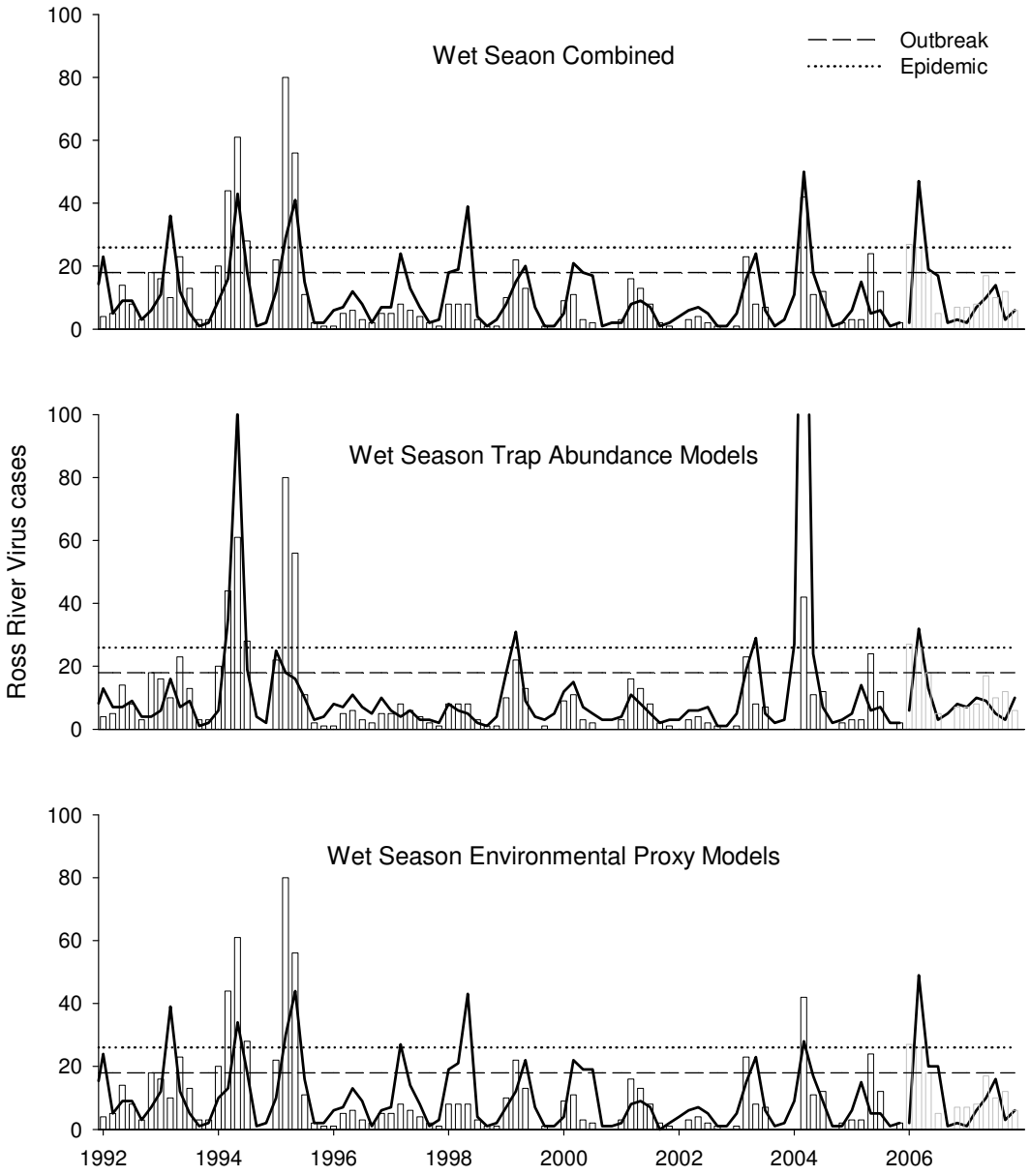

Figure 4 Model-averaged predictions for Ross River virus (lines) and actual Ross River virus cases (bars) for the time series data used to develop models (black bars) and the validation data (grey bars), wet season only (November to April). Model sets used for model averaging include only environmental proxy models, only vector trap abundance models and a model set combining both environmental proxy and vector trap abundance models. Dashed lines indicate the cut-off point for a wet season Ross River virus outbreak and epidemic.

Climate Change Projections

To gain insight into the projected effects of climate change on Ross River virus outbreaks in Darwin, I developed simplified versions of my wet season environmental proxy models (models that gave the most accurate predictions of wet season outbreaks), using only variables for which there are projected climate change predictions for northern Australia (www.climatechangeinaustralia.com.au). I included these variables in simplified models and found that the model-averaged predictions of wet season outbreaks were not accurate (Table 5). Therefore, I did not progress to predicting virus case incidence under full climate change scenarios.

Discussion

When attempting to predict highly complex systems such as vector-borne disease expression, there is no one answer. This is an excellent case for using multi-model inference and model averaging. Model averaged predictions incorporate the complexity of the system by allowing predictions to be made from every model considered, and these separate model predictions are weighted according to their distance from reality (Burnham and Anderson, 2002). Using full model uncertainty in my predictions, I conclude that while highly informative for vector ecology and pest management, adult vector abundance estimates derived from monitoring data might not be necessary to predict vector-borne disease risk. If one has a sound ecological understanding of the conditions driving local vector population dynamics, it is possible to use environmental proxies of adult vector abundance that are as effective (or more) at predicting disease epidemics. This particularly useful for the poverty-stricken tropical countries that carry 95% of the global burden of vector-borne disease, as, if there is already knowledge of the ecology of the local vector species, the limited available funding need not be diverted into costly on-going vector monitoring programs (World Health Organization, 2004).

In any given year in northern Australia, the majority (80%) of Ross River virus cases occur during the wet season (November to April). Therefore, predicting wet season outbreaks

is arguably more important than predicting annual cycles of case peaks and troughs, or accurately predicting dry season cases. While my models explained a relatively lower amount of variation in the case time-series data compared to other studies of Ross River virus (see Table 2; Gatton et al., 2005; Woodruff et al., 2006; also Jacups et al., 2008b), my wet-season specific environmental proxy models are the most accurate at predicting the highest priority health statistic: wet season outbreaks of virus cases. Adult vector monitoring systems attempt to capture information on fluctuating vector population sizes in areas of human habitation; however, the reality is that there is a disconnect between these measured vector numbers and conditions that are important for disease transmission such as adult vector survival, vectorial capacity, percentage of vectors infected, and their exposure to humans (Glass, 2005; Adams and Kapan, 2009). This is because these disease transmission conditions are highly affected by vector body size, which in turn is dependent on the larval development environment, rather than measurements of adult vector population size (Chapter 4; Agnew et al., 2002; Gimnig et al., 2002; Manoukis et al., 2006; Bevins, 2008; Gavotte et al., 2009; Reiskind and Lounibos, 2009). The environmental proxies used in my models actually represent the environmental carrying capacity, or adult vector production, of the larval habitats (Chapter 3). If the relationship between the environmental drivers of larval habitats and subsequent adult vector production has previously been quantified, environmental proxies may in fact be a more accurate representation of the adult vector population dynamics important for disease transmission (vector population size, adult survival and dispersal capacity) than a residential adult vector monitoring system (Chapters 2, 3, and 4).

For all my models, most relationships between Ross River virus case numbers and the vector variables were as expected, and have been previously identified. High incidence of the virus is associated with high adult abundance and increases in the environment's larval vector carrying capacity (higher tides, more rainfall) (Russell, 2002; Woodruff et al., 2002; Gatton et al., 2005, Tong et al., 2005; Hu et al., 2006a; Woodruff et al., 2006; Jacups et al., 2008a,b; Tong et al., 2008; Williams et al., 2009; McIver et al., 2010). *Aedes vigilax* populations

exhibit strong endogenous control, and previous studies of *Ae. vigilax* population dynamics in Darwin show patterns of strong negative feedback (Chapter 2; Yang et al., 2008a,b; Yang et al., 2009). Therefore, the negative relationships exhibited in my models (between 1 month lagged *Ae. vigilax* trap numbers and virus cases, and between the tidal proxies of 1 month lagged *Ae. vigilax* abundance and virus cases) are artefacts of these intrinsic population dynamics, emphasising the importance of fully understanding the complexities of vector population dynamics when modelling vector-borne disease.

Along with variables related to adult vector abundance, my models also incorporated variables related to other aspects of Ross River virus epidemiology, such as human exposure and residual host immunity. The major vertebrate hosts for Ross River virus in the Northern Territory are the new juvenile populations of agile wallabies *Macropus agilis* and dusky rats *Rattus colletti* (Jacups et al., 2008a). Here, juvenile recruitment and population growth in both reservoir species are driven by seasonal rainfall (Madsen and Shine, 1999; Shine and Brown, 2008). I contend that including lags of rainfall in my models provides surrogate information in this regard. The negative relationship between both measures of *Ae. vigilax* abundance (both trap and environmental proxy) and RRv incidence indicates that this vector species might be involved in zoonotic amplification cycles in the wallaby and rat populations, rather than acting as a major RRv vector to humans *per se*. Human exposure to infected vectors is an essential component of predicting incidence of any vector-borne disease, however it is difficult to quantify (Glass, 2005; Woodruff et al., 2006; Adams and Kapan, 2009; Carver et al., 2010). Nonetheless, rainfall lags and proxies of human exposure risk included in my models did not explain much of the variation in virus cases, even though they represent important aspects of Ross River virus epidemiology. Quantifying all sources of potential variation in annual RRv cases is impossible; however, two relevant sources of year-to-year variation not included in my models were: variability in public health efforts, and the cycles of zoonotic host immunity. Models incorporating data on these two effects might provide more accurate predictions of annual RRv incidence.

Most vector-borne diseases have a complex epidemiology and the relationships between the many drivers of disease incidence are difficult to define. Tools such as meta-model manager (Miller and Lacy, 2003) that can link demographic population models to epidemiological models of disease, allows one to parameterise the complex dynamics of disease transmission and then link this to ecologically realistic population models of vectors and hosts, giving a more holistic and accurate understanding of disease dynamics. A detailed knowledge of both the disease epidemiology and vector and host ecology is imperative for the development of sophisticated quantitative predictive tools such as this.

In epidemiological systems such as mosquito-borne disease, where the ecology of vectors is driven by climate conditions, the potential for climate change to alter dynamics is of concern. Indeed, there are many modelled projections of the effects of climate change on mosquito populations and pathogen activity of various vector-borne diseases (Zhang et al., 2008; Russell, 2009; Russell et al., 2009a; Tonnang et al., 2010; Ramasamy and Surendran, 2011). I found that climate-change simplified model predictions of current wet season outbreaks were not accurate, and therefore did not progress to predicting virus case incidence under full climate change scenarios. This example gives further evidence for the importance of accurately capturing the complexity of the regional ecology and epidemiology in models attempting to predict vector-borne disease either for the near future or for climate change (Jacups et al., 2008a; Russell, 2009).

I conclude that adult vector trapping abundances are not necessarily the most effective predictors of vector-borne disease outbreaks, and a detailed understanding of the intrinsic and extrinsic drivers of vector population dynamics and disease epidemiology can provide ecologically accurate environmental proxies that are a cheaper and effective alternative to adult vector monitoring for predicting outbreaks of vector-borne disease. Progress in successfully addressing the problem of global vector-borne disease now and in the future will rely upon our ability to understand this complexity to the extent that mitigation measures can be planned and implemented in an intelligent and cost-effective manner.

CHAPTER 6

CONCLUSIONS

Mosquito-borne disease systems are highly complex to monitor and manage, and although there is a wealth of knowledge on different vector species and correlated disease transmission, rarely is the effectiveness of different management options for these systems scrutinised in detail. Sophisticated models based on both the dynamics of long-term time series of species-specific larval and adult mosquito abundances, and the sensitivities of experimentally quantified mosquito vital rates to intrinsic feedback mechanisms and environmental variation, are excellent tools for both accurate prediction of disease outbreaks (Chapter 5) and evaluating different mosquito and disease management options (Chapter 2, Chapter 3, Chapter 4). Once the ecological knowledge required to parameterise models completely has been collated, models such as these can provide a cost-effective way of examining different mosquito control options (Chapter 3), or even negate the need for difficult and expensive ongoing larval and adult mosquito surveys (Chapter 2 and Chapter 5). This is particularly pertinent for poverty-stricken tropical developing countries that carry over 95 % of the global burden of mosquito-borne disease (Chapter 1) because rarely can extensive monitoring programmes be funded internally.

The importance of quantifying the different compensatory and depensatory intrinsic processes influencing mosquito population dynamics, and how these intrinsic processes interact with the local environmental variation and control programmes, is often overlooked. Organisms such as mosquitoes that have recruitment-driven population dynamics experience strong compensatory effects of population density on different vital rates, even at relatively low densities (Agnew et al., 2002; Sibly et al., 2005). Failing to account for these intrinsic feedback mechanisms can lead to vector control programs that do not suppress mosquito populations in the long term (Chapter 2), or might even produce mosquitoes capable of higher rates of disease transmission (Chapter 4).

Mosquito larvae are particularly vulnerable to control as they are restricted to ephemeral

aquatic habitats. Changes in local micro-climatic conditions affect the survival and development of larval stages and it is therefore important to quantify the relationships between mosquito production (emergence) and juvenile development, and local environmental conditions, and how these vary spatially and temporally (Chapter 2 and Chapter 3). Variation in habitats can also affect compensatory intrinsic feedback processes (e.g. larval competition for nutrients or adult competition for oviposition sites), as the amount of resources available can change across different habitat types or in different seasons (Chapter 2 and Chapter 3). This further emphasises the importance of developing a detailed understanding of species-specific vital rates and how these change with intrinsic processes (compensatory feedbacks) and extrinsic processes (environmental variation) and any interactions between the two, to develop effective and targeted control programs (Chapter 2, Chapter 3 and Chapter 4).

Recently there has been a focus on predicting changes in disease transmission and mosquito population size and range in relation to predicted climate change (Campbell-Lendrum and Woodruff, 2007; Zhang et al., 2008; Kearney et al., 2009; Lafferty, 2009; Jansen and Beebe, 2010; Tonnang et al., 2010). However, the projected variables available from climate change models based on 21st-Century emissions scenarios – changes in temperature, rainfall and sea levels – fall short of fully capturing the detailed environmental parameters needed to predict accurately changes in mosquito vital rates and population sizes (Chapter 5). Therefore, care should be taken when interpreting changes in mosquito-borne disease systems predicted from climate change models.

Darwin mosquito control: current and future

Mosquito control program

The current program of mosquito control in Darwin has been operating since the 1980s. As a result, many ecological data describing the local mosquito ecosystem, including long-term (> 10 years) time series of weekly adult trapping and monthly larval sampling abundance data,

are now available. To make reliable inference about larval habitat use, mosquito population dynamics, and the subsequent recommendations for efficient mosquito control and mosquito-borne disease management, long time series over a large spatial scale such as these are crucial.

Based on these data, my research exposed several essential aspects of this complex ecosystem that are directly applicable to current mosquito management programmes. First, my large-scale field experiments revealed that the main mosquito control method currently employed, aerial application of the microbial larvicide *Bti*, is effective at suppressing immediate adult emergence of *Ae. vigilax* across a range of habitat types. Other larval control options examined such as vegetation removal by burning or slashing, were not as effective as larvicide at controlling emergence (Chapter 3). I also determined that larvicide application methods should not ignore low-density larval sites because resource competition at the larval stage and resultant compensation means that low-density habitats have the potential to produce adult mosquitoes that live longer, fly farther and are more fertile. Rather, to benefit from the effects of high-density larval competition, larval control should be applied to the later stages of juvenile development, at the 4th instar or pupal stages (Chapter 4). *Aedes vigilax* and *Cx. annulirostris* populations respond differently to this type of control; for *Ae. vigilax*, larvicide application during the larval stage will ensure suppression of the entire generation, and therefore this method is relatively cost-effective for this species. However, for species such as *Cx. annulirostris* that slowly build population density over longer periods rather than exhibiting monthly peaks in emergence like *Ae. vigilax*, I recommend repeated applications of larvicide over at least fortnightly intervals to suppress each generation (Chapter 2; Chapter 3).

Despite the quantified effectiveness of larvicide application, the adult and larval population dynamics of both *Cx. annulirostris* and *Ae. vigilax* reveal little to no long-term effects of the current and past program of opportunistic larvicide application and environmental modification (Chapter 2; Yang et al., 2008a). This has likely arisen in part

from the strong compensatory density feedbacks in population size and growth rate exhibited by these species (Chapter 2; Chapter 4; Yang et al., 2008a,b; Yang et al., 2009). It might be the case that control reductions in larval density are reducing the density compensation pressure on adult survival and fertility, and therefore, actually increasing the population growth rate (Juliano, 2007). This hypothesis is supported by Yang *et al.* (2008a) who found phenomenological evidence for elevated survival or fertility in generations following periods of low larval density as drivers of population growth rates, and also my spatiotemporal models of larval abundance (Chapter 2) and manipulation experiments (Chapter 4); These provided direct evidence for the main mechanism of compensation: competition for resources during the larval stage at high densities results in smaller emerging adults with lower survival, longevity and fertility.

To explore further the complexities of over-compensatory mortality resulting from ineffective control programs, I suggest that a logical next step, although beyond the scope of my thesis, is to construct demographic matrix models that accurately incorporate quantified effects of compensatory density feedbacks and the estimated mortality from control measures for the populations of both these species. This would be necessary to design an effective mosquito control programme that results in active reduction of the adult mosquito population over time.

Adult and larval sampling methods

Monitoring data such as larval surveys and adult trapping that are collected to aid mosquito management programs are imperative for making immediate and informed decisions. However, monitoring data of this sort often fall short of quantifying the variability inherent in ecological systems in sufficient detail. Before reliable inference can be made from any sampling procedure that purports to measure relative abundance, it is important to quantify whether the sampling procedure is accurately capturing the variance of the sampled population. My experiment examining the effectiveness of the current larval sampling

procedure (dipping) (Chapter 3), revealed this sampling procedure does not adequately represent the variance in larval abundance or adult emergence. There was little correlation between sampled larval abundances and adult emergence numbers, and at best, the sampling method could only quantify larval presence or absence (Chapter 3). Therefore, future larval surveys that apply this sampling method should be aware of this limitation and restrict their inferences of abundance patterns accordingly. This is yet another argument for a broader application of larvicide across all possible larval habitats, rather than attempting to target high density larval habitats during control operations as has previously been suggested and practiced (Gu et al., 2008).

Sampling issues are common in ecological datasets; indeed, there are more problems associated with the time series I used to quantify spatiotemporal variation in larval habitats. Larval survey data are collected opportunistically based on unquantified information about adult population dynamics, tide and rainfall patterns, and larval habitat ecology. This dataset therefore lacks the necessary sampling rigour to allow sophisticated analyses aimed at decoupling the various drivers of the mosquito population dynamics within this system, as shown by the low explanatory power of the models (Chapter 2). These problems are also true of the weekly adult trapping time series in residential Darwin. While these data are collected following a more rigorous regime of weekly trap setting, the traps only provide a measure of relative abundance rather than true measures of total population size. Therefore, caution needs to be employed when interpreting the results from models of mosquito-borne disease patterns because there is a disconnect between these measures of relative mosquito abundance and the mosquito population parameters that are important for disease transmission, i.e., adult mosquito survival, vectorial capacity, percentage of vectors infected, and their exposure to humans (Chapter 5; Glass, 2005; Adams and Kapan, 2009). Indeed, when comparing models of relative trapped adult mosquito abundance to models that incorporated detailed ecological proxies of adult mosquito population size, I found that relative abundance from trapping programs were not the most effective predictors of Ross River virus outbreaks in humans

(Chapter 5). Rather, a detailed understanding of the intrinsic and extrinsic drivers of mosquito population dynamics and disease epidemiology can provide ecologically accurate environmental proxies of mosquito population parameters, with the corollary that these proxies are a cheaper alternative to adult mosquito monitoring for predicting outbreaks disease.

A population ecology five-step plan for mosquito control

Sophisticated, spatially explicit, demographic models of mosquito population dynamics require a large amount of data to estimate parameters and validate predictions. While much of this ecological knowledge is readily available, additional field and/or laboratory surveys and experiments are usually required to create accurate local models predicting the responses by particular mosquito species. Once constructed, such models can indicate potential control strategies that are most likely to be successful given local conditions, highlighting any potential problems before they occur, saving time and money, and identifying areas for further research. Given unlimited resources, this is the five-step plan I would propose to create an effective mosquito control programme:

Step 1. *Quantify species-specific vital rates to understand the contribution of different life history stages to potential population growth.*

a. Using both laboratory and field experiments, measure egg survival and development, larval survival and development, pupae survival and development, adult survival and longevity, sex ratio, proportion of autogenous females, fertility of autogenous and anautogenous females.

b. Using CO_2 -baited adult mosquito traps placed at emergence and oviposition sites, and at different distances in urban locations, conduct mark- recapture experiments and laboratory studies on captured females that quantify the size of the adult female population, the proportion of the population feeding, the

range of body sizes present in the population and the dispersal capacity of adults with different body sizes.

Step 2. *Quantify the sensitivity of various vital rates to compensatory and depensatory intrinsic feedback mechanisms to understand how population growth rate will respond to changes in adult or larval densities.*

a. Examine time series of relative abundances for evidence and strength of compensatory density feedback.
b. Using both phenomenological models and laboratory and field experiments, define mechanisms of density feedbacks (i.e., larval competition, competition for oviposition sites, etc.).
c. Using manipulation experiments, quantify effects of set densities on vital rates (e.g., per cent change in larval mortality, per cent change in adult survival and fertility).
d. Examine evidence for depensation, and the Allee threshold for the local population, using laboratory experiments that explore possible Allee effects that may be expressed, e.g. mate-limitation, larval predator satiation, oviposition site selection (Williams et al., 2008b; Tobin et al., 2011). Once depensatory mechanisms or Allee effects have been determined for a population, use long-term multi-generational laboratory experiments that manipulate these factors to determine the Allee threshold.

Step 3. Quantify the sensitivity of various vital rates to micro-climatic conditions and how these change in space and time to define habitats and months of high and low mosquito production and therefore, effectively target control programs.

a. Using field experiments, quantify any sampling measures (larval or adult) that will be used to help parameterise the model.

b. Using time series of larval data, construct regional larval habitat suitability predictions.

c. Using field experiments, define the average production of adults per unit area in the absence of control.

d. Using laboratory and field experiments, quantify predation rate and any effects of inter-specific competition on survival and development of juvenile stages (egg, larval, pupal) using laboratory experiments of different ratios of predators to larvae, and different densities of larval competitors, and also in the field, comparing emergence rates from predator exclusion traps (see Chapter 3) to similarly constructed traps made of wire or mesh with large enough holes to allow predator, competitor, and mosquito larval immigration and emigration.

e. Using laboratory and field experiments, quantify the effects of regional environmental conditions (temperature, humidity) and water qualities (temperature, pH, salinity, nutrients) on juvenile stage (eggs, larvae, pupae) survival and development, and adult stage survival, longevity and fertility.

Step 4. *Quantify the effects of control on targeted vital rates.*

Quantify the effects of any current or possible future control program, e.g., if control consists of the application of larvicide, quantify larval and pupal mortality rates and changes to emergence rates and sizes of adults; or if control is in the form of environmental manipulation (e.g., vegetation removal, draining swamp habitat, reducing water containers in and around residential areas), quantify changes in available larval habitat and larval densities, survival and development, and adult emergence rate per unit area.

Step 5. *Build fully parameterised, spatially explicit, stage-specific stochastic population models*.

a. Build a matrix population model that includes the various mosquito life stages (i.e., egg, larval, pupal, adult emergence, autogenous adult, anautogenous adult), and density-dependent vital rate parameters based on previously quantified vital rates and sensitivities to compensatory and depensatory feedback processes in the absence of control programs (see methods in Caswell, 2001). Attach this model to spatio-temporal habitat suitability models that incorporate changes in the micro-climatic conditions that affect vital rates (Brook et al., 2009). Perform stochastic sensitivity analysis to determine which vital rates most affect population growth, and therefore, how best to apply mosquito control and management systems. For example, if it was determined by sensitivity analysis that adult female survival was the most important life stage for population growth, then the best management option would be targeted effective larval control that suppresses adult emergence across all habitats, as the aquatic larval stage is easier to target than adult vectors that have the ability to disperse over a large spatial scale (Killeen et al., 2002a).

b. Incorporate previously quantified changes in vital rates and population size due to different control methods in models to (*i*) examine the effectiveness of current control methods, (*ii*) assess the effectiveness of any previously quantified novel methods, and (*iii*) determine if there is any possibility of using control methods to push the local population below an Allee threshold and into decline to extinction.

c. Use the model to examine changes in population size due to climate change predictions.

d. Couple the demographic model to epidemiological models to predict outbreaks of disease, and examine the sensitivity to disease transmission parameters to variation in different mosquito vital rates.

Improving the Darwin mosquito control programme

The ecology of the two main vector species in Darwin has been well-studied and along with the main environmental drivers of larval habitat for each species, there have been many studies defining various vital rates (Table 1, Fig 1).

Here I will follow my population ecology five-step plan for mosquito control, step by step, and define the areas that my research has contributed to such model building for the two major vector species in Darwin.

Step 1. *Quantify species-specific vital rates to understand the contribution of different life history stages to potential population growth.*

The egg, larval and pupal survival and development rates for *Ae. vigilax* and *Cx. annulirostris* have already been quantified in a range of different environmental conditions (Table 1). The sex ratio of emerging *Ae. vigilax* adults in Darwin is 1.2:1 (Chapter 4). Hugo *et al.* (2003) studied the relationship between body size (wing length) and fertility and autogeny rates of female *Ae. vigilax* adults. Extrapolating from this work using measured wing lengths (Chapter 4), I estimated the fertility and autogeny rates of female *Ae. vigilax* emerging from different habitats in the Leanyer/Holmes Jungle swamp complex (Table 2, Fig. 2). Adult survival of *Ae. vigilax* has been poorly quantified, and considering the importance of this parameter for disease transmission (Garrett-Jones, 1964), it will be important for future research to quantify the survival rates and longevity of adults, and how these vary with body size, using CO_2 trapping over time and mark-recapture experiments, where larvae and/or emerging adults are marked with dyes or fluorescent dust, and traps set up at various distances from the emergence site and from urban areas. Adult survival rates and population size can be calculated from the proportions of marked individuals in the population over time (Service, 1993).

