DEFINING THE MOSQUITO PROBLEM AT HAYS INLET, QUEENSLAND, AUSTRALIA

By
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In Partial Fulfillment of the Requirements for the Degree of

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Abstract

For many jurisdictions in Australia, the local Ross River virus vector problem has not been well defined. The aim of this thesis was to investigate the local vector problem at Hays Inlet, Queensland, Australia. Twenty-four 1-octen-3-ol and CO_2 baited-CDC style light traps were set at this location during February and March, 2003. Trap catches were identified to species, and were assessed with the Spearman's rank correlation and cluster analyses to see if contiguous traps had similar rankings for each species. These data were also characterized according to meteorological and habitat assessment data. The most abundant known vector species present in this area were *Ochlerotatus vigilax*, *Culex annulirostris*, *Verrallina funerea*, and *Ochlerotatus procax*. Both *Ve. funerea* and *Cx. sitiens* numbers each appeared to be clustered to a specific group of trap sites in locations with vegetation consistent with their known habitat. These preliminary findings will guide adjacent local governments to better target their vector control efforts and will facilitate future studies to promote movement towards a GIS-based system of surveillance and control.

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Table of Contents

Abstract i

Acknowledgements... ii

Table of Contents iii

List of Tables vii

List of Figures vii

Chapter 1: Introduction 1

1.1 Background 1

1.2 Study Rationale 2

1.3 Research Purpose and Objectives 3

1.4 Outline of Methods 4

1.5 Organization of Thesis 4

1.6 List of Key Terms 4

Chapter 2: Review of the Literature 6

2.1: Principles Relevant to Mosquito Surveillance and Control Program Design: Defining the Mosquito Problem 6

2.1.1 Vector Competence and Vector Incrimination: An Overview 8

2.1.2 Timing of Mosquito Abundance: Influence of Meteorological Variables ... 10

2.1.3 Location of Vector Abundance: Habitat Characteristics and Spatial Pattern 13

2.1.4 Interpretation of Vector Abundance Data 15

2.1.5 Mosquito Control Program Frameworks and Components 17

2.1.6 Current Trends in Mosquito Control Program Design 20

2.2: Defining the Local RRv Vector Problem in Australia 22

2.2.1 Background: Clinical Aspects, Epidemiology and Public Health Significance 22

2.2.1.1 Ross River Virus: Components of Transmission Cycle 23

2.2.1.2 RRv Vector Distribution 25

2.2.2 Past and Emerging Research related to RRv vectors in Australia: Implications for Mosquito Control Programs 26

2.2.2.1 General patterns in RRv vector distribution in Australia 27

2.2.2.2 Ross River virus Activity and Spatial Patterns 30
2.2.3 Vector incrimination and Vector status in Australia 31
2.2.4 Ross River Virus Vector Lifecycle in Relation to Habitat and Meteorological Variables 34
2.2.4.1 Lifecycle of Oc. vigilax in relation to landscape 34
2.2.4.2 Lifecycle of Oc. vigilax in relation to temperature, seasonality, and time 35
2.2.4.3 Lifecycle of Culex annulirostris in relation to landscape 37
2.2.4.4 Lifecycle and development of Culex annulirostris, in relation to temperature and rainfall 38
2.2.4.5 Lifecycle of Ve. funerea in relation to landscape 38
2.2.4.6 Lifecycle of Oc. procax in relation to landscape, and meteorological variables 39
2.2.5 Spatial Pattern of Vector Abundance 39
2.2.6 Ross River Virus Infection Risk 43
2.2.7 Mosquito Control in Queensland 46
2.2.7.1 Control of Ross River virus in Queensland 46
2.2.7.2 Mosquito Control at Hays Inlet 49
2.3 Chapter Summary 50

Chapter 3: Methods 52
3.1 Study Area 52
3.1.1 The Southeast Queensland Bioregion 52
3.1.2 The Hays Inlet Study Site 54
3.1.2.1 Urban Areas 55
3.1.2.2 Predominant vegetation and land use 56
3.1.2.3 Hydrological Characteristics 57
3.1.2.4 Designated Protected Areas 57
3.2 Data Collection 58
3.2.1 Study Period 58
3.2.2 Meteorological Variables 59

3.2.3 Habitat Assessment *59*
3.2.4 Mosquito Composition and Abundance *61*
3.2.4.1 Sampling device and role in relative population estimation *61*
3.2.4.2 Trap site designation *62*
3.2.4.3 Systematic sampling technique *63*
3.2.4.4 Light trap set-up and sampling schedule *63*
3.2.4.5 Sorting of light trap collections *63*
3.3 Data Analysis 64
3.3.1 Temporal pattern of Mosquito Abundance *64*
3.3.2 Spatial Pattern of Mosquito Abundance *64*
3.4 Chapter Summary 67
Chapter 4: Results 69
4.1 Characterization of Meteorological Conditions 69
4.2 Habitat Assessment 72
4.3 Mosquito and Vector Composition 77
4.4 Temporal Pattern of Vector Abundance 79
4.5 Spatial Pattern of Vector Abundance 83
4.6 Chapter Summary 90
Chapter 5: Discussion 92
5.1 Vector Composition 92
5.2 Spatial Patterns and Habitat 93
5.3 Temporal Patterns and Meteorological Variables 96
5.4 Relationship of Study to Cumulative and Emerging Literature 97
5.5 Relationship of Study to Mosquito Management Frameworks 99
5.5.1 Vector Composition *99*
5.5.2 Monitoring Vector abundance over time and space *100*
5.5.3 Relationship to emerging trends in Program Design:
Hays Inlet and Risk of RRv Infection *100*
5.6 Chapter Summary 101
Chapter 6: Summary, Conclusions, and Recommendations 103
6.1 Thesis Summary 103

6.2 Conclusions 104
6.2.1 Objective 1: The characterization of meteorological variables 104
6.2.2 Objective 2: Vector and general mosquito composition 107
6.2.3 Objective 3: Spatial patterns of abundance 108
6.2.4 Objective 4: Habitat assessment 110
6.3 Recommendations for Mosquito Management and Future Research 111
References 114
Appendix I: Glossary 125

List of Tables

Table 2.1: Table of mosquitoes in which virus isolation has been demonstrated for RRv in Queensland Australia....................33

Table 4.1: Estimated numbers of adult female mosquitoes collected in CO2 and octenol-baited CDC-style light traps around the Hays Inlet, during the sampling period, 2003....................78

Table 4.2: Mean wind-speed and total daily rainfall on nights traps were set for each main species collected at Hays Inlet, 2003....................83

Table 4.3: Spearman's correlation coefficient values for each main species collected at Hays Inlet, February-March, 2003....................86

List of Figures

Figure 3.1: Bioregions of Queensland, Australia....................53

Figure 3.2: Aerial Photo of Hays Inlet (1:50000)....................54

Figure 3.3: Mean monthly rainfall, and mean maximum and minimum temperatures collected from the Redcliffe weather station (1982-2003)....................55

Figure 3.4: Major components of the CO2- and 1-octen-3-ol -baited CDC light trap....................62

Figure 3.5: Example of cluster analysis....................67

Figure 4.1: Daily maximum and minimum temperatures collected from the Redcliffe weather station, from January 15 to March 31, 2003....................71

Figure 4.2: Tidal amplitude and precipitation collected from Redcliffe weather station, from January 15 to March 31, 2003....................71

Figure 4.3: Representative vegetation and strata levels surrounding trap site 4....................74

Figure 4.4: Representative vegetation and strata levels surrounding trap site 6....................75

Figure 4.5: Representative vegetation and strata levels surrounding trap sites 9-12....................75

Figure 4.6: Representative vegetation and strata levels surrounding trap site 14. a) Strata levels present at this site, b) wheel ruts c) sites at which tidally inundated water forms temporary pools....76

Figure 4.7: Representative vegetation and strata levels surrounding trap sites 19-20....76

Figure 4.8: Representative vegetation and strata levels surrounding trap site 15....77

Figure 4.9: Mean number of female *Oc. vigilax* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003)....80

Figure 4.10: Mean number of female *Cx. annulirostris* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003)....81

Figure 4.11: Mean number of female *Ve. funerea* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003)....81

Figure 4.12: Mean number of female *Oc. procax* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003)....82

Figure 4.13: Mean number of female *Cx. sitiens* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003)....82

Figure 4.14: Scatterplot of *Oc vigilax* rankings between period 1 (17-23 February) and period 3 (17-27 March). 87

Figure 4.15: Scatterplot of *Cx. annulirostris* rankings between period 1 (17-23 February) and period 3 (17-27 March)....88

Figure 4.16: Scatterplot of *Ve. funerea* rankings between period 1 (17-23 February) and period 3 (17-27 March)....88

Figure 4.17: Scatterplot of *Oc. procax* rankings between period 1 (17-23 February) and period 3 (17-27 March)....89

Figure 4.18: Scatterplot of *Cx. sitiens* rankings between period 1 (17-23 February) and period 3 (17-27 March)....89

Chapter 1: Introduction

Ross River virus (RRv) is an alphavirus vectored by several mosquito species around Australia. Research efforts have yielded a general understanding of the regional nature of disease epidemiology, especially pertaining to the relevance of vector-related factors such as vector composition, competence, abundance, and the relationship of these factors to RRv activity. It is possible that effective Ross River virus vector control in Australia minimally requires monitoring activities that include an understanding of the local vector composition and abundance in relation to vegetation and climatic factors. These assessments might be combined with assessments of local vector competence, virus isolations, and associations between RRv cases and mosquito abundance, vegetation, climate, etc. The research presented in this thesis is aimed to guide two local governments in Southeast Queensland, Australia, to understand the vector composition and adult female abundance in relationship to a shared mosquito breeding site. This study will help guide vector surveillance and control in this area.

1.1 Background

In Australia, a few arboviruses that have been implicated in human disease elevate mosquito control programs from the level of nuisance/pest control to public health control. The main arboviruses that cause human disease in Australia include the dengue suite of flaviviruses, Murray Valley/Kunjin encephalitis viruses, Barmah Forest virus, and Ross River virus. Although some of the above are considered to be life-threatening (Murray Valley encephalitis, Dengue Hemmhoragic Fever), RRv is considered to have the greatest public health significance in terms of the number of cases reported and economic impact. RRv is endemic and epizootic in Australia, and causes a characteristic syndrome of constitutional effects, rash, and rheumatic manifestations. The main vectors of RRv, *Ochelrotatus vigilax* (Skuse) and *Culex annulirostris* (Skuse), are found in all states of Australia, thus contributing to the widespread distribution of RRv and the need for vector control (Russell, 2002). These species are the most closely monitored of all mosquito species against which the greatest effort of control is directed. There is a shift in research priorities towards using landscape ecological approaches for comprehending RRv activity and a concurrent shift in vector control programs to operationalize the latest mosquito control best-practice activities.

1.2 Study Rationale

The Hays Inlet area located between the City of Redcliffe and Pine Rivers Shire, Southeast Queensland, has been identified as a productive breeding site for *Oc. vigilax* (Redcliffe City Council, unpublished data). In order to fulfill their obligations for mosquito control, Redcliffe City Council and its neighbouring Local Governments (Pine Rivers Shire Council, Caboolture Shire Council and Brisbane City Council) primarily apply liquid formulations of the microbial pesticide *Bacillus thurigiensis israelensis* (*B.t.i.*) to the saltmarsh habitats in which *Oc. vigilax* breed. These habitats are regularly inundated by high spring tides and a single aerial treatment of these areas can involve up to 5000 hectares. In close proximity to the inlet area are residences on the north and eastern portion of inlet, along with a golfcourse in the south-east portion, both of which represent areas of potential human contact with mosquitoes. On the western side of the inlet, which falls in the Pine Rivers Shire jurisdiction, very few people inhabit areas close to the inlet, so there is less incentive for mosquito control operators to treat the larval populations in habitat around it. Other concerns relevant to mosquito control include encroaching development activities (e.g. residential housing) into suitable breeding sites and habitat around the inlet. However, there is no general knowledge regarding what other putative vector species may be located within the Hays inlet area. Additionally, the spatial pattern of mosquito abundance in conjunction with relevant landscape features, climatic factors, etc. has never been investigated at this site.

1.3 Research Purpose and Objectives

The overall purpose of this study is to characterize the vector composition, abundance and general habitat characteristics in the Hays Inlet area, with the aim of improving vector surveillance and control in this vicinity. The specific objectives of this study related to this purpose are to

1) characterize the study in terms of relevant meteorological and seasonal features;
2) identify mosquito species in the area that are known Ross River virus vectors;
3) determine whether there are relevant patterns in adult mosquito abundance for each relevant vector and pest species; and
4) identify and describe landscape features around the inlet that may be relevant to the mosquito lifecycle (mosquito habitat).

1.4 Outline of Methods

The methods used in this study consisted of collecting meteorological information regarding temperature, rainfall, and tide, surveying the habitat at selected trap sites, and light trapping to assess the likely vector composition in the area, and spatial and temporal trends in vector and pest adult female mosquito abundance.

1.5 Organization of Thesis

The thesis is organized in the form of six chapters. The present introductory chapter gives some general background to the study, and states the study rationale and study objectives. Chapter 2 outlines the literature relevant to the study. Section 2.1 outlines general principles, while section 2.2 focuses specifically on the relevant literature in Australia. Chapter 3 presents the methods used in this study. Chapter 4 concisely summarizes the results of the study. In Chapter 5, the discussion of the results is presented. Finally, in Chapter 6, a summary, conclusions, limitations, and recommendations of the study are given.

1.6 List of Key Terms

vector: An organism, such as a mosquito or tick, that carries disease-causing microorganisms from one host to another. The efficiency of this transmission is tested via vector competence studies.

king tide: The term "king" tide has no scientific definition although in popular usage it refers to any high tide well above average height. They are typically designated as the highest of the spring tides that occur during the summer months of

December, January and February and also in the winter months of June, July and August (Maritime and Safety Queensland, 2005).

Chapter 2: Review of the Literature

Climate and topography restrict vector-borne infections to certain geographical areas (Bergquist, 2001), and within these areas different epidemiologies of pathogen transmission might be present. Additionally, pathogen activity is inextricably linked to the activity of the vectors in which they are carried. Therefore, in regions in which the mosquito composition, abundance, and location have not been characterized, a major approach to this characterization involves defining the mosquito problem. In section 2.1 of this literature review I shall outline general principles relevant to the definition of the mosquito problem, while in section 2.2, I will give a review of studies relating to RRv and its vectors in Australia that pertain to the characterization of the mosquito problem within a given jurisdiction.

2.1: Principles Relevant to Mosquito Surveillance and Control Program Design: Defining the Mosquito Problem

Defining the mosquito problem is of fundamental importance to the development and design of mosquito surveillance and control programs. In general terms, as outlined in numerous mosquito control guidelines and manuals, the mosquito problem encompasses both vector and pest mosquitoes present in a given jurisdiction. Mosquito problem definition involves three components: 1) defining which vectors and pests are present, 2) when they are present, and 3) where (MCAA Inc., 1998). Each component is related to specific biological determinants of mosquito control program design. The following discussion will focus on elements of vector problem definition.

The examination of the vector competence of those mosquitoes present in an area is typically the first phase of vector problem definition. Vector competence is the ability of a vector to acquire a pathogen and to transmit it successfully to another susceptible host, usually tested under laboratory conditions. Vector competence testing results are used by vector control agencies to define the target species of interest based on those vectors whose species' ranges and habitat preferences fall within the given jurisdiction. Defining the target species guides control program operators to further understand the biology of each species and its known relationship to pathogen transmission, and this understanding can then be linked to associated management options and interventions.

Defining where vectors are present involves an understanding of mosquito habitat, which is defined by the occurrence of potential breeding sites, suitable resting sites, and possible sources of blood meals. From a landscape ecology perspective, it is the specific landscape features or designated habitat types that often determine the spatial pattern of abundance of relevant vector species. Determining the landscape factors most suitable for vector development or activities could aid in the risk assessment of pathogen transmission and to plan more efficient mosquito control programs (Gleiser *et al.*, 2002).

An important part in defining the mosquito problem is the manner in which mosquitoes present in an area are sampled. Sampling the adult mosquitoes is of fundamental importance to vector control program operation. Entomologists are concerned with how to catch the particular vectors of interest, and in the way in which trap catches can be adapted or standardized in order to provide a close estimate of the changes in vector population density of the vector population as a whole, according to season, habitat, and various control measures applied (Muirhead-Thomson, 1968).

Finally, these and other detailed studies of the landscape ecology and relationship to sampling have culminated in a Geographical Information-based system for program operation in certain regions, such as the Coachella valley of southern California. Certain layers underpin the landscape features that influence arbovirus activity and mosquito abundance, such as habitat and soil (Lothrop and Reisen, 1999).

2.1.1 Vector Competence and Vector Incrimination: An Overview

Vector competence is generally defined as the intrinsic permissiveness of an arthropod for the infection, replication, and transmission of a pathogen (Goddard *et al.*, 2002). In general, the criteria to establish vector competence are based on the stages of virus amplification and transmission during biological transmission between female mosquitoes via a viraemic host. These include: field isolation, demonstration of susceptibility to infection, replication of virus, sequential movement from mesenteron through haemocoele to salivary glands, transmission by bite and field evidence confirming association of the infected arthropod with the vertebrate population in which infection is occurring (Sudia *et al.*, 1969).

Virus isolation studies by themselves provide preliminary evidence of involvement of several mosquito species in the transmission cycle. However, evidence of transmission is necessary before any species can be implicated as a vector. Additionally, the role of a vector in the transmission cycle is determined with consideration to population density, host preference, feeding behaviour, longevity, and seasonal activity of each species.

For the public health purposes of defining disease risk, the presence of virus and vectors and the vector competence of the virus-vector system are used, highlighting the importance of vector competence to the epidemiology of human arbovirus disease. For vector control programs, which are often the central line of defence against pathogen transmission, identifying the species which have the greatest potential for transmission is essential for formulating and focusing a prevention plan. The transmission cycle of vector-borne pathogens often involves more than one competent vector. In such cases, it is essential that surveillance and control activities determine the relative importance of each vector species and role in transmission. A method for classifying vectors involves distinguishing between maintenance and amplification vectors. Maintenance vectors determine long-term vector survival of the virus in nature, as they typically have low infection thresholds, have weak or no barriers to dissemination, and possess a high transmission rate. Amplification vectors, on the other hand, may only become infected from highly viraemic hosts and exhibit poor rates of dissemination and hence transmission. This poor transmission ability may be compensated by extremely high seasonal population densities and the ability to feed on a wide variety of vertebrate species. This explains how some mosquitoes are continually incriminated as competent vectors in various localities and why other species are only locally competent or implicated during epidemics.

2.1.2 Timing of Mosquito Abundance: Influence of Meteorological Variables

Three key variables that influence the development and population dynamics (in particular, abundance) of mosquito species within a breeding season include temperature, precipitation, and tide. Numerous regional studies from different parts of the globe exhibit how these variables might influence mosquito abundance, and especially in regions with a seasonal pattern of mosquito abundance, show similar general trends.

Typically, temperature sets the limits on the seasonal timing of emergence and adult activity (mating, oviposition, etc.), and the rate of development of both the vector and virus. Physiological time is a measure of the amount of heat required over time for an insect to complete its various stages of development. Expressed as day-degrees, it is the cumulative product of total development time (in hours or days) multiplied by the temperature above the development threshold, below which no development occurs (Gullan and Cranston, 2000).

The quantity of rainfall influences the number and location of ephemeral pools in which larval development can take place. The absence of rainfall within the appropriate breeding season limits larval development of freshwater species to more permanent breeding sites, which typically subject larvae to greater predation.

The timing and amplitude of spring tides determines the timing of the availability of coastal larval habitat. Typically, only the highest tides inundate saltmarsh habitat and occur only at specific times of the year (Lee *et al.*, 1984).

The interactions between these variables in influencing abundance levels are numerous and often subtle. The more obvious interactions between these variables can be viewed in relation to their seasonal timing and persistence of ephemeral larval habitat. The tidal inundation of an area followed by a rainfall event dilutes saltwater pools, which might affect the species composition of these pools to include salt-brackish species. Additionally, rainfall subsequent to tidal inundation increases the length of time that pools are around, and thus could promote continuous breeding (Lee *et al.*, 1984).

Sparse rainfall followed by periods of high temperature reduces the length of time salt and freshwater ephemeral breeding sites are available for larval development. Although temperatures may be high enough to promote optimal development through the various stadia of development, low precipitation relative to temperature reduces the temporal availability of breeding habitat, which may prevent the emergence of one generation, or might limit the number of generations that might emerge from the site. On the other hand, consistently high total monthly rainfall in conjunction with seasonal temperatures appears to augment abundance through the persistence of ephemeral breeding sites in addition to permanent sites.

The following studies illustrate the relationships between vector abundance and meteorological variables, in particularly rainfall. In a study conducted in two counties in New Jersey, U.S.A. during May to August (the main *Culex* breeding season), three trends in the data relating monthly *Culex* abundance to temperature and rainfall were found (DeGaetano, 2005). First of all, total precipitation during the month preceding catch data was the strongest predictor of catch data. Second of all, warm May minimum temperature enhanced the subsequent June catch, whereas warm July maximum temperature resulted in lower August catch. Finally, Maximum monthly rainfall decreased catch in June/July. The results of this study capture the potential interaction of these variables over the breeding season.

Raddatz (1982) showed that *Cx. tarsalis* trap catches used to infer population abundance depended upon preceding temperature and precipitation conditions. Overall, population peaks followed wet/warm conditions, while small peaks occurred if one of these conditions was inadequate.

In the Mar Chiquita region of Central Argentina, Gleiser *et al.* (2000) showed that over a four year seasonal sampling period (October-March/April, 1994-1997), the daily relative density (number of females caught if traps were operated over a 24 hr period) of *Ochlerotatus albifasciatus* (Macquart) positively correlated with rainfall accumulated over the previous 7-15 days and with an index combining rainfall and temperature. Additionally, traps set closest to larval habitat showed the highest correlations with both rainfall and this index. In general, the cohort size of *Oc. albifasciatus* appears to depend on the size of the area inundated that remains long enough for the insects to complete preimaginal development.

In many regions, especially temperate parts of the globe, it appears that during the primary breeding season, precipitation may be a more important limiting factor for hatching than temperature. Additionally, rainfall measurements such as total monthly rainfall tend to be better predictors of mosquito abundance than temperature (Gleiser *et al.*, 2000).

2.1.3 Location of Vector Abundance: Habitat Characteristics and Spatial Patterns

The spatial distribution of adult mosquitoes has been associated with the availability of oviposition sites, resting sites, patterns of vegetation, and the distribution of potential blood meal sources. The relatively new disciplines of landscape ecology/epidemiology are now providing the conceptual frameworks for the study of vector ecology and disease epidemiology under these considerations. Landscape features have long been recognized as important determinants of transmission of vector-borne diseases (Kitron, 1998). Landscape ecology studies have been typically focused on landscape features as determinants of habitats of reservoir hosts and vectors, and as foci for disease (Kitron, 1998). Under the landscape ecology paradigm, the epidemiology of vector-borne diseases is to be viewed as a dynamic interaction among vector populations, human and/or domestic animals, reservoir hosts, pathogens, and environmental determinants of transmission. Additionally, a thorough understanding of the landscape ecology of a zoonosis is now deemed to be important/critical in developing effective surveillance and control strategies as well as in ascertaining relative risk of human infection (Reisen *et al.*, 1995).

Study types used to delineate the spatial distribution and landscape ecology of vectors include the stratification of vector abundance data by habitat categories, proximity analysis of abundance to potential habitat determinants of spatial distribution, and spatial statistical analysis of abundance data.

Different habitat types might support different levels of vector abundance and provide an indication of vector habitat preferences. Studies in which the aim has been to detect differences in abundance among habitat categories have included categories derived from terrain features, vegetation and/or water surface characteristics. Reisen *et al.* (1995) examined *Cx. tarsalis* abundance collected from traps set in sites representative of nine different habitat categories in Southern California. The researchers used ANOVA analysis to assess differences in abundance attributable to the designated habitat categories, and with two-way ANOVA analysis, differences over time. Gleiser *et al.* (2002) stratified *Oc. albifasciatus* abundance into habitat types characterized by water surface dynamics and predominant plant coverage in the Mar Chiquita basin of Central Argentina. This group also used ANOVA analysis to detect differences in abundance among habitat types. Both of these studies found the abundance of the mosquito species of interest to be significantly higher in certain habitat types or categories compared to others.

