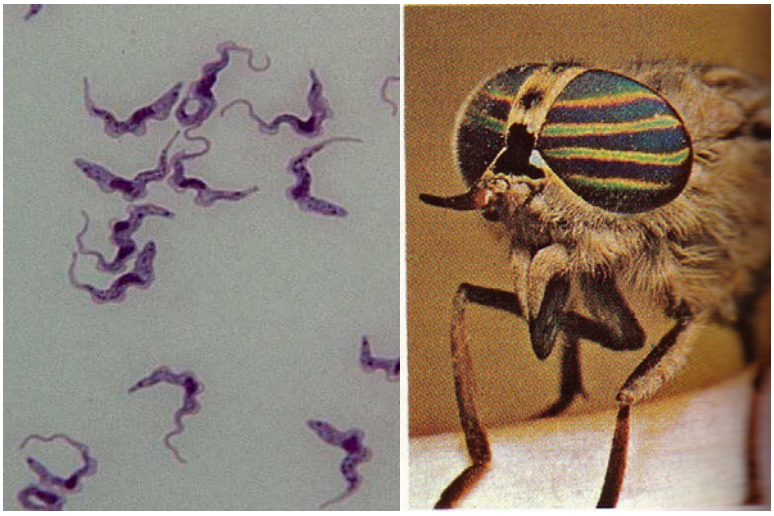
ASPECTS OF THE ECOLOGY OF TABANID FLIES (FAMILY: TABANIDAE) IN NORTH QUEENSLAND AND THEIR POTENTIAL TO TRANSMIT *TRYPANOSOMA EVANSI*



A thesis submitted by
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in August 2007

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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

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STATEMENT ON THE CONTRIBUTION OF OTHERS

Financial support for this project in the form of scholarships was provided by The School of Veterinary and Biomedical Sciences (Gluyas Fellowship), Biosecurity CRC and Graduate Research School (James Cook University Doctoral Completion Award). Project funding was obtained from the AB-CRC. The work was completed under the supervision of Dr Lee Skerratt, A/Prof Lee Fitzpatrick and Prof Rhondda Jones. The AB-CRC project was in collaboration with researchers at Murdoch University, Queensland Health (Brisbane), Department of Agriculture Fisheries and Forestry and School of Tropical Environmental Sciences and Geography, James Cook University.

Statistical support was provided by the School of Maths and Physics, James Cook University, Rhondda Jones and Reinhold Muller (School of Public Health and Tropical Medicine).

I acknowledge the assistance of Glenn Bellis (AQIS, Darwin) and Dave Spratt (CSIRO, Canberra) with tabanid identification and access to the National Insect Collection in Canberra for comparison with their reference specimens.

I acknowledge the assistance of National Parks and Wildlife (Environmental Protection Agency), Queensland Department of Primary Industries and Fisheries and Australian Quarantine and Inspection Agency (Department of Agriculture, Fisheries and Forestry) and the members of the public listed in the acknowledgements for their research assistance with tabanid collections in Cape York Peninsula.

Assistance with use of Geographical Information Systems analysis was provided by the School of Tropical Environmental Science and Geography, with thanks to James Moloney. I also acknowledge the technical assistance of Odwell Muzari with tabanid feeding behaviour and daily activity studies and taking wing length measurements on the Cape York data. I acknowledge the support of Andrew van den Hurk and "Clara" Wai Yuen Cheah at Queensland University for their assistance in training me in blood-meal analysis techniques. Kathleen Buick assisted with proof reading.

Kirsty van Hennekeler
August 2007

DECLARATION OF ETHICS

Relevant research reported in this thesis received approval from the James Cook University Ethics Review Committee (approval numbers A991, A1059 and A1060) and National Parks and Wildlife (Scientific Purposes Permit: WISP03550006).

Kirsty van Hennekeler
August 2007

ACKNOWLEDGEMENTS

The reason I became involved in this project was that it posed a great challenge: a cross-disciplinary project that was heavily oriented towards being a useful tool for key stakeholders. Being a "big picture" person, the only reason I managed to sustain interest in this PhD was that for me it fulfilled several criteria: it was original, I had a significant role in the process of defining its direction and scope and I would learn new skills and synthesise information in a way that would help me pursue an abiding interest in biosecurity issues. But great ideas cannot reach fruition without a lot of hard work and a good team. I was lucky that some wonderful, kind and knowledgeable people believed in the project and were patient enough to teach me aspects I was largely ignorant of. The inspiration for this project came from Bruce Copeman, who suggested the topic and told me that very little was known about possible tabanid vectors of surra in Australia. He also warned me that it would be difficult- very prophetic! In addition, Simon Reid offered his ideas and expertise during early discussions about which aspects I would explore and would have played a much larger role, except for the tyranny of distance. I am also grateful to Dick Copland for his encouragement and early involvement.

This thesis would not have been possible without the generosity and assistance of a large number of people, who offered all kinds of help ranging from being a shoulder to cry on when things weren't going well, to very hands-on, practical aid. I am very appreciative of all of you.

I would like to gratefully acknowledge the contribution of a number of people who facilitated the collection of tabanid data in remote locations throughout Cape York. Sincere thanks to several organisations including the Environmental Protection Agency, in particular Mark Peacock, Andrew and Lia Hartwig, Lenny Banjo, Carl and Andrea Goetze, Lance and Karin Spain; the Queensland Department of Primary Industries and Fisheries especially Robert Hedlefs, Jason Bode, Scott Templeton and Sara Wales; Northern Australia Quarantine Strategy, especially James Walker, Jackson Sailor, James Bond, James Matthews, Anita Barz, Lucy Picco, Bruce Lansdowne; and a number of the general public who were willing to be involved, despite the long-term commitment and low pay (well, no pay) including Mark and Isla Upham, Bill Rutherford, Judy Irwin, Jenny and Greg Ford, John Hardacre, Peter and Dee Friel and John Pritchard.

Chris Coleman played a very significant role in designing the trap shelter, which needed to be strong enough to withstand cyclones and light enough to be transported by light plane to these remote locations. The end result surpassed expectations, and was a tribute to Chris' ingenuity. Chris was also extremely helpful with other logistical support throughout the project- thanks Chris.

Tabanid identification is quite difficult and as I discovered, there are few people in Australia who have the skills to do it. I am indebted to Glenn Bellis, Bill Doherty and Dave Spratt for much assistance with tabanid identifications. Also, many thanks to Odwell Muzari for his technical assistance.

Much to my frustration, the blood-meal analysis work could not be completed in time to be part of this thesis, but nevertheless, I thank Andrew van den Hurk and "Clara" Wai Yuen Cheah for their help with learning some analysis techniques. Thanks also to Kathleen Buick for editorial assistance. Many thanks to Reinhold Muller, who conducted the CART analysis in Chapter Ten. James Moloney (TESAG) has been a wonderful teacher of all things GIS- many thanks to you James, I still owe you a few beers. Many thanks also to the staff at the Graduate Research School, especially Helene Marsh, for their kindness and support.

I also need to acknowledge the great support and camaraderie from the staff and fellow post-grads at Vet and Biomedical Sciences. I feel very fortunate to have gotten to know so many incredible, witty, lovely people and I will remember the often tangential morning tea and lunch-time conversations with great fondness and a few giggles.

I am very grateful for the financial support I received in the form of scholarships from the Gluyas Fellowship (School of Veterinary and Biomedical Sciences), Australian Biosecurity CRC and the Graduate Research School. In addition, I thank the AB-CRC for project funding. Thanks also to our project collaborators, including Robert Dobson and Simon Reid.

Many thanks to my supervisors: Lee Fitzpatrick, who safely flew us both around the Cape on many occasions in somewhat stressful circumstances and for supporting the project (and me) throughout its travails; Lee Skerratt for advice and editorial assistance; and of course, to Rhondda Jones- my mentor, stats guru and personal motivator. Rhondda, you have been so much more than a supervisor- your enthusiasm, intelligence, warmth, humour, perseverance and caring have made all the difference to my being able to complete this project. Words don't do justice to the influence you have had on my life, during the most difficult times I have ever experienced.

I am blessed with a number of wonderful friends who have been there for me, through thick and thin, who have listened, counselled, commiserated and celebrated with me. People who deserve special mention are Deon Barritt, Marg Ludlow, Nime Kapo, Tony Croke, Elsa Germain, Kim Nagle, Jane Day, Andrew Wright, Lisa Elliot, Ray Layton, Ruth Campbell, Donna Martin, Pam Megaw, Phil Summers, Leigh Owens, Ketheesan, Andrew Greenhill, Marshall Feterl, Rhonda Chesser and the Aquapella gang. I'm sure there are people I've forgotten to mention, so to all of you, thankyou from the bottom of my heart.

And of course, very special thanks to my wonderful family for all their love and support. Without you, so many things would not have been possible. To my Mum and Dad- thankyou for always believing in me, for never giving up, for all the financial and emotional support you have given me over the years. To my brother Erik, his partner Kate and my niece Ella- thankyou for being such awesome people, for being there for me throughout it all and for caring so much. To Yvette and Nikolaas also, thanks and much love to you both.

Lastly, I'd like to dedicate this thesis to my brother Dirk, who passed away tragically on the 19th March 2007 after a titanic battle with severe depression. Dirk- you are one of the most amazing, talented, loving, giving people I've ever met and I'm grateful that you were part of my life for 35 years. There isn't a day that goes by that I don't think of you and wish you were healthy, happy and here sharing this life with us. But I still feel your presence around and I'm glad you are finally at peace. Love always.

ABSTRACT

Surra, the disease caused by the protozoal parasite, *Trypanosoma evansi*, is characterised by weight loss, anaemia, dependent oedema and death in susceptible animals. It affects all mammalian species tested, and is known to cause acute disease with high mortalities in wallabies and kangaroos (Reid *et al.*, 2001). There is no evidence of presence of *T. evansi* in Australia, however it is considered a high biosecurity risk as it has the potential to cause significant economic loss due to livestock death and weight loss, as well as a possibly devastating effect on native wildlife (Reid, 2002; AFFA, 2003).

Tabanid flies (also called march flies or horse flies), especially the genus *Tabanus*, are considered the primary vectors of surra (Nieshulz, reviewed by Krinsky 1979). The distribution, abundance and population dynamics of insect vectors all influence the risk of *T. evansi* transmission. The risk of incursion is considered to be greatest in the northern-most parts of Queensland, Australia (Reid, 2002; Thompson *et al.*, 2003a). Disease surveillance is expensive and logistically difficult in this region due to the low population density and remote location. Little historical information was available on the ecology of tabanid flies in Australia, so the main aim of this study was to seek ecological data on tabanids that would promote understanding of the times and places that tabanid abundance occurred in northern Queensland. This information would then be extrapolated over the northern Australian region and used in the production of risk maps for surra in Australia.

In this study, data on tabanid flies was collected in north Queensland over 21 months, and the weather and other environmental factors that were significantly related to their abundance was determined. This information was then applied to a GIS and the annual and spatial abundance of likely vector species was mapped. These maps will be used in conjunction with additional data on host animal density and distribution and disease spread between animals to provide risk maps that will help focus disease surveillance activities in areas of highest risk.

The yearly abundance of *Tabanus spp.* was greatest in the most northern part of Cape York Peninsula, and was related to average annual minimum temperature and solar radiation values. This area of northern Queensland corresponds to a high geographical risk of surra incursion associated with the proximity to West Irian (Indonesia) and Papua New Guinea, which is thought to be the likely route of entry for surra into Australia. In addition, species of *Tabanus* are present for an average of 11 months of the year in this region, as a result of a wide variety of species present in this area, including the presence of *T. ceylonicus*, which is active during the dry season. This indicates that there is a confluence of risk factors in the most northern part of Cape York, which increases the risk of incursion and establishment of surra in this region.

Other aspects of tabanid behaviour and ecology were also studied. It was established that the Nzi trap was the most efficient means of trapping tabanids in Australia, and that attractants greatly improved capture rates. Also the times of greatest daily activity, and activity between days, differed among various tabanid species and this was related to variation in response to meteorological variables.

This study has established relationships among tabanid numbers and weather and environmental factors. This has elucidated the annual temporal and spatial abundance patterns of tabanids in the north Queensland region. This information will provide the basis for further studies that further establish the links between vector intensity and disease incidence in surra endemic countries, which will in turn allow a greater understanding of the epidemiology of this disease.

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COMMONLY USED ABBREVIATIONS

AGID	Agar Gel Immuno-diffusion Assay
ANOVA	Analysis of Variance
AQIS	Australian Quarantine and Inspection Service
AVHRR	Advanced Very High Resolution Radiometer
BOM	Bureau of Meteorology
CART	Classification and Regression Tree Analysis
CCD	Cold Cloud Duration
CO_2	Carbon Dioxide
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAFF	Department of Agriculture Fisheries and Forestry
ELISA	Enzyme-Linked Immuno-Sorbent Assay
EIAV	Equine Infectious Anaemia Virus
EPA	Environmental Protection Agency
GIS	Geographic Information Systems
JCU	James Cook University
LST	Land Surface Temperature
NAQS	Northern Australian Quarantine Strategy
NASA	National Aeronautics and Space Administration (USA)
NDVI	Normalised Difference Vegetation Index
NOAA	National Oceanic and Atmospheric Agency
Meteosat	Meteorological Satellite
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
s	Seconds

CHAPTER ONE
GENERAL INTRODUCTION

1.1 Background

Australia is fortunate to be free of many animal diseases that cause widespread deaths and production losses of livestock. Freedom from many of the world's most devastating diseases of livestock allows Australia broad market access for export livestock and meat products. This market access confers substantial economic prosperity on this country. The livestock industry is worth in excess of 13 billion dollars to the economy annually. In addition, our domestic market benefits from widespread consumer confidence in the quality and safety of meat products produced in the country.

Australia's disease-free status is underpinned by a substantial surveillance program, which employs both active and passive disease survey methods, in order to provide the most comprehensive testing possible. National strategies to deal with possible disease incursions (AusVetPlan) are in place and are updated whenever new information becomes available. A major challenge faced by Quarantine and Primary Industries authorities undertaking surveillance activities is the difficulty of monitoring disease in very large areas of sparsely populated land, especially in northern Australia, where the geographical risk of disease incursion from infected countries to the north, is thought to be greatest (Thompson *et al.*, 2003b).

Surra, the disease caused by the protozoon *Trypanosoma evansi* and transmitted by flies in the family Tabanidae, causes significant morbidity and mortality amongst livestock in countries where the disease is endemic (Reid, 2002). It does not occur in Australia, although an incursion did occur in a group of camels imported from Pakistan in 1907 (Cleland, 1907). The diseased animals were destroyed, and no further cases of surra have been reported in Australia, despite surveillance activities to test for its presence. Surra poses a significant risk to this country's livestock productivity and export markets, in addition to being a threat to our biodiversity, due its extensive mammalian host range.

1.2 The purpose of the study

Very little information was previously available on aspects of the ecology of Australian tabanid flies with respect to their potential transmission of *T. evansi*, despite recognition that they could be vectors of surra, if the disease were to enter Australia (Thompson *et al.*, 2003b).

Information relating to the spatial and temporal abundance of potential vectors of surra in northern Australia would enable the mapping of vector intensity at different times of year. When the areas and times of greatest transmission risk have been identified, this information could be combined with information on the density and distribution of mammalian host animals and areas of geographic risk, to produce risk maps for the introduction and establishment of *T. evansi* in northern Australia.

These spatial and temporal risk maps could be invaluable for focussing surveillance in high risk areas, thus reducing the cost and difficulty involved, while improving the chances of early detection of an incursion. Early detection in turn increases the likelihood of containment and eradication of the disease and therefore protection of Australia's disease-free status.

1.3 The study

The aim of this thesis was to look at how the risk of potential transmission of *T. evansi* by tabanid vectors varies in time and space. Different scales of time and area have been explored, as have the relationships of the more common tabanid species with environmental and meteorological variables in space and time. The development and activity of tabanids is intimately associated with the environment and aspects of weather such as temperature, humidity and rainfall, as is the case for most insects. Characterising these relationships would enable extrapolation of point data collected at 12 sites in northern Queensland throughout the region of northern Australia as well as into the future.

Chapter Two provides a review of available literature on tabanids as mechanical vectors of surra and outlines the sparse historical data available on Australian tabanids.

Initially, it was important to evaluate some sampling techniques to determine the most efficient and appropriate method of capturing tabanid flies. In Chapter Three, two different trap types and attractants were compared and the results provided the basis for future sampling methods used throughout the various studies presented in this thesis.

In Chapter Four, the daily activity patterns of local Townsville tabanids were examined in relation to meteorological variables. Variations in body size and the presence of visible developing eggs within and between days was evaluated to determine whether the tabanids active at different times throughout the day, or on different days throughout the flight season, were significantly different physiologically. This information was important to determine whether tabanids collected throughout the sampling period could be used in feeding behaviour experiments, without concern for possible physiological differences biasing the results.

The feeding behaviour of tabanids in Townsville was evaluated in Chapter Five. In particular, the sites and duration of landing and feeding were examined. In addition, host

defensive behaviours and the causes of fly departure were studied. These aspects of tabanid behaviour influence how well they can mechanically transmit pathogens. Landing and feeding sites influence feeding success and the likelihood that insecticides applied to the host animal's back will be efficacious for tabanid control.

The remaining four experimental chapters (Chapters Six to Ten) relate to a 21-month longitudinal study of tabanids at various sites in Cape York Peninsula and Townsville, north Queensland. Data were collected on the distribution, seasonal dynamics and relative abundance of various species of tabanids in this region. Chapter Six provides an overview of the data, including the species caught and the overall species succession, or timing of different species' flight seasons throughout the year. In Chapter Seven, the variation in body size of different species at different locations and times of year is examined. Tabanid body size is related to the quality of larval nutrition and larger sized tabanids produce more eggs. So these data can indicate the quality of a site for supporting tabanid development and optimal body size. Size is also related to fecundity, so larger sized tabanids should occur at sites where there are also higher numbers of that tabanid species. The data in Chapter Eight provides information on the temporal abundance of different tabanid species throughout the year. In particular, the times of emergence, peak abundance and duration of activity (flight season) are examined. These phenomena are collectively referred to as phenology, and the phenological pattern often differs among species. In Chapter Nine, the seasonal and spatial abundance patterns of tabanids were analysed in relation to weather variables. Geographic Information Systems (GIS) predictive maps of yearly abundance patterns were produced for each of the two study years using these predictive weather variables. An attempt was made to define variables that predict the monthly distribution of the six main *Tabanus* species and the genus *Tabanus* as a whole in Chapter Ten. Classification and Regression Tree (CART) analysis was performed to establish the environmental and meteorological variables that were associated with higher tabanid prevalence i.e. presence of tabanids at a greater proportion of site-month combinations. The findings of this study are summarised and their relevance to the risk of introduction and establishment of surra in

Australia are discussed in Chapter Eleven. In each chapter, the results are related to implications for the risk of potential transmission of *T. evansi*.

There are certain issues associated with modelling based on data extrapolation that should be kept in mind when evaluating the utility of the models (such as those produced in Chapter Nine). Namely, that abundance data recorded during a two year study period would not be expected to remain constant in subsequent years, or to exhibit the exact relationships with weather patterns that were seen during the study. Also, tabanid abundance data collected at a certain geographical location, with its associated soil type and other ecological parameters, can not be assumed to exhibit the same relationships with weather variables in other geographic regions. However, in the absence of more extensive longitudinal surveys with a more comprehensive spatial design, this information is currently the best available.

This thesis provides information that is useful for understanding and managing the risk of surra transmission and establishment on a broad spatial scale for a region in north Queensland where the risk of surra incursion is high. The seasonal vector dynamics and how this relates to temporal transmission risk are also discussed. In addition, the risk of transmission on a daily basis is presented and techniques for trapping and studying the behaviour of tabanids have been explored.

CHAPTER TWO
AUSTRALIAN TABANIDS AS POTENTIAL VECTORS OF SURRA: AN OVERVIEW OF THE SCENARIO

2.1 Introduction

The purpose of this review was to explore aspects of surra and its vectors that have been reported in the literature and evaluate the potential risk of incursion and establishment of surra posed to Australia. Gaps in current knowledge that were identified in the review for investigation as part of this PhD study are explored in more detail at the start of the relevant chapter in this thesis.

Surra is an exotic disease to Australia and potentially could have a major impact on Australia's economy and biodiversity if it were introduced (Reid, 2002). It is caused by the protozoan parasite *Trypanosoma evansi* and is transmitted mechanically by haematophagous diptera, principally by tabanid flies (Luckins1998). Surra is considered a high quarantine risk to Australia because it occurs in countries close to northern Australia, and because appropriate species of hosts and vectors for surra are present in northern Australia where incursion might occur. Experimental transmission of *T. evansi* has been demonstrated using more than 20 species of *Tabanus* (Dieleman, 1986; Luckins, 1998). After numerous experiments in Indonesia, Nieschulz concluded that all members of this genus would be capable of transmitting the parasite (Dieleman, 1986). Australia has 230 species of Tabanidae, including 25 species of *Tabanus*, many of which occur in northern Australia (Mackerras, 1970). Therefore, species likely to transmit *T. evansi* are present in northern Australia. In addition, other Australian tabanid species in northern Australia are possible vectors, depending on whether they possess the determinants for mechanical transmission. Appropriate host species, such as cattle, wild pigs and wallabies are also present in this area in large numbers.

Tabanids are important vectors of disease. Krinsky (1979) evaluated over 200 references in a landmark review of the disease agents transmitted by tabanids. Pathogens included

filarioid worms (*Loa Loa*, *Pelecitus roemeri),* bacteria *(Bacillus anthracis),* viruses (e.g. those responsible for Bovine Leukaemia, Equine Infectious Anaemia, Swine Fever) and protozoa (*Trypanosoma evansi).* High densities of tabanids, may also agitate stock and cause weight loss (Hughes *et al.*, 1981).

No Australian species of *Tabanus* is as yet known to transmit any infection of man or livestock, although Spratt showed in 1972, that *Pelecitus roemeri* (Linstow), a filarioid parasite of kangaroos and wallabies that inhabits the subcutaneous and inter-muscular connective tissue of the knee region, is transmitted by at least 11 species of tabanid in south-east Queensland, including *Dasybasis* (Diachlorini) as well as *Tabanus* species (Spratt, 1972; Spratt, 1974b).

Given the extensive list of pathogens mechanically transmitted by tabanids as reviewed by Krinsky (1979), and the paucity of Australian research in this area, the current importance of tabanids as vectors of disease in Australia may be substantially underestimated. Nonetheless, it is generally agreed that *T. evansi* would be transmitted in Australia by tabanids here, if it were to enter the country (Thompson *et al.*, 2003b).

An American tabanid researcher, Lane Foil (1989) deplored the limited nature of the research on Tabanidae, despite their importance to medical and veterinary disease worldwide. Most studies of tabanids have focused on biology and distribution. Foil considered that the lack of established laboratory colonies of any tabanid species was in large part to blame for research into other aspects such as vector behaviour. He also questioned generalisations made about the family from studies on single species, since the family Tabanidae is highly diverse, containing as many species as the Culicidae. He pointed out that the relationship between tabanid trap catches and the actual burden on animals has seldom been examined. In addition, clarification of the factors that influence activity periods of tabanids was needed. These gaps make the evaluation of control efforts directed towards tabanids difficult compared with the evaluation of similar techniques directed toward other, better known arthropod groups (Foil, 1989).

Eldridge and Edman (2000) attribute the lack of epidemiological studies on mechanical transmission by arthropods to the difficulty in assessment of the public health and veterinary significance of mechanical transmission, because pathogens transmitted mechanically by arthropods are nearly always transmitted by other means as well. While tabanids are accepted as the most important means of transmission of *T. evansi*, transmission of the protozoa is also possible by vampire bats, through milk, via coitus and by eating freshly killed infected animals (Brun *et al.*, 1998). Iatrogenic means of spread are also possible (Reid, 2002).

2.2 Surra: The Disease

2.2.1 Clinical signs

Clinical signs of surra include pyrexia, progressive anaemia, loss of condition and lassitude. Episodes of fever and parasitaemia recur during the course of the disease. Subcutaneous oedema occurs, especially in the lower parts of the body such as abdomen, ventral neck, chin and genitalia. Urticarial plaques and petechial haemorrhages of the serous membranes are commonly seen. Abortion has been noted in buffaloes in Asia. It is possible that immunodeficiencies also result from infection with *T. evansi* (Anon., 2004).

These clinical signs are indicative of surra, but are not pathognomonic, requiring diagnostic confirmation by laboratory tests.

Species affected by surra include cattle, horses, buffaloes, camels, pigs, sheep, goats, dogs, deer, capybaras and llamas. In fact, almost all mammals investigated appeared to be infected by *T. evansi*. Until recently it was thought that *T. evansi* was not able to infect humans, but a human case was recently detected in India (Joshi, 2005). It arose due to a lack of apolipoprotein L-1 (a lytic factor that prevents infection with *T. evansi*) in the patient's bloodstream (Vanhollebeke, 2006) rather than a mutation of the parasite, that might have caused it to resist the lytic factor.

Different strains of *T. evansi* vary considerably in their pathogenicity. Different host species also exhibit variation in their susceptibility to surra. Acute and chronic forms of the disease exist. In chronic cases, the disease may be present for many months. Surra is often rapidly fatal in cattle, buffaloes, dogs, horses, camels and llamas, but more mild forms of the disease may also occur in these species (Anon., 2004).

2.2.2 Vectors of Surra

Surra was first reported in Indonesia in 1897 (Penning 1900 in Dieleman 1986) and was found to be an important disease of animals, which prompted research into possible vectors of the disease because historically the disease had been associated with the presence of biting flies. It has been reported in most of the main islands of Indonesia.

Experimental transmission trials were conducted by Nieshulz, who worked alone and in collaboration with Ponto, between 1925 and 1930. The flies used in the experiments were collected while feeding and animals naturally infected with *T. evansi* such as dogs, horses and buffaloes were used as sources of the pathogen. Transmission studies were performed on horses, guinea pigs and buffaloes (Dieleman, 1986).

The experimental studies determined that species of *Tabanus* were the most efficient vectors of surra in Indonesia. Transmission of the parasite was still possible after a feeding interruption of one half to three hours and as long as six hours. The effect of interruption times between tabanid feeds on donors and recipients during laboratory transmission of *T. evansi* showed that the probability of transmission dropped precipitously from 0.05 within five minutes of an infective feed to 0.04 one hour later, 0.003 after three hours, 0.001 six hours later and 0.0003 after 24 hours (Leclerq 1952 in Luckins 1998). It was also established that one *Tabanus* fly can infect three animals successively (Dieleman, 1986).

At least 27 different species of *Tabanus* have been shown experimentally to transmit *T. evansi* including:

Tabanus albitriangularis, T. albimedius, T. bicallosus, T. bilateralis, T. brunnipes, T. ceylonicus, T. ditaeniatus, T. flavivittatus, T. fumifer, T. grisepalpis, T. hilaris, T. immanus, T. latifascies, T. macer, T. malayensis, T. minimus, T. nemocallosus, T. nemoralis, T. partitus, T. reducens, T. rubidus, T. rufiventris, T. stilatus, T. tropica, T. vagus, T. venecki and T. virgo (Luckins, 1998).

Of these species, *T. ceylonicus* occurs in Australia. None of the other species known to be surra transmitters is found in Australia. However, some Australian species of *Tabanus* may have near relatives in the region, for example *T. cinerescens* Macleay appears closely related to the Indonesian *T. rubidus* Wiedemann (Mackerras, 1970).

Although other species of biting fly, such as *Haematopota, Lyperosia* and *Chrysops,* have experimentally been found to transmit *T. evansi*, their role as vectors in field situations is not well understood. Also, it is not clear how important *Stomoxys* is, despite successful experimental transmission with *S. nigra* and *S. calcitrans* (Luckins, 1998). In Mauritius, *S. nigra* was considered the principal vector of surra, whereas studies in the Philippines found it was unimportant. Successful laboratory transmission from blood-soaked cotton wool to an uninfected mouse, using flies restrained in a mesh-covered vial, was demonstrated in Kenya using *S. taeniatus* and *S. n. niger* (Sumba *et al.*, 1998). While this trial proved the vector competence of these species, the forced movement of the flies meant that natural feeding behaviour and its effect on transmission could not be examined. Consideration of the feeding behaviour of certain species is an important factor in determining the significance of their role as vectors. It is likely that the tendency of *Stomoxys, Liperosia* and *Haematopota* to complete their blood meals on a single animal diminishes their effectiveness as vectors in the field (Luckins, 1998). A study comparing the feeding behaviour of *Stomoxys* and *Glossina* showed that *Stomoxys* was much less responsive to the host's defensive behaviour (Schofield and Torr, 2002). These authors discussed the likelihood that the persistent feeding behaviour of *Stomoxys* probably reflected their shorter lifespan and greater fecundity, compared to *Glossina*.

This feeding persistence reduces the probability of pathogen transmission, as transfer from an infected to a non-infected host needs to occur in a short time frame. Sumba *et al.* (1998) also noted that viable *T. evansi* only remained present on the proboscis of *Stomoxys* for 5-7 minutes after feeding, and indicates that tabanid mouthparts with their sponging labellae facilitate parasite survival better than do *Stomoxys*, which have needle like mouthparts. It would be useful to confirm the importance of *Stomoxys* as a vector of *T. evansi*, in comparison to *Tabanus*, under field conditions, utilising natural feeding behaviour, in order to clarify the issue of their comparative utility as vectors.

While the experimental evidence amassed does establish various aspects of the mechanical transmission of *T. evansi*, the dynamics of transmission are not well understood. Livestock owners were aware of the association between the presence of biting flies and the disease surra well before identification of the causative agent and its vectors. Despite this long-term circumstantial evidence, little experimental evidence establishes the relationship between outbreaks of disease and high numbers of probable vectors. Important features of the epidemiology of surra that need further investigation include the role of the host species, the level of parasitaemia and duration of infection. It would be useful to determine the relative efficiency of different vector species, their seasonal abundance and relative importance among those species feeding on hosts. Strong temporal and spatial associations of high incidence of surra, with high abundance of tabanid species that have been shown to be competent vectors would improve the evidence for vector incrimination and the role of high vector numbers preceding outbreaks (Luckins, 1998).

2.3 Surra: Biosecurity threat to Australia

An incursion of surra in northern Australia may not be immediately evident, as clinical signs of disease in livestock, feral or wild animals may not be readily detected due to the remote location and hence infrequent observation of animals by people.

An outbreak of surra in Australia would be very difficult, or impossible, to control once established. Large feral populations of animals such as camels, pigs, buffaloes, horses, donkeys, deer and goats would provide a substantial reservoir for carriers of surra (Anon., 2007a).

An outbreak of surra was detected in a group of 500 camels imported from Karachi, Pakistan into Port Hedland, Australia in 1907 (Cleland, 1907). The diagnosis was made by identifying trypanosomes in blood smears. The outbreak was controlled by culling of infected animals and no further reports of surra have been made in Australia (Anon., 2007a).

The presence of surra in Australia could be very costly, in both economic terms, and in terms of a likely negative impact on biodiversity. The livestock industry in Australia is worth over 13 billion dollars to the country's economy annually. Losses to this industry of millions of dollars would be incurred from deaths and reduced growth of livestock, the costs of treatment and finding alternatives to horses for management of cattle and adverse effects on live animal trade (Reid, 2002). Experimental evidence suggests that Australian marsupial populations could be devastated by *T. evansi* (Reid *et al.*, 2001). Australian dingoes are also likely to be highly susceptible to infection. An incursion could therefore mean a potentially highly injurious effect on wildlife populations and biodiversity.

2.3.1 Current world distribution of *T. evansi*

Surra is the most widely distributed of the pathogenic animal trypanosomoses (Anon., 2004). It is considered to have originated in North Africa and to have spread from there via movement of infected animals to India and the Asian continent and the islands of the Indian Ocean at the end of the 19th century (Hoare 1972 in Dieleman 1986). It is now distributed extensively throughout the tropical and subtropical regions of the globe, including northern Africa, the Middle East, China, India, South-east Asia, Russia and South America (Figure 2.01). There is also evidence indicating the presence of *T. evansi*

in West Papua, Indonesia (Anon., 2007a). However, there has been no recent confirmed presence of *T. evansi* in Papua New Guinea (Reid and Copeman, 2000).

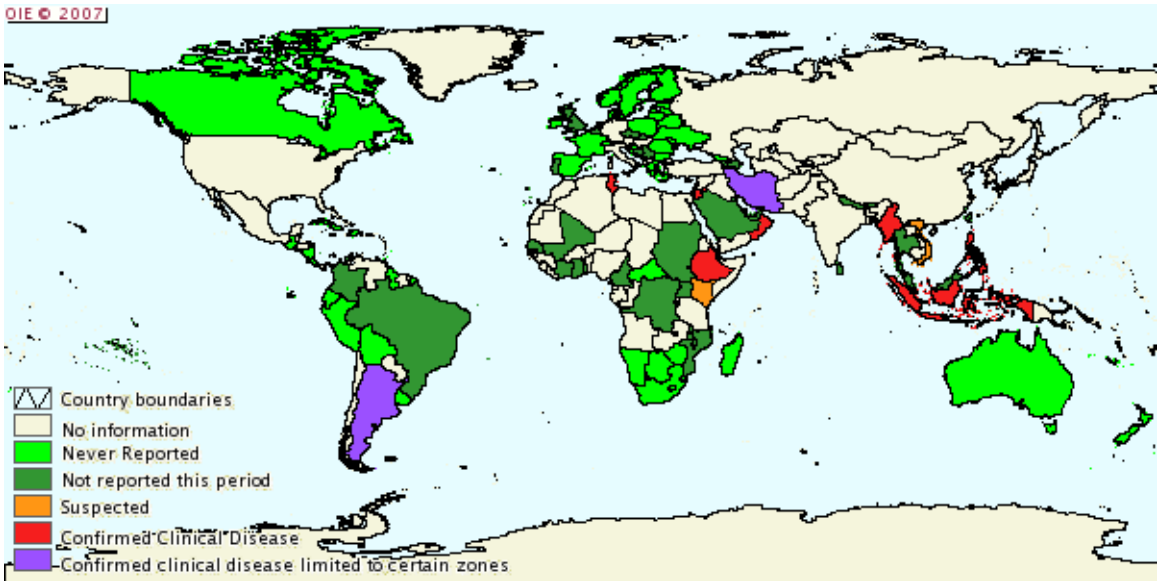


Figure 2.01: Map indicating world-wide surra cases for period July-December (Anon., 2006).

2.3.2 Potential route of entry into Australia

As the map below indicates, *T. evansi* is present in Indonesia and East Timor, both countries that are geographically in close proximity to Australia (Figure 2.02). The likely route of entry is from the province of Papua (Indonesia) through Papua New Guinea and the islands of the Torres Strait into northern Cape York Peninsula, or from East Timor into the northern part of the Northern Territory. These parts of Australia that are seen as the highest risk areas for incursion also contain potential hosts and vectors for *T. evansi* (Thompson *et al.*, 2003b).

Infected animals are more likely to bring *T. evansi* into Australia than wind-borne insects, since the protozoa do not survive for long outside the host e.g. on the mouthparts of mechanical vectors. Animals such as dogs or pigs could be moved with the aid of humans, through this region. Also, deer are known to swim and could move between the islands. Movement of infected animals into Australia on illegal vessels is also possible, and increasing numbers of unauthorised vessels have been noted along the northern coastline, especially near Darwin and Broome (Thompson *et al.*, 2003b).

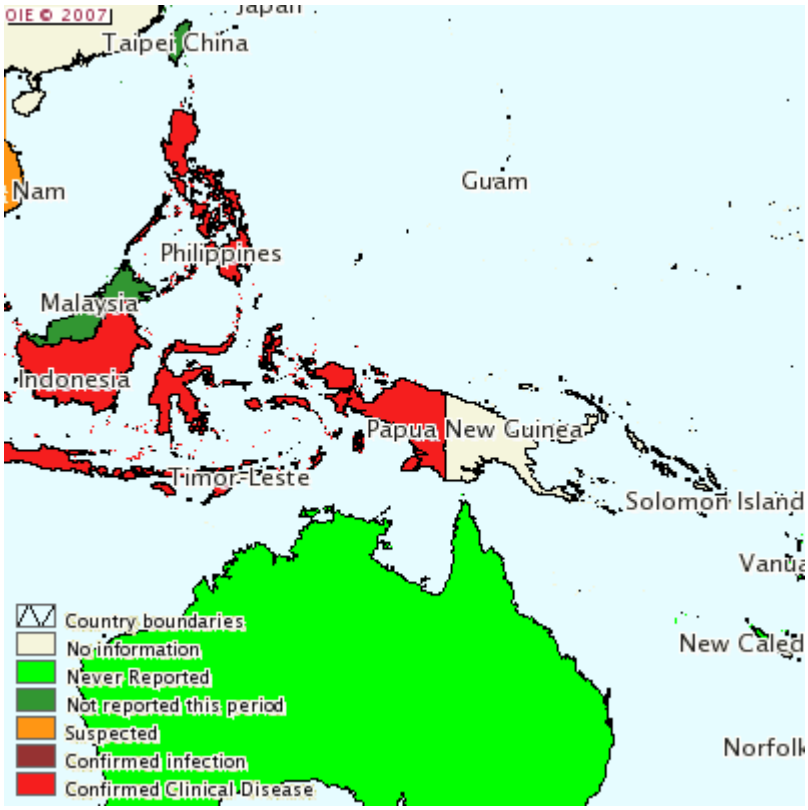


Figure 2.02: Map indicating surra cases in Australasian region for period July-December 2005 (Anon., 2006).

The northern part of Cape York is a particular risk area because of the proximity of the Torres Strait islands to both Australia and Papua New Guinea. There have long been trading and cultural interactions between indigenous people in the Torres Strait and PNG, which are explicitly protected by the Torres Strait Treaty between Australia and PNG. Movement of people, animals and goods in this region is carefully controlled by the Northern Australian Quarantine Strategy (NAQS) (Figure 2.03). Pre-border and post-border surveillance activities are also part of the Strategy. Pre-border surveillance in areas of close proximity to northern Australia, such as PNG, has the advantage of providing an early warning system that the risk of possible introduction is higher. Preventing the entry of this disease is advantageous, because it negates the need for expensive control and containment measures within Australia. Post-border surveillance is necessary to assess the disease status of the country and to provide evidence of freedom from disease. However, confirmation of a disease incursion during post-border surveillance necessitates the difficult task of effective control measures. If considerable

time has elapsed between the incursion and surveillance detecting the disease, establishment of the disease in wild, feral and domestic animal populations could mean disease control measures are extremely extensive or are not economically or environmentally viable. These surveillance efforts are severely hampered by both the remoteness of the regions and the large geographical area involved, however. Focusing surveillance efforts in areas of highest risk would help to reduce costs and increase the chances of discovering a disease incursion early in its course, which in turn would improve the likelihood of containing the disease.

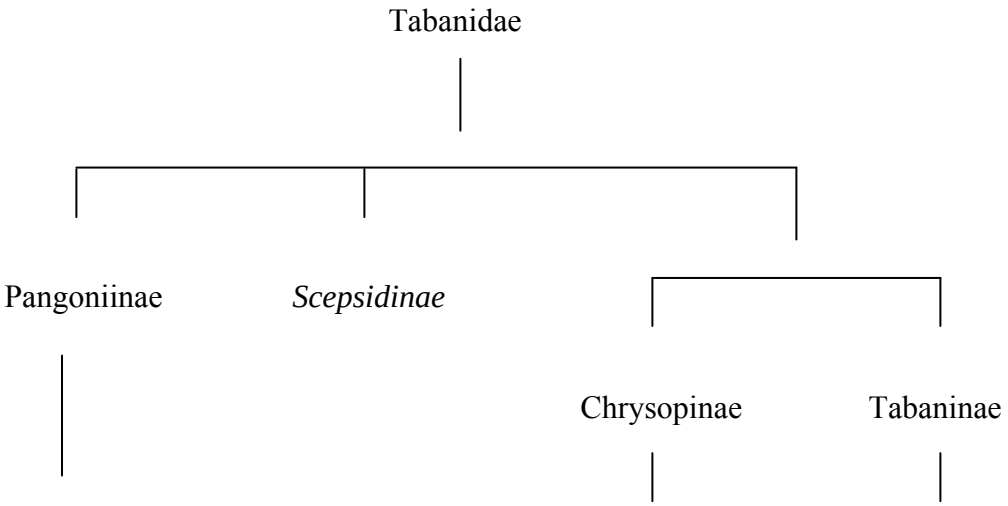
Figure 2.03: Torres Strait quarantine zones (Anon., 2007b)

2.4 General Overview of Tabanidae of Australia

Approximately 230 species of Tabanidae are found in Australia, Tasmania and the islands of the Torres Strait. Almost 90% of the total numbers belong to four ancient tribes: Pangoniini (18 Australian species), Scionini (63), Bouvieromyiini (38) and Diachlorini (92). The more recent Oriental element comprises 25 Tabanini and one Chrysops. About 190 specific names are accepted as valid, there are more than 120 synonyms, 28 described species remain unrecognised and about 40 species are undescribed (Mackerras, 1956).

The tribe Tabanini is represented in the region only by the genus *Tabanus*. Twenty-five species are recognised, of which one is inadequately described (Mackerras, 1970).

2.4.1 Classification of Tabanidae



(Mackerras, 1956; Daniels, 2007)

N.B. Italicised groups do not occur in Australia

2.5 Biology of Tabanids

2.5.1 Life cycle of Tabanidae

The early stages of four Australian species of *Tabanus* have been described: *T. particaecus* and *T. townsvilli* by Johnston and Bancroft (1920 in Mackerras 1970) and *T. pallipennis, T. townsvilli* and *T. dorsobimaculatus* by Hill (1921 in Mackerras 1970). Hill described the development of *T. townsvilli, T. pallipennis* and *T. dorsobimaculatus* in Townsville, north Queensland, where the larvae of the three species behaved in much the same way. The females lay masses of 250 – 700 eggs on vegetation above pools or swamps. Eggs hatch six to seven days later and the larvae then drop onto the water below. The first moult occurs within six hours of hatching and the second at 14 – 21 days. The entire developmental period lasts a little over three months in the laboratory, but could take longer in field conditions. They feed mainly on small molluscs and all stages are extremely cannibalistic, despite an abundance of other food being available.

Larvae migrate into relatively dry soil when they are fully grown and bury themselves 5 – 15cm underground. They remain quiescent for two to six months, with longer periods in the dry season. The pupal stage lasts 8 – 20 days (average 12 days). It was noted by Hill that adult flies appear promptly after rain, suggesting that the pharate adult, like the pre-pupal larva, may be able to remain quiescent until conditions are favourable.

In drier inland areas, larvae of *T. particaecus* and *T. townsvilli* have been found in different habitats, including wet mud, lagoons, sand, temporary creeks and waterholes, salt and freshwater lakes, swamps, artesian bore drains and water-tank overflows. Laboratory studies conducted by Alan Dyce (in Mackerras 1970) demonstrated that the final instar larva could withstand dessication in mud cells and if transferred to water, weeks later, would immediately become active and start feeding. Dyce also observed large numbers of immature larvae in the margins of temporary waters after summer rains

and surmised that the ability to survive dessication might exist at any stage of growth. This ability, combined with the short pupal period, would promote survival of the species in semi-arid environments with uncertain rainfall (Mackerras 1970). These observations on Australian tabanid biology are in general agreement with those found in other parts of the world (Dieleman, 1986; Kettle, 1995; Wall and Shearer, 1997).

Kettle reports that the time taken for hatching to occur and the speed of subsequent development is temperature dependent. At a constant high temperature e.g. 32 – 35˚C, *T. taeniola* completes its life cycle from egg to adult in 10 – 11 weeks, but at 22˚C, it takes 42 weeks (Kettle, 1995). The speed of development may be restricted less by temperature and more by moisture availability in the tropics.
North American species of tabanid have an adult life-span of three to four weeks in field conditions and produce five to six batches of eggs (Kettle, 1995). Dieleman (1986) wrote that studies on Indonesian species showed that tabanids lived for more than 70 days. *Tabanus striatus,* found in Indonesia and the Philippines, has a life cycle of three months and three weeks and produces two to three generations per year.

In Egypt, Hafez (1970) reported that the gonotrophic cycle, or time taken between blood meal consumption when eggs were laid, was temperature dependent, with faster times recorded in higher ambient temperatures. In temperature controlled laboratory studies on *T. taeniola*, he reported the gonotrophic cycle length was 2.7 days at 35˚C and 6.0 days at 22˚C.

2.5.2 Morphology of tabanids

Tabanid flies are characterized by their large body size (up to 25mm in length), their large, broad heads and protuberant eyes. Their body colour is usually dark, in shades of black, brown and grey, often with patterns of stripes or triangles. Colour can vary to green, yellow and metallic blue. The prominent antennae are made up of a scape, pedicel and enlarged flagellum. The antennal flagellum is composed of five segments, with the first segment bearing a horn-like projection. The eyes can be brilliantly coloured or

banded. Females are dichoptic (eyes are separated by a space, the frons) while males are holoptic (no space between the eyes) (Wall and Shearer, 1997).

Tabanid mouthparts incorporate both sponging and sucking components. They have a pair of mandibles with flat-saw like blades and a pair of maxillae that are narrow-toothed files (Wall and Shearer, 1997).

Wing venation of tabanids is very characteristic with R_{4+5} forked to form a large "Y" across the wing tip (Figure 2.04 below) (Wall and Shearer, 1997).

Figure 2.04: *T. townsvilli* indicating characteristic wing venation (Photo O. Muzari)

2.5.3 Adult mating and feeding

Both adult males and females feed on nectar and pollen of flowers (Daniels, 2007). Adult females mate before seeking a blood-meal. Many species require a blood meal for development of their eggs, but some do not for the first batch of eggs and are termed

"autogenous" (Kettle, 1995). However, there are no records of Australian Pangoniini being blood feeders (Daniels, 2007).

Tabanids are telmophagous, or pool feeders. A pool of blood and tissue fluids is created by the scissor-like actions of the mandibles in the subcutaneous tissue of the host. Saliva, containing anti-coagulant is forced into the wound, prior to the blood being sucked up through the food canal (Wall and Shearer, 1997; Kazimirova *et al.*, 2002).

The mouthparts are withdrawn on cessation of feeding and when the labellae close together, a small amount of blood is trapped between them. If pathogens are present in this trapped blood, they may remain viable for a period of time, e.g. an hour or more, allowing infection of additional hosts with subsequent feeds (Wall and Shearer, 1997).

Information on the size of the blood-meal, autogeny and frequency of feeding is not available for Australian species of tabanids, so the account below describes these parameters for tabanids in other parts of the world. On average tabanids feed every two days, although generally they feed every day for a few consecutive days, followed by a break of a number of days. Duration of feeding is approximately 10 minutes (Dieleman, 1986).

Tabanids feed mainly on large mammals and occasionally, birds. The bites of flies are deep and painful and may cause considerable disturbance (Kettle, 1995; Wall and Shearer, 1997). The amount of blood taken up during feeding is between 20 - 200mg but oozing after feeding means that blood loss is often greater (Kettle, 1995). Leprince and Foil (1993) found that *T. fusciostatus* consumes, on average, a blood meal of about 50mg. In the same study, 3.1 eggs were produced for each milligram of blood ingested by the fly, resulting in an average of 154 eggs per bloodmeal for this species.

2.5.4 Adult dispersal

Different tabanid species may prefer specific habitats such as forest or open land, although some are widespread through a variety of vegetation types. Larvae can occur in most semi-aquatic habitats and may be dispersed considerable distances by running water from where they were oviposited (Kettle, 1995).

The intrinsic flight range of tabanids has been measured at over 50 km, but they generally do not disperse far from the place of emergence. The salt marsh tabanids, *T. nigrovittatus*, *C. fuliginosus* and *C. atlanticus* in North America, disperse less than 200m. Other species are reported as dispersing 1 – 3 km, or up to seven kilometres from their source (Kettle, 1995).

2.5.5 Seasonal Distribution

Seasonal activity of Australian tabanids has been reported by Mackerras (1970). The season of adult activity was found to be slightly bimodal as a result of a secondary peak during late summer (Figure 2.05). Adults may be active throughout the year in tropical regions but not in temperate areas e.g. *Tabanus pallipennis* was collected throughout the year in northern Australia but only between October and April south of the Tropic of Capricorn.

Tabanid species are distributed throughout coastal Australia but are most numerous in the tropics, from the Kimberleys through to the Northern Territory and into northern Queensland (see Figure 2.06). The species present in coastal New South Wales also occur in Queensland (Mackerras, 1970).