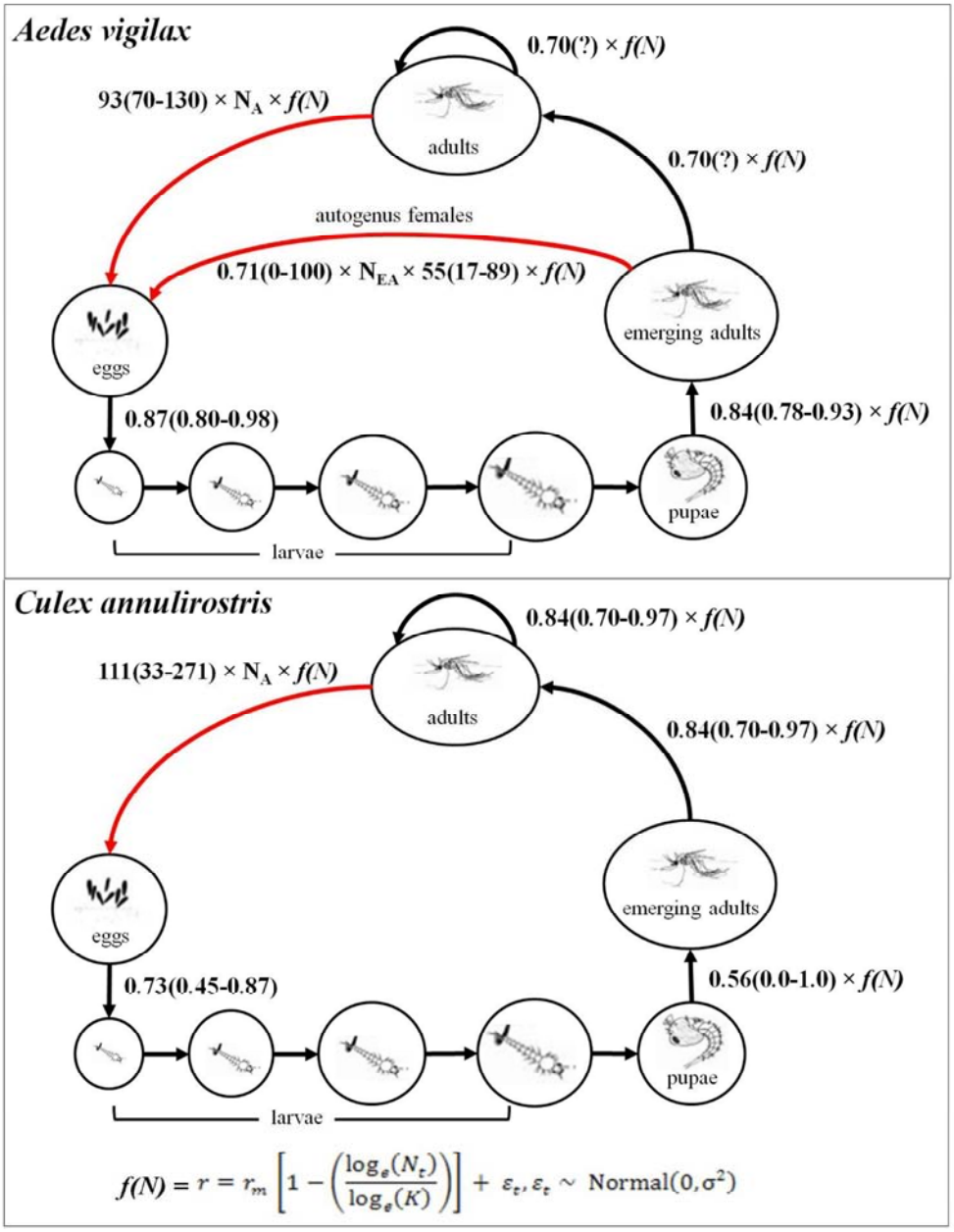

Figure 1 Demographic stage-structured life cycle graph for *Aedes vigilax* and *Culex annulirostris*. Mean values of parameters are reported, and the range is shown in parentheses. Note: parameter values of vital rates will vary with environmental conditions (e.g., temperature, humidity, etc.). N_A = number of females, N_{EA} = number of emerging females, and *f(N)* = Gompertz-logistic model of population growth, where *r*= realised population growth rate, r_m = maximal intrinsic *r*, *K* = carrying capacity, N_t = mosquito population size at time *t*, and ε_t = environmental variability. Autogeny (capability of developing the first batch of eggs without a blood meal) is negligible in *Culex annulirostris* (only 7% of females), and therefore was not included in the life cycle for this species.

	Aedes vigilax	*Culex annulirostris*
autogeny rate	70.6% (0-100)	7.1% (0-30.9)
gonotrophic cycle number	3 cycles	5.5 days (5-6)
gonotrophic cycle length	3 days (2-4)	
autogenous gonotrophic cycle length	3.1 days (2.3-3.6)	
fecundity (eggs per female)	93 eggs (70-130)	111 eggs (33-271)
autogenous fecundity	55 eggs (17-89)	
egg senescence	116 days	
egg development rate	2.1 days (2-2.3)	2 days (1-3)
egg survival	87.4% (79.8-98)	72.5% (45.0-86.7)
larval survival	83.6% (77.5-93)	56.3% (0-100)
larval density	6550/m2 (0-33300)	1295/m2 (8-9025)
1st larval instar to adult development rate	9 days (5-20)	16.1 days (7.1-37)
adult survival	70%	84.2% (70-96.5)
body size (wing length)	2.81 mm (2.00-3.48)	2.74 mm (1.18-3.80)
adult dispersal	52 km (0-100)	8.3 km (6.8-12)
density dependence	GL, 12.76% change in *r*	GL, 26.90% change in *r*

Table 1 Demographic life history parameters for *Aedes vigilax* and *Culex annulirostris* (see supplementary material for references). Mean values of parameters are reported, and the range, recorded over a range of temperatures, is shown in parentheses. GL = Gompertz-logistic model of population growth, *r* = population growth rate. Note: parameter values of vital rates will vary with environmental conditions (e.g., temperature, humidity, etc.). References for *Aedes vigilax* and *Culex annulirostris* vital rates: 21, 31, 37, 41, 59, 71, 88, 97, 98, 99, 106, 107, 111, 125, 127, 128, 129, 141, 142, 149, 185, 186, 192, 193, 194, 204, 210, 220, 228, 250, 256, 267.

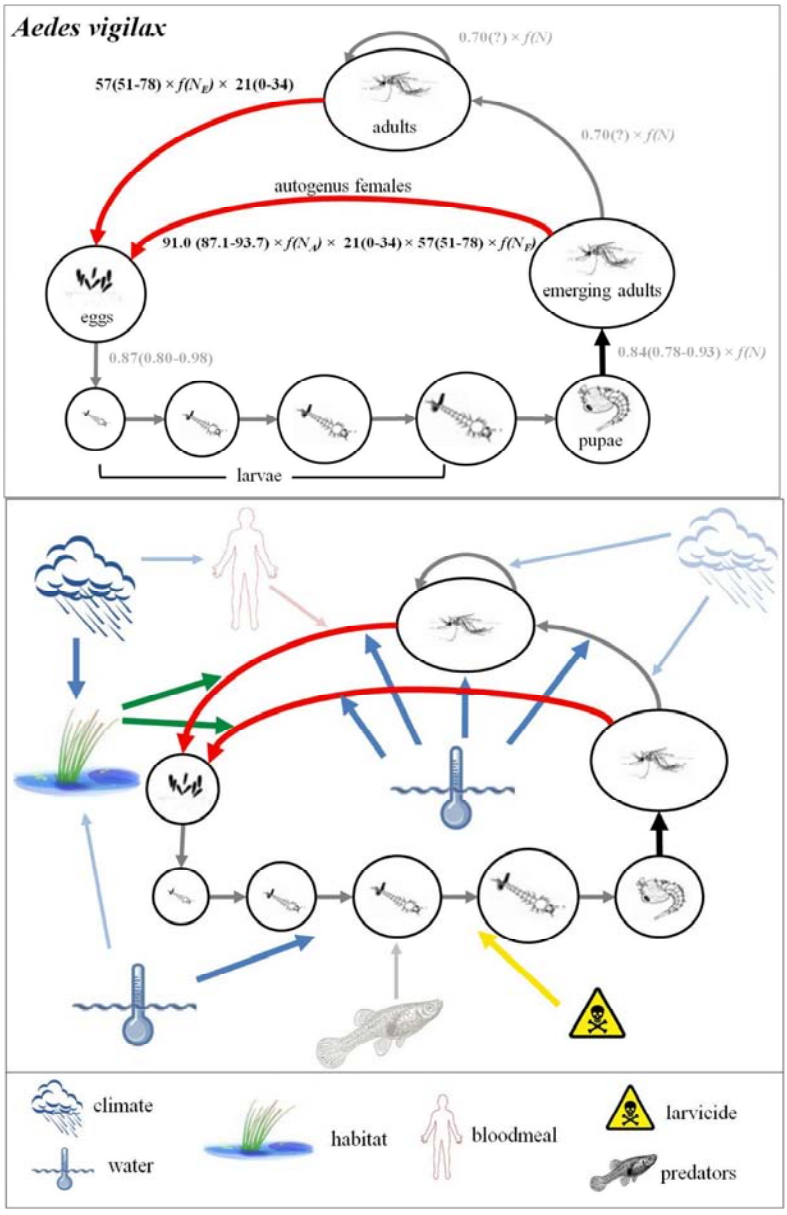

Figure 2. Demographic stage-structured life cycle graph for *Aedes vigilax.* Mean values of parameters are reported, and the range is shown in parentheses. $f(N_F)$ represents the quantified effects of larval density on fertility, $f(N_A)$ represents the quantified effects of larval density on autogeny rates, and $f(N)$ represents the quantified effects of larval density on survival (for values see Table 2). Note: parameter values of vital rates will vary with environmental conditions (e.g., temperature, humidity, etc.). The various environmental variables that affect vital rates are shown: **climate** = season, rainfall, temperature and tides; **water** = water qualities such as pH, salinity, temperature and nutrition level; **habitat** = available larval habitat (number of ephemeral pools, vegetation type); **bloodmeal** = probability of acquiring a blood meal (dispersal); **insecticide** = mosquito control measures (insecticides, larvicides); and **predators** = aquatic predators of larvae. Bold arrows, text and diagrams indicate vital rates and intrinsic and extrinsic processes quantified by my research.

Larval habitat	Emergence	Fertility	Autogeny
Eleocharis reeds	15.6 (16.8)	57	93.7
mangrove forest	15.2 (14.8)	51	91.4
mangrove edge	26.6 (32.9)	78	92.0
Schoenoplectus reeds	29.6 (25.7)	42	87.1

Table 2. *Aedes vigilax* vital rates across different larval habitats. Emergence = mean number of adults emerging m^{-2} (standard deviation in parentheses), Fertility = mean number of eggs per female, Autogeny = mean autogeny rate (%) of females rate per larval habitat (extrapolated from Hugo et al., 2003). See Appendix 5 for calculations of fertility and autogeny rates.

Step 2. *Quantify the sensitivity of various vital rates to compensatory and depensatory intrinsic feedback mechanisms to understand how population growth rate will respond to changes in adult or larval densities.*

Compensatory density feedbacks have previously been identified as explaining just under half of the total explainable variation in population growth of *Ae. vigilax*, and 27 % of the variance in population growth in *Cx. annulirostris* (Yang et al., 2008a,b). The population growth rates of both species best follows the Gompertz-logistic model, indicating rapid and strong feedback as soon as population size begins to increase (Yang et al., 2008b). It was previously hypothesised that the main mechanism of this compensation is reduction in survival and fertility in generations following periods of high density; however, these mechanisms had not been quantified (Yang et al., 2008a).

Spatio-temporal modelling of larval abundance for *Ae. vigilax* and *Cx. annulirostris* revealed that the strongest temporal drivers of larval abundance for both vector species were 1-month lagged logarithm of adult density and 1-month lagged rainfall (Chapter 2). There was

a positive relationship between adult numbers in the previous month and larval density in the following month, suggesting the major mechanism of compensatory density feedback in these species is not competition for blood meals, harbourage or oviposition during the adult life stage – such interactions would lead to lower larval densities after a peak in adult density. Instead, my models show that the main regulatory mechanism in *Ae. vigilax* and *Cx. annulirostris* occurs during the larval life stage where lower to medium larval densities will result in higher adult emergence, survival, and fertility (Chapter 2).

Using field manipulation experiments, I was able to verify the mechanism of compensatory density feedback in the life history of *Ae. vigilax* was indeed larval competition for resources leading to a reduced body size of emerging adults, with corollary effects on adult survival, longevity and fertility (Chapter 3). Although trap failure limited my ability to quantify the relationship between larval density and adult emergence size, the remaining results give some indication of how vital rates such as fertility and autogeny rates change in response to larval density (Table 3).

Density	Emergence	Wing length	Fertility	Autogeny
High	72	2.48 (0.07)	24	54.1
Low	34	2.64 (0.07)	36	80.0

Table 3. *Aedes vigilax* vital rates from high and low larval density. Emergence = number of adults emerging m^{-2}, Wing length = proxy for adult body size (mm) (standard deviation in parentheses), Fertility = mean number of eggs per female (extrapolated from Hugo et al., 2003), Autogeny = mean autogeny rate (%) of females rate per larval habitat (extrapolated from Hugo et al., 2003). See Appendix 5 for calculations of fertility and autogeny rates.

I did not attempt to identify any Allee effects or the Allee threshold for *Ae. vigilax* or *Cx. annulirostris*. Williams *et al.* (2008b) demonstrated an Allee effect of oviposition preference in the container-breeding species *Aedes aegypti*. Oviposition preference is unlikely

to be an Allee effect in the ephemeral pool-breeding *Ae. vigilax* and *Cx. annulirostris*. One way of determining the presence of Allee effects in these species would be to apply phenomenological depensation models to abundance time series (Fig. 3). If local population extinction is the aim of control measures, these would be important depensatory feedbacks to investigate in the future.

Figure 3 An example data set showing the fit of five population growth models to data for the lilac beauty moth (*Apeira syringaria*) where the Ricker-Allee model of population growth (unbroken blue line) is top-ranked. Taken from Figure 1 in Gregory *et al.* (2010).

Intrinsic (density feedback) and extrinsic (environmental) processes act together to moderate population size and growth rates. It is important to understand the degree of interaction between these processes to have a more complete understanding of the reasons for variation in population densities (Saether, 1997; Wang et al., 2006; de Little et al., 2007). Spatio-temporal models identified that intrinsic (adult density) and extrinsic (rainfall, elevation) were equally important in describing the variation in larval abundance of *Ae. vigilax* and *Cx. annulirostris* (Chapter 2). Field experiments examining the effect of different larval densities across habitat types on emergent adult size identified different relationships

between density and body size in different vegetation types (Chapter 3). The hypothesised reason for this is possible differences in available nutrients across habitat types. Further study investigating nutrient concentrations across the different habitat types would allow models to incorporate the interactions between different larval densities and available resources and how this affects adult survival, longevity and fertility. Methods such as those used by the container-inhabiting mosquito simulation models (CIMSiMs) parameterise this relationship effectively, where the interaction between available resources and larval density is modelled as 'available nutrients for weight gain per larva', and larval and pupal development weights are then directly proportional to both the survival of the juvenile stages, and the emerging body size of adults (Focks et al., 1993a,b; Focks et al., 1995; Williams et al., 2008a; Magori et al., 2009; Williams et al., 2010; Xu et al., 2010).

Step 3. *Quantify the sensitivity of various vital rates to microclimatic conditions and how these change in space and time to define habitats and months of high and low mosquito production and to design effective target control programs.*

To quantify the capacity of a landscape to support mosquito populations, the demographic population model of mosquito life history should ideally be linked to spatio-temporal habitat models that predict the distribution of suitable habitat (Brook et al., 2009). As expected, the most suitable habitats of the saltwater-breeding *Ae. vigilax* were defined spatially by lower elevations where the swamp complex is more influenced by tides, and temporally by seasonal tide height and frequency. During the late dry season, high tides regularly flood large areas of the swamp complex, and the ephemeral tidal pools allow enough time for the development of *Ae. vigilax* and the emergence of adults before drying to expose mud for oviposition (Chapter 2). My models revealed that the most suitable larval habitats of the obligate freshwater breeder *Cx. annulirostris* were defined spatially and temporally by the transition of the coastal swamp to freshwater wetlands. At higher elevations, areas in the swamp complex that are inundated by rainfall and freshwater runoff are not as affected by tidal regimens; once rainfall

reaches a certain threshold during the early wet season, the swamp complex becomes flooded with fresh water, and the wetlands become suitable for *Cx. annulirostris* breeding (Chapter 2).

For both species studied, elevation was the dominant spatial driver of larval abundance (Chapter 2). Elevation determines the fine-scale depths of larval habitats and the vegetation characteristics in each of the water depth zones (Shaman et al., 2002). Mosquito larvae are commonly associated with areas of ephemeral water because deeper or more permanent water can allow greater predator access and can subject the larvae to tidal flushing (Dale et al., 2002; Chase and Knight, 2003). The resolution of elevation data included in the analyses (10-cm increments) was much finer than that commonly available in geographic information system analyses (Shaman et al., 2002; Zeilhofer et al., 2007) indicating the importance of such fine-scale resolution for realistic and accurate larval habitat suitability models.

Both *Ae. vigilax* and *Cx. annulirostris* are generalists that can breed, develop and emerge from a wide variety of habitats (Sinclair, 1976; Lee et al., 1984; Lee et al., 1989). This is evidenced by the weak statistical support for vegetation type as a driver of larval abundance for either species (Chapter 2). This finding was further supported by the similar emergence rates across all vegetation types observed in field experiments (Chapter 3). I further defined the carrying capacity of various larval habitats through these field experiments (Chapter 3) where I quantified the average production of emerging adults m^{-2} in the absence of control measures and predators across different habitat types (Table 1, Fig 1).

Field experiments (Chapter 3) revealed that although there was some influence of different microclimate conditions (water temperature, pH, salinity and dissolved oxygen), my models did not identify one single microclimate attribute as the principal driver of larval presence or adult emergence of *Ae. vigilax* (Fig. 1). These experiments confirmed that during the spring tides, the Leanyer/Holmes Jungle swamp complex acts as an ideal *Ae. vigilax* larval incubating environment; *Ae. vigilax* are able to breed effectively in a wide range of temperatures and salinities (Sinclair, 1976; Lee et al., 1984), and the maxima and minima of the microclimate parameters measured fell well within these ranges. Therefore, generation

times for this species in Darwin will be as rapid as developmental instar progression allows (Chapter 3).

While my research did not quantify larval mortality due to predation, the emergence traps used for all experiments actively excluded predators (see Chapter 2 for diagram). Therefore, predation mortality did not contribute to the quantified emergence rates of adult *Ae. vigilax* in the absence or presence of control. It is important to quantify the different types of larval mortality (predation, resource limitation, artificial control) in isolation to parameterise population models seeking to explore the different effects of these mortalities on mosquito population size (Juliano, 2007; Lord, 2007).

Step 4. *Quantify the effects of control on targeted vital rates.*

Field experiments quantified the effects of aerial larvicide (*Bti*) application on adult *Ae. vigilax* emergence from several different habitats (Chapter 3). This method of larvicide application, if applied effectively to all habitats supporting larval densities, will suppress 95% of emerging adults (increase larval to adult mortality by 95%). Control methods involving disruption of oviposition sites and larval habitats through vegetation removal suppressed adult emergence by only 41 to 50 %, making these methods unsatisfactory as effective mosquito control due to the effects the strong compensatory feedbacks in larval densities exhibited by *Ae. vigilax* (Chapters 2 and 3), and therefore, the possibility that this control mortality will be over-compensatory (Juliano, 2007).

Further Research

Step 5. *Build spatially explicit, stage-specific stochastic population models.*

My research has made a major contribution to quantifying the species-specific vital rates and sensitivities of those vital rates to local intrinsic and extrinsic processes driving *Ae. vigilax* population dynamics in Darwin (Fig 2). This species is one of the two main vectors of Ross River virus, a mosquito-borne disease that is a major contributor to Australia's burden of

disease (Carver et al., 2010). My analyses of Ross River virus incidence in Darwin revealed that a sound understanding of the ecological drivers of mosquito population dynamics can predict disease outbreaks more effectively than relative adult vector abundances measured by a residential vector monitoring system (Chapter 5); this is further confirmation of the importance of building sophisticated models of mosquito populations that have locally quantified parameters to aid in the prediction and management of mosquito-borne diseases.

Further research should focus on quantifying the epidemiologically important vital rates for *Ae. vigilax* (adult survival, longevity, and feeding rates), and whether any depensatory feedbacks exist in these populations. Once these parameters have been defined, the next step is building a fully parameterised, spatially explicit, stochastic stage-structured population model. While well beyond the scope of my PhD, this step would culminate our current understanding into a global model capable of predicting mosquito and disease responses to a variety of management interventions. Sensitivity analysis of such models will further allow managers to determine the best mosquito life stages to target for control, and whether any control methods could force local vector population extinction. These models can also create a framework for examining possible changes in vector population size and range due to different climate change scenarios.

In particular, quantifying the percentage of larval density reduction required to avoid undesirable compensatory effects that outweigh the advantages of reduced adult emergence is an important step for any vector control program. This relationship is highly complex, and incorporates the effects of component density feedback (influence of larval density on adult survival and fertility), ensemble density feedback (influence of larval density on the population growth rate), the vectorial capacity of the population, and the influence of adult survival and body size on vector competence. A detailed understanding of all of these aspects of the vector-disease system and how they respond to environmental conditions would be essential to model these relationships. For a species such as *Ae. vigilax* that has no relationship between body size and vector competence (Jennings and Kay, 1999), and because

the Darwin populations exhibit a log-linear relationship between population density and growth rate (Yang et al., 2008a), the next step would be to determine the nature of the component density feedback: for example, whether adult survival is independent of larval density, declines linearly with larval density, or declines log-linearly with larval density. Once this relationship between adult survival and larval density is established, it will be possible to determine if there is a threshold point of larval density to target with control methods to acheive a desired adult survival rate/population size balance to minimise disease transmission risk.

Tools that can link sophisticated models produced by different methods (see Miller and Lacy, 2003) will allow the parameterisation of the complex dynamics of disease transmission to be linked to ecologically realistic population models of mosquitoes and hosts, and give a more holistic and accurate understanding of disease dynamics. A detailed knowledge of both the disease epidemiology and vector and host ecology is imperative for the development of sophisticated quantitative predictive tools such as this.

However, the practical application of this five-step plan is probably exceedingly ambitious for most areas of vector-borne disease management, due to the major research effort that would be required over many years to provide the necessary data to put this plan into action in contrast to the urgency with which vector management solutions are required in the face of increasing disease incidence. Although some small areas with manageable vector populations, such as Darwin, do lend themselves well to this approach, the reality is that vector control programs need immediate practical guidance for where, when, and how to intervene in the vector-disease-host cycle and reduce the possibility of a disease outbreak. Species- and location-specific studies relating mosquito abundance and life-history traits (e.g. frequency of female adult body size in the population), zoonotic host abundance and immunity cycles, and probability and intensity of human-vector contact, along with relatively simple heuristic models of transmission, might be more likely to provide less accurate, but important, answers in the short term to guide immediate vector management actions.

APPENDICIES

Appendix 1 Letters from the Department of Health and Community services stating the details of the $479172 in-kind contribution for the Australian Research Council Linkage Grant #LP0667619.

PART F—COLLABORATING ORGANISATION DETAILS

Part F must be completed for each Collaborating Organisation

F1 COLLABORATING ORGANISATION CONTACT DETAILS LP0667619

Organisation contact

Family name	Krause	**Title**	Dr
First name	Vicki	**Second name**	Lynne
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Organisation	Department of Health and Community Services						
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Postal address line 2							
Locality	Casuarina	**State**	NT	**Postcode**	0811	**Country**	Australia

Other organisation details

Australian Business Number (ABN)	84085734992	**ANZSIC**	863
Web page address (URL)	www.health.nt.gov.au		
Organisation Type	Government - State & Local		

F2 COLLABORATING ORGANISATION CONTRIBUTION

Amount committed ($)						
Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**TOTAL**	
10000	**10000**	**10000**	**0**	**0**	**30000**	**Cash**
159724	**159724**	**159724**	**0**	**0**	**479172**	**In-kind**
169724	**169724**	**169724**	**0**	**0**	**509172**	**Total**

F3 COLLABORATING ORGANISATION CERTIFICATION

- I certify that the above contribution will be made available to the project
- (For Government Agencies) No part of our cash contribution is drawn from funds previously appropriated from government sources for the purposes of research, evaluation and/or consultancy activity and
- I have read and understood the requirements in the Funding Agreement about collaborating organisation Agreements including the requirement to enter into arrangements for intellectual property.

F4 COLLABORATING ORGANISATION LETTER OF SUPPORT

Attach the letter of support from the collaborating organisation to this application.

Northern Territory Government
Department of Health and Community Services

Section: CENTRE FOR DISEASE CONTROL
Phone: 89228 510
Email: vicki.krause@nt.gov.au

Reference:
Facsimile: 89228 310
Mobile:

29 April 2005

Professor Robert Wasson
Pro Vice-Chancellor
Higher Education and Research
Northern Territory University
Darwin NT 0909

Dear Professor Wasson

I am writing to confirm that the Northern Territory Department of Health and Community Services supports the proposal *Modelling and control of mosquito-borne diseases in Darwin using long-term monitoring* (LP0667619) lodged with the Australian Research Council.