Additionally, both of these studies assessed mosquito abundance in relation to trap proximity to certain features to identify vector habitat preferences. In southern California, the effects of site distance from major aquatic landscape features (Whitewater Channel and Salton Sea) along with elevation on mosquito abundance were analyzed (Reisen *et al.*, 1995). The decrease in mosquito abundance with distance from Salton Sea was curvilinear, while the decrease in abundance exhibited a negative curvilinear function of elevation in meters. In central Argentina, the percentage of land covered by prairie types within 500m of the trap site, distance to woods and prairies, and land slope were the variables included in a multiple forward stepwise regression. This model indicated that mosquito density is higher in sites covered by prairies and lower in sites near woods and terrain with steeper slopes (Gleiser *et al.*, 2002).

Spatial statistical techniques in conjunction with GIS and GPS (Global Positioning Systems) are being used to assess the potential for distinguishing between areas with high levels of abundance versus those with low abundance. Spatial statistics are a set of tools to describe, explain, extrapolate, and predict the distribution of objects and processes in space. The spatial analytical method of kriging has been used to interpolate values to unsampled sites, while that of local spatial statistics has been used to detect spatial clustering around known or suspected foci, as well as the occurrence of hot spots (Liebhold *et al.*, 1993).

2.1.4 Interpretation of Vector Abundance Data

Adult mosquito trapping is a frequently used method in the design of research investigations into the nature of vector populations. In conjunction with sampling other stages of the vector lifecycle and depending on the design of the experiment, mosquito abundance can contribute to understanding the relationship to meteorological variables, spatial distribution of mosquito vector abundance, vector habitat characteristics and preferences (e.g. hydrology, soil characteristics, vegetation cover), and relationship to disease risk.

A number of general considerations apply to the interpretation of vector abundance data in both monitoring and research programs. These include the type of population estimate and what this estimate may perform, and the type of trap and sampling scheme used and what the chosen scheme can detect.

Mosquito abundance is a relative rather than an absolute population estimate. Multiple factors affect the size of relative estimates in addition to actual changes in the population. These factors make it difficult to estimate the absolute population. Therefore, what one is really estimating is the proportion of those members of the population that were in the right phase to respond and did so under the prevailing climatic conditions and efficiency of the trap (Southwood, 1978). Thus, instead of measuring density or absolute size, standardized trapping protocols produce data expressed as number of females per trap night (Reisen and Lothrop, 1999). While the relationship between the size of the trap collection and the mosquito population of an area is unknown, systematically collected data from trap collections may be compared over time and space regarding changes in the number of mosquitoes collected (Bidlingmayer, 1969).

The interpretation of trap data primarily depends on the type of trap used and the sampling protocol. Numerous traps are available to capture different segments of the mosquito population, either those that are in a different phase of activity, or those at a different physiological stage. For instance, light traps, such as the New Jersey light trap, possess an artificial light source, which attract various light-sensitive insects, including mosquitoes. These traps sample mosquitoes predominantly in appetential flight, since light does not fulfill any direct mosquito need. When CO_2 is added to such traps, host seeking females are presented with a definite olfactory signal with which to orient themselves.

In general, the interpretation of catch data is subject to a number of considerations. Meteorological variables also impact upon the size of the trap catch.

Trap catches by two traps situated in the same habitat may vary because mosquitoes respond to slight differences in terrain features such as variation in height and density of vegetation, land elevations, soil moisture, etc. which occur within the same habitat, so that numbers vary within the same habitat (Bidlingmayer, 1985). Each site has different physical characteristics and proximity to adjacent alternative habitats and physiological needs. As a consequence, each trap site has a mosquito population that is also unique.

For the purposes of operating a mosquito surveillance and control program, sampling is typically stratified to detect sensitively the change in abundance over time based on the distribution of target species, the occurrence of human cases, or other human populations with attributes such as density and socio-economic status. Stratified collection usually produces a sensitive indication of changes in abundance, but typically overestimates abundance throughout an area that is typically mosaic of both favourable and unfavourable habitats. If the objective is to monitor population changes, then population changes should be monitored at each site by determining the difference between the most recent catch and earlier catches.

2.1.5 Mosquito Control Program Frameworks and Components

A number of considerations are relevant to the design of current mosquito arbovirus vector control program frameworks. Arguably, the relative importance given to each consideration has been influenced by the role of pesticides versus other control options in program design, program goals and implied intervention targets, and research emphasis. Movement away from prioritizing vector longevity as the main intervention target over many potential targets led to the revitalization of research in various fields relevant to Medical Entomology and Epidemiology (Ault, 1994). Additionally, the development of Integrated Pest Management (IPM) approaches to mosquito control has led to the shift in applications from a calendar to an 'as needed' basis (Brenner *et al.*, 1998). As a result, monitoring and surveillance become the dominant activities. Monitoring schemes typically focus on when and where pests are expected to be found based on the known biology of the target organism. A surveillance system that is universally applicable does not exist. However, the basic structure of mosquito control program design as based on an ecological and IPM approach consists of program objectives, a standardized system of data collection, data analysis and decision making, and program and intervention evaluation.

Mosquito abundance data collected from various trapping systems can be developed to provide information on numerous elements of arbovirus activity and mosquito population ecology relevant to arbovirus transmission. For this reason, the U.S. Center for Disease Control (CDC) outlines that the major goals of mosquito surveillance in the overall context of West Nile virus surveillance are to:1) use data on mosquito populations and virus infection rates for human disease threat assessment; 2) identify high risk geographic areas; 3) assess the necessity for and timing of the application of interventions; 4) identify larval habitats for targeted control; 5) monitor the effectiveness of surveillance and improve prevention and control measures; and, 6) develop a better understanding of transmission cycles and potential vector species (CDC, 2003).

Comprehensive adult mosquito surveillance activities may be employed to obtain relevant mosquito population information. For example, data can be developed in relation to species diversity, abundance, seasonal range of activity, physiological age, blood meal determination, assessment of control intervention, flight range, virus infection, minimal infection ratios, and vector potential (White, 2001).

The specific role of vector control jurisdictions typically includes monitoring the adult population for the spatial assessment of abundance, timing of control, and often monitoring of virus activity in mosquito populations.

Programs operated through local government jurisdictions typically have two objectives: 1) to monitor and control nuisance mosquitoes, and 2) to monitor and control vectors that transmit pathogens (LGAQ, 2002). The species targeted through these objectives might be different, as well as the emphasis of surveillance and the timing and targeting of control activities.

Species composition of adult collections can be determined by accurate mosquito identifications and categorization of specific species into primary, secondary, or nuisance species, depending on the recognized contributions of that species to the mosquito-borne pathogen cycle. These data can be helpful to improve the ability to predict when secondary vectors may be in abundance and more likely to carry the virus out of the normal amplifying host cycle and increase risk of human infection.

Once local vector composition is defined, relative population estimates that are utilized by vector control agencies to compare abundance levels in time and space may be obtained specifically for these species. Standard guidelines for estimating mosquito abundance from traps include counting and identifying mosquito collections, and, over the sampling season, monitoring changes in relative numbers. Data analysis and decision-making regarding interventions are typically based on the numerical values of trap data, larval surveillance data, and weather variables. Control measures include the judicious use of biological control agents such as the bacteria *Bacillus thuringensis israelensis* (*Bti*) to target larvae, in addition to other classes of chemical compounds applied in accordance with the appropriate government legislation.

The program evaluation phase involves an assessment of the efficacy of interventions, as well as collection and analytical techniques. Programs may be revised based on new and applicable research findings, and on whether or not the framework appears to be matching the program objectives.

2.1.6 Current Trends in Mosquito Control Program Design

Risk assessment is an emerging approach in mosquito control program design. Hunsaker and Graham (1990) cite a two phased approach to risk assessment: 1) the definition phase, in which the endpoint, source terms, and reference environment are defined, and 2) the solution phase, in which exposure and effect are assessed and exposure levels are related to effect levels to determine risk, or the probability of a certain event happening (LGAQ, 2002). When disturbance is translated to imply disease, pathogen, or vector, the guidelines provided by ecological risk assessment can be adapted to the risk mapping of vector borne diseases and are particularly relevant to emerging diseases and invasion of exotic vectors.

The application of risk assessment to mosquito-borne virus surveillance to response plan formation has been developed for the arboviruses of California (Western Equine Encephalomyelitis virus, Saint Louis Encephalomyelitis virus, and West Nile virus). A conceptual model is used which is based on the calculation of risk from the averaging of risk from seven factors, including environmental conditions, vector abundance and virus isolations, sentinel chicken seroconversions, fatal infections in birds, equine and human infections, and proximity of virus activity to urban or suburban regions (Barker *et al.*, 2003). The average falls into three predetermined categories of risk and response: normal season, emergency, and epidemic.

The use of Geographic Information Systems (GIS) permits the visualization of vector abundance data in relation to various types of physical maps (Bergquist, 2001). Habitat categories enable program operators to detect when and under what conditions mosquitoes exploit each source. Soil classifications provide information regarding water surface persistence following rainfall, to better determine the location of larval habitat following a rainfall event. The assessment of adult mosquito abundance is conducted by a uniform sampling scheme, which, when visualized in relation to larval records, one can detect if a source has been overlooked relative to distribution and flight range (Lothrop and Reisen, 1999).

Overall, general efforts related to mosquito vector problem definition were outlined in this section. The next section will specifically deal with such efforts as related to RRv vectors in Australia.

2.2: Defining the Local RRv Vector Problem in Australia

2.2.1 Background: Clinical Aspects, Epidemiology and Public Health Significance

Ross River virus is most often the causative agent associated with the manifestation of epidemic polyarthritis in humans (Mackenzie *et al.*, 1998). Human infection can be manifest by varied constitutional disturbances, rash and rheumatic symptoms (Fraser, 1986). In 1980, the yearly incidence of epidemic polyarthritis in Australia was estimated to be 23.6 cases per 100 000 residents (Aaskov *et al.*, 1981). However, reporting was less efficient prior to 1990. Since this time, human cases of arbovirus infection have been monitored through the National Notifiable Diseases Surveillance System (NNDSS), Commonwealth Department of Health and Ageing. Using a common case definition, each state reports their Ross River virus cases, and typically a total of several hundred to several thousand cases of Ross River virus are reported annually (Mackenzie *et al.*, 1994). The average annual notified cases in Australia from 1991-2000 was 4 745, with a maximum number of 7 823 in 1996. This maximum number coincides with an outbreak in Queensland, at which time close to 5 000 cases occurred with a state notification rate of 148 per 100 000 (Harley *et al.*, 2001).

The majority of cases in Australia have onset dates from March and June, which corresponds to the late summer and early autumn months (Aaskov *et al.*, 1981). However, in Central and Northern Queensland, cases occur year-round. The incubation period is usually 7-9 days, but may vary from 3-21 days (Harley *et al.*, 2001).

Although the majority of patients are able to return to work within a month of onset of symptoms, a significant proportion of patients suffer residual arthralgia lasting more than a year. While the virus infection is not life-threatening, the morbidity caused by the debilitating polyarthritis syndrome is of considerable social and economic concern. The morbidity associated with infection is thought to cost a minimum of tens of millions of dollars annually in direct and indirect health costs nationally, although no authoritative investigations into the economic effects of patient morbidity have been undertaken (Russell, 2002). A more conservative estimate defined the yearly cost as between 2.8-5.7 million (Harley *et al.*, 2001). The risk of disease is greater for the rural residents in Queensland, as it appears to be also the case in the other states where data are available (Russell, 2002). However, from 1990 to 1998, increased virus activity in rural areas has resulted in the intrusion of Ross River virus into major metropolitan areas, such as Perth (1989), Brisbane (1992 and 1994), and Sydney and Melbourne (1997) (Mackenzie *et al.*, 1998).

2.2.1.1 Ross River Virus: Components of Transmission Cycle

RRV is maintained in nature enzootically and is transmitted to humans during epizootic and epidemic periods (Harley *et al.*, 2001). In arid parts of Australia the virus may be maintained by vertical transmission in certain mosquito species.

There are a number of virus strains which have been characterized and associated with particular regions and vectors, although movement of these strains appears to occur within the continent. Molecular epidemiological studies have been used to defined such strains: RNA fingerprinting studies have demonstrated the existence of four major genetic types of RRv, and sequencing of virus cDNA and construction of phylogenetic trees define three clusters of viruses that predominate in different geographic areas in Australia: genotype I in Charleville, genotype II in Queensland, NSW, and Victoria, and genotype III in Western Australia and the Northern Territory. Virological factors considered during outbreaks have mostly included vector/host competence and strain of virus, which has been anecdotally associated with different levels of virulence of different vectors (Harley *et al.*, 2001).

From antibody prevalence and laboratory infection/transmission studies, macropodid marsupials appear to play a significant role as reservoir hosts, and are considered to be the principal natural vertebrate host for RRv (Aaskov *et al.*, 1981, Rosen *et al.*, 1981, Mackenzie *et al.*, 1994). However, these animals have very low population levels in urban areas, so other potential reservoir candidates in urban areas have been investigated. These include dogs (*Canis familiaris* Linneaus), cats (*Felis catus* Linneaus), brush-tail possums (*Trichosurus vulpecula* Kerr), and flying foxes (*Pteropus* Iylei). So far, no conclusive evidence has been found to implicate any of these species as important urban RRv reservoir hosts. Overall, investigations of RRv reservoir hosts in Australia have yet to establish any clear links between infections in vertebrates with human disease (Harley *et al.*, 2001).

RRv has been isolated from wild-caught mosquitoes of 42 species from the genera *Aedes, Anopheles, Coquillettidia, Culex, Culiseta, Mansonia, Ochlerotatus, Tripteroides* and *Verrallina* (Russell, 2002). So far, only 10 have been shown to be capable of transmitting virus, although many have not been examined in laboratory transmission experiments. Overall, only a few species are deemed to be important in maintaining RRv transmission over the Australian continent as a whole. The status of these species is based on their widespread distribution, the consistency in virus isolation from the states in which they are found, and some evidence of vector competence. These species include the saltmarsh mosquito species, *Ochlerotatus vigilax* (Skuse) and *Ochlerotatus camptorhynchus* (Thomson), which are important in coastal settings, and the freshwater mosquito, *Culex annulirostris* Skuse, which is considered to be of prime importance in inland settings. Additional vectors have been confirmed in certain locations, although not to the same extent as the above vectors; these include *Ochlerotatus notoscriptus* (Skuse), *Ochlerotatus procax* (Skuse), and *Verrallina funerea* (Theobald).

2.2.1.2 RRv Vector Distribution

Ochlerotatus vigilax extends into the Oriental and Australasian regions, including Thailand, Papua New Guinea, New Zealand, and Australia. Within these regions, its distribution is coastal, and within Australia, *Oc. vigilax* is present along the coastline, except in Victoria and Tasmania. *Ochlerotatus vigilax* inhabits or is associated with saltmarsh pools, ponded water in mangroves, and low lying estuaries. *Culex annulirostris* is found in southern and western Australasia. In Australia, this species is widely distributed, except in Tasmania, and breeds in a wide range of habitat types, including freshwater, polluted water, or temporary pools, indicating that breeding areas represent a wide range of habitat types.

2.2.2: Past and Emerging Research related to RRv vectors in Australia: Implications for Mosquito Control Programs.

The above documentation provides sufficient scope for mosquito control agencies in Australia to pursue ecologically-based surveillance activities that are specific to the regional environment in which the relevant mosquito control jurisdictions exist. However, the research necessary to support this approach is in its infancy. Since RRv was discovered, most research efforts have focused on the general vector ecology and general vector competence of mosquitoes for this virus. This research has been important for the establishment of the general notion that there are different disease epidemiologies in Australia. In contrast, research efforts over the past decade have focused on testing causal hypotheses that attempt to establish linkages between the location of mosquito breeding or mosquito abundance and the measured risk of human disease (Harley *et al.*, 2001). Together, the foundational and emerging literature establish the knowledge base for mosquito control agencies to identify, monitor, and control relevant mosquito species according to vector related factors associated with disease risk.

Different regional settings possess different epidemiologies of RRv which depend on the locality of infection, virus strain, and vector species (Russell, 1994). Variation in RRv activity within and between regions is strongly influenced by vector related factors. These factors include vector distribution, vector composition, and vector competence of species present in an area, and the age and abundance of these species.

2.2.2.1 General patterns in RRv vector distribution in Australia

A cumulative review of studies from around the continent highlights a few general notions related to vector distribution and abundance. Different categorical divisions of vectors draw attention to different factors that affect vector distribution. For instance, dividing vectors into those that breed in saltwater versus freshwater sites illustrates the differential importance of tidal inundation in the former case in comparison with the importance of rainfall in the latter case. When distinguishing between temperate vs. tropical patterns of distribution, differences in the seasonality of abundance or temporal differences in distribution of adults of the same species comes into focus. For instance, in the temperate south, mosquito activity has a distinct seasonal association, whereas in the tropical north, breeding can occur year round. Additionally, general differences in climate and vegetation become important factors in delineating species tolerance ranges and habitat preferences. Distinct seasonal epidemic activity in the temperate south is usually associated with summer and autumn rainfall in inland areas, or tidal inundation of coastal marshes when vectors are most active (Russell, 2002). The most important vectors associated with this hypothesis include *Cx. annulirostris* and *Oc. camptorynchus*. In contrast, in the tropical north, seasonal activity is more closely associated with the highest spring tides, while some regions may have year-round activity. In coastal areas, the most important vector is *Oc. vigilax*, while in inland areas, *Cx. annulirostris*. There is also a distinct difference in urban vs. rural patterns of distribution, since there are abundant artificial containers in cities suitable for those species that prefer to breed in such places, for instance *Oc. notoscriptus*. Overall, there is significant ecological heterogeneity between different transmission settings that influence vector composition and the roles which these vectors might play in RRv transmission (Russell, 2002).

Additionally, the epidemiology of RRv outbreaks seems to vary as the environmental factors determining mosquito abundance vary within and between region and seasons (Russell, 2002). Mosquito abundance is an important factor for initiating outbreaks. The importance of vector abundance as a determinant of virus activity rests upon the probability that with greater mosquito abundance, the greater the likelihood of being bitten (reservoirs/hosts) and the greater the chance that the mosquito population will include old females that have bitten both a reservoir host and a human (Weinstein, 1997). Two broad categories of environmental factors include meteorological variables and breeding habitat/vegetation variables.

Weather variables, such as temperature and water availability are important determinants of the distribution and abundance of vectors. Weather conditions influence mosquito population biology, directly affecting the breeding, survival/longevity, abundance, and their extrinsic incubation period. Conditions which have been cited that specifically impact upon mosquito abundance in Australia include rainfall, temperature, and tide. Sea levels and the Southern Oscillation Index (SOI) have been additionally cited (Tong *et al.*, 1998). A number of investigations have linked these same conditions to RRv activity and epidemics, thus paving the way for the development of predictive models for epidemics using climate variables. However, different regions of Australia are affected by different climate conditions, i.e., different weather conditions and patterns exert influence in tropical, arid, and temperate regions. Additionally, transmission cycles are driven by different vector species (along with vertebrate hosts) in disparate regions of the country, so that a variation in influence of weather variables on habitats and populations also exists within and between regions and seasons. Therefore, the relative importance of climatic factors in the transmission of RRv potentially varies with geographic area, indicating that generalized predictive models are inappropriate for the application to RRv disease in Australia. Investigations of the key climatic factors that increase mosquito populations may elucidate important local and geoclimatic patterns to improve vector surveillance (Kelly-Hope *et al.*, 2004).

The proximity of breeding habitats to human populations influences the degree of RRv transmission. RRv activity appears to be driven by local vector activity, with outbreaks of RRv being associated with local increases in mosquito populations. The majority of outbreaks and clusters have been reported in residential areas close to vector breeding sites. The types and suitability of habitats also influence the species present and may coincide with vectors of differing biology and vector competence. The topography of outbreak regions has been described mainly in terms of natural features, particularly waterways and their capacity and susceptibility to retain water. Examples include river systems, tidal creeks, shallow tidal inlets, coastal lagoons, brackish wetlands, saltmarshes and mangrove swamps. Little is specifically documented on vegetation. However, the distinct geographical regions of outbreaks and the aforementioned characteristics of natural landscapes provide some clues to the kind of vegetation that may support local vectors and hosts

(Kelly-Hope *et al.*, 2004). Recent spatial associative studies have been carried out to identify local differences in the spatial distribution of RRv infection risk with regard to natural features (Muhar *et al.*, 2000).

2.2.2.2 Ross River virus Activity and Spatial Patterns

In Australia, the emerging literature over the past decade includes studies that have attempted to elucidate spatial patterns of RRv activity, and important environmental factors that are associated with these patterns. The emerging studies conducted over the past decade have been focused on specific study areas or jurisdictions and have attempted to define local vector composition, competence, and spatial differences in mosquito abundance as related to the spatial risk of RRv disease. Two main objectives that these studies have aimed to reach include: a) to define local RRv vectors, and b) to elucidate patterns in RRv case distribution in relationship to vegetation and mosquito abundance.

As a preliminary approach, virus isolation procedures conducted on field-collected mosquitoes have implicated multiple potential vectors for RRv, such as in Brisbane (Ritchie *et al.*, 1997). The more rigorous vector competence studies of locally abundant mosquitoes (e.g. Ryan *et al.*, 1999) have demonstrated that lesser known species that effectively transmit virus have the potential to play important roles in virus amplification and transmission before and during an outbreak event.

Additionally, studies that have related RRv activity to mosquito abundance and vegetation types have used techniques to define relationships between the above on smaller geographic scales, and have used a combination of case notification/place of residence information, $C0_2$ light-trap mosquito abundance data, and vegetation type and location data to expose patterns of cases/activity in relation to mosquito population levels and landscape features. Overall, the investigation of the nature of RRv vector and disease ecology without reference to location is not as generalizable as once thought.

The above research, in conjunction with a landscape-based approach to vector surveillance and control, has a number of potential implications for RRv vector control programs in Australia. These implications include expansion of programs to include freshwater species, prioritized control, and more effective monitoring activities. Therefore, research conducted in conjunction with local mosquito control agencies with specific reference to local vector populations and site conditions will help to establish better monitoring and surveillance of vectors in relation to disease risk, so that these programs will be able to better target vector populations from this perspective.

2.2.3 Vector incrimination and Vector status in Australia

Mechanical, biological, and transovarial transmission have been described for RR virus in mosquitoes (Kay *et al.*, 1981). Biological transmission has been identified as the main mode of transmission of RR virus.

Ten competent vector species have been confirmed in Australia (Russell, 2002). Of these species, eight have been confirmed as competent vectors in Queensland. As such, these species have been traditionally considered to be the main vectors associated with the RRv maintenance cycle in Australia as a whole. The recent additions of certain vectors to this list reflect the importance of regional competence testing. Demonstration of laboratory competence of a single species from a single region is not a generalizable measure since competence has not been tested for all potential vectors in all regions, and since regionally abundant vectors may warrant further competence testing. Recent investigations suggest the regional importance of amplification vectors during epidemics. Virus isolation results provide further clues regarding additional vectors that might play a role in transmission in regional environments, and if these species are abundant, may warrant local competence testing. In Queensland, virus isolations have been demonstrated for the species in Table 2.1. Studies to define local vectors have been carried out in Southeast Queensland. Ritchie *et al.* (1997) performed virus isolations from mosquitoes collected during the 1994 epidemic in Brisbane, Queensland, and repeated isolations and demonstration (to varying degrees) of infection were shown in *Ve. funerea*, *Oc. notoscriptus*, and to a lesser extent, *Oc. procax*. All three of these species have been shown to be very capable of transmission. The authors proposed that these vectors could have filled different roles in transmission during the epidemic. For example, the association of *Ve. funerea* with flying foxes could have provided a linkage for broader dissemination of RR via viraemic flying foxes. *Ochlerotatus notoscriptus* prevalence in suburban yards and its association with RRv in May and June point to the possibility that this species may prolong RR epidemics and provide a means of overwintering. Overall, this study demonstrated that some redirection in control efforts is necessary to include potential amplification vectors.

Ryan *et al.* (1999) attempted to define the important vectors of RRv in Maroochy Shire, Queensland, by comparing the vector competence of several mosquito species to that of *Oc. vigilax* and *Cx. annulirostris* collected from light traps in this jurisdiction. Based on adult abundance and vector competence, *Cx. annulirostris*, *Ve. funerea*, and *Oc. vigilax* appeared to be important vectors of RRv in the region, as did *Oc. procax*. In addition, *Oc. notoscriptus* from this region was not found to be competent for RRv, indicating that vector competence must be examined in different spatial and temporal situations. Although RRv was isolated from *Oc. notoscriptus* and *Oc. multiplex* (Theobald), and the former was quite abundant, they were not found to be subsequently competent, indicating that isolation is not a reliable indicator for ability of species to transmit virus.