Number of species

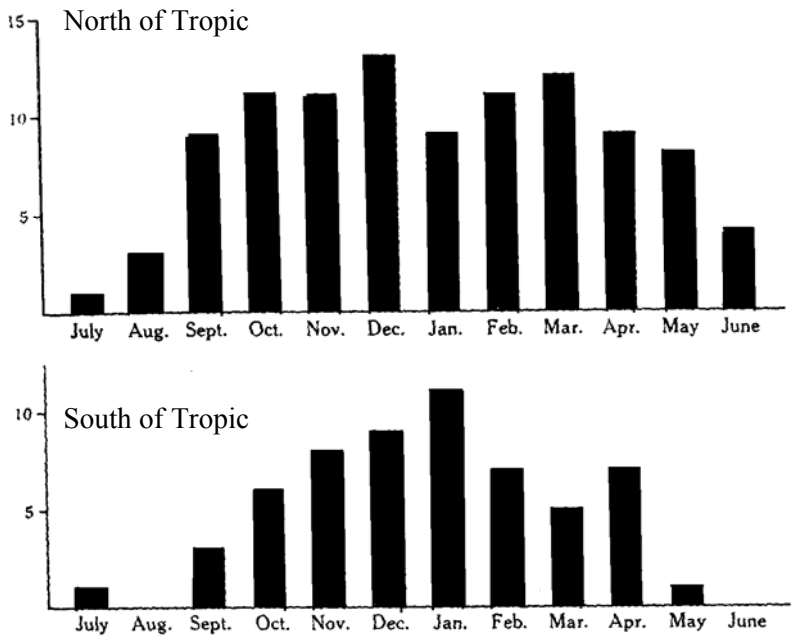


Figure 2.05: Seasonal distribution of *Tabanus spp.* north and south of the Tropic of Capricorn (Mackerras, 1970).

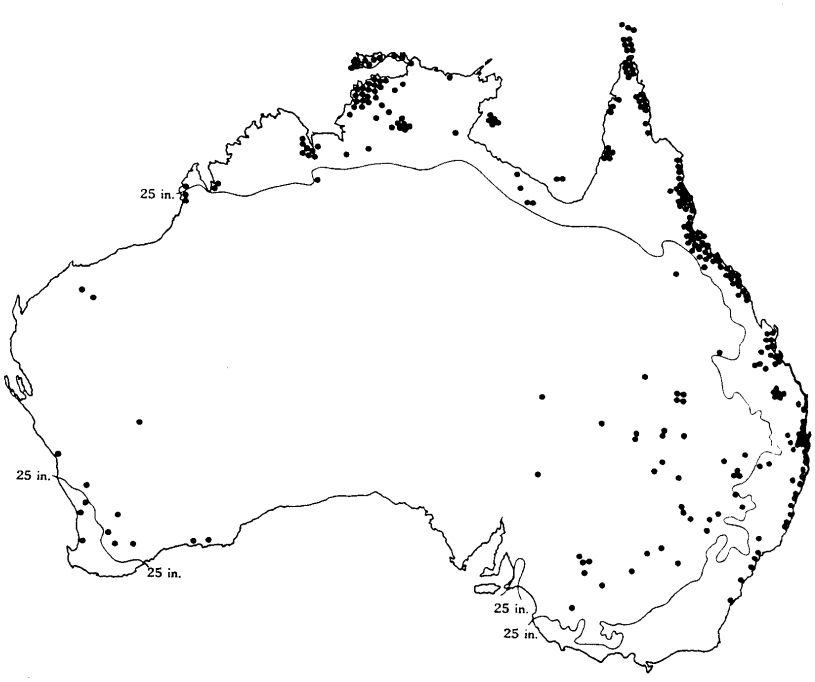


Figure 2.06: Distribution of the genus *Tabanus* in Australia. Each spot is a record of one species within the area enclosed by 1° of latitude and longitude. (Mackerras, 1970). The line marked "25 in." is the 625 mm isohyet.

2.6 Evaluating the Risk Australian Tabanids Pose to *T. evansi* Transmission

The vectorial capacity of Australian tabanids can be evaluated in two main ways. Firstly, the components of an individual species' ability to act as a mechanical vector is determined largely by the morphology of its mouthparts, by its feeding behaviour and by the amount of blood it can transmit. These aspects will be examined under the heading "2.6.1 Mechanical transmission". Secondly, the population dynamics and distribution of tabanids as a whole, contribute to the broader scale risk of transmission of disease over wide geographic areas. These aspects are explored under the heading "2.6.2 Epidemiology of vector-borne disease".

2.6.1 Mechanical transmission

2.6.1.1 Role of tabanids as vectors of disease

The role of tabanids as vectors of disease has been reviewed by Krinsky (1976) and Foil (1989). The mouthparts of tabanids lend themselves particularly well to mechanical transmission of blood pathogens between hosts. Mechanical transmission of pathogens is further enhanced in tabanids by virtue of their feeding behaviour, since they may readily transfer to another host when disturbed by host defensive behaviours.

Nieshulz's experiments with transmission of *T. evansi* between 1925 and 1930 (reviewed by Krinsky, 1976) showed that *Tabanus spp*. were more efficient vectors than *Haematopota* and *Chrysops* (which are other genera of Tabanidae) and that all tabanids were better vectors than mosquitoes or other biting muscids such as *Stomoxys*.

No epidemiological models are currently available to test for evidence of the mechanical transmission of pathogens of humans or other animals, but significant factors posited by Foil (1989) include: pathogen persistence, host proximity, vector mobility, feeding behaviour, host defences, blood residue on mouthparts, quantity of blood transferred, pathogen titre, vector density and host factors.

2.6.1.2 Specific aspects of mechanical transmission

Mechanical transmission is defined as the transfer of pathogens from an infected host or substrate to a susceptible host, without any biological development (cyclic, propagative or cyclo-propagative) of the agent within the vector: i.e. there is no development or multiplication of an agent within the vector (Eldridge and Edman, 2000).

With mechanical transmission, the vector, pathogenic agent and host are the primary components. The events that lead to the natural or experimental mechanical transmission of agents are:

1. Vector initiates feeding on the infected host
2. The vector's feeding is interrupted
3. The vector moves to a susceptible host, transporting the agent on or within the body parts that routinely contact the host
4. The vector feeds upon the susceptible host and introduces the agent via a wound (Foil, 1989).

The characteristics of a good mechanical vector are that it:

- Is frequently interrupted in feeding
- Is highly mobile
- Has large mouthparts to transfer agents.

All of the above are characteristics of tabanids. However, the basic question of how much infectious material is transferred among animals is also dependent on the density of tabanids (Foil, 1989).

The size and form of mouthparts associated with blood-feeding influence mechanical transmission. Haematophagous arthropods that are pool-feeders (telmophagous) and have blade-like mouthparts (e.g. tabanid flies) are more likely to be involved in mechanical transmission than vessel-feeding (solenophagous) arthropods with needle-like mouthparts (e.g. mosquitoes). This is because a larger surface area of the mouthparts is in contact with the blood meal resulting in the potential for a larger quantity of blood to be retained on the mouthparts (Eldridge and Edman, 2000).

Because mechanical transmission does not involve development of the pathogen within the vector, pathogen survival outside the host depends on vector behaviour, temporal aspects of transmission and the environmental stability of the infectious agent (Foil, 1989). For environmentally labile pathogens, successful transmission usually involves the interrupted feeding of the vector and re-feeding within a short time. The feeding

behaviour and flight capability of tabanids contribute to their accepted importance as mechanical vectors (Foil *et al.*, 1987).

2.6.1.3 Vector feeding behaviour

Certain features of feeding behaviour influence the likelihood of mechanical transmission of pathogens. Mobility and density of tabanids influences the probability of interrupted feeding, or taking partial feeds from a number of hosts. Persistence of feeding can be influenced by the age, nutritional state and reproductive status of the vector, in addition to inter-species variation (Eldridge and Edman, 2000).

Species of *Tabanus* found in Australia have similar behaviour to related species elsewhere in the world. Mackerras (1970) describes them as "robust, active, quick-moving and sometimes persistent in their attacks", although some, like *T. ceylonicus*, are described as "shy and relatively subtle in their approach". Many species will feed on people, but livestock are preferred and horses appear to be favoured over cattle (Mackerras, 1970). The locations where *Tabanus* appear to feed on horses are the nose, ears, rump and coronet. Mackerras observed that "most appear to feed at any time of day, but some may be crepuscular and a few northern species have been taken in houses at night, possibly being more attracted by the lights than the prospect of a blood meal" (Mackerras, 1970).

2.6.1.4 Factors influencing transmission probability

- Proximity of infected and susceptible hosts

  Regardless of the pathogen involved, the proximity of infected and susceptible hosts affects the probability of the mechanical transmission of agents. Foil (1983) designed a study to test the effect of spatial barriers on tabanid flight behaviour among horses. Flies were captured and marked, then released approximately 0.31m from the host. The change in the return rate of marked flies to the same host after

disturbance was measured, with increasing distances between alternate hosts. The number of flies returning to the host was proportionally related to distance between hosts. Distances of up to 36.6m between hosts were investigated in this study. At a distance of 36.6m 87.5% of tabanids returned to the same host. By extrapolation, it was determined that 99% of horse flies would be expected to return to the original host when separated as far as 49.3m from other hosts. This percentage would no doubt vary among different species of tabanids and in different environments (Foil, 1989). An additional study conducted in Brazil demonstrated that none of the 1274 tabanids captured transferred between animals separated by 50m (Barros and Foil, 2007). These findings indicate that spatial separation of 50m or more is an effective means of prevention of pathogen transmission.

Because *T. evansi* is transmitted mechanically by tabanid flies and the protozoa survives only a matter of hours on the mouthparts of the flies, only a single, interrupted blood-meal is capable of transmitting the disease. If tabanids will not fly more than 50m to complete a bloodmeal, then if host dispersal in such that there is greater than 50m between infected and non-infected animals the risk of transmission is greatly reduced. Given that livestock tend to group together, despite the extensive management systems such as those found in northern Australia, transmission is still likely to occur in these animals. It is possible that animals that tend to be widely dispersed and only come into contact with other animals for short periods of the day (such as at water holes), may be at a lower risk of becoming infected.

- Vector mobility and feeding persistence

Vector mobility is obviously important to the success of mechanical transmission and is directly related to host proximity (Foil, 1989).

"Feeding persistence" is defined as the tenacity with which different tabanid species pursue a single host until engorged. In field situations, the less persistent tabanid species are probably the most important in terms of mechanical transmission because

they more readily fly off to feed on different animals to complete the blood meal (Foil, 1989; Wall and Shearer, 1997).

Foil (1989) warned that laboratory transmission trials were often done with tabanid species which feed readily when trapped and brought into a laboratory environment. Such species are likely to be persistent feeders and are less likely to be important mechanical transmitters of disease in field situations. He noted that "such experiments simply demonstrate the potential of a pathogen being maintained on the mouthparts of tabanids long enough, and at high enough titres for mechanical transmission to occur" (Foil, 1989).

In his study on distance of re-location, Foil (1983) found that for flies that were marked but not disturbed, 3.2% of 750 flies dispersed to one of three other horses in a 9.1 metre square formation. Foil (1989) generalised that the larger the body size of the tabanid, the greater the likelihood of transfer between two host animals.

- Quantity of blood transferred

The quantity of bloodmeal residue that remains on mouthparts following an interrupted feed has been studied (Foil *et al.*, 1987). The amount of blood remaining on dissected mouthparts of *Tabanus fusciostatus* was estimated using an ELISA for equine IgG. Immediately after feeding it was found that there was a residue of approximately 10 +/- 5 nl remaining on the mouthparts. They estimated that 10% of the residue would be deposited during a second probe, meaning the range of deposition would be 10^{-6} to 10^{-5} ml. This estimate correlates well with actual transmission trials of Equine Infectious Anaemia Virus (EIAV) and Bovine Leukaemia Virus (BLV) using *T. fusciostatus* (Foil *et al.*, 1987).

The titre of infectious agent in the host is a key factor. For example in EIAV, when the titre reaches levels of 10^6 infectious doses per millilitre, a single tabanid can

transfer infection. In nature, seasonal changes in and persistence of circulating infectious agents are also important (Foil, 1989).

Similarly at least one in 10 tabanid flies can transmit infectious agents such as BLV when titres exceed 10^5 infectious doses per millilitre of blood (Eldridge and Edman, 2000).

The amount of infectious material deposited at the portal of entry is important and there have not been any studies with tabanids that adequately address this topic. Quantifying the percentage of natural regurgitative feeding of different insect species is an important area for future research (Foil, 1989). There have been no studies to determine whether tabanids regurgitate while feeding, however if this were the case, it would be useful to quantify the amount of blood that is involved.

Regurgitation is an important process in digestion for a number of flies e.g. *Musca spp*. Non-biting flies cannot consume solid food without first breaking it down using enzyme laden saliva. Blood sucking muscoids such as the stable fly regurgitate under artificial conditions, but no convincing studies support this as a natural means of pathogen transmission (Eldridge and Edman, 2000).

- Amount of pain perceived by the host during feeding

The amount of pain perceived by the host during feeding will influence the extent of host defensive manoeuvres and therefore the relative degree of feeding persistence (Foil, 1989; Eldridge and Edman, 2000). Most tabanids that feed upon tranquilised or restrained animals complete the blood meal, which is an important factor to consider in terms of experimental design. It has also been observed that older horses that have been exposed to heavy tabanid burdens for many years can actually sleep through thousands of tabanid bites. Host responses can actually be diminished during the acute stages of some infectious diseases, therefore this may be the most critical

time for transmission as pathogen titres are also highest during these periods (Foil, 1989).

- Site of feeding

Studies in Zimbabwe indicate that different tabanid species exhibit a preference for feeding on certain sites on a host (Kettle, 1995).
The preferred feeding site of a particular tabanid species on the body of the host can influence the persistence of feeding and hence opportunities for pathogen transmission. For instance, a feeding site on the back might limit the effectiveness of tail swipes from livestock (Eldridge and Edman, 2000).

- Host age

Host age also affects the tabanid burden of animals. Foals have as low as 1.4% of the tabanid burden of mares and a low incidence of EIAV infection in young horses has been attributed to this phenomenon (Foil, 1989). This was also found to be the case with cattle, in a study by Torr and Mangwiro (2000), who found that for cows and calves running together, cows are much more likely to be fed from by tsetse flies than calves. The authors postulated that cows were more attractive because of their increased size and decreased defensive behaviours compared with calves. This may partly explain why very young calves can survive long periods in tsetse-infected areas without contracting trypanosomiasis (Torr and Mangwiro, 2000).

- Host immunity

The prevalence of infection and immunity in a host population is another variable that must be considered when estimating the probability of the mechanical transmission of pathogens in nature (Foil, 1989).

Most horses with EIAV show no clinical signs of infection, but these animals serve as sources of infection for natural epizootics associated with tabanid population peaks (Foil *et al.*, 1984).

The role of host immunity on the outcome of contact with potentially infectious pathogens is important, but beyond the scope of this discussion which is focused on the role of the vector.

2.6.2 Epidemiology of vector-borne disease

The epidemiology of vector-borne disease is influenced by a number of factors in addition to those discussed above. These include abundance of vector species in relation to host density, the seasonal dynamics of vector populations, availability of breeding sites, climate and weather and how they relate to the life cycle of the vectors (i.e. if they are conducive to increased vector abundance).

2.6.2.1 Vectorial capacity

Vectorial capacity is defined as the average number of potentially infective bites delivered by all vectors feeding on a single host in one day. It describes the dynamic relationship between vectors of disease pathogens and their vertebrate hosts (Fine 1981 in Eldridge and Edman 2000). Reisen (1989) produced the equation shown below (Equation 1) as a formal description of vectorial capacity of arthropods. It has been applied primarily to mosquito-borne disease transmission, especially malaria, and assumes that an essential part of the pathogen life cycle takes place in the vector before a new infection can occur.

Equation 1:

$$C = \frac{ma^2VP^n}{-\log_e P}$$

Where:

C = Vectorial capacity, the number of infective bites received daily by a single host

m = Density of vectors in relation to density of hosts

a = Feeding habit (proportion of vectors feeding on a host divided by the length of the gonotrophic cycle in days)

V = Vector competence

P = Daily survival of vectors

n = Extrinsic incubation period

This equation takes into account the vector's susceptibility to infection and ability to transmit pathogens, which is known as the vector competence. Two lag periods are incorporated into the equation. The first is pathogen development within the vector, accounted for by the "extrinsic incubation period" which is the time from the uptake of a pathogen by the vector during a blood-meal, to the time a vector is capable of transmitting a pathogen. The second is the gonotrophic cycle of the vector, which determines when an infectious vector might next want to feed. The existence of these lags requires that the equation also needs to account for vector mortality (via the parameter P), which may be substantial.

In the case of mechanically transmitted pathogens such as *T. evansi*, the vector is immediately capable of transmitting the pathogen, and in fact is most effective at doing this within an hour of feeding from an infected host, due to the environmentally labile nature of the trypanosome. Hence the lags specified in Equation 1 do not apply. Mechanical vectors such as tabanid flies do not need to be susceptible to infection by the organism, so it is likely that "vector competence" relies on the size of the flies mouthparts and appropriate feeding behaviour i.e. regular switching between hosts.

Equation 1 could therefore be simplified to describe mechanical vectors as follows:

Equation 2:

$$C = taimV$$

Where:

t = tabanid fly density per host

a = feeding habit (bites per host per day per tabanid)

i = proportion of bites that are interrupted before a feed is completed

m = the proportion of interrupted feeds that result in a move to a new host

V = vector competence (the proportion of bites from infected flies which lead to a new infection)

However, consideration of the assumptions of Equations 1 and 2 suggests that equations of this form are unlikely to give realistic estimates of vectorial capacity for tabanids.

Some of the assumptions of Equation 1 are supported by available data: that all flies attempting a meal on a particular day, begin that day free of infection, as indicated by (Leclerq 1952 in Luckins 1998) and that an infected fly which bites an uninfected host can infect every subsequent host which it bites in the course of obtaining its meal. Nieshulz did establish that one *Tabanus* fly could infect three animals successively (Nieschulz in Dieleman, 1986), but it is unknown if this ability is widespread throughout the genus *Tabanus*. This equation, like its predecessor, also assumes that all flies which attack an infectious host become infectious. The variation in infectivity of these flies (conferred by variation in mouthpart size and feeding behaviour) is taken into account in evaluating their ability to transmit the disease i.e. their vector competence.

A more problematic assumption, however, is that Equation 2, like Equation 1, assumes that every host has an equal chance of being bitten. This is a defensible assumption in Equation 1, where the lag between infection and infectivity allows substantial vector movement. For tabanids, however the reality is certainly quite different, as infectivity is immediate and short-lived, so that host animals closer to an infected animal being

attacked by a tabanid are very much more likely to subsequently be bitten (and infected) than those further away.

Consideration of the assumptions of this family of equations suggests that more realistic descriptions of vectorial capacity for tabanids will need to take spatial pattern – in particular short-term tabanid movement, host herd sizes and host spacing behaviour - explicitly into account.

2.6.2.2 Host preference and host-feeding patterns

Haematophagous arthropods will often feed on certain host species, despite the availability of other vertebrate species in the area, a concept known as host preference (Eldridge and Edman, 2000). The relative temporal and spatial availability of hosts influences patterns of blood feeding by many insects. The term "host feeding pattern" has been used to describe the host selection by haematophagous arthropods at a given time and place. Host feeding patterns can be estimated by determining the host of origin of a blood-meal of some arthropods (Eldridge and Edman, 2000).

Different types of tests can be used to determine the host of origin of a tabanid blood-meal. Agar gel immunodiffusion (AGID), ELISA and molecular techniques have been used and different methods vary in terms of cost, sensitivity, ease of use and cross-reactivity. Caution should be exercised when interpreting the results of the host blood-meals. If an estimate of host preference is required, the relative density of different host species needs to be considered. A more sophisticated preference model involves consideration of three factors: (1) the temporal and spatial concurrence of hosts and vectors (2) the feeding success of vectors and (3) the relative body sizes of different hosts (Kay et al 1979 in Eldridge and Edman 2000). The complexity involved in estimating these factors concurrently means that host preference is exceedingly difficult to ascertain with any certainty.

Collection of samples for determining host feeding patterns also presents some problems. Common collection methods for tabanid flies involve the use of attractants (visual and olfactory) that are attractive to host-seeking female flies, which are likely to be looking for a blood meal. This may mean that flies collected in this manner contain small numbers of engorged females (Eldridge and Edman, 2000). In the absence of information on how long it takes for an engorged fly to digest a blood-meal, how often they seek hosts in order to feed and how many take partial blood-meals, it would be difficult to predict whether a particular type of collection technique or blood-meal assay would yield better results. Magnarelli and Anderson (1980) in Connecticut, found using capillary precipitin tests, that the prevalence of trap-caught tabanids (using dry ice baited canopy traps) that had consumed a partial blood-meal was 10% of 2559 tabanids caught,. Collection of samples using a method that does not select host-seeking females may improve the proportion of blood fed flies (Eldridge and Edman, 2000).

Sampling bias can also affect the results of analysis of host-feeding patterns. This may reflect a local concentration of a host species. Also, if the fly has ingested blood from more than one species, this may complicate the analysis (Eldridge and Edman, 2000).

Alternate methods for estimating host preference include field tests with animals semi-enclosed by electrified mosquito netting that kill flies on contact with the screen (Vale, 1974). Laboratory choice tests that employ the use of an olfactometer and observations of fly behaviour in response to odour cues from different animals can also be used.

2.6.2.3 Vector incrimination and vector competence

Vector incrimination is the term given to determining the relationship of a particular vector species or population with the transmission of a particular pathogen. Accurate determination of vectors is essential for appropriate vector control and to prevent and control disease (Eldridge and Edman, 2000). To date, little effort has been made to incriminate the vectors of surra in countries where it is endemic.

Vector competence is defined as “the ability of individuals in a population of arthropods to become infected by, and to transmit a given strain of pathogen”. Vectorial competence is an important component of vectorial capacity and is one of the underlying requirements of vector incrimination. Vector competence is estimated by infection and transmission experiments carried out in a laboratory (Eldridge and Edman, 2000). In the case of proving vector competence of Australian tabanids for *T. evansi*, high level containment, such as that at the Australian Animal Health Laboratory, Geelong, would be required.

2.6.2.4 Density of vectors in relation to density of hosts

The relative density of vectors to hosts is important to vectorial capacity, because it relates directly to the likelihood of vector-host contact (Eldridge and Edman, 2000).

Smaller tabanids move between hosts less frequently and individually transport less residual blood-meal. However, differences in population density may change the relative importance of different sized tabanid vectors. High density of smaller sized tabanids may be more important for disease transmission, in a particular region, than low density of large sized tabanids (Foil, 1989).

Foil (unpublished, cited in Foil 1989), throughout 10 years of study on Equine Infectious Anaemia Virus, has observed that although several tabanid species have been incriminated as vectors, the total tabanid population irrespective of species composition was the factor most consistently correlated with infection rates.

2.7 Surveillance for Arthropod-borne Diseases

A thorough knowledge of a disease’s epidemiology must underpin a surveillance system if it is to be useful for disease prediction. Eldridge and Edman (2000) have reviewed the requirements of surveillance systems for arthropod-borne diseases and the account below summarises their conclusions.

Various factors in nature, such as high vector population densities, may precede animal and human disease cases. Therefore, estimates of these phenomena can provide an early warning of outbreaks. Environmental factors such as weather patterns, which may influence vector abundance dynamics, may also be useful surveillance indicators.

Arthropod reproductive and developmental processes are intimately linked with certain weather factors. In temperate climates, increased temperatures are often associated with increases in vector populations. For vectors with aquatic immature stages, such as tabanids, a combination of warm temperatures and lots of standing water tend to favour increased rates of larval development (i.e. during the "wet season" in tropical north Queensland). Thus meteorological and environmental data that is related to increased vector abundance may be used to predict the probability of vector-borne disease. This data may b in the form of interpolated meteorological data (e.g. tempertaure, humidity or rainfall) or satellite-derived data (e.g. solar radition, Normalised Difference Vegetative Index or NDVI).

Meteorlogical and environmental data that are related to vector abundance can be incorporated into predictive or relative risk models using Geographical Information Systems (GIS). Understanding the elements that differ between outbreak years and non-outbreak years is critical and requires the analysis of historical data.

Periods of accelerated transmission of vector-borne disease (epizootics) are especially disruptive to animal populations. Epizootics are often related to changes in vector/ host populations which may be due to changes in weather or the biotic environment. In the tropics, transmission patterns may be consistently related to rainy seasons. Some epizootics occur in clusters, with several years between outbreaks. Reasons for unusual patterns such as outbreak clusters may include changes in herd immunity and/ or weather anomalies, e.g. El Nino years and cyclones.

Sampling of arthropods is often done in order to estimate vector density. This is because high vector densities are often associated with outbreaks of vector-borne disease. An important principal of systematic sampling is to use the same type of trap throughout the study to minimize the bias inherent in different trap types. Climate and environmental factors that are associated with the presence of vectors can also be used to map the potential distribution of a vector-borne disease using GIS to extrapolate this information to the wider area. This technique may be useful in countries where the distribution of a vector or disease is unknown, for example, where the remoteness of the region precludes extensive surveying. In such situations, findings from available surveys must be extrapolated to the wider region by the development of predictive models of either vector or disease distribution.

The accuracy of this technique needs to be taken into account however. Extrapolation assumes that vectors will respond in an identical way to their abiotic environment throughout the range of their distribution and that the processes that regulate their population size are also geographically uniform. These assumptions are more likely to hold true for species with a limited geographic distribution.

Arthropods may also be tested for the presence of pathogens using techniques such as microscopic examination or inoculation into microbiological media, tissue cultures or live animals. Immunoassays can identify pathogens from positive samples.

Surveillance for vector-borne disease will become more important in the future, as increased trade will increase the risk of entry of exotic pathogens. GIS based risk maps will enable useful predictions of potential future outbreaks of disease. Increased information on vector-pathogen-host interactions will improve the predictability of risk.

2.8 Conclusion

The following study aimed to address some of the knowledge gaps identified above. In particular, information was sought on the effect of weather on the seasonal dynamics of tabanids in Cape York Peninsula, northern Queensland. Knowledge of the temporal and spatial abundance patterns of tabanids in this region would contribute to our ability to assess the risk of surra incursion and transmission in Australia. GIS maps of tabanid abundance in northern Queensland would enable the incorporation of this information into a GIS-based predictive risk map for surra. Other aspects of tabanid behaviour and ecology that were examined were the responses of local tabanid species to trap and attractant alternatives, their daily activity patterns in relation to short-term changes in weather, the alighting and feeding behaviour of tabanids and the seasonal differences in tabanid body size.

CHAPTER THREE
TRAP AND ATTRACTANT COMPARISON

3.1 Introduction

It was envisaged that devices for trapping tabanids would be needed extensively throughout this project, in order to survey and collect tabanids for experiments. Therefore, it was important to assess the various methods of trapping, to determine the most appropriate methodology for the local conditions and tabanid populations of north Queensland.

The largest trapping study planned would involve a two year survey of tabanids in Cape York, far north Queensland. This area posed a number of challenges because of the remote location and the extreme weather conditions it can experience in the wet season, especially cyclones and heavy rain. The remoteness of the trapping sites in Cape York would necessitate the transport of all trap materials by light plane. Therefore, an efficient trap that was robust and easy to transport was required. Attractants would most likely increase the number of flies and diversity of species caught in the trap, but could compromise the ease of use of the trap in remote locations. Therefore, it was important to assess the difficulties and benefits of using attractants.

In addition, experiments were planned that required large numbers of tabanids for local studies on daily activity patterns and feeding behaviour of tabanids in the Townsville region. For these experiments, issues such as ease of transportation and frequent monitoring of attractant emissions were not as problematic, but the most effective trap and attractant combination was needed to maximize trap catches with the widest range of species.

Most traps primarily attract host-seeking female tabanids. Although trap catches do not directly measure the likely tabanid burdens on animals, in general trap catch numbers may be correlated with overall tabanid burdens. Leprince and co-workers (1992; 1994)

found that the parity rates and proportions of different species of trap-caught tabanids and those caught using an animal bait did not differ significantly, so they are probably representative of the same population. However, the present study did not seek to make a comparison between trap catches and tabanid burdens on animals, but merely used the traps as a sampling device.

A number of trapping methods have been used to monitor numbers and species of tabanids. Different trap types may capture different numbers of individuals and species (Roberts, 1972; Roberts, 1976; Mihok *et al.*, 2006).

Tabanids are known to be attracted to blue and black objects, although the ecological basis for this is not clear (Gibson and Torr, 1999). In addition, quite subtle variations in blue colour can have a substantial effect on the attraction of biting flies to traps (Mihok *et al.*, 2006). The effectiveness of visual traps for tabanid surveys and control purposes seems to depend on the trap's contrast with its background, which may be why certain colours and silhouettes are attractive (Allan and Stoffolano, 1986).

Canopy traps such as the Manitoba trap (Thorsteinson, 1965) and modifications of it (Thompson, 1969b; Catts, 1970; Adkins, 1972; Hribar *et al.*, 1992) are quite cheap to construct and are lightweight and easily transported. They are often used with a black sphere suspended beneath the canopy as a visual decoy. The black ball enhances trap catches by acting as a visual decoy for tabanids, simulating a host animal from a distance. It has been used successfully by several researchers including Thompson (1969b), Roberts (1977) and Schreck and co-workers (1993). Canopy traps are good for surveys and epidemiological studies, but are not very effective without the use of an attractant (Foil and Hogsette, 1994). This type of trap had previously been used in Australia by Spratt (1972) and Holland (1997). It had generally been used with dry ice as an attractant and appeared to be reasonably effective. However, no comparison with other trap types had been made in Australia.

The Nzi trap (Mihok, 2002) was developed in Kenya for trapping biting flies. It is economical, strong and captures a variety of biting flies, including tabanids. It uses coloured panels of blue and black fabric to attract tabanids. It has been used with excellent results in many tropical areas, including Burkina Faso (Desquesnes and Dia, 2003) and Kenya (Mihok, 2002), but had not been previously tested in Australia.

Olfactory stimuli, also known as host kairomones, are used by tabanids to locate hosts from a considerable distance (Vale and Phelps 1974 in Gibson and Torr 1999). These researchers also found that tabanids moved upwind in response to host odours (Vale and Phelps 1976 in Gibson and Torr 1999). Olfactory stimuli, such as carbon dioxide and octenol, have therefore been used as attractants to improve trap capture rates, but relative responses to different attractants may vary among species (Leprince *et al.*, 1994).

Octenol is a component of ox breath that acts as an olfactory stimulant for a number of haematophagous insects, including tabanids, and can significantly increase trap catches, depending on the tabanid species (French and Kline, 1989; Hribar *et al.*, 1992).

Carbon dioxide in the form of dry ice was originally reported to be effective in attracting tabanids by Wilson *et al.* (1966 in French and Kline 1989). CO_2 is also effective when released from cylinders as demonstrated by Roberts (1970). Since then it has been extensively used as an effective attractant for Tabanidae (Roberts, 1977; Leprince *et al.*, 1994; Gibson and Torr, 1999).

Because different tabanid species may respond differently to traps and attractants, monitoring techniques appropriate to the local tabanid community and the logistical requirements of collection needed to be determined.

Therefore, this study compared the canopy and Nzi traps, in conjunction with attractants that had been used successfully elsewhere, to determine the most appropriate combination for future use in the course of this research.

3.2 Materials and Methods

3.2.1 Traps

The two trap types compared were Nzi traps (Mihok, 2002) and canopy traps, similar to the design described by Thorsteinson (1965).

Nzi traps, 1-metre triangular cloth traps, were constructed as directed by Mihok (2001) (Figure 3.01). The fabric used in their construction was Sunbrella Pacific Blue and Sunbrella Black, robust awning fabric that is designed for prolonged use outdoors, with white polyester mosquito mesh (Mihok *et al.*, 2006). The collection apparatus at the apex of the trap consisted of a clear plastic bottle and plastic bag, as described by Mihok (2001).

The only modification to the original design was that webbing ties were sewn onto the corners of the trap and used to secure the trap to steel fencing posts, whereas on the original design, sleeves of material were used. The traps were made by Grid North (specialist equipment manufacturing shop, Hermit Park, Townsville) according to Mihok's specifications.

Canopy traps, were constructed from four pieces of two metre long dowel in a four-sided pyramidal structure with white polyester mosquito mesh enclosing the top half (Figure 3.02). An inflatable plastic ball (61cm diameter) painted glossy black, was suspended beneath the canopy. A collection container made from a circular wire frame with mesh covering was placed at the apex of the trap.

Once tabanids are attracted towards the trap and are under the canopy, they are attracted upwards towards the collector at the apex by positive phototaxis.

Figure 3.01: Photo of Nzi trap

Figure 3.02: Photo of canopy trap

3.2.2 Attractants

The attractant treatments that were compared were:

- control (no attractant)
- octenol only
- carbon dioxide gas only and
- a combination of octenol and carbon dioxide gas.

Octenol lures (Biosensory Inc., Willimantic, CT, USA) were used in preference to liquid octenol due to ease of handling and longer duration of activity (30-60 days, depending on temperature). The lures contained octenol (1-octen-3-ol, 24.5% octenol, 3.72g octenol per strip) in a wax base to provide an even release rate.

Carbon dioxide gas was released from cylinders through 5mm diameter plastic tubing at a rate of 1 litre per minute via a regulator and then a flow meter (Roberts, 1975).

3.2.3 Study site

The study was conducted on grazing land, consisting of eucalypt-dominated savannah vegetation, adjacent to the School of Veterinary and Biomedical Sciences, James Cook University at Townsville, Queensland. There is no permanent water nearby, but there is an ephemeral creek within 500 metres of the study site. Townsville is located in the dry tropical region of north Queensland, Australia (latitude 19˚17'S, longitude 146˚48'E). The average annual rainfall is 1143mm, most of which falls during the "wet season" from November to April.

Four trap sites were set up, each containing a pair of traps (one canopy and one Nzi trap), one site for each attractant treatment. Attractant treatments were rotated among sites, as described later (Table 3.01). Sites were at least 218 metres apart, and the two traps at a site were approximately 30 metres apart. Around each trap, an area approximately 20 metres in diameter was cleared of grass. The sites were aligned along a line running NW to SE, so that each site was at right-angles to the prevailing north-

easterly wind (Figure 3.03). This alignment was chosen to provide the best opportunity for tabanids to encounter the traps when flying upwind in response to odour cues, as had been found by Phelps and Vale (1976). The octenol strips and the CO_2 outlet were placed on the north-easterly side of the trap.

The positioning of the trap pair was alternated between the four sites. At sites one and three, the Nzi was in the south-easterly position of the pair, whereas for sites two and four, the canopy trap was in the south-easterly position. This configuration was chosen so that if the wind direction was other than from the north-east, one trap would not be favoured over the other.

The sites were chosen to be as uniform as possible, free of overhead branches of trees, and in open country. Site three had several trees close by, whereas the other sites had no nearby trees. No attempt was made to place traps at the edge of a wooded area or at the foot of a hill. Some authors have described higher catches in such areas (Sheppard 1977), but there are probably species-specific differences in tabanid preference for wooded versus cleared land (Thornhill and Hayes, 1972; Sheppard, 1977). No such data are available for Australian species of Tabanidae.

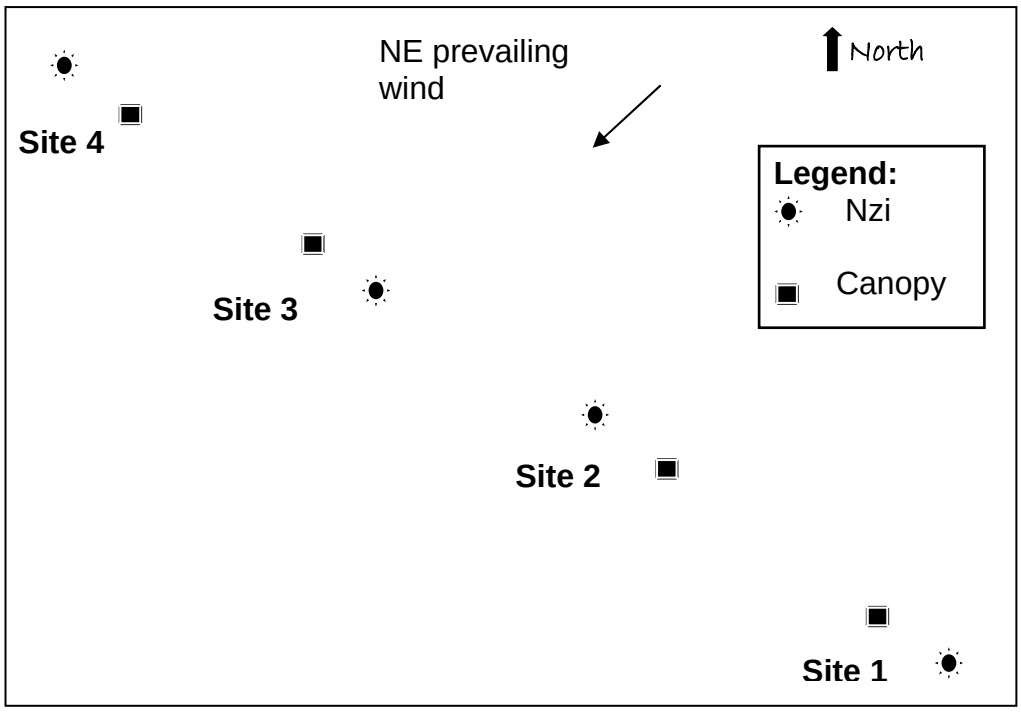


Figure 3.03: Layout of traps in relation to prevailing wind (not drawn to scale). Traps within a pair are 30m apart, sites are 218m apart.

3.2.4 Study design

The study was run for approximately four weeks, from February to March, 2004. Each trapping week included a four day trapping period and a three day 'rest' period (to avoid "trapping-out" tabanid populations). In each trapping period, the same attractant treatment was used with both traps in each pair for a period of four days, after which time, the attractant was rotated, while the traps remained at that site (Table 3.01). Thus by the end of the study, each attractant treatment had been used once at each of the four sites, so that any site bias did not affect the treatment comparisons.

The collection containers were placed on the traps and the CO_2 was turned on at 9am each morning. The CO_2 was turned off and collection containers removed each afternoon at 5pm. The octenol strips were left in place between collecting periods. Care was taken to ensure all traps were empty of tabanids prior to attaching collectors in the morning.

At the time of collection, any tabanids that were still in the trap canopy, but not in the collection container, were captured using a battery-powered handheld vacuum cleaner, with a small sleeve on the front to collect the tabanids. Following each daily collection period, containers of tabanids were labelled and placed in a freezer (-20˚C) overnight and the contents counted and identified the following day. Collections were not made on days that were very overcast or rainy, in order to reduce the variation in numbers caught due to inclement weather. Octenol strips were replaced 3.5 weeks after they were first unwrapped and 2.5 weeks after the start of the experiment, well within their effective period. Tabanids were identified using keys by Mackerras (1959; 1961; 1970) and Mackerras and Spratt (unpublished).

Table 3.01: Sequence of attractant rotations at each site

Site	**GPS co-ordinates**	**Attractant Rotation**			
		Week 1 **6th, 9th-11th February 2004.**	**Week 2** **16th -19th February 2004.**	**Week 3** **23rd -26th February 2004.**	**Week 4** **1st , 3rd -5th March 2004**
1	M1: 19° 19' 48.8" S 146° 46' 00.8" E	CO_2 + octenol	CO_2	Control	Octenol
	N1: 19° 19' 49.5" S 146° 46' 01.6" E				
2	M2: 19° 19' 42.0" S 146° 45' 58.9" E	Control	Octenol	CO_2 + octenol	CO_2
	N2: 19° 19' 41.0" S 146° 45' 58.5" E				
3	M3: 19° 19' 33.1" S 146° 45' 55.1" E	CO_2	CO_2 + octenol	Octenol	Control
	N3: 19° 19' 34.0" S 146° 45' 55.5" E				
4	M4: 19° 19 25.3" S 146° 45' 52.3" E	Octenol	Control	CO_2	CO_2 + octenol
	N4: 19° 19 24.3" S 146° 45' 52.0" E				

3.2.5 Statistical analysis

The statistical significance of differences observed between numbers of tabanid flies (summed over four day periods) collected using the different trap and attractant types was determined in two ways: with a Mann-Whitney non-parametric test to compare trap types for each attractant separately (and vice versa), and with a two-way fixed factor analysis of variance using log-transformed counts (which successfully stabilised the variance and normalised the distribution). Because attractant location and week are unavoidably confounded by the study design, it is not possible to do a four-way fixed

factor ANOVA. In each case the sample size (i.e. number of trap-days) is 128. Statistical analysis was performed using SPSS (SPSS Inc., version 12.0.1).

3.3 Results

3.3.1 Trap and attractant efficiency

The traps caught 1780 tabanids over the period of the study, all but two of them female. The two males caught were a *Tabanus townsvilli* Ricardo, and a *Pseudotabanus spp*. (included below grouped with the unidentified species).

The most abundant of the 17 species caught during the trapping period was *Tabanus townsvilli* Ricardo, which comprised 21.4% of the total. Other abundant species were *Tabanus pallipennis* Macquart (19.6%), *Pseudotabanus silvester* (Bergroth) (15.5%), *Dasybasis clavicallosa* (Ricardo) (15.1%) and *Tabanus strangmannii* Ricardo (15.1%).

Other species trapped in lower numbers include: *Tabanus dorsobimaculatus* Macquart (5.2%), *Tabanus notatus* Ricardo (2.1%), *Tabanus parvicallosus* Ricardo (1.8%), *Pseudotabanus distinctus* Ricardo (1.3%), *Tabanus obscurilineatus* Taylor (1.0%) and *Lilaea fuliginosa* (Taylor) (0.9%).

Rare species that were not statistically analysed on an individual basis were *Tabanus concolor* Walker (2 specimens), *Tabanus particaecus* Hardy (1), *Dasybasis oculata* (Ricardo) (1), *Cydistomyia brevior* (Walker) (2), *Cydistomyia musgravii* (Taylor) (2) and unidentified (7). This group also includes a distinct but unidentified species of *Tabanus* (3).

A complete list of species caught is provided in Appendix 1.

3.3.1.1 Effects of trap type on capture rates

Both trap type and attractant treatment had substantial effects on the total capture rates (Figure 3.04).

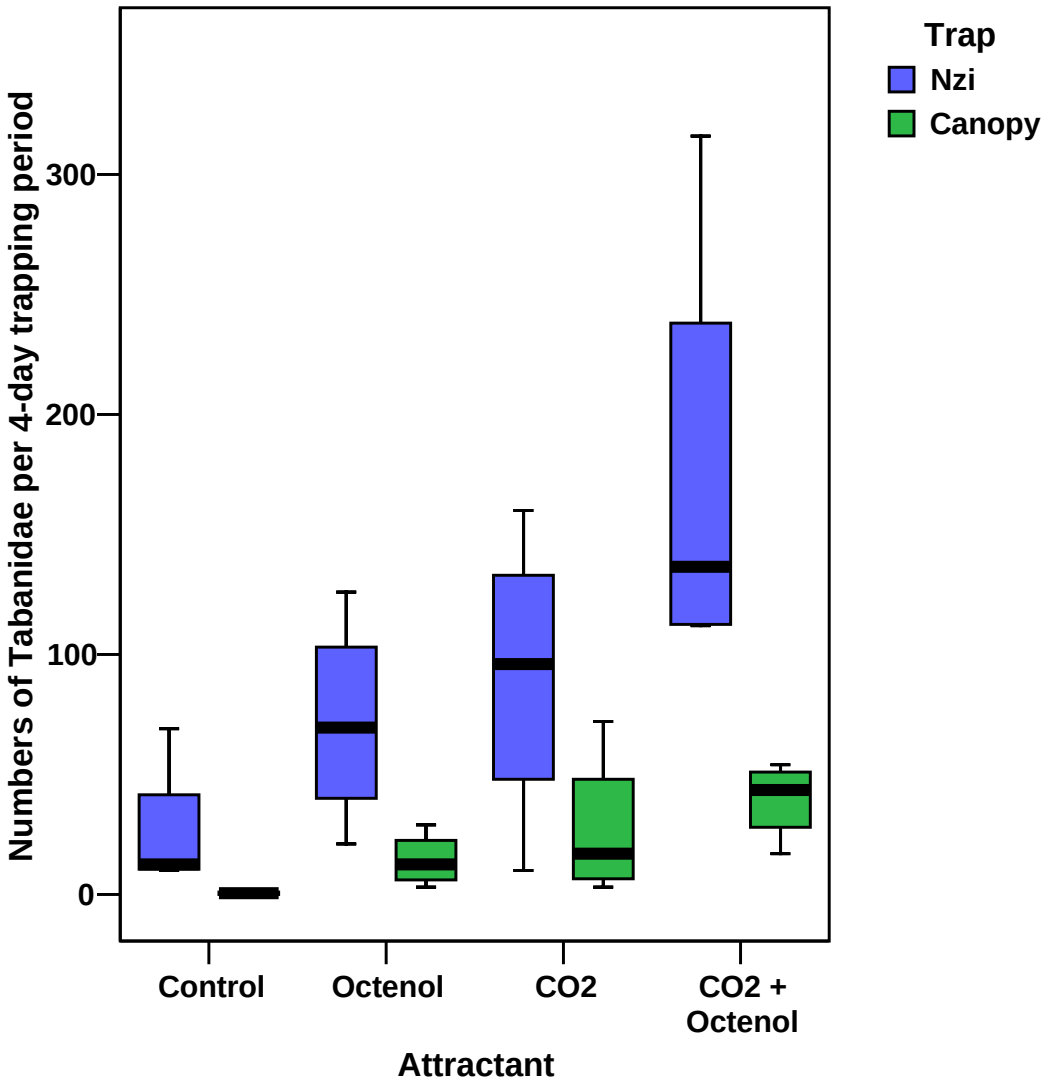


Figure 3.04: Total Tabanidae capture rates for each trap and attractant combination per 4-day trapping period.

The Nzi trap consistently caught more individual tabanids than the canopy trap (Figure 3.04). Averaged over attractant types, Nzi traps averaged 90.8 tabanids per trapping period (four days), whereas the canopy traps averaged 20.4 tabanids per trapping period ($F = 34.98$, $df = 1x\ 24$, $p < 0.0005$). There was no interaction between trap type and

attractant type: that is, the difference between trap types (for log-transformed data) was similar for all attractant types ($F = 0.97$, df = 3x 24, $p = 0.43$).

Table 3.02 shows the average capture rates of each of the 11 most common species for the two trap types. For seven species, capture rates were significantly higher in the Nzi trap. For three species there was no significant difference in average capture rate between trap types, and for one species (*T. obscurilineatus*, the least common of the 11), average capture rates were significantly higher in the canopy trap.

Table 3.02: Average capture rates of the two trap types per four day trapping period for each of the most common species trapped. Asterisks indicate those species for which the difference in capture rates between trap types achieved statistical significance using ANOVA.
NB:* P = 0.01- 0.05, ** P = 0.001- 0.01, *** P < 0.001.

Species	**Mean number of each species caught per four days of trapping (± standard deviation)**	
	Canopy Trap	Nzi Trap
T. townsvilli ***	2.3 (± 3.4)	21.5 (± 20.6)
P. silvester ***	0.5 (± 0.7)	16.7 (± 18.8)
D. clavicallosa ***	1.8 (± 3.8)	15.1 (± 18.2)
T. strangmannii ***	2.1 (± 3.1)	14.6 (± 20.1)
T. pallipennis	9.3 (± 11.4)	12.5 (± 15.1)
T. dorsobimaculatus	2.1 (± 3.8)	3.6 (± 6.1)
T. notatus *	0.1 (± 0.3)	2.3 (± 5.6)
P. distinctus **	0.1 (± 0.3)	1.4 (± 1.9)
T. parvicallosus	1.1 (± 2.6)	0.9 (± 1.8)
L. fuliginosa **	0.1 (± 0.3)	0.9 (± 1.2)
T. obscurilineatus *	0.9 (± 1.4)	0.2 (± 0.8)
TOTAL	20.4 (± 22.3)	90.8 (± 79.4)

3.3.1.2 Effects of attractant treatment on capture rates

Averaged over trap types, the attractant treatments all caught significantly more tabanids than the controls ($F = 14.37$, df = 3 x 24, $p < 0.0005$). Figure 3.04 shows that for the total catch, the combination of octenol and CO_2 was the most successful of the attractants. Carbon dioxide alone was the next best, followed by octenol and then no attractant. Table 3.03 shows that this ranking is followed by all individual species except *T. obscurilineatus*, *P. distinctus* and *P. silvester*.

Without use of an attractant, the Nzi trap caught a mean of 26 tabanids over four days of trapping while the canopy trap caught a mean of 0.5 over the same period. The Nzi trap without attractant caught 9 of the 11 most common species trapped during the experiment (those not caught were *T. parvicallosus* and *T. obscurilineatus*), while the control canopy trap caught only one of the 11 most common species trapped (*T. pallipennis*).

Table 3.03: Average capture rates of each of the 11 most common species for each attractant treatment (standard deviations in brackets). Asterisks indicate those species for which the difference in capture rates between attractant treatments achieved statistical significance. NB:* P = 0.01- 0.05, ** P = 0.001- 0.01, *** P < 0.001.