I confirm that my Department will provide cash support of $30,000 in the first year of the three year program. The cash will contribute to the employment of a gathering and analysing the relationship of notifiable mosquito-borne with environmental and ecological variables in the Darwin region.

Additionally my department will provide in-kind support exceeding $100,000 over 3 years as detailed in the proposal. In-kind support will include: (1) provision of departmental health data, (2) logistic support from the Medical Entomology Branch to undertaken field survey to map major engineering works designed to destroy mosquito habitats, (3) assistance in the compilation of mosquito trapping records, (4) undertaking targeted controlled spraying programs and associated monitoring of mosquito numbers to provide experimental verification of the prediction of the ecological models, and (5) the expert intellectual contributions of departmental health staff including medical entomologists, public health physicians and epidemiologists in the collection and interpretation of these data.

This level of support in time of special budgetary stringency is testament to the Northern Territory Department of Health and Community Services commitment to this project. We look forward to a successful outcome.

Yours sincerely

Dr Vicki Krause
Director, Centre for Disease Control
Northern Department of Health and Community Services

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Appendix 2 Comparison the full generalized linear mixed-effects model set used to assess monthly change from November 2000 to December 2006 in (a) *Aedes vigilax* and (b) *Culex annulirostris* larval densities. Explanatory variables are fixed effects: log*N* = log of adult abundance in the previous month, rain1 = rainfall in the previous month (mm), tide = monthly max tide (m), tide.f1 = frequency of tides above 7.4 m in the previous month, elevation = elevation above sea level at 10-cm resolution, spray = total spraying hours in the previous month, rain = total monthly rainfall (mm), and the random effect: (1|veg) = vegetation type (Mangrove, Mangrove Edge, Reeds and Grasses, Forest). Shown are median values from 1000 randomisations of the percent deviance explained by the fixed effects of the model (%DE), the Bayesian information criterion (BIC) and Akaike's corrected information criterion (AIC_c), and for each of these information criterions, the difference from best model (ΔBIC, ΔAIC_c), model weights (*w*BIC, *w*AIC_c) scaled relative to a total sum of 1, and the % of times each model was t ranked by *w*BIC and *w*AIC_c in 1000 randomisations.

(a) Model for *Aedes vigilax* larval presence	Median BIC	Median *w*BIC	Median ΔBIC	% top BIC	Median AIC_c	Median $wAIC_c$	Median ΔAICc	% top AIC_c	Median % DE
~ logN + rain1 + (1|veg)	67.50	0.524	0.000	0.923	46.91	0.105	0.157	0.459	23.0
~ tide + logN + rain1 + (1|veg)	70.18	0.113	3.020	0.064	46.70	0.099	0.502	0.332	28.8
~ tide.f1 + logN + rain1 + (1|veg)	71.58	0.060	4.315	0.013	48.11	0.059	1.859	0.077	25.4
~ elev + logN + rain1 +(1|veg)	71.93	0.053	4.681	0	48.46	0.048	2.052	0.007	24.1
~ spray + logN + rain1 + (1|veg)	72.03	0.050	4.838	0	48.56	0.045	2.105	0.008	24.1
~ rain + logN + rain1 + (1|veg)	72.23	0.048	4.909	0	48.75	0.042	2.112	0.003	23.6
~ rain + tide + logN + rain1 + (1|veg)	74.57	0.012	7.452	0	48.22	0.045	2.117	0.040	30.4
~ elev + tide + logN + rain1 + (1|veg)	74.65	0.012	7.559	0	48.31	0.044	2.183	0.014	29.9
~ spray + tide + logN + rain1 + (1|veg)	74.66	0.012	7.531	0	48.32	0.044	2.128	0.018	30.0
~ tide.f1 + tide + logN + rain1 + (1|veg)	74.75	0.012	7.563	0	48.41	0.042	2.128	0.002	29.6
~ spray + tide.f1 + logN + rain1 + (1|veg)	75.94	0.007	8.770	0	49.59	0.026	3.495	0.011	26.8
~ elev + tide.f1 + logN + rain1 + (1|veg)	76.08	0.006	8.754	0	49.73	0.026	3.467	0.012	26.4
~ rain + tide.f1 + logN + rain1 + (1|veg)	76.32	0.006	9.059	0	49.97	0.023	3.793	0.001	26.1
~ elev + spray + logN + rain1 + (1|veg)	76.51	0.005	9.215	0	50.17	0.020	3.888	0	25.3
~ rain + spray + logN + rain1 + (1|veg)	76.61	0.005	9.547	0	50.27	0.018	4.104	0	25.0
~ elev + rain + logN + rain1 + (1|veg)	76.70	0.005	9.430	0	50.36	0.019	4.030	0	24.8
~ rain + tide.f1 + tide + logN + rain1 +(1|veg)	78.99	0.001	11.941	0	49.79	0.019	3.952	0.005	31.1
~ elev + rain + tide + logN + rain1 + (1|veg)	79.00	0.001	11.919	0	49.80	0.020	3.929	0.001	31.6
~ elev + spray + tide + logN + rain1 + (1|veg)	79.03	0.001	11.869	0	49.83	0.020	3.891	0	31.4
~ spray + tide.f1 + tide + logN + rain1 +(1|veg)	79.04	0.001	11.958	0	49.84	0.019	3.963	0.004	31.0
~ rain + spray + tide + logN + rain1 + (1|veg)	79.06	0.001	11.925	0	49.86	0.020	3.936	0.002	31.5
~ elev + tide.f1 + tide + logN + rain1 + (1|veg)	79.16	0.001	11.965	0	49.96	0.018	4.022	0.002	30.7
~ elev + spray + tide.f1 + logN + rain1 + (1|veg)	80.27	0.001	13.137	0	51.07	0.012	5.007	0	28.2
~ rain + spray + tide.f1 + logN + rain1 + (1|veg)	80.57	0.001	13.484	0	51.37	0.010	5.339	0	27.6
~ elev + rain + tide.f1 + logN + rain1 + (1|veg)	80.79	0.001	13.525	0	51.59	0.010	5.417	0	27.1

(a) continued Model for *Aedes vigilax* larval presence	Median BIC	Median *w*BIC	Median ΔBIC	% top BIC	Median AIC_c	Median *w*AIC_c	Median ΔAICc	% top AIC_c	Median % DE
~ elev + rain + spray + logN + rain1 + (1|veg)	81.02	<0.001	13.938	0	51.82	0.008	5.780	0	26.3
~ rain + spray + tide.f1 + tide + logN + rain1 + (1|veg)	83.41	<0.001	16.406	0	51.37	0.009	5.539	0.001	32.7
~ elev + rain + spray + tide + logN + rain1 + (1|veg)	83.43	<0.001	16.238	0	51.39	0.009	5.421	0	33.1
~ elev + rain + tide.f1 + tide + logN + rain1 + (1|veg)	83.56	<0.001	16.415	0	51.52	0.009	5.542	0	32.6
~ elev + spray + tide.f1 + tide + logN + rain1 + (1|veg)	83.57	<0.001	16.273	0	51.53	0.009	5.512	0.001	32.4
~ elev + rain + spray + tide.f1 + logN + rain1 + (1|veg)	84.88	<0.001	17.804	0	52.84	0.005	6.852	0	29.0
~ elev + rain + spray + tide.f1 + tide + logN + rain1 + (1|veg)	87.79	<0.001	20.702	0	52.92	0.004	7.038	0	34.3
(b) **Model for *Culex annulirostris* larval presence**	**Median BIC**	**Median *w*BIC**	**Median ΔBIC**	**% top *w*BIC**	**Median AIC_c**	**Median *w*AIC_c**	**Median ΔAICc**	**% top *w*AIC_c**	**Median % DE**
~ logN + rain1 + (1|veg)	54.30	0.463	0	0.725	33.71	0.082	1.176	0.299	5.7
~ elev + logN + rain1 +(1|veg)	55.57	0.200	1.717	0.275	32.10	0.149	0.000	0.630	21.7
~ tide + logN + rain1 + (1|veg)	58.70	0.045	4.866	0	35.23	0.036	2.848	0.002	7.2
~ rain + logN + rain1 + (1|veg)	58.71	0.045	4.959	0	35.24	0.035	2.968	0.009	7.7
~ tide.f1 + logN + rain1 + (1|veg)	58.73	0.048	4.885	0	35.25	0.038	2.890	0.005	8.2
~ spray + logN + rain1 + (1|veg)	59.08	0.042	4.936	0	35.61	0.032	3.065	0	7.0
~ elev + rain + logN + rain1 + (1|veg)	60.15	0.020	6.257	0	33.81	0.065	2.042	0.026	24.0
~ elev + tide + logN + rain1 + (1|veg)	60.23	0.020	6.339	0	33.88	0.062	2.098	0.000	23.2
~ elev + tide.f1 + logN + rain1 + (1|veg)	60.28	0.020	6.329	0	33.93	0.061	2.087	0.028	23.6
~ elev + spray + logN + rain1 + (1|veg)	60.35	0.020	6.370	0	34.00	0.059	2.091	0	23.1
~ tide.f1 + tide + logN + rain1 + (1|veg)	63.12	0.005	9.321	0	36.77	0.018	4.328	0	10.5
~ rain + tide + logN + rain1 + (1|veg)	63.15	0.005	9.480	0	36.81	0.017	4.516	0	10.1
~ rain + tide.f1 + logN + rain1 + (1|veg)	63.28	0.004	9.756	0	36.94	0.015	4.736	0	9.9
~ spray + tide + logN + rain1 + (1|veg)	63.40	0.004	9.683	0	37.06	0.014	4.744	0	8.6
~ rain + spray + logN + rain1 + (1|veg)	63.47	0.004	9.708	0	37.13	0.014	4.857	0	9.1
~ spray + tide.f1 + logN + rain1 + (1|veg)	63.48	0.005	9.635	0	37.14	0.015	4.706	0	9.9

(b) continued **Model for *Culex annulirostris* larval presence**	**Median BIC**	**Median *w*BIC**	**Median ΔBIC**	**% top *w*BIC**	**Median AIC_c**	**Median $wAIC_c$**	**Median ΔAICc**	**% top $wAIC_c$**	**Median % DE**
~ elev + rain + tide + logN + rain1 + (1|veg)	64.66	0.002	10.707	0	35.46	0.028	3.697	0.001	26.4
~ elev + tide.f1 + tide + logN + rain1 + (1|veg)	64.84	0.002	10.888	0	35.64	0.025	3.947	0	25.7
~ elev + rain + spray + logN + rain1 + (1|veg)	64.85	0.002	10.979	0	35.65	0.026	3.879	0	25.4
~ elev + spray + tide.f1 + logN + rain1 + (1|veg)	64.87	0.002	11.021	0	35.67	0.024	3.987	0	25.3
~ elev + rain + tide.f1 + logN + rain1 + (1|veg)	64.88	0.002	11.014	0	35.68	0.025	3.983	0	25.3
~ elev + spray + tide + logN + rain1 + (1|veg)	64.89	0.002	10.976	0	35.69	0.024	4.115	0	24.9
~ rain + tide.f1 + tide + logN + rain1 +(1|veg)	67.31	0.001	13.878	0	38.11	0.008	6.083	0	14.0
~ spray + tide.f1 + tide + logN + rain1 +(1|veg)	67.85	<0.001	13.985	0	38.65	0.007	6.233	0	12.3
~ rain + spray + tide + logN + rain1 + (1|veg)	67.88	<0.001	14.178	0	38.68	0.006	6.344	0	11.4
~ rain + spray + tide.f1 + logN + rain1 + (1|veg)	68.04	<0.001	14.451	0	38.84	0.006	6.579	0	11.6
~ elev + rain + tide.f1 + tide + logN + rain1 + (1|veg)	69.14	<0.001	15.211	0	37.10	0.012	5.501	0	29.1
~ elev + rain + spray + tide + logN + rain1 + (1|veg)	69.33	<0.001	15.379	0	37.29	0.011	5.536	0	28.2
~ elev + spray + tide.f1 + tide + logN + rain1 + (1|veg)	69.36	<0.001	15.458	0	37.32	0.010	5.842	0	27.8
~ elev + rain + spray + tide.f1 + logN + rain1 + (1|veg)	69.56	<0.001	15.715	0	37.52	0.010	5.843	0	26.7
~ rain + spray + tide.f1 + tide + logN + rain1 + (1|veg)	71.94	<0.001	18.430	0	39.90	0.003	7.989	0	16.3
~ elev + rain + spray + tide.f1 + tide + logN + rain1 + (1|veg)	73.74	<0.001	19.755	0	38.88	0.005	7.219	0	31.6

Appendix 3a Comparison of the full model sets used to assess change in *Aedes vigilax* (a) larval presence and (b) adult emergence across four habitat types. Explanatory fixed effects are vegetation = vegetation type (mangrove forest, mangrove edge, *Schoenoplectus, Eleocharis*), larvae = median larval presence per trap, water = median trap water depth (mm), pc1 = first principal component of a principal components analysis of local environmental conditions, and larval models include trap ID as a random effect (1|trapID). Statistics shown are Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight ($wAIC_c$) scaled relative to a total sum of 1, and percent deviance explained by the fixed effects of the model (%DE).

Model	***k***	**AIC_c**	**ΔAIC_c**	***w*AIC_c**	**%DE**
(a) *Aedes vigilax* larval presence models					
water + vegetation + (1|trapID)	6	155.25	0	0.390	9.1
water + (1|trapID)	3	155.86	0.62	0.287	4.5
water + vegetation + pc1 + (1|trapID)	7	157.50	2.26	0.126	9.1
water + pc1 + (1|trapID)	4	157.91	2.66	0.103	4.6
vegetation + (1|trapID)	5	160.03	4.78	0.036	4.6
Null + (1|trapID)	2	160.82	5.58	0.024	0
vegetation + pc1 + (1|trapID)	6	161.36	6.12	0.018	5.2
pc1 + (1|trapID)	3	161.78	6.53	0.015	0.7
(b) *Aedes vigilax* adult emergence models					
water + larvae	3	406.55	0.00	0.267	28.1
water + larvae + pc1	4	406.70	0.15	0.247	28.8
larvae + pc1	3	406.85	0.30	0.229	28.0
vegetation + larvae	5	407.89	1.34	0.136	29.3
larvae	2	409.49	2.94	0.061	26.8
vegetation + larvae + water	6	410.90	4.35	0.030	29.6
vegetation + larvae + pc1	6	411.20	4.65	0.026	29.5
vegetation + larvae + water + pc1	7	415.57	9.02	0.003	29.7
vegetation	4	500.60	94.05	<0.001	7.6
vegetation + pc1	5	502.63	96.08	<0.001	8.0
vegetation + water	5	503.34	96.79	<0.001	7.8
vegetation + water + pc1	6	506.68	100.13	<0.001	8.0
pc1	2	523.08	116.53	<0.001	1.2
water + pc1	3	523.93	117.38	<0.001	1.7
water	2	524.25	117.70	<0.001	1.0
Null	1	525.97	119.42	<0.001	0

Appendix 3b Comparison of the full generalised linear mixed-effects model set used to assess change in *Aedes vigilax* (a) larval presence and (b) adult emergence with respect to different vector control methods. Fixed effects are time = before or after aerial larvicide application, spray = trap exposure to aerial larvicide application, state = vegetation state (burned, slashed or control), water = water depth (mm), pc1 = first principal component of a principal components analysis of local environmental conditions and larvae = median of total larvae sampled per trap. Larval models random effect = trap ID nested in vegetation type, adult models random effect=vegetation type. Statistics shown are Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight ($wAIC_c$) scaled relative to a total sum of 1, and percent deviance explained by the fixed effects of the model (%DE).

Model	*k*	AIC_c	ΔAIC_c	$wAIC_c$	%DE
(a) *Aedes vigilax* larval presence models					
water + spray	5	231.06	0	0.266	12.7
water + pc1 + time + spray	7	231.46	0.40	0.218	14.2
water + time + spray	6	231.77	0.71	0.187	13.3
water + pc1 + spray	6	232.07	1.01	0.161	13.2
water + pc1 + time + spray + spray*time	8	233.35	2.29	0.085	14.3
water + time + spray + spray*time	7	233.37	2.31	0.084	13.5
water + pc1	5	256.69	25.63	<0.001	2.6
pc1	4	256.84	25.78	<0.001	1.7
water	4	258.40	27.34	<0.001	1.1
Null	3	259.07	28.01	<0.001	0
(b) *Aedes vigilax* adult emergence models					
larvae + water + pc1 + spray + state	8	356.46	0	>0.999	57.1
larvae + pc1 + spray + state	7	387.18	30.71	<0.001	52.8
larvae + water + spray + state	7	390.52	34.06	<0.001	52.3
larvae + spray + state	6	425.53	69.07	<0.001	47.5
water + pc1 + spray + state	7	466.35	109.89	<0.001	42.7
water + spray + state	6	474.48	118.02	<0.001	41.2
pc1 + spray + state	6	522.61	166.15	<0.001	35.1
spray + state	5	534.18	177.72	<0.001	33.3
larvae + water + pc1	5	541.89	185.42	<0.001	32.3
larvae + pc1	4	561.62	205.16	<0.001	29.4
larvae + water	4	599.13	242.67	<0.001	24.6
Larvae	3	620.59	264.12	<0.001	21.6
water + pc1	4	765.33	408.87	<0.001	3.4
pc1	3	766.03	409.57	<0.001	3.0
Water	3	785.11	428.65	<0.001	0.5
Null	2	787.01	430.55	<0.001	0

Appendix 3c Comparison of the full model sets used to assess change in *Aedes vigilax* (a) larval presence and (b) adult emergence with respect to combinations of vector control methods. Fixed effects are T = time (before or after aerial larvicide application), Sp = spray (trap exposure to aerial larvicide application), state = vegetation state (burned, slashed or control), W = water depth (mm), pc1 = first principal component of a principal components analysis of local environmental conditions and larvae = median of total larvae sampled per trap. Larval models random effect = trap ID. Statistics shown are Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight ($wAIC_c$) scaled relative to a total sum of 1, and percent deviance explained by the fixed effects of the model (%DE).

Model	***k***	**AIC_c**	**ΔAIC_c**	**$wAIC_c$**	**%DE**
(a) *Aedes vigilax* larval presence models					
W + pc1 + Sp	5	155.60	0	0.495	25.9
W + pc1 + T + Sp + Sp*T	7	156.10	0.49	0.387	27.9
W + pc1 + T + Sp + state + Sp*T + Sp*state	11	158.56	2.96	0.113	31.2
W + T + Sp + Sp*T	6	165.85	10.24	0.003	21.8
W + T + Sp	5	167.62	12.02	0.001	19.8
W + + T + Sp + state + Sp*T + Sp*state	10	167.96	12.36	0.001	25.2
W + pc1	4	180.76	25.16	<0.001	12.0
pc1	3	182.59	26.99	<0.001	10.0
W	3	196.09	40.49	<0.001	3.2
Null	2	200.21	44.61	<0.001	0
(b) *Aedes vigilax* adult emergence models					
larvae + W + Sp + state	6	317.82	0	0.538	72.4
larvae + W + pc1 + Sp + state	7	318.33	0.51	0.417	72.7
larvae + W + Sp + state + Sp*state	8	323.79	5.96	0.027	72.5
larvae + W + pc1 + Sp + state + Sp*state	9	324.63	6.81	0.018	72.9
W + pc1 + Sp + state	6	352.62	34.79	<0.001	68.0
W + Sp + state	5	352.86	35.04	<0.001	67.5
W + Sp + state + Sp*state	7	356.82	38.99	<0.001	67.9
W + pc1 + Sp + state + Sp*state	8	357.45	39.62	<0.001	68.3
larvae + pc1 + Sp + state	6	371.05	53.22	<0.001	65.7
larvae + pc1 + Sp + state + Sp*state	8	375.81	57.98	<0.001	66.0
larvae + Sp + state	5	384.59	66.77	<0.001	63.6
larvae + Sp + state + Sp*state	7	389.89	72.07	<0.001	63.7
Sp + state	4	454.66	136.83	<0.001	54.4
pc1 + Sp + state	5	457.53	139.70	<0.001	54.4
Sp + state + Sp*state	6	459.43	141.61	<0.001	54.5
pc1 + Sp + state + Sp*state	7	462.86	145.03	<0.001	54.5
larvae + W + pc1	4	548.53	230.71	<0.001	42.5
larvae + pc1	3	572.79	254.96	<0.001	39.1

(b) adult emergence models continued	***k***	**AIC$_c$**	**ΔAIC$_c$**	***w*AIC$_c$**	**%DE**
larvae + W	3	585.43	267.60	<0.001	37.5
larvae	2	607.61	289.78	<0.001	34.4
pc1	2	876.03	558.21	<0.001	0.6
W + pc1	3	876.07	558.24	<0.001	0.9
Null	1	878.68	560.85	<0.001	0
W	2	878.72	560.89	<0.001	0.3

Appendix 4a Principal Components Analysis Results

For the full dataset, the first principal component (PC1) explained 76% of the variance in the *temperature* variable cloud, and all temperature variables (average daily temperature, average maximum temperature, absolute maximum temperature, average minimum temperature and absolute minimum temperature) increase with increasing values of the PC1 (Fig 1). For the wet season data, the first and second principal components (PC1 and PC2) explained 57 and 26% of the variance, respectively. Average daily temperature, average maximum temperature and absolute maximum temperature increase with increasing values of the PC1, while average minimum temperature and absolute minimum temperature increase with decreasing values of the PC2 (Fig 1). The PC1 of the *temperature* PCA for the dry season data explained 88 % of the variance, and again, all temperature variables increase with increasing PC1 values (Fig 1).

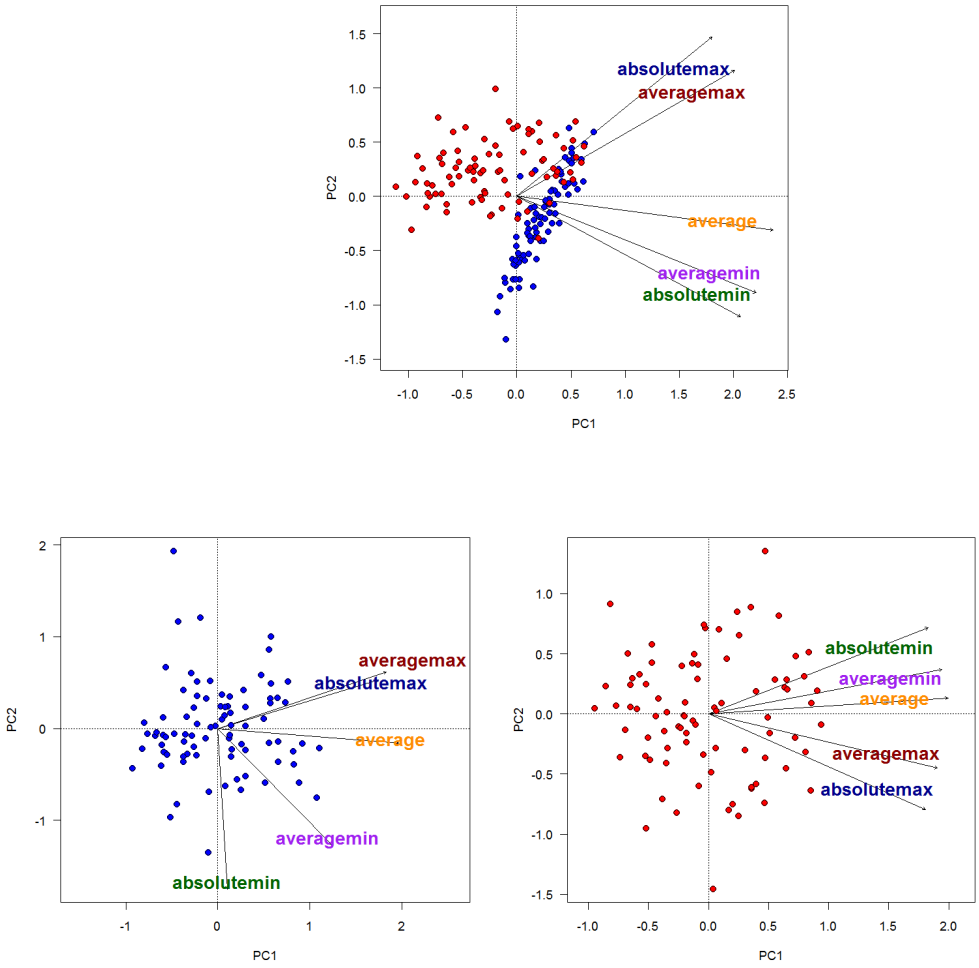

Figure 1 Principal components analysis of *temperature* data including: average monthly maximum temperature, maximum monthly temperature, average monthly temperature, average monthly minimum temperature and minimum monthly temperature; all temperatures measured in ^{o}C, for the full dataset, wet season dataset and dry season data set. Red points represent dry season data, blue points represent wet season data.

For the *wetness* PCA, results varied across the three different datasets. The PC1 of full dataset *wetness* PCA explains 78% of the variance, and rainfall, number of rain days and vapour pressure (humidity) all decrease with increasing values of PC1, while evaporation increases with PC1 (Fig. 2). For the wet season *wetness* PCA, the PC1 and PC2 explain 72% and 16% of the variance. Humidity, rainfall and rain days increase with increasing PC1, and evaporation increase with increasing PC2 (Fig. 2). In contrast, rainfall, rain days and humidity

all decrease with increasing PC1 in the dry season dataset and evaporation decrease with increasing PC2 (Fig. 2). The PC1 and PC2 explain 66% and 22% of the variance, respectively.