Table 2.1: Species of mosquitoes in which virus isolation has been demonstrated for RRv in Queensland, Australia. (Adapted from Russell, 2002).

Aedes aegypti (Linneus)
Ochlerotatus alternans (Westwood)
Ochlerotatus vigilax (Skuse)*
Verrallina carmenti (Edwards)
Ochlerotatus imprimens (Edwards)
Ochlerotatus kochi (Donitz)
Ochlerotatus lineatus (Taylor)
Ochlerotatus multiplex (Theobald)
Ochlerotatus normanensis (Taylor)*
Culex annulirostris Skuse*
Culex sitiens Weidemann
Culex gelidus Theobald
Verrallina funerea (Theobald)
Ochlerotatus notoscriptus (Skuse)*
Ochlerotatus procax (Skuse)*
Culex australicus Dobrotworsky & Drummond*
Mansonia septempunctata Theobald
Mansonia uniformis (Theobald)*
Anopheles amictus Edwards

*species for which laboratory infection and transmission have been demonstrated.

From the cumulative body of evidence, overall, in southeast Queensland, the species that have been shown to have a potential role in RRv transmission and that may then be candidates for further study in regional investigations and vector control activities (in the absence of local transmission cycle data) include *Oc. vigilax, Cx. annulirostris, Ve. funerea,. Oc. procax,* and *Oc. notoscriptus.*

2.2.4 Ross River Virus Vector Lifecycle in Relation to Habitat and Meteorological Variables

2.2.4.1 Lifecycle of Oc. vigilax in relation to landscape

Ochlerotatus vigilax oviposition occurs at the edge of depressions where a cover of vegetation reduces moisture loss. Also, eggs are laid in crevices in moist soil or on the bases of plants. For aedine mosquitoes, selection of oviposition sites seems to be affected by shade, ground features, vegetation and terrain (Kerridge, 1971). The saltmarsh species, *Oc. vigilax,* completes its pre-imaginal development stages in ephemeral brackish pools common to saltmarsh environments. Saltmarshes are natural grassy wetlands that occur behind mangroves. Typical vegetation includes the salt tolerant species *Salicornia* and *Sporobolus.* Saltmarshes are typically above the mean daily high tide mark and are inundated by only the highest monthly tides. Therefore, these environments are characterized by shallow depressions that form pools after tidal inundation or rainfall in addition to poor drainage. The pools are usually ephemeral, drying out in the days following inundation. Larval development of *Oc. vigilax* is well suited to the stressful environment of saltmarsh pools. These stresses include a short period of water availability, fluctuating salinity levels from tidal inundation and rainfall and high larval densities. *Ochlerotatus vigilax* can develop in fresh to hypersaline pools remaining after days of evaporation (Lee *et al.*, 1984).

Once emerged, *Oc. vigilax* disperse. Adults have been recorded up to 64 km from larval habitats, showing that dispersal is relatively extensive. Dispersal is aided by prevailing winds. Attacks on humans occur in the vicinity of saltmarshes after peaks of emergence, but appetitive dispersal often brings large numbers of females into populated areas. Mangroves adjacent to breeding areas provide shelter for a great number of adults, but breeding seldom occurs in dense mangrove (Marks, 1982).

2.2.4.2 Lifecycle of Oc. vigilax in relation to temperature, seasonality, and time

Tidal inundation of saltmarshes exerts an influential effect on the maturation of *Oc. vigilax* eggs. Characteristics of oviposition and egg dormancy ensure that prolific hatching of eggs occurs when saltmarsh depressions are inundated. Eggs often accumulate to large densities at oviposition sites subject to periodic flooding and drying out. Eggs show variable expression of dormancy following maturity. Some eggs will hatch if flooded within a short time after reaching maturity if kept under moist conditions. Other eggs require drying and attain a state of desiccation-resistance, hatching in installments with subsequent floodings. Kerridge (1971) also found that eggs could remain viable for at least 116 days (almost four months) and that installment hatching resulted from successive floodings of samples at intervals of 7, 24, and 85 days from collection. Flooding with 100% seawater appeared to give the highest hatching rates. These characteristics generally ensure a large reserve of eggs to repopulate saltmarsh pools with larvae following inundation.

The characteristics of egg hatching result in bursts of adult emergence. These bursts are synchronized with the inundation of the saltmarsh pools by the highest monthly tides. The periodic emergence of adults is characteristic of a brooded mosquito type. However, on coastal flats where semi-permanent to permanent ponds occasionally subject to tidal influx occur, rather more contiguous breeding takes place in water which may be up to three feet deep.

In the lab, eggs require a minimum period of 48-54 hours at 25°C to complete embryonic development after being laid. *Ochlerotatus vigilax* shows rapid development in ideal conditions (Lee *et al.*, 1984). In one study, Oc. *vigilax* required 6 days until emergence in January, but 9 days until pupation in May (Kerridge, 1971). During a colder winter, larval development was extended to 20 days and some pools dried out before adult emergence. On Coomera Island, Queensland, Dale *et al.* (1986) recorded a scantiness of hatching from March (autumn) onwards. During this period, there were also few larvae or adults in the study area or adjacent marshland. These results are consistent with Reynolds (1961) and Kerridge (1971). They suggested that quiescence, diapause, or both, caused the temporal variation in the hatchability of eggs. Kerridge (1971) suggested that some, but not all, eggs entered facultative diapause which, in her study, was induced by low temperatures. During the summer in southeast Queensland, development to adulthood usually takes from 6-8 days. Newly emerged adults show limited activity over a 24 hour period during which time the cuticle hardens. Adults may remain at the saltmarsh for a period of time and then disperse in large numbers in search of suitable hosts. Females begin biting activity commencing at, and reaching a peak about an hour after sunset, then subsequently increasing again before sunrise. In Australia, biting activity occurs both day and night, especially during crepuscular periods.

2.2.4.3 Lifecycle of Culex annulirostris in relation to landscape

Culex annulirostris occupies a variety of habitats under the broad heading of freshwater wetlands, with some breeding in nutrient rich waters (Dale and Morris, 1996). These habitats may be scattered, and small in size. Specific categories of larval habitat include fresh water swamps, lagoons, and transient grassy pools, and streams. Dale and Morris (1996) created a classification system for breeding sites in Brisbane following a larval survey: temporary pools in grassy fields, permanent ponds with floating vegetation, and major natural/semi-natural drains. It also has been collected with *Cx. sitiens* within saltmarsh pools and, following prolonged heavy rainfall, *Cx. sitiens* may displace *Oc. vigilax* as the dominant species in the flooded saltmarsh. Also, irrigation channels with marginal vegetation, rock pools with leaf litter and surface algae, temporary ground pools with marginal vegetation, drainage ponds, and permanent ground pools act as suitable larval habitat.

The eggs are not resistant to desiccation. They are laid on the water surface in rafts of 50-200 eggs. *Culex annulirostris* collections from investigations of adult resting sites in Queensland were obtained outdoors, from shaded ground cover, such as overgrown couch grass and flood debris in trees, and rabbit burrows (Lee *et al.*, 1989).

2.2.4.4 Lifecycle and development of Culex annulirostris in relation to temperature and rainfall

With respect to temperature requirements, larval survival is greatest in the laboratory at 25°C, while prohibited at 10 and 40°C. The period for complete juvenile development ranged from 8.57 days at 35°C to 37 days at 15°C. Development from egg to adult required 196 day-degrees above 9.7°C with incubation temperatures between 15 and 30°C. For Queensland mosquito populations, development times were estimated as 1-2 days for egg hatch in summer, with 1-2 days for each of the 4 larval instars (Kay *et al.*, 1981).

2.2.4.5 Lifecycle of Ve. funerea in relation to landscape

Larval habitats of *Ve. funerea* have been reported as slightly brackish to freshwater temporary pools in swampy areas of tea tree and sedges adjoining tidal areas, usually well shaded with abundant and overhanging vegetation (Marks, 1982). Additionally, larvae have been sampled from puddles, ponds and borrow pits that provided temporary breeding in well-shaded areas. *Verrallina funerea* breeding occurs just inland of *Oc. vigilax* breeding, although the two simultaneously occur together. Adults have been sampled from a variety of forest and farmland in Northern Queensland (Standfast and Barrow, 1969).

2.2.4.6 Lifecycle of Oc. procax in relation to landscape and meteorological variables

Due to its recent implication as a vector in Southeast Queensland, little is known about the biology of *Oc. procax*. It is known to breed in temporary pools. This species breeds in freshwater pools but can tolerate low to moderate saline levels (Ryan and Kay, 2000).

2.2.5 Spatial Pattern of Vector Abundance

The summer of 1991-1992 outbreak of epidemic polyarthritis in Brisbane and other areas of Southeast Queensland suggested that multiple vectors were involved due to the wide case distribution in Brisbane. Following the 1994 outbreak in the same region, Ritchie *et al.* (1997) reported that other vectors besides *Oc. vigilax* were involved in the outbreak, and implicated certain freshwater and brackish water species as important suburban vectors of RRv. Although a non-standardized trapping system was used which precluded the use of statistical analysis, trends were observed in individual species abundance over space. For instance, *Ve. funerea* abundance appeared to be concentrated in the suburb of Indooroopilly, *Oc. notoscriptus* was widespread, and both *Cx. annulirostris* and *Oc. procax* abundance appeared to be concentrated in the western suburbs of Brisbane. Recent investigations in Australia have utilized the approach of spatial analysis. Lee *et al.* (2003) performed a spatial analysis on the abundance of vectors encircling Indooroopilly Island in Brisbane. The results using contour maps for each species showed a spatial expansion and decline of *Oc. vigilax* abundance originating from the island, which potentially represented the emergence, increase, and decline of one *Oc. vigilax* cohort originating from the island and corresponding with the previous tide.

In order to investigate the spatial distribution of known and potential vectors, Jeffery *et al.* (2002) mapped the numbers of mosquitoes using local spatial statistics, and found that they could define areas with high versus low numbers using this method. There was a consistently strong spatial autocorrelation associated with the southern-central distribution of saltmarsh and mangrove areas on Russell Island. This area may contain more suitable adult harbourage sites and/or experiences sub-optimal control (e.g. is not treated with appropriate larvicides or other control techniques are not administered to this area). Numbers of *Oc. vigilax* were consistently higher on the southern half of Russell Island, probably because this area has a low elevation and is periodically inundated by high tides. Additionally, the brackish water species, *Ve. funerea*, had a spatial distribution strongly skewed towards the southern half of the island, as many northern traps did not contain any of this species. *Culex annulirostris* exhibited a weak spatial pattern.

Both of these studies used a systematic trapping system to collect mosquitoes. However, the nature of each study area demanded a different set of questions to be investigated, a different time scale, and a different interpretation of results. Previous to the study centred at Indooroopilly Island, Brisbane (Lee *et al.*, 2003), it had been suggested that this island might be similar to a point-source from which certain mosquito species might spread to nearby residences. Encircling the island with concentric rings of traps thus served to assess if, where, and when mosquitoes that probably originate from Indooroopilly Island impact local residences. The timing of this study coincided with a high tide event, with trapping commencing near the end of a series of high tides. The timing was relevant in this study because *Oc. vigilax* numbers are expected to increase after such a tidal event, and dispersal is carried out shortly after. In the study at Russell Island (Jeffery *et al.*, 2002), the researchers were more concerned with whether or not areas of low and high vector abundance were consistent over approximately two months (February and March, which typically correspond with high seasonal abundance). As such, trapping was performed once a week

Collectively, these studies highlight that from the perspective of spatial dependence, certain species exhibit strong spatial dependence, that maps can be made to depict areas with high versus low numbers of a particular vector's abundance, that these regions may or may not be stable over time, and that a sampling grid is useful for answering different types of questions related to space. Compared to the studies presented in section 2.1.4, these studies emphasize geocoded location of trap catches rather than placement of traps in different habitat types.

In Australia, the main work carried out regarding habitat identification has been in regard to the location of larval habitat. Investigations into the distribution of pre-imaginal stages of *Oc. vigilax* have shown that different associations occur at different spatial scales, and that potential breeding areas might be predicted using remote sensing. Dale *et al.* (1986) investigated the distribution of the immature stages of *Oc. vigilax* on a coastal saltmarsh in Southeast Queensland. At the macroscale, the largest number of hatched larvae was taken in areas with relatively low open vegetation adjacent to drainage channels. Additionally at this scale, most larvae were found associated with two habitat types: *Sporobolus virginicus* (L) Kunth at elevated positions and in depressions dominated by the succulent *Sarcocornia quinqueflora* (Bunge ex Ung.-Stern). At the micro-scale, most hatching occurred in narrow bands at specific elevations. At the meso-scale, the distribution of larvae appeared to be related to water movement, more larvae being found in areas of slower water movement.

Vegetation types might also be a useful indicator of mosquito breeding. Muhar *et al.* (2000) identified seven vegetation types relevant as mosquito habitats from the City of Brisbane's database. Of these, two of the three associated with the highest infection rates, littoral/ephemeral wetlands and riparian wetlands, are also known vegetation types specifically suitable for mosquito breeding.

In a study conducted to assess the emergence of mosquitoes from three distinct habitat types along the same drainage channel (Ryan and Kay, 2000), the numbers of each species that had emerged were greater in some habitat types compared to others, and that the emergence from different types was asynchronous. Additionally, the salinity of pools was determined at each site, and it was found that *Oc. vigilax* and *Cx. sitiens* emerged from pools with the highest mean salinities, followed by *Ve. funerea* in brackish pools. As rainfall occurred during this study in the study area, changes in salinity were noted, and these changes also changed the suitability of larval habitat for different species.

In Australia, the above trends have also been observed regarding the influence of meteorological variables on mosquito abundance. During the king tide season in

Australia (approximately Nov.-April), these tides occur on a monthly to bimonthly basis.

2.2.6 Ross River Virus Infection Risk

There was a generally held belief that the risk of contracting RRv disease is not related to place of residence but is randomly distributed in space (Muhar *et al.*, 2000). This is at least in part because the mosquitoes which transmit the disease in Brisbane are widely dispersed from their breeding sites by the prevailing onshore winds during the main breeding season.

Prior to 1999, little research work had been conducted in Queensland to identify differences in the spatial risk of RRv infection, either with regard to natural features or mosquito abundance. Lindsay *et al.* (1999) noted that people residing within 3 km of larval habitat were more likely to contract RRv than people residing greater than that distance away, thereby suggesting a risk associated with residence.

In Brisbane, a study was conducted to investigate whether there were spatial differences in RRv infection, and if these differences are related to any specific vegetation types (Muhar *et al.*, 2000). The researchers examined whether or not RRv cases in Brisbane were clustered into areas of high and low infection rates, and if so, whether there was any spatial relationship with vegetation types, as indicators of mosquito breeding habitats. They demonstrated that the risk of RRV disease was spatially associated with certain vegetation types, and particularly with whose which were obviously related to mosquito breeding such as wetlands.

Given that the known and potential vectors are associated with different habitat types, Ryan and colleagues (1999) embarked on a study using a similar approach to assess whether or not there were spatial differences for RRv case notification in a local government jurisdiction north of Brisbane. In addition to finding that certain suburbs do in fact possess significantly higher case notification rates, the researchers also found that, around a suburb with higher than expected notification rates, the number of RRv cases was independent of *Oc. vigilax* abundance. In contrast, light trap indices of *Cx. annulirostris* abundance were correlated closely with RRv disease notifications, and therefore this species may play an important role in the ecology of RRv at Maroochy Shire.

Others have attempted to deduce risk factors that have a temporal element. For instance, the seasonality of RRv infection risk is reasonably well documented in certain parts of Australia. In Maroochy Shire, the seasonality of RRv disease activity was reflected by the above average overall number of notifications during either summer or autumn (Ryan *et al.*, 1999).

Additionally, the timing of outbreaks in relation to meteorological variables has also been investigated. For instance, in Brisbane, the significant differences between years in infection rates were significantly related to monthly rainfall, particularly monthly rainfall and the number of cases two months later (Muhar *et al.*, 2000). The timing of rainfall events also appears to be important for the prediction of Ross River virus epidemics. In a study conducted at two different sites in the temperate southeast of Australia, the aggregation of RRv case notifications into regions and the calculation of monthly spatial summary value for an extensive list of weather variables in each region were used to identify the probability of an outbreak in each site by a logistic regression model (Woodruff *et al.*, 2002). Two predictive models emerged from the analysis: an early warning and a late warning model. The former implicated high precipitation in late winter/early spring (July-December), and low maximum temperatures in late spring and low rainy days in the preceding spring as being predictive of an epidemic. The latter model was slightly different between the two regions. For both regions, low maximum temperatures in early summer were associated with epidemics, while high minimum temperatures in late summer and high maximum temperatures were associated with each region respectively.

In a related study at the same study site, the researchers examined the vector competence of the suspected and confirmed vectors of RRv and found that indeed, the saltmarsh species *Oc. vigilax*, freshwater species *Cx. annulirostris* and *Oc. procax*, and the brackish water species *Ve. funerea* appear to be important vectors of RR virus in this jurisdiction. This study gave insight into the diversity of RRv vectors, regional differences in vector competence, and the need to conduct these kinds of studies to develop effective programs for mosquito control (Ryan *et al.*, 2000).

Based on an extensive review of the literature, it appears that there are three categories of risk factors associated with RRv outbreaks in Australia (Kelly-Hope, 2004). These categories include environmental, virological, and human (host) factors. Within these categories, the risk factors most obviously concerning space and most likely related to vector abundance include place of residence as a human factor, and vegetation/terrain of the outbreak region as an environmental factor. In light of the above studies, place of residence is an important risk factor when proximal to breeding habitat, or associated with increased mosquito abundance. Obviously, increased proximity of residences to breeding habitat might only occur in an outbreak region whose general topography supports immature mosquito development and potentially RRv transmission ecology.

2.2.7 Mosquito Control in Queensland

Regional bodies aimed at mosquito control in Southeast Queensland include the North Eastern Moreton Mosquito Organization (NEMMO), Contiguous Local Authorities Group (CLAG), and the Sunshine Coast Mosquito Organization (SCMO). Such groups have been formed to help coordinate treatments of larvicide in Southeast Queensland. NEMMO representatives from Brisbane City Council, Caboolture Shire Council, Redcliffe City Council and Pine Rivers Shire Council collaborate by coordinating treatment efforts. With significant growth projected for the majority of coastal areas, urban development in the coastal zone is a major challenge. Mosquito control activities are required to expand with expanding population centers, given the health threat that close proximity of humans to breeding sites poses, especially along coastal areas where saltmarsh mosquito breeding is prevalent.

2.2.7.1 Control of Ross River virus in Queensland

Queensland Health is responsible for communicable disease control under the Health Act, 1937, including mosquito-borne diseases such as Barmah Forest virus infection, dengue fever, and Ross River virus infection. However, this responsibility has been delegated to local government to enforce through the *Health Regulation*

1996-Part 8 Mosquito Prevention and Destruction. This regulation requires local government to take action to prevent mosquito breeding on land within local government jurisdiction. Guidelines available that specifically relate to mosquito surveillance and control in Queensland are outlined the Mosquito Management Code of Practice (MMCP) (LGAQ, 2002), as well as the Australian Mosquito Control Manual (AMCM) (MCAA Inc., 1998). The MMCP was published as a set of practices to follow to safeguard local jurisdictions from legal action, and outlines the basic scope of a sustainable mosquito management program that would meet the code of practice. This includes strategies for mosquito management such as the identification of each problem breeding area, the development of thresholds of abundance, surveillance of each species towards which control is warranted, an integrated mosquito management program, and a review process. Additional guidelines for surveillance provided by the AMCM include defining the mosquito problem and the components of the mosquito surveillance programs, and the identification of potential breeding areas. These documents, combined with others that variously describe regulations for runnelling, use of larvivorous fish, etc. provide enough of an impetus for mosquito control program operators to understand the vector problem in relationship to the local landscape.

In coastal areas in southern Queensland, *Oc. vigilax* (common saltmarsh mosquito) is the primary species targeted for control. Each local government is responsible for the treatment of *Oc. vigilax* breeding sites within their area, and priority is given to areas where human populations are located within the pest range of adult *Oc. vigilax*, i.e., distance away from the breeding site at which adult mosquitoes are unlikely represent a significant pest or disease risk to humans. However, although adult *Oc. vigilax* have been collected up to 50 km away from the nearest larval habitat (Marks 1982), the pest range has not been defined, and it is likely to vary according to the natural and built environment, and local weather conditions. Therefore, cooperation between mosquito control programs in adjacent local governments is required.

Southeast Queensland, and particularly coastal areas of this region, has been a major focus of research activities in addition to mosquito control related policy and program reformulation. Locally competent vectors are present in the region, in addition to associations between RRv activity, vegetation, and mosquito abundance. Also, policy and mosquito control programs in the region are taking into consideration staggering population growth, residential development that are encroaching on breeding sites, as well as legislation and protection of areas that are also mosquito breeding sites. There are significant correlations of Ross River virus disease incidence with the abundance of freshwater species *Ve. funerea*, and *Cx. annulirostris*, specifically within the jurisdiction of coastal Maroochy Shire (Ryan *et al.*, 1999). During the 1994 activity in Brisbane, RRv was isolated from *Ve. funerea* for the first time. This species yielded multiple isolates and was implicated as a possible important vector, although it was subsequently found to be not highly competent (Russell, 2002). However, control programs that target these species are not as well developed as those for the saltmarsh species *Oc. vigilax*, which is often presumed to be the major vector of Ross River virus. In some areas, *Cx. annulirostris* and *Ve. funerea* can be present in large numbers, but their pest or vector status may be unrecognized because surveillance activities target *Oc. vigilax* near saltmarsh and mangrove areas.

2.2.7.2 Mosquito Control at Hays Inlet

Given that Hays Inlet falls within both the jurisdictions of the City of Redcliffe and Pine Rivers Shire (Fig. 3.2), these two jurisdictions are responsible for its treatment, and generally coordinate the timing of control activities. The two local governments use light traps to determine the level of adult mosquito activity. Timing of control activities corresponds with the timing of king tide or rainfall events during the spring and summer season: September to March, when seasonal temperatures permit larval development. Once a tide reaches 2.35m or more the city's saltmarsh areas become flooded, creating temporary pools of water once the tide recedes. Aerial treatments occur at these times to kill larvae present that have hatched as a result of tidal and/or rainfall related flooding of saltmarsh depressions at Hays Inlet. The field staff also conducts ground treatments of areas that could not be accessed by the helicopter.

Both jurisdictions apply the larvicides *Bti* and methoprene in their treatment of the saltmarsh areas. In cases of high adult mosquito activity the Council will apply chemical adulticides to the perimeter of saltmarsh areas (Redcliffe City Council, 2005).

Treatment efficacy is assessed using assay rings. Five rings consisting of 100 larvae are placed in different locations around Hays Inlet. One ring is used as a control ring and is covered when the helicopter passes over. The effectiveness of the treatment is ascertained by the amount of dead larvae in the assay rings versus the control ring. (Redcliffe City Council, 2005).

2.3 Chapter Summary

The current thrust in the study of vector management as related to vector ecology and vector-borne disease epidemiology is the understanding of regional differences in the composition and abundance of vectors and how these influence the transmission of vector-borne pathogens. A number of studies relevant to defining the mosquito problem in a given jurisdiction focus variously on the definition of local vector composition and competence, vector habitat preferences within a region, the spatial statistical analysis of count data to define areas with higher vs. lower abundance, and the meteorological variables that influence mosquito abundance. Since these studies are regionally based, the results are relevant to proximal jurisdictions, and they can be incorporated in a meaningful fashion into the adult mosquito sampling scheme and into the interpretation of vector abundance data. In southeast Queensland, studies relevant to local and regional mosquito problem definition have included studies on the regional vector competence and relative importance of various mosquito species in the transmission of RRv. Additional studies have focused on analysing the spatial dependence of these vectors, while others have aimed at understanding the risk of RRv infection in relation to mosquito abundance and vegetation characteristics. Overall, these studies have helped vector control agencies in the region to most appropriately design and interpret the results of adult vector monitoring activities, so that they might then more appropriately and effectively target control activities.

The methods used in this study to define the mosquito problem at Hays inlet are based on viewing the temporal and spatial components of problem definition in terms of meteorological variables and habitat.

Chapter 3: Methods

This chapter is an outline of the methods used to address each objective of this study. A description of the study area is presented followed by an outline of the methods used for data collection and analysis.