Species	**Mean number of each species caught per four days of trapping (± standard deviation)**			
	No attractant	Octenol	CO_2	CO_2 + octenol
T. townsvilli **	2.0 (± 2.6)	7.1 (± 10.9)	15.4 (± 20.3)	23.1 (± 22.7)
T. pallipennis ***	0.8 (± 0.7)	8.3 (±7.2)	11.9 (± 16.2)	22.6 (± 13.5)
D. clavicallosa **	1.3 (±2.4)	5.4 (±9.3)	10.0 (± 14.0)	17.0 (± 22.3)
T. strangmannii	3.0 (± 6.2)	6.6 (± 6.9)	7.5 (± 8.1)	16.4 (± 28.3)
P. silvester	5.0 (± 12.2)	12.3 (± 22.0)	5.4 (± 9.6)	11.8 (± 16.6)
T. dorsobimaculatus **	0.1 (± 0.4)	1.4 (± 1.4)	3.4 (± 6.8)	6.6 (± 6.3)
T. notatus	0.1 (± 0.4)	0.8 (± 1.2)	0.8 (± 1.4)	3.1 (± 8.0)
T. parvicallosus *	0.0 (± 0.0)	0.1 (± 0.4)	1.9 (± 3.6)	2.0 (± 2.1)
P. distinctus	0.5 (± 0.9)	0.3 (± 0.7)	0.5 (± 1.1)	1.8 (± 2.4)
T. obscurilineatus	0.0 (± 0.0)	0.3 (± 0.7)	1.1 (± 1.4)	0.9 (± 1.7)
L. fuliginosa	0.3 (± 0.7)	0.3 (± 0.5)	0.8 (± 1.4)	0.8 (± 1.2)
TOTAL	13.3 (± 23.2)	42.9 (± 42.6)	58.9 (± 56.7)	107.4 (± 96.8)

3.3.2 Effects of trap type and attractant on the species richness of the catch

The Nzi trap caught a greater range of species than the canopy trap for each of the attractant treatments (Figure 3.05). In general, patterns of species richness follow the patterns in total abundance for each trap and attractant combination. The lowest species richness was caught using the control canopy trap (only one species, *T. pallipennis* was caught), while the highest species richness was caught with the Nzi trap using the combination of octenol and carbon dioxide as an attractant (16 species).
The only species not caught by the combination of the Nzi trap with octenol and CO_2, was *T. obscurilineatus*. Eighteen specimens of *T. obscurilineatus* were caught in total. Only three of the 18 were caught in Nzi traps (all using CO_2 as an attractant). The remaining 15 were caught with canopy traps using octenol (two specimens), CO_2 (six specimens) and combined CO_2 with octenol (seven specimens).

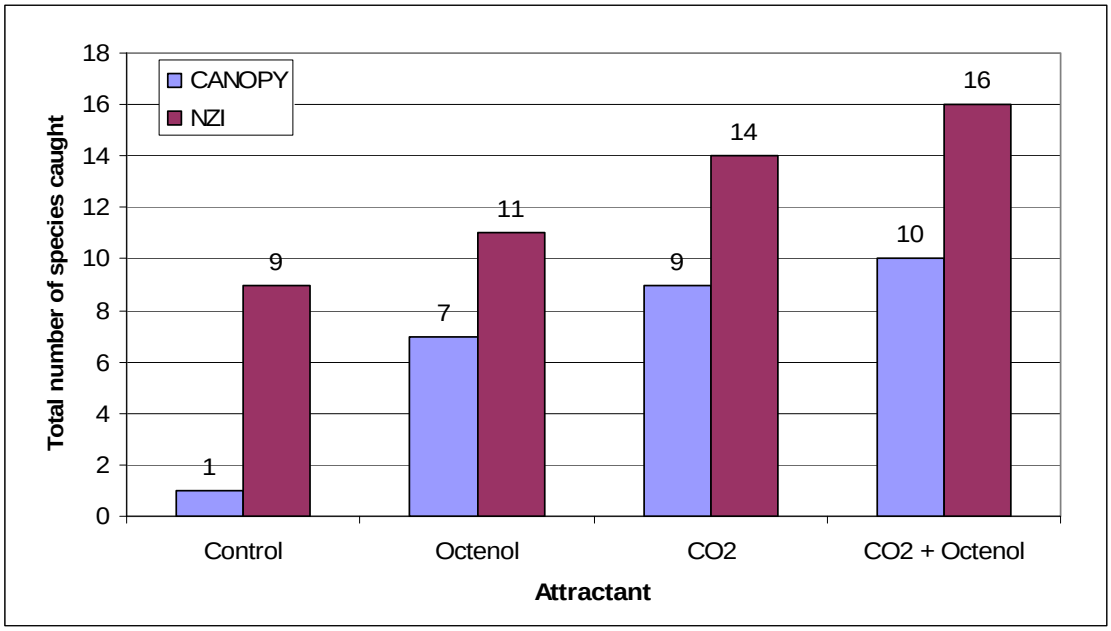


Figure 3.05: Total species richness for each trap and attractant combination.
Note: No error estimation is provided as these figures are totals overall.

Each trap and attractant combination moved to a different site every four days. The total number of species caught in that four day period is represented in Figure 3.06.

On average, approximately two-thirds of the overall total number of species for a particular trap and attractant combination were caught during a four day trapping period.

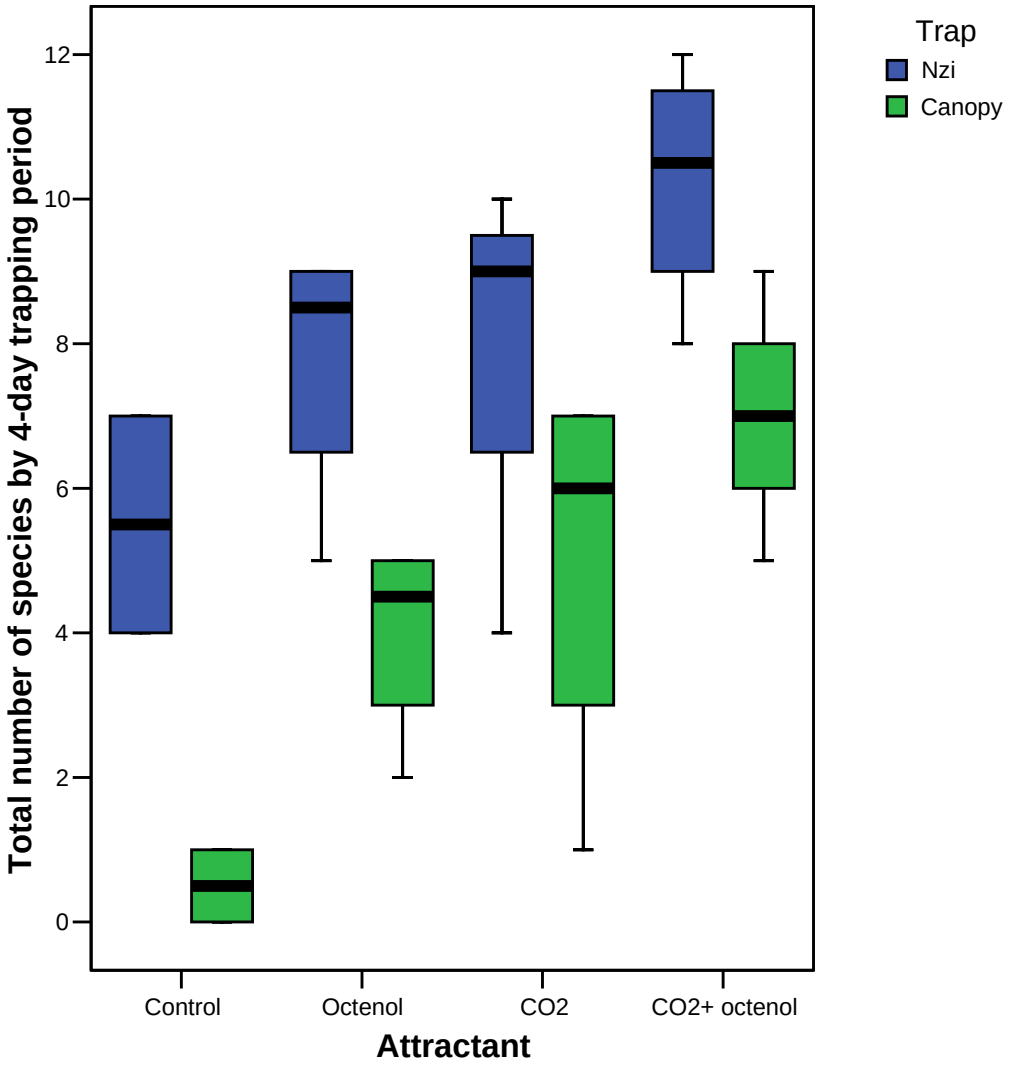


Figure 3.06: Total number of species caught with each trap and attractant combination per four-day trapping period.

3.3.3 Site and week effects

There was some variation in tabanid abundance between sites and weeks in the study, but their effects were small: trap type and attractant type accounted for 77% of the total variance in the total catch. Moreover, for every site individually, and in every week, the Nzi trap performed better than the canopy trap, and traps using attractants performed better than those without attractants.

The effects of site and week cannot be separated with certainty, as the experimental design does not allow this. In addition, sites changed differentially over time in the experiment due to grass growth, which may have obscured traps and affected trap catch. This was not predicted in the experimental design. This means that any apparent site effect may be a week effect and vice versa. This should be kept in mind when referring to Figure 3.07, which shows fluctuations in trap catch over the course of the study.

3.3.3.1 Fluctuations in trap catch over time

The total numbers of Tabanidae caught in traps fluctuated over the four weeks of the study (Figure 3.07). The highest numbers were caught on the first day of collection, and gradually decreased to a low on collection day nine (in week three). Tabanid numbers increased again thereafter.

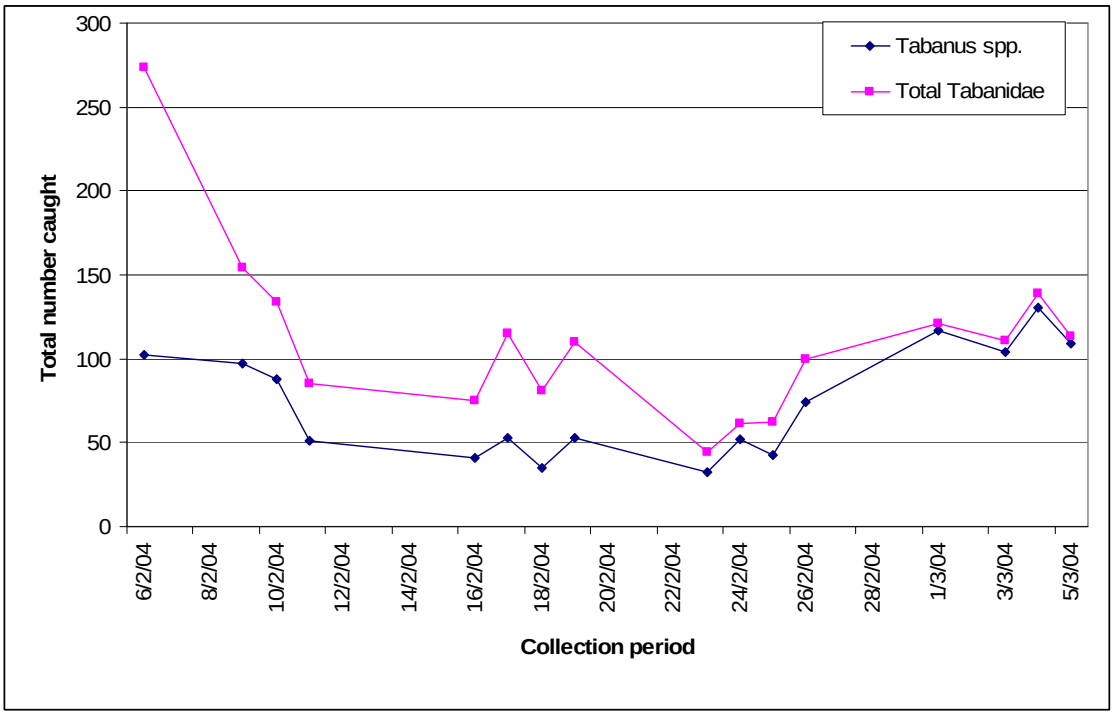


Figure 3.07: Fluctuations in numbers of total Tabanidae and *Tabanus spp*. collected over the course of the study.

3.3.3.2 Effect of local vegetation on trap site

The four trap sites were contained within an area of several square kilometres, so it was not envisaged that they would be substantially different from each other, in fact they were chosen to be as uniform as possible. However there were some obvious differences in sites due to the variation in local vegetation, which may have influenced trap catch.

Site three had some trees nearby which may have shaded the traps at certain times of the day. Also, the rapid growth of tall grass over the course of the study had obscured site four by the final week of collection. Other sites were not affected as greatly by the grass growth and this may have introduced a site effect that became more influential over time. However, the effect of site cannot be examined in more detail as previously mentioned.

3.4 Discussion

The results of the trap and attractant comparison study may be summarised as follows: The Nzi trap caught more tabanids than the canopy trap. The Nzi trap caught a greater number of species of tabanid. Seven of the 11 most common species were caught in higher numbers in the Nzi trap, three were caught in comparable numbers in both trap types, and one species was more likely to be caught in the canopy trap. The Nzi trap performed better in all attractant treatments. Use of attractants improved both capture rates and the species richness of the catch, for both trap types. The attractant treatment that caught both the greatest number of individual tabanids and the greatest number of species was the combination of CO_2 and octenol, followed by CO_2, then octenol, then the control.

This study demonstrates that whether or not attractants were used, the Nzi trap caught more tabanids and a more diverse range of species of tabanids, compared with the canopy trap, in Townsville, Queensland. This finding agrees with that of Mihok and co-workers, who tested the Nzi trap at various sites in Canada and the USA, and found it caught five times more tabanids than the canopy trap (Mihok *et al.*, 2006). However these authors noted differences in the relative performance of the traps among tabanid species and geographic locations, as had been recorded by Roberts (1972; 1976).

The Nzi trap caught all of the 17 species trapped during this study when results were averaged over attractant types, while the canopy trap caught only 11 species. The Nzi trap also caught significantly more of seven common species of tabanid than the canopy trap (*T. townsvilli*, *P. silvester*, *D. clavicallosa*, *T. strangmannii*, *T. notatus*, *P. distinctus* and *L. fuliginosa*). For three species (*T. pallipennis*, *T. dorsobimaculatus* and *T. parvicallosus*), there is no significant difference in average numbers trapped between trap types and for one species (*T. obscurilineatus*), average capture rates were significantly higher in the canopy trap.

Most of the tabanid species caught during this study are widely distributed in northern Queensland, so these data suggest that Australian tropical tabanid species assemblages may be more readily monitored with Nzi traps than with canopy traps.

Use of attractants certainly improved capture rates and the species richness of the catch, when averaged over trap types. The most effective attractant was the combination of CO_2 and octenol. It caught the highest numbers and the most species of tabanids. For the 11 most common species trapped, the combination of CO_2 and octenol caught the highest numbers, followed by CO_2, octenol and no attractant. The only species that did not follow these rankings were *T. obscurilineatus*, *P. distinctus* and *P. silvester*. In none of these three was the difference in catch rate between attractant treatments statistically significant. *T. obscurilineatus* and *P. distinctus* were caught in relatively small numbers but *P. silvester* was a common species and the data suggest that if it responded at all to attractants, the response was to octenol alone. This indicates that if *P. silvester* were the primary focus of monitoring, octenol may be the attractant of choice.

For studies which need to detect smaller changes in abundance, or which need to detect rarer species, the combined attractant should be used. These findings are in general agreement with those of French and Kline (1989) and Hayes and co-workers (1993). Holland (1997) also found a significant increase in tabanid catch with use of dry ice as an attractant, when it was used with canopy traps in Townsville. In addition, increasing the rate of CO_2 release above one litre per minute might improve capture rates as previously described by Roberts (1975). The rate of octenol release could be increased as well, by dispensing liquid octenol from a vial, as described by French and Kline (1989).

The Nzi trap with the combination of CO_2 and octenol caught greater numbers of tabanids than any other trap and attractant combination, and the greatest range of tabanid species (16 of a total of 17 species). This trap and attractant combination is best for situations where very large numbers of tabanids are required, for example when they are

required for studies of daily activity patterns, to achieve statistically significant results during a relatively short flight season.

The only species not caught with the Nzi trap and combination of octenol and CO_2 was *T. obscurilineatus*. Only 18 specimens of *T. obscurilineatus* were caught during the study, three in an Nzi trap with CO_2 and the remainder in the canopy trap. The results indicate that the canopy trap and the combined attractant (CO_2 and octenol) is the most effective combination for trapping *T. obscurilineatus*.

For long-term monitoring, especially in remote areas, it may not be feasible to use attractants because it is difficult to ensure a consistent rate of emission of attractants over time and transport of CO_2 cylinders is costly and difficult. If attractants are not used, this study suggests the Nzi trap should still provide a satisfactory monitoring mechanism for common species. If a species can be trapped with attractants, it is very likely that an Nzi trap without attractants will also catch it, albeit in smaller numbers. This result is consistent with experience elsewhere: the Nzi trap has been used to catch a variety of haematophagous Diptera in Kenya, Canada and a number of other countries, without the use of an attractant (Mihok 2002, 2006).

By contrast, the canopy trap without attractants caught very few tabanids and only one species, and clearly is not suitable as a monitoring device in this region. The canopy trap has been previously used in Australia for trapping Tabanidae (Spratt, 1972; Holland, 1997), but the present study suggests that it is not effective without the use of an attractant. This finding is in agreement with Foil and Hogsette (1994).

Mihok (2001; 2006) described catches of tabanids in Canada up to 67 per day and in Kenya, Burkina Faso and the USA, hundreds of tabanids have been caught daily using the Nzi trap. Daily tabanid catches during this study were generally lower than these (range of 1 – 103 overall, or 1 – 34 without use of attractant). The apparently lower tabanid density in Townsville might be due to climate and vegetation-related factors in

the region. Greater numbers have been caught in higher rainfall areas of Queensland to Townsville's north (van Hennekeler, unpublished data).

The orientation of the Nzi trap may also have an effect on the numbers of tabanids caught. Mihok (2006) found that a west-facing trap caught more tabanids than east-facing, which he had originally chosen after considering earlier findings by other researchers, including upwind movement in response to odour plumes (Vale and Phelps 1976 in Gibson and Torr 1999) and predominantly westerly winds in the region. Mihok suggests this was due to the effect of the afternoon sun on the trap face inducing larger numbers of tabanids to investigate the trap. In the present study, the traps faced towards the south-west, for the same reasons provided by Mihok (and north-easterly prevailing winds). Thus the afternoon sun would have fallen on the trap face, at least partially. This orientation may have favoured those tabanids that are more active in the afternoon, versus those active in the morning, when the trap face may have been partially shaded. The daily activity patterns of local tabanid species is covered in more detail in subsequent chapters.

The effects of trap site and the week of collection could not be examined separately, as referred to earlier in the results. However, it is possible that the fluctuations in the numbers of tabanids caught over time were real, and were the result of meteorological factors, such as rainfall, influencing the total abundance of tabanids in the area (Gibson and Torr, 1999). Meteorological effects on tabanid numbers collected in traps are examined in more detail in subsequent chapters.

It is also possible that the effects of site were genuine, and were due to the proximity of local vegetation, especially the obscuring effects of grass, which grew taller over the course of the study. The presence of local vegetation can alter trap catches, for example, by nearby trees shading traps, or long grass and shrubs obscuring trap entrances and decreasing the visual attraction of the trap (Foil and Hogsette, 1994). Visual stimuli are important for precise location of a host and are likely to play a role in the success of tabanids in locating a trap (Vale and Phelps 1974 in Gibson and Torr 1999). However,

even if the visual attraction of the trap were diminished, the attractants would still be effective to some extent, because they influence the host-seeking behaviour of tabanids from a greater distance (Vale and Phelps 1974 in Gibson and Torr 1999). Future studies on the effect of local vegetation on trap catch would serve to elucidate the nature and magnitude of this effect. Another source of potential site bias is that the "best" treatment may have unintentionally been applied to the "best" site in the first week. However it is not possible to test this possibility, because of the study design as described previously.

Comparisons that have been conducted elsewhere show that some trap and attractant types can offer higher catches with greater species richness than others. However, no comparison of trapping methods, using readily available materials, had been done under Australian conditions. This study suggests, that for Australian tropical tabanids, the combination of the Nzi trap with CO_2 and octenol is best for intensive studies where large numbers of individuals and a large range of species are required. The Nzi trap without any attractant is useful for long-term tabanid surveillance in remote locations, as may be required for monitoring potential vectors of surra in northern Queensland. An additional advantage of the Nzi trap is that its robustness makes it ideal for use in the tropics of Australia.

CHAPTER FOUR
DAILY ACTIVITY PATTERNS OF TABANIDS IN TOWNSVILLE

4.1 Introduction

This study aimed to determine the daily activity patterns of tabanids in Townsville, and whether there was any evidence of inter-specific or intra-specific variation in fly activity.

This information is required to design future experiments on feeding behaviour, as well as to identify the periods when risk of pathogen transmission is highest.

Blood-feeding diptera have developed strategies to help them locate hosts, which are mobile and often difficult to find. They set a course in relation to environmental factors, such as wind direction, in a way that maximises their chances of encountering host cues while actively ranging. Their search tactics are species-specific, and may include the use of visual, tactile, auditory, and especially olfactory cues, often simultaneously (Gibson and Torr, 1999). Different species may therefore exhibit variation in their responses to the same set of environmental conditions, and may have different daily patterns of activity.

A study of tabanid feeding activity on cattle in Uganda indicated that most Ugandan species observed (15 of 19 species) were active during daylight hours, with peak activity between 11am and 2pm. However, four species of *Haematopota* had a bimodal pattern of biting, with the first peak a few hours after sunrise and the second, an hour after sunset (Harley 1965 in Muirhead-Thomson 1982). Studies in the Cameroons indicated that there was variation in activity patterns among species: *Chrysops silacea* and *C. dimidiata* exhibited bimodal activity from approximately 10am - 12pm and 4 - 6pm, while *C. langi* and *C. centurionis* had peak activity from 5 - 7 pm i.e. at dusk and the early hours of darkness (Duke 1958 cited in Muirhead-Thomson 1982). Observations in Zambia showed peak tabanid activity (for all five *Tabanus* species and five of seven

Haematopota species) from 12 - 3pm (Okiwelu, 1975). The remaining two species of *Haematopota* exhibited peak activity from 9am - 12pm.
In America, daily activity patterns in Oklahoma, using dry ice baited Malaise traps and observations on cattle, showed that for four of eight tabanid species (three *Tabanus* and one *Hybomitra*), highest activity tended to occur between 3 - 6pm, for the remaining four *Tabanus* species, peak activity was noted from 6 - 9pm. Sunset in this region occurred at 8.30pm (Hollander and Wright, 1980). A study on tabanid daily activity patterns in Mississippi found two peaks of activity: the major peak was from 5 - 9pm and the minor one was from 6am - 10am. The lowest activity occurred between 11am and 2pm (Roberts, 1974).

The data produced by these researchers in Africa and America show that most tabanid activity in these areas occurs during daylight hours, however they report species that have activity patterns that peak at different times of the day, indicating that activity in certain geographic areas can vary quite markedly among species.

The limited data from Australia suggest that tabanids in south-east Queensland are most active between 10am and 3pm, especially on hot sunny days (Spratt, 1974a). However, no significant differences in activity between times of day were identified during a small number of observations in Townsville (Holland, 1997).

Tabanid activity may vary with the time of year and time of day (Burnett and Hays, 1974) . This has led a number of researchers to investigate the effects of various meteorological factors on tabanid activity patterns. Research conducted in South Carolina, USA, where the highest mean activity occurred around midday, found that the overall activity of tabanids was affected by barometric pressure, temperature, relative humidity, time of day and cloud cover. Light intensity had no effect on flight activity, but higher numbers were captured during periods of partial cloudiness. These authors note that the importance of meteorological factors affecting activity is partially dependent on the location of sampling studies, the type of trap used and the species involved (Alverson and Noblet, 1977). Similarly, data from Alabama, USA, indicated

that barometric pressure had the most significant effect on tabanid activity. Temperature, evaporation and evaporative change were less important and total sky radiation change, total sky radiation, wind velocity change, temperature change and relative humidity were least important. Seasonal and daily activity varied among the 34 species collected over both years of the study. In general, the mean tabanid catch increased with a decrease in barometric pressure, cloud cover and relative humidity and an increase in evaporation. Daily activity was influenced to different degrees, depending on species, by the weather factors examined (Burnett and Hays, 1974).

Dale and Axtell (1975) examined the meteorological effects on three tabanid species in North Carolina and found similarly, that the relative influence of the different weather factors on daily activity differed among species, which probably accounted for the variation in daily activity patterns observed among them. *Tabanus nigrovittatus* was caught in highest numbers in conditions with intermediate light intensity (40,000 lux), temperatures of around 25°C, and no wind. Highest activity of *Chrysops atlanticus* was related to low light intensity (5,000 lux), high temperatures (30°C) and air moisture equivalent to a vapour pressure deficit of -8 mmHg. *C. fuliginosus* activity was greatest in conditions of high light levels (100,000 lux). No literature is available on the effect of meteorological variables on daily activity patterns for Australian tabanid species.

This study reports on the daily activity patterns and changes in size and ovarian status of several local species of tabanids that were collected in March and April 2006. The relationship between the activity patterns of different species and meteorological variables is also examined. The implications of these data for the design of tabanid feeding experiments and modelling of activity patterns are discussed.

4.2 Materials and Methods

4.2.1 Traps

Nzi traps (Mihok, 2002) were used at both sites. The traps were very similar to those described in Chapter Four, except that white Phifertex mesh was used instead of white polyester mosquito mesh, and the collection container was modified: a rectangular, wire-framed collection container covered in white polyester mosquito mesh was placed directly on the outlet, instead of using a bottle connected to a bag. (This modification was introduced to improve the survival of flies in the trap).

4.2.2 Attractants

Both CO_2 gas and liquid octenol were used at both trap sites. Food grade CO_2 gas was released from a G size cylinder, via a regulator and then a flow meter, at a rate of three litres per minute. Liquid octenol was dispensed from a 50 ml vial with two wicks. Ten millilitres of octenol was added to each jar at the start of the day and the amount remaining at the end of the day was measured. The average release rate overall was 0.37 ml of octenol per hour.

4.2.3 Study sites

The trap sites were located on grazing land adjacent to the School of Veterinary and Biomedical Sciences, James Cook University. The location of the two sites was close to one of the sites described in the trap and attractant chapter, in the most south-eastern corner of the paddock. Site one GPS co-ordinates were 19° 19’49.5” S, 146° 46’ 01.6” E. Site two was located approximately 50 metres north of site one. The front of each trap faced south-west, and the attractants were positioned on the north-east side, since the prevailing winds were north-easterly.

4.2.4 Study design

The study period started on 3rd March 2006 and finished on 19th April 2006. Collections were made from site one on all but one day of the 20 day study period. The second site operated on 12 of the 20 days.

On each day collections were made, the CO_2 was turned on and octenol dispensers were attached to the trap at 7am. The collection containers were removed at 3 hour intervals until 7pm at night. After each collection, the tabanids were kept alive in an incubator (25-26°C, 60% relative humidity) in individual vials, then counted and identified to species. When not used in later experiments, the tabanids were killed by freezing at -20°C and dissected to determine the physiological status of their ovaries.

4.2.5 Wing length measurements

The wing length measurement from costa to the intersection of the R4 and R5 wing veins, has been found to be proportional to the entire wing length (Leprince and Foil, 1993). Wing length is also a good indicator of body length, and is easier to measure. Wing length measurements from the costa to the branch of the R4 vein were made using an eye piece graticule and dissecting microscope.

4.2.6 Ovarian dissection

Ovarian status was determined by dissection of the abdomen with forceps and exteriorisation of the ovaries in order to examine them for the presence or absence of eggs, using a dissection microscope. As noted earlier, only flies not required for other purposes were dissected. The ovaries were separated from other abdominal structures and examined without opening them up. The ovaries were classified as having “visible developing eggs” if there were dark, opaque portions evident (Figure 4.01) and if they appeared as narrow, transparent tubes they were classified as having “no visible developing eggs” (Figure 4.02). The dark opaque portions are developing eggs (including fully-developed eggs), with accumulating yolk.

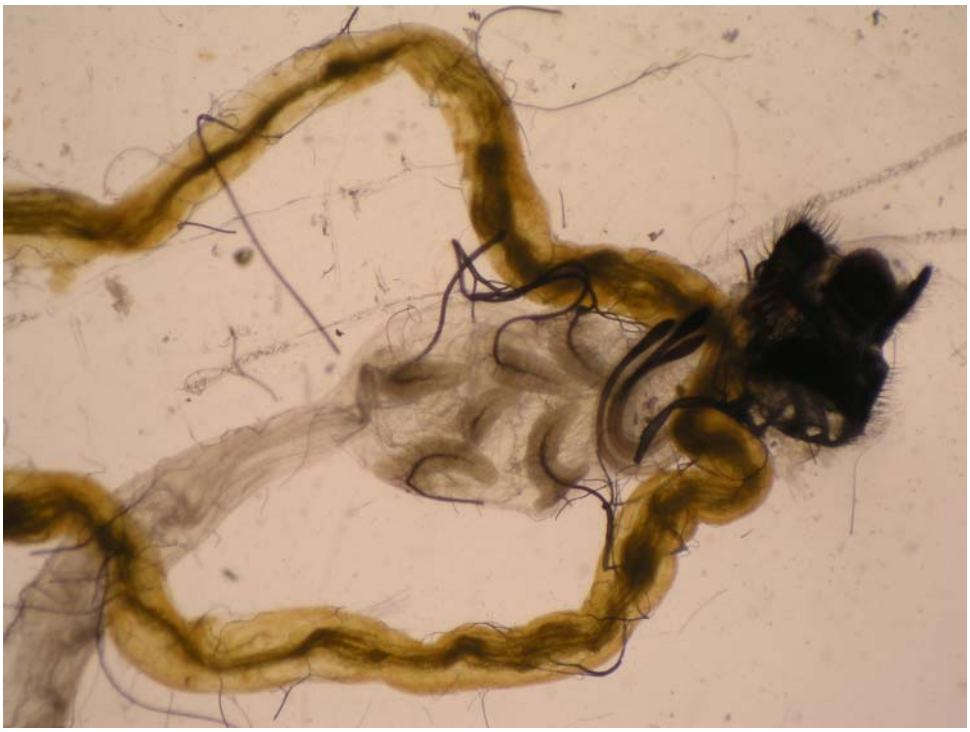


Figure 4.01: *T. pallipennis* –visible developing eggs

Figure 4.02: *T. pallipennis* –no visible developing eggs

4.2.7 Weather data

Weather data was received in two formats. In one format, weather values on a half-hourly basis were provided by the regional Bureau of Meteorology centre in Brisbane (Meteorology, 2006). These data were used for analysing weather effects on activity patterns during collection periods (which were three hours in duration). A table of the weather variables and how the values over the collection period were calculated is provided in Appendix 2, Table A2.10.

When daily abundance patterns over the six weeks of the study were examined, weather data in a daily format was used. A table of the weather variables used for the analysis of activity on a daily basis, and how they were calculated, is provided in Appendix 2, Table A2.11 (Meteorology, 2006). The weather data was collected from the Townsville airport site, several kilometers from the study site.

4.2.8 Statistical analysis

Data analyses used SPSS (SPSS Inc, Version 12.0.1) and S-Plus (Insightful Corp, Version 6.4). Chi-squared analyses and logistic regressions were used to examine categorical data, including analysis of activity patterns and ovarian status. Analyses of variance were performed on wing length data. Linear regression, correlation analysis and analysis of covariance were used to analyse log transformed counts of tabanids. Factors affecting the total daily catch of each species were analysed via stepwise multiple regression analyses and analyses of covariance. The slopes of the linear relationships expressed in graphs indicate the effect of change in weather variables on tabanid abundance. A log transform (log10 [N+1]) was applied to the total catch, which successfully stabilized the variances. Sites one and two were analysed separately for those species with significant differences in abundance between sites.

4.3 Results

A total of 1071 tabanids were captured during the course of the study. Of the total, 809 were trapped at site one (BP) and 262 were caught at site two (CP).

The most numerous species caught were *Tabanus pallipennis* Macquart (43.6%), *Tabanus townsvilli* Ricardo (34.7%) and *Pseudotabanus silvester* (Bergroth) (14.4%). Other species caught in lower numbers include *Tabanus dorsobimaculatus* Macquart (2.8%), *Tabanus strangmannii* Ricardo (2.1%), *Lilaea fuliginosa* (Taylor) (1.7%) and *Dasybasis clavicallosa* (Ricardo) (3 specimens). Six other specimens were caught, including *Tabanus cohaerens* (1 specimen) and 5 unidentified tabanids (Figure 4.03).

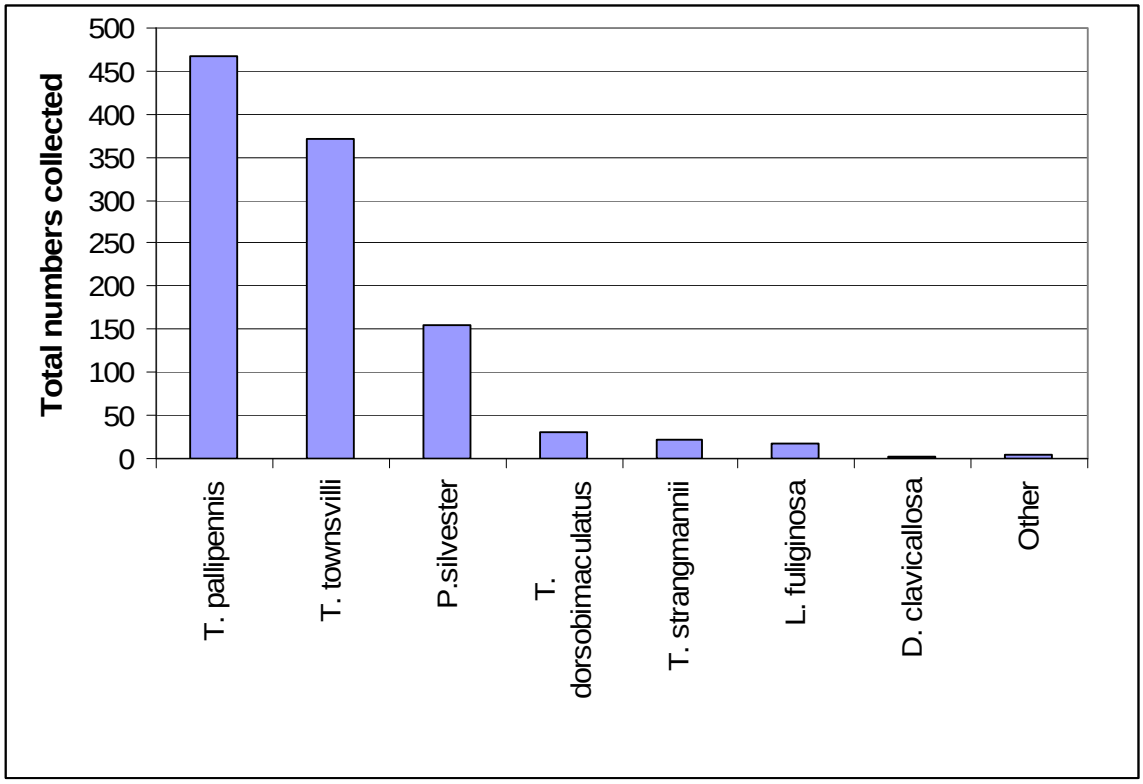


Figure 4.03: Total number of each species collected.

4.3.1 Species variation in overall patterns of daily activity

Each species had a different pattern of activity throughout the day (Figure 4.04). The distribution of flies among collection periods differed significantly among species, when all catch days were combined ($\chi^2 = 365.41$, df = 9, n = 1023). There were also significant differences among species on 14 out of 20 days, using Fisher's exact tests.

Tabanus pallipennis was mainly active in the late afternoon from 4-7pm. The lowest activity of this species was recorded in the morning, and activity progressively increased throughout the day.

Tabanus townsvilli had its main activity period from 10am to 4pm, with less than 20% of specimens caught outside these times.

Pseudotabanus silvester had its peak activity (52% of total caught) between 4-7pm, with a smaller peak from 7-10am (21% of total caught). Activity was lower in the middle two collection periods.

Tabanus dorsobimaculatus was most active from 10am to 1pm (47% of total caught) with lower activity from 7am-10am (30% of total caught) and declining activity in the afternoon collection periods.

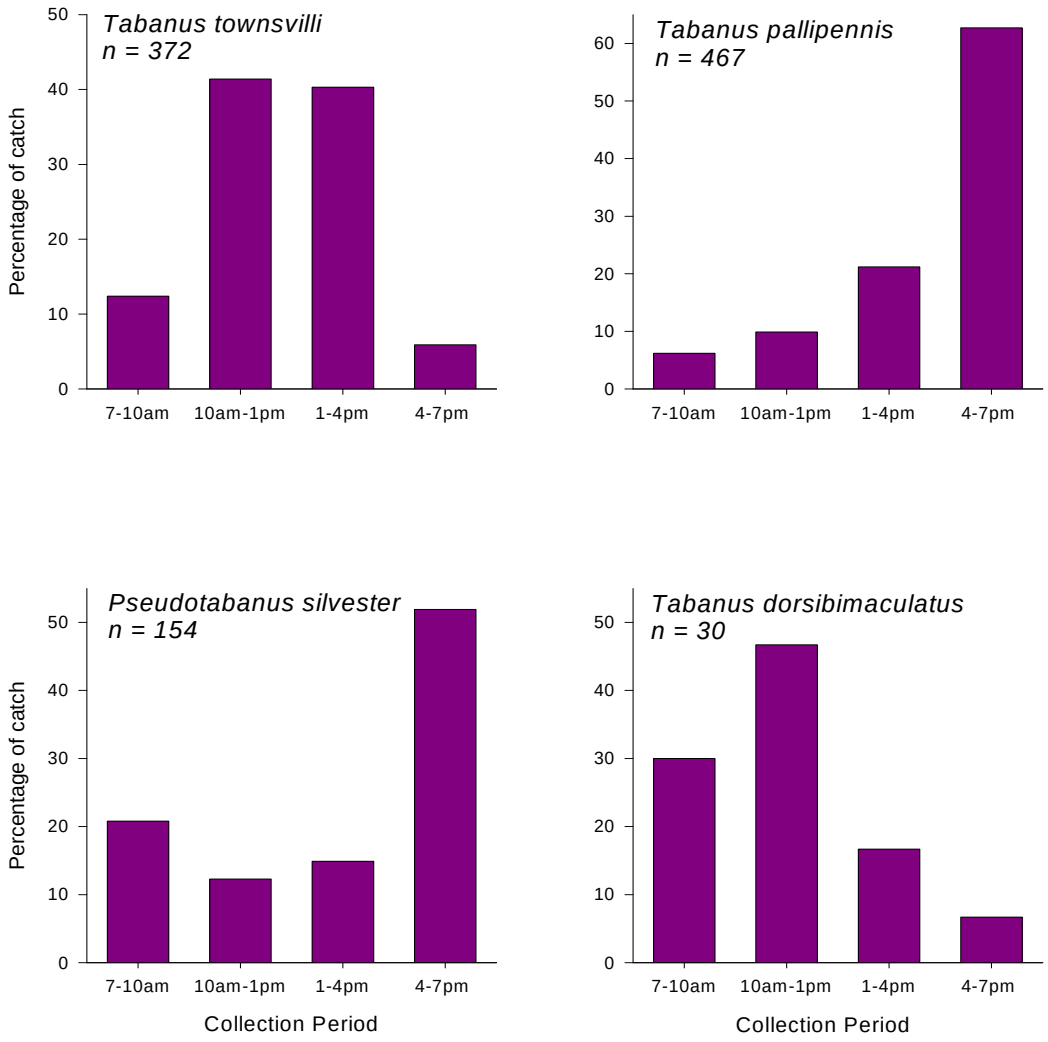


Figure 4.04: Overall activity patterns of the four most numerous species as a percentage of the total species catch.

4.3.2 Species variation in daily activity patterns between days

There were significant differences in the pattern of activity among days for all three of the common species.

4.3.2.1 *Tabanus townsvilli* activity pattern variation among days

For *T. townsvilli*, although the two middle periods of the day consistently have the highest activity, days vary in which of the two is greatest, and in the extent to which activity occurs early and late in the day ($\chi^2 = 47.653$, df = 16, n = 292). Collection periods one and four were pooled, to keep expected values within the limits for Chi-squared analysis. The activity of *T. townsvilli* during the different collection periods on a sample of days is provided in Figure 4.05, indicating the variation in the daily cycle.

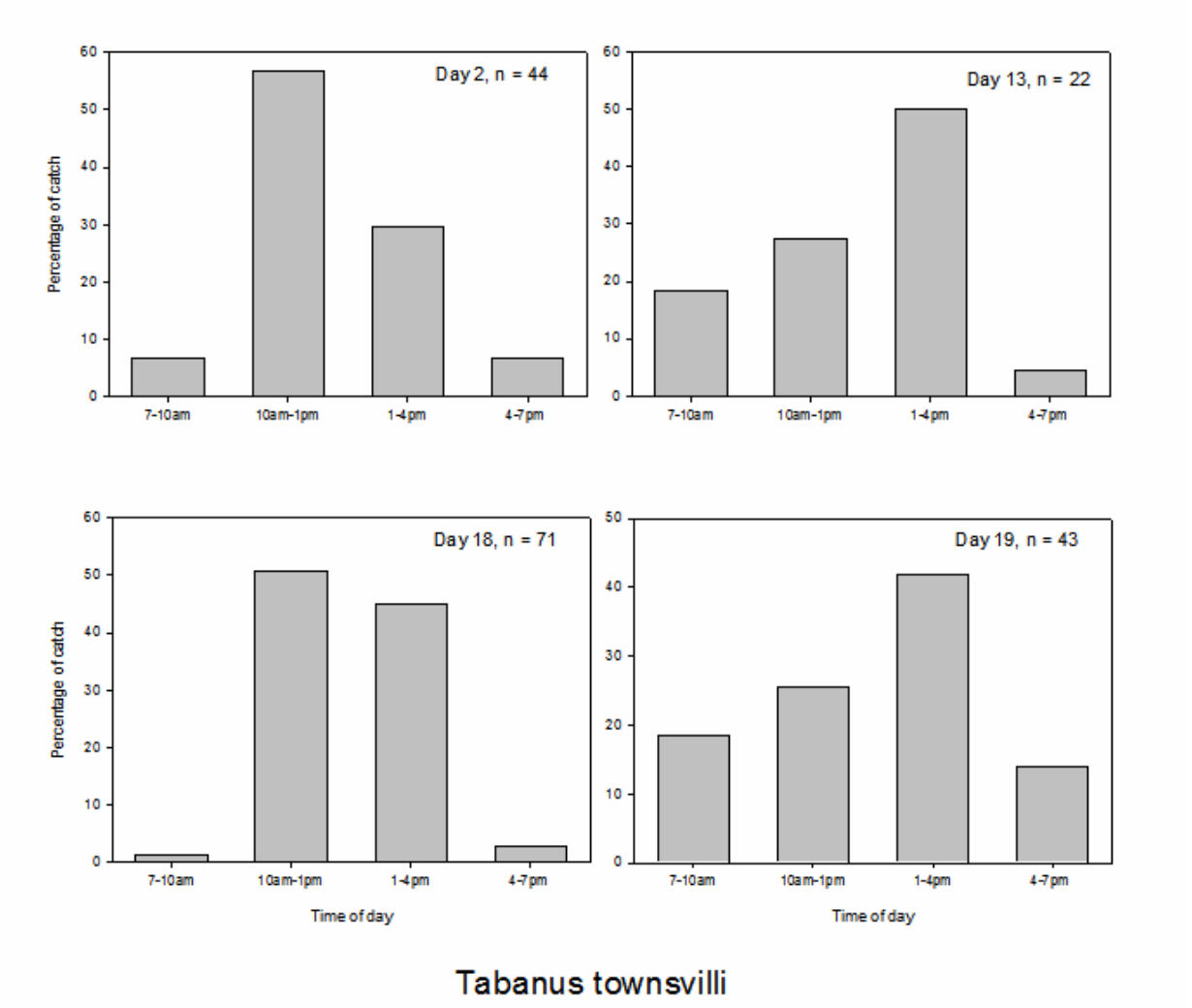


Figure 4.05: Samples of days on which the activity of *T. townsvilli* in different collection periods varied.

4.3.2.2 *Tabanus pallipennis* activity pattern variation among days

For *T. pallipennis*, the highest activity consistently occurred late in the day, and variation was in the extent to which the final period dominated the day's catch. Differences between collection periods were significant ($\chi^2 = 25.117$, df = 10, p = 0.005, n = 254). Note that collection periods one and two were combined for this analysis to keep the expected values sufficiently high for a valid test. The variation in the daily cycle is shown in Figure 4.06.

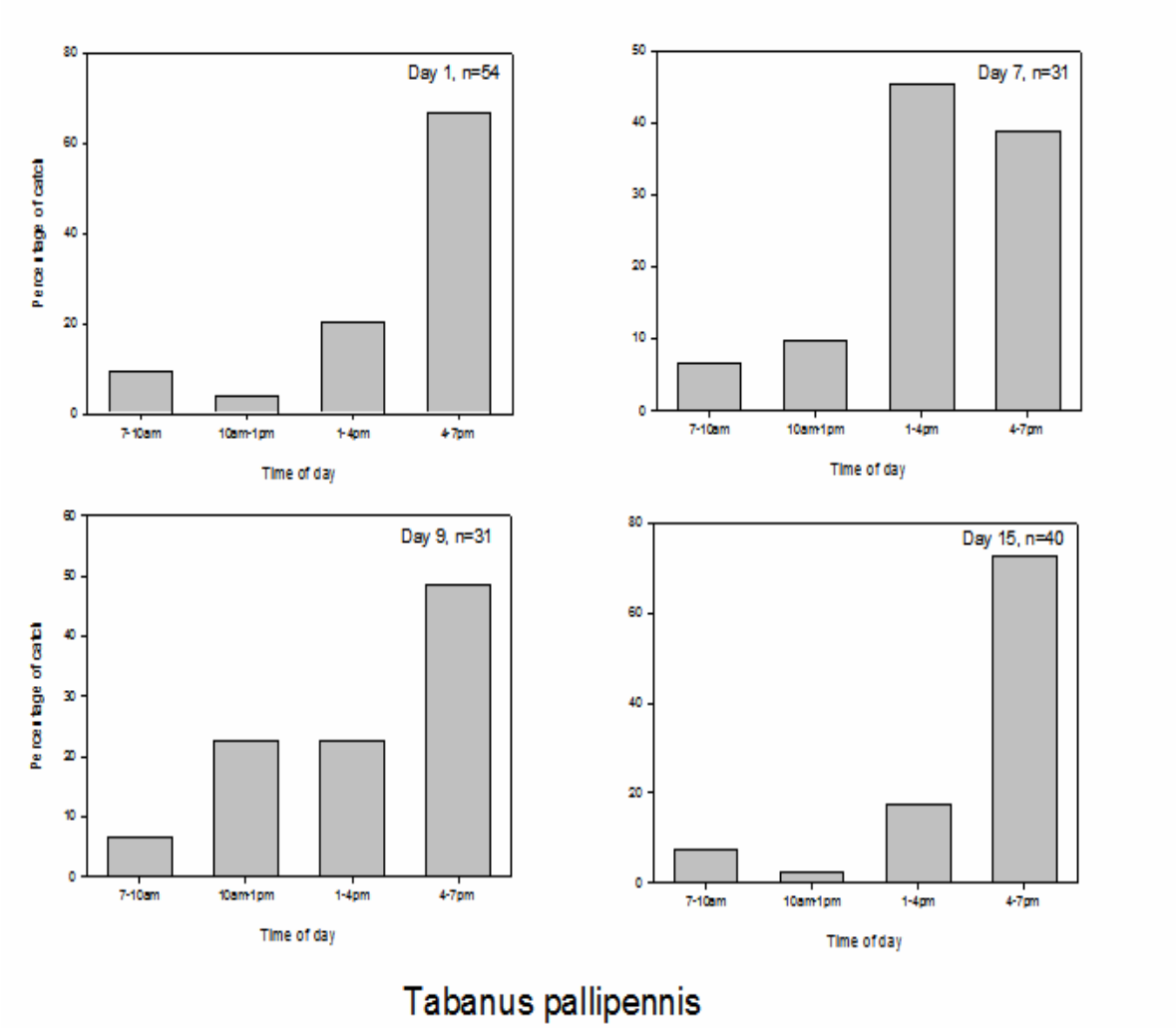
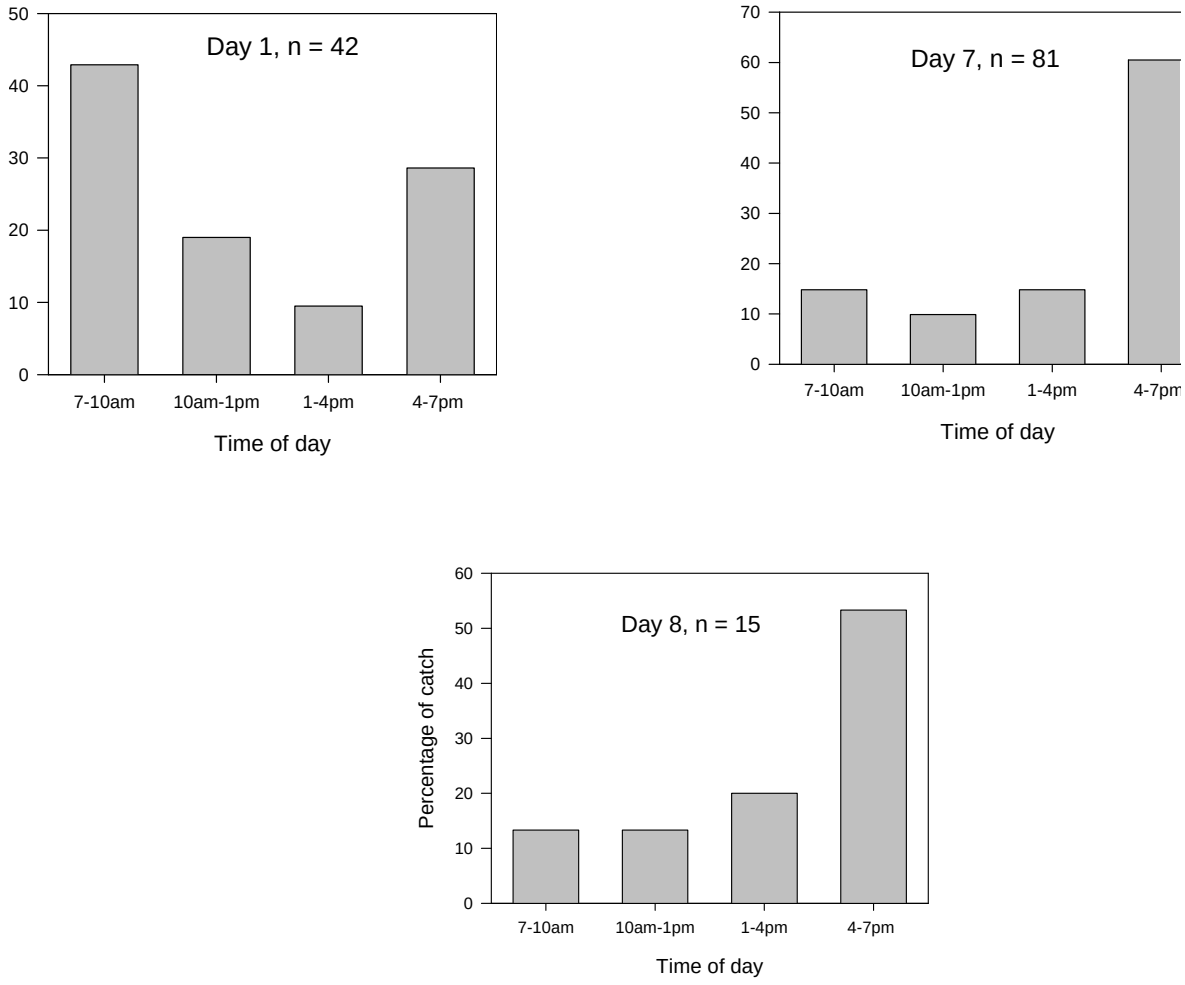


Figure 4.06: Samples of days on which the activity of *T. pallipennis* in different collection periods varied.