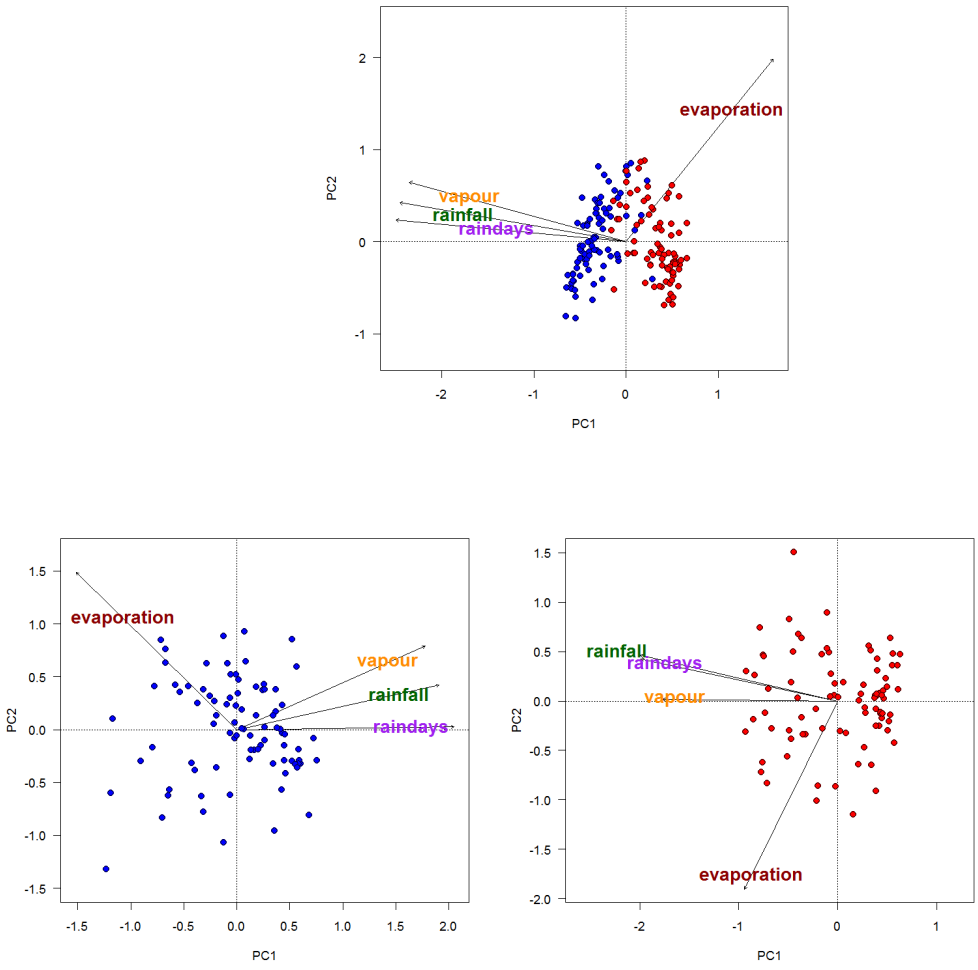

Figure 2 Principal components analysis of *wetness* data including: humidity measured as average vapour pressure (hPa), evaporation (mm), total monthly rainfall (mm), and number of rain days per month, for the full dataset, wet season dataset and dry season data set. Red points represent dry season data, blue points represent wet season data.

As expected, dataset-specific relationships between *rain* variables are also apparent. For the full dataset *rain* PCA, PC1 explained 98% of the variance and rainfall and rain days both increase with increasing values of PC1 (Fig. 3). The wet season PC1 (that explains 92% of the variance) also increases with increasing rainfall and rain days. However, for the dry season

dataset, rainfall and rain days decrease with increasing PC1, which explains 97% of the variance (Fig. 3).

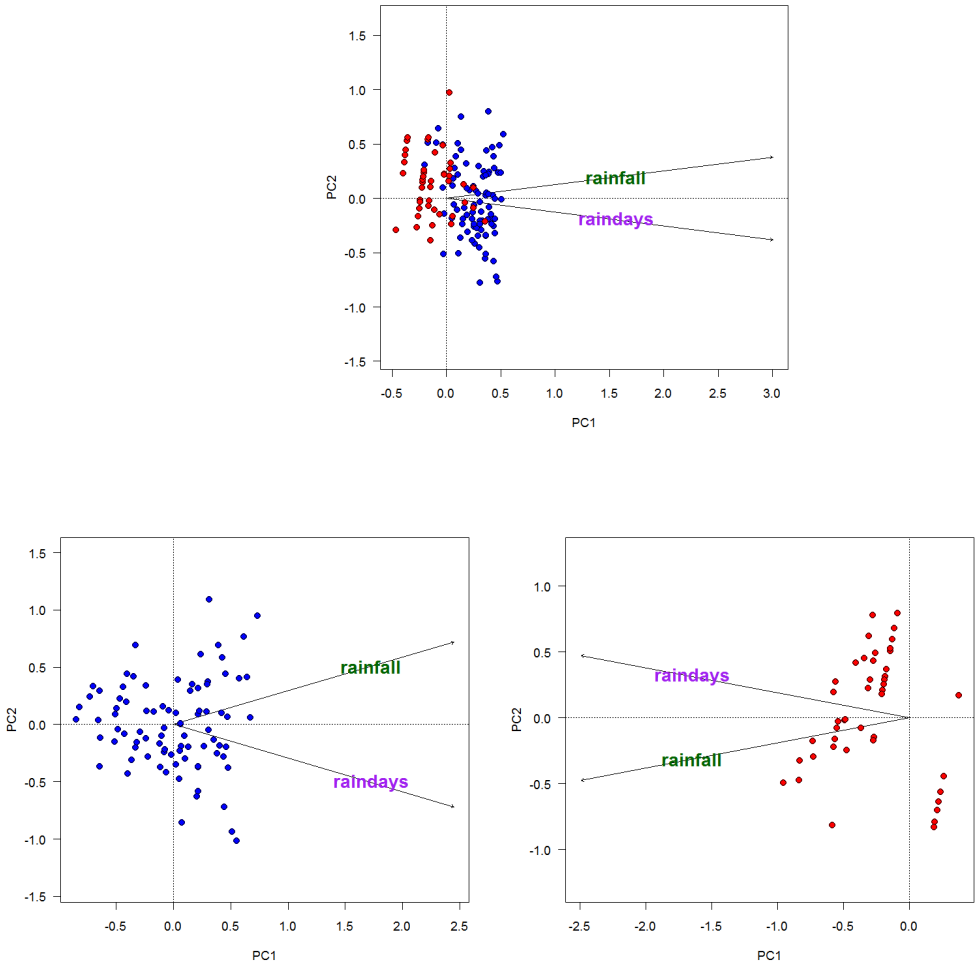

Figure 3 Principal components analysis of *rain* data including: total monthly rainfall (mm), and number of rain days per month, for the full dataset, wet season dataset and dry season data set. Red points represent dry season data, blue points represent wet season data.

The PC1 of each of the three *tidal* PCAs explained 83, 80 and 80% of the variance in the full, wet season and dry season datasets, respectively. For all three datasets, mean monthly tide height, maximum monthly tide height, and frequency of high tides all increase with increasing PC1 (Fig. 4).

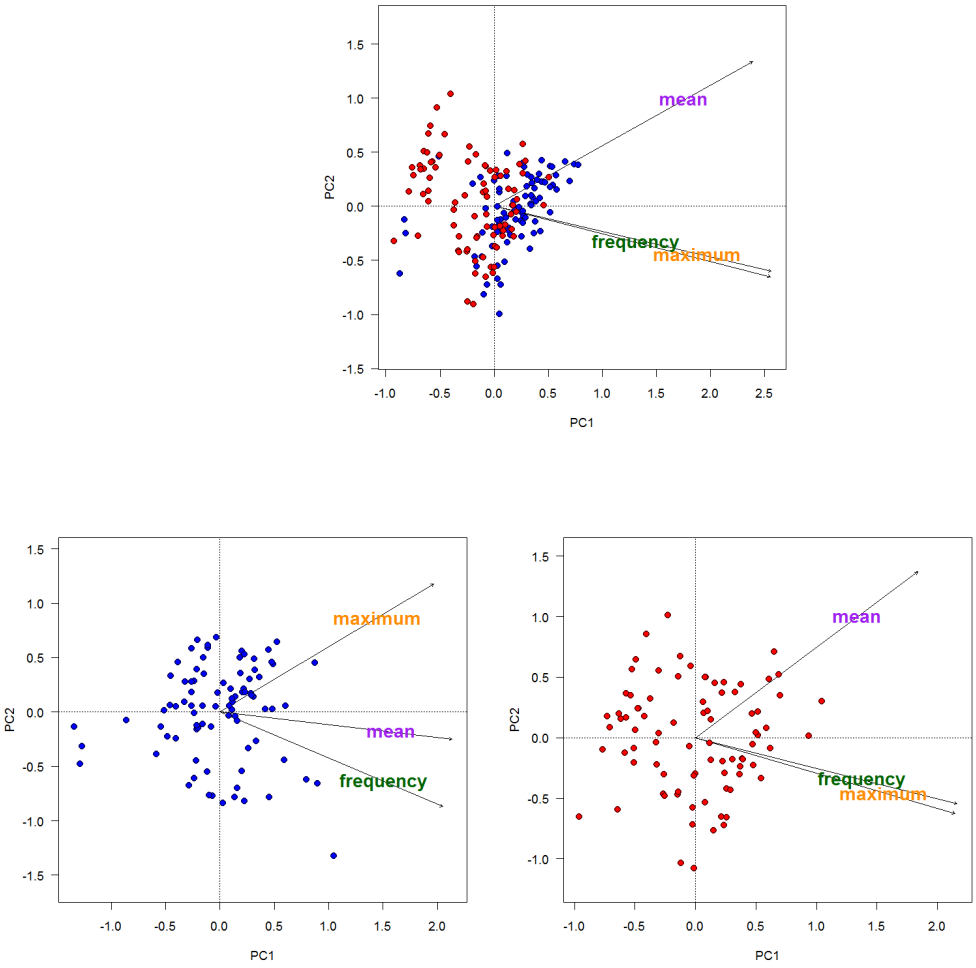

Figure 4 Principal components analysis of *tidal* data including: maximum and mean monthly tide heights (m), and frequency of tides higher than or equal to 7.4 m per month, for the full dataset, wet season dataset and dry season data set. Red points represent dry season data, blue points represent wet season data.

Appendix 4b Comparison of the full model sets and vector trap models and the null models used to assess change in monthly Ross River virus (January 1991 to December 2005) cases for (a) & (b) environmental proxy models, (c) & (b) vector trapped abundance models, and (e) combined environmental proxy and vector trap models. Explanatory variables considered are: $tide_{lag2}$ = 2 month lag of tidal first principal component, $tide_{lag1}$ = 1 month lag of tidal first principal component, $rain_{lag2}$ = 2 month lag of rain first principal component, $rain_{lag1}$ = 2 month lag of rain first principal component, CxA_{lag1} = 1 month lag of mean monthly *Culex annulirostris* trap abundance, CxA = mean monthly *Culex annulirostris* trap abundance, AeV_{lag1} = 1 month lag of mean monthly *Aedes vigilax* trap abundance, AeV = mean monthly *Aedes vigilax* trap abundance, temp = temperature principal components, wet = wetness principal components, season = wet or dry, holidays = number of public holidays per month and RRv_{lag} = peak Ross River virus cases from the previous wet season. Shown are the number of fitted model parameters (*k*; includes intercept), and the median score (range of score in parentheses) from 100 block bootstrap iterations for Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE).

(a) Environmental proxy models	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season	6	838.92	0.000	0.277 (0.224-0.338)	39.91 (37.01-44.07)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + season	5	839.43	0.380	0.2 (0.157-0.245)	39.61 (36.63-43.93)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$	5	840.22	0.493	0.191 (0.138-0.236)	39.28 (36.7-43.86)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$	4	840.77	1.405	0.14 (0.08-0.172)	39.1 (36.28-43.6)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag2}$ + season	5	845.26	3.428	0.051 (0.015-0.098)	38.89 (35.51-42.65)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag2}$	4	845.35	4.333	0.032 (0.008-0.061)	38.39 (35.31-42.13)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + season	4	846.54	7.051	0.01 (0.002-0.041)	37.44 (34.11-41.16)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$	3	847.07	8.221	0.006 (0.001-0.025)	36.97 (33.67-41.05)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$ + $rain_{lag2}$ + season	5	851.84	10.901	0.001 (0-0.008)	35.96 (32.46-40.06)
RRv ~ $tide_{lag1}$ + $rain_{lag2}$ + season	4	853.42	11.427	0.001 (0-0.006)	35.64 (32.03-39.89)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + season	4	854.14	12.156	0.001 (0-0.003)	35.45 (31.76-39.01)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season	5	856.56	14.426	0 (0-0.005)	33.88 (31.34-39.66)
RRv ~ $rain_{lag2}$ + season	3	855.49	14.873	0 (0-0.002)	34.68 (31.23-38.5)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$	4	857.26	15.375	0 (0-0.003)	33.27 (31.1-39.38)
RRv ~ $tide_{lag2}$ + $rain_{lag1}$ + season	4	863.03	20.819	0 (0-0)	31.64 (28.69-35.9)
RRv ~ $tide_{lag1}$ + $rain_{lag1}$ + season	4	858.24	19.250	0 (0-0.001)	32 (30.16-37.7)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$ + season	4	878.97	37.766	0 (0-0)	25.49 (21.81-28.34)
RRv ~ $tide_{lag2}$ + season	3	882.09	39.137	0 (0-0)	24.44 (21.19-27.35)
RRv ~ $rain_{lag1}$ + season	3	863.31	23.036	0 (0-0)	31.45 (27.56-35.46)
RRv ~ $tide_{lag1}$ + season	3	884.39	45.066	0 (0-0)	22.02 (19.2-25.41)

RRv ~ $tide_{lag2}$ + $tide_{lag1}$ + $rain_{lag2}$	4	857.61	19.472	0 (0-0)	33.13 (29.23-37.45)
RRv ~ $tide_{lag1}$ + $rain_{lag2}$	3	861.56	20.776	0 (0-0)	32.63 (28.78-36.51)
RRv ~ $tide_{lag2}$ + $rain_{lag1}$	3	863.55	21.339	0 (0-0)	31.31 (28.44-35.47)
RRv ~ $tide_{lag1}$ + $rain_{lag1}$	3	858.97	19.542	0 (0-0.001)	31.61 (29.82-37.26)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$	3	858.61	19.981	0 (0-0)	32.94 (28.94-37.19)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$	3	911.95	69.905	0 (0-0)	9.95 (7.16-13.25)
RRv ~ $rain_{lag2}$	2	862.09	21.921	0 (0-0)	31.82 (28.32-36.24)
RRv ~ $tide_{lag2}$	2	913.79	71.546	0 (0-0)	8.9 (6.34-11.83)
RRv ~ $rain_{lag1}$	2	864.06	24.093	0 (0-0)	30.86 (27.33-35.15)
RRv ~ $tide_{lag1}$	2	917.87	74.068	0 (0-0)	8.06 (5.81-10.69)
RRv ~ season	2	885.06	47.855	0 (0-0)	21.54 (18.73-24.83)
Null	1	933.20	89.098	0 (0-0)	0 (0-0)
(b) Environmental proxy models with human exposure and RRv immunity	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays + RRv_{lag}	8	812.19	0.000	0.256 (0.198-0.354)	46.06 (39.95-49.9)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + season + holidays + RRv_{lag}	7	813.35	0.619	0.167 (0.121-0.222)	45.77 (39.58-49.76)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + RRv_{lag}	7	814.51	0.742	0.154 (0.094-0.213)	45.14 (39.81-49.48)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + holidays + RRv_{lag}	6	815.77	1.782	0.098 (0.049-0.135)	44.97 (38.85-49.23)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + RRv_{lag}	7	818.14	4.993	0.017 (0.004-0.045)	44.3 (37.09-48.38)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + season + RRv_{lag}	6	819.06	6.011	0.014 (0.003-0.042)	44.16 (37-48.16)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag}	6	821.24	7.249	0.008 (0.002-0.028)	43.74 (36.48-47.41)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + RRv_{lag}	5	822.43	7.635	0.006 (0.001-0.023)	43.59 (36.01-47.05)

RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season + holidays	7	815.61	3.336	0.055 (0.013-0.098)	44.33 (37.99-48.73)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + season + holidays	6	817.05	4.240	0.033 (0.009-0.069)	43.95 (37.84-48.43)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays	6	819.47	4.951	0.023 (0.004-0.065)	42.73 (37.94-48.17)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + holidays	5	820.04	6.030	0.014 (0.002-0.035)	42.45 (37.41-48.05)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + season	6	823.44	9.486	0.002 (0-0.01)	42.57 (35.66-46.51)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + season	5	824.01	9.922	0.002 (0-0.006)	42.47 (35.48-46.45)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$	5	827.63	11.814	0.001 (0-0.005)	40.54 (34.54-45.69)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$	4	828.41	12.428	0.001 (0-0.004)	40.44 (34.4-45.64)
RRv ~ holidays + RRv_{lag}	3	911.23	102.527	0 (0-0)	1.9 (0.78-3.62)
RRv ~ RRv_{lag}	2	911.57	103.247	0 (0-0)	1.13 (0.38-2.94)
RRv ~ holidays	2	913.42	105.006	0 (0-0)	0.29 (0.11-0.79)
Null	1	913.85	106.209	0 (0-0)	0 (0-0)
(c) Vector trapped abundance models	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + season	6	832.96	0.000	0.501 (0.428-0.558)	43.27 (39.25-47.97)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV + season	5	834.39	0.947	0.324 (0.216-0.432)	42.52 (39.11-47.5)
RRv ~ CxA_{lag1} + CxA + AeV + season	5	840.91	3.594	0.089 (0.017-0.226)	41.51 (36.71-46.13)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + season	5	843.55	7.959	0.009 (0.002-0.029)	39.62 (35.01-44.92)
RRv ~ CxA_{lag1} + CxA + season	4	847.11	8.992	0.006 (0.001-0.016)	39.35 (34.67-44.85)
RRv ~ CxA_{lag1} + AeV + season	4	848.03	10.611	0.003 (0-0.012)	39.2 (35.25-43.4)
RRv ~ CxA_{lag1} + AeV_{lag1} + season	4	848.85	12.192	0.001 (0-0.003)	38.39 (34.11-43.88)
RRv ~ AeV_{lag1} + CxA + AeV + season	5	853.06	14.697	0 (0-0.003)	37.59 (32.68-42.53)

RRv ~ CxA_{lag1} + season	3	851.03	14.894	0 (0-0.001)	36.79 (32.89-42.27)
RRv ~ CxA + AeV + season	4	856.61	15.581	0 (0-0.002)	36.9 (32.13-42.38)
RRv ~ AeV_{lag1} + AeV + season	4	868.00	26.827	0 (0-0)	32.95 (27.81-36.74)
RRv ~ All_{lag1} + All + season	4	860.56	21.156	0 (0-0)	35.83 (29.81-39.99)
RRv ~ AeV_{lag1} + CxA + season	4	864.81	22.780	0 (0-0)	33.63 (27.55-40.61)
RRv ~ AeV_{lag1} + season	3	886.71	48.030	0 (0-0)	24.48 (18.47-28.73)
RRv ~ All_{lag1} + season	3	862.94	23.216	0 (0-0)	34.83 (28.93-38.98)
RRv ~ AeV + season	3	876.33	36.149	0 (0-0)	28.7 (23.73-34.02)
RRv ~ CxA + season	3	868.13	28.133	0 (0-0)	32.22 (26.21-39.56)
RRv ~ All + season	3	882.20	41.776	0 (0-0)	26.27 (20.01-31.44)
RRv ~ season	2	887.72	48.405	0 (0-0)	23.85 (18.39-28.43)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV	5	869.47	36.661	0 (0-0)	27.47 (23.32-33.72)
RRv ~ CxA_{lag1} + CxA + AeV	4	876.90	45.446	0 (0-0)	25.12 (20.66-30.11)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV	4	870.26	38.273	0 (0-0)	27.14 (22.98-33.63)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA	4	873.11	45.385	0 (0-0)	24.49 (20.93-30.79)
RRv ~ AeV_{lag1} + CxA + AeV	4	893.63	59.028	0 (0-0)	18.8 (16.26-21.71)
RRv ~ AeV_{lag1} + AeV	3	909.22	75.437	0 (0-0)	11.04 (9.56-13.1)
RRv ~ CxA_{lag1} + CxA	3	877.31	49.422	0 (0-0)	23.79 (19.71-28.97)
RRv ~ All_{lag1} + All	3	897.37	65.381	0 (0-0)	15.15 (11.86-20.85)
RRv ~ CxA_{lag1} + AeV_{lag1}	3	874.63	48.073	0 (0-0)	23.41 (19.68-29.89)
RRv ~ AeV_{lag1} + CxA	3	903.39	65.830	0 (0-0)	16.44 (13.84-19)
RRv ~ CxA_{lag1} + AeV	3	887.70	54.498	0 (0-0)	21 (17-25.52)

RRv ~ CxA + AeV	3	895.79	60.550	0 (0-0)	18.5 (15.59-21.57)
RRv ~ AeV_{lag1}	2	927.48	91.252	0 (0-0)	2.88 (1.66-4.8)
RRv ~ CxA_{lag1}	2	889.46	57.838	0 (0-0)	19.68 (16.38-24.97)
RRv ~ All_{lag1}	2	897.99	66.996	0 (0-0)	14.74 (10.96-20.54)
RRv ~ AeV	2	923.47	94.165	0 (0-0)	1.84 (0.76-3.36)
RRv ~ CxA	2	903.82	67.558	0 (0-0)	15.55 (12.74-18.18)
RRv ~ All	2	921.04	86.018	0 (0-0)	6.12 (3.95-8.79)
Null	1	929.95	97.512	0 (0-0)	0 (0-0)
(d) Vector trapped abundance models with human exposure and RRv immunity	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag}	8	824.82	0.000	0.378 (0.276-0.435)	45.18 (41.45-50.23)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag}	7	825.10	0.224	0.29 (0.223-0.38)	45.12 (41.04-50.12)
RRv ~ CxA_{lag1} + CxA + AeV + season + holidays + RRv_{lag}	7	828.82	2.269	0.105 (0.053-0.18)	44 (40.36-49.5)
RRv ~ CxA_{lag1} + CxA + AeV + season + RRv_{lag}	6	829.85	3.065	0.077 (0.028-0.124)	43.97 (39.88-49.42)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays	7	832.93	4.983	0.031 (0.005-0.116)	43.77 (39.46-48.03)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + season	6	832.96	5.472	0.024 (0.005-0.086)	43.27 (39.25-47.97)
RRv ~ CxA_{lag1} + CxA + AeV + season + holidays	6	840.43	9.826	0.003 (0-0.022)	41.78 (37.39-46.21)
RRv ~ CxA_{lag1} + CxA + AeV + season	5	840.91	10.517	0.002 (0-0.017)	41.51 (36.71-46.13)
RRv ~ holidays + RRv_{lag}	3	929.76	98.383	0 (0-0)	1.84 (0.86-3.31)
RRv ~ RRv_{lag}	2	929.82	98.662	0 (0-0)	1.19 (0.3-2.83)
RRv ~ holidays	2	929.88	102.077	0 (0-0)	0.22 (0.06-0.58)
Null	1	929.95	102.925	0 (0-0)	0 (0-0)

(e) Combined models with temperature and wetness principal components	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + season + holidays + RRv_{lag} + temp + wet$	10	822.74	0.000	0.135 (0.078-0.203)	48.89 (44.88-52.52)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + season + holidays + RRv_{lag} + temp$	9	822.96	0.556	0.098 (0.059-0.146)	48.75 (44.69-52.21)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + season + holidays + RRv_{lag} + temp + wet$	9	822.81	0.557	0.097 (0.068-0.149)	48.7 (44.7-52.42)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + season + holidays + RRv_{lag} + temp$	8	823.04	1.184	0.075 (0.048-0.11)	48.53 (44.52-52.17)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays + RRv_{lag} + temp + wet$	9	823.70	2.431	0.048 (0.012-0.09)	47.91 (44.23-51.81)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + holidays + RRv_{lag} + temp + wet$	8	823.78	2.905	0.041 (0.009-0.07)	47.73 (43.69-51.61)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays + RRv_{lag} + temp$	8	824.58	3.462	0.035 (0.007-0.067)	47.54 (43.69-51.02)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + holidays + RRv_{lag} + temp$	7	824.71	3.784	0.031 (0.004-0.051)	47.32 (43.44-50.88)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + season + holidays + RRv_{lag} + wet$	9	827.29	5.789	0.012 (0.002-0.044)	47.04 (43.14-50.6)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + season + holidays + RRv_{lag} + wet$	8	827.95	6.360	0.009 (0.001-0.039)	46.98 (42.9-50.49)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + season + holidays + RRv_{lag}$	8	827.88	6.335	0.008 (0.001-0.038)	46.97 (42.87-50.47)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + season + holidays + RRv_{lag}$	7	828.49	6.646	0.007 (0.001-0.028)	46.86 (42.71-50.24)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag} + temp + wet$	10	823.29	7.183	0.006 (0-0.117)	46.74 (42.63-51.1)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag} + temp$	9	823.57	7.712	0.004 (0-0.069)	45.93 (42.07-51.06)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag} + temp + wet$	9	824.38	7.847	0.004 (0-0.068)	46.57 (42.39-51.03)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays + RRv_{lag} + wet$	8	828.26	7.468	0.004 (0-0.019)	46.07 (42.84-50.02)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag} + temp$	8	824.51	8.082	0.004 (0-0.046)	45.86 (41.89-51.01)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + holidays + RRv_{lag} + wet$	7	829.09	8.175	0.003 (0-0.013)	46 (42.65-49.9)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays + RRv_{lag}$	7	828.88	8.217	0.003 (0-0.019)	45.93 (42.62-49.99)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + holidays + RRv_{lag}$	6	829.54	8.570	0.003 (0-0.01)	45.85 (42.51-49.86)

$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag} + wet$	9	824.56	8.886	0.002 (0-0.018)	45.62 (41.98-50.27)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag} + wet$	8	824.90	9.669	0.002 (0-0.016)	45.4 (41.44-50.16)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + holidays + RRv_{lag}$	8	824.82	9.714	0.002 (0-0.017)	45.18 (41.45-50.23)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + season + RRv_{lag}$	7	825.10	10.311	0.001 (0-0.013)	45.12 (41.04-50.12)
RRv ~ temp + wet	3	884.66	66.373	0 (0-0)	23.27 (20.75-26.33)
RRv ~ temp	2	928.01	113.400	0 (0-0)	0.8 (0.22-2)
RRv ~ wet	2	896.57	73.113	0 (0-0)	20.71 (17.61-24.3)
Null	1	929.95	115.419	0 (0-0)	0 (0-0)

Comparison of the full model sets and vector trap models and the null models used to assess change in monthly Ross River virus during the wet season (November to April, 1991 to 2005) cases for (a) & (b) environmental proxy models, (c) & (b) vector trapped abundance models, and (e) combined environmental proxy and vector trap models. Explanatory variables considered are: $tide_{lag2}$ = 2 month lag of tidal first principal component, $tide_{lag1}$ = 1 month lag of tidal first principal component, $rain_{lag2}$ = 2 month lag of rain first principal component, $rain_{lag1}$ = 2 month lag of rain first principal component, CxA_{lag1} = 1 month lag of mean monthly *Culex annulirostris* trap abundance, CxA = mean monthly *Culex annulirostris* trap abundance, AeV_{lag1} = 1 month lag of mean monthly *Aedes vigilax* trap abundance, AeV = mean monthly *Aedes vigilax* trap abundance, All_{lag1} = 1 month lag of mean monthly trap abundance for all mosquito species, All = mean monthly trap abundance for all mosquito species, $temp_{pc1}$,$temp_{pc2}$= temperature principal components, wet_{pc1},wet_{pc2}= wetness principal components, holidays = number of public holidays per month and RRv_{lag} = peak Ross River virus cases from the previous wet season. Shown are the number of fitted model parameters (*k*; includes intercept), and the median score (range of score in parentheses) from 100 block bootstrap iterations for Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE).