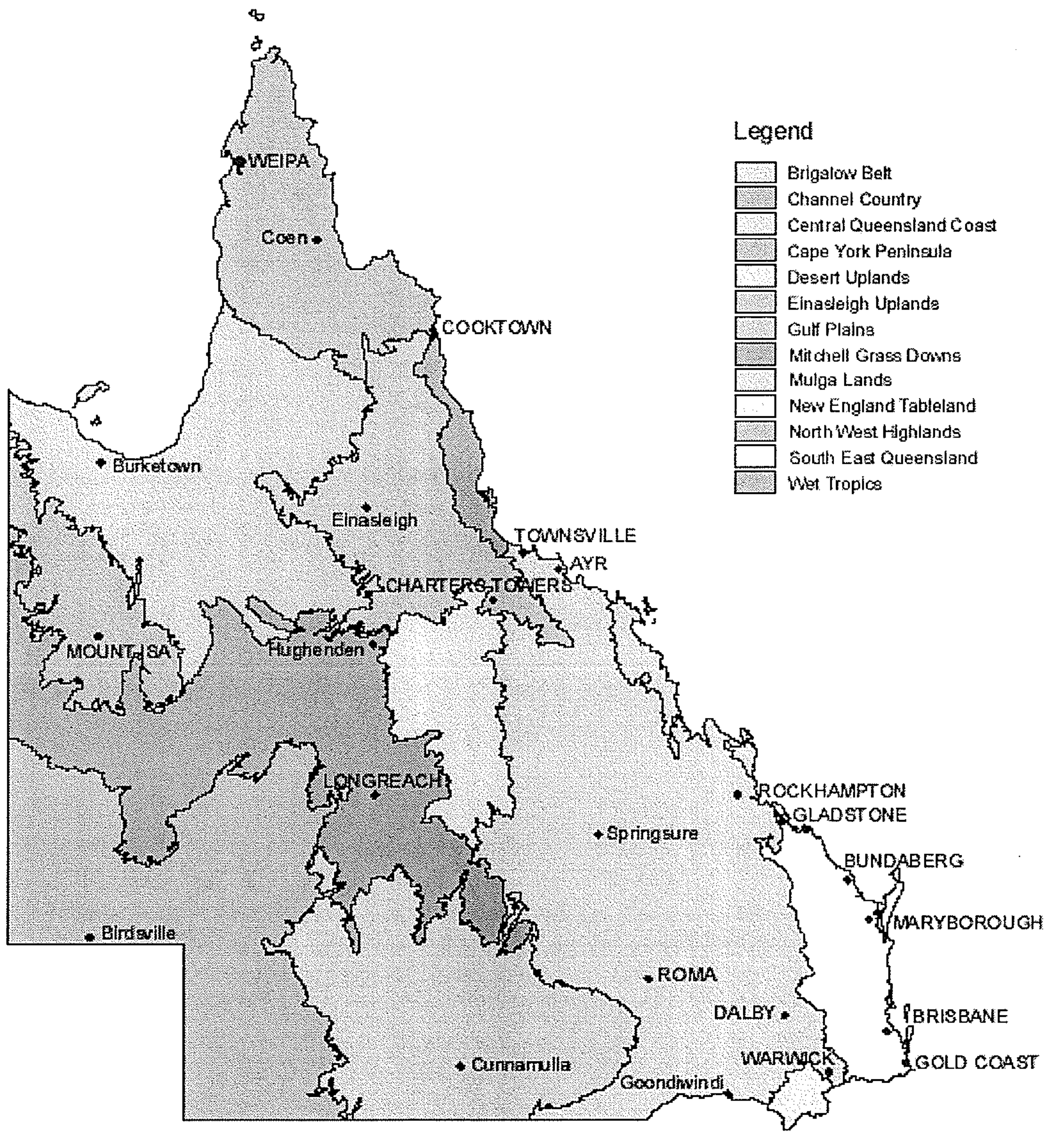
3.1 Study Area

3.1.1 The Southeast Queensland Bioregion

Hays Inlet (24°10'S, 151 °50'E) is situated in southeast Queensland, Australia. Southeast Queensland is recognized as one of thirteen bioregions in Queensland, Australia. The coastal part of this region includes numerous protected marine parks, wetlands, etc., as well as large urban centers such as Brisbane, and Gold Coast (Figure 3.1). With significant growth projected for the majority of coastal areas, urban development in the coastal zone is a major challenge. Under the Ecozone Classification System, a number of different regional ecosystems fall within this bioregion. Those of relevance to the Hays Inlet study site include saltpan vegetation on marine clay pans (regional ecosystem 12.1.2), and mangrove shrubland to low closed forest (regional ecosystem 12.1.3) (EPA/QPWS, 2005a).

Based on the Koeppen Climate Classification System, Southeast Queensland exhibits a subtropical climate, with coastal areas additionally characterized by a distinct dry season (Australian Government Bureau of Meteorology, 2005). The study site is in a warm humid summer zone, in which average January maximum temperature is less than or equal to 30 degrees °C and average 3 pm January water vapour pressure greater than or equal to 2.1 kPa (Australian Government Bureau of Meteorology, 2005a).

Figure 3.1: Bioregions of Queensland, Australia. Adapted from the Environmental Protection Agency/Queensland Parks and Wildlife Service website (EPA/QPWS, 2005b).



3.1.2 The Hays Inlet Study Site

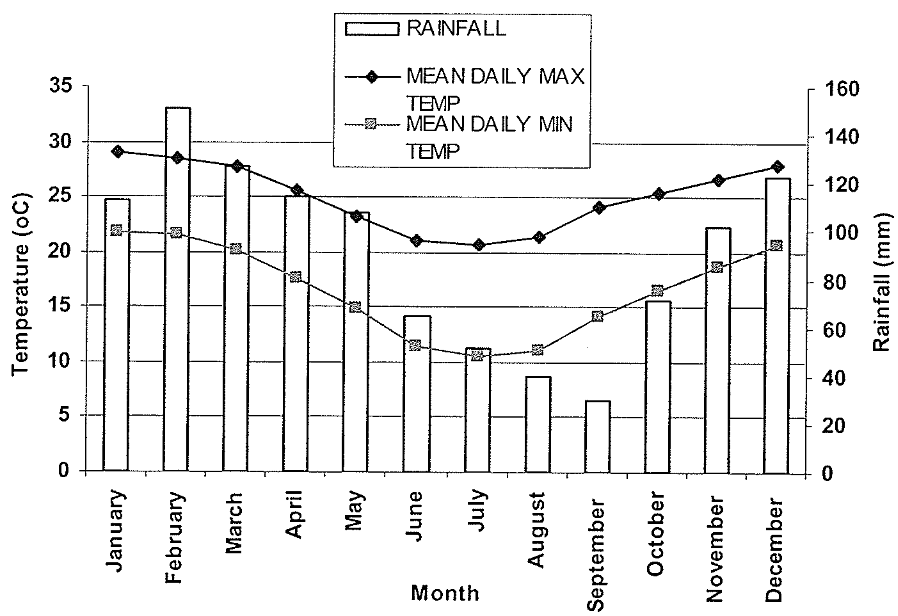
Figure 3.2: Aerial Photo of Hays Inlet (1:50000). (____: Boundary lines between Pine Rivers Shire and the City of Redcliffe. Boundary lines in Redcliffe represent boundaries between communities; ○ : trap locations; Numbers represent the trap number at the given location).

3.1.2.1 Urban Areas

Hays Inlet borders the southwestern half of the Redcliffe City Council Peninsula, and the northeastern portion of Pine Rivers Shire Council. The City of Redcliffe is 37 square kilometers in area, and has a population of approximately 50 000. Although having a greater population than Redcliffe at 113 000 residents, Pine Rivers Shire has a more dispersed population distribution, with residents living in pockets in a rural setting.

A summary of mean monthly weather variables from the Redcliffe Weather Station since 1982 is presented below in Figure 3.3.

Figure 3.3 Mean monthly rainfall, and mean maximum and minimum temperatures collected from the Redcliffe weather station (1982-2003) (Australian Government Bureau of Meteorology, 2005b).



3.1.2.2 Predominant vegetation and land use

The terrestrial flora that borders the inlet includes predominantly mangroves, saltmarshes, and mixed- eucalyptus forest. The dominant terrestrial tree species surrounding Hays Inlet (Chenoweth & Associates, 1995) include *Eucalyptus tereticornis* (F. Muell) (forest red gum), *Eucalyptus drepanophylla* F. Muell. ex Benth. (grey ironbark) and *Eucalyptus tesselaris* (F.v.M.) (Moreton Bay ash). Shrubs including *Breynia oblongifolia* Willgar (coffee bush) and the weeds *Baccharis halimifolia* L. (groundsel) and *Lantana camara* Linn. (lantana) constitute the dominant understory species. The groundcover consists of both native and exotic grasses, thickets of *Pteridium esculentum* (Forst. f.) (bracken fern) and other herbs such as *Lomandra longifolia* Labill. (mat rush) (Chenoweth & Associates, 1995).

The waterway is fringed by mangroves, with *Avicennia marina* (Vierh.) dominating the community, with *Aegiceras corniculatum* (L.) Blanco present in places. For the most part, the mangroves are most dense and expansive along the southern bank of the inlet. Landward of the mangroves, saltmarsh / claypan extends for a few metres to several hundred metres. *Sporobolus virginicus* (L) Kunth (salt couch) dominates to landward with patches. Chenopods, *Sarcocornia quinqueflora* (Bunge ex Ung.-Stern), *Suaeda australis* (R.Br.) all commonly occur, both close to the inlet and in areas prone to greater inundation.

Additionally, human land use areas border the site, which include agricultural land and a golfcourse. Off-road, four wheel drive vehicle tracks are also present that cut through the vegetation.

3.1.2.3 Hydrological Characteristics

Hays Inlet is the terminal discharge point of the Freshwater Creek system, which includes the two main tributaries, Freshwater Creek North and Freshwater Creek South. The creek rises in a low, hilly area to the west and flows, generally from west to east, discharging directly into Hays Inlet, which ultimately discharges to Moreton Bay.

3.1.2.4 Designated Protected Areas

Hays Inlet Conservation Park (CP) 1 and 2 are protected areas designated by the Environmental Protection Authority (EPA) Queensland. Additionally, the Hays inlet embodies a Fish Habitat Reserve A, which is managed by the Fisheries Group, Queensland Department of Primary Industries (QDPI) under the *Fisheries Act 1994.* Management level 'A' is assigned to locations where strict (rather than flexible) management arrangements are achievable. These sites allow the protection of inshore and estuarine fish habitats that are important for sustaining local and regional fisheries from physical disturbance and coastal development (Queensland Government, Department of Primary Industries, 2005). Moreton Bay is designated as a Marine Park. Marine parks are established over tidal lands and waters to protect and conserve special areas while allowing for the planned use of marine resources. This was declared in 1993 and extended in 1997 to cover most of Moreton Bay's tidal lands and tidal waters seawards to the limit of Queensland waters. Along the mainland and around the islands, the boundary is the line of the highest astronomical tide.

3.2 Data Collection

3.2.1 Study Period

The seasonal timing of sampling was chosen in its association with peak annual mosquito abundance and RRv activity. In Southeast Queensland, February and March coincide with late summer. Additionally, RRv peak monthly case notifications reported in Queensland typically relate to RRv transmission at this time. Summer also coincides with the occurrence of king tides, the highest high tides which occur monthly/bimonthly from approximately November to April, and correspond with tides that flood temporary saltmarsh breeding sites.

The temporal component of this study or, the commencement of trapping and spacing between consecutive trapping events is due to the general nature of *Oc. vigilax* development in relation to tidal events. This mosquito species is a brood species that, after an egg-containing habitat is flooded, produces a large cohort of developmentally synchronized individuals. Larval development and pupation are completed approximately 7-8 days after tidal inundation of breeding sites (P. Ryan, personal communication). The greatest habitat flooding occurs typically after a spring tide, so this investigation commenced at such a tide, after which time, peak numbers of the emerging cohort are likely to occur. Because of the timing of the study, I anticipated that *Oc. vigilax* was likely to be the most important species; however, this approach may also be useful for other species such as *Cx. annulirostris* and *Ve. funerea*, depending on the amount of rainfall. The reliability of samples in an extensive survey may be particularly sensitive to current weather conditions.

3.2.2 Meteorological Variables

Temperature variables influence development rates, which impact upon the timing of emergence of an individual cohort. Rainfall variables affect the presence of and level of breeding in ephemeral freshwater pools. Emergence of freshwater species, such as *Cx. annulirostris,* may be correlated with rainfall events and level of precipitation. Combined with temperature, one can predict approximately how long ephemeral breeding is expected to persist. Overall, these climate variables will facilitate an interpretation of trap catch data related temporally to tide, temperature, and rainfall events.

Meteorological data were obtained from the Australian Bureau of Meteorology, which was collected at the Redcliffe Council Weather Station (27°14'42"S, 153°06'02"E, Elevation: 25 m). Temperature and precipitation variables were collected as Daily Maximum and Minimum temperature (°C), and daily total rainfall (mm to 0900), respectively. Data were obtained from the beginning of January, 2003, until the end of April, 2003. Estimated tide heights were obtained from Brisbane Bar, in the 2003 Official Tide Tables and Boating Safety Guide (Maritime Safety Queensland, 2003).

3.2.3 Habitat Assessment

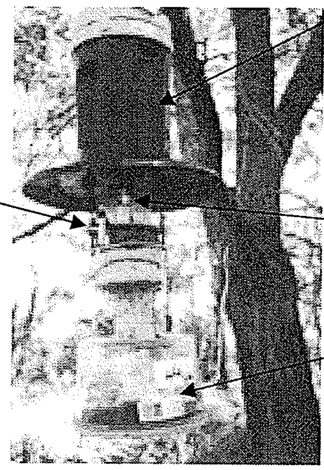
After becoming familiar with the study area during the trapping phase of the research, five of the trap sites were surveyed intensively to determine their floral characteristics, as would be relevant to the lifecycle of the primary vector species trapped in this area. The sites were chosen based on the fact that they are representative of surrounding vegetation, and/or that high abundance levels of certain or all vector species during the light-trapping phase of the research were observed. Trap sites 4, 6, 9-12, 14, 15, 19-20 were chosen for closer scrutiny. Trap site 4, 14, 15, 19-20 were chosen primarily because of the high abundance of certain or all mosquito species at these sites compared to other sites, while trap sites 6 and 9-12 were primarily chosen due to the fact that they are representative of certain habitat types found at Hays Inlet (saltmarsh/mangrove, and woodland, respectively). These observations were collected to relate patterns in mosquito abundance to specific habitat, and to give insight into the presence of vector species in the study site in terms of their known habitat preferences. A visual inspection of the vegetation and topography surrounding the trap sites was used to perform the habitat assessment. Sites were first assessed for the main habitat type present (mangrove, saltmarsh, forest/woodland), in order to establish the likely presence of saltwater versus freshwater larval habitat. Within each habitat type, dominant floral species, co-dominant floral species, and strata levels were noted, since certain types of vegetation are indicative of freshwater larval habitat or of suitable adult mosquito harbourage sites. Finally, sites were assessed for the presence of water or local topographical features that would indicate locations in which temporary bodies of water might pool (e.g. ground surface depressions) to form suitable freshwater mosquito larval habitat.

3.2.4 Mosquito Composition and Abundance

3.2.4.1 Sampling device and role in relative population estimation:

The trap type chosen for use in this study is the CO_2 (in the form of dry-ice) and octenol-baited CDC-style light trap, as examined by Kemme *et al.* (1993), who tested the response of *Oc. vigilax*, *Ve.funerea*, *Cx. sitiens*, and *Cx. annulirostris* to CO_2, octenol, and CO_2-octenol baited light traps. CO_2 traps additionally baited with octenol were more sensitive to *Ve. funerea* and *Oc. vigilax*, and slightly less sensitive to *Cx. annulirostris*. The CO_2 (dry-ice), light, and octenol attractants used in this study are geared towards attracting the female, host-seeking portion of the population. The trap consisted of a 1.5 L cylindrical cooler containing dry-ice pellets attached to a collection bucket, with a fan to draw mosquitoes into the bucket, and an attached miniature light bulb and vial of 2-3 drops of 1-octen-3-ol as attractants. The release rate of carbon-dioxide was not calculated for the traps used in this study. The trap used is shown in Fig. 3.5. In this project, the availability of the population is the result of the response to the stimuli, the activity, and the abundance of each mosquito species. This relative trapping method enables one to estimate the proportion of those members of the population that are in a particular phase to respond to the trap and that did so under the prevailing climatic conditions and the current level of efficiency of the trap (Southwood, 1978). Time of day that samples are taken will also affect population estimates (Southwood, 1978). Since host-seeking behaviour is typically expressed by *Oc. vigilax* from dusk to dawn (therefore a function of its diurnal cycle), its general level of activity should be the highest at this time, while the expression of this activity will be conditioned by the prevailing climatic conditions. In general, measures of availability may be used for the immediate assessment of the attacking or colonization potential of a population.

Fig. 3.4 Major components of the CO_2- and 1-octen-3-ol -baited CDC light trap.



3.2.4.2 Trap site designation

The designation of trap sites was based on the study of aerial photographs of the study site, followed by extensive ground truthing. Ground truthing was used to select sites with vegetation to provide a hanging site at 1.5 m above ground, and sufficient shelter from wind. Trap sites were initially chosen to contour the inlet, in order to be in the closest proximity with saltmarsh breeding sites. However, northern trap sites were moved back into wooded areas following extensive rainfall before sampling that made a number of sites closer to the saltmarsh inaccessible. Trap sites were chosen in order to accommodate the systematic sampling routine. Traps were set approximately 500 m apart, but this distance varied slightly depending upon whether or not the above conditions were met.

3.2.4.3 Systematic sampling technique

Given that the information regarding mosquito abundance is desired for the Hays inlet as a whole, a systematic sampling design was employed in this study. The actual trap interval of 500 m was determined by such factors as the number of actual traps that may be used for this project, feasibility of trap maintenance and operation, and a requirement for a fairly fine level of resolution.

3.2.4.4 Light trap set-up and sampling schedule

A series of spring high tides was predicted to commence on 15 February, 2003. Actual trapping commenced on 17 February and continued every two days for four days in total. Trapping then continued for one night a week until the second series of high tides predicted to commence on 16 March, 2003 occurred, at which time trapping again took place every two days for approximately two weeks. Traps were set from1200 to 1600 (before sunset), and retrieved the next morning from 0800 to 1100. The mosquitoes were transported to the Queensland Institute of Medical Research (QIMR), then were subsequently killed on dry ice, transferred into 250 mL containers, and stored at -20°C until they were ready to be sorted.

3.2.4.5 Sorting of light trap collections

Once ready to be sorted, adult mosquitoes were identified to species according to Russell (1996), and subsequently counted. Large numbers of mosquitoes were present in most of the trap catches, so subsampling was performed on the majority of them.

Subsamples of 500 randomly selected females from each catch which exceeded 1000 individuals were identified, dried for 24 hrs at 65^{0}C, and then weighed. The remainder of the catch was also dried and weighed in order to estimate the total number of mosquitoes and each counted mosquito species by direct proportion. The number of each species in each trap was then estimated by multiplying the total number of mosquitoes by the % of each species in the subsample (Jeffery *et al.*, 2002).

3.3 Data Analysis

3.3.1 Temporal pattern of Mosquito Abundance

1) For each species, estimated catch data from all traps were pooled into three sample periods: 17-23 February, 3 March and 10 March, 17-27 March. For each period, the mean numbers per species per trap night were calculated.
2) For each species, the mean estimated count per trap and standard error were calculated for each of the 12 trap nights. These means were compared from night to night and changes were assessed in relation to changes in wind speed during the time when the trap was set, and total daily rainfall for each trapping date.

3.3.2 Spatial Pattern of Mosquito Abundance

A Lilliefors test was used to assess whether the distributions of numbers for each species per trap in trap period one and three were normal. A result of 1 indicates that the distribution is likely non-normal and a result of 0 indicates that the distribution is likely normal. For results of 1, the data were log transformed to see if this adjusted the results to normality. For most traps, the distributions were not normal, and many were not adjusted to normality via the log transformation. Therefore, non-parametric methods were used to detect the presence of spatial pattern using rankings.

For each species, the mean number of estimated adult female mosquitoes for each trap site was calculated for the period of trapping from Feb. 17-23, and from March 17-27. For each of these sampling periods, the traps were ranked from 1 to 24, the highest rank going to the trap with the highest mean catch. Trap sites were grouped into those with a consistently high rank (consistently ranked in the top 10), low rank (consistently ranked in the bottom 10). Change in ranking relative to change in mean count was also noted for each trap.

A Spearman's rank correlation test was performed to assess the degree to which trap site rankings between the former sampling period agreed with those of the latter sampling period. The Spearman's rank test statistic can be expressed as follows:

$$r_s = 1 - \frac{6\sum_{i=1}^{n} d_i^2}{n^3 - n}, \qquad (3.1)$$

where d_i is the difference between rankings, and n is the total number of rankings.

The r_s value obtained for each species was used to infer whether rankings between the two time periods were consistent. A high positive r_s value indicates a strongly positive correlation and would be interpreted as an overall pattern of consistent and stable rankings of mosquito numbers per trap for the given species. An r_s value approaching zero indicates that no correlation between the two sets of rankings exist, and that the overall pattern of rankings was inconsistent and unstable.

Additionally, the Spearman's test statistic was used to test if the correlations were significant at the 0.05 confidence level:

$$t = r_s \sqrt{\frac{n - 2}{1 - r_s^2}} \quad (3.2)$$

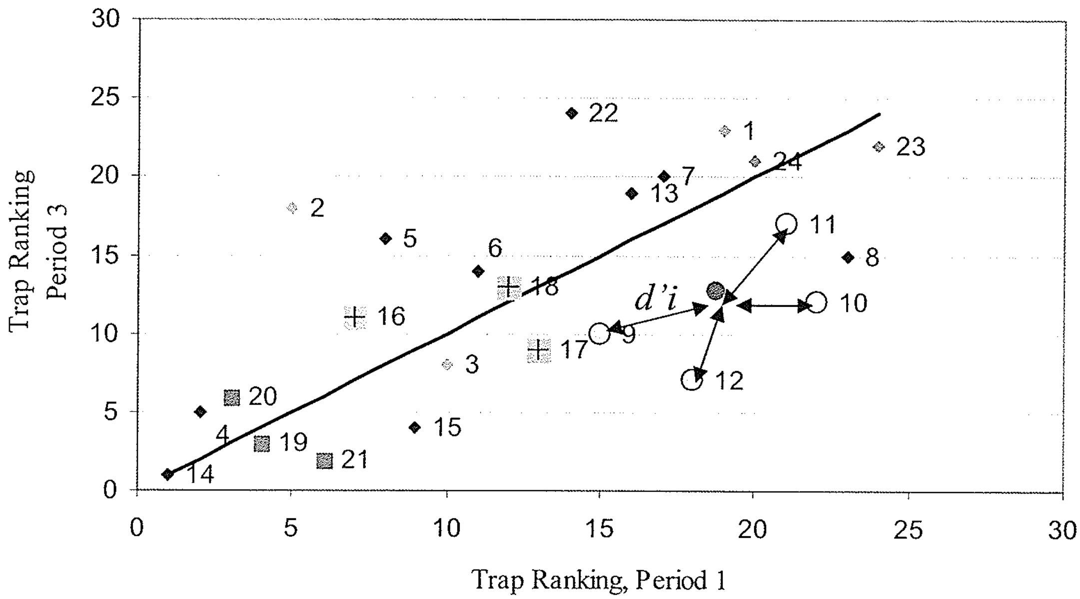
In order to examine the potential for clustering of areas with consistently high and low mosquito numbers, the scatterplots of rankings for each species were examined to identify contiguous trap sites with both stable and proximal rankings. Additionally, trap sites in well defined habitat identifiable from the aerial photo were examined for ranking contiguity. These sites included traps 1-3 along the golfcourse, sites 9-12 in woodland to the north of the inlet, and sites 16-18 in the saltmarsh to the west of Hays Inlet. Cluster analysis was performed on the above trap sites by computing the mean ranking m (dark green dot on figure 3.5) for each of these identified sites followed by computing the mean distances of trap rankings d_i' to the above computed mean. Finally the mean squared distance was calculated as:

$$CA_{MSD} = \frac{1}{N}\sum_{i=1}^{N} d_i', \quad (3.3)$$

where N is the total number of traps for the particular cluster.

These values were used for each species as a quantitative way to verify that the traps in a particular cluster were ranked more consistently when compared to the other clusters. Therefore, the value that is looked for is just the minimum among the clusters within the same species data and not a particular numerical one.

Figure 3.5 Example of cluster analysis. Three clusters can be identified in this example: the red, blue and yellow ones. Arrows indicate the distance from each trap rankings to the average mean value *m*.