4.3.2.3 *Pseudotabanus silvester* activity pattern variation among days

For *P. silvester*, where overall there were activity peaks early and late in the day, the early peak dominated on some days and the late peak on others. Differences between collection periods were significant ($\chi^2 = 18.581$, df = 8, p = 0.005). Again, collection periods one and two were combined to ensure a valid test.

A sample of days for *P. silvester* shows the variation in the daily cycle (Figure 4.07).



Pseudotabanus silvester

Figure 4.07: Samples of days on which the activity of *P. silvester* in different collection periods varied.

4.3.3 Variation in daily activity patterns with catch size

Each of the three most numerous species was analysed to determine whether daily activity patterns, or proportions of the catch within each collection period, varied with the size of the total daily catch of the species. The data were grouped according to the daily catch sizes: group one had a total daily catch of less than 10, group two had a catch size of 11-30 and group three had a catch size of 31 or greater. A significant difference was found in the daily activity patterns of *T. townsvilli* with different catch sizes, but there was no relationship for *T. pallipennis* and *P. silvester*. Catches from the two sites on the same day were treated as replicates.

4.3.3.1 Variation in daily activity patterns with *T. townsvilli* catch size

For *T. townsvilli*, as catch size increased, the proportion of the catch in each collection period changed and the two middle collection periods were more heavily represented (Figure 4.08). The differences in the proportions in collection periods for days with different catch sizes is significant ($\chi^2 = 16.97$, df = 6, $0.005 < p < 0.01$).

When the daily catch size was low (10 or less), the catch was relatively evenly distributed throughout the first three collection periods. As the catch size increased, the percentage of the catch caught outside the two middle periods decreased, from 30% in the low catch sizes, to 17% in the middle catch sizes, and 13% in the highest catch sizes.

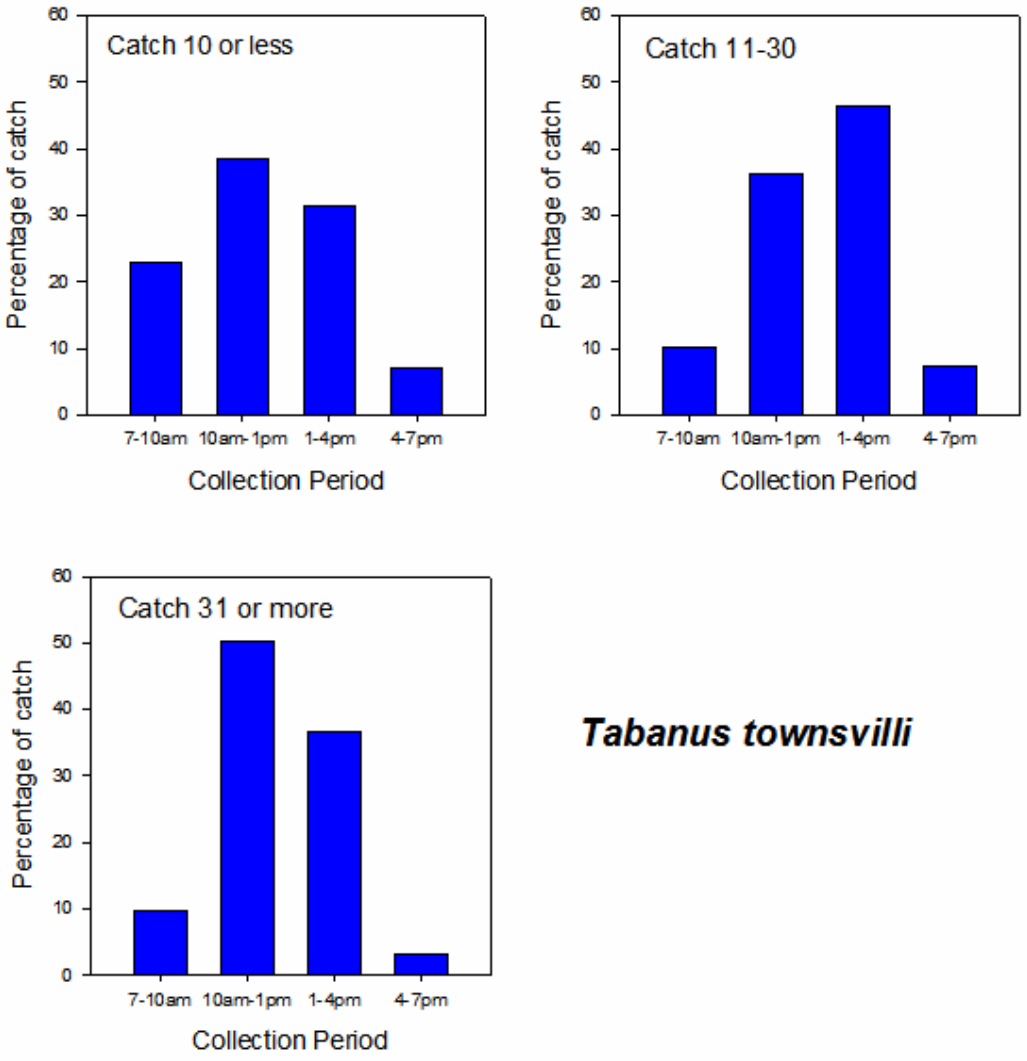


Figure 4.08: Differences in daily activity patterns of *T. townsvilli* with different catch sizes

4.3.4 Variation in size

The largest of the tabanids caught in sufficient numbers to analyse for variation in size was *T. dorsobimaculatus*, with a mean wing length measurement of 10.2mm (+/- 0.4mm, n = 12). The next largest was *T. strangmannii* with 9.5mm (+/- 0.6mm, n = 18), followed by *T. townsvilli* with 8.6mm (+/- 0.6mm, n = 141), *T. pallipennis* with 7.4mm (+/- 0.5mm, n = 209) and *P. silvester* with 5.9mm (+/- 0.3mm, n = 144) (Figure 4.09).

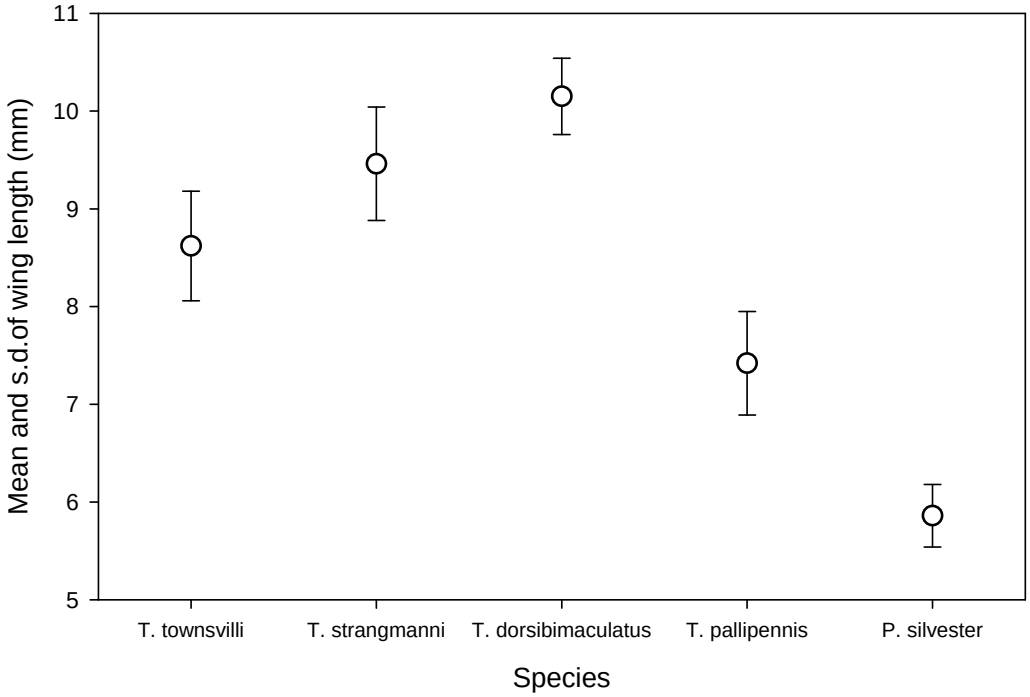


Figure 4.09: Mean wing lengths (and standard deviations) of the five most numerous species.

There was no significant difference among collection periods in the average size of any of these species (Table 4.01).

Table 4.01: Mean wing lengths (mm) per collection period (+/- S.D.).

Collection period	**1**	**2**	**3**	**4**
	Mean +/- S.D.	**Mean +/- S.D.**	**Mean +/- S.D.**	**Mean +/- S.D.**
T. townsvilli (n =141)	8.6 +/- 0.6	8.7 +/- 0.6	8.6 +/- 0.5	8.5 +/- 0.7
T. pallipennis (n =209)	7.8 +/- 0.5	7.3 +/- 0.5	7.4 +/- 0.5	7.4 +/- 0.5
T. dorsobimac-ulatus (n =12)	10.1 +/- 0.4	10.4 +/- 0.2	-	9.9 +/- 0.4
P. silvester (n =144)	5.9 +/- 0.3	5.8 +/- 0.3	5.8 +/- 0.3	5.8 +/- 0.3
T. strangmannii (n =18)	9.4 +/- 0.7	9.2 (no SD, n=1)	9.4 +/- 0.6	9.9 +/- 0.6

Analysis of variance was performed on each of the three most numerous species to determine if their daily mean size changed throughout the experimental period. A significant change in body size would indicate there was probably some population turnover during the course of the study.

The daily mean size of *T. townsvilli* did vary significantly over the course of the study ($F = 2.328$, $df = 9$, $p = 0.018$), while *T. pallipennis* variation in size was close to significance ($F = 1.740$, $df = 10$, $p = 0.074$) (Figure 4.10). The variation in daily mean size of *P. silvester* was not significant ($F = 1.954$, $df = 4$, $p = 0.105$). Variances were homogeneous in all cases.

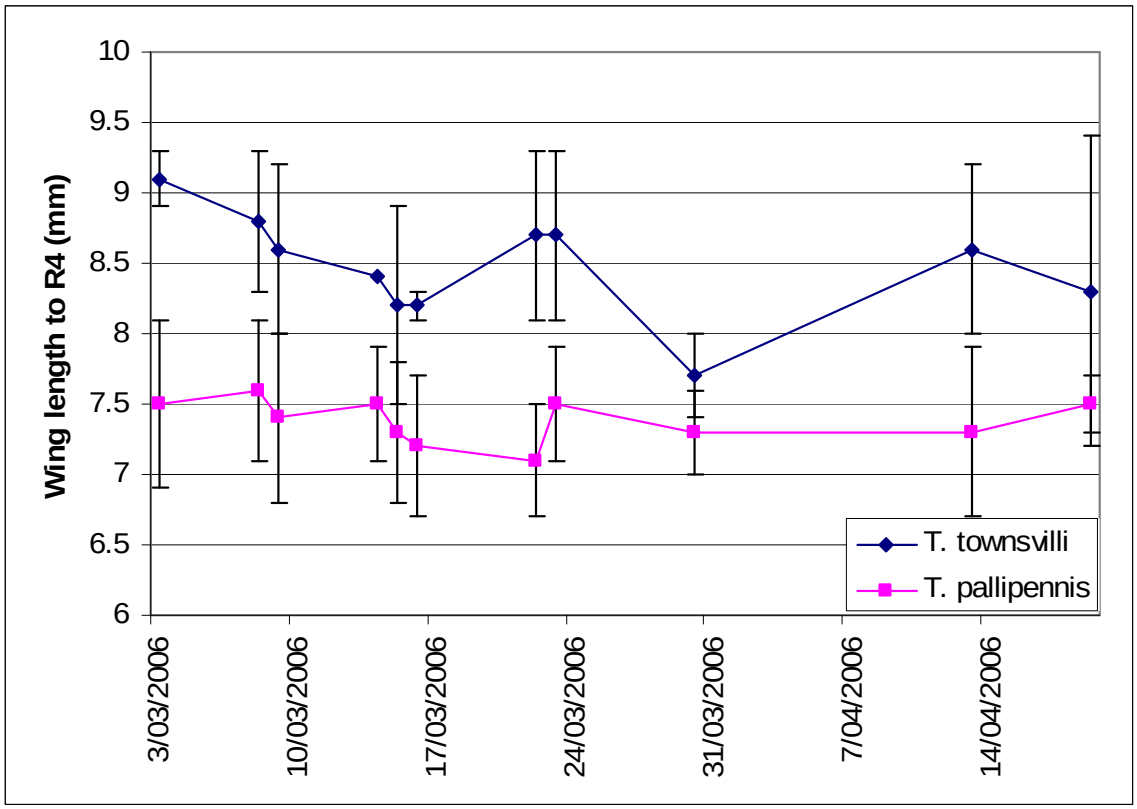


Figure 4.10: Variation in daily mean wing length (mm) over time

4.3.5 Ovarian status

The proportion of each of the five most numerous species with visible developing eggs was determined (Figure 4.11). There was a significant difference between species in the proportion with visible developing eggs, when data from all days were combined ($\chi^2 = 47.481$, df = 4).

Pseudotabanus silvester had the highest proportion of specimens with visible developing eggs (62%), followed by *T. pallipennis* (46.1%), *T. dorsobimaculatus* (36.4%), *T. strangmannii* (27.8%) and *T. townsvilli* (20.7%).

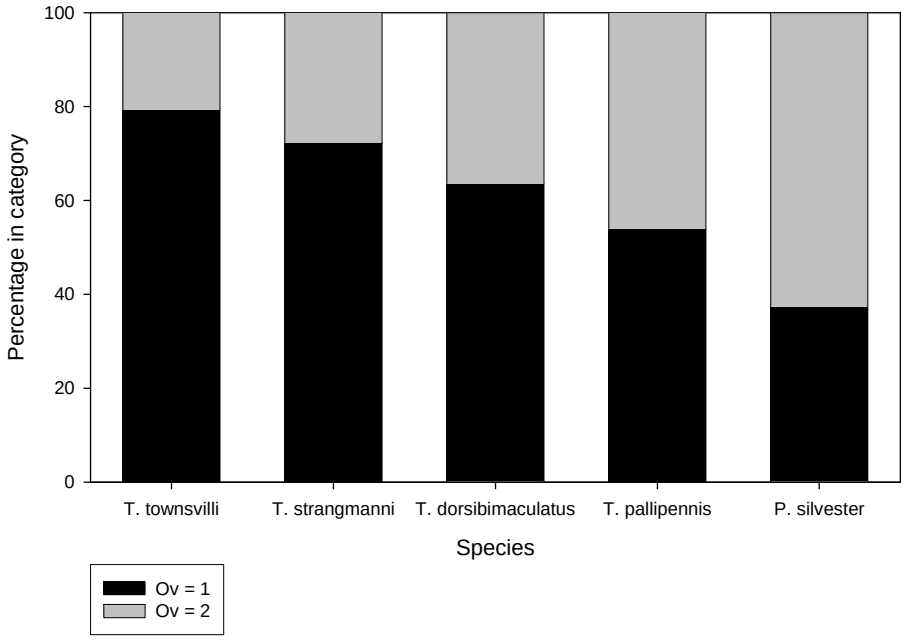


Figure 4.11: Species differences in percentage with visible developing eggs.
NB: 1= no visible developing eggs, 2= visible developing eggs.

The three most numerous species were analysed using logistic regression to determine if the proportion with visible developing eggs varied by either collection period or day. For *T. townsvilli* and *T. pallipennis*, there was no significant effect of either collection period or day.

For *P. silvester*, logistic regression gave a marginally significant effect (with both coefficients just significant). The distribution of *P. silvester* ovarian status in each collection period is indicated in Table 4.02 (B = -0.420, p = 0.040). Ovarian activity by day appeared significant also (B = 0.203, p = 0.015). Additional collections would be useful to confirm this result, since only 99 flies were available for dissection for this species, and no significant differences were found when collection period and day were analysed separately using Chi-squared tests.

Analysis of collection period and ovarian status indicates that flies caught earlier in the day were more likely to have visible developing eggs than those caught later.

Table 4.02: Ovarian status of *P. silvester* in each collection period

Collection period	**Presence of visible developing eggs**		**Total**
	No	**Yes**	
1	7 30.4%	16 69.6%	23 100%
2	3 23.1%	10 76.9%	13 100%
3	6 54.5%	5 45.5%	11 100%
4	21 40.4%	31 59.6%	52 100%
Total	37 37.4%	62 62.6%	99 100%

4.3.6 Effect of weather variables on total daily catch

The total daily catch of each of the three common species varied over the experimental period (Figure 4.12). In general, the three species appeared to vary independently: their abundances showed no significant correlations (Table 4.03).

Table 4.03: Correlation coefficients between log (catch+1) of each of the three common species: *T. pallipennis*, *T. townsvilli*, and *P. silvester*.

	T. pallipennis	*T. townsvilli*	*P. silvester*
T. pallipennis	1.00	0.22	0.30
T. townsvilli	0.22	1.00	-0.14
P. silvester	0.30	-0.14	1.00

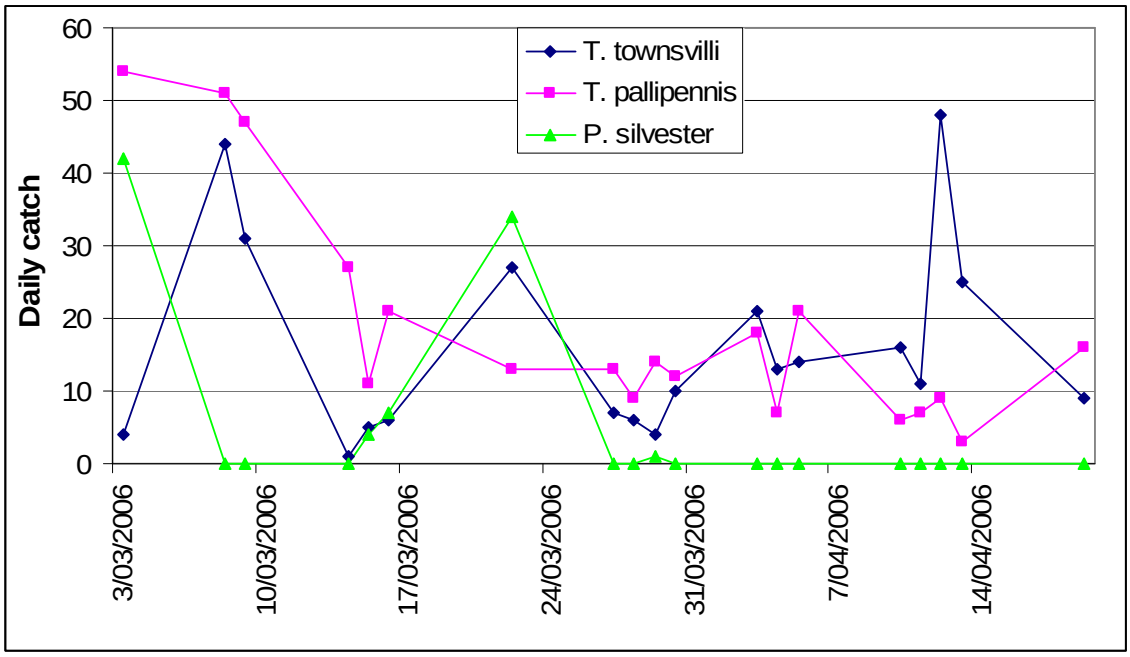


Figure 4.12: Fluctuations in daily catch (number of tabanids) over time

Meteorological variables affecting the total daily catch of each species were analysed. They included temperature, humidity, barometric pressure, rainfall on the sampling day, hours of sunshine, and wind speed. The range of values for each of the meteorological

variables during the sampling period is presented in Appendix 2, Table A2.12. These analyses suggest that each of the three species responds differently to meteorological variables, as indicated in Table 4.04.

Table 4.04: Meteorological variables affecting the total daily catch

Species	**Significant factors**	**Comment**
T. pallipennis	Site (1 > 2) p = 0.034 Temperature (+) p < 0.0005	The temperature variable used was (max + min) /2
T. townsvilli	Site (1 > 2) p = 0.001 Wind speed (-) p = 0.0163 *OR* Humidity (+) p = 0.0123	The wind speed variable used was (WS at 9am + WS at 3pm) / 2 Relative humidity variable: (9am RH + 3pm RH) / 2 NB: The significance for humidity is for site 1 only
P. silvester	Barometric pressure (-) p < 0.0005 Humidity (-) p = 0.001	The barometric pressure variable used was (9am pressure + 3pm pressure)/2. Relative humidity variable: (9am RH + 3pm RH) / 2 NB: There was no significant difference between sites for this species.

4.3.6.1 *Tabanus pallipennis*

Temperature is the main weather variable exerting an effect on *T. pallipennis*. Higher temperatures result in higher catches, and the response to temperature appears similar at the two sites (Figure 4.13).

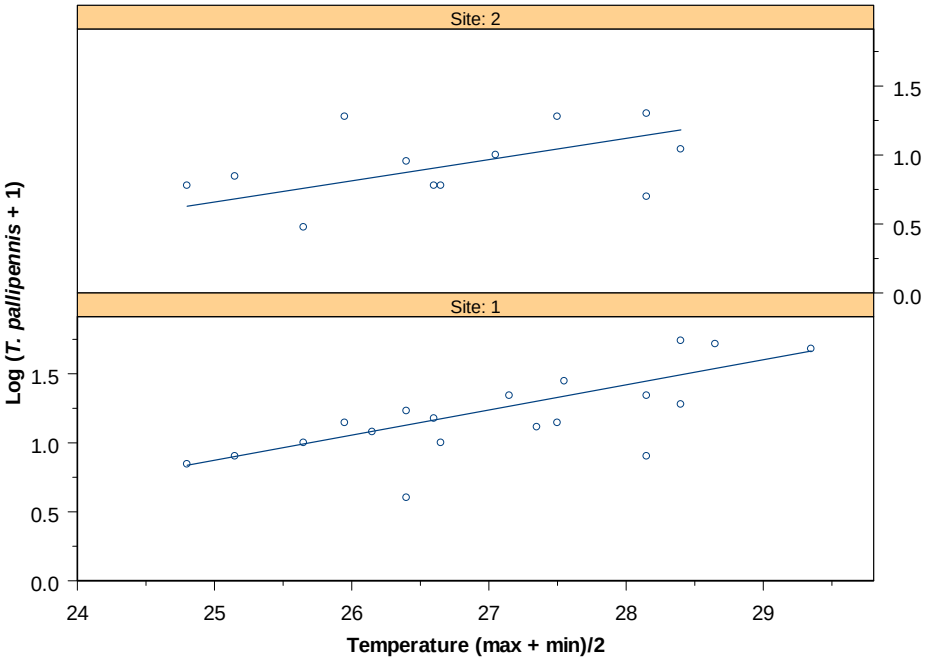


Figure 4.13: Effect of temperature on *T. pallipennis* daily catch

4.3.6.2 *Tabanus townsvilli*

Both wind speed and humidity give some degree of prediction of *T. townsvilli* catch, but because these two variables are themselves inversely correlated during the experimental period, they are alternative predictors of the catch rather than joint predictors.

Figure 4.14a shows the relationship between wind speed and *T. townsvilli* catch at each site, and Figure 4.14b shows the relationship with relative humidity. At both sites, catch rate decreases as wind speed increases. Humidity shows an equally strong relationship with catch rate at site one, but its effect is weak or absent in the site two data, where few *T. townsvilli* were caught.

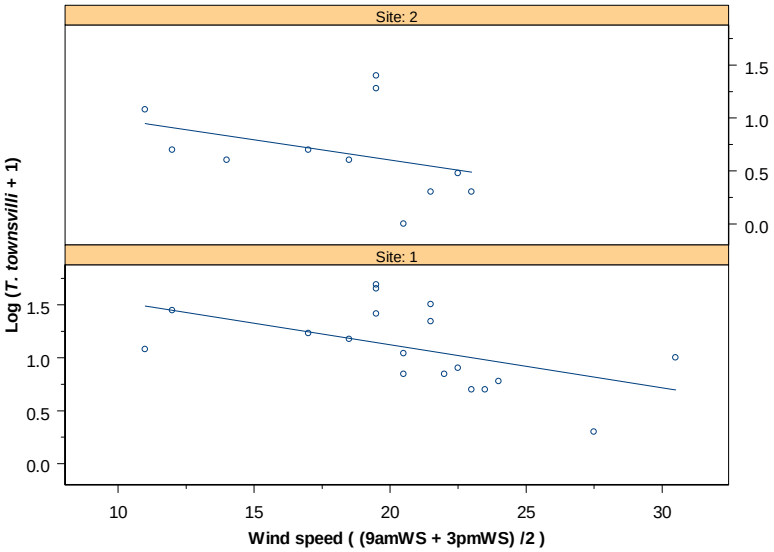


Figure 4.14a

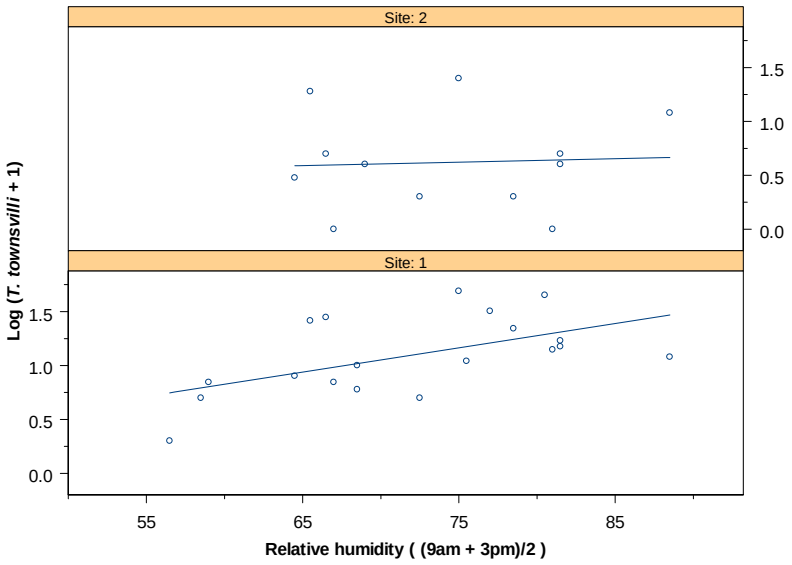


Figure 4.14b

Figure 4.14: Relationship of wind speed and relative humidity with *T. townsvilli* catch at each site

4.3.6.3 *Pseudotabanus silvester*

The abundance of this species was similar at the two sample sites. Figure 4.15 shows the effect of barometric pressure on *P. silveste*r catch rates at high and low humidities (dividing the sample days into two approximately equal groups). No individuals were caught on days with high humidity (greater than or equal to 75%: note that most points in the high humidity graph actually represent two data points, one for site one and one for site two). On days with low humidity, the catch decreased as the barometric pressure increased.

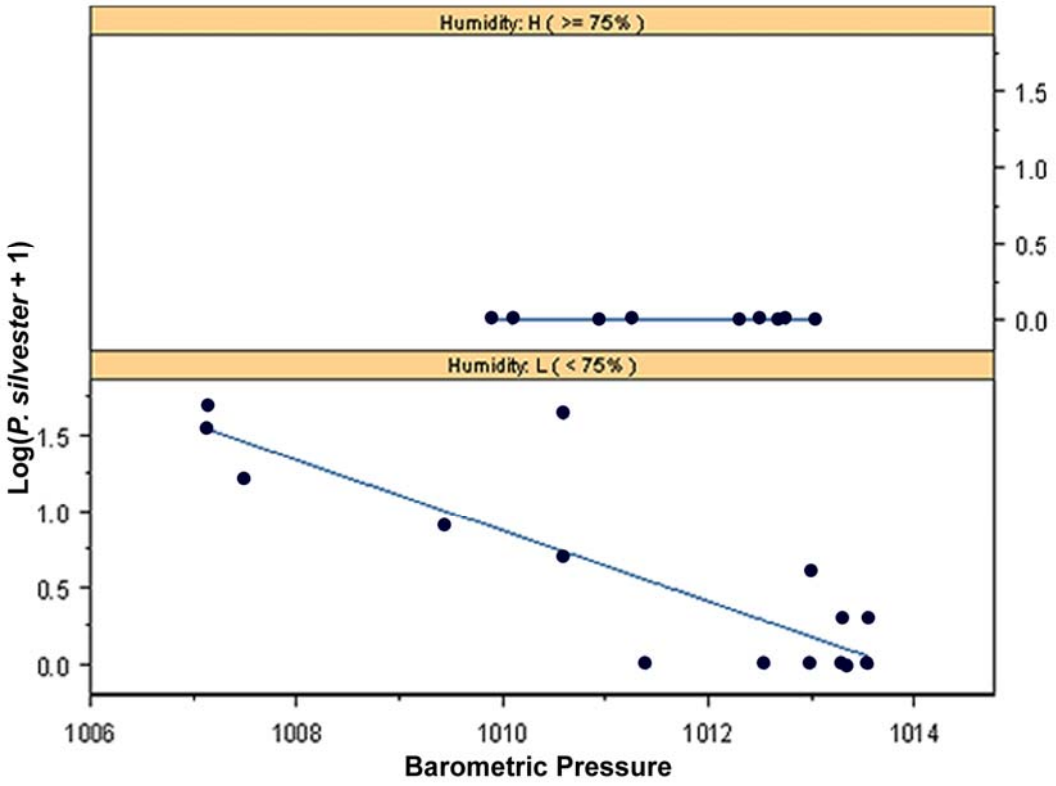


Figure 4.15: Effects of barometric pressure on *P. silvester* catch at high and low humidities.

4.3.7 Weather variables and activity during each collection period

Linear regression and analysis of covariance were also used to determine which weather variables had significant effects on the catch in each collection period, for each of the most numerous species (Table 4.05). In these analyses, the meteorological variable represents conditions during the collection period rather than the whole sample day.

Table 4.05: Weather variables and activity during collection periods indicating factor, direction and significance. N.B. Effect of rain during collection period could not consistently be examined because too many combinations had no periods with rain.

Collection period	**7-10am**	**10am-1pm**	**1pm-4pm**	**4pm-7pm**
T. pallipennis	**Site 1** RH (-) 0.026	W speed (+) 0.025	Air temp (+) 0.015	Air temp (+) <0.005
	Site 2 None significant	None significant	RH (-) 0.0454	Air temp (+) 0.042
T. townsvilli	**Site 1** Air temp (+) 0.004	Air temp (+) 0.016 Rain 9am (-) 0.004 RH (+) 0.012 W speed (-) 0.015	Air temp (+) 0.019 RH (+) 0.025 W quad (+) 0.021	None significant
	Site 2 None significant	None significant	None significant	None significant
P. silvester (NB no significant difference between sites)	**Both sites** Pressure (-) 0.0002 RH (-) 0.0081	Pressure (-) 0.00009 RH (-) 0.0676	Pressure (-) 0.0001 RH (-) 0.0084	Pressure (-) 0.0324 RH (-) 0.0077

4.3.7.1 *Tabanus pallipennis*

As for the whole-day catches, the primary influence on *T. pallipennis* catch rates was temperature, especially in the afternoon collection periods where catch rates were highest. Although significant only in the afternoon collection periods, the trends are similar, although weaker, earlier in the day (Figure 4.16). As noted in Table 4.05,

humidity and wind speed also appeared to affect catch rates in the 7 - 10am and 10am - 1pm periods respectively, but so few *T. pallipennis* were caught in these periods that these effects, if real, would have little influence on the daily catch rate.

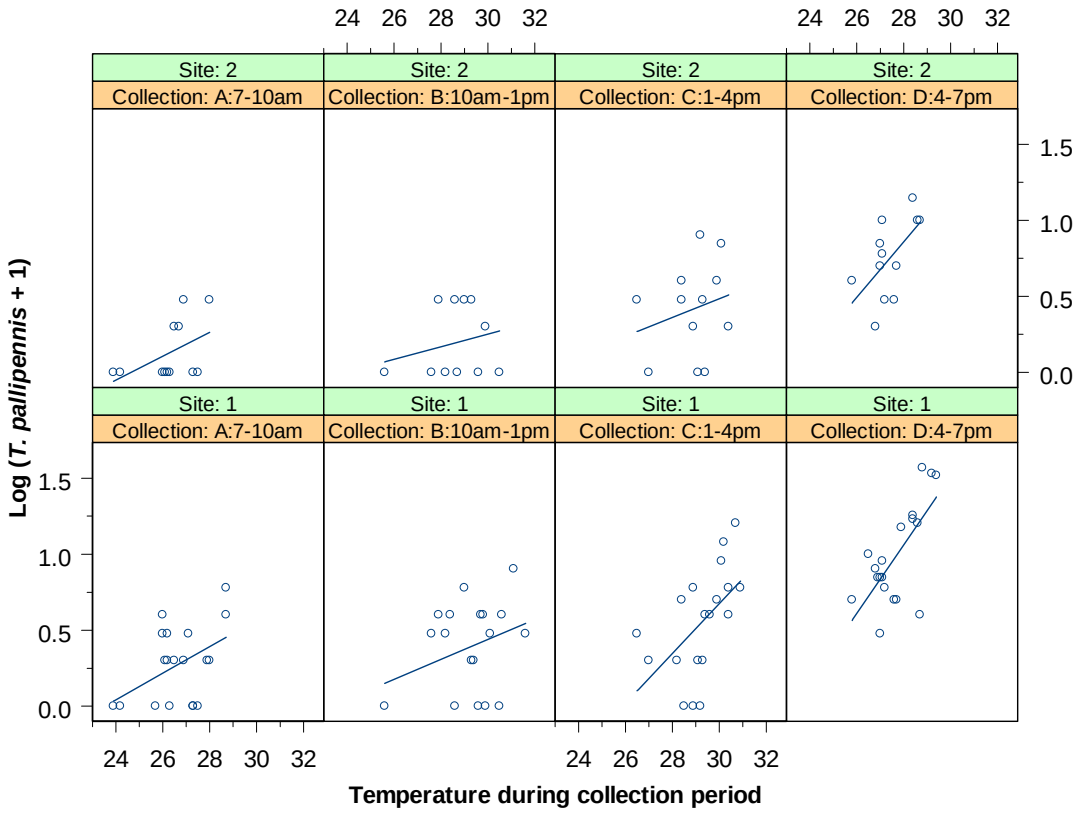


Figure 4.16: Effects of temperature on *T. pallipennis* catch rates in different collection periods.

4.3.7.2 *Tabanus townsvilli*

Analyses of collection periods separately make it clear that the factors influencing *T. townsvilli* catch throughout the day are complex. Because so few *T. townsvilli* were caught at site two, the figures below illustrate the effects observed at site one only.

- Temperature and humidity effects

To illustrate the interaction between the effects of temperature and humidity in each collection period, the effect of humidity is plotted at temperatures above and below 28°C (which divides the data into two approximately equal temperature groups). In general, higher temperatures were associated with higher catch rates (Table 4.05). When temperatures are highest – primarily during the two peak-activity collection periods during the middle of the day – *T. townsvilli* catch rates increase as humidity increases (Figure 4.17). But when temperatures are lower, in the early morning and late afternoon, increasing humidity has no effect on catch rate.

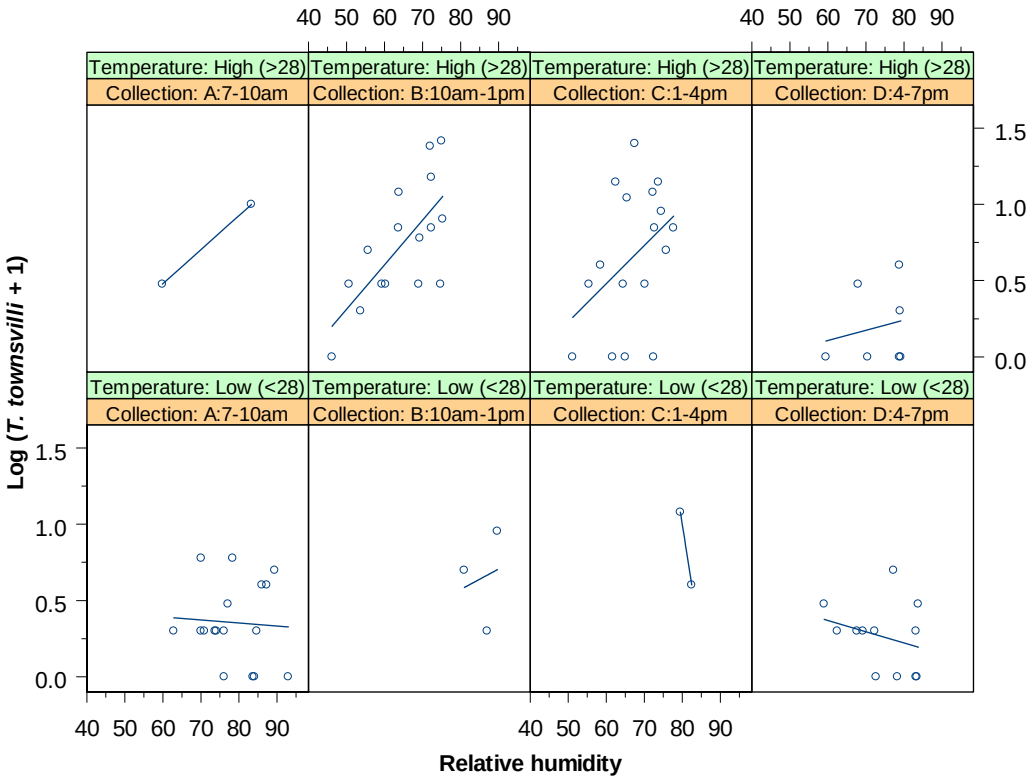


Figure 4.17: Effects of humidity on *T. townsvilli* catch rates at different temperatures and collection periods.

- Wind effects

Wind speed and direction appeared to affect catch rates during some collection periods but not others (Table 4.05). Although wind speed had a significant effect only in the 10am to 1pm collection period, with catches decreasing as wind speed increased, a weaker wind speed effect may be present in later collection periods as well (Figure 4.18). Early in the morning, however, when wind speeds tend to be lower, there was no significant effect. Afternoon collections (1 - 4pm) also tended to be higher when the winds were south-easterly.

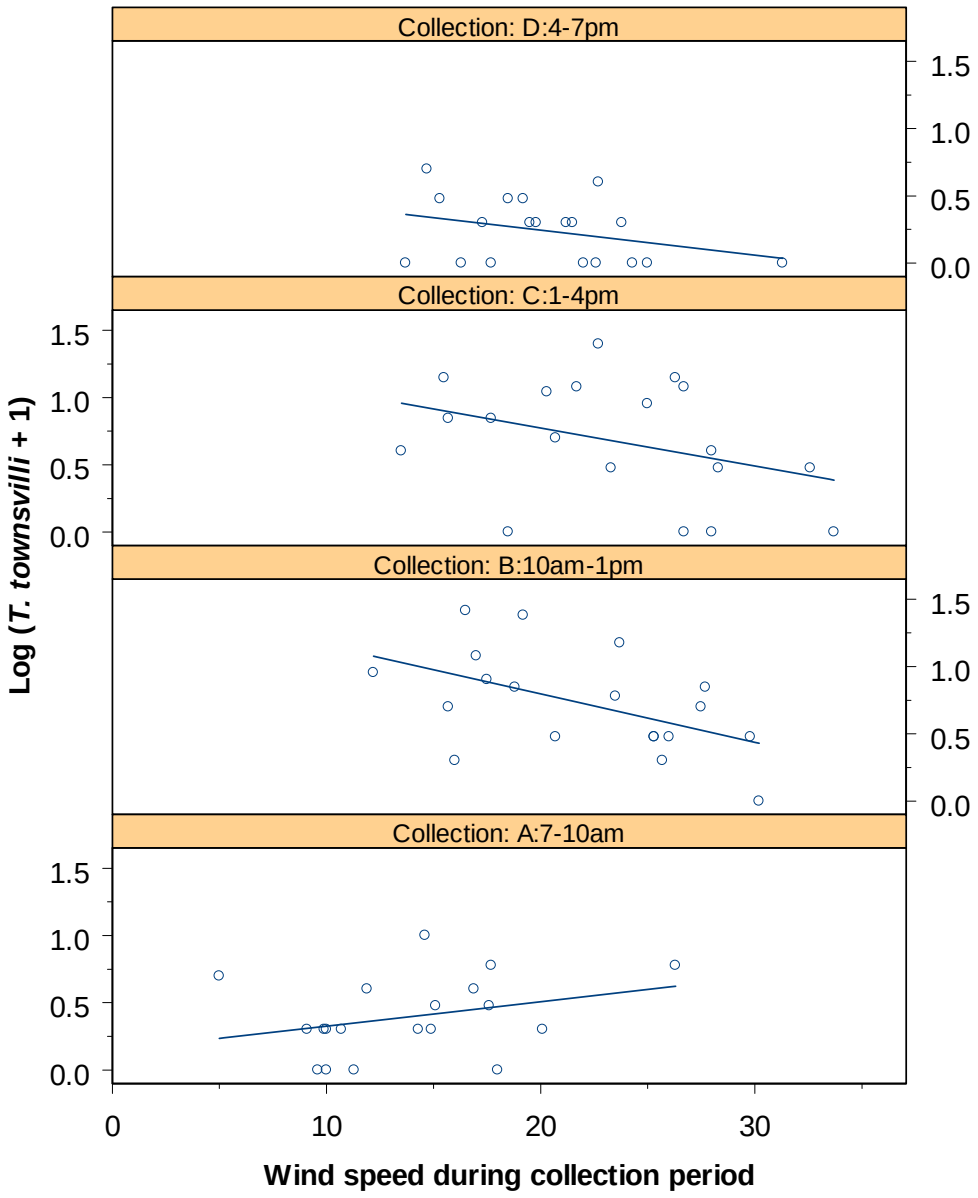


Figure 4.18: Effects of wind speed on *T. townsvilli* catch rates during different collection periods

4.3.7.3 *Pseudotabanus silvester*

As with the total day's catch, the key variables affecting *P. silvester* catch rates during each collection period were barometric pressure and relative humidity. These effects are illustrated in Figure 4.19 below. In all collection periods, high humidity appeared to diminish fly activity. At low humidity, there was a consistent decrease in catch rate as the barometric pressure increased.

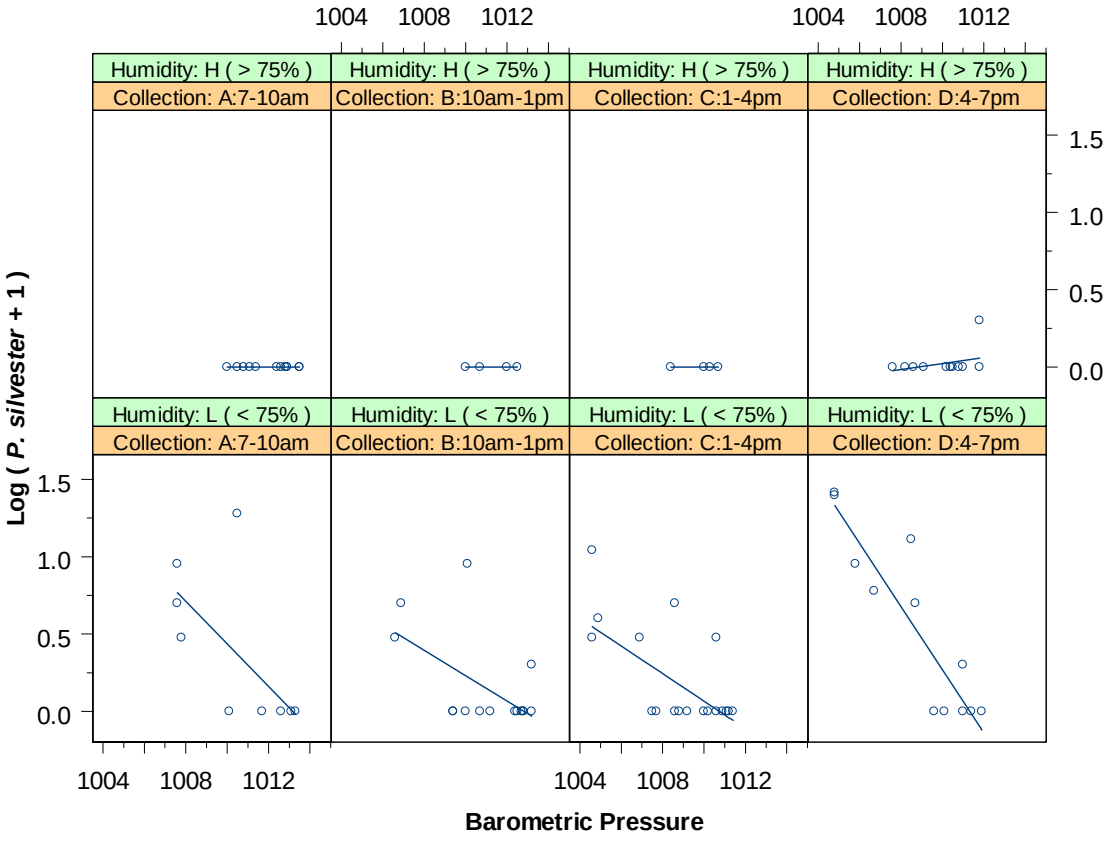


Figure 4.19: Effects of barometric pressure on *P. silvester* catch rates at different levels of relative humidity and in different collection periods

4.4 Discussion

Each tabanid species has a different pattern of activity throughout the day, so the proportions of each species of tabanids biting at one time of day are likely to be different from those biting at another. Therefore, if vectorial capacity for a disease such as surra varies between species, the risk profile would fluctuate throughout the day. The result also suggests that experiments designed to examine feeding behaviour should be timed to match the peak activity period of the particular species being examined: for example, 4 - 7pm for *T. pallipennis* and *P. silvester* and 10am - 4pm for *T. townsvilli*. Species differences in activity patterns have been described previously (Roberts, 1974; Okiwelu, 1975; Hollander and Wright, 1980; Muirhead-Thomson, 1982).

The pattern of activity for any one species also differed somewhat among days and collection periods. Analysis of weather variables has clarified their effect on temporal variation in activity patterns of different species of tabanids.

Roberts' (1974) qualitative observations in Mississippi, USA, indicated that certain weather conditions appeared responsible for the marked change in the daily activity patterns of different species of tabanids. Burnett and Hays (1974) quantitatively analysed the within-day variation in different species in relation to weather variables and found for the six most abundant species, the relative effects of different factors varied. In addition, variation between species' responses to different weather factors was found by Dale and Axtell (1975).