(a) Environmental proxy models	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$	5	443.57	0.000	0.44 (0.349-0.496)	47.89 (44.09-51.79)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$	4	443.98	0.173	0.367 (0.27-0.435)	47.81 (43.81-51.28)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$ + $rain_{lag2}$	4	448.81	2.994	0.097 (0.027-0.213)	45.26 (42.47-48.84)
RRv ~ $tide_{lag1}$ + $rain_{lag2}$	3	448.98	3.520	0.08 (0.023-0.153)	45.16 (42.22-48.63)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag2}$	4	460.52	12.642	0.001 (0-0.005)	37.95 (34.24-43.11)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$	4	458.54	14.364	0 (0-0.002)	37.74 (33.42-42.16)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$	3	463.05	15.040	0 (0-0.002)	37.07 (33.16-41.08)
RRv ~ $tide_{lag1}$ + $rain_{lag1}$	3	461.93	16.559	0 (0-0.001)	36.07 (31.81-39.54)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$	3	465.30	18.296	0 (0-0.001)	34.13 (30.85-39.29)
RRv ~ $tide_{lag2}$ + $rain_{lag1}$	3	471.97	24.791	0 (0-0)	28.6 (25.19-33.61)
RRv ~ $tide_{lag2}$ + $tide_{lag1}$	3	496.08	50.367	0 (0-0)	4.29 (2.74-6.56)
RRv ~ $rain_{lag2}$	2	467.83	20.283	0 (0-0)	32.73 (29.16-36.91)
RRv ~ $tide_{lag2}$	2	498.05	51.324	0 (0-0)	2.88 (1.28-5.54)
RRv ~ $rain_{lag1}$	2	472.70	26.167	0 (0-0)	27.64 (24.35-32.53)
RRv ~ $tide_{lag1}$	2	498.89	54.224	0 (0-0)	0.41 (0.12-1.16)
Null	1	499.45	55.116	0 (0-0)	0 (0-0)
(b) Environmental proxy models with human exposure and RRv immunity	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**

RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays	7	444.46	0.000	0.352 (0.261-0.422)	51.45 (45.98-55.68)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + RRv_{lag} + holidays	6	446.47	0.301	0.239 (0.182-0.316)	51.23 (45.76-55.19)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays	6	449.07	1.918	0.121 (0.06-0.195)	49.27 (44.45-53.5)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + holidays	5	450.38	2.638	0.094 (0.041-0.138)	48.82 (44.35-53.21)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag}	6	449.14	3.784	0.053 (0.023-0.093)	48.62 (42.72-52.56)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + RRv_{lag}	5	450.35	4.814	0.041 (0.016-0.073)	47.96 (42.53-52.03)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$	5	452.21	6.164	0.015 (0.005-0.039)	46.63 (41.19-51.46)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$	4	452.68	7.073	0.011 (0.004-0.032)	46.42 (40.95-50.81)
RRv ~ holidays + RRv_{lag}	3	498.11	51.035	0 (0-0)	7.18 (4-11.98)
RRv ~ RRv_{lag}	2	500.97	54.693	0 (0-0)	3.84 (1.45-8.65)
RRv ~ holidays	2	501.77	56.568	0 (0-0)	3.66 (1.9-4.74)
Null	1	505.58	60.029	0 (0-0)	0 (0-0)
(c) Vector trapped abundance models	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV	5	470.80	0.000	0.415 (0.337-0.492)	40.25 (35.94-48.02)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV	4	470.84	0.590	0.297 (0.185-0.398)	39.52 (35.07-47.42)
RRv ~ CxA_{lag1} + CxA + AeV	4	477.08	3.312	0.078 (0.019-0.168)	37 (32.03-43.19)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA	4	478.65	4.076	0.054 (0.009-0.111)	35.36 (29.77-43.89)
RRv ~ CxA_{lag1} + CxA	3	480.23	4.952	0.034 (0.004-0.062)	34.78 (28.22-42.58)
RRv ~ CxA_{lag1} + AeV_{lag1}	3	480.18	6.388	0.017 (0.004-0.038)	34.06 (28.35-41.32)
RRv ~ CxA_{lag1} + AeV	3	481.57	8.901	0.005 (0.001-0.014)	32.46 (29.6-38.44)
RRv ~ CxA_{lag1}	2	483.08	10.712	0.002 (0-0.006)	31.32 (27.13-37.19)

RRv ~ All_{lag1} + All	3	483.31	11.754	0.001 (0-0.013)	30.8 (26.59-37.22)
RRv ~ AeV_{lag1} + CxA + AeV	4	490.67	20.320	0 (0-0)	23.11 (19.07-28.79)
RRv ~ AeV_{lag1} + AeV	3	500.80	28.477	0 (0-0)	13.26 (11.3-16.65)
RRv ~ AeV_{lag1} + CxA	3	498.81	25.734	0 (0-0)	18.68 (14.54-24.03)
RRv ~ CxAM + AeV	3	494.09	21.515	0 (0-0)	22.42 (18.74-28.17)
RRv ~ AeV_{lag1}	2	515.69	43.185	0 (0-0)	0.62 (0.16-1.51)
RRv ~ All_{lag1}	2	488.69	19.197	0 (0-0)	24.12 (18.38-32.95)
RRv ~ AeV	2	511.49	37.167	0 (0-0)	6.48 (5.06-10.3)
RRv ~ CxA	2	502.37	29.924	0 (0-0)	15.45 (11.32-19.32)
RRv ~ All	2	513.75	41.318	0 (0-0)	2.46 (0.76-4.79)
Null	1	516.52	44.038	0 (0-0)	0 (0-0)
(d) Vector trapped abundance models with human exposure and RRv immunity	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}	7	467.41	0.000	0.259 (0.205-0.343)	44.98 (38.52-55.54)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag}	6	467.58	0.303	0.183 (0.128-0.238)	44.17 (38.15-53.73)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV + holidays + RRv_{lag}	6	467.66	0.693	0.142 (0.084-0.201)	44.18 (36.93-52.4)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV + RRv_{lag}	5	467.92	1.257	0.116 (0.058-0.156)	43.93 (36.71-50.68)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays	6	469.94	1.565	0.106 (0.037-0.136)	42.24 (36.56-52.14)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV	5	469.98	3.052	0.057 (0.017-0.112)	41.77 (36.22-51.17)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV + holidays	5	470.89	4.025	0.04 (0.01-0.094)	41.65 (35.44-50.89)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV	4	470.95	4.894	0.027 (0.006-0.065)	41.29 (35.14-49.57)
RRv ~ holidays + RRv_{lag}	3	509.15	44.733	0 (0-0)	4.57 (2.66-7.71)

RRv ~ RRv_{lag}	2	512.85	47.672	0 (0-0)	1.6 (0.57-3.67)
RRv ~ holidays	2	511.84	46.438	0 (0-0)	2.98 (1.65-4.37)
Null	1	514.32	50.004	0 (0-0)	0 (0-0)
(e) Combined models with temperature and wetness principal components	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ $tide_{lag2}$+$rain_{lag2}$+$tide_{lag1}$+$rain_{lag1}$ + RRv_{lag} + holidays +$temp_{pc1}$+$temp_{pc2}$+wet_{pc1}+wet_{pc2}	11	434.99	0.000	0.212 (0.002-0.314)	58.18 (53.65-62)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + RRv_{lag} + holidays + $temp_{pc1}$ + $temp_{pc2}$ + wet_{pc1} + wet_{pc2}	10	435.30	0.848	0.174 (0.001-0.251)	58.06 (53.26-61.64)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + $temp_{pc1}$ + $temp_{pc2}$ + wet_{pc1} + wet_{pc2}	10	436.36	3.064	0.069 (0-0.202)	57.09 (52.95-60.96)
RRv ~ CxA_{lag1}+ AeV_{lag1}+ CxA + AeV + holidays + RRv_{lag} +$temp_{pc1}$+$temp_{pc2}$+wet_{pc1}+wet_{pc2}	11	442.27	3.935	0.036 (0-0.298)	54.91 (49.82-61.58)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag} + $temp_{pc1}$ + $temp_{pc2}$ + wet_{pc1} + wet_{pc2}	10	443.07	5.874	0.023 (0-0.176)	54.16 (49-61.43)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + $temp_{pc1}$ + $temp_{pc2}$	9	439.63	7.704	0.007 (0-0.084)	56.07 (50.68-59.5)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + RRv_{lag} + holidays + $temp_{pc1}$ + $temp_{pc2}$	8	440.25	7.882	0.007 (0-0.071)	55.87 (50.51-59.47)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + $temp_{pc1}$ + $temp_{pc2}$ + wet_{pc1} + wet_{pc2}	10	444.79	9.022	0.005 (0-0.062)	53.54 (48.68-59.44)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV + holidays + RRv_{lag} + $temp_{pc1}$ + $temp_{pc2}$ + wet_{pc1} + wet_{pc2}	10	445.06	9.693	0.003 (0-0.034)	52.91 (48.5-59.79)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + $temp_{pc1}$ + $temp_{pc2}$	8	441.07	9.367	0.002 (0-0.024)	54.38 (50.23-58.4)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV + RRv_{lag} + $temp_{pc1}$ + $temp_{pc2}$ + wet_{pc1} + wet_{pc2}	9	447.07	11.008	0.001 (0-0.022)	52.5 (47.2-59.34)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + wet_{pc1} + wet_{pc2}	9	443.80	14.626	0 (0-0.006)	51.52 (47.9-56.06)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + $temp_{pc1}$ + $temp_{pc2}$	9	452.08	15.823	0 (0-0.004)	50.54 (43.58-56.17)
RRv ~ $rain_{lag2}$ + $rain_{lag1}$ + $tide_{lag1}$ + RRv_{lag} + holidays + wet_{pc1} + wet_{pc2}	8	444.36	15.005	0 (0-0.005)	51.42 (47.32-55.83)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag} + $temp_{pc1}$ + $temp_{pc2}$	8	453.02	17.050	0 (0-0.002)	49.19 (42.91-56.01)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + holidays + wet_{pc1} + wet_{pc2}	8	445.20	17.228	0 (0-0.003)	50.83 (46.1-54.6)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV + holidays + RRv_{lag} + $temp_{pc1}$ + $temp_{pc2}$	8	453.23	18.304	0 (0-0.001)	49.18 (42.87-55.33)

$RRv \sim CxA_{lag1} + AeV_{lag1} + AeV + RRv_{lag} + temp_{pc1} + temp_{pc2}$	7	454.28	19.427	0 (0-0.001)	48.63 (41.84-55.23)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + temp_{pc1} + temp_{pc2}$	8	453.36	19.290	0 (0-0.001)	48.4 (41.8-54.64)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + wet_{pc1} + wet_{pc2}$	9	456.83	18.544	0 (0-0.001)	47.73 (42.21-54.54)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag} + wet_{pc1} + wet_{pc2}$	8	458.08	20.034	0 (0-0.001)	47.54 (42.07-54.23)
$RRv \sim CxA_{lag1} + AeV_{lag1} + AeV + holidays + RRv_{lag} + wet_{pc1} + wet_{pc2}$	8	457.14	21.197	0 (0-0)	46.31 (41.48-52.92)
$RRv \sim CxA_{lag1} + AeV_{lag1} + AeV + RRv_{lag} + wet_{pc1} + wet_{pc2}$	7	458.81	21.939	0 (0-0)	45.82 (41.33-52.15)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + wet_{pc1} + wet_{pc2}$	8	459.73	23.549	0 (0-0)	45.56 (39.73-50.96)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}$	7	462.63	24.171	0 (0-0)	44.17 (39.54-51.49)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag}$	6	463.68	24.946	0 (0-0)	43.21 (38.53-50.94)
$RRv \sim CxA_{lag1} + AeV_{lag1} + AeV + holidays + RRv_{lag}$	6	463.17	25.520	0 (0-0)	42.9 (39.24-50)
$RRv \sim CxA_{lag1} + AeV_{lag1} + AeV + RRv_{lag}$	5	464.10	27.174	0 (0-0)	41.97 (37.86-49.6)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays$	6	464.89	27.745	0 (0-0)	41.85 (37.08-47.77)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag} + holidays$	7	446.05	17.599	0 (0-0.001)	49.78 (46.12-53.15)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + RRv_{lag} + holidays$	6	446.92	18.205	0 (0-0.001)	49.7 (45.47-53.1)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays$	6	447.45	19.308	0 (0-0)	48.36 (44.13-52.3)
$RRv \sim temp_{pc1} + temp_{pc2} + wet_{pc1} + wet_{pc2}$	5	479.00	46.143	0 (0-0)	27.63 (21.99-33.89)
$RRv \sim temp_{pc1} + temp_{pc2}$	3	482.05	49.429	0 (0-0)	24.98 (20.76-30.41)
$RRv \sim wet_{pc1} + wet_{pc2}$	3	499.61	69.214	0 (0-0)	6.33 (4.04-9.86)
Null	1	506.35	75.461	0 (0-0)	0 (0-0)

Comparison of the full model sets and vector trap models and the null models used to assess change in monthly Ross River virus during the dry season (May to October 1991 to 2005) cases for (a) & (b) environmental proxy models, (c) & (b) vector trapped abundance models, and (e) combined environmental proxy and vector trap models. Explanatory variables considered are: $tide_{lag2}$ = 2 month lag of tidal first principal component, $tide_{lag1}$ = 1 month lag of tidal first principal component, $rain_{lag2}$ = 2 month lag of rain first principal component, $rain_{lag1}$ = 2 month lag of rain first principal component, CxA_{lag1} = 1 month lag of mean monthly *Culex annulirostris* trap abundance, CxA = mean monthly *Culex annulirostris* trap abundance, AeV_{lag1} = 1 month lag of mean monthly *Aedes vigilax* trap abundance, AeV = mean monthly *Aedes vigilax* trap abundance, All_{lag1} = 1 month lag of mean monthly trap abundance for all mosquito species, All = mean monthly trap abundance for all mosquito species, $temp_{pc1}$ = temperature principal components, wet_{pc1},wet_{pc2}= wetness principal components, holidays = number of public holidays per month and RRv_{lag} = peak Ross River virus cases from the previous wet season. Shown are the number of fitted model parameters (*k*; includes intercept), and the median score (range of score in parentheses) from 100 block bootstrap iterations for Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight scaled relative to a total sum of 1 ($wAIC_c$) and percent deviance explained (%DE).

(a) Environmental proxy models	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1}$	5	323.73	0.000	0.216 (0.171-0.277)	12.72 (10.68-15.1)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2}$	4	324.23	0.494	0.146 (0.117-0.2)	11.96 (9.9-14.31)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1}$	4	325.77	0.395	0.146 (0.118-0.185)	11.77 (10.07-14.34)
$RRv \sim tide_{lag2} + tide_{lag1} + rain_{lag2}$	4	326.12	1.667	0.094 (0.056-0.12)	10.76 (9.01-13.46)
$RRv \sim rain_{lag2} + rain_{lag1}$	3	329.07	2.169	0.074 (0.029-0.105)	9.79 (8.16-11.42)
$RRv \sim tide_{lag2} + rain_{lag2}$	3	326.30	2.393	0.07 (0.04-0.096)	9.91 (8.18-12.15)
$RRv \sim tide_{lag1} + rain_{lag2}$	3	327.72	2.535	0.057 (0.029-0.098)	9.91 (8.07-12.18)
$RRv \sim rain_{lag2}$	2	329.92	3.625	0.035 (0.013-0.058)	8.37 (6.89-10.26)
$RRv \sim tide_{lag2} + tide_{lag1} + rain_{lag1}$	4	330.16	3.993	0.029 (0.008-0.053)	7.76 (5.87-11.06)
$RRv \sim tide_{lag2} + rain_{lag1}$	3	331.20	5.624	0.012 (0.004-0.032)	6.75 (4.4-9.11)
$RRv \sim tide_{lag1} + rain_{lag1}$	3	330.72	5.980	0.011 (0.003-0.03)	6.49 (4.34-8.89)
$RRv \sim rain_{lag1}$	2	332.55	7.499	0.005 (0.001-0.017)	5.18 (3.48-6.66)
$RRv \sim tide_{lag2} + tide_{lag1}$	3	335.52	8.751	0.003 (0.001-0.011)	3.18 (1.66-5.32)
$RRv \sim tide_{lag2}$	2	335.60	9.732	0.002 (0.001-0.006)	1.91 (0.41-4.49)
$RRv \sim tide_{lag1}$	2	335.73	11.153	0.001 (0-0.003)	0.95 (0.21-2.16)
Null	1	336.44	12.430	0 (0-0.001)	0 (0-0)

(b) Environmental proxy models with human exposure and RRv immunity	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag} + holidays$	7	308.10	0.000	0.253 (0.192-0.362)	26.61 (21.9-31.43)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2} + RRv_{lag} + holidays$	6	308.95	0.471	0.17 (0.119-0.225)	25.67 (20.83-30.78)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + RRv_{lag} + holidays$	6	309.60	0.750	0.139 (0.082-0.19)	24.47 (19.96-29.72)
$RRv \sim tide_{lag2} + tide_{lag1} + rain_{lag2} + RRv_{lag} + holidays$	6	311.44	2.425	0.073 (0.024-0.13)	24.22 (20.04-27.8)
$RRv \sim rain_{lag1} + rain_{lag2} + RRv_{lag} + holidays$	5	309.88	2.416	0.072 (0.028-0.132)	23.07 (19.19-28.54)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag}$	6	313.52	5.351	0.019 (0.006-0.053)	21.44 (16.74-25.94)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + RRv_{lag}$	5	313.73	6.481	0.01 (0.002-0.042)	20.4 (15.34-24.52)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2} + RRv_{lag}$	5	313.94	6.503	0.009 (0.002-0.032)	20.15 (14.73-24.73)
$RRv \sim tide_{lag2} + tide_{lag1} + rain_{lag2} + RRv_{lag}$	5	316.04	7.390	0.006 (0.001-0.018)	19.79 (14.89-23.89)
$RRv \sim rain_{lag1} + rain_{lag2} + RRv_{lag}$	4	314.31	8.822	0.003 (0.001-0.016)	18.46 (13.53-22.83)
$RRv \sim holidays + RRv_{lag}$	3	316.04	9.230	0.003 (0.001-0.008)	17.76 (13.78-22.51)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + holidays$	6	316.98	9.637	0.003 (0-0.05)	17.44 (13.49-22.48)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2} + holidays$	5	317.38	9.993	0.002 (0-0.023)	16.41 (12.78-20.52)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + holidays$	5	319.59	10.799	0.001 (0-0.012)	15.86 (12.49-18.71)
$RRv \sim tide_{lag2} + tide_{lag1} + rain_{lag2} + holidays$	5	320.88	11.523	0.001 (0-0.018)	14.44 (10.54-18.42)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1}$	5	322.39	13.657	0 (0-0.002)	13.31 (10.65-16.01)
$RRv \sim rain_{lag1} + rain_{lag2} + holidays$	4	321.02	13.259	0 (0-0.006)	14.04 (11-15.98)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2}$	4	322.90	14.765	0 (0-0.002)	12.56 (10.07-14.95)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1}$	4	324.56	14.940	0 (0-0.002)	12.08 (10.05-14.87)
$RRv \sim tide_{lag2} + tide_{lag1} + rain_{lag2}$	4	324.14	15.870	0 (0-0.001)	10.73 (8.81-13.32)

RRv ~ $rain_{lag1}$ + $rain_{lag2}$	3	327.78	17.907	0 (0-0.001)	9.71 (7.73-11.26)
RRv ~ RRv_{lag}	2	325.44	20.390	0 (0-0)	6.24 (1.86-12.63)
RRv ~ holidays	2	327.67	19.027	0 (0-0.001)	8.75 (5.21-10.62)
Null	1	336.08	28.503	0 (0-0)	0 (0-0)
(c) Vector trapped abundance models	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV	5	332.59	0.000	0.205 (0.133-0.306)	10.25 (6.74-12.87)
RRv ~ AeV_{lag1} + CxA + AeV	4	334.30	1.806	0.099 (0.058-0.152)	8.06 (5.69-11.85)
RRv ~ CxA_{lag1} + CxA + AeV	4	333.68	2.086	0.076 (0.034-0.138)	7.33 (4.62-9.76)
RRv ~ All_{lag1} + All	3	335.63	2.184	0.063 (0.018-0.171)	7.31 (4.26-9.98)
RRv ~ CxA_{lag1} + AeV_{lag1} + AeV	4	334.37	3.146	0.06 (0.027-0.097)	7.19 (4.78-9.66)
RRv ~ All	2	335.93	2.859	0.049 (0.013-0.139)	6.71 (3.94-9.11)
RRv ~ AeV_{lag1} + AeV	3	335.73	3.727	0.046 (0.02-0.071)	6.11 (4.17-8.48)
RRv ~ CxA + AeV	3	335.19	3.469	0.039 (0.017-0.063)	5.7 (3.52-8.81)
RRv ~ CxA_{lag1} + AeV	3	336.80	5.057	0.022 (0.006-0.04)	4.89 (2.93-7.25)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA	4	335.40	4.834	0.02 (0.008-0.054)	4.56 (2.5-7.62)
RRv ~ AeV	2	337.08	5.772	0.017 (0.004-0.029)	3.95 (2.25-5.97)
RRv ~ AeV_{lag1} + CxA	3	336.17	5.622	0.015 (0.006-0.033)	3.24 (1.51-7.23)
RRv ~ CxA_{lag1} + CxA	3	336.58	6.987	0.009 (0.003-0.026)	2.72 (1.58-4.47)
RRv ~ CxA	2	337.44	7.735	0.007 (0.002-0.016)	1.42 (0.83-3.72)
RRv ~ CxA_{lag1} + AeV_{lag1}	3	338.59	7.774	0.005 (0.002-0.014)	1.42 (0.51-3.92)
RRv ~ All_{lag1}	2	338.67	8.333	0.005 (0.001-0.013)	1.92 (0.47-3.02)

RRv ~ AeV_{lag1}	2	338.84	8.394	0.005 (0.001-0.012)	0.56 (0.11-2.53)
RRv ~ CxA_{lag1}	2	338.88	9.871	0.002 (0.001-0.006)	0.22 (0.05-0.85)
Null	1	339.61	10.450	0.002 (0-0.005)	0 (0-0)
(d) Vector trapped abundance with human exposure and RRv immunity	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}	7	311.92	0.000	0.492 (0.404-0.555)	23.2 (18.72-27.51)
RRv ~ AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}	6	313.68	1.488	0.231 (0.165-0.291)	21.55 (16.91-25.97)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays	6	322.26	5.104	0.036 (0.003-0.139)	16 (13.23-20.53)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + RRv_{lag}	6	321.07	5.881	0.027 (0.007-0.1)	16.93 (12.96-22.1)
RRv ~ holidays + RRv_{lag}	3	320.27	6.198	0.021 (0.003-0.068)	16.08 (13.94-19.86)
RRv ~ AeV_{lag1} + CxA + AeV + holidays	5	323.91	6.875	0.016 (0.001-0.058)	13.71 (11.52-19)
RRv ~ AeV_{lag1} + CxA + AeV + RRv_{lag}	5	322.02	6.874	0.015 (0.005-0.056)	15.84 (11.69-21.21)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV	5	330.34	13.817	0.001 (0-0.003)	8.61 (6.13-12.13)
RRv ~ AeV_{lag1} + CxA + AeV	4	331.62	15.316	0 (0-0.001)	6.96 (4.68-10.01)
RRv ~ holidays	2	331.13	14.466	0 (0-0.002)	7.6 (4.72-11.15)
RRv ~ RRv_{lag}	2	331.51	16.924	0 (0-0)	5.32 (2.05-9.8)
Null	1	338.04	23.439	0 (0-0)	0 (0-0)
(e) Combined models with temperature and wetness principal components	***k***	**Median AIC_c**	**Median ΔAIC_c**	**Median $wAIC_c$**	**Median %DE**
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + $temp_{pc1}$ + wet_{pc1}+ wet_{pc2}	10	297.72	0.000	0.185 (0.069-0.335)	36.46 (30.51-41.54)
RRv ~ CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + wet_{pc1}+ wet_{pc2}	9	298.43	1.456	0.109 (0.028-0.23)	35.07 (30-39.82)
RRv ~ $tide_{lag2}$ + $rain_{lag2}$ + $tide_{lag1}$ + $rain_{lag1}$ + RRv_{lag} + holidays + $temp_{pc1}$ + wet_{pc1}+ wet_{pc2}	10	300.50	1.557	0.081 (0.01-0.169)	32.95 (28.59-37.04)