Finally, the spatial patterns in the rankings as identified from above were compared to the habitat assessment data as outlined in the previous section, in order to determine whether these patterns are consistent with habitat features known to be relevant to the biology of each species.

3.4 Chapter Summary

The location of this study, Hays Inlet, is situated in Southeast Queensland, Australia. The primary data collection to occur during this study, the mosquito sampling using CO_2 (dry-ice) and octenol-baited light traps, is relevant to the determination of vector composition via mosquito identification of each trap catch in the lab, and to the determination of spatial and temporal trends in abundance through the procedures outlined in the data analysis. Meteorological information in the form of tide, temperature and rainfall obtained will be used to characterize these trends, as will the habitat assessment that occurred after sampling. The following chapter will summarize the results for each part of the data collection outlined in this chapter.

Chapter 4: Results

This chapter concisely summarizes the results of this study in terms of each objective described in section 1.3 and associated method used. The results from the acquisition of meteorological conditions data, from the collection of habitat assessment information, and from light-trapping including vector composition, temporal trends in abundance, and the data analysis of spatial trends in abundance will be given. The discussion in Chapter 5 will relate these results to the actual purpose of the study and relevant literature.

4.1 Characterization of Meteorological Conditions

From the collection of the tidal information and the temperature and rainfall information, the key results relevant to the interpretation of mosquito abundance data are as follows:

Tide: The first series of tides of the king tide season high enough to inundate saltmarsh depressions (2.4 m) occurred from 5 to 7 November, 2002. Two more series of such tides occurred in December, 2002 (4-7; 21-23 December). Three subsequent tides preceeded the commencement of sampling (1-5, 18-22 January; 30 January-3 February). As such, six king tides preceded sampling. The two high tides that occurred within this study were consecutive, the first commencing thirteen days following the preceding high tide, and the two tides occurred approximately 1 month (27 days) apart (16-19 February; 17-19 March). The amplitude of these tides is shown in Figure 4.2.

Rainfall: Total rainfall for the month of January was 8.6 mm, with a maximum daily rainfall of 5 mm on 3 January. The total rainfall for February was 330.3 mm, average total daily precipitation of 12.7mm. In February, 2003, days with over 10 mm of rainfall included 3-5, 23-25, 27 February. Two significant rainfall events occurred prior to and during sampling. The first event occurred from 2-7 February, 2003, with a cumulative precipitation of 127.8 mm. The second event occurred from 20 February to approximately 2 March, with a cumulative precipitation of 208.3 mm.

Temperature: In January 2003, the mean maximum daily temperature recorded was 28.8°C, while the mean daily minimum temperature was 20.9°C (Figure 4.1). The highest maximum daily temperature (30.9°C) occurred on 31 January, the lowest (27.5°C) on 8 January, and the highest minimum daily temperature (23.5°C) occurred on both the 23 and 29, with the lowest (17.6°C) on 12 January. In February, the mean daily maximum temperature recorded was 28.1°C, while the mean daily minimum temperature was 21.9°C. The maximum daily temperature ranged from 22.8 to 30.5°C, and the mean daily minimum temperature ranged from 19.5°C to 24.0°C. In March 2003, the mean maximum daily temperature was 27.0°C, while the mean minimum daily temperature was 20.0°C. The maximum daily temperature ranged from 22.2°C to 31.0°C, and the daily minimum temperature ranged from 17.4°C to 23.8°C.

Figure 4.1 Daily maximum and minimum temperatures collected from the Redcliffe weather station, from 15 January to 31 March, 2003.

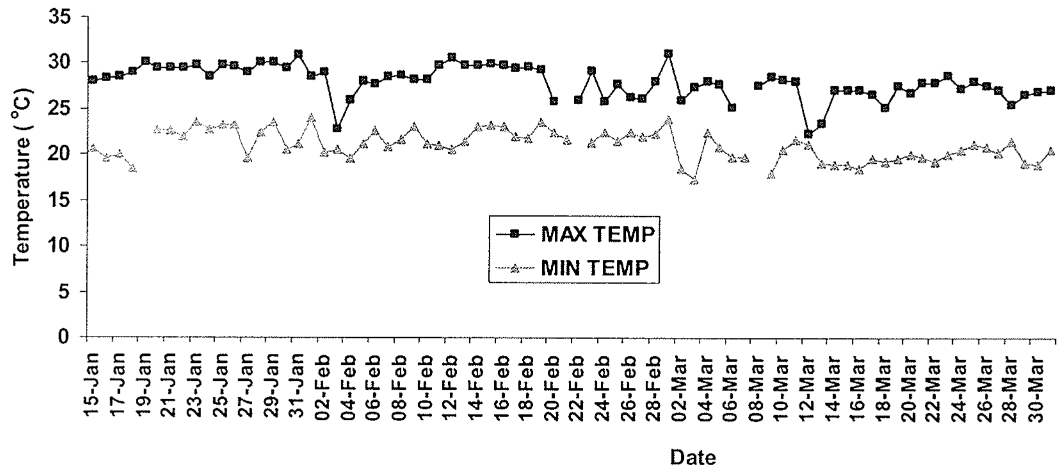
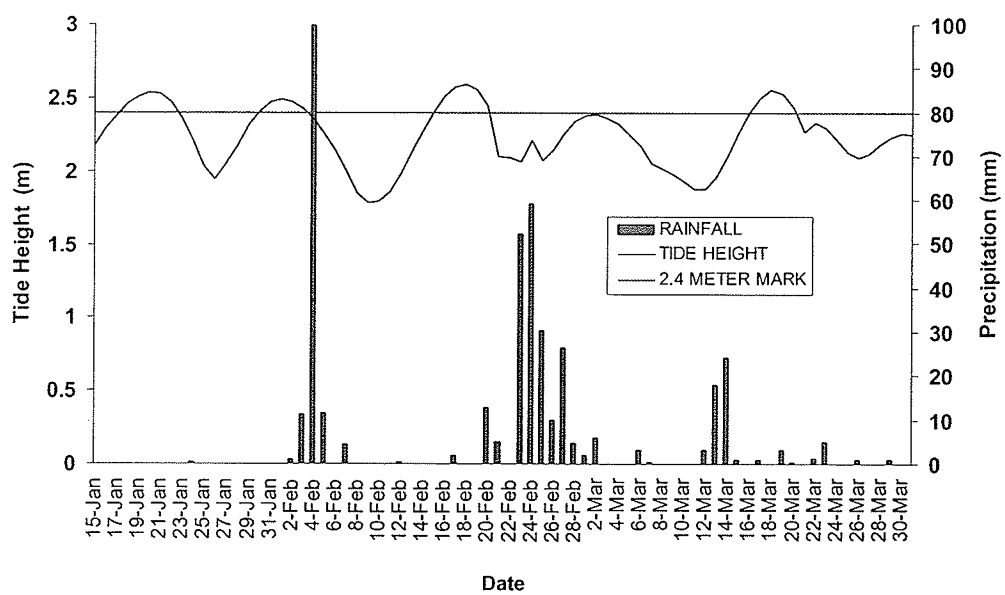


Figure 4.2: Tidal amplitude and precipitation collected from Redcliffe weather station, from 15 January to 31 March, 2003.



4.2 Habitat Assessment

Pictures of habitat characteristics for each of the six site assessments are illustrated in Figures 4.3- 4.8. Trap site 4 was situated in an area characterized by forest woodland habitat type, adjacent to mangrove and saltmarsh (Figure 4.3). Six dominant tree species were present in the area. By strata level, these include: Tree layer to 25 m *Eucalyptus tereticornis* (20% cover), *Melaleuca quinquenervia* (Cav.) Blake (10% cover), 6 m: *Acacia melanoxylon* (R. Br) (5% cover); Shrub layer to 0.5 m *Lomandra longifolia* (2% cover), and *Pteridium esculentum* (5% cover). The main codominant species present was *Lantana camara* (2% cover), within the shrub layer to 1 m in height. Depressions in the soil were additionally present.

Trap site 6 was characterized by mangrove and saltmarsh habitat type (Figure 4.4). The mangrove species *Avicennia marina* was present in both the tree (to 7 m 90% cover) and shrub (1.5-2 m; 90% cover) layers in the mangrove cover. *Sarcocornia quinqueflora* was present at the saltmarsh in the ground layer (0.2 m; 20% cover). *Sporobolus virginicus* bordered the site at the highest astronomical tide mark. Finally, present at the edge between these two habitat types, *A. marina* was present in the shrub layer (2m; 90% cover), and *Sarcocornia quinqueflora* was present in the ground layer (0.2 m; 40% cover).

Trap sites 9-12 (Figure 4.5) were situated in an area covered by a forest/woodland habitat type. The tree layer comprised 60% of the vegetative cover in the area, to 25 m, including the species *Eucalyptus tereticornis*, *Lophostemon confertus* (R. Br.), and *Eucalyptus siderophloia* Benth. The thick undergrowth of grass present in the ground layer included *Lantana camara*, *Lomandra longifolia*, and *Dianella longifolia* (R. Br.) species (0.6m; 25%). The site adjoined forest of *Allocasuarina glauca*, which then adjoined saltmarsh and mangrove. Wheel ruts and other depressions were present in this area.

Trap site 14 was situated in an area with the predominant habitat type being saltmarsh and mangrove (Figure 4.6). The dominant species in the tree layer included *Avicennia marina var. australasica* (Walp.), and *Allocasuarina glauca* Sieber, *A. marina* to a height of 5m, and the remaining two to a height of 7 m and 40 and 5% cover, respectively. The shrub layer to 2.5 m consisted of *Aegiceras corniculatum*, with a 5 % cover. Finally, the ground layer (to 30 cm) was primarily composed of *Sporobolus virginicus* (90%) and *Sarcocornia quinueflora* (5%). Many depressions, clay pans and interconnected clay pans were present at this site, and site was bordered by stands of *A. glauca* with depressions beneath.

Trap site 15 was present in forest/woodland habitat type adjoining mangrove and saltmarsh (Figure 4.8). The dominant species in the tree layer included *Allocasuarina glauca*, *Acacia melanoxylon* and *Eucalyptus sideroxylon* A. Cunn. (together, up to 15 m height; 40% cover). To 0.5 m, *Eucalyptus* species regrowth and *Lantana camara* were present in the shrub layer. Numerous species were present in the ground layer. These included *Pteridium esculentum*, *Brachiaria mutica* (Forsk.) and *Lophostemon confertus* (0.2 m; 80%). The saltmarsh and mangrove adjoining site 15 consisted of *Avicennia marina*, and *Sporobolus virginicus*, and is similar to the habitat presented for trap site 6.

Trap sites 19-20 were situated at a site with a main habitat type of forest and woodland (Figure 4.7). The per cent cover of the tree layer was 40-50%, and the dominant species include *Melaleuca quinquenervia* and *Allocasuarina glauca.* The latter species was also present in the shrub layer to a height of 3m and 5% cover, while the ground layer to a height of 30cm and 90% cover was dominated by *Sporobolus virginicus*. Mangrove and saltmarsh habitat was present at a distance of 100m from trap site placement. Many melonholes ((depressions characteristic of gilgai soil landscapes where the dominant soil process is characterized by a seasonal shrink-swell (Department of Infrastructure, Planning, and Natural Resources, 2004) and other depressions were present at this site.

Figure 4.3: Representative vegetation and strata levels surrounding trap site 4.

Eucalyptus tessellaris
Melaleuca quinquenervia

Acacia melanoxylon
E. tessellaris juvenile regrowth

Lomandra longifolia
Lantana camara
Pteridium esculentum

Figure 4.4: Representative vegetation and strata levels surrounding trap site 6.

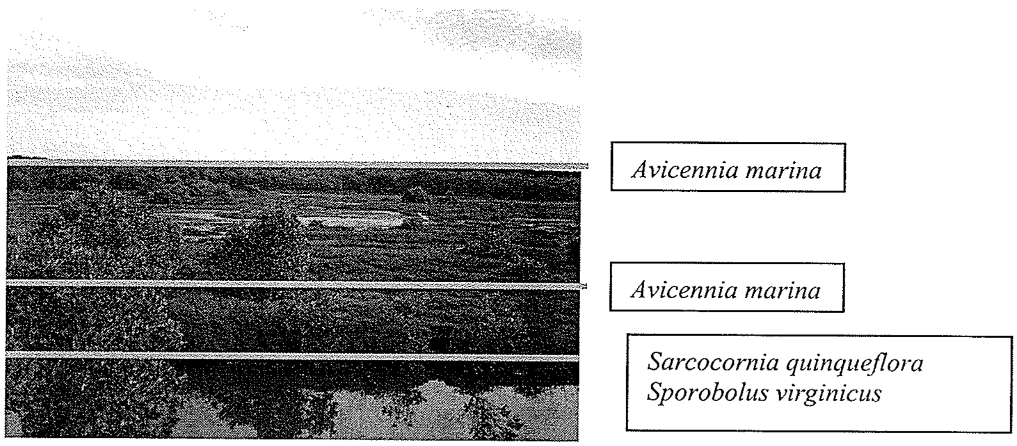


Figure 4.5: Representative vegetation and strata levels surrounding trap sites 9-12.

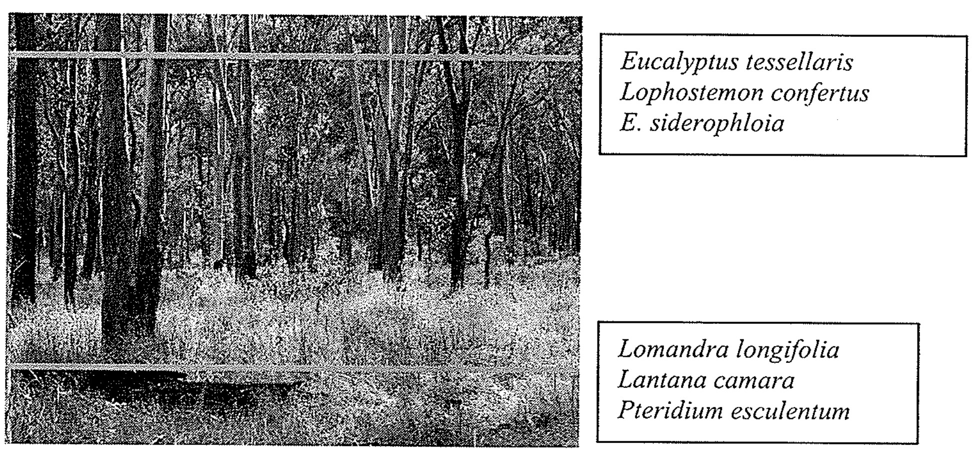


Figure 4.6: Representative vegetation and strata levels surrounding trap site 14. a) Strata levels present at this site, b) wheel ruts c) sites at which tidally inundated water forms temporary pools.

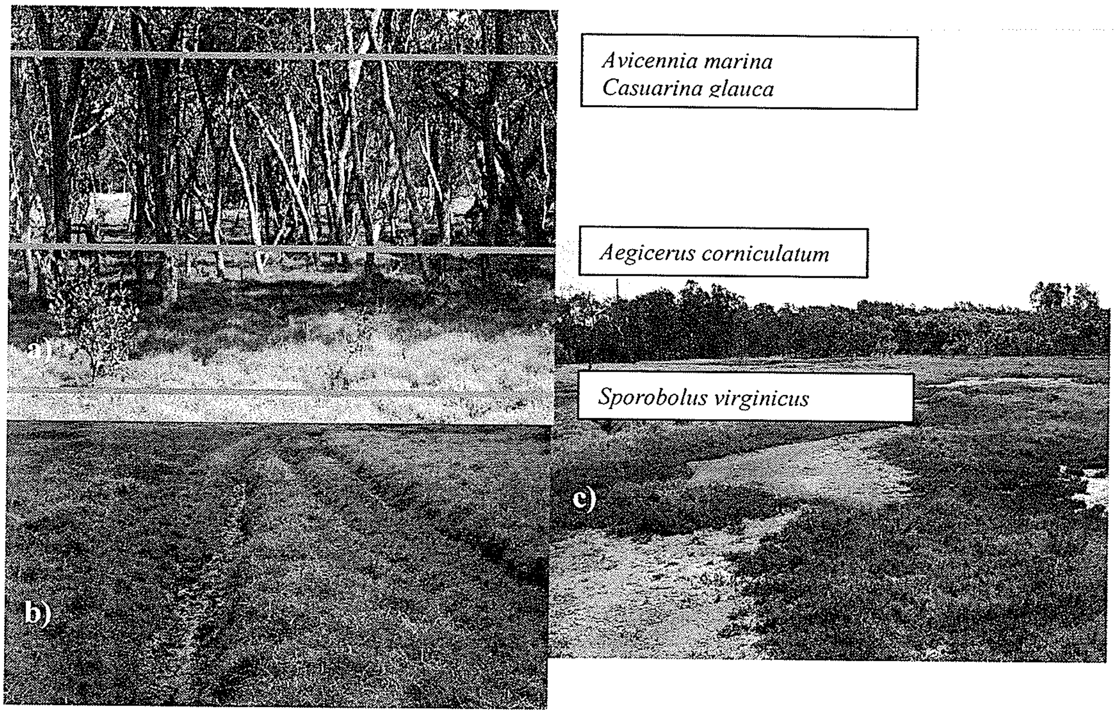


Figure 4.7: Representative vegetation and strata levels surrounding trap sites 19-20.

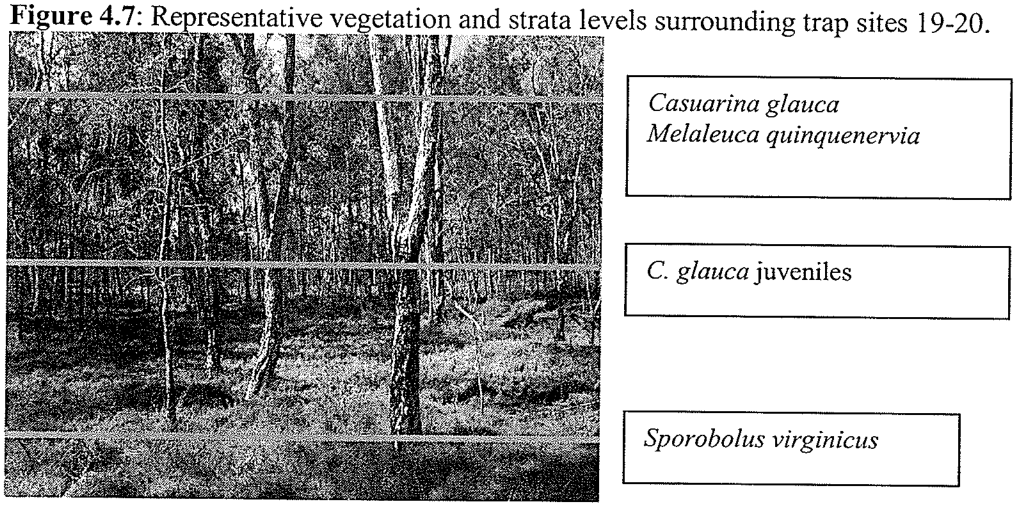
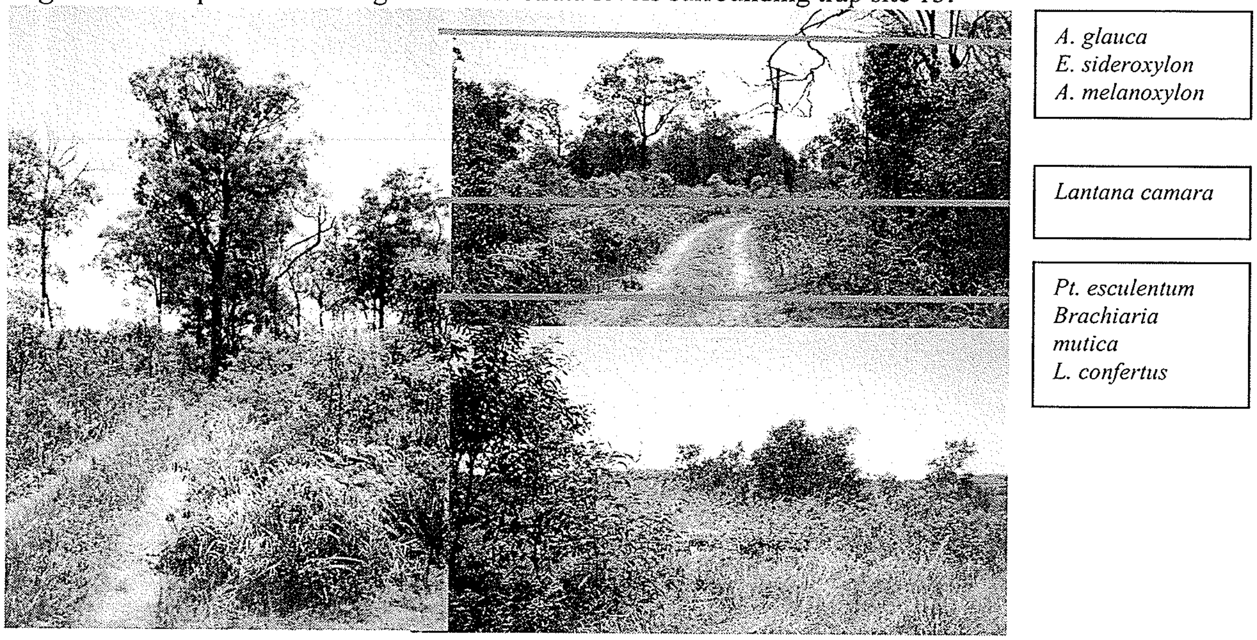


Figure 4.8: Representative vegetation and strata levels surrounding trap site 15.



4.3 Mosquito and Vector Composition

An estimated total of 1 263 949 mosquitoes was collected from over 12 nights of trapping. A total of 22 different species was captured in the traps over the sampling period (Table 4.1).

Table 4.1 Estimated numbers of adult female mosquitoes collected in CO2 and octenol-baited CDC-style light traps around the Hays Inlet, during the sampling period, 2003.

Species	Total Number Collected	% Total Catch
Ochlerotatus vigilax	904 054	71.52614543
Culex annulirostris	131 196	10.37984918
Culex sitiens	105 045	8.31085747
Verrallina funerea	58 567	4.63365214
Ochlerotatus vittiger	24 000	1.89881079
Ochlerotatus proca	20 792	1.64500308
Verrallina Marks sp. 52	7 493	0.59282455
Ochlerotatus alternans	3 776	0.29874623
Mansonia uniformis	3 507	0.27746372
Culex orbostiensis	2 216	0.17532352
Coquillettidia xanthogaster	1 181	0.09343731
Ochlerotatus aculeatus	495	0.03916297
Anopheles annulipes	396	0.03133037
Coquillettidia linealis	363	0.02871951
Ochlerotatus notoscriptus	304	0.02405160
Ochlerotatus mallochi	205	0.01621900
Ochlerotatus burpengaryensis	156	0.01234227
Ochlerotatus linneatopennis	121	0.00957317
Culex quinquefasciatus	49	0.00387673
Culex australicus	12	0.00094940
Uranotaenia sp.	11	0.00870288
Culex bitaeniorhyncus	10	0.00791171
Total	1 263 949	100%

Of the 288 scheduled trap catches, estimates of adult numbers were only made on 256 of these traps, since 32 trap catches were lost due to trap operational difficulties, or due to infestations of ants. Lost catches represent 11% of the total catch. Six species, *Oc. vigilax*, *Cx. annulirostris*, *Cx. sitiens*, *Ve. funerea*, *Oc. vittiger*, and *Oc. procax* were the most abundant, and comprised 98.4% of the total catch; the remaining comprised 1.6%. Four of the six most abundant species are implicated as vectors for RRv in Southeast Queensland. In order of mean trap abundance, these include *Oc. vigilax*, *Cx. annulirostris*, *Ve, funerea*, and *Oc. procax*.

4.4 Temporal Pattern of Vector Abundance

During sampling that corresponded with the first sampling period (17-23 February), the mean numbers of *Oc. vigilax* were 5394 females per trap. Subsequently, mean numbers decreased slightly during the second sampling period, to 4652 per trap, then dropped more substantially to 1998 mosquitoes per trap during trapping corresponding with the third sampling period, 17-27 March (see Figure 4.9). *Verrallina funerea* and *Oc. procax* exhibited a similar trend (see Figures 4.11 and 4.12). *Verrallina funerea* mean trap numbers were 320 during the first sampling period, 303 during the second sampling period, and 147 during the third sampling period. Mean numbers of *Ochlerotatus procax* were 158, 61, and 40 females per trap during the first, second, and third periods of sampling. Mean trap numbers for *Cx. annulirostis* (Figure 4.10) and *Cx. sitiens* (Figure 4.13), on the other hand, increased slightly between sampling periods. *Culex annulirostris* mean trap catches started at 498, decreased slightly to 446, then increased to 543 for each consecutive sampling period. *Culex sitiens* mean numbers commenced at 498 per trap, followed by 446, and 543 per trap, during each consecutive sampling period.