Given the short duration of the present study, variations in catch rate are likely to be due primarily to variation in activity levels rather than variation in population size; although there is evidence that some population turnover occurred during the experimental period. The key result of this study is that the activity of different species appears to be responsive to different weather variables, so it is not surprising that catch rates of different species were uncorrelated. The slopes of linear relationships between weather variables and tabanid abundance are generally moderate. However, because tabanid

abundance is expressed in a log scale, the slopes often indicate a substantial increase in the number of tabanids with changes in weather variable.

Tabanus pallipennis, favoured higher temperatures during its period of peak activity late in the day (temperatures ranged from 23.9 – 31.6°C during the study). This effect is evident both in the total daily catch and in the catch rates for individual collection periods. This finding is similar to that of Burnett and Hays (1974), who determined that temperature was a significant factor affecting the activity patterns of six temperate tabanid species. Of the six species, five preferred lower temperatures and one preferred higher temperatures. Temperatures in that study ranged from 5.2°C to 35°C. Other factors (particularly wind speed and humidity) may also influence catch levels of *T. pallipennis* outside the most active period.

Tabanus townsvilli, which is most active during the middle part of the day, showed a complex pattern of response to weather variables. In examining factors able to predict the total daily catch, daily average values of either wind speed or humidity could be used: wind speed had a significant negative effect and humidity, a significant positive effect. But individual collection periods were jointly affected by the temperature and humidity levels during that collection period: at high temperatures there was a positive response to increasing humidity which was not present at low temperatures. In general, *T. townsvilli* appears to favour warmer conditions but only in circumstances which do not induce rapid water loss (i.e. low wind speeds and high humidity).

Wind is known to influence haematophagous diptera in two ways. Firstly, if wind speed exceeds the flight speed of an insect, then its ability to make a directed response is impaired. Secondly, variation in wind speed affects the odour plumes i.e. host kairomones that provide the cue for tabanids to travel upwind and encounter a host or trap. At higher wind velocities, greater wind turbulence occurs which breaks up the odour plume, decreasing the strength of the signal and inhibiting tabanid response and therefore diminishing trap catch (Gibson and Torr, 1999).

Burnett and Hays (1974) found that only large changes in wind speed affected tabanid activity, and suggested that wind velocities strong enough to inhibit flight were not reached during that study (range 0 – 13.8km/hr). These researchers did find that the activity of one species, *T. pallidescens*, was affected by wind velocity change, but it is not clear whether high or low wind speeds were favoured. However another study found that flight activity of *T. nigrovittatus* decreased with increasing wind velocity and predicted maximum activity would occur when there was no wind (Dale and Axtell, 1975). Mean wind velocity in this study ranged from 7 – 158 m/min (0.42 – 9.48 km/hr).

Wind velocities in the present study were in the range of 5.0 – 33.7 km/hr, with a mean of 18.7 km/hr. At low wind speeds in the morning, a positive effect of increasing wind speed was noted on *T. townsvilli* activity, whereas later in the day, this trend was reversed. The effect of wind speed was not significant in other species.

Wind from the south-east also tended to increase afternoon trap catches in this species. This might indicate that the preferred habitat for *T. townsvilli* lay to the north-west of the trap, given that wind from this direction would carry odour cues from the trap's attractants in this direction. An ephemeral creek lies in that direction and would constitute a likely tabanid habitat. This result is similar to that of Burnett and Hays (1974) who found increased mean catch rate with westerly winds was possibly due to wind from that direction enhancing the movement of CO_2 odours downwind towards the wooded area that was a suspected tabanid habitat.

Pseudotabanus silvester, which has both morning and afternoon peaks of activity, was most responsive to barometric pressure and humidity, with these variables accounting for most variation in both the total daily catch and individual collection periods. In general, this species is most active when the barometric pressure is low, and when humidity is also relatively low. Indeed, no activity was recorded when the daily humidity measure was above 75%, and almost none occurred in any individual collection period when the humidity during that period exceeded 75%. That is, this species appears most active when rain might be anticipated in the future (low barometric

pressure) but current conditions are not excessively moist. The effect of barometric pressure is in agreement with that of Burnett and Hays (1974), who found that overall and for each of the six most numerous tabanid species, increases in barometric pressure exerted a significant negative affect on activity levels. These researchers noted a species difference in response to relative humidity: four of the six species responded to conditions of low humidity, while two preferred high humidity.

The meteorological data used in this analysis was collected at the Townsville Aero BOM site, which is several kilometres from the sites at which specimens were trapped. The distance between the sites of meteorological data collection and tabanid collection may have introduced some inaccuracy into the calculations, but it is unlikely to be great.

During this study, continuous sampling may have caused selective depletion of some species in the area, which is a potential source of bias. It is not possible to measure the magnitude of this effect or to gauge its significance. However the study used only two traps and the duration was approximately two months, so it is unlikely that this effect was large.

The diversity of response shown by these three species suggests that any attempt to predict what proportion of the total tabanid population may be active on any given day is unlikely to be generally successful, since total activity will be entirely dependent on the species composition of the tabanid community at the time and place being considered. It would therefore be more useful to identify which species are of interest as potential vectors, and to characterise and model the response of those species to weather variables along the lines used here.

It is also apparent that for species like *T. townsvilli*, better prediction of total daily activity may be obtained from modelling catch rates at different times of day and summing them. However, since this requires more detailed meteorological monitoring (in order to use weather conditions at specific times of day in generating predictions), it is probably not practicable for predicting activity levels over wide areas.

The proportion of activity in each collection period of *T. townsvilli* varies with the total catch size. For *T. townsvilli*, as catch size increases, the proportions of the catch in each collection period changes and the two middle collection periods are more heavily represented, i.e. the catch becomes more "peaky". This result may indicate that the factors that increase catch size also dictate a change in the daily pattern of activity in *T. townsvilli*. No comparable relationship with catch size occurred in either *T. pallipennis* or *P. silvester*.

No significant difference in size was found between the collection periods for any of the four main species. That is, there is no evidence from the size data that different classes of flies are active at different times of day.

Each of the three most numerous species were analysed to determine if their size changed throughout the experimental period. It was determined that the size of *T. townsvilli* did vary significantly over the course of the study, while *T. pallipennis* variation in size was close to significance. This indicates that there was probably some population turnover during the experimental period, and consequently that some of the day-to-day variation in catch may be due to variation in fly abundance rather than variation in activity.

There was a significant difference among species in the proportion with visible developing eggs. *Pseudotabanus silvester* had the highest proportion of visible developing eggs with 62%, followed by *T. pallipennis* (46.1%), *T. dorsobimaculatus* (36.4%), *T. strangmannii* (27.8%) and *T. townsvilli* (20.7%). Perhaps these differences are the result of different times of emergence i.e. increasing proportion with visible developing eggs is perhaps correlated with time since emergence - a function of "maturity". This is supported by findings of Leprince and Lewis (1986b) who found that peak abundance of *Tabanus quinquevittatus* occurred in mid-July in south-western Quebec, and in proportion, parous tabanids were more abundant in the second half of the flight period. Differences in proportions of visible developing eggs between species may

also be a function of speed of ovarian development, with faster development resulting in a bigger proportion of any sample with visible egg development. Autogeny of certain species is another possible explanation for these differences: for example, *P. silvester* might be able to partly develop its eggs without a prior blood meal while *T. townsvilli* may not.

For *T. townsvilli* and *T. pallipennis*, there is no effect of either collection period or day number on the proportion with visible developing eggs. This indicates that reproductive and not-yet-reproductive flies do not differ in their daily activity patterns, and that the proportion of reproductive flies remained similar throughout the observational period. For *P. silvester*, there appears to be some variation between collection periods and days in the proportion of flies with visible developing eggs, but sample sizes were small. If the effect is real, *P. silvester* from the collection period with the lowest proportion with visible developing eggs (from 1 - 4 pm) may be the best choice for feeding experiments, if we assume that tabanids with no visible developing eggs are more likely to be searching for a blood meal and therefore are more likely to feed.

The primary aim of this study was to determine the peak activity periods of different tabanid species during daylight hours when studies on tabanid behaviour would normally be held, in order to ensure these studies were carried out at appropriate times. Therefore, tabanid collections in this study began at 7am and finished each night at 7pm. No collections were made after 7pm, so we have not determined whether any of the species were crepuscular or nocturnal. *Tabanus pallipennis* and *P. silvester*, whose peak activity was between 4pm and 7pm, may well remain active past sunset. Researchers in Oklahoma report one species, *T. equalis*, with a definite crepuscular activity pattern (Hollander and Wright, 1980). A low level of nocturnal activity was also noted between 6pm and 6am in Zambia (Okiwelu, 1975). In Uganda, Haddow and Corbet (1960 in Muirhead and Thomson 1982) have found crepuscular species of *Tabanus*. Mackerras (1970) reported that *T. pallipennis* had been caught at night on board a ship in the Torres Strait. He also refers to unnamed northern tabanid species that have been caught in houses at night. In addition, some of the species captured during this study may have

begun their daily activity earlier than 7am. Further studies are required to confirm whether this was the case.

A secondary aim was to determine whether tabanids caught during different collection periods, or on different days, were significantly different physiologically from each other. If they were different, this may have influenced the results of the behaviour experiments.

Thirdly, the trap catches of tabanids are known to be influenced by meteorological variables, both on a large scale (influencing abundance) and a small scale (influencing daily activity). The results described here certainly show day-to-day variation in the pattern of activity associated with changes in weather variables.

It is interesting to note that the activity patterns of trap-caught tabanids have been well correlated with the feeding behaviour of tabanids on nearby animals. Tabanids caught using Malaise traps baited with CO_2 accurately reflected the host seeking activity of eight species of tabanids on a nearby cow. The percentage of the total trap catch at each time interval was very similar to percentage of the total number of observed landings on the cow in the same period (Hollander and Wright, 1980). Spratt (1974a) also found that numbers and species of tabanids caught in a modified Manitoba trap baited with dry ice were very well correlated with tabanids feeding on a nearby grey kangaroo ($R = 0.98$). Observations were made simultaneously on 13 occasions for a period of four to nine hours on each occasion.

It is likely, therefore, that the daily variation in catch rates seen here reflects the daily pattern of feeding activity in the flies and further studies in Townsville would confirm this for local species.

The daily activity patterns of the most common local species of tabanids in Townsville have been clarified, and have shown substantial differences between species. Besides contributing to our understanding of tabanid behavioural ecology, this information will

be useful in future studies on tabanid feeding behaviour, to ensure that experiments are carried out at times that match the peak activity of these species. The relationship between the activity patterns of the three most abundant species caught in relation to meteorological factors has also been clarified.

CHAPTER FIVE
TABANID FEEDING BEHAVIOUR IN TOWNSVILLE

5.1 Introduction

Observations of tabanid feeding behaviour on different host species were performed to investigate aspects that contribute to their success as mechanical vectors of *T. evansi* and other pathogens. Behaviours that contribute to an increased ability to act as a disease vector include imbibing a large blood meal from more than one host (polyphagy), having large mouthparts that facilitate the transfer of blood containing pathogens to a subsequent host and low feeding persistence i.e. readily moving to another host when disturbed during feeding. Landing and feeding positions on the host are also important when considering where and how to apply topical insecticides to livestock for biting fly control.

Tabanids are known to be highly selective for landing (and presumably feeding) sites on hosts (Mullens and Gerhardt, 1979), which suggests that surface cues of the host animal play a role in the selection of landing site (Gibson and Torr, 1999). These surface cues may be influenced by chemical and physical factors such as carbon dioxide, body heat, skin odours, colour and length of hair and body size (Magnarelli and Anderson, 1980).

Mullens and Gerhardt (1979) found definite landing area preferences for 19 tabanid species in eastern Tennessee. The trends noted for tabanid distribution were related to height on the host. There was a positive correlation between mean hair length at the point of landing and the length of tabanid mouthparts. Larger species tended to feed on the upper torso, while smaller species attacked areas with thin hair and easily accessible skin. Also, many species, especially those feeding on the torso fed more often on the thoracic area than in sacral and gluteal regions, presumably because they were less accessible to tail swipes. These findings were supported by Magnarelli and Anderson (1980) who found that *Hybomitra* and *Tabanus* species generally favoured the backs and sides of cattle, while 82% of *Chrysops* selected the head region. Hafez (1970) noted that

the preferred biting sites for *T. taeniola* were the lower parts of the host, as well as the back loins and legs, with the inner legs preferred. *Atylotus agrestis* preferred similar feeding sites, while *T. sufis* attacked only the belly and shoulders. None of the tabanids studied attacked the head region (Hafez *et al.*, 1970).

Torr and Hargrove (1998) measured the landing and feeding responses of tsetse to a stationary ox semi-enclosed in electric nets. They discovered that an increased density of tsetse increased the grooming responses (defensive behaviours) but had no significant effect on the proportion of tsetse that engorged. The preferred landing site varied with fly density: approx imately 50% preferred the legs when density was <20 flies/ox, but at >40 flies/ox, 80% preferred the legs. This may have been due to the defensive behaviours of the ox which were directed at the torso. The practical implications of this research indicate the requirement for sufficient insecticide at areas where biting flies tend to feed e.g. insecticide impregnated ear tags may not be effective for foot feeders (Torr and Hargrove, 1998).

In Zimbabwe, Schofield and Torr (2002) compared the feeding behaviour of tsetse and stable flies (*Stomoxys*). Flies were judged to be fully fed when their abdomen was distended and they left without apparent disturbance from other flies or host. Data recorded included reasons for flies leaving hosts: (1) feed completed, (2) disturbed by other flies (3) disturbed by host behaviour (4) left without feeding.
The causes for disturbance of both species were similar, with host defences accounting for 69% and other flies 31%. Non-biting diptera e.g. *Musca spp*. gain access to blood by dislodging biting flies as they feed. Landing times for tsetse were approximately 30 seconds, regardless of cause of departure e.g. host defenses, other flies, etc. However, fully fed flies spent an average of 126 seconds on hosts (Schofield and Torr, 2002). Hafez (1970) found the time of engorgement for *T. taeniola* in field observations, ranged from 2.05 – 9.17 minutes with an average of 3 – 5 minutes, while in laboratory conditions on a guinea pig host, engorgement time was 4 – 18.13 minutes (mean 5 – 8 mins). When this species was fed on citrated blood-soaked cotton wool, engorgement

time decreased to 49 – 60 seconds (average: 55 seconds). The average amount of blood ingested by this species was 133.75mg.

Feeding interruptions produce repeated probings of the host, which increases the biting rate and probability of disease transmission. In fact, biting rate is one of the most important parameters in the epidemiology of trypanosomiasis (Torr and Hargrove, 1998). In a study by Schofield and Torr (2002), higher rates of feeding interruption were correlated with greater abundance of non-biting diptera and frequency of host defensive actions, which were mostly produced by large numbers of *Stomoxys*. This means increased rates of disease transmission could result from larger numbers of non-biting diptera and *Stomoxys.* Feeding success is diminished in young hosts and this may increase the chances of older animals running with calves becoming infected, which would have an effect on the epidemiology of trypanosomiasis (Schofield and Torr, 2002). Magnarelli and Anderson (1980) found 83% of *Chrysops* fed without interruption, while 65% of *Tabanus* and *Hybomitra* engorged continuously, in observations made by humans at close quarters in Connecticut.

Factors that influence the choice of host or host species are the size of host, group size, type of body covering, defensive behaviour and activity rhythms (Moore, 1993). Vale (1977) in Zimbabwe, studied the effects of defensive behaviour in different host species, on the feeding success of tsetse by comparing sedated and non-sedated animals. He had noted feeding success in non-sedated animals was lower on impalas, bushbucks, goats and sheep, compared to oxen. He found a 15 fold increase in feeding success with goats following sedation, but with the normally more complacent ox, there was little effect on feeding success following sedation. He also noted that the presence of men observing the feeding behaviour halved the attraction of tsetse to the ox and decreased the proportion of engorging flies by about three-quarters.

This study attempted to explore several aspects of the feeding behaviour of tabanid species in Townsville on four different host species: landing and feeding site preferences and times; host defensive behaviours; and feeding success.

The study was not successful, and recommendations for alternative methodology for use in future observational studies are provided.

5.2 Materials and Methods

5.2.1 Study design

Tabanids were trapped using the methods outlined in Chapter Four: Daily Activity Patterns of Tabanids in Townsville. The tabanids were kept alive in an incubator (25 - 26°C, 60% relative humidity) in individual vials, before use in feeding behaviour observational studies. Variations in the numbers and percentages of species used in the observational studies reflect those caught in traps in the 24 - 36 hours preceding the study.

The types of behaviours under investigation included the area of the host on which tabanids fed or landed, the length of time they spent feeding or alighting, the reason for tabanid departure from the host, and the host defensive behaviour.

Tabanids were released in a screened area around the host animal for a period of one hour, on average, with a maximum of two hours, and the landing and feeding behaviour of different tabanid species was recorded. The host defensive responses were also noted. The host species used were: cattle, sheep, pigs and agile wallabies (*Macropus agilis*) (Appendix 3, Table A3.01). Tabanids were classed as "feeding" when the mouthparts of the tabanid were engaged within the host's skin. If they had alighted, but did not have their mouthparts engaged, they were classed as having "landed".

Use of animals in observational studies on tabanid feeding behaviour was conducted in accordance with Animal Ethics guidelines (Approval number: A1060). Approval for studies on native species was gained from National Parks and Wildlife (Permit number: WISP 13550006)

Heifer studies were conducted in a screened pen (Figure 5.01) at the rear of the School of Veterinary and Biomedical Sciences, Douglas, Townsville (approximately $3m^2$). The pen was partially enclosed with shadecloth. The heifer was tethered in the centre of the

pen with a halter, within steel bars that limited movement and tabanids were released in the enclosure. Sheep, pig and wallaby studies were carried out in a mesh crate (Figure 5.02) (approximately 1m x 1.5m x 1.5m) covered with an olive green polyester mosquito net. Sheep and pig studies were carried out at the rear of the School of Veterinary and Biomedical Sciences, Douglas, Townsville. Wallaby studies were carried out at the home of the wallaby carer, at Rupertswood, Townsville. The wallabies were hand-reared and ready for release.

Figure 5.01: Heifer in screened pen.

Figure 5.02: Releasing tabanids into mesh crate, covered with mosquito net.

5.2.2 Observations recorded

Areas on the bodies of host animals where tabanid landing and feeding took place were divided into numbered sections for ease of data recording (Figure 5.03).

For each fly observed landing, the following data were recorded:

- Landing position of fly
- Genus of fly and species if possible
- Time of arrival
- Duration fly remained on animal
- Duration of feeding
- Duration of probing, other behaviour prior to feeding
- Type of host defensive behaviour that dislodged fly e.g. ear twitching, tail swishing, foot stamping, skin flicking
- Apparent cause of departure
    - Feed completed (i.e. abdomen fully distended and fly left without disturbance)
    - Disturbed by other flies (i.e those already present on the animal)
    - Disturbed by host behaviour
    - Left without feeding

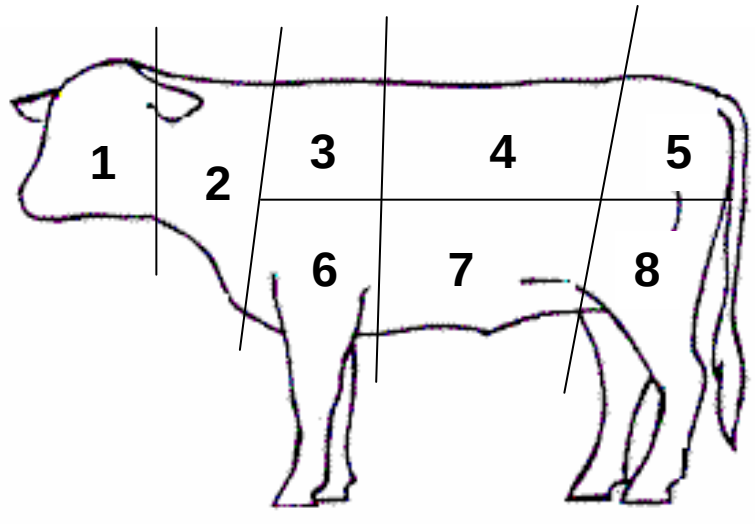


Figure 5.03: Areas on cattle for landing/feeding data recording

5.3 Results

5.3.1 Overall results

The study methodology used did not produce convincing results, since in more than 18 hours of observation, only 100 of the 659 tabanids released landed on hosts, and even fewer (19 individuals) attempted to feed.

Twelve species of tabanids were released around host animals, the majority of which was made up of *T. pallipennis* 41% and *T. townsvilli* 43.6.0% (n = 658). Of the tabanids that did feed (n = 19), 53 % were *T. pallipennis* and 26 % were *T. townsvilli*. The remainder was made up of *Cydistomyia* (16 %) and *L. fuliginosa* (5%).

In total, 15 feeding behaviour observational studies were performed, using one host per study. These included five studies with a heifer, three each using a wallaby and a sheep and four using a pig. A total of 658 tabanids were released and the total study duration was 18 hours and 40 minutes. During this time, 100 tabanids landed on the various hosts and 19 fed. The feeding success rate overall was 2.9% (Table 5.01, Appendix 3, Tables A3.01 – A3.02).

The highest rates of landing and feeding occurred on the pig and sheep, with low success on the heifer and wallaby. The sections below summarize landing and feeding events observed in these trials.

Table 5.01: Summary of tabanid feeding behaviour studies (see Appendix 3, Table A3.02 for list of tabanid species name corresponding to abbreviations used).

Host species		Tabanid species									
		TC	CA	CD	TT	TP	LF	TD	TS	Other	Total
PIG	#RELEASED				63	100	4	5	1	3	176
	#LANDED				30	26	2	4	0	0	62
	#FED				4	7	0	0	0	0	11
% released that	landed				48	26	50	80	0		35
% released that	fed				6	7	0	0	0		6
% landed that	fed				13	27	0	0			18
SHEEP	#RELEASED				132	40	3	9		1	185
	#LANDED				22	4	1	1			28
	#FED				1	1	1	0			3
% released that	landed				17	10	33	11			15
% released that	fed				1	3	33	0			2
% landed that	fed				5	25	100	0			11
HEIFER	#RELEASED	26	24	5	30	87		2	8		182
	#LANDED		3	1	1	1		0	0		6
	#FED		3	0	0	0		0	0		3
% released that	landed		13	20	3	1		0	0		3
% released that	fed		13	0	0	0		0	0		2
% landed that	fed		100	0	0	0					50
WALLABY	#RELEASED				62	43	4	2	2	2	115
	#LANDED				0	4	0	0	0	0	4
	#FED				0	2	0	0	0	0	2
% released that	landed				0	9	0	0	0		3
% released that	fed				0	5	0	0	0		2
% landed that	fed					50					50
OVERALL	#RELEASED	26	24	5	287	270	11	18	11	6	658
	#LANDED	0	3	1	53	35	3	5	0	0	100
	#FED	0	3	0	5	10	1	0	0	0	19
% released that	landed	0	13	20	18	13	27	28	0		15
% released that	fed	0	13	0	2	4	9	0	0		3
% landed that	fed		100	0	9	29	33	0			19

N.B. TC= *Tabanus concolor*, CA= *Cydistomyia* A (unidentified species), CD= *Cydistomyia doddi*, TT= *T. townsvilli*, TP= *T. pallipennis*, LF= *L. fuliginosa*, TD= *T. dorsobimaculatus*, TS= *T. strangmannii*. "Other" includes *P. silvester*, *Dasybasis*, *T. parvicallosus* and unidentified tabanids.

5.3.1.1 Feeding and landing times for tabanid species

Even with the limited data set collected, feeding and landing times varied considerably between individuals (Tables 5.02, 5.03). The data are too few to determine whether any apparent differences between tabanid species are genuine.

Table 5.02: Descriptive statistics of feeding times (seconds) for different tabanid species

Feeding times	***T. pallipennis***	***T. townsvilli***	***L. fuliginosa***	***Cydistomyia A***
Mean	131.27	258.40	27.00	570.00
Median	120.00	238.00	27.00	360.00
Standard Error	35.29	68.75	-	320.78
Standard Deviation	117.04	153.73	-	555.61
Minimum	4.00	69.00	27.00	150.00
Maximum	387.00	483.00	27.00	1200.00
Sum	1444.00	1292.00	27.00	1710.00
Count	11.00	5.00	1.00	3.00

Table 5.03: Descriptive statistics of landing times (seconds) for different tabanid species

Landing times	***T. pallipennis***	***T. townsvilli***	***T. dorsobi-maculatus***	***Cydistomyia A***	***L. fuliginosa***
Mean	51.54	70.30	42.60	3.00	144.50
Median	21.00	6.00	15.00	3.00	144.50
Standard Error	13.95	29.77	26.68	0.00	6.50
Standard Deviation	68.34	204.11	59.67	-	9.19
Minimum	2.00	1.00	2.00	3.00	138.00
Maximum	230.00	1200.00	145.00	3.00	151.00
Sum	1237.00	3304.00	213.00	3.00	289.00
Count	24.00	47.00	5.00	1.00	2.00

5.3.1.2 Feeding and landing times on different host species

The data in Table 5.04 suggest that host species differ in the likelihood that defensive behaviour will impact on feeding, but again, the data are too few to be convincing.

Table 5.04: Feeding times by host species (all tabanid species combined) (seconds)

Feeding Times	Pig	Sheep	Heifer	Wallaby
Mean	195.45	159.67	457.50	7.00
Median	148.00	189.00	255.00	7.00
Standard Error	44.24	69.69	253.19	3.00
Standard Deviation	146.73	120.70	506.38	4.24
Minimum	4.00	27.00	120.00	4.00
Maximum	483.00	263.00	1200.00	10.00
Sum	2150.00	479.00	1830.00	14.00
Count	11.00	3.00	4.00	2.00

Table 5.05: Landing times by host species (all tabanid species combined) (seconds)

Landing times	Pig	Sheep	Heifer	Wallaby
Mean	93.27	8.60	3.00	35.50
Median	20.00	3.00	3.00	35.50
Standard Error	27.52	2.57	0.00	32.50
Standard Deviation	196.51	12.85	-	45.96
Minimum	2.00	1.00	3.00	3.00
Maximum	1200.00	50.00	3.00	68.00
Sum	4757.00	215.00	3.00	71.00
Count	51.00	25.00	1.00	2.00

5.3.1.3 Preferred landing position by host

The preferred landing positions on the pig and sheep were analysed using a chi-squared test to determine if the favoured body region varied between host species. Body areas were combined for this purpose: head and neck, all upper body areas, all lower body areas (Table 5.06). Results of the analysis indicate that there were significant differences in the region of the body favoured for landing between the two species (χ^2= 6.121, df= 2, p= 0.047). For the pig, the upper body had more landings than expected, whereas for the sheep, observed landings on the head/ neck and lower body exceeded expected results.

Table 5.06: Landing positions by host (total number of landings)

Landing position	**Head and Neck**	**Upper body**	**Lower body**	**Total (n)**
Pig- observed	8	36	14	58
- expected	**11.47**	**31.02**	**15.51**	
Sheep- observed	9	10	9	28
- expected	**5.53**	**14.98**	**7.49**	

5.3.1.4 Preferred landing position by tabanid species

The preferred landing positions for *T. pallipennis* and *T. townsvilli* were analysed for the combined body regions using a chi-squared test to determne whether the preferred landing sites differed between species of tabanid (Table 5.07). There was no significant difference in preferred landing site for *T. pallipennis* compared to *T. townsvilli* (χ^2= 2.22, df= 2, p= 0.330).

Table 5.07: Landing position by tabanid species (total number of landings)

Landing position	**Head and Neck**	**Upper body**	**Lower body**	**Total (n)**
T. pallipennis - observed	5	13	10	28
- expected	**6.18**	**14.55**	**7.27**	
T. townsvilli - observed	12	27	10	49
- expected	**10.82**	**25.45**	**12.73**	

5.3.2 Notes on feeding behaviour on different host species

5.3.2.1 Pig

The pigs were much less responsive to tabanids than some of the other hosts. They tended to lie down in the cage for much of the time and did not to respond immediately to tabanids' probing. Tabanids appeared to have trouble probing through the coarse hairs on the pig's body. Many *T. townsvilli* landed on the pig and attempted to feed, but had trouble getting to skin level because of the coarse hair – they were trying to feed on the upper side of pig, whereas *T. pallipennis* seemed much more successful as they tended to move to less haired areas further down body e.g. the sides and limbs. Defences employed by the pigs were mainly shaking the body or movement e.g. getting up and lying down.

Types of defences employed by pigs (for feeding flies and non-feeding flies, more than one type of defensive behaviour may have been used per fly) were (n = 20):

Head movement (including ear movement/flicking):	40%
Leg kicking/stamping/movement:	30%
Body movement/getting up/lying down:	38%
Skin flicking on body:	5%

Table 5.08: Reasons for tabanids leaving (landed but did not feed)

Host	**Disturbed by host**	**Left without feeding**	**Disturbed by another fly**
Pig (n = 52)	27%	63%	10%
Sheep (n = 25)	20%	76%	4%
Wallaby (n = 2)	50%	50%	

5.3.2.2 Sheep

All three successful feeds were on limbs of the host animal. Tabanids that attempted to feed elsewhere on the body of the sheep were not successful in getting through the thick wool, even though the sheep had been recently shorn (1-2 weeks prior to study). Two tabanids left because they had completed the feed and were engorged, while the third left due to host disturbance.

The majority of defensive actions by the sheep were foot stamping and leg kicking (88%, n = 8), as well as head shaking (22%). Sheep appeared quite reactive to the presence of flies and exerted vigorous defensive behaviours, which may have interfered with ability of tabanids to begin feeding. A number of tabanids landed, but not many of these attempted to probe. Many of the tabanids that landed left quickly, without host defences causing them to leave.

5.3.2.3 Heifer

The five studies using a heifer as the host animal took place over a total of 6 hours 15 minutes. During this time 176 tabanids were released, resulting in five tabanids landing and four tabanids feeding (3 *Cydistomyia A* and 1 *T. pallipennis*). The heifer did not appear to react very much to the presence of tabanids, although there were very few that landed.

5.3.2.4 Wallaby

Wallaby studies, using a male Agile wallaby (*Macropus agilis*), approximately 14 months old, were undertaken on three occasions for a total of three hours and 10 minutes. A total of 115 tabanids were released. Of these, four tabanids landed (all *T. pallipennis*) and two fed on the wallaby. The host's defensive moves were the most intense of all the host animals studied. The two successful feeds took place on the tail and the thigh area. Both feeding tabanids left because of disturbance by the host. The remaining two landings that did not feed were also on the hind leg and tail. The causes

of departure were disturbance by the host in one case and the other left without feeding. Types of host defences employed were manual disturbance of tabanids with front paws, biting at the tabanid and body movement. Tabanids appeared to be able to feed anywhere on the host's body, as the host's fur did not seem to be a deterrent to feeding. The wallaby was highly mobile within the cage and was very reactive to tabanid bites.

5.4 Discussion

There was a very low feeding success rate observed in the course of this study (2.9% of 658 tabanids released). This figure is much lower than the success rate of naturally attracted tabanids feeding on cattle (Muzari, unpublished, 2007). Possible reasons for the low number feeding were: the presence of observers, the tabanids not being host-seeking i.e being in the wrong physiological state when trapped, stress caused by the trapping and storage conditions, or environmental conditions that did not favour feeding by the tabanids e.g. decreased visibility of host due to low UV light caused by the use of shade-cloth and mosquito net, inappropriate time of day (see Chapter Four: Daily Activity Patterns) or unfavourable weather conditions (see Chapter Four).

The species of tabanids used in these studies were determined by those abundant when trapping took place. Timing of the behaviour studies coincided with high seasonal tabanid abundance.

Tabanus townsvilli had longer mean feeding and landing times compared to *T. pallipennis*, although *T. pallipennis* had greater feeding success. Greater feeding success in *T. pallipennis* may have been due to the timing of studies which were largely carried out in the afternoon. This timing would have favoured the daily activity patterns of this species, which are more active between 4 - 7pm, compared to *T. townsvilli* (most active from 11am - 4pm). The areas on the host's body preferred by these two species are similar, both tending to alight on areas of the upper body. This means that application of insecticide by back-rubbers or back-line sprays would be expected to be efficacious for these species.

The longest feeding times were observed on the heifer, followed by the pig, sheep and wallaby. These results are in keeping with the intensity of observed defensive behaviours in these species, which were greatest in the wallaby, followed by the sheep, pig and heifer. The landing positions favoured on the pig and sheep were significantly different, with the upper body preferred in the pig and the head/neck and lower body in

the sheep. The greater percentage of tabanids landing on the head and neck and lower forelimbs of sheep probably reflects the shorter hair (and ease of access to the skin) in these areas.

These results need to be confirmed by observations on tabanids that were unconstrained and naturally attracted to the host animals, however. The methodology used in this study may have adversely affected feeding preferences and host responses, as well as feeding probabilities.

The limitations of the data in this study do not support firm conclusions on the feeding behaviour of the different species of tabanids or the effect of the host defensive behaviours on feeding success. However, if the observations from this study were further supplemented and shown to be consistent over larger sample sizes, some generalisations could be made regarding the effect of vector and host behaviours on the risk of disease transmission. For example, the shorter mean feeding time recorded for *T. pallipennis*, indicates a lower persistence in feeding and therefore increased likelihood of host switching, compared to *T. townsvilli*. Higher rates of host switching favour an increased possibility of disease transmission. In addition, the intensity of host defensive behaviours also influence the likelihood of disease transmission. Animals exhibiting a high degree of intensity of defensive behaviour increase the rate of tabanid host switching. For example, the results of this study indicate the intense defensive behaviour of wallabies would promote high rates of host switching and therefore increased risk of disease transmission compared to cattle.

Observations by humans in close proximity can affect the attraction of biting flies to hosts (Hargrove, 1976), so methods such as observations from a tower or ventilated pit have been used to circumvent this problem (Schofield and Torr, 2002). Use of these methods, in addition to use of an incomplete ring of electric nets (Vale, 1974), in an outdoor, unscreened environment, using tabanids that were naturally attracted to the host animals, may have improved the feeding rate of local tabanids in Townsville. The use of attractants such as carbon dioxide gas and octenol may also improve the numbers of

tabanids attracted to the hosts. It is recommended that these methods be employed for future studies on tabanid feeding behaviour. In addition, studies should be carried out on warm, sunny days, with the timing of studies appropriate to the daily activity patterns of the main species present.

This study provides some preliminary measurements of landing and feeding behaviour of local Townsville tabanid species. Recommendations for alternate methodology to be used in future feeding studies may improve feeding success, allowing more extensive data collection and analysis of vector feeding behaviour and host defensive behaviour on the likelihood of *T. evansi* transmission.

CHAPTER SIX
SEASONAL AND SPATIAL VARIATION IN TABANIDS IN CAPE YORK AND TOWNSVILLE

6.1 Introduction

The seasonal dynamics of tabanids in Cape York and Townsville were studied over two wet seasons, in order to examine the spatial and temporal abundance and diversity of tabanids in an area deemed a possible route of incursion for *Trypanosoma evansi* (Thompson *et al.*, 2003b).

The risk of transmission of *T. evansi* is likely to be highest when abundance of vectors (and therefore biting rate) is highest. Therefore, characterisation of the seasonal and spatial abundance of probable vector species will enable a better understanding of the areas and times of greatest risk.

This chapter describes the overall patterns of abundance and species richness which were observed – later chapters will examine and model the factors which may affect those patterns (see Chapters Eight, Nine and Ten).

The constraints of early life stages restrict the abundance of temperate and tropical tabanid species to defined seasonal periods, although the meteorological variables primarily responsible for this seasonality appear to be different in tropical and temperate zones, as discussed below. In temperate areas, the key determinant of tabanid seasonality appears to be seasonal changes in temperature, whereas in the tropics, tabanid seasonality appears to be regulated by the rainfall pattern. This is consistent with observations on other insect groups (Jones, 1987).

In the African tropics, tabanids are most abundant during the wet season. Larval habitats are often aquatic or require wet soil, and few adults are caught in the dry season (Lehane, 2005). In Zambia, Okiwelu (1975) found that periods of high abundance of five species of *Tabanus* and seven species of *Haematopota* coincided with the warm,

rainy season from November to April, and that a seasonal succession of species was seen during this period. He notes this seasonal abundance had been previously documented by a number of researchers, including Vanderplank (1944), Glasgow (1946), and Chapman (1960) in Tanzania and Clarke (1968) in Zambia. He asserts that the wet season abundance peaks are related to the "well-known association of tabanid immature stages with water".

In temperate areas, winter temperatures are more likely to limit population growth, with tabanid abundance peaking in summer (Lehane, 2005).

In New Jersey, Thompson (1969a) found 37 species in three genera occurred in highest numbers in summer from June to September. Sofield and co-workers (1985) reported the same seasonal distribution for the *Tabanus nigrovittatus* complex in this area.

Similar seasonal abundance patterns were observed in eastern Croatia, where tabanids were most abundant from the second half of June to the end of August, and are sometimes collected to the end of September. This is later than in central Europe, where the first species emerge in the second half of May. The later appearance of tabanids in Croatia is due to the low temperature of the soil during the Spring months, inducing late pupation of larvae (Chvala 1972 in Krcmar, 2005). Krcmar (2005) compared the seasonal abundance of tabanids from two locations in eastern Croatia for two years. Four species that were trapped at both locations and in both years showed temperature-related differences in total abundance between locations and in the time of year that peak abundance was reached.

Similarly in Guelph, Canada, the main period of abundance is from the end of May to mid-September, with highest numbers caught in June and July (Golini and Wright, 1978). These workers related the abundance to the degree of soil wetness at four different sites near Guelph. Nearly equal numbers of species were trapped at all four locations in 1971, but the relative abundance of the more numerous species appeared to vary between habitats. This author notes that significant changes in temperature between successive fly seasons are known to influence the date of first appearance of adult

tabanids, but this study did not demonstrate any significant differences in the flight periods of individual species, because there was little difference in the seasonal temperatures during the study (Golini and Wright, 1978).

These data suggest that in temperate climates, seasonal changes in temperature may regulate the beginning of flight activity of tabanids, as well as the duration of flight activity, and the timing of peaks of abundance. The Canadian data demonstrate that moisture availability may also influence patterns of abundance in temperate areas.

Australia includes both temperate and tropical regions, but seasonal patterns of tabanid abundance are better documented in temperate areas. Mackerras (1970) has reported on the seasonal presence of *Tabanus* species north and south of the Tropic of Capricorn in general terms. He noted that the season of adult activity in the tropics was mildly bimodal, with a peak in December and a secondary peak in March, and that adult seasons were shorter in southern localities than northern ones e.g. *T. pallipennis* has been collected in every month of the year in the north, but only between October and April south of the Tropic of Capricorn. He reports that the seasons of most species are long e.g. September-June for *T. innotabilis* (Mackerras and Spratt, 1971).

Spratt (1974a) studied the seasonal succession and abundance of tabanid vectors of the filarioid nematode *Pelecitus roemeri* in the subtropical/warm temperate areas of south-east Queensland. The seasonal abundance of tabanids in this region was greatest from November to April, with higher levels of transmission of *P. roemeri* being associated with the peaks of abundance of several species of *Dasybasis* that were incriminated as vectors. A difference in the relative abundance of some species was noted at the two locations at which tabanids were trapped (Durakai and Allan, from 1970 - 1971), although the months in which they were present were similar (Spratt, 1974b).

Australia has a different suite of tabanid species to those in neighbouring countries with endemic surra. The exception is *T. ceylonicus* which is common to many countries in South-East Asia and Australasia. *Tabanus ceylonicus* is reported to transmit *T. evansi* in experimental trials (Luckins, 1999). There is also evidence in the literature to suggest

that all *Tabanus spp.* are likely vectors of surra (Dieleman, 1986). It is certainly possible that other genera could also transmit surra in Australia.

This study examines the seasonal patterns of abundance and species composition of tabanids in tropical northern Queensland, specifically in Cape York and Townsville. The implications of abundance patterns for the potential risk of *T. evansi* transmission in the region are discussed.

6.2 Materials and Methods

6.2.1 Sample sites

Tabanid collections were made at eleven sites on Cape York and one site at Townsville (North Queensland, Australia) over a 21 month period from September 2004 to June 2006 (Figure 6.01, Table 6.01). All tabanids caught in the trap were removed once a week from December to the end of May each year (during the wet season, when abundance was high) and every four weeks at other times.

Table 6.01: Co-ordinates of trapping sites

Site	**Site code**	**Latitude (decimal degrees south)**	**Longitude (decimal degrees east)**
Bamaga	BA	10.893	142.399
Coen	CO	13.773	143.128
"Valley View" Cooktown	CK	15.351	145.030
Heathland National Park	HE	11.750	142.581
Karumba	KA	17.493	140.831
Lakefield National Park	LA	14.925	144.200
Lockhart River Airport	LR	12.783	143.305
Old Mapoon	MA	11.967	141.893
"Rutland Plains"	RP	15.639	141.823
"Stirling"	ST	17.183	141.710
Weipa	WE	12.661	141.858
Townsville	TV	19.324	146.765

6.2.2 Traps

Tabanids were collected using Nzi traps (Mihok, 2002) without an attractant. Translucent polycarbonate ("Laserlite®"; Laserlite Australia Pty Ltd, Cheltenham, Victoria, Australia) roofs were added to protect trapped tabanids from damage and mould due to heavy rain that occurs during the wet season on Cape York (Figure 6.02).

The mesh parts of the trap and the collection bags were constructed from white PVC-coated polyester mesh ("Phifertex®"; Phifer, Tuscaloosa, Alabama, USA). The coloured fabric used was "Sunbrella®"(Glen Raven Inc, North Carolina, USA) in Pacific Blue and Black colours (Mihok *et al.*, 2006).

6.2.3 Tabanid species identification

Species identification used the descriptions and keys of Mackerras (1959; 1961; 1964; 1970), (Mackerras and Spratt, unpublished)and comparisons with specimens in the Australian National Insect Collection (CSIRO Entomology, Canberra). Data analysis used Microsoft Excel (2003, version 11) and SPSS (SPSS Inc, versions 12 and 14).

6.2.4 Analysis of long-term weather and species richness and abundance

For analysis of total abundance and species richness against long-term weather variables, the total figures per site for the entire collection period from 1st September 2004 to 31st May 2006 were used in a separate database. Interpolated weather data ("drill data") for each site were averaged over the period from 1990- 2005 for the analysis of total species richness and total abundance at each site.

6.2.5 Ultra-violet radiation transmission through Laserlite® roofing

A comparison was made between the amount of measured ultra-violet (UV) radiation transmitted through new, unused Laserlite® roofing and roofing that had been subjected to field conditions for the length of the data collection period (21 months). The comparison was made between the amount of UV transmitted during the first and last month of the study. The Laserlite® roofing is purported to provide 99.9% protection from UV radiation, with a lifetime warranty. So the comparison was performed to ensure that there was no significant difference in the amount of UV transmitted through the Laserlite® throughout the course of the 21 month study, as this may have influenced the trap catch. Measurements of UV radiation were made using Safesun® UV meters (Optix

Tech Ltd, Washington, USA). Only two Safesun® UV meters were available, so the Laserlite® roofing was tested on separate occasions (three readings each for the new and used roofing). On each day that readings were made, one UV meter recorded the UV radiation underneath the roofing and one meter recorded the ambient UV radiation at a site approximately 1m away. Because preliminary testing revealed that zero readings for UV were common under the Laserlite, it was necessary to have a comparison with the ambient reading without the Laserlite, to provide a denominator.

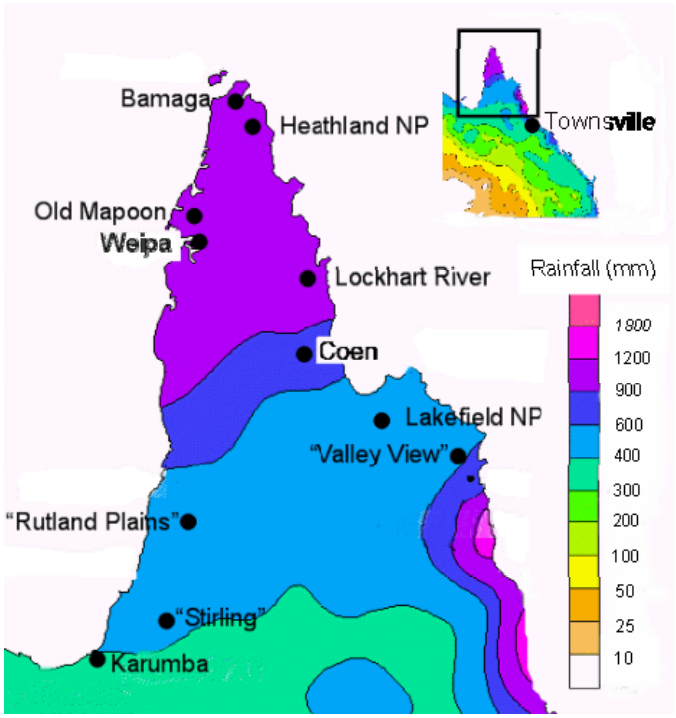


Figure 6.01: Map showing trap sites and rainfall zones (northern wet season rainfall totals from 1st October 2004 to 30th April 2005) in north Queensland (Bureau of Meteorology, 2006).

Figure 6.02: Trap with Laserlite® roof at Lakefield National Park

6.3 Results

6.3.1 Abundance and species richness over time

Tabanids were most abundant (more than 100 individuals caught) from December 2004 to the end of March 2005 and November 2005 to January 2006 (Figure 6.03). Species richness was highest (at least 10 species caught) from December 2004 to April 2005 and November to February 2006. There was also a small peak (10 species) in August 2005 (Figure 6.04). These peaks generally coincided with the wet seasons of each year.

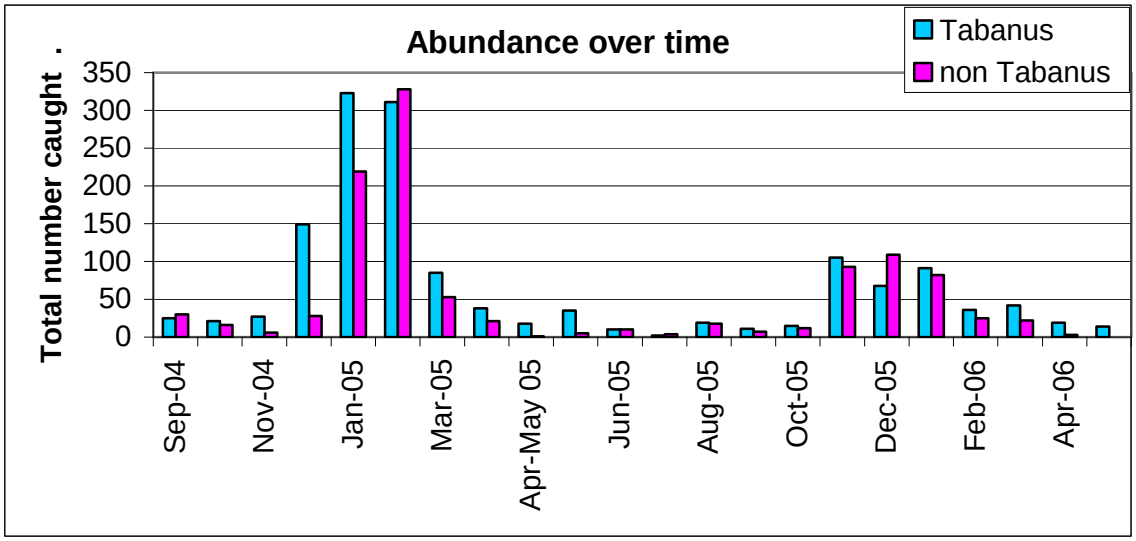


Figure 6.03: Total abundance over time (all sites combined) for *Tabanus* and non-*Tabanus* species of tabanid.

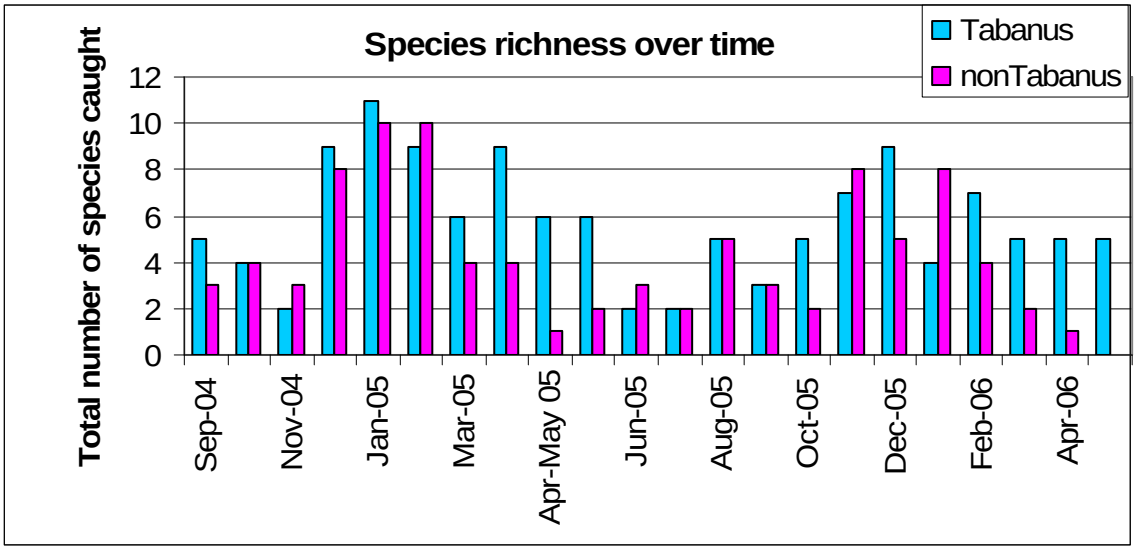


Figure 6.04: Species richness over time (all sites combined) for *Tabanus* and non-*Tabanus* species of tabanid.