$RRv \sim AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + temp_{pc1} + wet_{pc1} + wet_{pc2}$	9	297.98	2.718	0.073 (0.028-0.153)	35.19 (29.38-40.26)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag} + holidays + wet_{pc1} + wet_{pc2}$	9	301.51	2.618	0.056 (0.004-0.129)	32.16 (27.74-36.53)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2} + RRv_{lag} + holidays + temp_{pc1} + wet_{pc1} + wet_{pc2}$	9	300.92	3.037	0.045 (0.005-0.093)	31.99 (28.16-36.34)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + RRv_{lag} + holidays + temp_{pc1} + wet_{pc1} + wet_{pc2}$	9	301.20	3.560	0.043 (0.006-0.112)	31.47 (27.49-36.67)
$RRv \sim AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + wet_{pc1} + wet_{pc2}$	8	299.37	3.575	0.039 (0.009-0.096)	33.75 (28.87-37.82)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2} + RRv_{lag} + holidays + wet_{pc1} + wet_{pc2}$	8	302.04	4.291	0.026 (0.003-0.073)	31.21 (26.78-36.19)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + RRv_{lag} + holidays + wet_{pc1} + wet_{pc2}$	8	302.53	4.696	0.023 (0.003-0.067)	30.67 (26.6-36.14)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + temp_{pc1}$	8	303.41	8.300	0.004 (0.001-0.021)	28.88 (24.47-34.48)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag} + holidays + temp_{pc1}$	8	304.57	8.359	0.003 (0-0.017)	28.27 (23.31-30.9)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2} + RRv_{lag} + holidays + temp_{pc1}$	7	306.23	9.333	0.002 (0-0.012)	27.32 (22.53-30.87)
$RRv \sim AeV_{lag1} + CxA + AeV + holidays + RRv_{lag} + temp_{pc1}$	7	304.53	9.491	0.002 (0-0.012)	28.33 (23.05-34.02)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + RRv_{lag} + holidays + temp_{pc1}$	7	307.01	10.643	0.002 (0-0.009)	26.63 (22.11-29.91)
$RRv \sim tide_{lag2} + rain_{lag2} + tide_{lag1} + rain_{lag1} + RRv_{lag} + holidays$	7	306.87	11.061	0.001 (0-0.01)	26.43 (22.21-29.43)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag2} + RRv_{lag} + holidays$	6	307.58	12.242	0.001 (0-0.004)	25.28 (21.35-28.63)
$RRv \sim rain_{lag2} + rain_{lag1} + tide_{lag1} + RRv_{lag} + holidays$	6	309.45	13.343	0 (0-0.003)	24.69 (20.61-28.31)
$RRv \sim CxA_{lag1} + AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}$	7	308.60	13.771	0 (0-0.001)	24.01 (20.07-29.24)
$RRv \sim AeV_{lag1} + CxA + AeV + holidays + RRv_{lag}$	6	310.47	15.748	0 (0-0.001)	22.61 (18.33-27.22)
$RRv \sim temp_{pc1} + wet_{pc1} + wet_{pc2}$	4	329.38	36.625	0 (0-0)	3.93 (1.88-6.39)
$RRv \sim temp_{pc1}$	2	332.25	39.004	0 (0-0)	0.87 (0.17-2.22)
$RRv \sim wet_{pc1} + wet_{pc2}$	3	332.97	38.302	0 (0-0)	1.43 (0.47-2.96)
Null	1	335.23	40.319	0 (0-0)	0 (0-0)

Appendix 5 Calculation of fertility and autogeny rates for *Aedes vigilax.* Hugo *et al.* (2003) published relationships between the adult body size (wing length (mm)) of *Ae. vigilax* and fertility and autogeny rates. Using a data extraction program (Plot Digitizer, 2010), I fit several different linear and non-linear models to the relationship between the *Ae. vigilax* fertility (number of eggs) and wing lengths (mm) collected by Hugo *et al.* (2003) in the field (Table 1).

Model	k	AIC_c	ΔAIC_c	$wAIC_c$	**%DE**
Log	2	3390.81	0	0.439	53.2
Linear	2	3391.48	0.68	0.314	53.1
Quadratic	3	3392.68	1.87	0.173	53.2
Cubic	4	3394.36	3.55	0.075	53.2
Null	1	3702.19	311.38	0.000	0

Table 1 Comparison of different models of the relationship between adult female *Aedes vigilax* fertility and wing length (mm). Statistics shown are Akaike's corrected information criterion (AIC_c), difference from best model (ΔAIC_c), Akaike weight ($wAIC_c$) scaled relative to a total sum of 1, and percent deviance explained by the fixed effects of the model (%DE).

I then used model-averaging (Burnham and Anderson, 2002) to predict the fertility rates of the *Ae. vigilax* adult females from the wing length data I collected during my two field experiments.

Appendix 6

Responses to reviewer's comments on Chapter 3, which was submitted as a journal paper to and accepted for publication in Pest Management Science on 7th September 2011.

Editor An important observation is that sampling using "five dips" does not provide an accurate picture of the number of larvae in the trap. This point is highlighted several times in the text, but I suggest it is included in the title which could be: Improved sampling techniques confirm that aerial larvicides are more effective than habitat modification for controlling disease-carrying *Aedes vigilax* mosquitoes.

Response: We have changed the title to "Improved sampling techniques confirm that aerial larvicides are more effective than habitat modification for controlling disease-carrying *Aedes vigilax* mosquitoes".

Reviewer 1 Comments regarding the 'slash and burn' experiments:

1. The title and abstract make no mention of the fact that the "slash and burn" treatments are nothing more than a pair of shears and a blow torch applied to 1 m quadrats. I'm really not very sure if such limited experiments can be extrapolated into grander claims about having conducted true field comparisons.

The very limited scale of these experiments is eventually discussed at the end of the paper, but I think the limitations need to be reflected in the title and abstract too.

The title made me believe that these were large scale trials, not semi-field experiments performed in 1m2 quadrats. The title a bit assertive for something done on such a closely controlled scale. How about "Small-scale comparisons of larviciding and habitat modifications as means for controlling Aedes vigilax"

Response: We have removed any reference to 'large-scale field experiment' and this has been

replaced with ‘controlled field experiment’. We have also renamed the ‘slash and burn’ treatments as ‘vegetation removal treatments (via shears or localised burning)’ throughout the body of the paper, including the Abstract. We have changed the title to remove the reference to field experiments: “Improved sampling techniques confirm that aerial larvicides are more effective than habitat modification for controlling disease-carrying *Aedes vigilax* mosquitoes”. It is also important to remember that while the cages were small relative to landscape extent, there are few, if any, such experiments done in field situations world-wide.

Comments regarding the presentation of results and Tables 3-5

Editor

Also the format used in Tables 3-5 to present the results is difficult to understand, and the relevant text doesn't help. Please try and find a more straightforward way to present these key results, as this would greatly increase the impact with a wider readership.

Reviewer 1

2. The results are presented in such an opaque way that I'd defy anyone who wasn't involved in the analysis to interpret them without putting half a day aside. I want to be able to see meaningful measures attached to treatments and their interactions. This section needs completely rewritten and the tables presented in a different way.

Table 3 is about abundance and emergence across habitats, Table 4 is about impacts of vector control methods and I can see that Table 5 is about something else but I couldn't tell you what it was. None of the tables leave me any the wiser and nor does a reading of text associated with them in the results section.

I would remove table 2 which goes into great deal of detail on measures which turned out not to have any impact.

The figures that describe the work are great - could we lose / cut down tables 3-5 and keep the figures?

Response: We acknowledge that the statistical methods used in our paper might appear

complex for the less-numerate reader; however, the complex nature of the data collected from our experiments required state-of-the-art statistical techniques (multi-model inference based on information-theoretic approaches). We can assure the editor and reviewer that we are applying the latest and most accepted approaches to test hypotheses from complex datasets – we use such approaches in all our hundreds of ecological publications. We have also written papers (e.g., Elliott & Brook 2007 *Bioscience* 57:608-614) and book chapters (e.g., Bradshaw & Brook 2010. The conservation biologist's toolbox. *Conservation Biology for All.* 313-334 Oxford Univ Press) on the techniques, and rigorously defend our approaches because classical Neyman-Pearson null-hypothesis testing are not only inadequate, they often result in incorrect conclusions.

However, to increase the accessibility of our results to readers, we have removed the majority of the tables (Tables 3-5) from the body of the manuscript and included them instead as supplementary information. We have re-written the results section to be clearer, focusing on the results from the adult emergence model sets (see Pages 13-15 in the revised manuscript). We have also included a variable-ranking statistic that allows readers to examine directly the explanatory strength of different predictor variables (see new Table 2).

Minor Comments

Editor

The format of the journal requires that the full stop at the end of a sentence comes before superscript with references.

Response: We have reviewed all sentences with reference superscripts and moved the full stop as necessary.

In Section 2.3 the wording should be: ...methods outlined by Service - not in Service.

Response: We have changed the wording as suggested.

In Section 4.3 ...current practice - not practise.

Response: We have changed the wording as suggested.

across appears twice in the heading for Table 2.

Response: We have removed this table from the manuscript.

Reviewer 1

The referencing at the start of the paper is a bit inadequate (see comments on marked up pdf)

Response: We address the comments from the marked pdf individually below:

Page 2, Line20, marked up pdf. "This suggests that there are drugs for dengue and yellow fever."

Response: We have changed this line to "While there are many ways to treat and prevent these diseases, pharmaceutical-based solutions, where they exist, ultimately become intractable during large outbreaks" Page 2, Line 20, revised manuscript.

Page 3, Line 2, marked up pdf. "Make it clear that in very few instances has resistance made much of an impact on transmission. The threat of resistance is greater than the actual impacts to date."

Response: We have changed this line to "The efficacy of insecticide-based adult-vector-control tools will be potentially compromised by the evolution of resistant vector populations" Page 3, Line 3, revised manuscript.

Page 3, Line 3, marked up pdf. "And are just as vulnerable to resistance evolution"

Response: We have changed this line to "however, non-insecticide-based methods that target vector larvae have had some success in reducing vector populations and the concomitant pathogens they transmit" Page 3, Line 4, revised manuscript.

Page 3, Line 10, marked up pdf. "but look at examples from agriculture. Bti is not immune to the evolution of resistance."

Response: We do not assert that microbial larvicides such as *Bti* are immune to the evolution of resistance, only that it is less likely than the evolution of resistance to chemical insecticides.

Page 3, Line 10, revised manuscript "Microbial larvicides are highly effective at suppressing vector numbers, are environmentally benign for non-target organisms, and due to the complex of insecticidal proteins present, are less likely to result in resistance than chemical insecticides."

Page 3, Line 16, marked up pdf. "This doesn't add up to great evidence Ref 6 is about Anopheles and ref 14 is about artificial environments"

Response: At Page 3, Line 18, in the revised manuscript, we have added the following reference that shows evidence for the effectiveness of environmental management in controlling populations of *Aedex vigilax* in the field:

Turner PA and Streever WJ. Changes in productivity of the saltmarsh mosquito, *Aedes vigilax* (Diptera: Culicidae), and vegetation cover following culvert removal. *Australian Journal of Ecology*; **24**: 240-248 (1999).

Page 3, Line 19, marked up pdf. "There's only one reference here that actually supports that statement. That's very slight evidence for one of your central hypotheses."

Page 3, Line 22, marked up pdf. "Again, I am not convinced that this is all adding up to evidence for the suggestion that seasonal vegetation removal might be a viable alternative to aerial larviciding."

Response: At Page 3, Line 22, in the revised manuscript, we have added the following references as evidence to support our central hypotheses:

Brogan B, Whelan PI, Carter J and Lamche G, Rectification and control practices in a major

salt marsh mosquito breeding site, Darwin, NT. *The Northern Territoy Disease Control Bulletin*; **9**: 16-21 (2002).

Whelan PI, Integrated mosquito control in Darwin. *Arbovirus Research in Australia*; **5**: 178-185 (1989).

Yang G-J, Brook BW, Whelan PI, Cleland S and Bradshaw CJA, Endogenous and exogenous factors controlling temporal abundance patterns of tropical mosquitoes. *Ecological Applications*; **18**: 2028-2040 (2008).

Page 4, Line 9, marked up pdf. "I'm concerned about your referencing, This Gu and Novak reference is a modelling paper that says nothing empirical about how larval and adult densities match up. Why don't you read something out of Scott and Morrisons lab where they look to see whether larvae or pupae are better indicators of adult density?"

Response: We thank the reviewer for the suggestion, at Page 4, Line 12, in the revised manuscript, we have added the following reference:

Getis, A., Morrison A. C., Gray, K. and Scott, T. W. Characteristics of the spatial pattern of the dengue vector, Aedes aegypti, in Iquitos, Peru. *American Journal of Tropical Medicine and Hygiene* **69**(5): 494-505 (2003).

Page 4, Line 14, marked up pdf. "you mean density dependent effects?"

Response: This line has been changed to: "Although monitoring adult numbers can track local vector population dynamics and quantify population trends resulting from control, monitoring only the adult population cannot detail the absolute effect of larval control on vector numbers due to the confounding influences of density feedback on larval survival, larval habitat availability, and the alternating activities of blood-meal seeking and oviposition." Page 4, Line 17, revised manuscript.

Page 4, Line 24, marked up pdf. "But the biggest problem is that it is a vicious biter and

a real nuisance."

Response: This line has been changed to: "*Ae. vigilax* is recognised as vicious biter and is also a major vector of Ross River virus and Barmah Forest virus in coastal and sub-coastal areas. Therefore, effective control of a major nuisance species that is one of the primary vectors of these pathogens is a high priority for the Northern Territory public health management." Page 5, Line 1, revised manuscript.

Page 5, Line 3, marked up pdf. "How effective were they, and why don't you use these to back the hypothesis that environmental management of this mosquito might be an alternative to larvicides?"

Response: This line has been changed to: "although there have been previous successful attempts to remove larval habitats permanently in some areas of Darwin through environmental modification." Page 5, Line 5, revised manuscript.

These references have been also used to back the hypothesis that environmental management of *Aedes vigilax* might be an alternative to larvicides (see previous comments).

Page 5, Line 11 "why were they abandoned?"

Response: To emphasise that previous environmental modification methods used to control *Aedes vigilax* populations were only applied once, and were major, disruptive engineering works that removed some, but not all of the larval habitats, this line has been changed to: "previously, large-scale engineering environmental modification methods, such as drain infilling and culvert removal, that aim to increase tidal flushing in coastal swamps, have been used for this species to alter pooling and vegetation characteristics, with some success at reduction of larval habitats." Page 5, Line 13, revised manuscript.

Page 5, Line 14 "so why is it used - presumably because of evidence that environmental management is not effective or too difficult or too expensive."

Response: The reviewer is right to point out that these are reasons against the use of aerial larvicide as a control measure; however, this method is used because the previous engineering methods did not remove all larval habitats, and therefore, ongoing larval control is needed. See previous response.

Page 5, Line 17 "Interesting point, can you say what has been done elsewhere in the world to quantify Bti effects on adult mosquitoes (e.g. Fillinger et al 2009 Bull WHO & Lardeux et al 2002 ATMP)."

Response: We have changed this line to "Although several studies have found evidence for reductions in surveyed larvae numbers and indoor resting adult populations in conjunction with local larvicide application, this outcome has never been quantified experimentally", Page 5, Line 23, revised manuscript. We thank the reviewer for their suggestions and have also added the following reference:

Fillinger, U., Ndenga, B., Githeko, A. and Lindsay, S. W. Integrated malaria vector control with microbial larvicides and insecticide-treated nets in western Kenya: a controlled trial. *Bulletin of the World Health Organisation* **87**(9): 655-665.

Page 6, Line 11 "avoid I, we, they etc."

Response: We recognise that the reviewer has asked that we amend our methods and results section so it is written in the third person; however, with all due respect, we prefer to write papers in the first person because it is less archaic, more succinct and we believe it reflects better the notion that we did the work we describe. Pest Management Science does not specify as to whether the methods need to be written in the first or third person. We have left the paper as written (in first person).

Page 8, Line 13 "The specifics here (time to the minute, tides to two decimal places) are unnecessary"

Response: We have removed the specifics, and simply reported date and tide to one decimal place, Page 8, Line 18, revised manuscript.

Page 11, Line 19 "standardise the way you write this. Previously it was: before-after/control-impact"

Response: After the first instance, we have changed references to the before-after/control-impact design as the BACI design, Page12, Line 9, revised manuscript.

Page 11, Line 24 "define these please"

Response: This line has been changed to "local environmental conditions (first principal component from the PCA of pH, conductivity, dissolved oxygen and mean daily temperature)", Page 12, Line 14, revised manuscript.

Page 12, Line 23 "Is any form of larval sampling a true measure of habitat

Response: We have changed this line to "indicating that this form of larval sampling is not an accurate measure of mosquito production from a given habitat", Page 13, Line 23, revised manuscript.

Page 13, Line 9 "So spell out the relationship between water depth and larval abundance."

Response: We thank the reviewer for raising the confusing language we previously used to describe our results. We have rewritten the results section to make our key results clearer to the reader. Water depth was included in models as a control of some of the external confounding variation present between traps (see Results section, Page 10, Line 24, revised manuscript).

Page 13, Line 13 "To what extent do you think that shears and a blowtorch applied to

1m quadrats represent what happens during slashing and burning?"

Response: We agree that shears and a blowtorch are a poor approximation of the agricultural practises of slashing and burning. We have renamed the 'slash and burn' treatments as 'vegetation-removal treatments (via shears or localised burning)' throughout the body of the paper. See previous response to major comments regarding the 'slash and burn' experiments.

Page 14, Line 18 "but no impact on larval abundance (line 12 page 13) "

Response: The reviewer is right to point out that we have made no reference to our larval results here; however, as we have previously quantified that our measures of larval abundance are poor (see Results section, Page 13, Line 22, revised manuscript), in the Discussion we decided to concentrate on the results of the more accurate representation of mosquito numbers per treatment: adult emergence.

Page 14, Line 21 "and at these experimental scales"

Response: We have changed this line to "we provide direct confirmation that larvicide application is the most effective control method (of those considered at these experimental scales)", Page 16, Line 13, revised manuscript.

Page 15, Line 21 "Again this Gu and Novak paper is a modeling exercise with, as far as know, no reference to empirical measures or operational decision making. "

Response: We have included the following reference:

Getis, A., Morrison A. C., Gray, K. and Scott, T. W. Characteristics of the spatial pattern of the dengue vector, Aedes aegypti, in Iquitos, Peru. *American Journal of Tropical Medicine and Hygiene* **69**(5): 494-505 (2003).

Page 16, Line 18 "Blanket spraying with Bti will, of course, affect all mosquitoes, blackflies and chironomids. Some of them are non-targets."

Response: We support the reviewer's assertion that *Bti* will affect non-target species, although in this paragraph, we are referring to the possible negative effects of environmental manipulation. Nowhere in the manuscript do we assert that *Bti* does not affect non-target species.

Page 17, Line 3 "although of course we know that it is extremely difficult to predict the levels of vector control necessary to impact on disease. The only strategy is to go for elimination."

Response: We have changed this line to: "The end goal of any vector control programme is the reduction, or if possible, elimination of the adult vector population and so by logical extension, the reduction in incidence of vector-transmitted pathogens and disease", Page 18, Line 16, revised manuscript.

Page 17, Line 4 "Very true, so why not add a couple of references to the argument."

Response: We have added the following reference:

Fillinger, U., Ndenga, B., Githeko, A. and Lindsay, S. W. Integrated malaria vector control with microbial larvicides and insecticide-treated nets in western Kenya: a controlled trial. *Bulletin of the World Health Organisation* **87**(9): 655-665.

Page 23, Line 9 "Spelling"

Response: We have corrected the spelling of "Linthicum", Page 25, Line 9, revised manuscript.

Page 29, Table 1 title "there are 4 dates in the table"

Response: We have amended the Table 1 title to: "(a) Numbers of larvae averaged over 5 sampling dips 1 taken each day for 4 days", Page 30, revised manuscript.

Page 29, Table 1(b) "nomenclature is not very useful here. Any way these results might be made more meaningful? The discussion of AIC in section 2.9 doesn't do anything like enough to make this accessible. All I want to know is which treatment worked!"

Response: We acknowledge that the statistical methods used in our paper might be more difficult for the less-numerate reader – see response above. With all due respect, we have used the common and widely used nomenclature for referring to Akaike's information criterion and related statistics (see examples in the references at the end of this response). We believe that readers will be able to determine that there is no statistical support for the model/hypothesis that larval sampling (via dipper) represents actual larval densities, from the title of this table: "Table 1 (a) Numbers of larvae averaged over 5 sampling dips taken each day for 4 days, and (b) Comparison of two models used to assess the ability of larval sampling dips to predict actual larval numbers per trap, using information-theoretic model ranking. Variables included in models are density: actual larval numbers per trap, and dip: larval numbers indicated by larval sampling using dipper", and the description of Akaike weights and statistical support for various models from the methods section: "the strength of evidence ($w\text{AIC}_c$) for any particular model varies from 0 (no support) to 1 (complete support)", Page 13, Line 6, revised manuscript.

References for Akaike's information criterion nomenclature:

Yang GJ, Brook BW, Whelan PI, Cleland S, Bradshaw CJA, Endogenous and exogenous factors controlling temporal abundance patterns of tropical mosquitoes., *Ecological Applications* **18**(8):2028-40 (2008).

Yang GJ, Bradshaw CJA, Whelan PI, Brook BW, Importance of endogenous feedback controlling the long-term abundance of tropical mosquito species, *Population Ecology* **50**(3):293-305 (2008).

Jacups SP, Whelan PI, Markey PG, Cleland SJ, Williamson GJ, Currie BJ, Predictive indicators for Ross River virus infection in the Darwin area of tropical northern Australia, using long-term mosquito trapping data, *Tropical Medicine & International Health*

13(7):943-52 (2008).

Yang GJ, Brook BW, Bradshaw CJA, Predicting the timing and magnitude of mosquito population peaks for maximizing control efficiency. *PLoS Neglected Tropical Diesases* **3**(2) (2009)

de Little SC, Bowman DMJS, Whelan PI, Brook BW, Bradshaw CJA, Quantifying the drivers of larval density patterns in two tropical mosquito species to maximize control efficiency, *Environmental Entomology* **38**(4):1013-21 (2009).

Russell TL, Lwetoijera DW, Knols BGJ, Takken W, Killeen GF, Ferguson HM, Linking individual phenotype to density-dependent population growth: the influence of body size on the population dynamics of malaria vectors, *Proceedings of the Royal Society B-Biological Sciences* (2011).

Page 30, Table 2 "Unless the authors see some trends in here which can be reported on, then I don't need to see all this. Just report this in the text as ranges. "

We removed Table 2: *Average local environmental conditions across measured across different habitat types and treatment levels* from the manuscript. We reported the values of the different environmental conditions in the Results text as ranges. See Page 14, Line 6, revised manuscript.

Page 31, Table 3 "I'm sure this a great analysis, but all this detail means very little if the reader can't interpret the table. What is the null scenario? nomenclature is not very useful here. Any way these results might be made more meaningful? The discussion of AIC in section 2.9 doesn't do anything like enough to make this accessible. All I want to know is which treatment worked!"

See previous response to major comments regarding the presentation of results and Tables 3-5. We have relocated Table 3 to the supplementary material.

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Addendum

Response to thesis examiners

Examiner 1:

Formatting of in-text referencing: the sequence for in-text references is neither alphabetical nor chronological, and seems to follow no particular rule. A chronological order should be established and applied uniformly throughout. Also references by different authors should be separated by a semi-colon. Authors who have published multiple papers in the same year should be cites as (for example) Smith (1998a,b) not Smith 1998a, Smith 1998b.

I have formatted the in-text referencing in chronological order, separated different authors by a semi-colon and cited authors who published multiple papers in the same year as (for example) Smith (a,b) rather than Smith1998a, Smith 1998b.

Use of genus name abbreviations: the genus name *Aedes* (abbrev. as *Ae.*) has not been applied with consistency, even within a chapter. I can understand that *Aedes* will be written in full on first mention in a chapter but thereafter should be abbreviated. This rule has not been adhered to throughout and should be remedied.

Further, the correct Australian system is for the genus name to be spelled out in full when used at the start of a sentence. I can't see why this shouldn't be applied to unpublished chapters.

I have searched the thesis and edited all occurrences of genus names and changed them according to these suggestions.

Contents pages: there are no chapter numbers given on the Contents page.

Chapter numbers have been added to the Contents pages.

Use of the term 'larvae' (pl. Noun) and 'larval (possessive): on numerous occasions through this thesis, the term 'larvae' has been used incorrectly. The term 'larval should be used as the possessive when using such terms as, "larval habitat", "larval density" etc. It shouldn't be written as "larvae density". That would be akin to writing "tadpoles density" or "fishes habitat".

I have searched the thesis and edited all occurrences where the term 'larvae' was used incorrectly, and replaced it with 'larval'.

Superscripts and subscripts: ensure that all superscript and subscript numerals are correct throughout.

I have searched the thesis and ensured that all superscript and subscript numerals have been corrected.