Average windspeed during the time traps were set up around the inlet over the sampling period varied from 9.6 to 20.6 km/hr. The most substantial change in windspeed occurred between 17 and 19 February, 2003, decreasing from a mean of 17.1 km/hr to 10.5 km/hr. A similar decrease in mean windspeed occurred between

17 and 19 March, 2003, from a mean of 20.6 km/hr to that of 9.6 km/hr (Table 4.2). A substantial rainfall event occurred on 23 February, 2003, with a total rainfall of 52.3mm (Table 4.2). All species experienced a mean increase in numbers between 17 and 19 February, which may have corresponded to the decrease in windspeed. However, this same result was not present between 17 and 19 March.

Figure 4.9: Mean number of female *Oc. vigilax* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003).

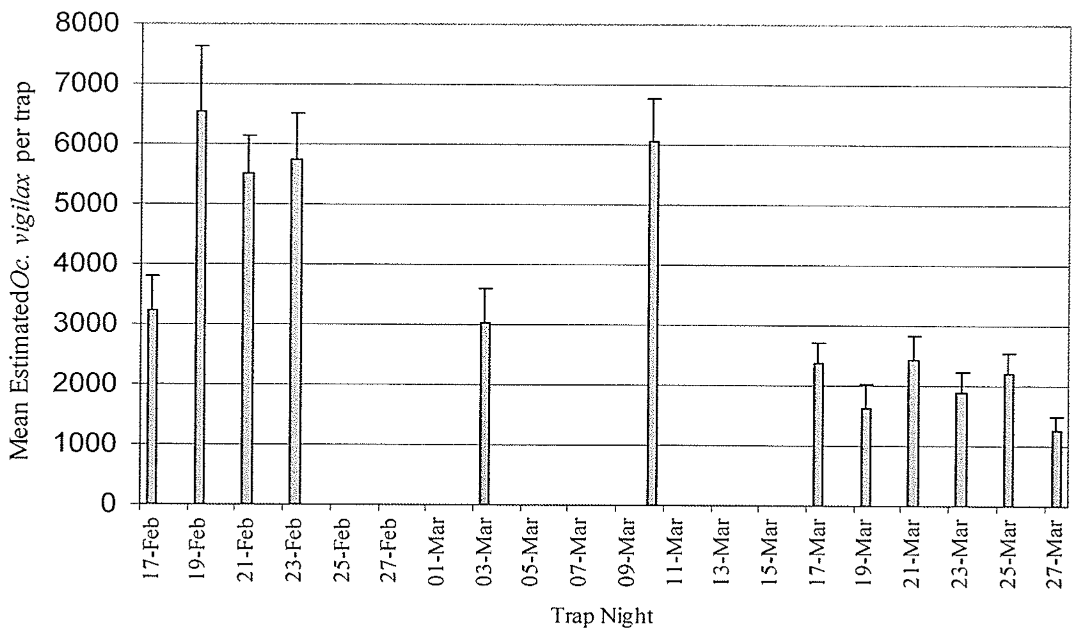


Figure 4.10: Mean number of female *Cx. annulirostris* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003).

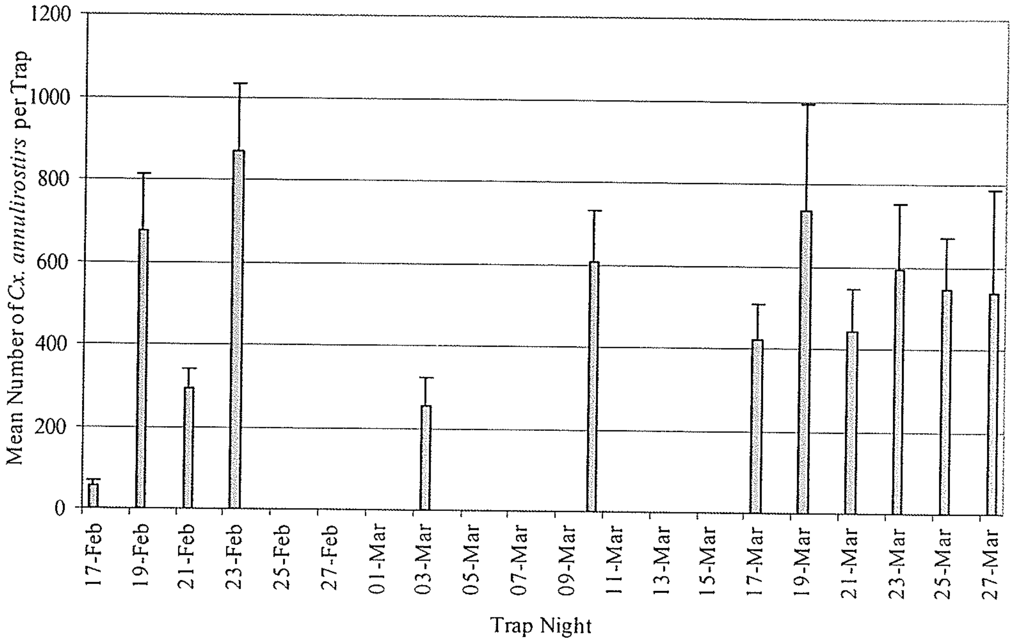


Figure 4.11: Mean number of female *Ve. funerea* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003).

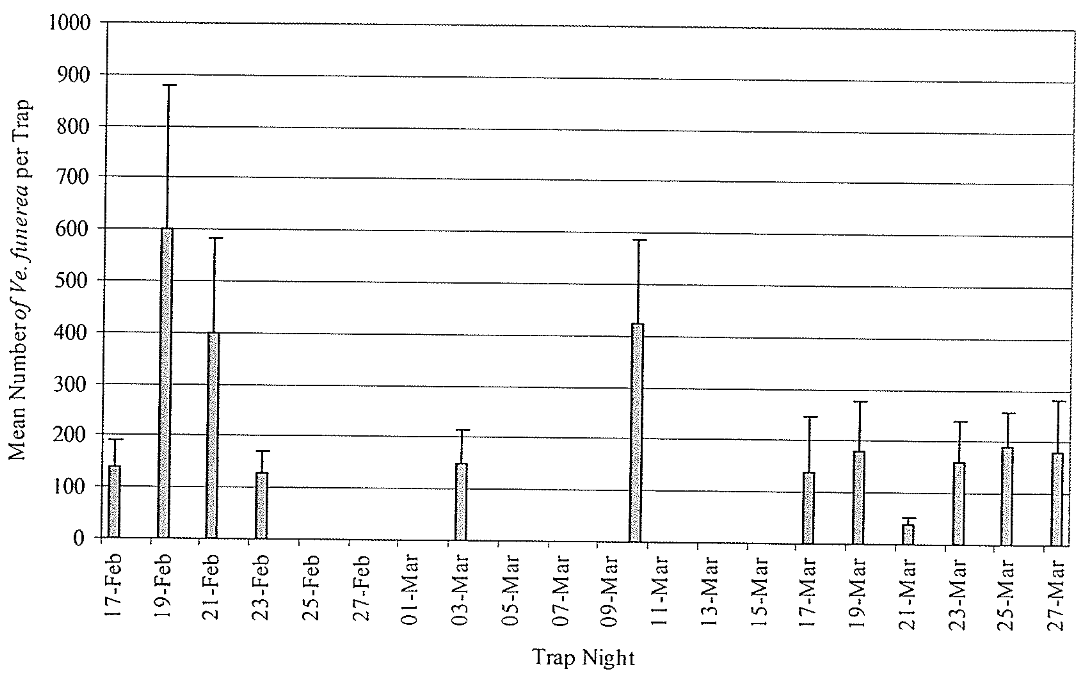


Figure 4.12: Mean number of female *Oc. procax* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003).

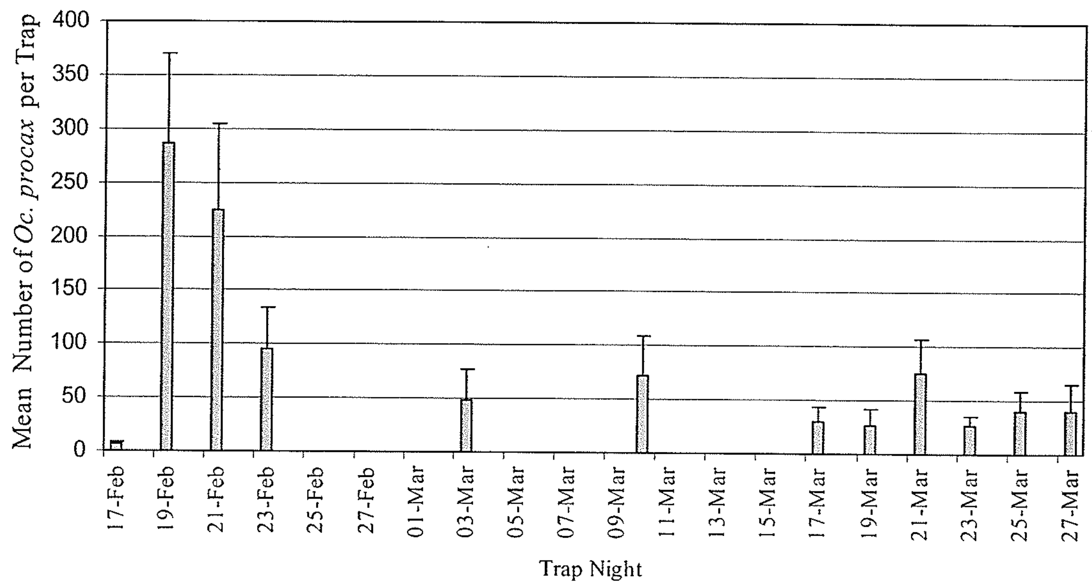


Figure 4.13: Mean number of female *Cx. sitiens* per trap for each trap night during sampling at Hays Inlet, Queensland, Australia (17 February-27 March, 2003).

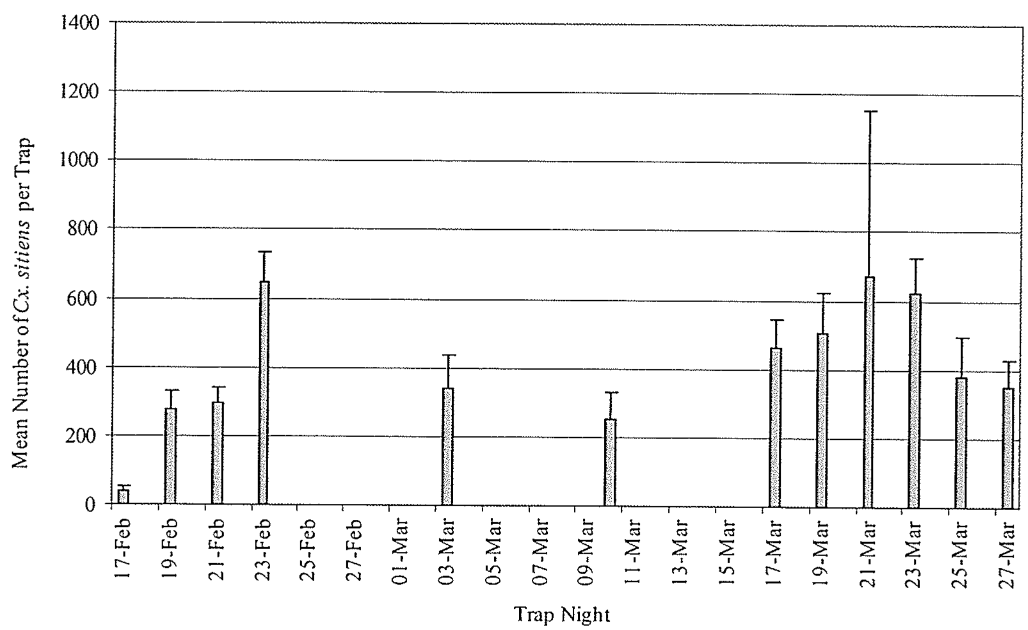


Table 4.2: Mean wind-speed and total daily rainfall on nights traps were set for each main species collected at Hays Inlet, 2003.

Date	Mean Windspeed (km/hr)*	Total Daily Rainfall (mm)
2/17/2003	17.1	1.8
2/19/2003	10.5	0.0
2/21/2003	13.9	5.0
2/23/2003	17.6	52.3
3/3/2003	15.5	0.0
3/10/2003	12.5	0.0
3/17/2003	20.6	1.0
3/19/2003	9.6	3.0
3/21/2003	11.6	.0.
3/23/2003	9.6	4.8
3/25/2003	11.6	0.0
3/27/2003	10.6	0.0

*Average from 12:00 PM to 9:00 AM

4.5 Spatial Pattern of Vector Abundance

Ochlerotatus vigilax: All traps experienced a decrease in mean numbers of mosquitoes between the two periods. The overall variation between mean catches among trap sites was less in the third sampling period compared to the first. Between sampling period 1 and 3 (17-23 February; 17-27 March), trap numbers 4, 8, 12, 15, and 17 were consistently ranked in the top ten trap sites with the highest mean abundance. Traps 14, 18, 19, 22, 23, 24 were consistently low-ranking traps (in the bottom 15). Contiguous traps, both with low rankings, included traps 18-19 and 23-24. Trap 15 was consistently in the top two trap sites for both periods (Figure 4.14).

The Spearman rank correlation coefficient for the *Oc. vigilax* trap rankings between sampling periods 1 and 3 resulted in a value of 0.361 (Table 4.3). The test statistic yielded a value of $t = 1.819955$. From this value, the level of confidence is p= 0.05. Therefore, because this case is the one with the lowest r_s it can be concluded that higher values of r_s can be considered correlated with even a higher confidence level. Cluster analysis results based on equation (3.3) for traps within predefined areas were: Traps 1-3: $CA_{MSD} = 5.4$, Traps 9-12: $CA_{MSD} = 3.5$, Traps 16-18: $CA_{MSD} = 7.6$ (Figure 4.14). Clearly the second set of traps (9-12) is the one that visually as well as quantitatively clustered closely together at low ranking values.

Culex annulirostris: Fifteen of the twenty-four traps experienced a mean increase in *Cx. annulirostris* numbers. Traps 4, 5, 12, 15, and 19 were consistently ranked in the top ten trap sites, trap sites 4 and 15 consistently in the top four sites. Traps 7, 9, 10, 13, 17, 18, 23, 24 were consistently low ranking traps. Trap sites 1-3 were consistently mid-ranking traps, while trap sites 8-11 fluctuated greatly (Figure 4.15). The Spearman's rank correlation between rankings in the first and third periods was slightly higher than that for *Oc. vigilax* (r_s =0.425, p= 0.05), demonstrating a positive correlation (Table 4.3). The cluster analysis showed that the average distance from the mean rankings for each predefined site was: Traps 1-3: $CA_{MSD} = 3.6$, traps 9-12: $CA_{MSD} = 9.2$, traps 16-18: $CA_{MSD} = 6.1$.

Verrallina funerea: Seventeen out of twenty-four traps experienced a mean increase in *Ve. funerea* numbers. Traps 3, 4, 14, 16, 19, 20, 21 were consistently ranked in the top ten trap sites. Traps 1, 7, 8, 11, 13, 23, and 24 were consistently low-ranking traps. Contiguous traps with similar rankings include: trap sites 19-21, which were consistently in the top ten rankings, while traps 23-24, which were consistently low-ranking (bottom fifteen). Trap 14 was consistently ranked as number one in both sampling periods (Figure 4.16). *Verrallina funerea* exhibited a moderate Spearman's rank correlation (r_s =0.650, p=0.05) between rankings in the first and third sampling periods (Table 4.3). Cluster analysis indicated that the predefined traps 1-3 had an average distance from the mean ranking of CA_{MSD} = 8.4, traps 9-12 an average distance of CA_{MSD} = 4.5, traps 16-18 and average distance of CA_{MSD} = 3.0. Additionally, traps 19-21 had the smallest distance from the mean ranking and therefore cluster analysis was also performed on these traps yielding the value CA_{MSD} = 2.7.

Ochlerotatus procax: Twenty-two out of twenty-four traps experienced a mean decrease in *Oc. procax* numbers. The means of the two remaining traps did not change. This species exhibited the highest values for Spearman's correlation coefficient (0.768, p=0.05) (Table 4.3). Traps 4, 5, 9, 10, 11, 12, 15, 16, 21 were consistently ranked in the top ten trap sites. Traps 1, 6, 7, 13, 17, 23, 24 were consistently low ranking traps (Figure 4.17). The cluster analysis showed that the average distance from the mean rankings for each predefined site was: Traps 1-3: CA_{MSD} = 6.3, traps 9-12: CA_{MSD} = 4.6, traps 16-18: CA_{MSD} = 7.6.

Culex sitiens: Seventeen out of twenty-four traps experienced a mean increase in *Cx. sitiens* numbers. Traps 1, 2, 3, 4, 19 were consistently ranked in the top ten trap sites. Traps 9, 10, 13, 16, 23, 24 were consistently low-ranking traps. Traps 1-4 were in the top five trap sites for both sampling periods (Figure 4.18). The Spearman's rank correlation between the first and third periods was significant r_s =0.546, (see Table 4.3). The cluster analysis showed that the average distance from the mean rankings for each predefined site was: Traps 9-12: $CA_{MSD} = 5.6$, traps 16-18: $CA_{MSD} = 8.2$. For this particular case, because trap 4 was visually very close to traps 1-3 (see Figure 4.18), it was added to the site cluster consisting then of Traps: 1-4, yielding a value of $CA_{MSD} = 1.5$.

Although trap sites 23 and 24 appeared to cluster at low rankings for certain species, these traps were not analyzed because results at site 24 are likely not reliable. This trap was placed in an unsheltered area that was highly exposed to wind.

Table 4.3: Spearman's correlation coefficient values for each main species collected at Hays Inlet, February-March, 2003.

Species	r_s value
Oc. vigilax	0.361
Cx. annulirostris	0.425
Ve. funerea	0.650
Oc. procax	0.768
Cx. sitiens	0.548

Figures 4.14- 4.18: Scatterplots of rankings between sampling periods 1 (17-23 February) and 3 (17-27 March). Line indicates perfect correlation of rankings between the two trapping periods. Numbers indicate trap sites. Different coloured points indicate either predefined sites or those of interest for cluster analysis.

Figure 4.14: Scatterplot of *Oc vigilax* trap rankings between period 1 (17-23 February) and period 3 (17-27 March).

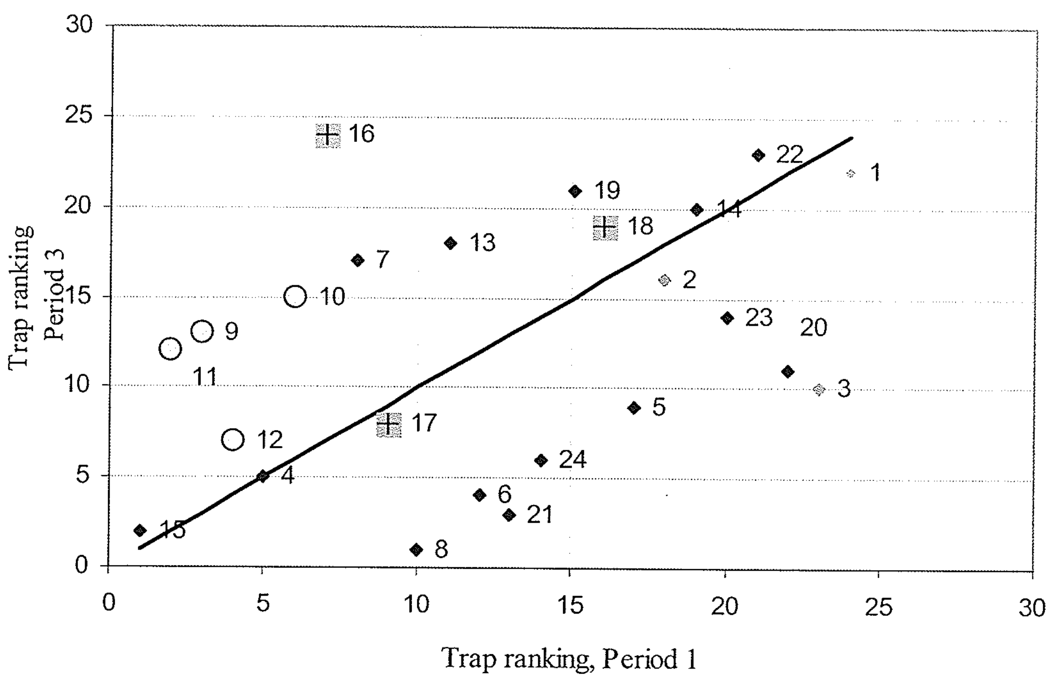


Figure 4.15: Scatterplot of *Cx. annulirostris* trap rankings between period 1 (17-23 February) and period 3 (17-27 March).

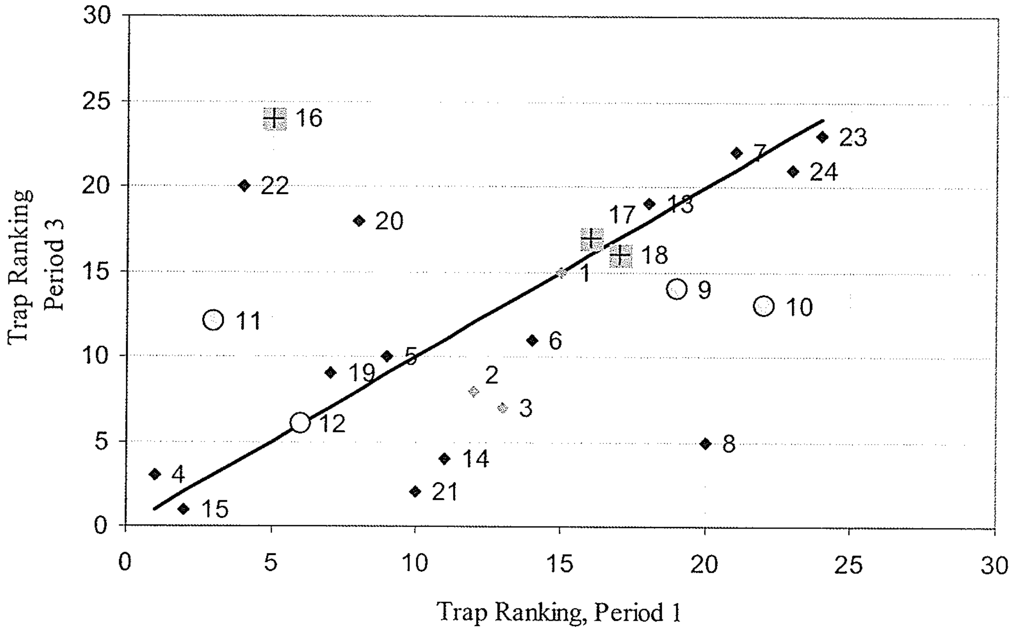


Figure 4.16: Scatterplot of *Ve. funerea* trap rankings between period 1 (17-23 February) and period 3 (17-27 March).

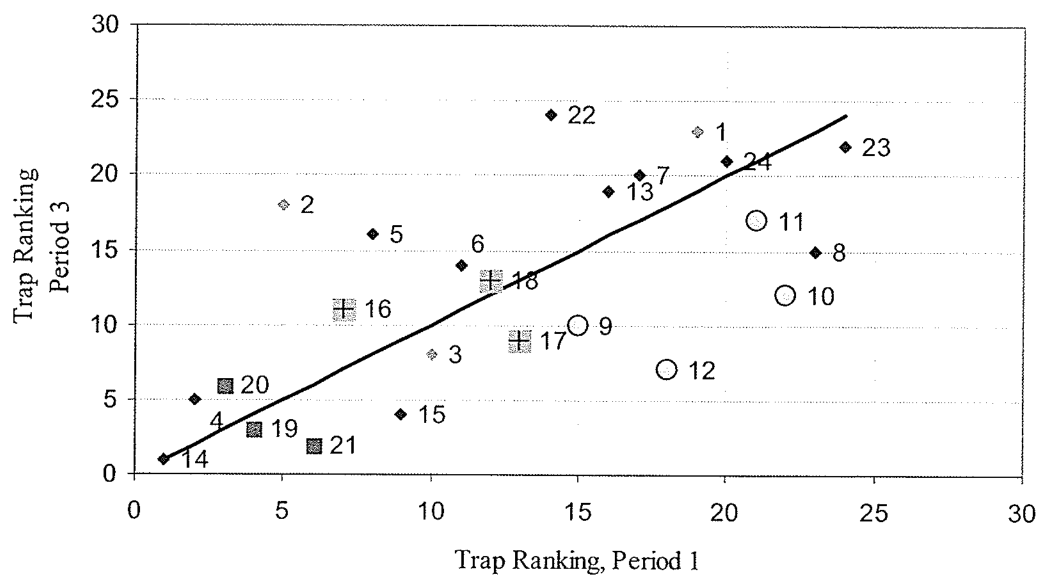


Figure 4.17: Scatterplot of *Oc. procax* trap rankings between period 1 (17-23 February) and period 3 (17-27 March).