6.3.2 Abundance and species richness by site

There does not appear to be a consistent relationship between total abundance and species richness (see for example the high species richness and low abundance at the Weipa site) (Figures 6.05, 6.06). Overall the correlation is not significant (0.548, p= 0.065, Table 6.05). Tabanidae were most diverse at Bamaga with 16 species in total (Figure 6.06), and least diverse at Karumba with only four species, three *Tabanus* and one *Dasybasis*. Abundance was lowest at "Stirling" with a total count of 26, and highest at Lockhart River with a total count of 525 (Figure 6.05, Tables 6.03, 6.04). A total of 38 species was captured overall, which included 15 *Tabanus* species (Tables 6.02, 6.03 and 6.04).

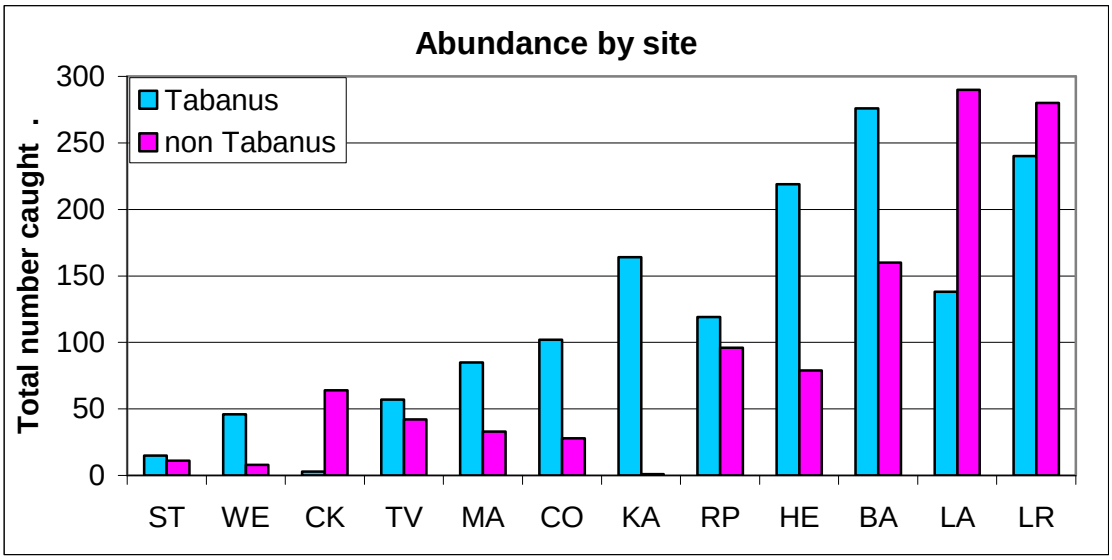


Figure 6.05: Total abundance by site (all collection data combined) for *Tabanus* and non-*Tabanus* species of tabanid.

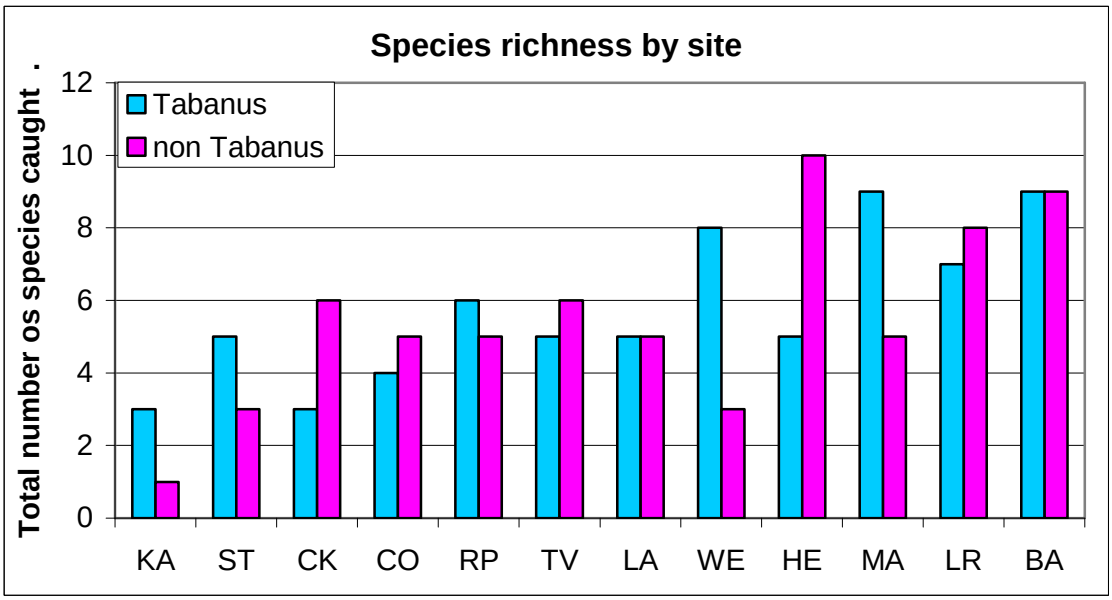


Figure 6.06: Species richness by site (all collection data combined) for *Tabanus* and non-*Tabanus* species of tabanid.

Table 6.02: Complete list of species and total numbers of each caught
***** Specimens badly damaged

Species	**Species code**	**Total number collected**
Cydistomyia cf. *bancroftae* Mackerras	Cyd-ban	1
Cydistomyia doddi (Taylor)	Cyd-dod	1
Cydistomyia musgravii (Taylor)	Cyd-mus	2
Dasybasis clavicallosa (Ricardo)	Das-cla	1
Dasybasis dixoni (Darwinensis form) (Ferguson)	Das-dix	2
Dasybasis cf. *griseoannulata* (Taylor)	Das-gri	4
Dasybasis nemotuberculata (Ricardo)	Das-nem	67
Dasybasis oculata (Ricardo)	Das-ocu	271
Dasybasis Unknown A	Das-unA	1
Dasybasis Unknown B	Das-unB	4
Lilaea demeijerii (Ricardo)	Lil-dem	70
Lilaea fuliginosa (Taylor)	Lil-ful	192
Lilaea mansoni (Summers)	Lil-man	1
Mesomyia (Eucompsa) sp. Enderlain	Mes-euc	1
Mesomyia (Pseudotabanus) ater (Taylor)	Mes-ate	1
Pseudotabanus distinctus Ricardo	Pse-dis	1
Pseudotabanus eyreana (Mackerras)	Pse-eyr	83
Pseudotabanus cf frontalis (Ricardo)	Pse-fro	27
Pseudotabanus fuscipennis (Ricardo)	Pse-fus	4
Pseudotabanus montanus (Ricardo)	Pse-mon	2
Pseudotabanus queenslandii Ricardo	Pse-que	25
Pseudotabanus silvester (Bergroth)	Pse-sil	234
Scaptia Unknown A	Sca-xxx	1
Tabanus ceylonicus (Schiner)	Tab-cey	70
Tabanus cohaerens Walker	Tab-coh	3
Tabanus concolor Walker	Tab-con	34
Tabanus dorsobimaculatus Macquart	Tab-dor	81
Tabanus innotabilis Walker	Tab-inn	114
Tabanus nigrimanus Walker	Tab-nig	16
Tabanus notatus Ricardo	Tab-not	628
Tabanus obscurilineatus Taylor	Tab-obs	4
Tabanus pallipennis Macquart	Tab-pal	77
Tabanus particaecus Hardy	Tab-pat	2
Tabanus parvicallosus Ricardo	Tab-par	2
Tabanus praepositus Walker	Tab-pra	1
Tabanus strangmannii Ricardo	Tab-str	259
Tabanus townsvilli Ricardo	Tab-tow	31
Tabanus muruensis Mackerras	Tab-mur	3
Indeterminate *Cydistomyia spp.*	Cyd-xxx	1
Indeterminate * *Dasybasis spp.*	Das-xxx	37
Indeterminate * *Mesomyia spp.*	Mes-xxx	58
Indeterminate * *Tabanus spp.*	Tab-xxx	139
Indeterminate * *Tabanidae*	xxx-xxx	83
Total		2639

Table 6.03: Total number of non-*Tabanus* species caught at each site (species abbreviations are explained in Table 6.02)

Species code	**BA**	**CK**	**CO**	**HE**	**KA**	**LA**	**LR**	**MA**	**RP**	**ST**	**TV**	**WE**	**Total**
Cyd-ban	0	0	0	0	0	0	0	0	1	0	0	0	1
Cyd-dod	0	1	0	0	0	0	0	0	0	0	0	0	1
Cyd-xxx	0	0	0	0	0	0	1	0	0	0	0	0	1
Cyd-mus	0	0	0	0	0	0	0	0	0	0	2	0	2
Das-cla	0	0	0	0	0	0	0	0	0	0	1	0	1
Das-dix	0	1	0	0	0	0	1	0	0	0	0	0	2
Das-gri	0	0	0	0	0	0	0	1	0	0	0	3	4
Das-nem	1	55	0	0	0	0	0	10	1	0	0	0	67
Das-ocu	1	0	6	5	1	2	189	15	49	0	0	3	271
Das-unA	1	0	0	0	0	0	0	0	0	0	0	0	1
Das-unB	0	1	0	1	0	0	2	0	0	0	0	0	4
Das-xxx	1	1	1	0	0	1	21	2	8	0	2	0	37
Lil-dem	14	0	0	30	0	0	26	0	0	0	0	0	70
Lil-ful	5	1	1	0	0	123	12	2	32	7	9	0	192
Lil-man	0	0	0	0	0	0	0	0	1	0	0	0	1
Mes-ate	0	0	0	1	0	0	0	0	0	0	0	0	1
Mes-euc	0	0	0	1	0	0	0	0	0	0	0	0	1
Mes-xxx	15	2	3	1	0	20	9	1	4	1	1	1	58
Pse-dis	0	0	0	0	0	0	0	0	0	0	1	0	1
Pse-fro	1	0	0	24	0	2	0	0	0	0	0	0	27
Psu-eyr	13	0	4	2	0	56	6	0	0	2	0	0	83
Psu-fus	0	0	1	0	0	0	1	2	0	0	0	0	4
Psu-mon	0	0	0	0	0	0	0	0	0	0	2	0	2
Psu-que	19	0	0	6	0	0	0	0	0	0	0	0	25
Psu-sil	89	2	12	7	0	86	12	0	0	1	24	1	234
Sca-xxx	0	0	0	1	0	0	0	0	0	0	0	0	1
Totals	**160**	**64**	**28**	**79**	**1**	**290**	**280**	**33**	**96**	**11**	**42**	**8**	**1092**

Table 6.04: Total number of *Tabanus* species caught at each site (species abbreviations are explained in Table 6.02)

Species	BA	CK	CO	HE	KA	LA	LR	MA	RP	ST	TV	WE	Total
Tab-cey	53	0	0	3	0	0	4	2	0	0	0	8	70
Tab-coh	0	0	0	0	0	0	0	2	0	0	0	1	3
Tab-con	6	1	0	6	0	14	0	0	3	3	0	1	34
Tab-dor	3	0	0	0	0	6	0	45	9	0	15	3	81
Tab-inn	2	0	1	0	98	0	2	2	0	0	0	9	114
Tab-mur	1	0	0	0	0	0	0	0	0	0	0	2	3
Tab-nig	0	0	0	0	0	0	1	15	0	0	0	0	16
Tab-not	108	0	92	57	1	84	190	11	79	2	2	2	628
Tab-obs	0	0	0	0	0	0	1	1	1	1	0	0	4
Tab-pal	10	1	2	2	0	21	6	1	22	1	8	3	77
Tab-par	2	0	0	0	0	0	0	0	0	0	0	0	2
Tab-pat	0	0	0	0	0	0	0	0	1	0	1	0	2
Tab-pra	0	0	0	0	1	0	0	0	0	0	0	0	1
Tab-str	52	1	4	149	0	12	33	2	0	6	0	0	259
Tab-tow	0	0	0	0	0	0	0	0	0	0	31	0	31
Tab-xxx	39	0	3	2	64	1	3	4	4	2	0	17	139
Totals	**276**	**3**	**102**	**219**	**164**	**138**	**240**	**85**	**119**	**15**	**57**	**46**	**1464**

6.3.3 Species partitioning

Individual *Tabanus* (Figure 6.07) and non-*Tabanus* (Figure 6.08) species partitioned each wet season in a consistent manner. Different species peaked at different times, and the sequence was consistent between years. For the *Tabanus* species, *T. innotabilis* preceded the emergence of *T. notatus* and *T. ceylonicus*. They were followed by *T. dorsobimaculatus*, with *T. strangmannii* the last to peak. This series was repeated in the second wet season. Interestingly, there was another small peak in *T. ceylonicus* numbers in May-July 2005 and September-October 2005, traditionally the drier, cooler season. Sites that contributed to these later, small peaks of *T. ceylonicus* were Bamaga and Weipa.

Lilaea demeijerii was the first of the non-*Tabanus* species to peak in 2005, followed by *P. silvester, D. nemotuberculatus, D. oculata* and *P. eyreana* which emerged almost concurrently, followed by *L. fuliginosa*. The pattern was less pronounced, though still evident, in 2006. The data also show striking differences in the relative abundance of different species between 2005 and 2006.

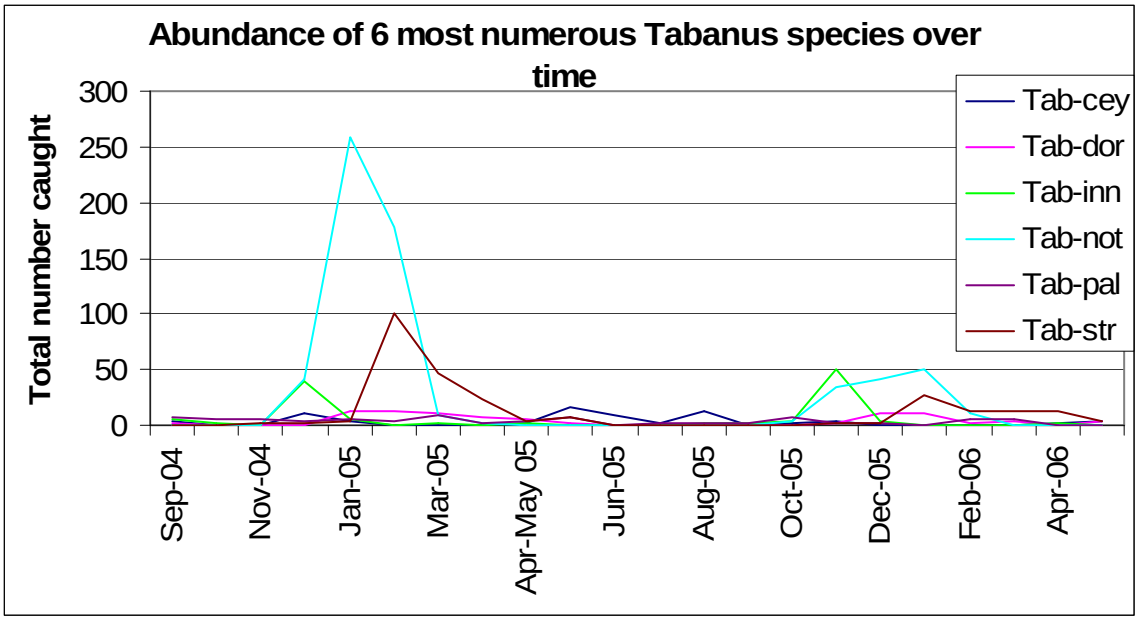


Figure 6.07: Abundance of six most numerous *Tabanus* species over time (all sites combined).

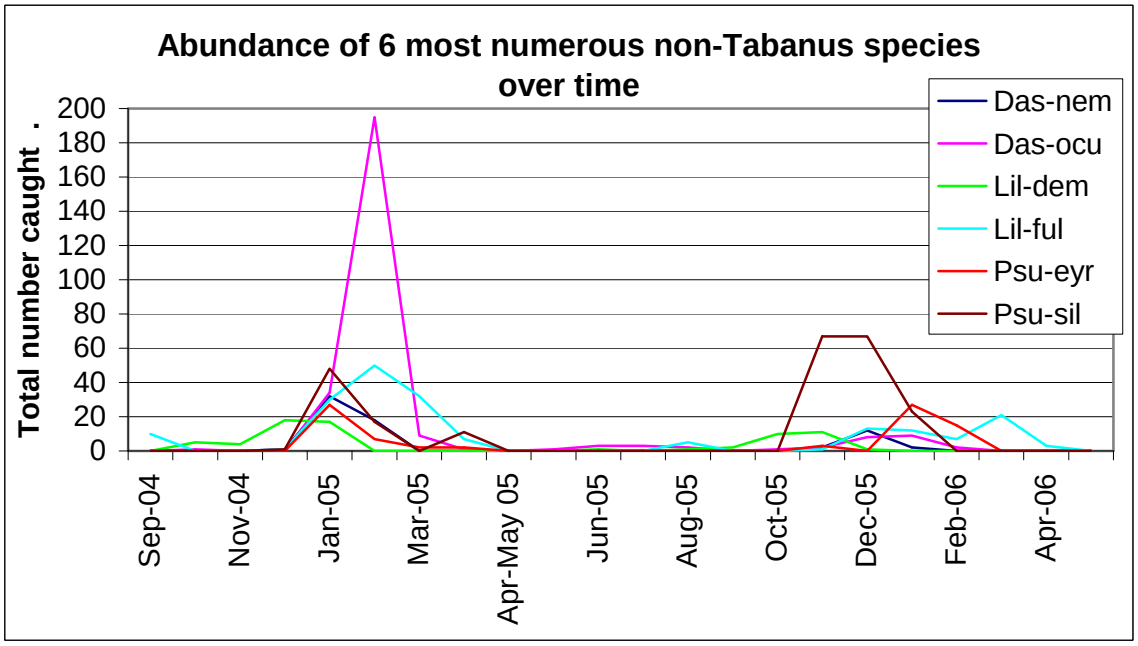


Figure 6.08: Abundance of the six most numerous non-*Tabanus* species over time (all sites combined).

6.3.4 Abundance and species richness: correlation with long-term weather

Total tabanid abundance and species richness were analysed against long-term average weather variables for each site. Correlations were significant between some of the weather factors and species richness, but not total abundance (n = 12 in each case) (Table 6.05). The weather factors that were significantly correlated with species richness were mostly inter-correlated and relate to moisture in the environment. Species richness was significantly correlated with average annual rainfall, solar radiation, vapour pressure and relative humidity (minimum and maximum). Species richness was most highly correlated with vapour pressure (Pearson correlation coefficient = 0.837, p = 0.001). The correlation between abundance and diversity at a site was not significant (Pearson correlation coefficient = 0.548, p = 0.065). An explanation of weather variables is provided in Chapter Nine (Section 9.2.2.3).

Table 6.05: Correlations between total abundance and species richness of tabanids (at each trap site) with long-term averages of weather data. Weather data (daily) were averaged over the last 16 years (1990-2006). N=12 in each case.

	Total number of species per site	**Average annual rainfall**	**Solar radiation**	**Vapour pressure**	**RH (max)**	**RH (min)**
Total number of species per site	1.000	0.758 (p=0.004)	-0.791 (p=0.002)	0.837 (p=0.001)	0.800 (p=0.002)	0.647 (p=0.023)
Abundance	0.548 (p=0.065)	0.302 (p=0.340)	-0.380 (p=0.223)	0.495 (p=0.102)	0.502 (p=0.096)	0.360 (p=0.250)

6.3.5 UV radiation transmission through Laserlite® roofing

The amount of UV radiation transmitted through both new Laserlite and Laserlite subjected to field conditions for 21 months was negligible in each case (all readings were 0.00 Minimal Erythemal Dose). These results meant that a comparison of UV readings taken underneath the new and used Laserlite using a paired t-test could not be undertaken (since all values were zero). Therefore to examine whether the amount of UV present on the days when the new Laserlite was tested, was the same as the days on

which the used Laserlite was tested, paired t-test was performed on the ambient UV readings. The amount of ambient UV radiation measured for the duration of readings for both new and used Laserlite was not significantly different ($T = -0.30$, $df = 6$, $p = 0.77$) (Appendix 4, Table A4.03). Measurements of ambient UV radiation by the two meters were not significantly different either ($T = 2.98$, $df = 2$, $p = 0.10$) (Appendix 4, Table A4.04). This result ensured that the calibration of the UV meters was not responsible for errors.

6.4 Discussion

Thirty-eight species of Tabanidae, including 15 species of *Tabanus*, were captured at 11 locations on Cape York and at Townsville, suggesting that appropriate species for transmission of surra may be present in northern Australia.

Tabanids are known to be sensitive to UV radiation (Hribar *et al.*, 1991), so it was important to determine whether the amount of UV radiation transmitted through the Laserlite roofing changed during the course of the study, as this could have altered the trap catch. A comparison of UV readings under new and used Laserlite roofing revealed that even after 21 months in field conditions, no UV was detectable. This meant that no degradation of the Laserlite roofing occurred to any detectable effect, which may have altered the amount of UV light under the roof and caused changes the attractiveness of the trap over time.

Tabanus species (Tribe: Tabanini) are considered the most likely vectors of *T. evansi* (Dieleman, 1986). The 15 *Tabanus* species found on Cape York and at Townsville include *T. ceylonicus*, a species known to transmit *T. evansi* in Indonesia (Luckins, 1998). This species was trapped at a number of locations on Cape York, including Bamaga, Heathlands, Lockhart River, Old Mapoon and Weipa. *Tabanus ceylonicus* was trapped in greatest numbers at Bamaga, which is located at the tip of Cape York. The presence of these known vectors at a location that is possibly at greatest risk of incursion of *T. evansi,* given its proximity to Papua New Guinea and the substantial traffic of small vessels between the islands of the Torres Strait, underlines the importance of disease surveillance in this area (Reid, 2002; Thompson *et al.*, 2003b).

Species richness at the 12 sites was highly correlated with average annual rainfall, vapour pressure, solar radiation and relative humidity but total abundance was not related to any of the long-term average climate factors. Vapour pressure is related to both temperature and humidity, so it is not surprising that the highest correlation was produced with this variable. It is likely that abundance data at each site are more closely allied to the short-term fluctuations in local weather, while species richness is a product

of the long-term weather conditions in a region, as has been the case with other insect groups (Schowalter, 2006). Some sites were much drier during the study years than their long-term averages, which is likely to have adversely affected the abundance of some or all species. The impact of annual variation in weather variables is examined further in Chapter Nine.

Tabanus species occurred in abundance at many of the sites studied, and were most prevalent in the wet season from December to March. Other genera, which could also be vectors of *T. evansi*, occurred in substantial numbers coincidently with *Tabanus*. These data are consistent with experience in south-east Queensland (Spratt, 1974a). This may indicate that the risk of *T. evansi* transmission is highest during December - March, when vector abundance is highest, as was the case in Spratt's epidemiological study of *P. roemeri* (Spratt, 1974a). The total abundance and therefore the potential biting rate may vary with weather factors (discussed in Chapters Nine and Ten). The presence of *T. ceylonicus* in significant numbers during the dry season implies that there is also potential for *T. evansi* transmission to occur during this time, which was an unexpected finding.

In this study, the peaks of abundance coincided with the wet seasons of each year. This is not surprising, since wet season conditions are ideal for larval development, which is aquatic, and also provide the right conditions for emergence of pupae that have over-wintered in a state of arrested development (Mackerras, 1970). This is consistent with reports from the African tropics (Okiwelu, 1975; Lehane, 2005).

The abundance and species richness appeared to be higher in 2005, compared to 2006. This may have been due to differences in certain weather factors between the two seasons. The impact of weather will be examined further in Chapters Nine and Ten.

Two species that are described as Papua New Guinean species were found in the most northern points of Cape York, suggesting possible migration through the Torres Strait. Three *Tabanus muruensis* specimens were collected from Bamaga and Weipa. Also, a

darker version of *Tabanus dorsobimaculatus*, which matches the description from Papua New Guinea was found (one specimen) (Mackerras, 1964).

This chapter defines the seasonal dynamics of tabanid species richness and abundance in an area of northern Australia. Analysis and modelling of factors affecting spatial and temporal patterns of abundance is covered in Chapters Eight, Nine and Ten.

CHAPTER SEVEN
VARIATIONS IN TABANID BODY SIZE AMONG SITE AND FLIGHT SEASON

7.1 Introduction

Dipteran body size is governed by a number of factors including the level of larval nutrition, density of larvae, environmental temperature during larval development and whether diapause occurs (Schowalter, 2006). Differences in size during a flight season usually indicate there has been some turnover in population, while differences during the same season at different sites or between years may indicate environmental conditions that are more or less conducive to growth.

A population might decrease in size over a flight season for a number of reasons:

- Emergence of different classes of flies at different times during the season: for example a batch might emerge following diapause over a dry season, that were larger in size because of a longer larval development period, followed by a batch that was produced within the same flight season, having gone through its whole life cycle from egg-laying to adult while the environmental conditions for development and emergence were appropriate. Thus, a significant difference in size during the course of the flight season may indicate that there is heterogeneity in the age structure of the species.
- Differential survival of flies of different sizes: for example if smaller flies tended to have shorter life-spans, the average size of flies might increase during the flight season.
- Differential dispersal into or out of the population as the season progresses: that is, the immigration or emigration of flies having a different average size to the local population as a whole.

Studies elsewhere indicate the first of these may be the most common.

Leprince and Bigras-Poulin (1988) refer to an "emergence gate", a theoretical barrier that is opened or closed according to environmental factors, that either prevents or allows the transformation from larvae to pupae to adults, and may influence the final body size of adults. Larvae may reach their potential maturity after the "emergence gate" has been closed, in which case they will continue their larval development and emerge as bigger adults the following year, at the beginning of the next emergence period. If larvae reach potential maturity while the "emergence gate" is open, they can pupate and emerge. Some larvae can pupate without achieving full potential size, and they emerge as smaller adults. These authors found that the mean size of *Tabanus quinquevittatus* decreased over the course of the flight season, probably as a result of these smaller flies emerging (Leprince and Bigras-Poulin, 1988).

The majority of *T. quinquevittatus* required two years to complete their life cycle, indicating that if conditions were not appropriate, larvae pursued a further year of larval development, resulting in emergence of adults that were equally competent to complete gonotrophic cycles (Logothetis and Schwardt 1948 in LePrince and Bigras-Poulin 1988). This strategy is different from mosquitoes, which have very small adults if the larvae are starved. These small adults may require two blood-meals to initiate the first egg batch (Feinsod and Spielman 1980 in LePrince and Bigras-Poulin 1988).

Body size (as determined by wing length) is correlated with the number of ovarioles per ovary (Leprince and Lewis, 1983) and egg production is correlated with wing length (Leprince and Foil, 1993). This means that the larger the adult body size of a species, the more offspring it will produce. Leprince and Jolicoeur (1986a) found that two species they studied in south-western Quebec did not have significantly different body size or number of ovarioles between sampling years. However, *T. lineola* did have a significant difference in these parameters between sampling years. Certain environmental conditions can affect larval development by altering body size and potential fecundity of adults. It would be expected that this would result in alterations in the total egg production and therefore annual population levels. This effect may be seen within the same sampling year at different sites, or between sampling years where larval nutritional levels have been quite different.

This chapter examines the differences in the body size of different tabanid species, to see if there are significant differences between sites, and between six month periods assigned arbitrarily as “flight seasons”. If differences in body size are found they might indicate environmental conditions that are more or less conducive to larval growth and potential fecundity. The sites with the largest sized tabanids would be expected to also have the highest populations of tabanids.

7.2 Materials and Methods

Tabanids were caught in various sites in Cape York and Townsville over 21 months (methods of tabanid capture and sites used are presented in Chapter Six). Wing length measurements were carried out as described in Chapter Four. Analysis of variance was performed on the wing lengths of each of the six most numerous *Tabanus* and non-*Tabanus* species, to determine whether wing length varied either by site of capture or flight season of capture. The flight seasons were defined as follows:

- Flight season one: October 2004- March 2005
- Flight season two: April 2005- September 2005
- Flight season three: October 2005- March 2006
- Flight season four: April 2006- May 2006

These groupings attempted to categorise species that were active in the wet season and dry seasons, for each of the study years, separately.

7.2.1 Statistical methods

Univariate analysis of variance (SPSS, SPSS Inc, version 14) was performed on wing length measurements, with wing length as the variable and site and flight season as fixed factors. Tests for homogeneity of variance were also performed. If there were less than five specimens in a group (either for site or flight season), those specimens were removed from the analysis, in order to remove the variation due to very small group sizes. Scatter plots and tables of the results were produced. Tests for homogeneity of variance may not have been passed due to a large discrepancy in the number of specimens "n" among groups. The sample sizes were determined by the catch of each species at a site and thus could not be controlled. Therefore the results are plotted even when the test for homogeneity of variance was not passed. Post-hoc tests were performed to determine where significant differences occurred.

7.3 Results

7.3.1 *Tabanus spp*. body size variation among sites

The body size of *T. pallipennis* varied significantly between sites. The test for homogeneity of variance was satisfied for this species (Table 7.01). The scatter plot (Figure 7.01) indicates the general trend for decreasing wing length with decreasing annual average rainfall. Individuals of this species were significantly larger at Lockhart River than Bamaga ($p = 0.046$), Lakefield ($p < 0.0005$) and Townsville ($p < 0.001$). *Tabanus pallipennis* was also significantly larger at Rutland Plains than Lakefield ($p = 0.012$) and Townsville ($p = 0.023$). (Details of post-hoc tests are found in Appendix 7.01).

While tests for homogeneity of variance were not satisfied for the remaining *Tabanus* species analysed, the body size of *T. notatus*, *T. dorsobimculatus*, *T. innotabilis* and *T. strangmannii* all varied significantly between sites, with the same general trend for decreasing body size with decreasing average annual rainfall (Figure 7.01, Appendix 7.01).

7.3.2 *Tabanus spp.* body size variation among flight seasons

Significant differences in body size were determined among flight seasons for *T. ceylonicus*, *T. dorsobimaculatus*, *T. innotabilis* and *T. strangmannii*, although these differences were not consistent among flight seasons (Figure 7.02, Appendix 7.02). Significant interaction was present between flight season and site for *T. notatus*, *T. ceylonicus* and *T. strangmannii*. That is, the differences between flight seasons were affected by site.

Body size of *T. ceylonicus* varied significantly between flight seasons but not sites (Table 7.01, Appendix 7.02). Significantly larger average body size was recorded in flight season two (April- September 2005) than flight season one (October 2004- March

2005). Body size of *T. innotabilis* was significantly larger in flight season one than flight season three (October 2005- March 2006) (Appendix 7.02). *Tabanus dorsobimaculatus* was significantly larger in flight season three than during either flight season one or two and significantly larger in flight season two than one (Appendix 7.02). Body size of *T. strangmannii* was significantly larger in flight season four than either flight seasons one or two and significantly larger in flight season three than flight season one (Appendix 7.02).

Table 7.01: Summary of ANOVA results of site and flight season effects for six *Tabanus* species. (NB empty cells in this table indicate that no test could be performed due to inadequate numbers of specimens in groups).

Species	**Site**	**Flight season**	**Flight season*site**	**Levene's test for homogeneity of variance passed**
T. notatus	F = 36.5 df = 6 p < 0.0005	F = 1.9 df = 1 p = 0.174	F = 13.1 df = 6 p < 0.0005	No
T. ceylonicus	F = 1.2 df = 1 p = 0.285	F = 19.5 df = 3 p < 0.0005	F = 25.1 df = 1 p < 0.0005	No
T. pallipennis	F = 8.0 df = 4 p < 0.0005	F = 0.9 df = 2 p = 0.425	F = 1.3 df = 4 p = 0.267	Yes
T. dorsobimaculatus	F = 7.08 df = 2 p = 0.002	F= 16.82 df = 2 p < 0.0005	F = 0.90 df = 1 p = 0.348	No
T. innotabilis	F = 24.3 df = 1 p < 0.0005	F = 8.2 df = 1 p = 0.006	-	No
T. strangmannii	F = 12.6 df = 4 p < 0.0005	F = 6.4 df = 3 p < 0.0005	F = 4.2 df = 6 p = 0.001	No

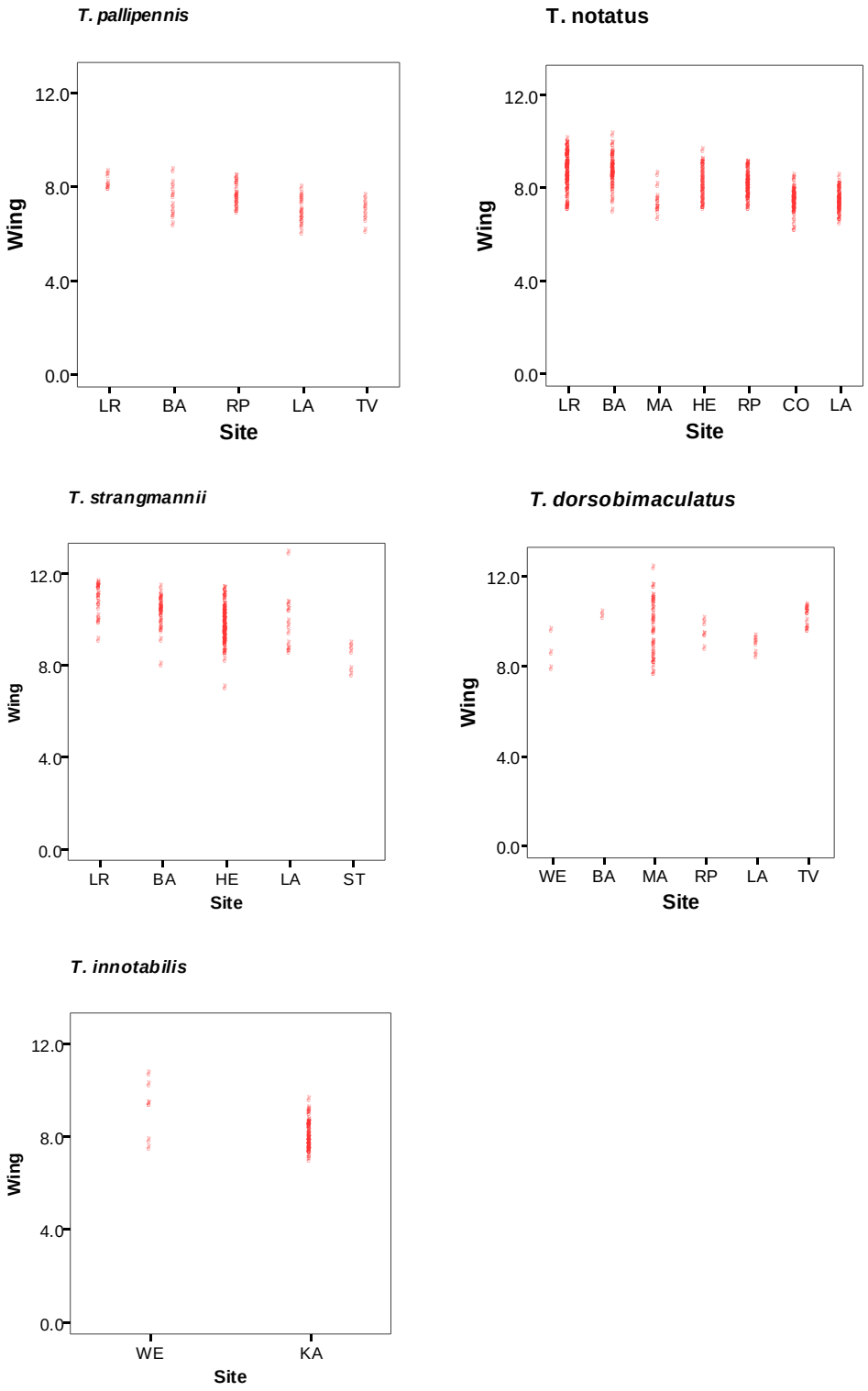


Figure 7.01: Scatter plots of wing lengths (mm) between sites for *T. pallipennis*, *T. notatus*, *T. strangmannii, T. dorsobimaculatus* and *T. innotabilis* (sites are in order of decreasing average annual rainfall).

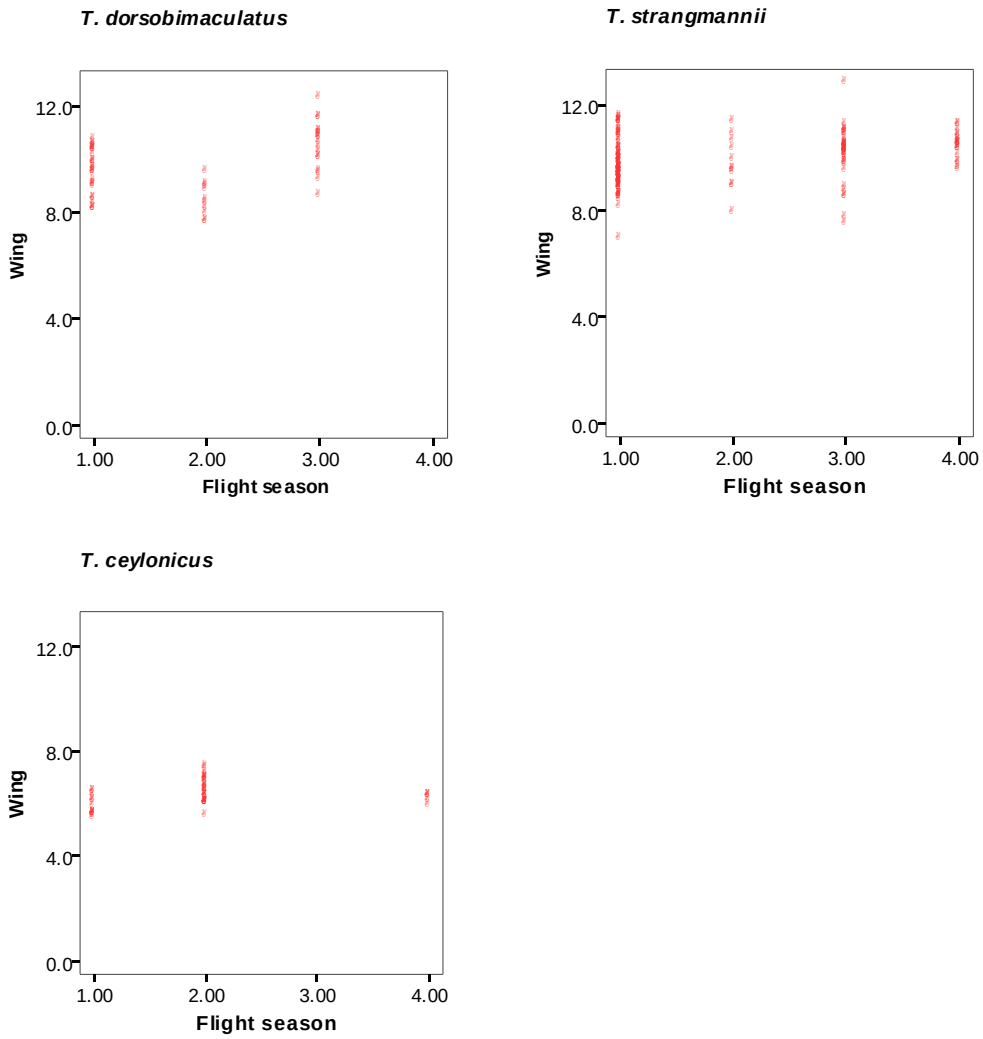


Figure 7.02 Scatter plots of wing lengths (mm) of *Tabanus* species between flight seasons for (sites are in order of decreasing average annual rainfall).

7.3.3 Non-*Tabanus spp*. body size variation among sites

Significant differences in body size among sites were noted for *D. oculata*, *L. fuliginosa*, *L. demeijerii*, *P. eyreana* and *P. silvester* (Table 7.02). No readily apparent trend for increasing body size with increasing average annual rainfall at a site was noted for the non-*Tabanus* species analysed (Figure 7.03, Appendix 7.03). Tests for homogeneity of variance were passed in all cases.

Table 7.02: Summary of ANOVA results of site and flight season effects for six non-*Tabanus* species.

Species	**Site**	**Flight season**	**Flight season*site**	**Levene's test for homogeneity of variance passed**
D. oculata	F = 7.39 df = 4 p < 0.0005	F = 4.78 df = 2 p = 0.009	F = 0.28 df = 1 p = 0.599	Yes
D. nemotuberculata	F = 0.05 df = 1 p = 0.823	F = 7.36 df = 1 p = 0.009	F = 2.55 df = 1 p < 0.116	Yes
L. fuliginosa	F = 6.13 df = 5 p < 0.0005	F = 3.05 df = 2 p = 0.050	F = 1.86 df = 5 p = 0.105	Yes
L. demeijerii	F = 24.83 df = 2 p < 0.0005	F= 5.53 df = 1 p = 0.022	F = 6.05 df = 2 p = 0.004	Yes
P. eyreana	F = 8.27 df = 2 p = 0.001	F = 6.63 df = 1 p = 0.012	F = 5.57 df = 1 p = 0.021	Yes
P. silvester	F = 14.35 df = 5 p < 0.0005	F = 8.11 df = 2 p < 0.0005	F = 6.59 df = 3 p < 0.0005	Yes

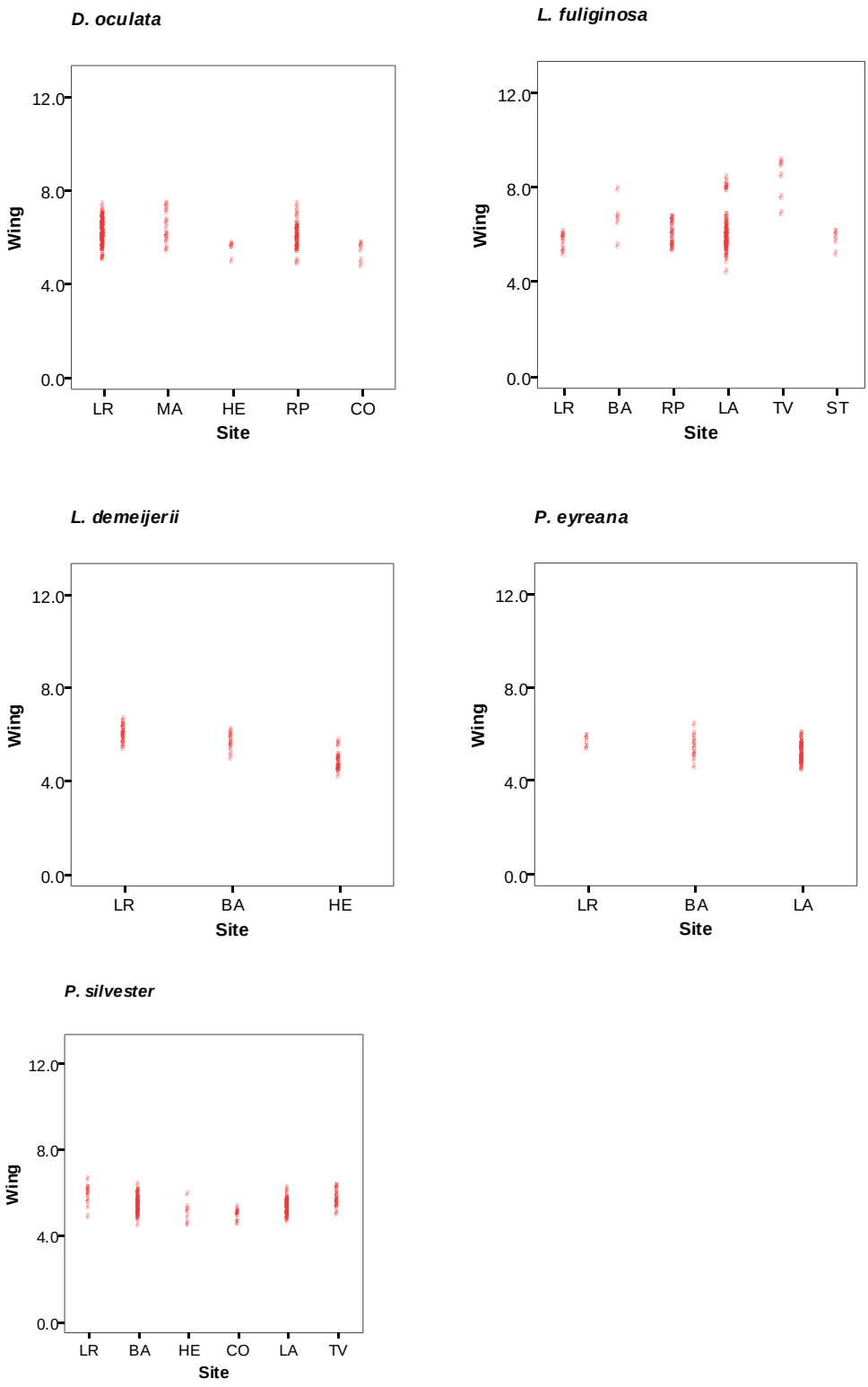


Figure 7.03 Scatter plots of non-*Tabanus spp*. body size variation among sites (sites are in order of decreasing average annual rainfall).

7.3.4 Non-*Tabanus* spp. body size variation among flight seasons

Significant differences in body size were observed among flight seasons for all six non-*Tabanus* species (Figure 7.04, Appendix 7.04). No consistent pattern between flight seasons was found for these species. Significant interaction between flight season and site occurred for *L. demeijerii*, *P. eyreana* and *P. silvester* which meant that differences between flight seasons were influenced by site.

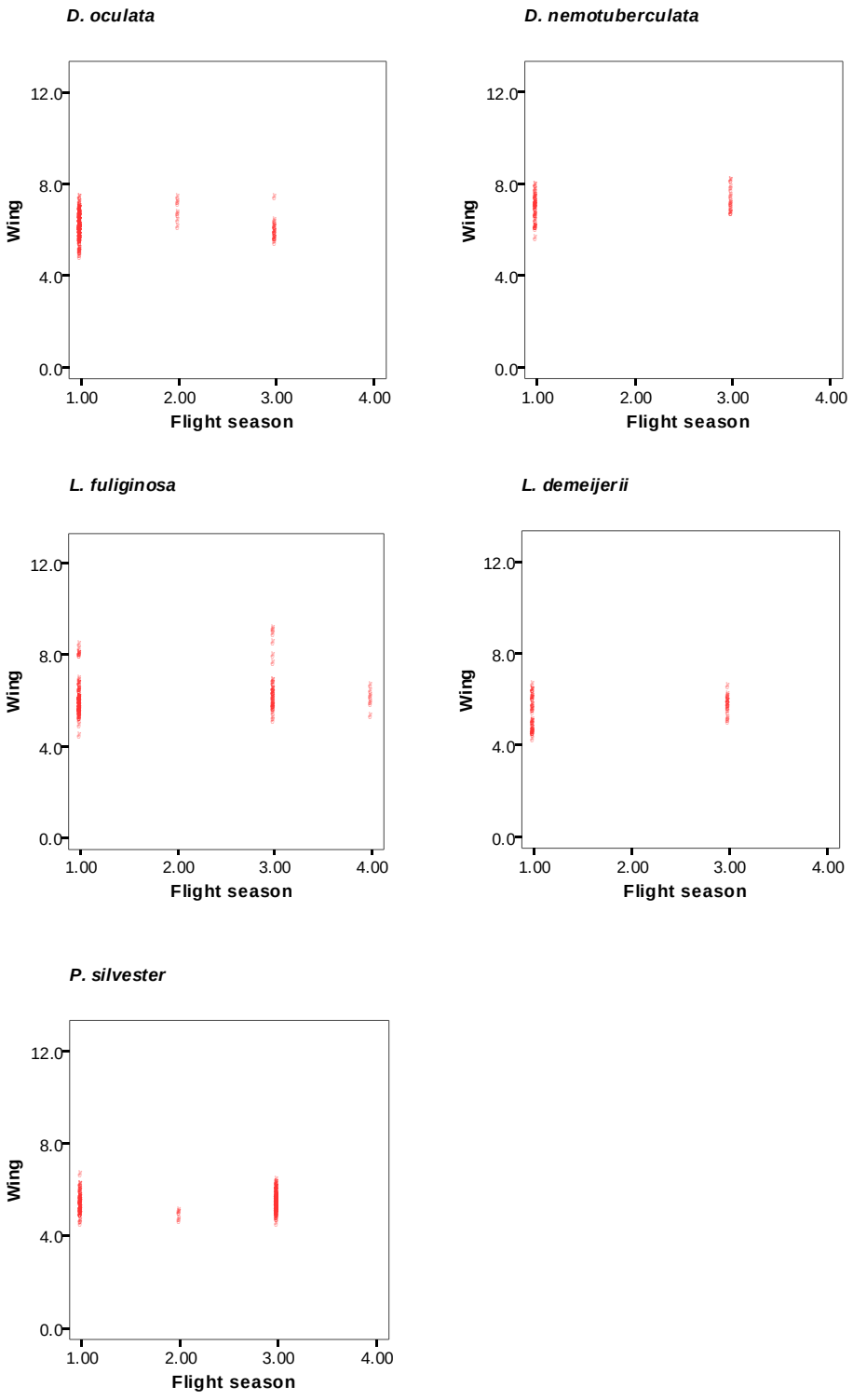


Figure 7.04: Scatter plots of body size differences among flight seasons for non-*Tabanus spp.*

7.4 Discussion

Differences in mean body size between sites were found for *T. pallipennis*, *T. innotabilis*, *T. dorsobimaculatus*, *T. strangmannii, T. notatus, D. oculata, L. fuliginosa, L. demeijerii, P. eyreana and P. silvester*. In general, body size increased with increasing annual average rainfall at a site for the *Tabanus* species. This result is consistent with reports in the literature that indicate certain environmental conditions can contribute to differences in body size of adult tabanids (Leprince and Jolicoeur, 1986a). Increased body size of tabanid species in areas of higher annual average rainfall indicates that higher fecundity of these species would also be expected to occur in these areas (Leprince and Foil, 1993). This is consistent with results reported in Chapter Nine which indicate higher species abundance tended to occur in areas of higher rainfall. If body length is proportional to mouthpart length, then mouthpart length would be expected to increase significantly under the same conditions as increased body size. There was no consistent trend between body size and average annual rainfall at a site noted for the non-*Tabanus* species however.