Chapter 1

Pg 14 last para: 'thusfar' is one word not two.

'thus far' is two words, and therefore has not been changed.

Pg 18 para 1: Should read 'surrogate'

'surrogates' has been corrected to 'surrogate'

Chapter 2

Methods: field estimates of larval density are fraught with error due to being heavily influenced by inter-operator viability. Some discussion of how important/unimportant this is needs to be provided. It's worth noting that the NT is exceptional in Australia for being a jurisdiction in which larval density data are routinely used. Many in this country don't view larval density measures as reliable or very usable.

My final models accounted for the possible errors in the larval density scores, by modelling

the relative larval densities as weighted presence/absence data, rather than abundance data. I have added this explanation in the methods section (pg 27):

> "To account for sampling error, either due to inter-operator variability or sampling process, the relative larval densities were modelled as weighted presence/absence scores rather than abundance scores. The completed dataset spans November 2000 - December 2006."

Pg 26 para 1: CO2 should be written with 2 as subscript.

'CO2' has been corrected to 'CO_2'

Pg 27 para 1: start all sentences with upper case. Should read 'My...'

'my' has been corrected to 'My'

Pg 27 last para: 100-m2 should have the 2 as a superscript

'100-m2' has been corrected to '100-m^2'

Pg 31 para 1: should read 'Cx. annulirostris'

'*Cx. Annulirostris*' has been corrected to '*Cx. annulirostris*'

Pg 32 last para: I don't understand the use of the word 'insecticides' in the first line.

The word 'insecticides' has been removed from the first line of the last paragraph on pg 31.

Pg 38 para 1: last sentence: should read, 'This is shown...'

'This is show' has been corrected to 'This is shown'

Pg 39 para 1: sentence starting with lower case.

'my' has been corrected to 'My'

Pg 42 para 1: sentence starting with lower case.

'my' has been corrected to 'My'

Chapter 3

Methods: how were dead larvae (killed by Bti) considered in counts? They should have been visible and dipped in the days after spraying

Dead larvae were not considered in counts. They were visible in the traps, and occasionally sampled in the dipper, but only live larvae were counted. I have made this clearer by adding the following to the Methods section (pg 54):

'...I did five dips (one in each corner of the trap and one in the centre) using a standard dipper (190 ml volume). I counted live larvae and then returned them to the traps...'

Pg 48 para 2: suggest reword '...high priority for public health management in the Northern Territory...'

'high priority for the Northern Territory public health management' (pg 48, paragraph 2) has been reworded as 'high priority for public health management in the Northern Territory'

Pg 58 para 2: Results line 1 – subheadings should be in italics.

Subheading 'Larval sampling, larval presence and adult emergence across habitat types'

(pg 55, paragraph 4) subheading has been italicised to '*Larval presence and adult emergence across habitat types*'

Pg 69 para 2 line 5: sentence to start with upper case

'my' has been corrected to 'My'

Pg 69 para 2 line 6: suggest replacing 'via' with 'by'

'via' has been replaced with 'by'

Pg 70 last para: sentence to start with upper case

'my' has been corrected to 'My'

Pg 71 last para and Pg 72: the discussion on the limitation of larval survey is fine, but this needs to be linked with the decisions currently (commonly?) made regarding application of larvicide. If applications are simply made based upon presence of larvae, then the limitations of the larval survey are not so relevant.

Before the research findings I describe in this chapter, decisions about the application of larvicide to particular swamp areas were based on *Ae. vigilax* larval 'densities' recorded during routine larval surveys. My research shows that the sampling methods currently used accurately represent actual larval density, and therefore control decisions are based on false assumptions. I have now made this distinction clearer in the final paragraph of my conclusions (pg 69):

> "Quantification of the relationship between larval sampling measures, larval abundance and/or density, and adult vector emergence is essential to determine whether control methods are indeed reducing the productivity of larval habitats. Larval densities measured during sampling surveys are routinely used to direct control efforts; therefore, extreme care needs to be taken when interpreting the findings of such surveys. Using larval sampling surveys as an indication of larval presence or absence only, or better still, supplementing larval sampling surveys with models of the climatic, environmental and intrinsic factors that drive vector population dynamics to determine optimum control strategies can assist, with the corollary that good predictive models based on the ecology of local vector populations might eventually supplant expensive and time-consuming larval surveys."

Chapter 4

Pg 76: the term is 'vectorial capacity' I believe

'Vector capacity' (pg 73, last paragraph) has been corrected to 'Vectorial capacity', and this correction has been applied to all other occurrences of the term 'vector capacity' in the thesis.

Pg 77: para 1 should read, '...pathogens with long incubation periods...'

'pathogens with a long extrinsic incubation periods' (pg 74, paragraph 1) has been corrected to 'pathogens with long extrinsic incubation periods'

Pg 78: last para – delete extra period after, '...(e.g.)'

'(e.g.,' has been corrected to '(e.g.'

Pg 79 para 1: I don't understand the difference between 'emerging' and 'adult' mosquitoes – both are adults

The 'emerging' population refers to recently emerged adults that have not yet started feeding, whereas the 'adult' population was sampled using CO_2 traps, indicating that these females were already feeding. I have made this less confusing by changing:

'by examining female body size of both emerging and adult populations' (pg 76, paragraph 2) to 'by examining female body size of both emerging and feeding adult populations'

Pg 80: some clarity is required around the definitions of larval density manipulations. The resultant larval densities are not shown and they need to be. It's hard to know if increasing or decreasing larval density by 15-30 per trap is significant, particularly if the total number goes from say 1000 to 1030. From the results, I suspect the density change probably IS significant but we need clarity here.

I have included the average larval density and adult emergence rate per trap that was determined in Chapter 3 to give an idea of how important any reduction or supplementation of

larval density was (pg 77).

> "Ten other emergence traps were also established in this vegetation type, and once the larvae hatched, the larval densities of five of these traps were reduced and the collected larvae used to supplement the density of the five remaining traps. The average density of *Ae. vigilax* in the *Schoenoplectus* habitat of the LHJ swamp was 78 (± 25) larvae 1 m^{-2} larval trap, and average emergence was 30 (± 26) adults per trap (Chapter 3). It is often difficult to achieve exact replication of manipulation in the field (Pedersen et al., 2004; Fordham et al., 2009), and it is especially difficult to determine larval density from larval sampling procedures (Chapter 3; Workman and Walton, 2000; Thullen et al., 2002); therefore, I reduced the larval density (removed larvae) in the traps where a low count of larvae was observed during sampling. On average, trap larval density was reduced by 15-20 larvae, or increased (supplemented) by 30 larvae per trap."

Pg 82 adult size variability and distance from LHJ – a) how do you know these mosquitoes were from LHJ? You would need to do a mark-release-recapture study to tell (why didn't you do one? There are a lot of precedents for saltmarsh mosquitoes). b) assuming they WERE all from LHJ, how do you measure distance from a swamp complex? Where is the point of measurement from? Because the source of the mosquitoes is unverified (can you provide some assurance on this?) it is then hard to know the duration since emergence. A mark-release-recapture would have solved these probems. What were the actual distances – these are never stated, making it hard to interpret the results.

Unfortunately I did not have the timing or the funding to undertake a mark-release-recapture study to verify the emergence sites of the adult *Ae. vigilax* females caught in the CO_2 monitoring traps situated around the LHJ swamp edge. As the LHJ swamp is the nearest *Ae. vigilax* breeding site to the monitoring traps, I assumed that the mosquitoes caught in these traps emerged there. Distance to emergence site was measured as the shortest distance

between the CO_2 trap and the edge of the tide-inundated breeding area of LHJ. I have included this description and the distances to traps in the methods section (pp 78-79):

> "Four of these trap locations were selected as study sites (Karama, Palm Creek, Holmes Jungle and Marrara Round Swamp) (Fig. 1). These sites were chosen to sample variability in sizes of the female *Ae. vigilax* adults at different distances from their probable emergence site (LHJ swamp). The LHJ swamp is the nearest *Ae. vigilax* breeding site to the four CO_2 traps selected, so I assumed that most of the mosquitoes caught in these traps emerged there. Distance to emergence site was measured as the shortest distance between the CO_2 trap and the edge of the tide-inundated breeding area of LHJ swamp (Karama = 1.58 km, Palm Creek = 2.33 km, Holmes Jungle = 0.99 km, Marrara Round Swamp = 2.75 km). On two occasions, a sample of 50 adult female *Ae. vigilax* were selected randomly from the total trapped adults at the four sites."

Pg 87 figure – poor resolution on this figure – please rectify

I am unable to rectify the resolution on this figure because I lost the image files that would allow me to do this when a virus infected my computer and back-up hard drive. For any publications arising, I will have to redo this figure from scratch.

<u>Chapter 5</u>

Pg 96: Ross River virus is not best described as tropical/subtropical. Very high incidence occurs in temperate Australia.

'a widespread tropical and subtropical disease in Australia – Ross River virus' has been changed to 'a widespread mosquito-borne disease in Australia – Ross River virus'

Pg 100 para 2: should read, '...here I examine...'

'Here examine' has been changed to 'Here I examine'

Pg 101. Please briefly explain the significance of 7.4m tides.

I have included an explanation of why 7.4 m tides are important for creating *Ae. vigilax* breeding habitat, including the relevant references (pp 98-99):

> "The Australian Bureau of Meteorology provided monthly data for climatic variables covering the same interval as the Ross River virus dataset (www.bom.gov.au). These included summed rainfall (mm), number of rain days (where a rain day is defined as ≥ 1 mm rain), humidity measured as average vapour pressure (hPa), evaporation (mm), average daily maximum temperature (^{o}C), maximum temperature (^{o}C), average temperature (^{o}C), average daily minimum temperature (^{o}C), minimum temperature (^{o}C), maximum tide height (m), mean tide height (m), and frequency of tides higher than or equal to 7.4 m (tides > 7.4 m generate temporary saltwater habitats ideal for the oviposition and larval development of *Ae. vigilax* in the swamps around Darwin (Whelan 1987, Yang et al., 2008b))."

Pg 103: another reason for including time-lagged variables for disease models is to account for zoonotic amplification cycles that precede human cases

I have included a sentence indicating this further reason for including time-lagged variables in disease models (pp 100-101):

> "The response variable was monthly Ross River virus case counts, and explanatory variables included were the first *tidal* and *rain* principal components. In the Northern Territory, the average time from inundation of *Ae. vigilax* and *Cx. annulirostris* larval habitats to adult emergence is 9 – 13 days (Sinclair, 1976; Lee et al., 1989). Therefore, I included lags of 1 and 2 months of the environmental proxies in the models. These lagged variables also account for zoonotic amplification cycles that can precede human cases. Season (wet/dry), human exposure (number of public holidays per month) and Ross River virus immunity (peak Ross River virus case count from the previous year) were also included in the models."

Pg 103 last para: always write in past tense when describing research. Should read, '...I used...'

'I use' has been changed to 'I used'

Pg 106 last para: should read, '...there was a positive correlation...'

'there is a positive correlation' has been changed to 'there was a positive correlation'

Pg 106: the negative relationship between *Ae. vigilax* abundance and RRV incidence may be because although *Ae. vigilax* is involved with zoonotic amplification cycles it is not the major vector to humans (*Cx. annulirostris* is). This modelling reveals some interesting insights. And pg 111 – the finding that high tide frequency lagged 2 mo. Lends further credence to the idea of *Ae. vigilax* being the zoonotic cycle amplifying vector.

I included a sentence on the idea of *Ae. vigilax* as a zoonotic cycle amplifying vector as opposed to a human vector in the discussion on page 123:

> "The major vertebrate hosts for Ross River virus in the Northern Territory are the new juvenile populations of agile wallabies *Macropus agilis* and dusky rats *Rattus colletti* (Jacups et al., 2008a). Here, juvenile recruitment and population growth in both reservoir species are driven by seasonal rainfall (Madsen and Shine, 1999; Shine and Brown, 2008). I contend that including lags of rainfall in my models provides surrogate information in this regard. The negative relationship between both measures of *Ae. vigilax* abundance (both trap and environmental proxy) and RRv incidence indicates that this vector species might be involved in zoonotic amplification cycles in the wallaby and rat populations, rather than acting as a major RRv vector to humans *per se*."

Why didn't temperature make it into the environmental proxy models? Temperature

speeds up extrinsic incubation period, thereby increasing vectorial capacity. It is also likely to influence biting rate.

Temperature affects biting rate, human exposure and extrinsic incubation period, so it was included in the final combined model sets (see pg 99 for description of variables, pp115-116 for highest-ranked models, and Appendices 4a&b, pp 157-181 for description of PCA and full model sets), rather than the environmental proxy model sets that include variables driving mosquito population dynamics (determined in previous Chapters) as opposed to the disease dynamics.

Chapter 6

Pg 134 and onwards: I'm not sure about the use of the term 'recipe' here. It trivializes what is a serious issue that has been treated seriously by the candidate. Further, it is not a great analogy in this case anyway.

I have removed all occurrences of the term 'recipe' and in all cases replaced it with a grammatically correct version of 'five-step plan'.

Pg 138: line 1 – delete 'the'

'therefore, the how best' has been changed to 'therefore, how best'

Pg 138 pts c) and d): 'model' should be used with a definite article here, i.e. '...the model...' or if not, then you should refer to 'models' (pl.)

'model' has been changed to 'the model'.

Pg 140: there are numerous problems with the parameters used in the model(s) because so many of these are temperature-dependent yet are treated as absolutes. So, rather than a single value being used, there should be functions. This will be especially important for any developmental rates, gonotrophic cycle lengths, larval survival (this

will also be density dependent). For egg senescence, is instalment hatching taken into account here? Most *Aedes* (*Ochlerotatus*) hatch off roughly 55% on first inundation then small amounts out to about 5-6 more inundations. Data are available for some Australian species (e.g. *Ae. camptorhynchus* – southern saltmarsh mosquito). The limitations of these quoted 'vital rates' need to be explained.

In Table 1 on pg 137, I specify that I am reporting mean values for the different vital rates, not absolute values. I also include the range of the various parameters that I sourced from the literature under varied environmental conditions such as temperature and humidity. The values of the vital rates under a range of conditions provide the standard deviation (in parentheses). Also, for tropical mosquitoes, during the breeding season, annual temperatures don't vary very highly compared to conditions experienced by in temperate areas. I have included a note to indicate that these various vital rates will change subject to environmental conditions in the table title:

> "Table 1 Demographic life history parameters for *Aedes vigilax* and *Culex annulirostris* (see supplementary material for references). Mean values of parameters are reported, and the range, recorded over a range of temperatures, is shown in parentheses. GL = Gompertz-logistic model of population growth, r = population growth rate. Note: parameter values of vital rates will vary with environmental conditions (e.g., temperature, humidity, etc.)."

In Figures 1 and 2 (pp 136 and 148), again, I report the mean values for the vital rates and the range within which they will vary, given different environmental conditions. I have included a sentence in the figure titles to make this more explicit:

> "Figure 1 Demographic stage-structured life cycle graph for *Aedes vigilax* and *Culex annulirostris*. Mean values of parameters are reported, and the range is shown in parentheses. Note: parameter values of vital rates will vary with environmental conditions (e.g., temperature, humidity, etc.). N_A = number of females, N_{EA} = number of emerging females, and $f(N)$ = Gompertz-logistic model of population growth, where r= realised

population growth rate, r_m = maximal intrinsic r, K = carrying capacity, N_t = mosquito population size at time t, and ε_t = environmental variability. Autogeny (capability of developing the first batch of eggs without a blood meal) is negligible in *Culex annulirostris* (only 7% of females), and therefore was not included in the life cycle for this species."

"Figure 2. Demographic stage-structured life cycle graph for *Aedes vigilax.* Mean values of parameters are reported, and the range is shown in parentheses. $\boldsymbol{f(N_F)}$ represents the quantified effects of larval density on fertility, $\boldsymbol{f(N_A)}$ represents the quantified effects of larval density on autogeny rates, and $\boldsymbol{f(N)}$ represents the quantified effects of larval density on survival (for values see Table 2). Note: parameter values of vital rates will vary with environmental conditions (e.g., temperature, humidity, etc.). The various environmental variables that affect vital rates are shown: **climate** = season, rainfall, temperature and tides; **water** = water qualities such as pH, salinity, temperature and nutrition level; **habitat** = available larval habitat (number of ephemeral pools, vegetation type); **bloodmeal** = probability of acquiring a blood meal (dispersal); **insecticide** = mosquito control measures (insecticides, larvicides); and **predators** = aquatic predators of larvae. Bold arrows, text and diagrams indicate vital rates and intrinsic and extrinsic processes quantified by my research."

Within the thesis text, whenever I refer to these vital rates I specify that they are temperature- and environment-dependent (see Figure 2., pg 138), and in Steps 2 and 3 of my five-step vector control plan, I explicitly state the importance of defining the sensitivity and dependence of these various vital rates on compensatory and depensatory intrinsic feedback mechanisms and environmental conditions (see pp 139-144).

Pg 141: end of last para – extra period should be deleted.

The extra period at the end of the last paragraph has been deleted.

Pg 145: Fig 3 – need to explain axes

Fig 3 axes have been labelled.

Pg 146: para 1 – should read, '...weight gain per larva...'

'weight gain per larvae' has been corrected to 'weight gain per larva'

Pg 147 Line 1 – replace 'costal' with 'coastal'

'costal' has been corrected to 'coastal'

Pg 148 Step 4 – Discussion – what would be nice to know, from a control perspective, is what percentage larval reduction is required to avoid undesirable compensatory effects that don't outweigh the advantages of reduced adult emergence. THAT modelling would be vital to know – is too little control a bad thing? How much is too little?

I have included a paragraph discussing how I would go about determining the percentage larval reduction required to avoid undesirable compensatory effects (pp 145-146):

> "In particular, quantifying the percentage of larval density reduction required to avoid undesirable compensatory effects that outweigh the advantages of reduced adult emergence is an important step for any vector control program. This relationship is highly complex, and incorporates the effects of component density feedback (influence of larval density on adult survival and fertility), ensemble density feedback (influence of larval density on the population growth rate), the vectorial capacity of the population, and the influence of adult survival and body size on vector competence. A detailed understanding of all of these aspects of the vector-disease system and how they respond to environmental conditions would be essential to model these relationships. For a species such as *Ae. vigilax* that has no relationship between body size and vector competence (Jennings and Kay, 1999), and because the Darwin populations exhibit a log-linear relationship between population density and growth rate (Yang et al., 2008a), the next step would be to

determine the nature of the component density feedback: for example, whether adult survival is independent of larval density, declines linearly with larval density, or declines log-linearly with larval density. Once this relationship between adult survival and larval density is established, it will be possible to determine if there is a threshold point of larval density to target with control methods to acheive a desired adult survival rate/population size balance to minimise disease transmission risk."

Examiner 2:

Introduction (Ch. 1)

pp. 1-3. Climate change and vector borne disease. The potential negative effects of climate change on vector borne disease are probably not described in sufficient detail to give a full appreciation of the complexity of what is expected. Most climate change models predict that changes are likely to be highly variable in space and localized effects are perhaps the most important aspects of climate change for vector borne disease. It is not that anything in this introduction is incorrect; rather, it is too simple and does not convey the true range of possibilities that arise because of variation in disease ecology, variation in local patterns of climate change, and variations in human responses to climate change. Land use change and human population change and movement are also likely to interact with climate change and it would be necessary to know a lot of detailed information about particular disease systems at particular locations to be able to make even approximate forecasts on how climate change will affect vector borne disease. In some ways this short chapter is an improvement over some of the very general, rather naive attempts to predict climate change effects on disease risk. But in attempting to speak generally about "vector borne diseases" collectively, it makes the same mistake as

past general reviews. On the plus side, this section at least addresses effects of climate on mortality of egg, larval, and adult stages, and the effects (however uncertain and variable) on habitat for larvae. Despite mentioning human responses early on, very little is said about this important aspect of global change and disease.

I have expanded my discussion about the effects of climate change on vector populations and disease transmission to include possibly synergies with human population movements, density and land-use changes, and also the possible varied effects of aspects of climate change (pp 3-4):

> "The projected increase of 1.4 to 5.8 ^{o}C in mean global temperature by 2100 is predicted to allow the expansion of vector population ranges into areas that have previously been disease-free (Campbell-Lendrum and Woodruff, 2007; Kearney et al., 2009; Lafferty, 2009; Jansen and Beebe, 2010; Tonnang et al., 2010). Increasing temperatures year-round will also increase the seasonal period when disease transmission is possible, and through the reduction of pathogen incubation time, increase the likelihood of vector infection and transmission (Lafferty, 2009; Paaijmans et al., 2009). Rising sea levels will increase the larval habitats available for saline water-breeding vectors such as *Anopheles sundaicus*, *Culex sitiens*, *Culex tarsalis* and *Aedes camptorhynchus*, and there is the possibility that freshwater-breeding species will adapt to increased salinity in the presence of larger availability of brackish and saline water habitats, along with changes in precipitation that will affect the availability of freshwater habitats (Zhang et al., 2008; Chaves and Koenraadt, 2010; Ramasamy and Surendran, 2011). These climatic effects on vector population sizes and ranges will, however, be tempered by the effects of changes in human activities and movements.
>
> Changes in human population sizes, movements, socio-economic status and land use are all factors likely to interact with climate change, and synergies between these different variables and changes in local climatic patters will contribute to the spread and intensity of vector-borne disease (Campbell-Lendrum and Woodruff, 2007; Kearney et al., 2009).

Anthropogenic influences on current distributions of vector species and parasite reservoirs include size of human settlements, housing conditions, type and size of water supply, vector control practices, and deforestation and other land-use changes (Sutherst 2004; Campbell-Lendrum and Woodruff, 2007; Yasuoka & Levis 2007; Kearney et al., 2009). In particular, poor socio-economic conditions, which are often linked to lower housing conditions and open, untreated water supplies, continue to enable disease transmission to occur (Sutherst 2004). Increasing human population density, coupled with the possiblity of environmental refugees, will lead to large poverty-ridden areas without the necessary infrastructure for the safe storage and distribution of clean and waste water, providing more breeding sites (such as used containers and tires) for vectors within urban areas. In addition, increasing pressure on the currently under-resouced public heath infrastruces in many tropical developing countries will exacerbate the morbidity and mortality associate with vector-borne disease (Sutherst 2004; Campbell-Lendrum and Woodruff, 2007).

Synergies between land use and climate change might also affect vector populations. Deforestation has had both positive and negative affects on vector populations due to increases in surface temepratures, drier conditions, and shifts to sun-tolerant species (Sutherst 2004; Yasuoka & Levis 2007). The deadly combination of habitat fragmentation and/or destruction and climate change is also likely to increase extinction rates, and this could alter existing vector-host-parasite relationships (Sutherst 2004).

Variation in local patterns of climate change will differently affect vector species and diseases. For example, changes in temperatures affect vector development, vector survival and many other individual components of the infection transmission cycle. For temperate climates, this could mean that threshold temperatures needed for malaria might become more common; however, the extremely high temperatures that slow parasite development could also become more common, particularly in tropical environments (Pascual et al., 2006; Campbell-Lendrum and Woodruff, 2007; Paaijams et al., 2009).

Some of the other potential negative effects of climate change on vector species

include: increasing temperatures will result in higher adult and larval mortality and egg desiccation, and changes in precipitation and sea levels will influence the ecology of seasonally ephemeral saltwater and freshwater swamplands, resulting in large changes in the available larval habitat (Yang et al., 2008a; Zhang et al., 2008; Kearney et al., 2009; Lafferty, 2009; Russell, 2009; Russell et al., 2009a; Ramasamy and Surendran, 2011). Predicting the risk of potential outbreaks and spread of vector-borne disease under climate change scenarios therefore depends critically on a sound knowledge of the ecology of the current climatic envelopes of vector species, the endogenous and exogenous drivers of vector population dynamics, and the vector-disease ecology and transmission cycle."

p. 4. From a historical perspective, it seems you are down-playing the importance of vaccination. Yellow fever vaccination has played and continues to play an important role in control of this VB disease

http://www.who.int/csr/disease/yellowfev/massvaccination/en/indew.html

I have included a sentence discussing the success and importance of vaccination as a method of reducing some vector-borne diseases (pg 5, paragraph 1):

"While some progress has been made towards vaccination against vector-borne disease (Amina et al., 2010; Appaiahgari and Vrati, 2010; Kumar et al., 2010), and in some cases, has been a highly effective method of breaking the host-parasite-disease cycle (World Health Organization, 2011), for most vector-borne diseases, the large-scale, practical applications of this method still remain some years away due to the high associated costs (Tediosi et al., 2009)."

p. 11. Typo

"However, high densities do not generally elicit higher larval mortality; instead, they tend to give rise to ~~larger~~ smaller body sizes in emerging adults, with resultant decline in adult survival rates, longevity, dispersal capacity and fertility..."

'larger body sizes' has been corrected to 'smaller body sizes'

In a general way this discussion of compensatory mortality effects is good and appropriate for the thesis. I think, however, that it may be too simple for reality. Density effects will also modify development times, which is not mentioned, and this too may contribute to compensation. More importantly, there is a major literature component missing from this discussion – that density may affect adult vector competence (the propensity of an individual to become infected when taking an infectious blood meal). Several recent investigations of arbovirus transmission indicate that females that were stressed (e.g., crowded) as larvae are MORE prone to acquiring arboviral infections than are individuals from benign larval environments. See papers by Barry Alto and co workers (listed below) among others. This complicates a central theme of your thesis – that low densities yield super mosquitoes (large, long lived, good vectors). That is likely true but how these two effects (longevity, competence) combine could be critical for forecasting disease risk. This could be incorporated into your sensitivity analyses, and could prove a really useful contribution. I view missing this point in the literature as a significant omission.