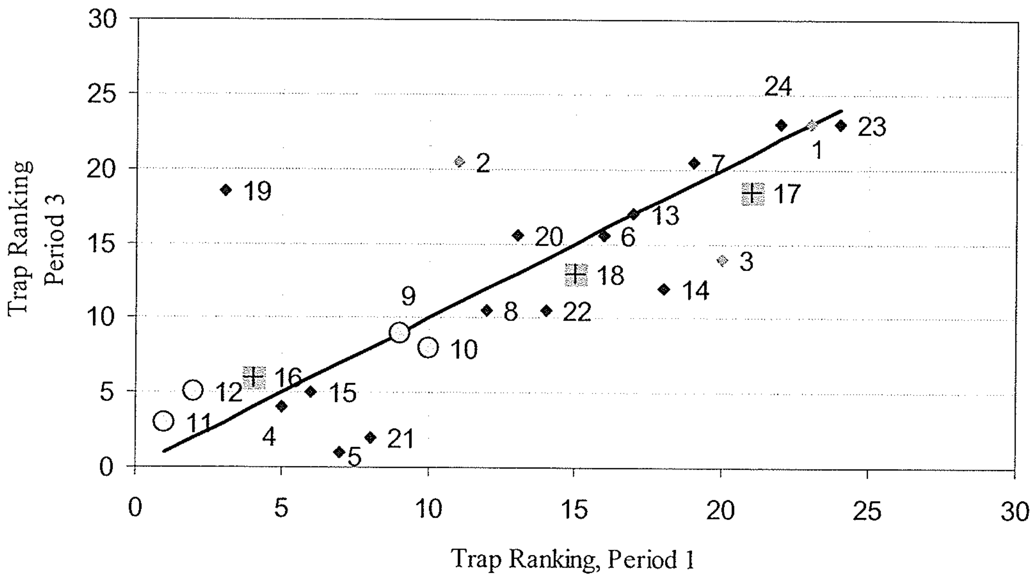
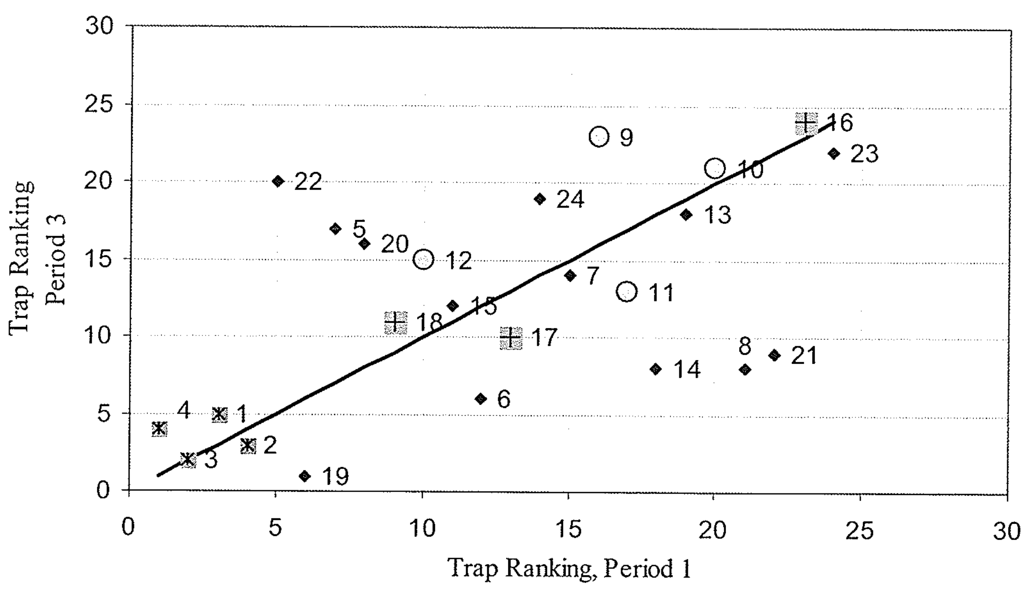


Figure 4.18: Scatterplot of *Cx. sitiens* trap rankings between period 1 (17-23 February) and period 3 (17-27 March).



4.6 Chapter Summary:

The key results from this study that will guide the discussion in the subsequent chapter are addressed as follows.

The mosquito identification of trap catches yielded the results that the most abundant vectors present at Hays Inlet were *Oc. vigilax*, *Cx. annulirostris*, *Ve. funerea*, and *Oc. procax*, while the most abundant pest species were *Cx. sitiens* and *Oc. vittiger*. The trap catch data for each of the above species were further analyzed. The rankings of all species were, overall, statistically consistent between sampling periods 1 and 3. The smallest values obtained from cluster analysis for contiguous trap sites were for *Ve. funerea* at trap sites 19-21, and for *Cx. sitiens* at trap sites 1-4. Other results related to general ranking consistency are that trap sites 4 and 5 were consistently ranked in the top ten trap sites for *Cx. annulirostris*, and trap site 15 was consistently the top ranked trap for *Oc. vigilax*.

The key results of the habitat assessment that will be compared with the above results related to trap rankings include the habitat characteristics at site 4, 15, 19-20. The interval between the preceeding tide and the high tide during the first sampling period was approximately two weeks, while the interval between the latter high tide and the subsequent tide during the third sampling period was approximately one month. A significant rainfall event occurred two weeks prior to sampling, while a second rainfall event occurred approximately two weeks later during the first sampling period. These results will compared with the following results related to changes in adult mosquito abundance over the study period.

The overall mean abundance of *Oc. vigilax* was higher during the first sampling period compared to the third, while the mean abundance of freshwater species remained stable between these two sampling periods.

Using these main results, the following discussion will discuss the vector composition results in relation to those from studies conducted in proximal locations in southeast Queensland, will compare the results from the habitat assessment with the results from trap ranking analyses, will compare the results from the collection of meteorological data with those from the analysis of temporal patterns in species abundance.

Chapter 5: Discussion

The purpose of this study was to define the mosquito problem around Hays Inlet. The following discussion reviews the results in relation to this purpose and to the related literature.

5.1 Vector Composition

The vector composition of species found at Hays Inlet is similar to that found in the proximal locations of Brisbane (Ritchie *et al.*, 1997) and Maroochy Shire (*Ve. funerea*, *Cx. annulirostris*, *Oc. vigilax*, *Oc. procax*, and *Oc. notoscriptus*) (Ryan *et al.*, 1999). The relative abundance of each vector species, however, differed from these locations. The lack of *Oc. vigilax* dominance in trap catches from these locations is likely due to the positioning of at least a portion of traps away from coastal saltmarsh and mangrove habitat due to different study objectives. *Ochlerotatus vigilax* comprised 56% of the total catch in Redcliffe over nine trap nights (Ritchie *et al.*, 1994), compared to 71.5% of the catch in this study. Though trap locations within Redcliffe were not specified, it is possible that at least some of these traps were set in the City of Redcliffe itself and not necessarily close to the inlet. It is likely that *Oc. notoscriptus* and *Cx. annulirostris* would become more important in areas away from areas flooded by seawater.

Of the remaining species captured in the light traps, *Coquilletidia linealis* (Skuse) and *Mansonia uniformis* (Theobald) have been additionally found to be competent vectors for Ross River virus in Queensland. *Mansonia uniformis* completes its pre-imaginal development in semi-permanent to permanent swamps and in its larval and pupal stages it attaches by modified siphon or trumpet to an aquatic plant from which it obtains oxygen (Marks, 1982). On Russell Island, *Cq. linealis* is the most abundant species captured in CO_2 and octenol-baited CDC light traps. Given that this species is attracted to this trap type when present in an area, it was likely not present in high numbers at Hays Inlet during the study period. *Ocherotatus notoscriptus*, the container-breeding mosquito species, likely would be present in higher numbers within the more urbanized Redcliffe city and in the developed residential pockets of Pine Rivers Shire.

The vector competence of *Cx. sitiens* has been tested previously, but its vector status is unconfirmed, as is that of *Ochlerotatus vittiger* (Skuse). Neither of these species is thought to play a significant role in RRv transmission. However, given the overall mean abundance for these species, they may likely prove to be pests to residents in the area. The remaining species rely on freshwater habitat for pre-imaginal development. *Ochlerotatus mallochi* typically breeds in tree-holes filled with a thick liquid, while *Culex quinquefasciatus* is a container-breeding species (Lee *et al.*, 1984). The rest are found in permanent, temporary, or both types of freshwater habitat.

5.2 Spatial Patterns and Habitat

In general, the sites surveyed via the habitat assessments are consistent with those known to produce mosquitoes. Vegetative cover specifically associated with larval development includes *Melaleuca spp.*, *Casuarina glauca*, and various mangrove (*Avicennia*) species. *Melaleuca quinquenervia* stands, present at sites 4 and 19-20, are often associated with freshwater mosquito breeding, because water is known to pool at the base of these trees. *Casuarina glauca* forests, containing fresh to brackish temporary pools, have been previously identified as productive larval habitat (Bick, 1951). Site 15 contained a high percent cover of species at the ground layer, which may have served as a major resting place for mosquitoes during sampling. Additionally, all sites, both woodland and saltmarsh, contained depressions that would likely fill after heavy rain and /or tidal inundation.

Ochlerotatus vigilax exhibited the smallest correlation value between the rankings of trap sites in each time period, although consistently high ranking traps for *Oc. vigilax* were not contiguous as can be seen in Figure 4.14, and were in different habitat types. Trap sites 4, 12, and 15 were characterized by forest woodland habitat, whereas trap sites 8 and 17 were adjacent to saltmarsh and mangrove habitats. *Ochlerotatus vigilax* requires both saltmarsh depressions for oviposition and larval habitat, and tree cover (mangrove or otherwise) for resting sites (Lee *et al.*, 1984). In general, these habitat types are adjacent and proximal to each other on most sides of the inlet (i.e., the distance of woodland to proximal saltmarsh is fairly small: less than 500 m). On a broad scale, *Oc. vigilax* habitat and spatial distribution are much simpler to designate compared to the freshwater species, given that this species can only complete immature development in saltmarsh depressions and other saline environments. Thus on Russell Island (Jeffery *et al.*, 2002), the distribution of *Oc. vigilax* abundance is skewed towards the southern half which is subject to flooding by high tides, compared to *Cx. annulirostris*, which exhibited a weak spatial pattern of abundance. However, in this study, the fact that the location of all traps were very close to Hays Inlet, even in different habitat types, and that the traps exhibited no distinct areas of high versus low abundance, suggests that when suitable *Oc. vigilax* oviposition, larval habitat, and resting sites are adjacent to each other, the distribution of adult female mosquitoes will be relatively uniform. Mean numbers of *Oc. vigilax* were fairly high for the twenty-four trap sites in both time periods, which indicate that the Hays Inlet is likely a very productive larval habitat.

The weak correlation in *Culex annulirostris* rankings in mean trap numbers between the two time periods suggests a trend towards stability of spatial pattern during the sampling period. Although consistently high ranking traps were not contiguous, these traps were present in areas either designated as forest or woodland by the habitat assessment. Traps 4 and 5 were both present in woodland habitat type designated by the habitat assessment at site 4, which contains *Melaleuca spp.* characteristic of one type of *Cx. annulirostris* larval habitat site.

Verrallina funerea exhibited a spatial pattern of abundance consistent with its habitat preferences and flight activity. Although Hays Inlet was recognized previously for its larval habitat and harbourage of *Oc. vigilax*, this study detected proximal harbourage and potential larval habitat for *Ve. funerea*. The area at which *Ve. funerea* were caught in greatest numbers, sites 19-21, based on the small value resulting from the cluster analysis, is likely related to the presence of known suitable and characteristic *Ve. funerea* habitats at this site. This site presented dense vegetation of *Casuarina glauca*, melonholes likely to fill with rainwater and in an area likely to be influenced by some degree of tidal inundation since it adjoins tidal areas. The presence of these features at this site is consistent with Bick (1951). Additionally, the clustering of sites with high *Ve. funerea* numbers is consistent with the finding of Jeffery *et al.* (2002), which was that a strong spatial autocorrelation of *Ve. funerea* on Russell island is present, heavily skewed toward the south of the study area. *Verrallina funerea* is not a very dispersive species, indicating that it will likely only be found very proximal to suitable larval habitat.

The low value from the cluster analysis indicates strong clustering of high *Cx. sitiens* rankings of mean trap catches between the two time periods for the contiguous traps set adjacent to the golfcourse (traps 1-4). Meaningful clustering was not found for traps in other contiguous trap sites. Permanent ponded saline water present in mangrove stands at this site is potentially the reason for such clustering compared to other sites. *Culex sitiens* larvae are found with *Oc. vigilax* in brackish pools left by high tides, but have also been found in permanent saline sites, such as saltings in irrigation areas (Marks, 1982). Compared to other brackish species, this species can tolerate higher salinity levels, as exhibited in Maroochy Shire (Ryan and Kay, 2000).

5.3 Temporal Patterns and Meteorological Variables

The presence of six tides high enough to inundate saltmarsh pools prior to sampling indicates that *Oc. vigilax* development and emergence had likely previously commenced in the Hays Inlet for the season. Additionally, the occurrence of a series of high tides two weeks before sampling combined with the high *Oc. vigilax* numbers already present at the site during the first two trap nights indicates that the presence of *Oc. vigilax* is in part due to hatchings from previous high tides. The numbers of *Oc. vigilax* dropped substantially between sampling periods 1 and 3, in between which approximately one month had lapsed between the series of relevant high tides. Given that the adult longevity of *Oc. vigilax* is 2-3 weeks, it is likely that fewer adults from the previous emergence coinciding with the first tide were still remaining in the area during the third sampling period, compared to the numbers that would have been remaining in the first from the tidal event two weeks before.

Although meteorological variables typically impinge upon trap efficiency (Bidlingmayer, 1985), the influence of these variables was not consistent between nights for each species.

The rainfall event prior to sampling combined with the presence of suitable freshwater larval mosquito habitat is consistent with the presence of such species in trap catches during the first sampling period. The second rainfall event 15 days later likely ensured that temporary freshwater pools would not dry out. Overall, these two rainfall events were likely responsible for maintaining a fairly consistent overall mean catch for freshwater species. Also, the fairly consistent rainfall might have lowered the salinity of certain pools inundated by saltwater during the high tide events. The many so-called freshwater species have salinity tolerance according to Ryan and Kay (2000), and brackish water species thrive in moderately saline waters, which might have contributed to the lack of a strong spatial pattern of abundance between sites.

5.4 Relationship of Study to Cumulative and Emerging Literature

Although this study was descriptive in nature, the results can be discussed in terms of both descriptive studies and on those that focused on testing causal hypotheses regarding the location or abundance of mosquito breeding and the measured risk for human disease.

The very high per cent composition of *Oc. vigilax* from the total mosquito catch during the study period highlights the notion of different vector distribution patterns related to coastal versus. inland (or, saltwater vversus freshwater) locations. As seen in southeast Queensland, *Oc. vigilax* is often presumed to be the major vector. However, significant correlations of disease incidence with the abundance of the freshwater species, *Ve. funerea* and *Cx. annulirostris*, have been reported (Ryan *et al.*,1999). These species were also present at Hays Inlet; the latter most likely of the two to be present due to rainfall, while the former due to the influence of both tidal inundation and rainfall. Thus, at Hays Inlet, tidal inundation of saltmarsh depressions and rainfall together influence the composition of mosquito species present in this area. The occurrence of habitat types associated with both saltwater and freshwater floral species in this area also point to the importance of both so called coastal and inland influences on the pattern of abundance and vector composition at Hays Inlet.

Also, the distinction between rural versus urban transmission ecology is highlighted in this study. Although relatively intact mangrove and forest stands are present around the inlet, urban development up to the margins of the inlet likely make it an important feature in the urban transmission ecology of the virus in the city of Redcliffe. Examples from the emerging literature related to RRv infection risk and proximity of residences to certain vegetation types (i.e. Muhar *et al.*, 2000) indicate that the so-called rural determinants of transmission may also be important in urban settings.

5.5 Relationship of Study to Mosquito Management Frameworks

5.5.1 *Vector Composition*

I identified six species of mosquitoes present in the area that are competent RRv vectors and two pest species that were abundant at the time of the study. The host preferences, biting activity, and status as primary or amplification vector of those species found at Hays Inlet serve as the starting point for identifying trends in abundance during periods of risk. *Ochlerotatus vigilax* and *Cx. annulirostris* are the two maintenance vectors found within the region. *Ochlerotatus vigilax* is mainly an evening biting mosquito, although heavy diurnal attacks on people may occur close to breeding grounds or in adult harbourage areas. The dispersal distance of this species is 20-30 miles from breeding places. *Culex annulirostris* feeds on mammals, including people, and birds, and its highest biting intensity apparently occurs after sunset hours. This species is known to disperse up to 7 km from breeding grounds. From studies conducted in southeast Queensland, the regionally competent amplification vectors include *Verrallina funerea*, *Ohchlerotatus procax*, *Ochlerotatus notoscriptus*, *Coquilletidia linealis*, and *Mansonia uniformis*. *Verrallina funerea* is a daytime pest species which attacks humans and other animals, and its bite apparently stings greatly (Lee *et al.*, 1987). This species does not disperse very widely; it typically stays within 1 km from where it emerged. *Ochlerotatus notoscriptus*, a container-breeding mosquito, is an avid biter at all times over a 24 hr period provided that a blood source is close to its vegetative cover (Lee *et al.*, 1982). The pest species *Cx. sitiens* and *Oc. vittiger* are human-biting mosquitoes, and may be an annoyance when present in high numbers during their breeding season.

5.5.2 Monitoring Vector abundance over time and space

The results of this study point to the utility and importance of interpreting the spatial pattern in catch data both by habitat stratification and uniform sampling. This understanding will aid in the establishment of trap sites around Hays Inlet. In the case of *Oc. vigilax*, the lack of a well defined and consistent spatial pattern by habitat type or similar contiguous trap ranking indicates that Hays Inlet does not present areas of high and low abundance. As related to vector surveillance, the above points to the possibility that a trap distance of 1 km, as recommended by Ryan *et al.* (2004), will likely be the smallest trap distance to detect spatial changes in abundance if points around the inlet were to serve as part of a broader grid sampling scheme. On the other hand, for other species, such as *Ve. funerea*, this trap distance is not reflective of the species dispersal capacity. In this case, the addition of habitat information and placement of traps to coincide with these habitats is an important factor for trap placement.

5.5.3 Relationship to emerging trends in Program Design: Hays Inlet and Risk of RRv Infection

Given that no apparent pattern of high or low abundance was found for *Oc. vigilax*, and that all traps exhibited relatively high numbers of this species at Hays Inlet, the risk of exposure to *Oc. vigilax* is likely the same for all residences situated on either side of the inlet. Temporal risk of *Oc. vigilax* exposure over the duration of the trapping season thus may be a more important indicator of risk since the mean numbers present from traps set in each tidal period was markedly different. However, the risk of exposure to *Ve. funerea* is very localized to certain areas. *Verrallina funerea* is an avid human biter and its abundance has been correlated with RRv cases in Maroochy Shire (Ryan *et al.*, 1999).

The topography of Hays Inlet is low-lying, and is subject to tidal inundation. The presence of depressions at various sites, in addition to the presence of saltmarshes and mangrove swamps indicates the potential capacity of this site to hold water. This sort of topography appears common to most RRv outbreak regions, as outlined in Kelly-Hope *et al.* (2004).

Place of residence appears to be a significant risk factor for RRv infection. Those residences immediately adjacent to the inlet are mainly present on the north and east sides of the inlet. People living in these residences have the potential to be at greater risk of contracting RRv compared to those in residences further away and not additionally proximal to another productive habitat. Most outbreaks have been reported in residential areas close to vector and host breeding sites (Kelly-Hope *et al.*, 2004). The land cover present at Hays inlet can be classified as both littoral and ephemeral wetlands, both of which showed high significance with RRv infection rate in Brisbane (Muhar *et al.*, 2000).

5.6 Chapter Summary

In sum, this discussion highlights certain parallels between the results of this study and studies conducted in the region. First of all, the vector composition in this study site is similar to those found in proximal locations, although the per cent composition varies between species for each site. Of the most abundant vector and pest species, there is consistency between the habitat preferences of *Ve. funerea*, and the localization of high ranking traps for this species to an area in which this habitat is present. Additionally, these results point to patterns in abundance attributable to both freshwater and saltmarsh habitat influences, and to both rural and urban patterns in transmission. Finally, the discussion outlines the potential relationships between the results of this study and RRv risk at Hays inlet, in addition to the potential implications of this study on the management of mosquito vectors in this region.

Chapter 6: Summary, Conclusions, and Recommendations

6.1 Thesis Summary

From the literature review and as reflected in the objectives described in section 1.3, a set of themes pervade this study. The first theme is the relevance of mosquito problem definition to the surveillance and control of vectors within a given jurisdiction, and the relevant biological concepts that underpin the process of problem definition. Another theme relates to the development of these biological concepts towards a landscape ecological approach to vector surveillance and control. A third theme is the relationship of this perspective to the development of novel management approaches. Finally, given the regional nature of vector-borne disease transmission in general, of RRv transmission in Australia, and more specifically in southeast Queensland, numerous comparisons can be drawn.

The methods used in this study included the collection of tide, rainfall and temperature data to characterize the study in terms of meteorological variables, a habitat assessment in order to characterize likely sites of mosquito breeding and or harbourage, and light-trapping around the inlet in order to characterize the adult mosquito vector composition, and spatial and temporal patterns of abundance.

The known vectors within the vicinity included *Oc. vigilax*, *Cx. annulirostris*, *Ve. funerea*, and *Oc. procax*. Given that sampling occurred during the typical mosquito breeding season, these vectors were present during this time. Ranking trap sites in order of mean *Oc. vigilax* abundance during each of the two sampling periods and performing a Spearman's rank test and cluster analysis did not detect a clear spatial pattern of abundance. For *Cx. annulirostris*, the trap sites consistently ranked in the top 10 sites were present in forest/woodland habitat type. For *Ve. funerea*, contiguous trap sites 19-21 were consistently ranked in the top 10 traps, and traps 19-20 were present in an area consistent with *Ve. funerea* habitat preferences. For *Cx. sitiens*, contiguous traps 1-4 were consistently ranked in the top five trap sites. Potential habitat includes a permanent saltwater site in the vicinity of these traps. For *Oc. procax*, trap sites ranked in the top 10 over the two sampling periods were in areas characterized by woodland habitat type. Between the two sampling periods, the Spearman's rank test showed the highest correlation in rankings. Overall these results are relevant to mosquito management and to the formulation of RRv transmission risk in the area.

6.2 Conclusions

The purpose of this study was to define the mosquito (and particularly the vector) problem around Hays Inlet, Queensland, Australia. For each objective related to this purpose, the conclusions that can be drawn from the results of this study, the limitations of these conclusions, and the management and research directions that stem from the conclusions and limitations are addressed below.

6.2.1 Objective 1: The characterization of meteorological variables

***Conclusions*:** Based on the results from the meteorological data and on the changes in mean abundance of each species during the study period, the following conclusions can be drawn:

1) Tide: The frequency of tidal events influenced the mean abundance of *Oc. vigilax* in trap catches during each sampling period. This conclusion is based on the fact that, in general, the frequency of king tides determines the frequency of generations, with one generation corresponding to each king tide. Adult longevity of *Oc. vigilax* is 2-3 weeks, therefore if two king tide events occur within this time period, the mosquito population consists of two overlapping generation, whereas if tidal events are spaced further apart (one month or more), the mosquito population consists primarily of one generation corresponding to a single series of king tides. The mean mosquito abundance was higher in the first sampling period compared to the third, and this can be attributed to the king tide previous to the first sampling period occurred roughly two weeks prior to sampling, while the king tide previous to the third sampling period occurred roughly one month prior to this sampling period.