The differences in body size found between flight seasons in this study, for four of the six *Tabanus* species and all of the non-*Tabanus* species examined, indicates that there are likely to be significant differences in the environmental factors relevant to larval nutrition between flight seasons. The differences between flight seasons were not consistent between species, however, indicating that there may be a confounding effect of site, or other unknown factors, on this result. The implication of a difference in body size between flight seasons is the number of eggs produced will vary and therefore the population will also fluctuate between years. It is likely that conditions of higher rainfall are more conducive to larger body size at a site, between flight seasons. This means that fecundity and therefore population growth are likely to occur in seasons of high rainfall, although the population growth may not be seen until the following season, or longer, depending on the length of time required for maturation. This appeared to be the case in Chapter Nine.

For *T. ceylonicus*, there was a significant difference in body size among flight seasons. A uniform decrease in body size following emergence would have been expected, as was the case in data collected in south-west Quebec, for *Tabanus quinquevittatus* over the course of the flight season (Leprince and Bigras-Poulin, 1988). However, *T. ceylonicus* appeared throughout the year, with no specific time of emergence identified (see Chapter Eight). Body size has been found to be related to potential fecundity. Leprince and Bigras-Poulin (1988) reported that the seasonal decrease in body size found in their study represented a decrease of more than 10% of potential fecundity. No measurement of fecundity was performed on specimens in the present study, however if the relationship between body size and fecundity hold true for other species of *Tabanus*, this might indicate that *T. ceylonicus* could have significant variation in potential fecundity between flight seasons, with highest fecundity in the periods from April- September 2005 and October 2005- March 2006.

These results indicate that higher rainfall areas produce larger tabanids, which are likely to have larger mouthparts and higher abundance. All of these factors are related to increased risk of *T. evansi* transmission. Larger tabanids were considered by Foil (1989) to be more likely to transfer between hosts when feeding. It is assumed that larger mouthparts are likely to be capable of transmitting a larger volume of residual bloodmeal, which is therefore more likely to contain pathogens. So areas of higher abundance are also likely to have an increased rate of transmission per tabanid bite. This compounds the effect of high tabanid abundance on the potential transmission of surra.

CHAPTER EIGHT
PHENOLOGY OF *TABANUS* IN CAPE YORK PENINSULA AND TOWNSVILLE

8.1 Introduction

This chapter examines the temporal abundance patterns of the genus *Tabanus,* and the six most abundant species of *Tabanus* individually, at each of the collection sites in further detail.

Information on the calendar months of the year during which *Tabanus* species are active is of critical importance to our understanding of the potential timing of *T. evansi* transmission. Because there is no trans-ovarial transmission of the pathogen within the vector, infectivity is not passed vertically to offspring so transmission between host species is restricted to periods when adult females of the genus are active and seeking a bloodmeal. Therefore there is a loss of ability to transmit *T. evansi* during periods when adult *Tabanus* are not active or biting hosts. This means that in the event of an incursion of surra, if effective treatment or culling of infected host animals is timed for periods when *Tabanus* are not active, an outbreak could potentially be controlled. If *Tabanus* species are active all year round at a location, however, the chance of controlling an incursion would be much reduced.

While it is the variation in overall abundance (in time and space) of the genus *Tabanus* as a whole that is most critical to the risk of *T. evansi* transmission, each site was dominated by the presence of one or two main species, so that the emergence and fluctuations in abundance of the genus *Tabanus* at a site was dependent largely on the activity dynamics of the dominant species at that site.

Each species of *Tabanus* peaks at different times during the season, indicating that species respond differently to weather and environmental variables, a phenomenon known as phenology. Characterising these responses will allow us to identify the

seasonal activity patterns of *Tabanus* species in Townsville and the Cape York Peninsula region.

Mackerras (1970) indicated that the phenology of tabanids (in particular adult emergence) in north Queensland was strongly influenced by rainfall. Therefore, the effect of rainfall is examined in further detail to establish the relationship between this variable and the time of emergence, peak abundance and length of flight season of the genus *Tabanus* (overall) and the six most numerous species of *Tabanus*. These factors will influence the timing and duration of the risk period of potential surra transmission. It is possible that the emergence of a species is governed by factors other than the onset of the wet season, for example, day length. In this chapter, a qualitative examination of the calendar month of commencement and duration of the flight season of the main species of *Tabanus* is presented.

This information on the calendar months of emergence and duration of activity of potential vectors will contribute to the development of models for potential surra transmission in northern Queensland, which is thought to be the most likely route of incursion for *T. evansi*.

8.2 Materials and Methods

8.2.1 Trapping method and experimental design

The materials and methods used in tabanid capture, experimental design and the sites used were described in detail in Chapter Six: Seasonal and Spatial Variation in Tabanids in Cape York and Townsville.

The calendar months of the flight periods of six species of *Tabanus* were examined qualitatively only, in order to define these periods over the course of this study and therefore the potential periods during which surra transmission could occur. In addition, we examined whether there was consistency of these flight periods for a given species between flights seasons or sites, and whether there were consistent emergence patterns in relation to the onset of the wet season.

The presence of a species at a study site was also compared to the known places at which this species had previously been confirmed.

8.2.2 Graphing methods

Plots of species succession and species activity over time (catch per day) in relation to rain were produced using SPSS (version 14.0, SPSS Inc, 2005). Interpolated rainfall data values ("drill data" from Bureau of Meteorology) used in the graphs were daily values, averaged over the calendar month.

8.3 Results

The months of the year during which the genus *Tabanus* was found at each site are presented in Figure 8.01. Northern sites such as Bamaga and Mapoon had *Tabanus* activity during 11 months of the year, while southern sites such as Karumba and Townsville had *Tabanus* activity for only four months of the year. Lockhart River, which has the highest average annual rainfall of the sites, had a very restricted period of *Tabanus* activity (three months of the year), despite having the highest abundance overall.

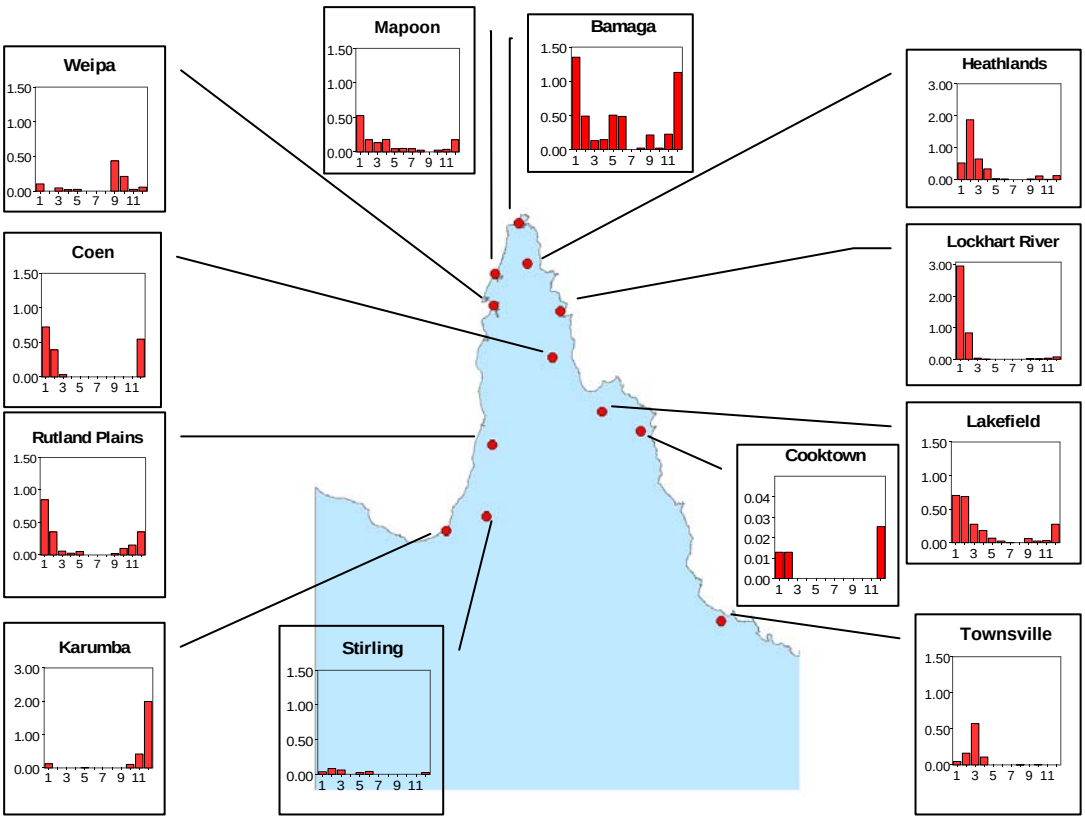


Figure 8.01: Months of the year during which *Tabanus* was captured at each of the collection sites (mean catch per day in each month for both years of the study).

8.3.1 Species composition of *Tabanus* at each site

The species composition of the six most numerous *Tabanus* species differs among sites, and the species occurring in greatest abundance at each site also varies (Figure 8.02). At each site, between one and three species are more numerous than the others, and their patterns of abundance (such as response to rainfall) dictate the overall patterns of *Tabanus* abundance.

While *T. ceylonicus* appears to be restricted to the most northern sites, other common species cover a wide latitudinal range, and the reasons for their dominance or absence at particular sites are not obvious but could depend on larval requirements which were not considered in this study.

Overall, greater numbers were caught in the first year (September 2004 - May 2005), compared with the second year (September 2005 - May 2006). For all sites combined, the total numbers of *Tabanus* caught in year two amounted to 41% of the previous year's total.

Tabanus notatus was the most abundant and widespread species overall and was the most numerous species at five of the 12 sites (Bamaga, Coen, Lakefield, Lockhart River and Rutland Plains). *Tabanus strangmannii* was the most numerous species at Heathlands and Stirling. *T. dorsobimaculatus* was the most numerous species at Mapoon and Townsville. At Weipa, *T. innotabilis* and *T. ceylonicus* occurred in highest abundance and in approximately equal numbers. At Karumba, *T. innotabilis* was the only species of *Tabanus* present in substantial numbers. Only three specimens of *Tabanus* were caught at Cooktown, including one each of *T. pallipennis* and *T. strangmannii*.

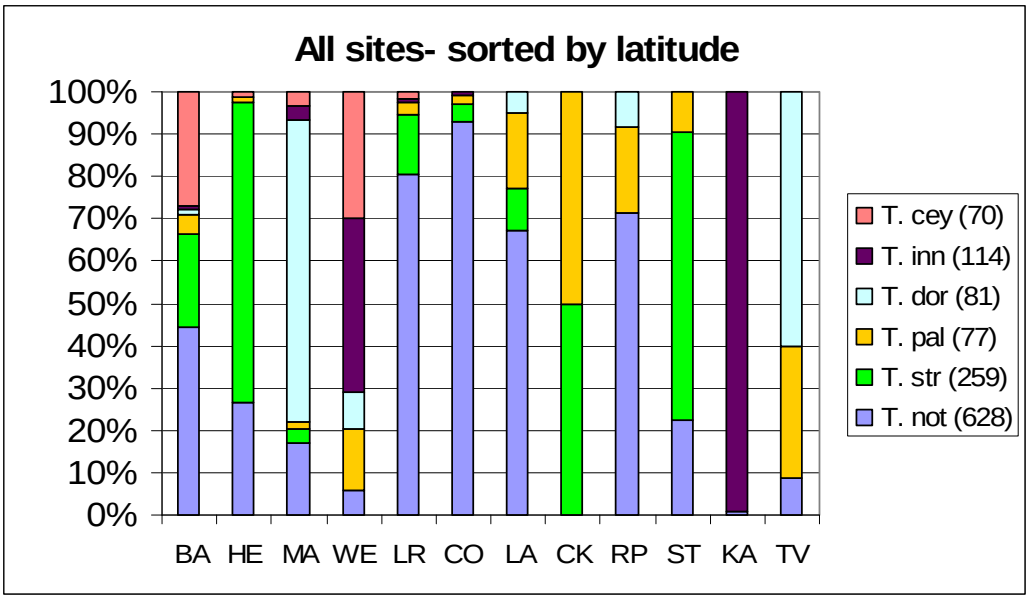


Figure 8.02: Species composition of six most numerous *Tabanus* species at each site (BA= Bamaga, HE= Heathlands, MA= Mapoon, WE= Weipa, LR= Lockhart River, CO= Coen, LA= Lakefield, CK= Cooktown, RP= Rutland Plains, ST= Stirling, KA= Karumba, TV= Townsville. T. cey= *T. ceylonicus*, T. inn= *T. innotabilis*, T. dor= *T. dorsobimaculatus,* T. pal= *T. pallipennis*, T. str= *T. strangmannii*, T. not= *T. notatus.*)

8.3.2 Dominant species at each site

The dominant species at each site in each year, described below, are summarised in Table 8.01 (see also Appendix 5, Table A5.02).

At Bamaga, *T. notatus* was the most numerous species (approximately 40% of the total year's catch of *Tabanus*), followed by *T. strangmannii* (20%) and *T. ceylonicus* (17%). The proportions of these species remained consistent between years.

Numbers of *Tabanus* species at Cooktown were too low to be of value for analysis.

Tabanus notatus was the most numerous species at Coen, other species did not occur in sufficient numbers to analyse. The percentage of *T. notatus* remained high during both years (92% in year one, 84% in year two).

At Karumba, *T. innotabilis* was the dominant species. Although the percentage of *T. innotabilis* in relation to total abundance appeared to be quite different between years (43% in year one versus 85% in year two), this may be the result of large numbers of damaged specimens in year one (probably *T. innotabilis*) that could only be identified to genus, so they were included in the denominator.

At Lakefield, *T. notatus* was dominant and considerably larger percentages occurred in year one (74% versus 44% in year two). Conversely, the next two most numerous species occurred in greater proportions in year two compared with year one *T. pallipennis* (11% versus 21%) and *T. strangmannii* (5% versus 14%).

At Lockhart River, despite problems with the trap in the second year causing a precipitate drop in catch numbers, the proportions of the two most numerous species remained similar between years (*T. notatus* approx 80% in each year, *T. strangmannii* 14% in year one and 7 % in year two).

At Heathlands, *T. strangmannii* was the dominant species in each year, with a lower percentage of the total in year two (77% in year one and 51 % in year two). *T. notatus* percentages increased in the second year (17% versus 44% in year two).

At Mapoon, *T. dorsobimaculatus* was dominant and percentages of this species were slightly higher in the second year (50%, 61%). *T. notatus* percentages were slightly lower in the second year (15%, 10%).

At Weipa, *T. innotabilis* (27%, 6%) and *T. ceylonicus* (7%, 38%) shared dominance, depending on the year. In year one, *T. innotabilis* was dominant and in year two, it was *T. ceylonicus*.

At Townsville, *T. dorsobimaculatus* was dominant (32%, 17%), with *T. pallipennis* being the next most numerous species (24%, 0%).

At Stirling, there were very low numbers of *Tabanus* species in both years. The majority of those present consisted of *T. strangmannii* (14%, 63%) and *T. notatus* (0, 25%). Percentages of both increased in the second year.

At Rutland Plains, *T. notatus* was the most numerous (74%, 49%), although relative abundance was lower in the second year. The percentage of *T. pallipennis* was higher in the second year (16%, 26%).

Table 8.01: Dominant species at each site. Total *Tabanus* numbers include all *Tabanus* species, including those specimens unable to be identified to species because of damage. (BA= Bamaga, HE= Heathlands, MA= Mapoon, WE= Weipa, LR= Lockhart River, CO= Coen, LA= Lakefield, CK= Cooktown, RP= Rutland Plains, ST= Stirling, KA= Karumba, TV= Townsville. T. cey= *T. ceylonicus*, T. inn= *T. innotabilis*, T. dor= *T. dorsobimaculatus,* T. pal= *T. pallipennis*, T. str= *T. strangmannii*, T. not= *T. notatus.*)

SITE	SEASON	Dom Sp 1	% *Tabanus*	Dom Sp 2	% *Tabanus*	Dom Sp 3	% *Tabanus*	Total *Tabanus* numbers
BA	1	T. not	40.3	T. str	18.3	T. cey	16.2	191
BA	2	T. not	40.3	T. str	22.1	T. cey	18.2	77
CK	1	T. pal	33.3	T. str	33.3			3
CK	2	T. pal	0.0	T. str	0.0			0
CN	1	T. not	91.6					83
CN	2	T. not	84.2					19
KA	1	T. inn	42.9					98
KA	2	T. inn	84.8					66
LA	1	T. not	73.8	T. pal	11.3	T. str	5.0	80
LA	2	T. not	43.9	T. pal	21.1	T. str	14.0	57
LR	1	T. not	79.1	T. str	14.2			225
LR	2	T. not	80.0	T. str	6.7			15
HE	1	T. str	77.1	T. not	16.7			144
HE	2	T. str	50.7	T. not	44.0			75
MA	1	T. dor	49.1	T. not	15.1			53
MA	2	T. dor	61.3	T. not	9.7			31
WE	1	T. inn	26.7	T. cey	6.7			30
WE	2	T. inn	6.3	T. cey	37.5			16
TV	1	T. dor	32.4	T. pal	23.5			34
TV	2	T. dor	17.4	T. pal	0.0			23
ST	1	T. str.	14.3	T. not	0.0			7
ST	2	T. str.	62.5	T. not	25.0			8
RP	1	T. not	73.8	T. pal	15.5			84
RP	2	T. not	48.6	T. pal	25.7			35

8.3.3 Species succession at each site

The species succession at each site, over the two trapping years, was examined to determine the relationship of each of the dominant species with time of year and rainfall: e.g. whether that species peaked in the same months of each year, whether the peak abundance coincided with rain or lagged after it and the length of the lag period if it was present. The results were then tabulated to clarify and summarise the relationships (details for all sites are given in Appendix 5, Table A5.02). The plots of species succession at Bamaga and Coen, shown below, demonstrate the impact of species richness on the contrasting patterns of abundance at these two sites. The phenology of individual species is similar at the two sites, but at Bamaga, there is a prolonged period of succession of different species that contribute to overall activity of *Tabanus* for many months of the year. The number of species present at Coen is fewer and the period of *Tabanus* activity is much more restricted at this site as a result (Figures 8.03- 8.04).

8.3.3.1 Species succession at Bamaga

At Bamaga, the dominant species were *T. notatus*, *T. strangmannii* and *T. ceylonicus*. In season one, *T. ceylonicus* and *T. strangmannii* emerged first, followed by *T. notatus* and then a larger peak of *T. strangmannii* with *T. dorsobimaculatus*. *Tabanus ceylonicus*, *T. strangmannii* and *T. pallipennis* occurred quite late in the season (May). There were small dry season peaks of *T. ceylonicus* and *T. pallipennis* (September). This pattern was repeated in the second season, with the exceptions of the first peak of *T. strangmannii*, and the fact that *T. dorsobimaculatus* did not appear. The amplitude of the peaks of each species was greater in season one compared with the second season (Figure 8.03).

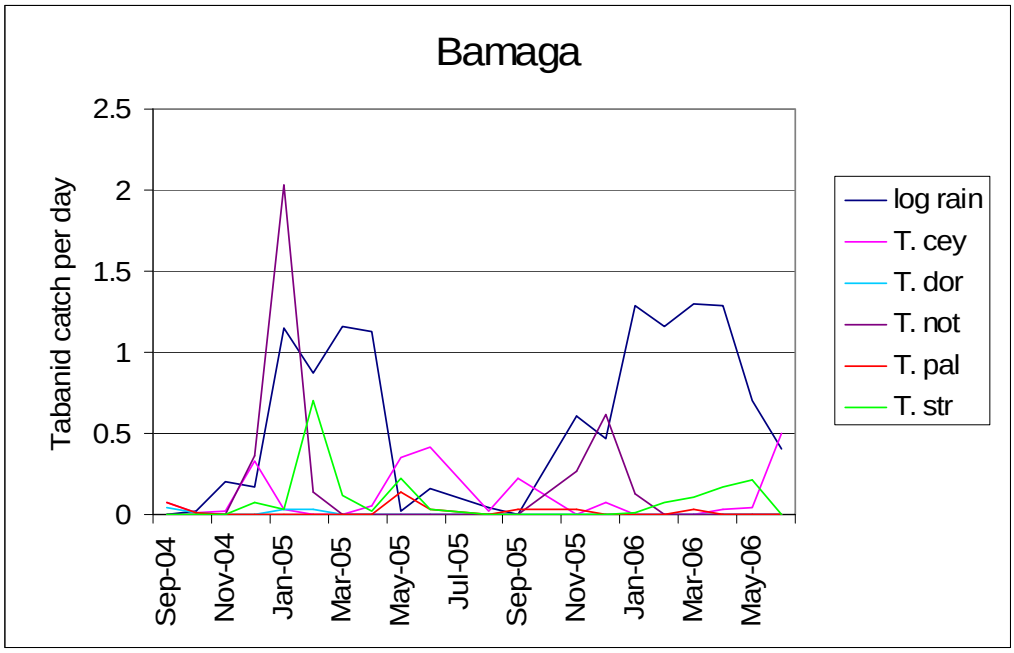


Figure 8.03: Species succession at Bamaga

8.3.3.2 Species succession at Coen

At Coen, *Tabanus notatus* was the dominant species. It emerged first in both seasons, with small peaks of *T. strangmannii* and *T. pallipennis* occurring later (Figure 8.04).

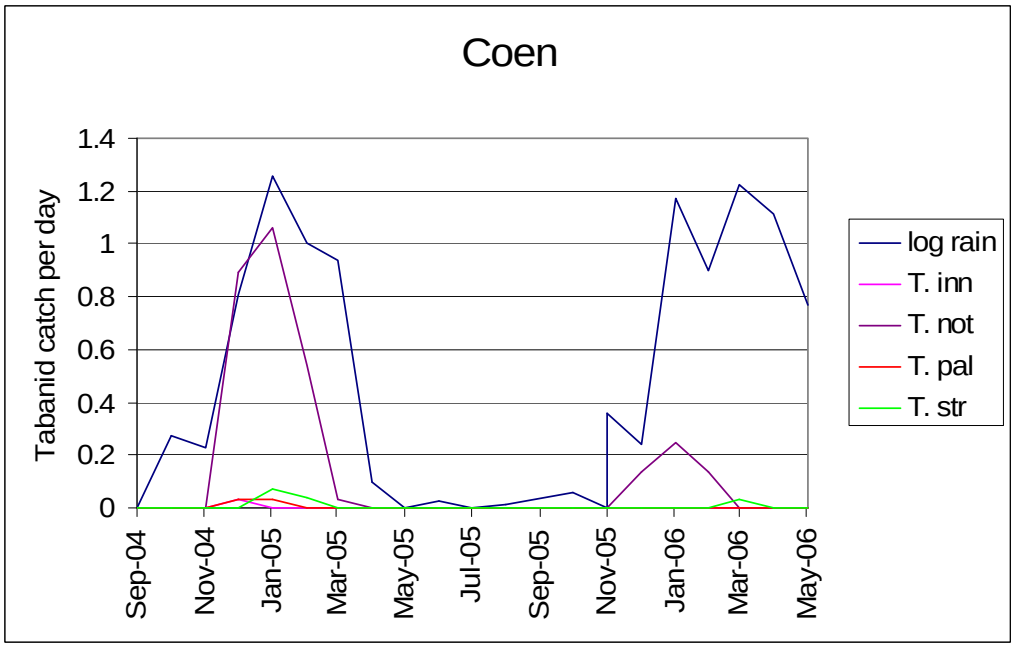


Figure 8.04: Species succession at Coen

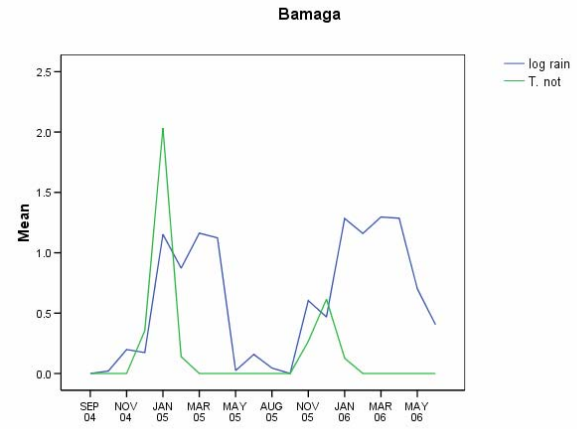
8.3.4 Species phenology - comparison of sites and years

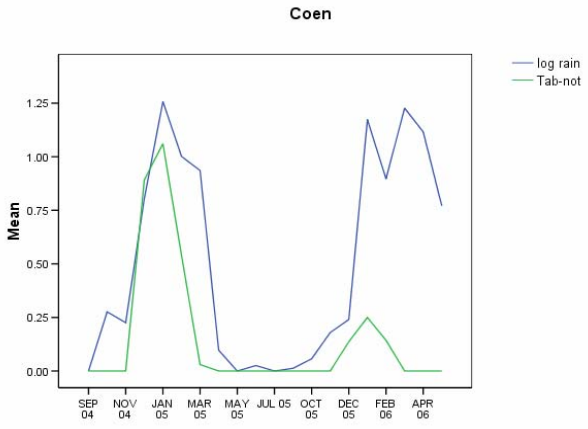
Line graphs of tabanid abundance for each calendar month over the two years of the study were used to examine the phenology of total *Tabanus* numbers and the six most numerous *Tabanus* species at each site where they were present. Rainfall patterns are also shown on each plot to examine whether peak abundances showed a consistent relationship with peak rainfall.

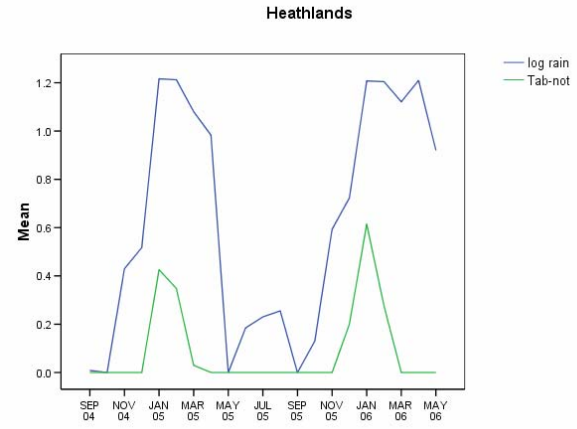
As Figures 8.03 - 8.04 (species succession) indicate, each species has a different phenological pattern. The timing of abundance peaks for each species might be influenced by weather (especially rainfall) or may be regulated by photoperiod or other factors.
Details for all species, including plots, are provided in Appendix 5, Table A5.01. The degree of variation between individual species is illustrated below using *T. notatus* and *T. ceylonicus* (Figure 8.05 - 8.06).

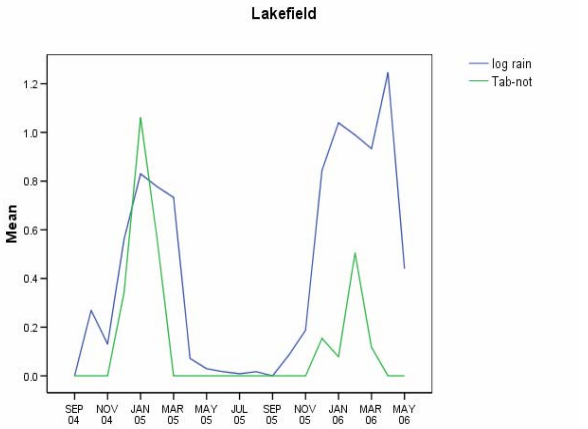
8.3.4.1 *Tabanus notatus*

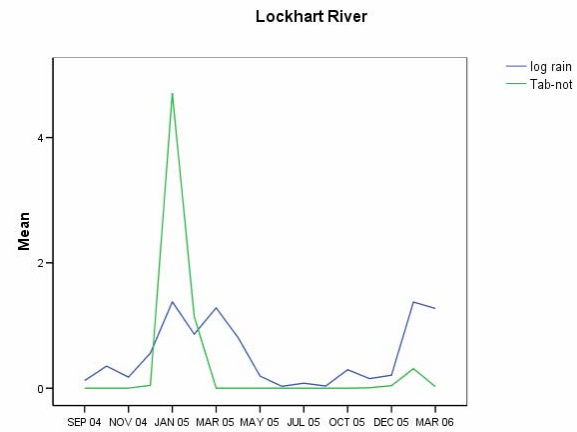
Numbers of this species generally peaked in January-February or February-March. While this is generally the wet season in tropical Australia, emergence variously occurred just before the wet season rain started, at the same time as rain, or, if the wet came early at a site, activity lagged one to two months after rain commenced. There was no recorded dry season activity of *T. notatus* after April-May. Late rain did not appear to prolong duration of activity past April-May (see Lakefield and Heathlands). Duration of activity was from January to April-May (Figure 8.05).

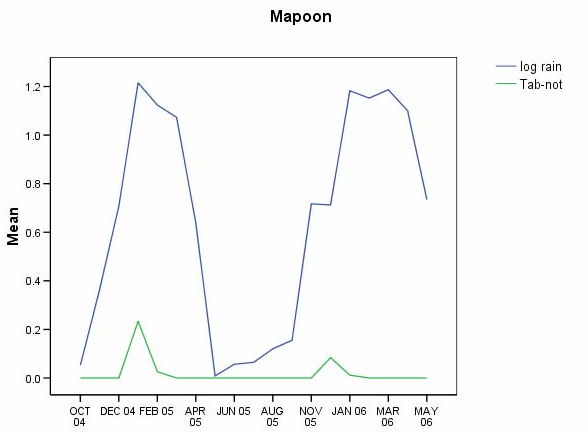

Bamaga
log rain
T. not
Mean
2.5
2.0
1.5
1.0
0.5
0.0
SEP 04
NOV 04
JAN 05
MAR 05
MAY 05
AUG 05
NOV 05
JAN 06
MAR 06
MAY 06


Coen
log rain
Tab-not
Mean
1.25
1.00
0.75
0.50
0.25
0.00
SEP 04
NOV 04
JAN 05
MAR 05
MAY 05
JUL 05
OCT 05
DEC 05
FEB 06
APR 06


Heathlands
log rain
Tab-not
Mean
1.2
1.0
0.8
0.6
0.4
0.2
0.0
SEP 04
NOV 04
JAN 05
MAR 05
MAY 05
JUL 05
SEP 05
NOV 05
JAN 06
MAR 06
MAY 06


Lakefield
log rain
Tab-not
Mean
1.2
1.0
0.8
0.6
0.4
0.2
0.0
SEP 04
NOV 04
JAN 05
MAR 05
MAY 05
JUL 05
SEP 05
NOV 05
JAN 06
MAR 06
MAY 06


Lockhart River
log rain
Tab-not
Mean
4
2
0
SEP 04
NOV 04
JAN 05
MAR 05
MAY 05
JUL 05
OCT 05
DEC 05
MAR 06


Mapoon
log rain
Tab-not
Mean
1.2
1.0
0.8
0.6
0.4
0.2
0.0
OCT 04
DEC 04
FEB 05
APR 05
JUN 05
AUG 05
NOV 05
JAN 06
MAR 06
MAY 06

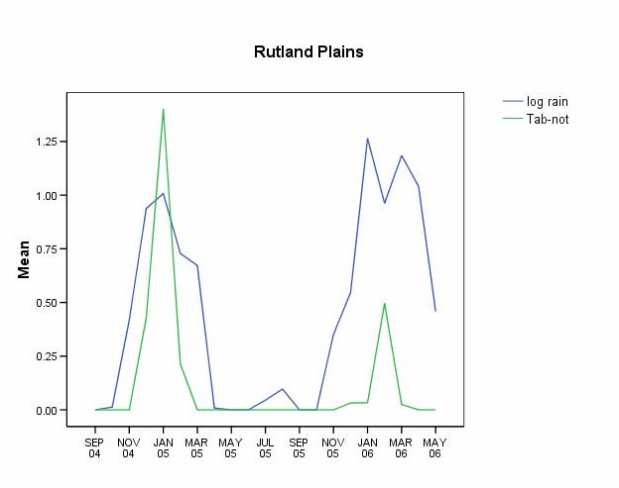
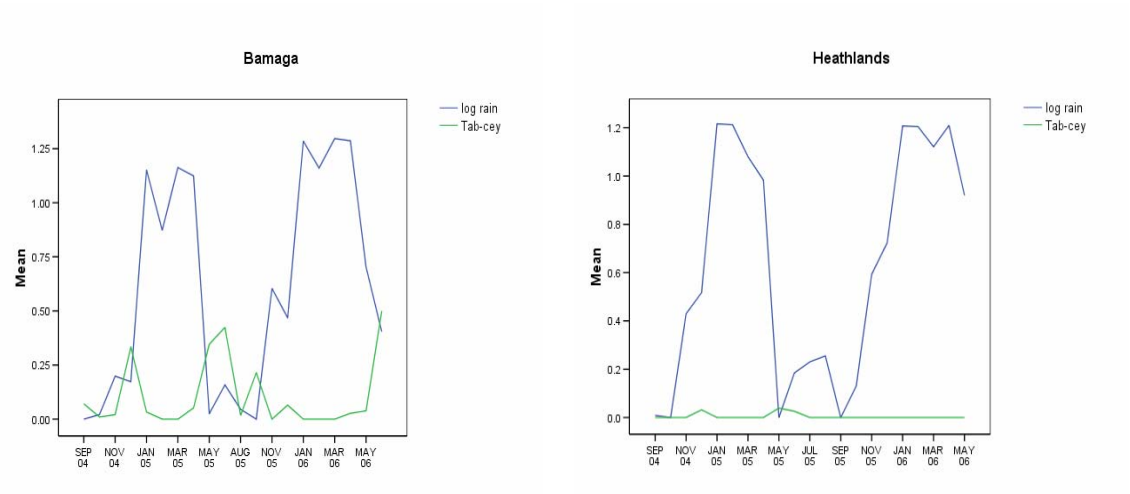


Figure 8.05: Activity of *T. notatus* at different sites

8.3.4.2 *Tabanus ceylonicus*

This species had multiple peaks throughout the year, and the timing of abundance peaks had no consistent relationship with the rainfall pattern. During the wet season in November, December and January abundance tended to coincide with peak rainfall. Dry season activity occurred in September, May-June, and August and did not appear to be associated with rain. Even during the wet season, emergence at times lagged up to one month after rain. It is possible that this species is active all year round, albeit with varying levels of abundance (Figure 8.06).



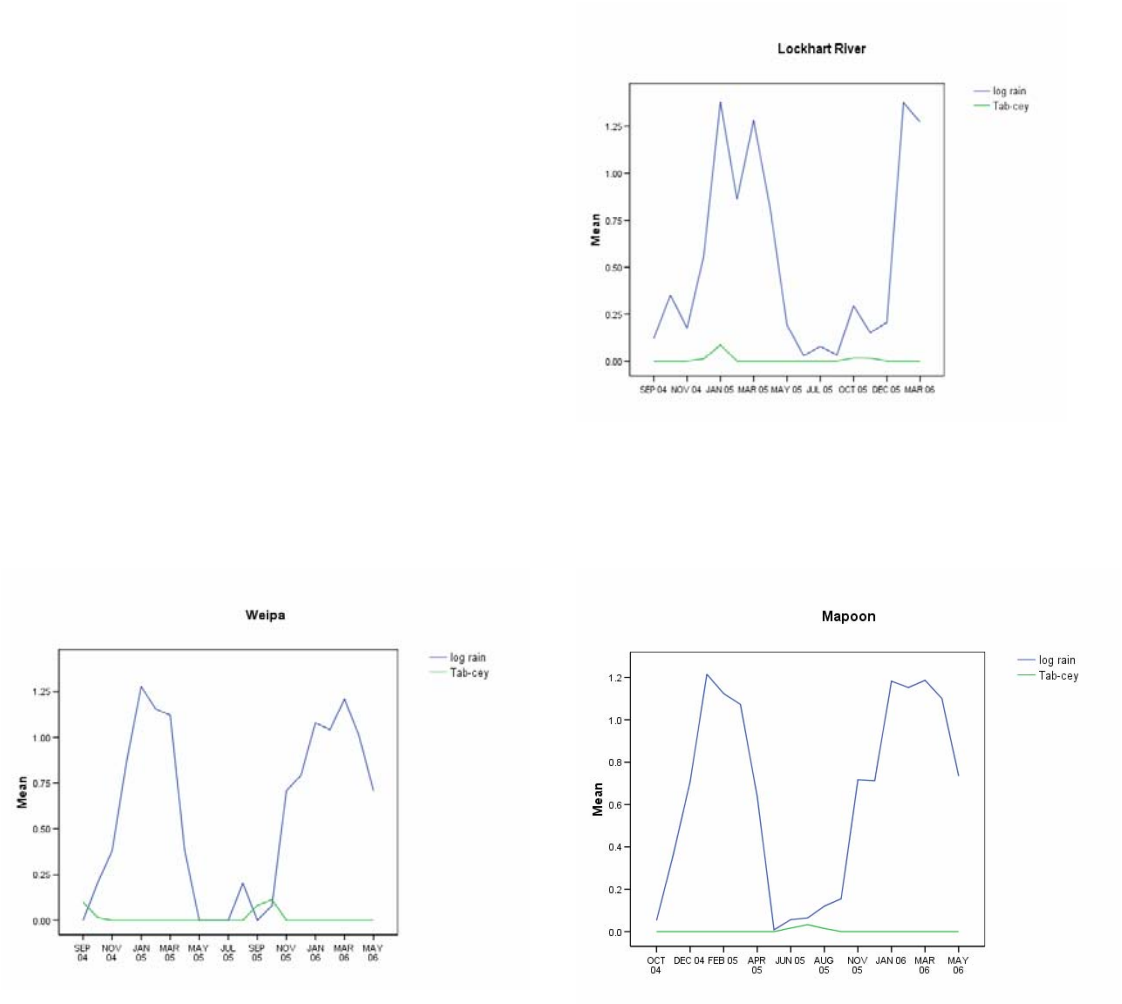


Figure 8.06: Activity of *T. ceylonicus* at different sites

8.3.4.3 *Tabanus strangmannii*

Abundance commonly peaked in February or February-March and duration of activity was from January-May. The only recorded dry season activity for this species occurred in May (Bamaga). Seasonal activity was possibly prolonged with rain (see Bamaga and Heathlands). Abundance peaks coincided with the peak of the rainy season. Emergence tended to lag two to four months after the onset of rain.

8.3.4.4 *Tabanus dorsobimaculatus*

Abundance generally peaked in January-February or February-March, usually at the same time as peak rainfall. Emergence usually lagged two to three months after rain. The only dry season activity recorded was in Bamaga in September. Duration of activity was from September-May.

8.3.4.5 *Tabanus pallipennis*

There were multiple peaks of activity throughout the year during both wet and dry seasons: October-November, January, February-March, March-April, September, May-June. This species did not appear to follow a monthly pattern or consistently respond to rain. Activity could occur before significant rainfall (Rutland Plains) or lag one to three months after.

Tabanus pallipennis was captured at 11 of the 12 sample sites and the dynamics at each site are described in further detail in Figure 8.07. The dry season peaks tended to occur in more northern latitudes such as Bamaga and Mapoon. The duration of activity of this species was quite short at most sites, occurring for four months of the year at seven of the 11 sites it was caught. However at Bamaga, Lakefield and Rutland Plains, there was an extended duration of activity of 7-8 months.

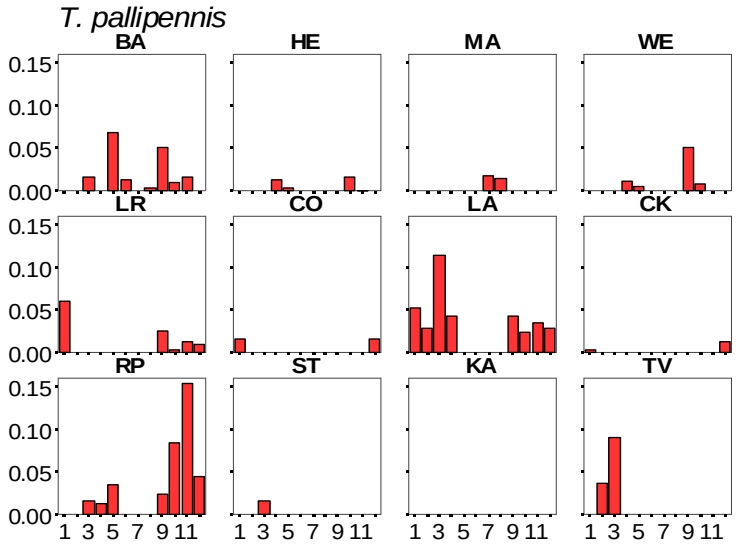


Figure 8.07: Months of the year *T. pallipennis* was present at different sites (mean catch per day in each month for both years of the study).

8.3.4.6 *Tabanus innotabilis*

Peak *T. innotabilis* abundance differed markedly between sites. Highest numbers of this species occurred at Karumba. There was a distinct peak in December at this site, which occurred before the rain peaked in both seasons. In Weipa, peaks occurred in September, March and April-May. There was some dry season activity in September in Weipa. Emergence variously occurred before rain, at the same time as rain began (Karumba) or had little apparent relationship to rain (Weipa).

8.4 Discussion

This study confirmed the previous known distribution of six *Tabanus* species in Cape York Peninsula and Townsville, based on information compiled by Mackerras (1970), and in some cases, contributed additional locations to the distribution. In most cases, the calendar months during which these species were caught were in agreement with those reported previously (Mackerras, 1970).

Tabanus notatus was previously found in Queensland only, from Cape York (the tip of the Peninsula, near Bamaga) to Mackay. In the current study it was widely distributed (11 of the 12 sites, not found at Cooktown) from Bamaga to Townsville. The months it had previously been collected were from December to March. In the present study this species was collected from September to May, with the majority found in December to March. This species had not previously been recorded at Cooktown, Heathlands, Karumba, Lakefield, Lockhart River, Mapoon, Rutland Plains, Stirling or Weipa.

The distribution of *T. dorsobimaculatus* was previously known to range from WA, NT, PNG and Queensland from Cape York to Mt Larcom. The present study found similarly, that this species was collected from Bamaga to Townsville. It had not previously been recorded at Lakefield, Mapoon, Rutland Plains or Weipa. Months of the year in which this species had previously been collected were November to April. The present study found this species from September to May, with the majority from January to March.

Historical records for *T. innotabilis* indicate a wide distribution: it was recorded in PNG, Torres Strait Islands, NT, Solomon Islands and Queensland from Cape York to Tin Can Bay (south-east Queensland). In the present study it was found in six of the 12 sites. It had not previously been recorded at Heathlands, Karumba, or Mapoon. It had previously been collected in the months from September to April, which agrees closely with this study's findings of September to May.

The known distribution for *T. strangmannii* included WA, NT, Torres Strait Islands, NSW and Queensland from Bamaga to Charleville. In the current study it was collected at eight of the 12 sites. It had not previously been found at Cooktown, Coen, Heathlands, Lakefield, Mapoon or Stirling. Months of the year in which this species had been collected previously were September to May. In the current study this species was collected from January to May.

The abundance dynamics and wide distribution of *Tabanus pallipennis* contribute to its apparent importance for the potential transmission of surra in Australia. Previous records indicate a very wide distribution for this species, including PNG, Torres Strait Islands, NT, NSW and in Queensland it had been collected from Bamaga to Brisbane. This study supported the previous findings: it was caught at 11 of the 12 sites (not Karumba). It had not previously been recorded at Cooktown, Coen, Heathlands, Lakefield, Mapoon, Rutland Plains, Stirling or Weipa. In this study, this species was collected all year round, as was the case in historical records. So this species is not only geographically widely distributed throughout northern Queensland and elsewhere in Australia, but it has quite prolonged periods of activity, including dry season activity in certain areas. These characteristics contribute to the likelihood that this species could play an important role in surra transmission.

Tabanus ceylonicus distribution was previously known to be very extensive internationally: it had been recorded from Sri Lanka, Indonesia, PNG and the Solomon Islands to Australia, where it has been collected in Queensland only, from Bamaga to Cardwell. In the current study, it was found in Bamaga and Lockhart River (where there were previous records of its presence) as well as Heathlands, Mapoon and Weipa (no previous records). Months of the year in which this species had been collected previously were February to May and September to October. During the present study, this species was collected all year round. This species appeared to prefer higher rainfall areas (e.g Daintree, Cairns, Babinda, Innisfail), and perhaps the vegetation associated with high rainfall.

Tabanus pallipennis and *T. ceylonicus* were collected all year round. The lack of seasonality and obvious response to rain indicates that at least in parts of their range, these species may be present as adults all year round, with activity determined by immediate local conditions (see Chapter Four). The presence of either of these species at a site is more likely to be associated with dry season activity than other species, although several species have extended durations of activity (September to May). However, the peak activity of most *Tabanus* species is more restricted (December to March). *Tabanus ceylonicus* is the only known transmitter of surra that occurs in Australia. This study shows that not only does this species occur in the most northern part of Cape York, but it also occurs all year round, making this area especially high risk for surra transmission.

It is generally accepted however, that most species of *Tabanus* are likely to transmit surra, so the activity of *Tabanus* as a group is important to the assessment of risk in Cape York Peninsula. Generally, it appears that sites in northern latitudes have *Tabanus* activity for more of the year than the southern latitudes. On average, Bamaga and Mapoon recorded *Tabanus* activity for 11 months of the year. The longer activity period appears to be due to the presence of *T. ceylonicus* at these northern sites. *Tabanus* activity was also prolonged at Lakefield and Rutland Plains (nine months of the year). This was due to the large proportion of *T. pallipennis* at these sites.

Overall patterns of *Tabanus* abundance at each site, including when activity commenced in the calendar year, how long it lasted and when peak abundance occurred, were strongly influenced by which species were present at the site, and in what proportions. This study indicates that the dominant species often had fairly consistent patterns of seasonal activity among sites. In addition, within a site, the same species remained dominant in successive seasons in nine of 11 sites (where both season's data were available). This might indicate that the species composition, including the dominant species, is determined by site characteristics, while the relative abundance of the species is influenced by other factors, including weather. This finding suggests that if the dominant species at a site could be predicted, the patterns of seasonal *Tabanus* activity

could also be deduced. This would allow a more accurate picture of the temporal risk at any given site. Prediction of abundance of different *Tabanus* species, and the genus as a whole, is discussed further in Chapter Ten.

The months of the year during which *Tabanus* are active indicates the times that surra transmission may be possible. Further studies to calibrate the trap catch with tabanid biting rate on nearby animals and information on the number of tabanid bites required for surra transmission to occur, will help translate this data into a more accurate and quantitative risk model.

CHAPTER NINE
ANNUAL TABANID ABUNDANCE AND GIS MAPPING

9.1 Introduction

9.1.1 GIS applications to vector-borne disease epidemiology

Vector-borne diseases of animals have a devastating economic and social impact worldwide. The applications of GIS for monitoring the impact of environmental influences on the distribution and abundance of disease vectors have obvious implications for the control and containment of episodic disease outbreaks among animal populations (Goetz *et al.*, 2000).

Vector population dynamics - the seasonal and annual variations in vector numbers and disease infection rates at monitored locations - can be correlated with remotely sensed or interpolated environmental data such as rainfall, temperature, humidity and environmental "greenness" or Normalised Difference Vegetation Index (NDVI). If these environmental factors can successfully predict vector dynamics, then the potential for pathogen transmission can be monitored by means of meteorological and satellite data analysed within a GIS.

Similarly, the climatic and environmental factors governing the distribution of vectors, and the diseases they transmit can be partially defined by spatial data. Integration of spatial and temporal processes provides an effective tool for analysis of disease vector dynamics with respect to disease patterns and assessment of control measures (Thomson and Connor, 2000).