I have reviewed the suggested literature and included the following sections in my introduction:

Pg 12-13

> "The relationship between compensatory density feedback in vector populations and their capacity for subsequent disease transmission is complex. While reduction in density can lead to higher adult survival and an ensuing increase in vectorial capacity (Garrett-Jones, 1964), smaller adults (produced either from high-density larval conditions or reduced development rates arising from stressful environmental conditions) of some vector species are more susceptible to disease infection and dissemination (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et

al., 2011), whereas other species become more susceptible to infection and dissemination as body size increases, and some species display no effect of body size on susceptibility (Nasci et al., 1994; Sumanochitrapon et al., 1998; Jennings and Kay, 1999; Alto et al., 2005; Westbrook et al., 2010; Muturi et al., 2011). A detailed understanding of species and disease-specific infection and dissemination relationships is therefore crucial for predictions of vector-borne disease prevalence and prevention."

Pg 15

"Sensitivity analyses can also be applied to coupled models of vector population dynamics and epidemiology to examine the sensitivity of disease transmission rates to various mosquito life-history traits, such as adult body size and survival, further informing integrated vector management programmes (Styer et al., 2007; Ruan et al., 2008; Stolk et al., 2008)."

pp. 17-18 Hypotheses. Nicely structured listing of topics for the chapters. However I do not find the statements of the hypotheses to be very useful. Aim 1 is OK, because you really do state a specific hypothesis (hypothesis = proposed explanation for a phenomenon; a description of the biology). This is distinct from the predictions (description of the data that will result if the hypothesis is true). For aim 1, your hypothesis is strong compensatory mortality, and the predictions follow. But consider Aim 3. You hypothesize that "...farther from emergence sites there will be a higher proportion of larger female adult *Ae. vigilax* mosquitoes caught, and as time progresses, smaller mosquitoes will die faster and therefore the mean body size of the female adult *Ae. vigilax* population will increase." This is really a <u>prediction</u> based on the hypothesis that <u>longevity increases with body size.</u> That hypothesis predicts larger size further from the emergence site because larger females live longer and can move farther in that greater time. Further, mean adult body size will increase over time is a prediction based on the longevity hypothesis. As you state it, your hypotheses are simply descriptions of

the data you expect. The hypothesis should describe the biology behind the data. For Aim 4 it is not clear <u>why</u> you expect environmental proxies to be better predictors of RRV cases. You need to explain the logic behind this.

I have re-written Aims 3 and 4 to state more clearly the biology behind the data in the hypotheses.

I have re-written the Aim 3 hypothesis as follows:

> "I hypothesise that adult *Ae. vigilax* female longevity increases with body size, and therefore predict that as the distance from emergence sites increases, there will be a higher proportion of larger female adult *Ae. vigilax* mosquitoes caught, and as time progresses, smaller mosquitoes will die faster, leading to an increase in the *mean* body size of the female adult *Ae. vigilax*. Also, I predict that as larval densities increase, female *Ae. vigilax* body size at emergence will decline, and that emergent *Ae. vigilax* body size will vary with habitat type and environmental conditions regardless of larval density **(Chapter 4)**"

I have re-written Aim 4 hypothesis as follows:

> "I hypothesise that mosquito trapping programs do not accurately measure the adult female mosquito population size or vectorial capacity; as such, I predict that environmental proxies for mosquito population dynamics constructed from quantified ecological relationships defined in the previous chapters will more accurately predict RRv incidence than measures of vector abundance from trapping programs **(Chapter 5).**"

<u>Chapter 2.</u>

p. 27. Typo "My" capitalize

'my' has been corrected to 'My'

p. 29. Logit-link function. Your data are larvae per sample, a quantitative variable. What is the justification for using logit-link, as opposed to any other? Did that just work out best or are you basing this on some theoretical background? Seems to me that logit

functions are usually used for presence/absence data, and, of course, that is not how this data set is structured.

I modelled the dataset of relative larval densities as *weighted* presence/absence data, rather than abundance data to account for any sampling error (see previous comment); therefore, the use of the logit link function is justified because my data are, in fact, presence/absence. I have included the following explanation in the methods section to make this clearer (pg 27):

> "To account for sampling error, either due to inter-operator viability or sampling process, the relative larval densities were modelled as weighted presence/absence scores rather than as raw abundance scores. The completed dataset spans November 2000 – December 2006."

p. 30-31. Time series data. Did you use an autoregressive model? ARIMA or otherwise? I know you used lagged adult abundances as a predictor, but that is a somewhat different issue (as they are different stages).

I accounted for possible temporal autocorrelation in the dataset by using bootstrapping rather than an autoregressive model. Autoregressive models require a (often only assumed) underlying correlation structure, whereas new bootstrap techniques control for temporal non-independence by maintaining the inherent (unknown) structure in the time series and resampling to avoid dependency issues. See pp 31:

> "To overcome the possibility of systematic changes in larval density associated with vegetation, I included "vegetation type" as a random factor in the GLMM. I also took 1,000 random subsets of 50% of the larval data to account for any temporal autocorrelation given that both *Cx. annulirostris* and *Ae. vigilax* populations in Darwin show a strong positive temporal autocorrelation in adult mosquito density (Yang et al., 2008b). I calculated the median values of BIC, AIC_c, ΔAIC_c, ΔBIC, $wAIC_c$, $wBIC$, and percent deviance explained for each model across the 1,000 subsets for final model comparisons."

p. 31. Typo annulirostris (no capital)

'*Cx. Annulirostris*' has been corrected to '*Cx. annulirostris*'

p. 32 typo. "insecticides" in the first sentence of the last paragraph, for no apparent reason.

The word 'insecticides' has been removed from the first line of the last paragraph.

In this chapter, you have weak but sig predictive ability. How does this advance our understanding of the biology of these species? I can see that elevation is both informative and has a mechanism that is plausible. What kinds of questions are adequately answered by the data? The predictive power is not very good. I would be somewhat hesitant to make recommendations for mosquito control based on the limited data set.

I discuss how my findings advance the understanding of the biology of the two species, despite the low predictive power of the models on pp 38-39:

> "Despite drawbacks in data quality, some generalizations can be made from my models. I found the strongest temporal drivers of larval density for both vector species were lagged logarithm of adult density and total rainfall from the previous month. I partially expected this result because adult mosquito populations in tropical north Australia are strongly density-dependent (Yang et al., 2008a,b). My results take this density relationship one step further by providing a clear mechanistic link between the two main life phases in mosquito development. Yang et al. (2008b) found a negative relationship between the adult mosquito population rate of change (r) and adult density, and there are several mechanisms by which this dynamic can be realized. Competition during the adult life stage for blood meals, harbourage, or oviposition sites can lead to lower larval densities after a peak in adult density. During a population peak, competition at the larval stage for nutrients and/or other stresses in the high-density larval habitat will result in higher

mortality at the larval and pupal stages, and the emergence of smaller adults with lower survival and fertility (Agnew et al., 2002). My results show a positive relationship between adult numbers in the previous month and larval density in the following month, which suggests that the major mechanism of density feedback in these species is not competition during the adult life stage – if this were the case, there would be a negative relationship between lagged adult density and larval density. I hypothesize that the main regulatory mechanism in *Ae. vigilax* and *Cx. annulirostris* occurs during the larval stage where lower to medium larval densities will result in higher adult emergence, survival, and fertility than would occur at high larval densities. It is therefore important that, during control operations, both low- and high- density larval habitats and seasons are targeted for control to reduce population size and disease transmission effectively."

I have also added a paragraph discussing the implications of elevation in terms of climate change and related management recommendations pg 40:

"This finding also has a particular importance with regards to climate change. In terms of elevation, even subtle differences in a superficially flat environment have potentially large implications for climate change as precipitation changes and sea levels rise. Even incrementally small increases in high spring tides will profoundly increase the available breeding habitat for *Ae.vigilax*, and therefore, any long-term vector management plans will need to incorporate these interacting effects of subtle elevation gradients and predictions of climate change."

Chapter 3. Effectiveness of control efforts

pp. 51-52. Description of treatments is overly complicated and could be greatly condensed to make it simpler to follow. It starts out sounding like there is no control (unmanipulated) and that the combination treatments (bti+burn, bti+slash) will not be there. Just list all the treatments simply. This section is very verbose.

As I am describing the design of several experiments in this section: (i) quantifying the

relationship between larval sampling and uncontrolled emergence, (ii) comparison of uncontrolled mosquito emergence across vegetation types, and (iii) quantifying the effect of different vector control measures, this section is necessarily long. I have, however, re-written this section so it reads more simply, and added different headings regarding the different experiments so the section reads more easily (pp 52-54).

"*Experimental design: Larval Traps*

I established larval traps before the highest monthly high tide events in October 2007 and November 2008 when all the sites were dry. The larval traps consisted of 1-m^2 galvanized metal frames, 20 cm in height, which had vertical rectangular holes on two sides that were covered with fine mesh that allowed water to flood the trap, but prevented the movement of mosquito larvae into or out of the trap (Fig. 2). I dug larval traps 0.05 m into the muddy substratum and attached a pyramid-shaped mosquito net to the top of the traps to prevent oviposition of non-target species in the plots, and also to capture emerging adult *Ae. vigilax*. During the aerial spraying event, traps that were not exposed to the *Bti* 'spraying treatment' were covered with plastic sheets and had the holes on the side of the traps blocked to prevent *Bti*-contaminated water from moving inside of the non-sprayed traps (Fig. 2).

Experimental design: Quantifying mosquito emergence and larval sampling.

To measure uncontrolled mosquito emergence, I placed five traps in October 2007 in each of the four vegetation types (Mangrove forest, Mangrove edge, *Schoenoplectus Eleocharis*). To examine the effectiveness of the current larval sampling procedure in relation to uncontrolled mosquito emergence, I placed 10 traps in the *Schoenoplectus* habitat during the November 2008 high tide event.

Experimental design: Spraying, Slashing and Burning as vector control.

I only applied the different mosquito control treatments to traps within the vegetation

types known to produce the highest numbers of emerging *Ae. vigilax* at that time of year, namely *Schoenoplectus litoralis* and *Eleocharis dulcis* (Russell and Whelan, 1986). I placed five trap replicates each within the *Schoenoplectus* and *Eleocharis* habitats in October 2007 for each of the three different treatments of burning, slashing, and spraying:

(i) Spraying: I exposed traps to Vectobac (*Bti* larvicide) sprayed from a Jetranger helicopter at a concentration of 1.5 L ha^{-1} at a height of approximately 2 metres on 29 and 30 October 2007

(ii) Burning: I removed vegetation within the frames by burning prior to the October 2007 high tide event. I achieved this within the traps by igniting the vegetation using a hand-held blow torch. I burned vegetation to ground level where possible but did not remove charred vegetation remains from the trap.

(iii) Slashing: I removed vegetation within the larval traps using pruning shears prior to the October 2007 high tide event to determine the effects of vegetation removal only. I trimmed the vegetation as close to ground level as possible and removed it from the frames.

To test whether *Bti* application had an interactive or additive effect with burning and/or slashing within the *Schoenoplectus* habitat, I also placed five traps in October 2007 for each of the treatment interactions: burning and spraying, and slashing and spraying."

p. 55, middle "...vegetation was also included as a random effect..." I don't think this makes any biological sense. What is the justification for thinking that vegetation should be random? As opposed to fixed? Or do you mean trap nested in vegetation was random? If so, that is not what the paragraph says. By P. 56 it is clear that you mean vegetation is random. I cannot see the justification for this. Those two vegetation types don't represent a random sample of all possible vegetation types – they are what was present in your study area. They are the kinds of vegetation that are relevant for this set of questions. Thus vegetation should be a fixed effect.

Vegetation was included as a random effect rather than a fixed effect as there was no apparent replication for vegetation type, as this was not a testable hypothesis. Including 'vegetation type' as a random effect was a way of controlling for the fact that emergence traps within a single vegetation type were more likely to give similar responses than traps between different vegetation types (i.e., traps were non-independent within type). Therefore, the random effect is simply controlling statistically for non-independence in the models, an assumption often violated when failing to control for random effects in simple linear models.

Chapter 4.

Abstract. This is a semantic point but "virulent" is the wrong word here. In the disease literature "virulence" pertains to the rates of morbidity and mortality cause by a pathogen. The mosquitoes are the vector. They may be more effective vectors, leading to greater infection rate, but there isn't much reason to suspect they change the rate of morbidity/mortality caused by the pathogen. And, in any case, the mosquito doesn't cause the mortality. The pathogen does. What you mean here is that the larger mosquitoes are more effective vectors. But see further comments below.

'vector-control should not ignore low-density larval sites because of their potential to produce more virulent mosquitoes,' (pg 72) has been corrected to 'mosquito-control should not ignore low-density larval sites because of their potential to produce more effective vectors', and this correction has been applied to all other occurrences of the term 'virulence' in this context in the thesis.

p. 76 more semantics. "vectorial capacity" is the term usually applied to what you are talking about in the last paragraph.

'Vector capacity' has been corrected to 'Vectorial capacity', and this correction has been applied to all other occurrences of the term 'vector capacity' in the thesis.

pp. 76-77. You have missed a potentially important component of the literature on effects of the larval environment on vectorial capacity. Recent work by Barry Alto and others has shown that for multiple arboviruses, vector competence (=susceptibility of the vector to infection when they take a pathogen-laden blood meal) is greater for females that have been reared at greater densities. Size of the female seems to be inversely related to susceptibility, though in some cases that relationship may be reversed.

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environmental temperature and the susceptibility of *Aedes albopictus* Skuse (Diptera: Culicidae) to chikungunya virus. Vector-Borne and Zoonotic Diseases 10:241-247

Relationships of competence to size or density or food have been documented for multiple arboviruses (Dengue, LaCrosse, Sinbis, Ross River, Chikungunya) and several vector species (*A. aegypti, A. albopictus, A. triseratus*). Thus, they seem to be general. The main point: you cannot ignore the effect of larval density on susceptibility and only focus on longevity and fecundity of the vector.

I have reviewed the suggested literature and included the following sections:

Pg 74

> "Some traits are also linked to vector body size: susceptibility to infection and dissemination are higher in smaller females of some vector species (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et al., 2011), and higher in larger females of other species (Nasci et al., 1994; Sumanochitrapon et al., 1998; Westbrook et al., 2010), and survival and dispersal are higher in larger females of many vector species (Agnew et al., 2002; Gimnig et al., 2002; Manoukis et al., 2006; Bevins, 2008; Gavotte et al., 2009; Reiskind and Lounibos, 2009)."

Pp 74-75

> "Effective and sustainable vector control therefore requires knowledge of whether the control mortality is additive to natural mortality or if it is compensated by increased vector survival or reproduction (Chapter 2; Juliano, 2007; Yang et al., 2008a,b; Yang et al., 2009), and also a detailed understanding of specific vector/disease relationships to determine if infection and dissemination is higher in smaller or larger females (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Nasci et al., 1994; Sumanochitrapon et al., 1998; Jennings and Kay, 1999; Alto et al., 2005; Alto et al., 2008a,b; Westbrook et al., 2010; Muturi et al., 2011)."

Hypotheses. See comments above on first chapter

I have re-written the hypothesis as follows:

> "I hypothesized that (1) adult *Ae. vigilax* female longevity, and consequent dispersal capacity, increases with body size, so larger adult female *Ae. vigilax* mosquitoes will be caught farther from emergence sites; over time, smaller mosquitoes will die faster giving rise to an increase in the *mean* body size of adult females; (2) higher densities of larvae will result in smaller female *Ae. vigilax* body size at emergence; and (3) emergent *Ae. vigilax* body size will vary with habitat type and environmental conditions regardless of larval density."

p. 85-86. Effects of time and distance on wing length are really small. You present the data starting out saying you found evidence. But it is a real stretch to believe that there is something real going on, either for time or distance. A 1% increase is not impressive. Combination of small effects, limited replication, and loss of some replicates mean this is not a strong paper. Having said that, it is a strong effort, even if it didn't work out. My real complaint is that you are, in my opinion, trying to make more of the data than you should.

I acknowledge the limits of my data in the discussion:

pg 88, paragraph 1

> "Unfortunately, the loss of five background emergence (control) replicates, three supplemented replicates and four reduced replicates (because no females survived to emergence), limited my ability to define clearly the relationship between larval density and adult emergence size for this species."

And I have added a further sentence acknowledging my small effect sizes (pg 88, last paragraph):

> "Measurements taken from weekly adult monitoring stations around Darwin at different times since emergence demonstrate that larger female adults do indeed live longer (Fig. 4). However, the effect size of time on frequency of body size is small (time since

emergence explains 2.5% of the deviance in body size, Table 1), and more experimental evidence is required to test the body size/density trade-off further."

p. 91. Effects of high density. Given the many papers documenting effects of density or size on vector competence, you should bring this competing effect into the discussion. Yes it is expected to be linear, whereas the effects on longevity are expected to be nonlinear. But there is fairly strong statistical evidence for the effects on vector competence, and you data do not really yield strong evidence for effects on longevity. The points you bring up in the Discussion about incorporating effects of larval density on vectors and impact of control are really important, and it is great you tried to address those questions. Your data did not really let you get at these issues, but your ideas are great.

I have reviewed the suggested literature on the relationship between vector competence and body size and included the following sections:

Pg 88

"There is a large body of work examining the effects of adult vector size on vectorial capacity traits. In some cases, larger individuals are more susceptible to viral infection from a bloodmeal (Nasci et al., 1994; Sumanochitrapon et al., 1998; Westbrook et al., 2010), although in others, smaller individuals are more susceptible (Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et al., 2011). This relationship appears to be species- and disease-specific, and previous research has shown no correlation between Ross River virus infection rates and *Ae. vigilax* female body size variation from larval nutrition changes (Jennings and Kay, 1999)."

Pg 90

"The corollary is that high-density larval environments should produce smaller, shorter-lived adults with lower disease-transmission potential – offset to a degree by the fact that there are more potential vectors available. While this theory holds for species where

vector competence (susceptibility to viral infection and dissemination) is positively correlated with, or unaffected by, vector body size (Nasci et al., 1994; Sumanochitrapon et al., 1998; Jennings and Kay, 1999; Alto et al., 2005; Westbrook et al., 2010; Muturi et al., 2011), in cases where vector body size is inversely related to vector competence (i.e., smaller females are more susceptible to infection and dissemination; Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2005; Alto et al., 2008a,b; Muturi et al., 2011), this relationship will also need to be taken into account, further emphasing the importance of defining the relationship between vector life history and disease infection."

Pg 91

"A metric that combines body size (as a proxy for adult survival) and adult abundance could therefore represent a better predictor of disease transmission potential. However, depending on species, this would mean that any changes to vector size might increase vector competence. A detailed knowledge of the species-disease relationship is therefore crucial to understand these complex relationships."

Chapter 5.

p. 100. Last paragraph of introduction. Typo. "Here we examine..."

'Here examine' has been changed to 'Here I examine'

More importantly, it is totally unclear why you postulate that environmental proxies will be better at forecasting RRV than will mosquito abundances. I can think of reasons, but you have not explained what your reasons are.

I state on pg 95 why I believe mosquito abundances measured by CO_2 trapping programs are inaccurate, and therefore, possibly inappropriate for use in predictive disease models. I also state why environmental proxies might more accurately represent the complexity of mosquito population dynamics and vectorial capacity:

"Estimates of adult abundance from trap programs are commonly used

phenomenologically to infer demographic patterns in vector populations (Glass, 2005; Adams and Kapan, 2009; Carver et al., 2010; Sullivan, 2010). However, accurate measurements of the highly vagile adult vector populations are economically and energetically costly, and not always obtainable (McIver et al., 2010). Also, the relationship between vector population dynamics and vector-borne disease epidemiology is complex, depending on many more elements than simple adult vector abundance, of which vector behaviour, longevity and dispersal ability, disease incubation period, and host population density and distribution are a few (Glass, 2005; Adams and Kapan, 2009; Hu et al., 2010a; Stresman, 2010, Sullivan, 2010). A basic first step for models seeking to make inference about the relationship between vector abundance and disease incidence is defining the ecological factors affecting vector population dynamics (Fish, 2008; Adams and Kapan, 2009). Such models might identify environmental variables that better predict disease occurrence than data from trap programs. This is because mosquito population dynamics are highly complex and intricately modified by stochastic environmental conditions (Chapter 2; Yang et al., 2008b; Yang et al., 2009; Russell et al., 2011). Because mosquitoes require ephemeral aquatic habitats for the development of their larvae and pupae, the relative amount of standing water in an environment due to rainfall or tidal patterns is often used as a predictor of vector population size or carrying capacity (Chapter 2; Chase and Knight, 2003; Yang et al., 2008a; Russell et al., 2011). Some models of vector-borne disease already use these environmental predictors of vector population size or 'environmental proxies' in place of unavailable measures of adult vector abundance; for example, incidences of both malaria and dengue are commonly modelled using rainfall as a proxy for adult vector abundance (Carver et al., 2010; Hu et al., 2010b; Sullivan, 2010)."

I have re-worded my hypothesis (pg 98), so that my reasoning is clearer:

"I hypothesise that the current mosquito trapping programs do not accurately measure the adult female mosquito population size or vectorial capacity, so combining environmental

proxies for mosquito population dynamics will represent a more cost-effective and accurate method of predicting disease incidence than measures of vector abundance from trapping programs."

Results.

Overall, these are interesting if somewhat idiosyncratic results. One consistent element across years is that there appears to be two peaks of cases each year, one in March, one in Sept. Years also vary in the sizes of the peaks. How did public health efforts vary over the years of the study, and would they explain some of the inter-year variation? Another possible source of variation among years might be immunity in the zoonotic host, which could affect the build-up of virus. Do you have any data on these things?

Some of the idiosyncrasies and oddities (e.g., negative RRV relationship to *A. vigilax* – p.106) in the data may arise because of trends in some of these other variables. Not sure if you can resolve this with this data set, but it might be worth investigating via alternative models.

I have included a discussion of which of my variables are linked to zoonotic amplification cycles, and how these cycles are related to the patterns of RRv cases:

Pg 100, paragraph 2

"The response variable was monthly Ross River virus case counts, and explanatory variables included were the first *tidal* and *rain* principal components. In the Northern Territory, the average time from inundation of *Ae. vigilax* and *Cx. annulirostris* larval habitats to adult emergence is 9 – 13 days (Sinclair, 1976; Lee et al., 1989). Therefore, I included lags of 1 and 2 months of the environmental proxies in the models. These lagged variables also account for zoonotic amplification cycles that can precede human cases. Season (wet/dry), human exposure (number of public holidays per month) and Ross River virus immunity (peak Ross River virus case count from the previous year) were also included in the models."

Pg 123, paragraph 2

> "The major vertebrate hosts for Ross River virus in the Northern Territory are the new juvenile populations of agile wallabies *Macropus agilis* and dusky rats *Rattus colletti* (Jacups et al., 2008a). Here, juvenile recruitment and population growth in both reservoir species are driven by seasonal rainfall (Madsen and Shine, 1999; Shine and Brown, 2008). I contend that including lags of rainfall in my models provides surrogate information in this regard. The negative relationship between both measures of *Ae. vigilax* abundance (both trap and environmental proxy) and RRv incidence indicates that this vector species might be involved in zoonotic amplification cycles in the wallaby and rat populations, rather than acting as a major RRv vector to humans *per se*."

I have also included the following sentences discussing other potential sources of variation:

Pg 123, paragraph 2

> "Quantifying all sources of potential variation in annual RRv cases is impossible; however, two relevant sources of year-to-year variation not included in my models were: variability in public health efforts, and the cycles of zoonotic host immunity. Models incorporating data on these two effects might provide more accurate predictions of annual RRv incidence."

<u>Conclusions</u>

p.130. First full paragraph. You phrase your conclusions as being about 'vectors', but this is a rather large leap. You have investigated mosquitoes that are vectors of RRV in N. Australia. I think it would be more prudent to try to generalize only to mosquitoes (rather than all vectors, as implied) and to qualify this (as you have, in a way) by noting that the stability of the habitats used by larvae influences whether or not the trends you observed in these studies are likely to hold for other mosquito species or other locations. You note the specificity of knowledge needed for these efforts later in this paragraph, but still I would prefer to see the beginning of the paragraph (and other locations in the

text) be more specific to, mosquitoes, as opposed to any vector.

I have reviewed the concluding chapter (Chapter 6) and replaced most occurrences of the term 'vector' with 'mosquito'.

Five-step plan. I love the intellectual effort you outline here, and fully agree this would be a scientifically useful research program. I hope you pursue it in your career as a scientist. However, I have to express some scepticism that this will be an effective plan for current vector control in any specific case. The plan you outline would require major research effort over many years to provide an answer. The practical problems associated with disease won't wait. Much as I think the steps you outline would be incredibly important for understanding a disease, and would, in the long run, help to improve control, in the immediate term of a few years, what control programs need is practical guidance for where, when, and how to intervene to reduce the possibility of an outbreak; I suspect that for this kind of problem, locality and species specific correlative studies relating mosquito abundances and perhaps adult trait distributions (e.g. size), zoonotic host abundances and immunity, and human-vector contact, along with relatively simple heuristic models of transmission, might be more likely to provide answers in the short term. Those answers might not be as accurate, but they would serve as a guide for action now. Perhaps I am overly pessimistic about the potential for your five-step plan to lead to improved control in the short term. If so, perhaps you can make some convincing arguments for this in your oral defence?

I have added a paragraph discussing the practicality of my five-step plan:

Pg 146, last paragraph.

> "However, the practical application of this five-step plan is probably exceedingly ambitious for most areas of vector-borne disease management, due to the major research effort that would be required over many years to provide the necessary data to put this plan into action in contrast to the urgency with which vector management solutions are

required in the face of increasing disease incidence. Although some small areas with manageable vector populations, such as Darwin, do lend themselves well to this approach, the reality is that vector control programs need immediate practical guidance for where, when, and how to intervene in the vector-disease-host cycle and reduce the possibility of a disease outbreak. Species- and location-specific studies relating mosquito abundance and life-history traits (e.g. frequency of female adult body size in the population), zoonotic host abundance and immunity cycles, and probability and intensity of human-vector contact, along with relatively simple heuristic models of transmission, might be more likely to provide less accurate, but important, answers in the short term to guide immediate vector management actions."