2) Rainfall: The presence and consistent mean abundance of all and specifically the most abundant freshwater species during the study period at Hays Inlet is temporally related to rainfall. During the month prior to sampling, very little rainfall had occurred. The presence of freshwater species during the study period, especially those that undergo larval development in temporary pools is related to the significant rainfall event from 2-7 February. The maintenance of a fairly consistent mean abundance during sampling period 1 compared to 3 for each freshwater species was temporally related to the second rainfall event from 20

February to approximately 2 March. Complete *Cx. annulirostris* development can occur within ten days in the summer subsequent to a rainfall event, and that the sustenance of temporary pools by more rainfall extends the number of consecutive generations that can emerge from a temporary larval development site.

3) Temperature: The seasonal and stable temperatures during the study period contributed to the seasonal presence and abundance of mosquitoes in this study.
4) Wind-speed did not play a consistent role in reducing or augmenting nightly mean trap abundance during the study period.

Limitations: The above conclusions are limited because meteorological data were not collected directly at the study site. Additionally, given the short duration of the study period, no correlations or related types of analyses could be carried out to statistically assess the relationship between each meteorological variable and the abundance of each species. Additionally, the local vector control district treated larval habitat with *Bti* prior to both sampling periods.

Recommendations: An understanding of the influence of meteorological variables on the pattern of vector and mosquito abundance at Hays Inlet would require long-term data collection of meteorological variables and of mosquito abundance within this area. Over a number of years and over consecutive months, correlation analysis of meteorological variables with mosquito abundance is often used to determine whether there is a significant association between rainfall, temperature, and/or tide, and vector/pest numbers (e.g. Gleiser *et al.*, 2000, DeGaetano, 2005). Such analyses are important for predictive modeling of mosquito abundance, and can be used by vector control agencies to predict mosquito abundance from meteorological events that transpire over the RRv transmission season.

6.2.2 Objective 2: Vector and general mosquito composition

Conclusions: Based on the results of vector composition data, this study demonstrates that:

1) The primary vectors of RRv in the vicinity of Hays inlet were *Oc. vigilax* and *Cx. annulirostris*,
2) The secondary vectors of RRv in this area were *Ve. funerea*, and *Oc. procax*. Additional vectors making up a very small percent of the mosquito composition were *Ma. uniformis* and *Cx. quinquefasciatus*.
3) The major non-vector pest species present at Hays inlet were *Cx. sitiens* and *Oc. vittiger*.

Limitations: These results are limited to the Hays Inlet study site, since proximal locations, such as the City of Redcliffe would potentially yield different faunal composition results because of the greater presence of both natural and artificial freshwater larval habitat. Additionally, these results are limited by the short duration of sampling. I could not take into account differences in composition/relative composition that may be attributable to differences in individual mosquito species' lifecycle in relation to seasonality. Thus, if sampling had occurred during a different point in the mosquito and RRv transmission season, it is possible that different pest or vector species may have been present in high abundance in addition to or in the place of those identified in the above conclusions.

Recommendations: The use of CO_2 and octenol-baited light traps set in the region on a weekly or monthly basis over the whole year for a number of consecutive years, and the identification of the mosquitoes in these trap catches to species would be important for firmly establishing the mosquito composition at Hays Inlet, and potential differences in mosquito composition over the RRv transmission season and over the whole year.

Given that the monitoring and control of vector species requires a specific understanding of which vectors are present in an area, the local governments which have jurisdiction over Hays Inlet might target control efforts towards the above vector species present in the area and understand the biology of these species specifically in relation to the regional ecology of each species. The first important step in targeted control is the identification of species of mosquitoes present in the area.

6.2.3 Objective 3: Spatial patterns of abundance

Conclusions: Based on the interpretation of the results of the Spearman's rank correlation and cluster analyses, the following conclusions can be drawn:

1) The ranking of mean abundance of traps is consistent over time for each species.

2) Contiguous trap sites did not exhibit similar rankings of mean *Oc. vigilax* abundance at the Hays Inlet study site. Trap site 15 consistently had the highest mean abundance of *Oc. vigilax* compared to other trap sites.

3) *Ve. funerea* abundance was similar and consistently higher in the vicinity of contiguous trap sites 19-21 than other sites during the breeding season. Additionally, *Ve. funerea* abundance around trap site 14 was also higher than other sites.

4) *Cx. sitiens* abundance around contiguous trap sites 1-4 was similar and consistently higher than other sites during the breeding season.

5) *Oc. procax* rankings of mean abundance for each trap were the most consistent of all the species examined using the Spearman rank correlation test.

6) *Cx. annulirostris* abundance was consistently the highest in the vicinity of trap sites 4 and 15.

Limitations: A main limitation to (and threat to the reliability of) the above conclusions is that the efficiency of trapping was not monitored during the sampling period. The octenol and dry-ice release rate was neither calculated nor calibrated. Additionally, the short duration of sampling is a limitation to the above conclusions.

Recommendations: The number of traps used in this study would be too great for vector control operators use for monitoring due to the length of time and effort required to set and retrieve them. However, given the results of this study and the presence of spatial differences in abundance for certain species, a systematic sampling scheme that is capable of detecting spatial differences in mosquito abundance is necessary for the monitoring of the main vector and pest species. A trap interval of 1 km would cover all habitat types in the area and would take into account the small dispersal of *Ve. funerea*. As mentioned in the discussion, care must be taken to include within the sampling scheme traps set in productive *Ve. funerea* habitat, *Cx. sitiens* habitat, and in other locations where habitat appeared to play a role in vector abundance.

6.2.4 Objective 4: Habitat assessment

Conclusions: Based on the results of the habitat assessment, this study demonstrates that:

1) Two main habitat types are present at Hays Inlet: saltmarsh/mangrove, and forest/ woodland.
2) The *Melaleuca quinquenervia* dominance at trap site 4 in conjunction with a consistently top ranking of *Cx. annulirostris* abundance at this site, point to the conclusion that this stand was suitable *Cx. annulirostris* habitat.
3) The high mosquito abundance of many species (especially *Oc. vigilax* and *Cx. annulirostris*) in this area was related to the presence of and high per cent cover of floral species in the ground layer.

4) The vicinity of Sites 19-20, characterized by dense *Allocasuarina glauca* coverage and in conjunction with trap data, may have suitable *Ve. funerea* larval habitat.

Limitations: The habitat preferences of a given mosquito species is dictated by the numerous factors not included in the habitat assessment. Additionally, the relationship of different stages of the lifecycle of each species to different habitat elements was not directly detected or measured in this study.

Recommendations: A main recommendation for research into the mosquito habitat identified in this study would be to verify the presence of larvae at each site in the habitat assessment. Specifically, the verification of the species that appeared to be in highest abundance at the site and that have been previously associated with the vegetation, etc., around those sites will be useful for establishing the habitat preferences for each species at Hays Inlet.

6.3 Recommendations for Mosquito Management and Future Research

6.3.2 Future research and management directions

Overall, future research that may be conducted in this jurisdiction involves studies that would facilitate the movement towards a GIS-based system of data coordination and surveillance. The fieldwork necessary for moving in this direction includes the assessment distribution of larval mosquitoes around Hays Inlet, a continuation of habitat mapping, and an assessment of the spatial pattern of abundance over the whole Redcliffe Peninsula.

The design of effective strategies for larval control requires knowledge of larval habitats and environmental conditions associated with pre-imaginal development (Ryan and Kay, 2000). Fieldwork including the characterization and mapping of standing water habitats will provide the basis for a comprehensive database for identifying mosquito breeding areas (White, 2001) that are associated with Hays Inlet. In particular, fieldwork to conduct includes surveying and mapping depressions that have filled after rainfall, larval sampling in the habitat types noted in this study, and an assessment of the salinity of pools.

The habitat assessment data for Hays Inlet could serve as a starting point for categorizing other habitat or vegetation types present in either local government jurisdiction. In effect, each habitat assessment performed at the Hays Inlet shows the emphasis of different vegetation cover at each site and how these differences relate to vector abundance. Each habitat type could be referenced back to the aerial photo of the inlet. From other aerial photos taken of adjacent land, particularly in the more rural parts of Pine Rivers Shire, hypotheses can be made regarding potential habitat or vegetation cover. Ground-truthing of these sites and the potential categorization of sites into different habitat types, and relating these to weather information and larval surveillance, delineate larval habitats requiring inspection and applicable treatment. If adjacent to human habitation, the geocoded habitat assessment information will aid in the delineation of risk areas for RRv infection.

Sampling at previously visited saltmarsh sites in addition to the newly identified freshwater-related habitats will facilitate an understanding of the species composition, diversity, seasonal population fluctuations, and efficacy of control activities (White, 2001). A continuation of habitat mapping in conjunction with the above assessment of larval distribution in both local government jurisdictions will facilitate the accumulation of historical data relating to larval occurrence and mosquito abundance within each type. These data will indicate when and under what conditions a given mosquito species exploits each habitat type. Salt, fresh, and brackish water species were present in this area. Due to the likely importance of the measurement of salinity in pools in relationship to rainfall, tidal inundation, and species emergence, salinity measures will give insight into what species will likely emerge from a given habitat type in the area based on their salinity tolerances (Ryan and Kay, 2000).

Overall, this study may support future research and data collection in the Hays Inlet such that surveillance and control activities in the area will be targeted to the relevant vector species, to their temporal relationships with meteorological variables, and to their spatial relationships with vegetation and habitat.

References

Aaskov, J. G., P. Ross, C. E. A. Davies, M. D. Innis, and R. W. Guard. 1981. Epidemic polyarthritis in northeastern Australia, 1978-1979. Medical Journal of Australia 2:17-19.

Ault, S. K. 1994. Environmental management: A re-emerging vector control strategy. American Journal of Tropical Medicine and Hygiene 50:35-39.

Australian Government Bureau of Meteorology. 2005a. Australian Climate Zones. Viewed July, 2005, <http://www.bom.gov.au/climate/environ/other/aus_clim_zones.shtml>.

Australian Government Bureau of Meteorology. 2005b. Climate averages for Australian sites- averages for Redcliffe Council. Viewed July, 2005, < http://www.bom.gov.au/climate/averages/tables/cw_040697.shtml>.

Barker, C. M., W. K. Reisen, and V. L. Kramer. 2003. California State mosquito-borne virus surveillance and response plan: a retrospective evaluation using conditional simulations. American Journal of Tropical Medicine and Hygiene 68:508-518.

Bergquist, N. R. 2001. Vector-borne parasitic diseases: New trends in data collection and risk assessment. Acta Tropica 79:13-20.

Bick, G. H. 1951. The ecology of mosquito larvae of New Guinea. Pacific Science 5:392-431.

Bidlingmayer, W. L. 1969. The use of logarithms in analyzing trap collections. Mosquito News 29(4): 635-640.

Bidlingmayer, W. L. 1985. The measurement of adult mosquito population changes-some considerations. Journal of the American Mosquito Control Association 1:328-348.

Brenner, R. J., D. A. Focks, R. T. Arbogast, D. K. Weaver, and D. Shuman. 1998. Practical use of spatial analysis in precision targeting for integrated pest management. American Entomologist 44:79-101.

CDC (Centers for Disease Control and Prevention). 2003. Epidemic and epizootic West Nile virus in the United States: Guidelines for surveillance, prevention, and control. Fort Collins, Colorado, U.S. Department of Health and Human Services.

Chenoweth & Associates. 1995. Hays Inlet Management Plan. Chenoweth and Associates, Brisbane.

Dale, P. E. R., K. Hulsman, D. H., and B. Congdon. 1986. Distribution of the immature stages of *Aedes vigilax* on a coastal salt-marsh in south-east Queensland. Australian Journal of Ecology 11:269-278.

Dale, P. E. R. and C. D. Morris. 1996. *Culex annulirostris* breeding sites in urban areas: Using remote sensing and digital image analysis to develop a rapid predictor of potential breeding areas. Journal of the American Mosquito Control Association 12:316-320.

DeGaetano, A. T. 2005. Meteorological effects on adult mosquito (*Culex*) populations in metropolitan New Jersey. International Journal of Biometeorology 49:345-353.

Department of Infrastructure, Planning, and Natural Resources. 2004. Soil Survey in New South Wales- terminology and definitions. Viewed July, 2005, <http://www.dlwc.nsw.gov.au/care/soil/ssu/termsdef.htm>.

EPA/QPWS (Environmental Protection Agency-Queensland Parks and Wildlife Service). 2005a. Regional ecosystems listing. Viewed June, 2005, <http://www.epa.qld.gov.au/projects/redd/listing.cgi?bioregion=12&landzone=12>

EPA/QPWS (Environmental Protection Agency-Queensland Parks and Wildlife Service). 2005b. Regional ecosystems. Viewed June, 2005, <http://www.epa.qld.gov.au/nature_conservation/biodiversity/regional_ecosystems/>

Fraser, J.R. 1986. Epidemic polyarthritis and Ross River virus disease. Clinics in Rheumatic Diseases. 12(2):369-88.

Gleiser, M. G., D. E. Gorla, and G. Schelotto. 2000. Population dynamics of *Aedes albifasciatus* (Diptera: Culicidae) south of Mar Chiquita Lake, Central Argentina. Journal of Medical Entomology 37:21-26.

Gleiser, R. M., G. Schelotto, and G. E. Gorla. 2002. Spatial pattern of abundance of the mosquito *Ochlerotatus albifasciatus*, in relation to habitat characteristics. Medical and Veterinary Entomology 16:364-371.

Goddard, L. B., A. E. Roth, W. K. Reisen, and T. W. Scott. 2002. Vector competence of California mosquitoes for West Nile Virus . Emerging Infectious Diseases 8:1385-1391.

Gullan, P. J. and P. S. Cranston. 2000. The Insects: An Outline of Entomology. London, Blackwell Science Ltd.

Harley, D., A. Sleigh, and S. Ritchie. 2001. Ross River Virus transmission, infection, and disease: a cross-disciplinary review. Clinical Microbiology Reviews 14:909-932.

Hunsaker, C. T. and R. L. Graham. 1990. Assessing ecological risk on a regional scale. Environmental Management 14:325-332.

Jeffery, J. A., P. A. Ryan, S. A. Lyons, P. T. Thomas, and B. H. Kay. 2002. Spatial distribution of vectors of Ross River virus and Barmah Forest virus on Russell Island, Moreton Bay, Queensland. Australian Journal of Entomology 41:329-338.

Kay, B. H., P. Sinclair, and E. N. Marks. 1981. Mosquitoes: Their interrelationships with man, CSIRO Press, Melbourne.

Kelly-Hope, L. A., D. M. Purdie, and B. H. Kay. 2004. Ross River virus disease in Australia, 1886-1998, with analysis of risk factors associated with outbreaks. Journal of Medical Entomology 41:133-150.

Kemme, J. A., P. H. A. van Essen, S. A. Ritchie, and B. H. Kay. 1993. Response of mosquitoes to carbon dioxide and 1-octen-3-ol in southeast Queensland, Australia. Journal of the American Mosquito Control Association 9:431-435.

Kerridge, P. 1971. Aspects of the ecology and biology of the salt marsh mosquito *Aedes vigilax* (Skuse). Master's thesis, University of Queensland, Brisbane.

Kitron, U. 1998. Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. Journal of Medical Entomology 35:435-445.

Lee, D. J., M. M. Hicks, M. Griffiths, R. C. Russell, and E. N. Marks. 1982. The Culicidae of the Australasian region. Commonwealth Institute of Health Monographic Series 2: 196-212.

Lee, D. J., M. M. Hicks, M. Griffiths, R. C. Russell, and E. N. Marks. 1984. The Culicidae of the Australasian region. Commonwealth Institute of Health Monographic Series 3: 207-233.

Lee, D. J., M. M. Hicks, M. Griffiths, M.L. Debenham, J.H. Bryan, R. C. Russell, M. Geary and E. N. Marks. 1987. The Culicidae of the Australasian region. Commonwealth Institute of Health Monographic Series 4: 276-282.

Lee, D. J., M. M. Hicks, M.L. Debenham, M. Griffiths, E. N. Marks, J.H. Bryan, and R. C. Russell. 1989. The Culicidae of the Australasian region. Commonwealth Institute of Health Monographic Series 7: 36-86.

Lee, K.J., P.A. Ryan, R.A. Hall, H. Field, S.A. Lyons, and B.H. Kay. 2003. Investigation of the Indooroopilly Island flying fox colony as a potential focus of Ross River virus activity. Mosquito and Arbovirus Research Committee Annual Report, 2002-2003 106-111.

LGAQ (Local Government Association of Queensland). 2002. Mosquito Management Code of Practice for Queensland. Local Government Association of Queensland, Queensland Government, Brisbane.

Liebhold, A. M., R. E. Rossi, and W. P. Kemp. 1993. Geostatistics and geographic information systems in applied insect ecology. Annual Review of Entomology 38:303-327.

Lindsay, M. D., A. K. Broom, N. Oliveira, E. Jasinska, B. van Heuzen, S. Caulfield, P. McMinn, D. W. Smith, and G. R. Shellam. 1999. Western Australian Arbovirus Surveillance and Research Program, annual report, 1997- 1998. The University of Western Australia, Perth, Western Australia, Australia.

Lothrop, H. D. and W. K. Reisen. 1999. A Geographical Information System to manage mosquito and arbovirus surveillance and control data in the Coachella Valley of California. Journal of the American Mosquito Control Association 15:299-307.

Mackenzie, J. S., M. D. Lindsay, R. J. Coelen, A. K. Broom, R. A. Hall, and D. W. Smith. 1994. Arboviruses causing human disease in the Australasian zoogeographic region. Archives of Virology 136:447-467.

Mackenzie, J. S., A. K. Broom, R. A. Hall, C. A. Johansen, M. D. Lindsay, D. A. Phillips, S. A. Ritchie, R. C. Russell, and D. W. Smith. 1998. Arboviruses in the Australian region, 1990 to 1998. Communicable Diseases Intelligence 22:93-100.

Maritime Safety Queensland. 2003. Official Tide Tables and Boating Safety Guide. Brisbane.

Maritime Safety Queensland. 2005. Tides - King tides. Viewed July 2005, <http://www.msq.qld.gov.au/qt/msq.nsf/index/tides_king>.

Marks, E.N. 1982. An Atlas of Common Queensland Mosquitoes. Queensland Institute of Medical Research, Herston, Australia.

MCAA. Inc. (Mosquito Control Association of Australia Inc.) 1998. Australian Mosquito Control Manual. Redland Bay, Queensland, Mosquito Control Association of Australia Inc.

Muhar, A., P. E. R. Dale, L. E. Thalib, and E. Arito. 2000. The spatial distribution of Ross River Virus infections in Brisbane: significance of residential location and relationships with vegetation types. Environmental Health and Preventive Medicine 4:184-189.

Muirhead-Thomson, R. C. 1968. Ecology of Insect Vector Populations. Academic Press, London.

Queensland Government Department of Primary Industries. 2005. Declared Fish Habitat Areas (FHAs). Viewed July, 2005, <http://www.dpi.qld.gov.au/fishweb/13401.html>.

Raddatz, R. L. 1982. Forecasts of *Culex tarsalis* populations in Winnipeg, Manitoba. Western equine encephalitis in Manitoba. Manitoba Health Services Commission, Winnipeg, Manitoba.

Reisen, W. K. and H. D. Lothrop. 1999. Effects of sampling design on the estimation of adult mosquito abundance. Journal of the American Mosquito Control Association 15:105-114.

Reisen, W. K., H. D. Lothrop, S. B. Presser, M. M. Milby, J. L. Hardy, M. J. Wargo, and R. W. Emmons. 1995. Landscape ecology of arboviruses in Southern California: Temporal and spatial patterns of vector and virus activity in Coachella Valley, 1990-1992. Journal of Medical Entomology 32:255-266.

Reynolds, J. L. 1961. The physiology and ecology of the immature stages of the saltmarsh mosquito *Aedes vigilax* (Skuse) (Diptera: Culicidae). University of New South Wales, Sydney.

Ritchie, S., I. D. Fanning, D. A. Phillips, H. A. Standfast, D. McGinn, and B. H. Kay. 1997. Ross River Virus in mosquitoes (Diptera: Culicidae) during the 1994 epidemic around Brisbane, Australia. Journal of Medical Entomology 34:156-159.

Rose, R. I. 2001. Pesticides and Public Health: integrated methods for mosquito management. Emerging Infectious Diseases 7:17-23.

Rosen, L., D. J. Gubler, and P. H. Bennet. 1981. Epidemic polyarthritis (Ross river virus) infection in the Cook Islands. American Journal of Tropical Medicine and Hygiene 30:1294-1302.

Russell, R. C. 1994. Ross River virus: Disease trends and vector ecology in Australia. Bulletin of the Society of Vector Ecology 19:73-81.

Russell, R. C. 1996. A colour photo atlas of mosquitoes of southeastern Australia. Department of Medical Entomology, University of Sydney and Westmead Hospital, Sydney.

Russell, R. C. 2002. Ross River virus: Ecology and distribution. Annual Review of Entomology 47:1-31.

Ryan, P. A., K.-A. Do, and B. H. Kay. 1999. Spatial and temporal analysis of Ross River Virus disease patterns at Maroochy Shire, Australia: Association between human morbidity and mosquito (Diptera: Culicidae) abundance. Journal of Medical Entomology 36:515-521.

Ryan, P. A., K.-A. Do, and B. H. Kay. 2000. Definition of Ross River Virus vectors at Maroochy Shire, Australia: Association between human morbidity and mosquito (Diptera: Culicidae) abundance. Journal of Medical Entomology 36:516-521.

Ryan, P. A. and B. H. Kay. 1997. Ross River virus ecology is complex. Arbovirus Research in Australia 7:247-251.

Ryan, P. A. and B. H. Kay. 1999. Vector competence of mosquitoes (Diptera: Culicidae) from Maroochy Shire, Australia, for Barmah Forest Virus. Journal of Medical Entomology 36:856-860.

Ryan, P. A. and B. H. Kay. 2000. Emergence trapping of mosquitoes (Diptera: Culicidae) in brackish forest habitats in Maroochy Shire, south-east Queensland, Australia, and a management option for *Verrallina funerea* (Theobald) and *Aedes procax* (Skuse). Australian Journal of Entomology 39:212-218.

Ryan P.A., S.A. Lyons, D. Alsemgeest, P. Thomas, and B.H. Kay. 2004. Spatial statistical analysis of adult mosquito (Diptera: Culicidae) counts: An example using light trap data, in Redland Shire, Southeastern Queensland, Australia. Journal of Medical Entomology 41:1143-56.

Southwood, T. R. E. 1978. Ecological methods, with particular reference to the study of insect populations, Second ed. Chapman and Hall, London.

Standfast, H. A. and G. J. Barrow. 1969. Mosquito collections in a high rainfall area of northern Queensland. Journal of Medical Entomology 6:37-43.

Sudia, W. D. 1962. Battery-operated light trap, an improved model. Mosquito News 22:126-129.

Sudia, W. D., V. F. Newhouse, and W. A. Chappell. 1969. Venezuelan equine encephalitis virus-vector studies following a human case in Dade County, Florida, 1968. Mosquito News 29:596-600.

Tong, S., P. Bi, K. Parton, J. Hobbs, and A. J. McMichael. 1998. Climate variability and transmission of epidemic polyarthritis. Lancet 351:1100.

Weinstein, P. 1997. An ecological approach to public health intervention: Ross River virus in Australia. Environmental Health Perspectives 105:364-366.

White, D. J. 2001. Vector surveillance for West Nile virus. Annals of the New York Academy of Sciences 951:74-83.

Woodruff, R. E., C. S. Guest, M. G. Garner, N. Becker, J. Lindesay, T. Carvan, and K. Ebi. 2002. Predicting Ross River Virus epidemics from regional weather data. Epidemiology 13:384-393.

Appendix I: Glossary

Aedine mosquitoes: Species of mosquitoes belonging to the genus *Aedes*. Includes all species that were formerly classified as *Aedes*, but whose nomenclature changed through the reestablishment of *Ochlerotatus* to the generic rank.

Appetential flight: Searching flight. Occurs in adult mosquitoes over 24 hrs of age and is undertaken in response to a physiological stimulus.

Crepuscular: A term used of animals to indicate they are active during the twilight, either morning or evening.

Ephemeral: Larval habitat that is transient due to its formation after rainfall or tidal inundation, followed by evaporation.

Kriging: A form of statistical modelling that interpolates data from a known set of sample points to a continuous surface.

Lilliefors test: A statistical test for the goodness of fit to a normal distribution.

Oviposition: Egg-laying or depositing. A Commonly used term for insect taxa.

Runnelling: A method of physical habitat modification used in saltmarshes to control pest mosquito populations. This method involves linking the tidal source to isolated mosquito breeding pools via shallow channels that enable slow water movement of low amplitude tides. Increased tidal flushing inhibits mosquito development.

Viraemic: A state in which the virus of interest is circulating in the blood of the given host.