In its most basic form, GIS can be used to overlay vector presence/absence data on topographical maps of rivers, roads, villages, towns and administrative areas in order to assist the planning and implementation of control procedures. Towns or farms within the flight range of vector breeding sites can be identified by use of simple distance

operators. It may not be possible in some countries to map the distribution of the vector or disease in sufficient detail, due to lack of resources and/or the extremely large land mass involved (e.g. Australia). In these cases, findings from localised surveys must be extrapolated to a wider region by the development of predictive models of either vector or disease distribution. For example, GIS database query commands are often used to extract information from environmental databases for specific locations where entomological data are available (Thomson and Connor, 2000). While these types of estimates of vector distribution might be the best prediction available, the limitations of accuracy need to be considered. Hightower and colleagues looked at small-scale correlations between proximity to mosquito breeding habitats and malaria incidence in Western Kenya. They found that for the month of June, the average household prevalence of malaria steadily decreased with increasing distance from larval habitat (but the difference was not statistically significant by linear regression). There was no difference for the month of September. Average numbers of mosquitoes caught in houses were related to the distance to the nearest breeding site for *Anopheles gambiae* for the dry month (June) but not the wet month (September) (Hightower *et al.*, 1998). This might indicate that during the wet month, mosquito breeding sites were ubiquitous, making population estimates by this means unfeasible.

Analysis of environmental factors associated with vector abundance (including statistical analysis) enables the predictors of vector abundance to be determined, which can then be applied to larger areas where field data are not available.

However, use of such extrapolation assumes that, throughout the range of each species, vectors respond the same way to their abiotic environment and that the processes that regulate their population size are also geographically uniform. This assumption may apply to species with a limited geographic distribution, but may not apply to those with a wide geographic distribution (Rogers and Williams, 1993).

9.1.2 Use of environmental proxies

Environmental factors influence the reproductive success, development, dispersal and survival of disease vectors and thereby their distribution and abundance. Therefore, remotely sensed environmental correlates, or proxies, can be used to study the temporal and spatial fluctuations in vectors. Generally speaking, insects increase in number in response to rain and high environmental temperatures (and therefore also with increasing NDVI) but this is not always the case, depending on the ecological niche to which the insect has adapted. Rogers and Randolph (1991) noted the difference in response between two species of tsetse to increasing NDVI. Rogers and Williams (1993) noted that sleeping sickness prevalence peaked at intermediate NDVI levels and declined to zero within a very narrow range of NDVI either side of the peak figure (Rogers and Williams, 1993).

Low resolution data (AVHRR NDVI) have been used quite widely to predict spatial and temporal variability of vectors. Other environmental indices that are relevant to epidemiological research include atmospheric humidity, vapour pressure deficit, soil moisture and air temperature, among others (Goetz *et al.*, 2000).

NASA's Centre for Health Applications of Aerospace Related Technologies (CHAART) defined some environmental variables that might have a direct or indirect bearing on the survival of pathogens, vectors, reservoirs or hosts. These factors are: vegetation/crop type, vegetation green-up, eco-tones, deforestation, forest patches, flooded forests, general flooding, permanent water, wetlands, soil moisture, canals, human settlement, urban features, ocean colour, soil surface temperature (SST) and soil surface humidity (SSH). Precipitation, humidity and surface temperature were not included because deriving these measurements from raw data required more in depth processing (Beck *et al.*, 2000).

Often these environmental variables are not independent, allowing spectral vegetation indices (SVI's) to be used as proxies for several related variables. For example

vegetation cover is affected by many of the variables and in fact, frequently changes in relation to them in a predictable manner. These same environmental variables often affect vector population changes in a predictable manner also. However, the relative importance of any single variable cannot be ruled out and may change rapidly if the breeding habitat is altered or a threshold relevant to vector development is reached. This may occur at short time scales even if correlated variables (e.g. vegetation cover) remain constant. So it is important to consider both integrative variables such as SVI's, as well as surface environmental variables that vary more rapidly, such as land surface temperature (Goetz *et al.*, 2000).

9.1.3 Disease modelling: Statistical versus biological models

Disease modelling may be based either on statistical relationships established between past case numbers and environmental predictors or on sets of equations that attempt to capture the biology of the transmission process (Myers *et al.*, 2000).

9.1.3.1 Statistical approach

This method requires samples from as wide a range of environmental conditions as possible. Predictions arising from this approach assume that other places are statistically similar to those for which the original models were developed i.e. the relationships already established between case numbers or vector numbers and environmental variables will persist into the future (Myers *et al.*, 2000; Rogers, 2000).

Such models can be used to make predictions about:

a) Presence/absence of a species (of vector) in areas inadequately surveyed in the past
b) Changes in distributions of the vector that will occur with environmental change in the future

In general, standard meteorological records are used as the predictor variables, occasionally with soil or vegetation information.

The key difficulty is to select those variables of apparent importance in determining the distribution pattern; the other variables can be discarded (Rogers and Williams, 1993).

Models based on the statistical approach can give impressive descriptions of spatial variation in risk but are generally poor in predicting temporal variation in risk. This is because the lag time between some environmental variables and a response that culminates in increased disease transmission is not easily shown by the statistical approach (Rogers, 2000).

Rogers and Randolph (1991) used AVHRR NDVI and trap catches of tsetse fly to examine correlations with mortality rates and abundance of tsetse, key determinants of disease transmission. They found a significant association between these factors in the northern part of Cote d'Ivoire. They noted the possibilities of extending this information to predict vector abundance and distribution over a much larger area of Africa to produce risk maps for trypanosomiasis. However, Rogers later tempers his enthusiasm for this approach (Rogers *et al.*, 1996).

Rogers et al (1996) used discriminant analysis of Fourier-processed surrogates for vegetation (AVHRR-NDVI), temperature (AVHRR-channel 4 brightness temperature) and rainfall (Meteosat-Cold Cloud Duration) to predict presence or absence of tsetse and abundance of tsetse. They found the most useful of the predictor variables in both cases to be the thermal data, followed by vegetation and rainfall indices. They interpreted this data to indicate that tsetse distribution limits in this part of Africa were more sensitive to temperature, while abundance within distributional limits appeared to be some function of rainfall, which determines vegetation growth (Rogers *et al.*, 1996).

Hendrickx and co-workers examined environmental variables in relation to tsetse numbers and disease prevalence, in particular land surface temperature (LST), NDVI and cold cloud duration (CCD) using AVHRR data in Togo. Contemporary observations of fly numbers, disease and cattle confirmed and extended relationships previously found elsewhere between mean trap catches of *Glossina tachinoides, G. palpalis* and

NDVI with further relationships between fly abundance and both CCD and LST estimates. Fly abundance, in categories of low, medium and high were described with accuracies greater than 70% (Hendrickx *et al.*, 1999; Hendrickx *et al.*, 2000; Hendrickx *et al.*, 2001)

9.1.3.2 Biological approach

This method requires details on all the biological parameters and variables considered to be important in disease transmission, or vector population dynamics. It requires detailed population measures and therefore is often restricted in the area that can be covered (because such measures are based on local intensive studies) and so are poor at describing large area spatial variation. In theory the biological approach predicts variation in time rather well (Rogers and Williams, 1993).

Biological models describe the conditions that favour birth and death of vectors, and hence relate to their population dynamics. Laboratory studies of temperature and humidity levels, for example, that favour vector reproduction can give only an approximate guide to field conditions however, given that tsetse (and probably other vectors) are effectively spatial opportunists and carefully select micro-environments that are most suitable i.e. that are moister and cooler than ambient (Rogers, 2000).

Biological models are thought to be more flexible and better predictive models because they can incorporate the effects of environmental change or vector control interventions if the effects of these changes on the key transmission parameters have been established (Myers *et al.*, 2000). However, while they are undoubtedly more powerful, biological models require much more work and testing to develop them.

9.1.4 Future uses for GIS in epidemiology

The applications for use of GIS as a means of demonstrating spatial aspects of disease epidemiology can be further extended to modelling disease, providing a means of displaying predictions of disease outbreaks.

Monitoring of environmental factors associated with disease outbreaks could provide information about the spatial variation in vector-borne disease risk, so that surveillance, control and containment measures could be directed accordingly (Rogers and Williams, 1993; Myers *et al.*, 2000). Epidemic preparedness in turn would allow prevention or amelioration of the size of the epidemic, and therefore its impact on people or animals.

In this chapter, the statistical relationships between the annual abundance and overall species richness of tabanids and meteorological and remotely sensed variables are explored. Using the results of this analysis, GIS maps have been produced which suggest how the variation in environmental variables would predict annual and spatial variation in abundance of the genus *Tabanus* and of the six most numerous species of *Tabanus*, individually.

9.2 Materials and Methods

9.2.1 Tabanid data

Tabanid collections were described in Chapter Six: Seasonal and Spatial Variation in Tabanids in Cape York Peninsula and Townsville. The total data collection period was 21 months in duration, which prevented comparisons being made between 12 month periods. The study was planned in order to study two wet seasons, which was when most of the tabanid activity occurred. In order for comparisons to be made between collection "years," nine month intervals were used. Total abundance for each of two nine-month "years" (September 2004 - May 2005 and September 2005 - May 2006) for the 12 sites was entered into a database. Data included abundance figures for total *Tabanidae*, *Tabanus*, non-*Tabanus* and the six most abundant individual *Tabanus* species. Data were only available for one dry season, and in the case of *T. ceylonicus*, it would have been desirable to additional dry season sampling.

9.2.2 Meteorological and remotely sensed data

A set of standard meteorological observations provided by the Bureau of Meteorology (BOM) for each site were included in the database (Appendix 6, Table A6.02). The values provided had been interpolated from the meteorological weather stations in the region by the BOM ("drill data"); the interpolated locations averaged 2.04 km from the trap site, compared to an average of 31.25 km for the distance between the trap site and the nearest weather station. The time period over which the weather data was averaged was the same as the tabanid data (nine months May - September for both collection "years" plus the previous "year"). The nine month period from the year previous to the data collection was included for two reasons. Firstly, when the annual weather data for the study years and the previous year were appraised (Appendix 6, Table A6.04), it appeared there may have been a relationship between the previous year's rainfall and abundance figures at each site. In addition, biological information compiled by Mackerras (1970) indicated that a development period of many months is required by

tabanids in Australia and that emergence of quiescent pupae appears to be encouraged by rainfall. So if a season particularly conducive to tabanid breeding occurs, (e.g. prolonged rainfall), the resultant increase in population may not be seen until the subsequent wet season.

Remotely sensed data, including satellite-derived solar radiation and Normalised Difference Vegetation Index (NDVI), were obtained as grids from the Bureau of Meteorology and CSIRO respectively (Appendix 6, Table A6.02). These grids provided data for the area of north Queensland contained in a rectangle bounded by latitudes -10 to -20˚S and longitudes 140 to 147˚E. Each grid cell contained a numeric value of the remotely sensed data. The size of the grid cells was 1 km^2 for NDVI and 5 km^2 for solar radiation (Appendix 6, Table A6.03). Remotely sensed data variables corresponding to each site during the two years of tabanid collection and the previous year (2003-2004) were extracted from the grids using Zonal Statistics within the Spatial Analyst tools (ArcGIS Version 9.1, ESRI). The solar radiation grids had already been averaged into the appropriate time frame, but the NDVI grids were in calendar month formats, so these had to be averaged over the nine month periods. The NDVI and satellite-derived solar radiation data values for each site were extracted from the grids and entered into the database with the tabanid data and meteorological data, in order for linear regressions to be performed. The entire grids were used to produce the GIS maps.

9.2.2.1 NDVI values

NDVI is a measurement of vegetation greenness. The data used in this analysis were obtained from NOAA AVHRR satellites. NDVI is calculated from different spectral reflectance measurements in the red and near-infrared regions: NDVI = (NIR-RED)/ (NIR+RED). The NDVI values vary between -1 and +1.

9.2.2.2 Generating nine-monthly average grids for NDVI

The monthly grids had occasional areas of cloud cover where there were no data in the grid cell. The method of averaging the grid cells ensured that only the months in which there was a data value for that grid cell were used.

9.2.2.3 Explanation of weather variables

The weather variables used in linear regression analysis of yearly tabanid abundance data are listed in Appendix 6, Table A6.02. Explanations are provided here for vapour pressure, evaporation and solar radiation.

Vapour pressure is the meteorological term for the atmospheric pressure which is exerted by water vapour and is a way of measuring the air humidity. For a particular temperature, an increase of water vapour in the air corresponds to an increase in the humidity of the air. Water vapour in the atmosphere comes from evaporation of water in oceans, lakes, wet land surfaces or from vegetation (transpiration). Water vapour absorbs the sun's radiation, so the sunlight received at the earth's surface will be more intense in a drier atmosphere (Meteorology, 2007).

Evaporation is sometimes used interchangeably with evapotranspiration-which is a collective term for the transfer of water, as water vapour, to the atmosphere from both vegetated and unvegetated land surfaces. It is affected by climate, availability of water and vegetation (Meteorology, 2007).

Solar radiation or solar exposure is the amount of solar energy reaching a specific location on the Earth’s surface. It is composed of two basic components: direct and diffuse solar energy. Direct solar energy is the energy that is produced from the sun’s beam, which is present at the Earth's surface. Diffuse solar energy results from the atmosphere attenuating, or reducing the magnitude of the Sun's beam. Some of this attenuated energy is redirected or scattered towards the ground. A computer model has now been produced which uses visible images from the geostationary meteorological satellites (currently MTSAT-1R) to estimate daily global solar exposures at ground level (Meteorology, 2007).

9.2.2.4 Long-term weather data and GIS mapping of species richness

A grid of long-term average rainfall (averaged over the period from 1990- 2005) for the area of north Queensland previously described was obtained from BOM and used in GIS mapping.

9.2.3 Statistical analysis

For the analysis of yearly abundance data, linear regressions were performed on catch data (transformed to Log10[Catch + 1]) using SPSS (Version 14.0, SPSS Inc., 2005) as described in Chapter Eight. A stepwise algorithm with backwards variable selection was used and the pattern of residuals was examined to evauate the fit. Variables for linear regression included interpolated weather data ("drill data" from the Bureau of Meteorology) plus NDVI and solar radiation values for each site. Weather variables for the same year as the data were collected were examined in the analysis ("same year") as well as weather values for the same period, but the previous year ("previous year").

For the analysis of total species richness and total abundance against long-term weather variables, correlations and linear regressions were performed. Plots of linear regressions were done using S-Plus (Insightful Corp, Version 6.4).

9.2.4 GIS mapping

GIS maps were created using ArcGIS (ArcMap Version 9.1, ESRI, 2005) using gridded data sets of weather and remotely sensed variables that were supplied by BOM (except NDVI- supplied by CSIRO) (Appendix 6, Table A6.03). The resolution of the final map was the same as the largest of the input grids. Input grids were converted to a common geographical projection prior to application of the prediction equation (determined by linear regression). Analysis was complicated by the unavailability of some environmental data in the grid form required to produce GIS maps. In particular, satellite solar radiation data were obtained from BOM because gridded values for

ground-based solar radiation data were not available. However, linear regression equations relating abundance to environmental variables used ground-based solar radiation data because satellite data for the 2005-2006 year were not available. A regression relating the satellite solar radiation values to the ground-based measurements was therefore calculated as an intermediate step in obtaining the GIS maps.

9.3 Results

9.3.1 GIS mapping of total species richness

The relationship between long-term annual average weather and total species richness and the total abundance of Tabanidae caught at a site over the entire course of the study was initially examined using correlations in Chapter Six (Section 6.3.4, Table 6.01). Correlations were significant between some of the weather factors and species richness, but not total abundance. These relationships were further explored using linear regression.

Gridded average annual rainfall was available from the BOM, so this variable was used in linear regression to predict species richness levels over the whole Cape York region, as it allowed GIS maps to be produced. The correlation co-efficient for vapour pressure was higher, indicating that it provided a better predictive relationship with species richness, but gridded vapour pressure was not available from the BOM, so it could not be used to produce GIS maps. The equation obtained from linear regression analysis is: Number of species = 2.274 + 0.006 * long-term average annual rainfall (interpolated) ($R^2 = 0.574$, $p = 0.004$) (Appendix 6, Table A6.01).

The GIS map showing the predicted variation in species richness in the north Queensland region is presented in Figure 9.01.

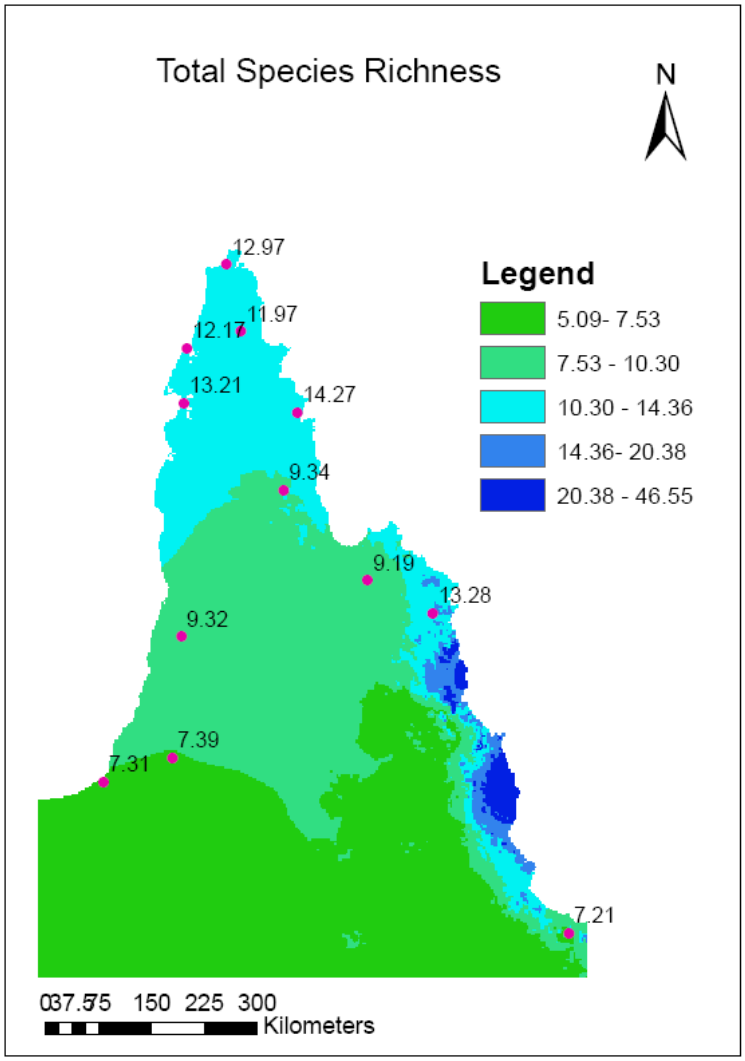


Figure 9.01: GIS map of predicted species richness in northern Queensland

Because of the relationship of species richness with the long-term annual rainfall, the map of species richness (Figure 9.01) predicts that greatest species richness will occur in high rainfall areas, such as the Mossman - Daintree area, between Cairns and Cooktown, and south of Cairns from Gordonvale to Tully. It is predicted that medium-level species richness will occur north of a line through Coen and down the eastern coast. Lowest species richness is predicted to occur in the south-west of the area covered by the map, south of a line through Normanton.

9.3.2 Effects of weather on total annual abundance of tabanids

Data used in this analysis were collected from September to May 2004-05 (year one) and 2005-06 (year two). A summary of the weather variables at all sites over three years (the two years collection were made and the one prior) is provided in Appendix 6, Table A6.02.

Correlations and linear regressions were performed with the weather variables for the same year as tabanid collections and the previous year (i.e. over the same time period September to May, in the year previous to the collections). The previous year's weather and NDVI had the most significant relationships (Table 9.01).

When total Tabanidae numbers (log 10 [tabanid+1]) were analysed against appropriate interpolated weather variables ("drill data" for each site), the previous year's solar radiation was significant. For *Tabanus*, overall, the previous year's solar radiation and minimum temperature were significant. For non-*Tabanus* species, mean NDVI (same year) and the previous year's minimum relative humidity were most significant. No significant associations were found for *T. dorsobimaculatus*.

The quality of the predictions provided by the linear regressions is reflected by the R^2 value. The R^2 value is the fraction of the variance in the variables that is shared. A value of 0.6 or above indicates that the linear regression equation has a very good predictive value, such as for *Tabanus strangmannii* ($R^2 = 0.641$). R^2 values of 0.4 - 0.5 indicate a good predictive value, such as those for *T. ceylonicus*, *T. notatus* and the non-*Tabanus* group. *Tabanus*, total Tabanidae and *T. pallipennis* all have dubious predictive value, with R^2 values ranging from 0.2 - 0.3. No worthwhile prediction can be gained from the linear regression results of *T. innotabilis* ($R^2 = 0.169$).

Table 9.01: Prediction equations of annual abundance obtained from linear regression (df = 21)

Taxa/ species Log 10 (x+1)	**Prediction variables**	**R^2**	**P value**
Non- *Tabanus*	-3.112 + 1.768 sy NDVI + 0.044 py RHmin	0.426	0.003
Tabanus	-0.379 + 0.230 py Tmin – 0.164 py Radn	0.281	0.031
Total Tabanidae	6.359 – 0.202 py SATRAD	0.208	0.025
T. pallipennis	7.120 - 1.035 Log sy Totseasrain – 0.174 py Radn	0.317	0.018
T. notatus	8.682 – 0.387 py Radn	0.538	<0.0005
T. strangmannii	11.252 -0.483 sy SATRAD +1.259 sy NDVI	0.641	0.010
Or	6.944 – 0.298 py SATRAD + 1.124 py NDVI * uses previous year's data (GIS maps)	0.401	0.005
T. innotabilis	-4.702 + 0.218 py Tmin	0.169	0.046
T. ceylonicus	-7.534 + 0.801 Log sy Totseasrain + 0.232 py Tmin	0.427	0.003
Or	-6.269 + 0.852 Log py Totseasrain + 0.171 pyTmin * uses previous season's data (GIS maps)	0.421	0.003

Table 9.02: Explanation of abbreviations

Abbreviation	**Meaning and units**
NDVI	Normalised Difference Vegetation Index (no units)
Tmin	Minimum temperature (˚C)
RHmin	Minimum relative humidity (%)
Radn	Solar radiation (ground-based values) (MJ/m^2)
SATRAD	Solar radiation (satellite values) (MJ/m^2)
totseasrain	Total rainfall for the "year" September - May (mm)
py	Previous "year" i.e. September – May, year previous to that when tabanid data were collected
sy	Same "year" i.e. September - May of same year that tabanid data were collected

An alternative equation is provided for *T. strangmannii* and *T. ceylonicus*. One equation gives a slightly better R^2 value, however, it uses "same year" weather values. The equation which uses "previous year" weather values was preferred for the GIS mapping, because this gives a predictive value for the subsequent year's vector intensity.

9.3.3 Plots of linear regression equations

Plots of *Tabanus* and *T. notatus* are provided to illustrate the relationship with the weather variables that is described by their linear regression equations in Table 9.01.

9.3.3.1 Plots of linear regression of *Tabanus*

Plots of log *Tabanus* linear regressions (Figure 9.02) illustrate a negative relationship with the previous year's solar radiation values and a positive relationship with the previous year's minimum temperature. The equation produced by linear regression was: Log (*Tabanus* + 1) = -0.379 + 0.230 (PY_minTemp) – 0.164 (PY_avRad) (R^2 = 0.281, df = 21, p= 0.031).

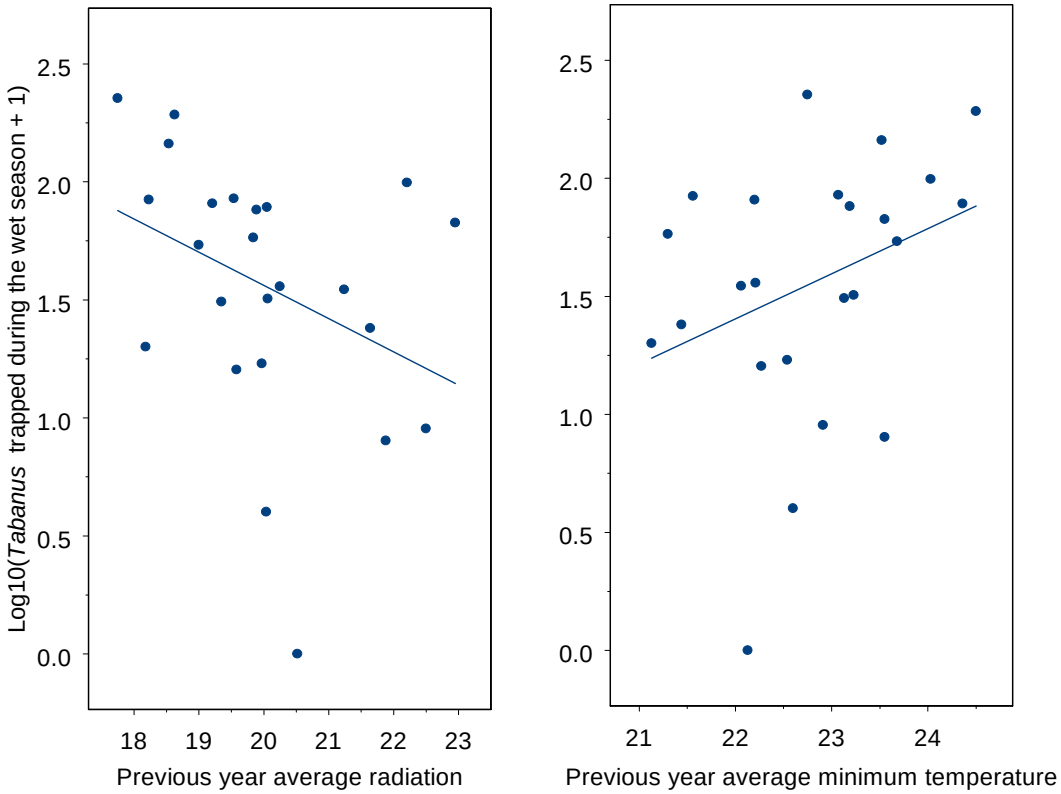


Figure 9.02: Plots of the relationship between *Tabanus* abundance and average solar radiation and average minimum temperature (units for solar radiation are MJ/m^2, units for temperature are °C)

9.3.3.2 Plot of linear regression of *T. notatus*

The plot of the linear regression of *T. notatus* shows a strong negative relationship between the annual abundance of this species and the previous year's solar radiation (Figure 9.03). The equation produced by linear regression was: Log (*Tabanus notatus*) = 8.862 – 0.387 (PY_avRad) ($R^2 = 0.538$, df = 21, $p<0.0005$).

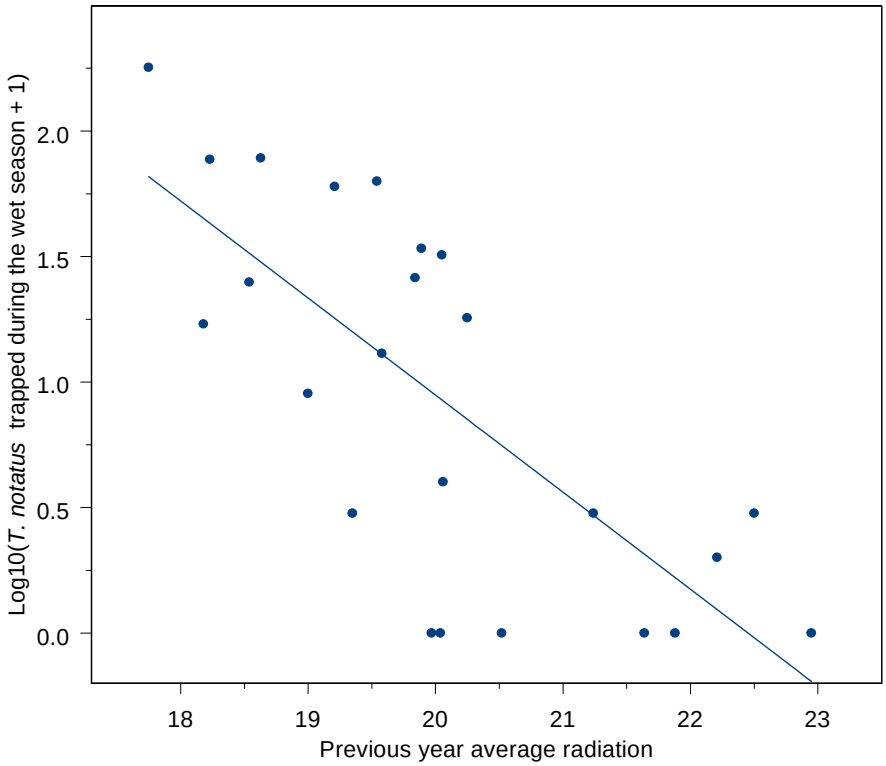


Figure 9.03: Plot of relationship between *T. notatus* abundance and average radiation

9.3.4 GIS maps of annual and spatial abundance patterns

9.3.4.1 *Tabanus* annual abundance GIS maps

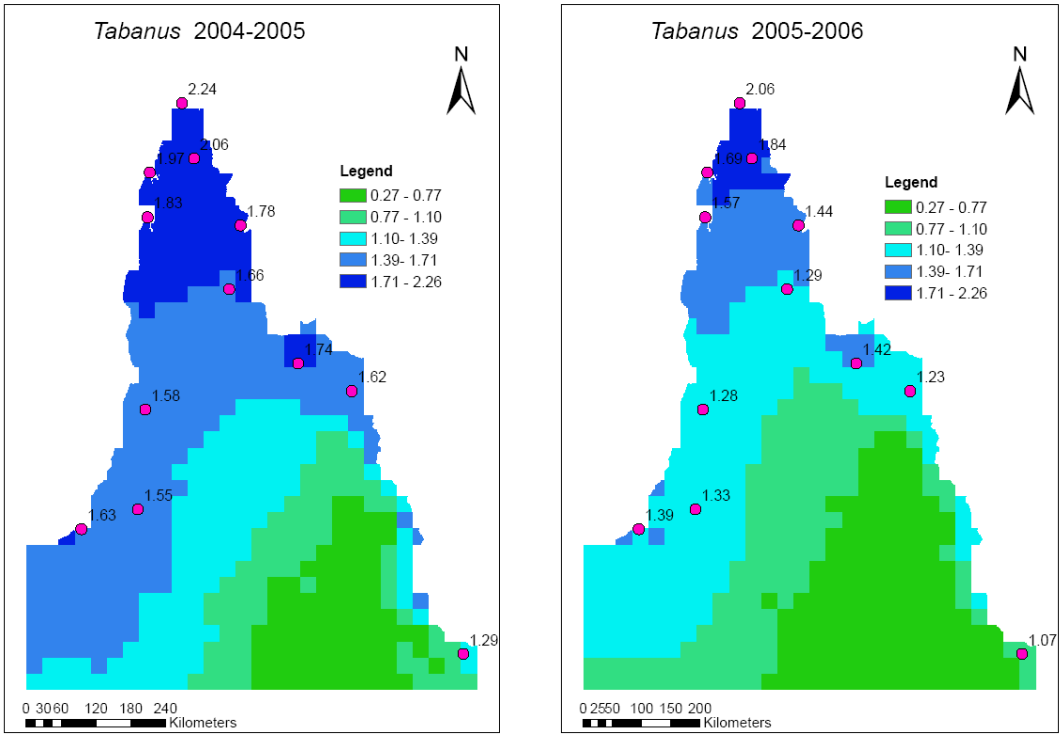


Figure 9.04: GIS maps of *Tabanus* annual abundance

Overall, prediction maps indicate there were more extensive areas with higher abundance of *Tabanus* in the year 2004-2005, compared to 2005-2006 (Figure 9.04). This is also true for the predictions of the five individual species of *Tabanus* that have been mapped in this manner (Figures 9.05 - 9.09) Highest levels of *Tabanus* abundance in 2004-2005 were predicted to occur in the most northern part of Cape York, north of a line through Coen and in the Lakefield National Park region. In 2005-2006, the highest level of abundance was predicted to be restricted to the area north of Weipa. In 2004-2005, the next highest level of abundance was predicted to extend south and west into the gulf country, whereas in 2005-2006, this level of abundance was predicted only north of Arukun and in the Lakefield National Park area. Lowest levels of abundance were predicted in the south-eastern part of the map, but did not extend to the coastline. Townsville was predicted to fall in the middle to low end of the abundance spectrum.

9.3.4.2 *Tabanus innotabilis* annual abundance GIS maps

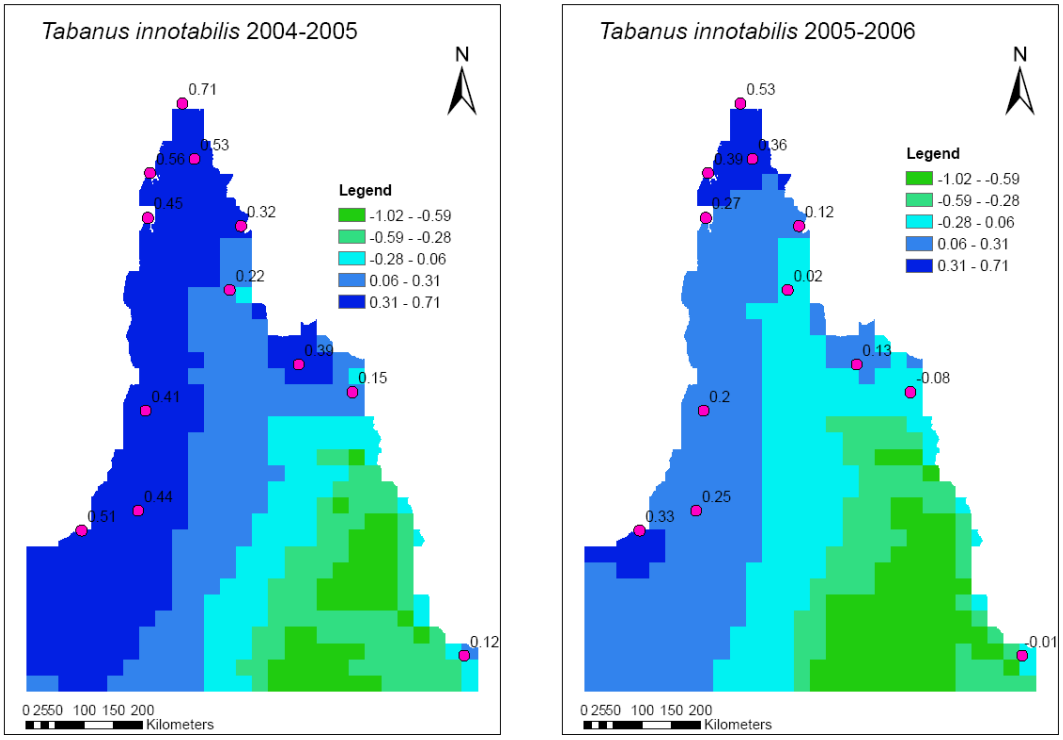


Figure 9.05: GIS maps of *T. innotabilis* annual abundance

The highest abundance of *T. innotabilis* in 2004-2005 was predicted to occur in the Lakefield National Park region, north of Lockhart River and in the western one-third of the Peninsula and southern gulf area (Figure 9.05). A zone east and south of the area of highest abundance was predicted to contain the next highest level of abundance.
In the year 2005-2006, the highest level of abundance was predicted to be restricted to the northern part of the peninsula, north of Weipa and a small area in the country adjacent to the Gulf of Carpentaria. Lowest abundance of this species was predicted to occur in the south-east of the map, just off the coastline.

9.3.4.3 *Tabanus notatus* annual abundance GIS maps

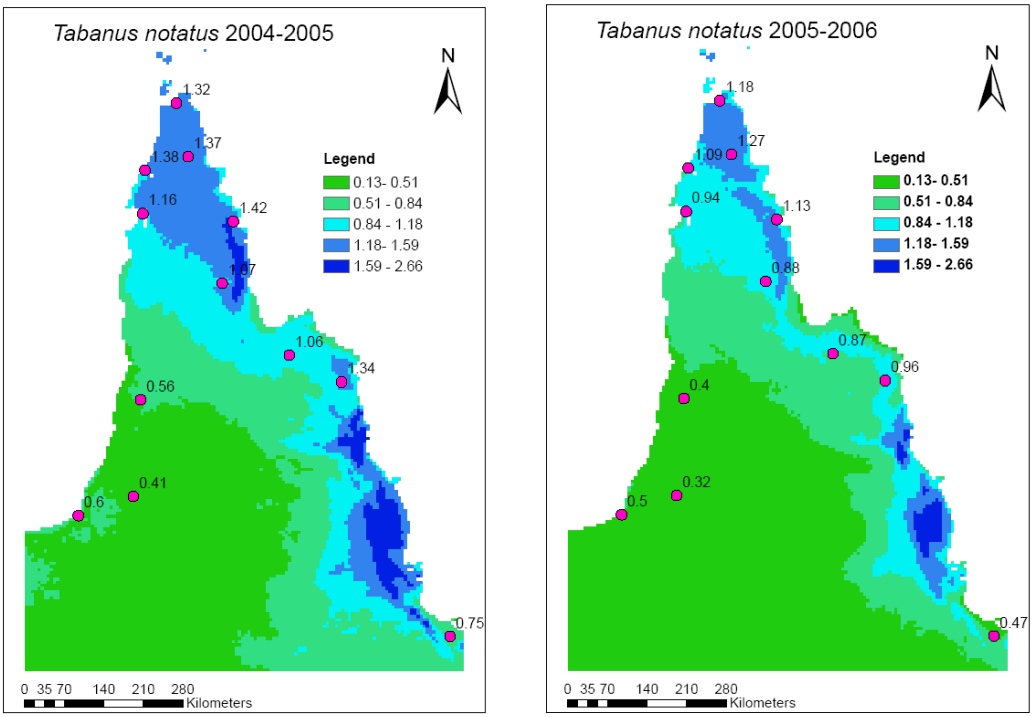


Figure 9.06: GIS maps of *T. notatus*

During the year 2004-2005, greatest abundance of *T. notatus* was predicted to occur in the area north of Coen, and on the eastern coast, between Cooktown and Tully (Figure 9.06). The abundance distribution was predicted to be similar for 2005-2006, although more restricted. Lowest abundance of this species was predicted in the south-western part of the peninsula and southern gulf area.

2.3.4.4 *Tabanus strangmannii* annual abundance GIS maps

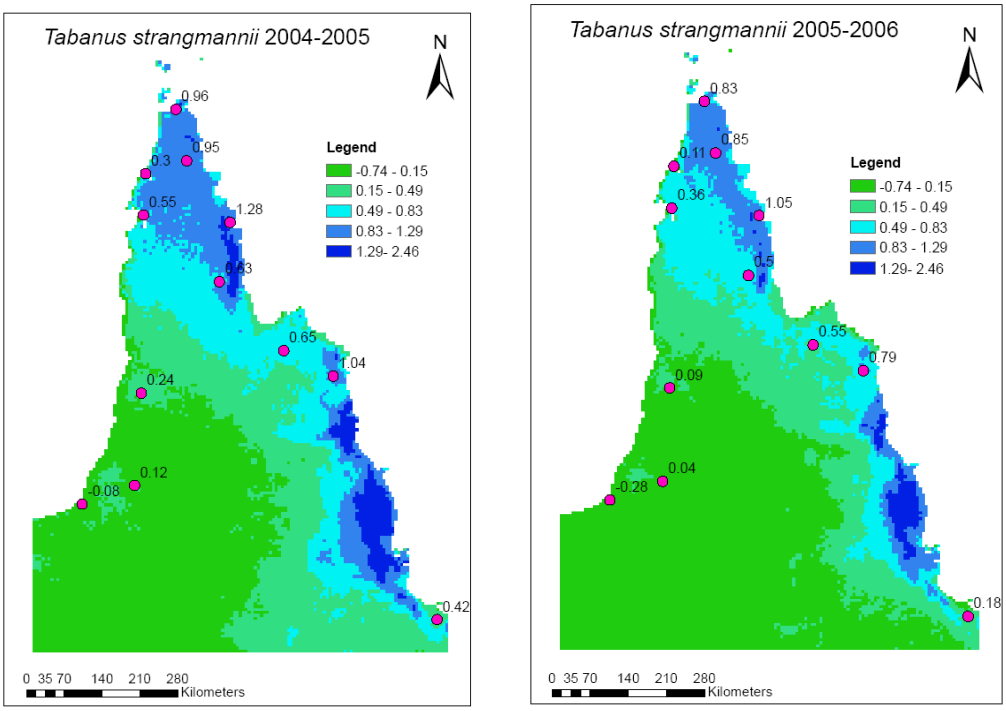


Figure 9.07: GIS maps of *T. strangmannii*

High abundance of *T. strangmannii* was predicted in 2004-2005 to occur north of Weipa and to extend slightly down the eastern coastline to just north of Princess Charlotte Bay (Figure 9.07). High abundance was also predicted on the eastern coastline from Townsville to Cooktown. This spatial pattern prediction was similar the following year, but did not reach the same extent. The lowest abundance was predicted in the south-west and southern gulf area.

2.3.4.5 *Tabanus ceylonicus* annual abundance GIS maps

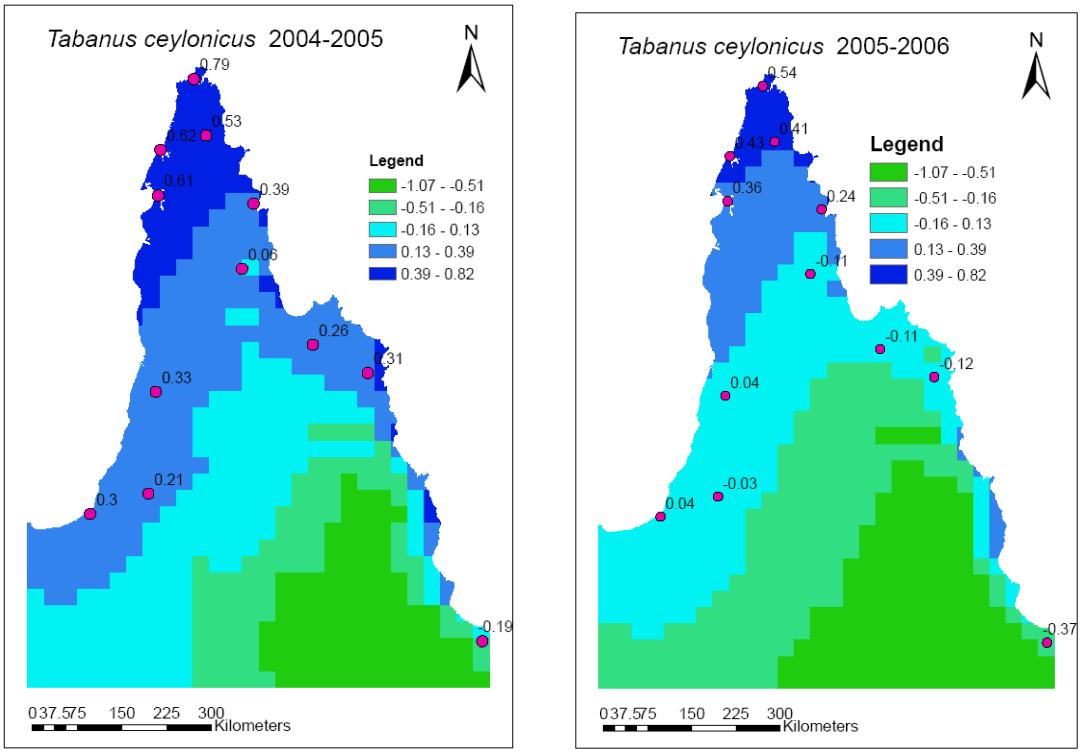


Figure 9.08: GIS maps of *T. ceylonicus*

The areas of highest predicted abundance of *T. ceylonicus* in 2005 - 2006 were in the far north of the peninsula, above a line between Lockhart River and Pompuraaw, with a few small areas on the eastern coastline around Princess Charlotte Bay, Cooktown and Innisfail (Figure 9.08). Abundance was also predicted to be high down the western and eastern edges of the peninsula. In 2005-2006, highest abundance was predicted north of Weipa, with the next highest level of abundance predicted to be north of a line between Kowanyama and Lockhart River. Lowest abundance in both years was predicted in the south-western part of the map, but not extending to the coastline.

2.3.4.6 *Tabanus pallipennis* annual abundance GIS maps

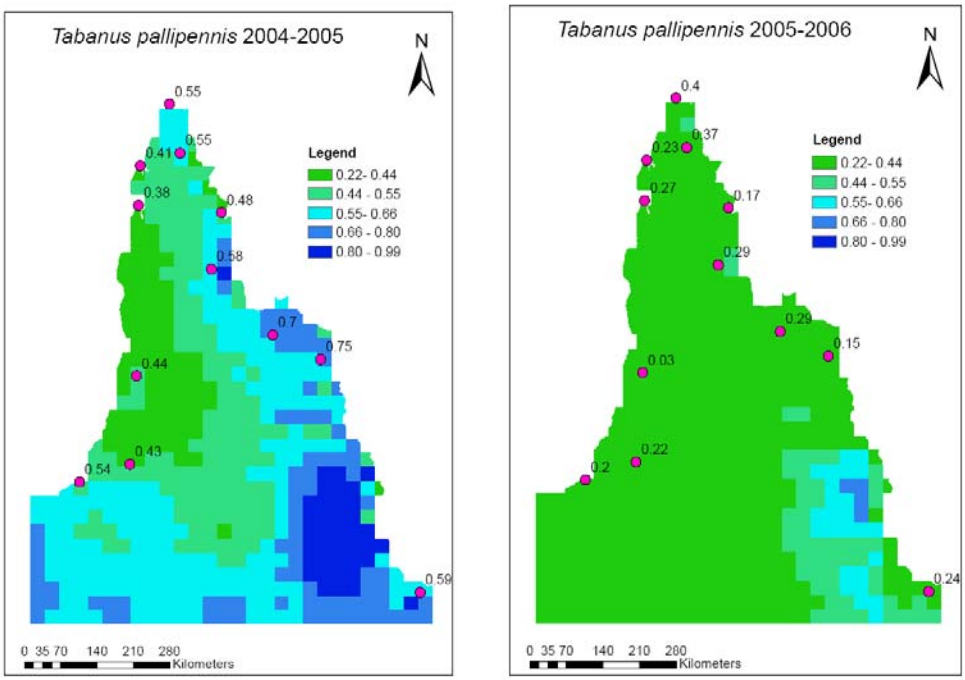


Figure 9.09: GIS maps of *T. pallipennis*

Higher abundance of *T. pallipennis* in 2004-2005 was predicted on the eastern coast, particularly from Cairns to Townsville (Figure 9.09). There were also some patches of high abundance predicted in the southern part of the map, extending west. In 2005-2006, the pattern was similar, but a much lower amplitude of abundance was predicted. In 2004-2005 the lowest abundance was predicted on the western half of the peninsula. In 2005-2006 the prediction of lowest abundance extended over most of the map extent, except for the south-eastern portion.

9.4 Discussion

The total species richness at each of the 12 sites was significantly related to a number of long-term weather variables that were mostly inter-correlated and relate to moisture in the environment. Information that is available regarding the breeding habits of Australian Tabanidae, indicates most or all species require an aquatic environment in order to breed (Mackerras, 1970). Greater diversity of tabanid species is therefore more likely in areas where this habitat is consistently present, that is, in areas of high rainfall. This relationship between species diversity and long-term rainfall is supported by the data labels at each of the study sites (Figure 9.01) which indicate that the species richness at each site falls within the range of species richness predicted by the GIS map.

Total tabanid abundance at the study sites was not significantly related to any of the long-term weather variables. However, the overall annual tabanid abundance, and abundance of five of the six most numerous species were significantly related to weather variables and NDVI from the previous year. This result indicates that the weather conditions during the breeding season of the previous year dictate the population size. This is useful for predicting the vector intensity that will be present during the following year, allowing management decisions regarding vector control and/or animal movement control to be implemented.

The abundance prediction GIS maps for individual species should be interpreted cautiously, as they do not represent the distribution of the species, and further work needs to be done to determine distribution maps for each species. While abundance of individual species is significantly related to weather variables and NDVI in some cases, the presence or absence of a species at a particular location is probably related to a number of factors and requires further clarification.

The R^2 values for the linear regression equations indicate the prediction value provided. For some species, the value of R^2 was quite low, indicating that no useful predictive value could be gained. This was the case for *T. innotabilis*, which was detected at only

two sites. The restricted distribution of this species and its short flight season mean that this species would have had many sites and collection periods with zero values, which contributed to the limited ability to predict its abundance.

Total *Tabanus* numbers are significantly positively related to minimum temperature and negatively related to solar radiation values, from the previous year. The genus *Tabanus* represents the group posing the highest risk to *T. evansi* transmission in Australia, as has been described previously. In the absence of further information regarding the vectorial capacity and vector competence of individual *Tabanus* species, it is most useful to look at the spatial and temporal variation of the genus as a whole. The area of greatest *Tabanus* abundance is the northern part of Cape York, north of Coen and the Lakefield National Park area. This region is also the closest geographically to Papua New Guinea and thus poses the greatest risk of an infected animal entering this part of Australia (Thompson *et al.*, 2003b). Therefore, the coincidence of these two risk factors indicates a higher probability of surra becoming established in north Queensland in the event of an incursion of one or more infected animals. If such an incursion occurred during the wet season, when vector numbers were highest, this would limit the possibility of detection prior to spread of the disease. In addition, *T. ceylonicus*, a species known to transmit *T. evansi* in Indonesia, occurs in highest numbers in the northern part of Cape York Peninsula and the GIS maps indicate the abundance patterns for this species are similar to that of *Tabanus* as a whole. *Tabanus ceylonicus* is known to be active during the dry months of the year, so the temporal risk in the north of Cape York may be present during much of the year.

Further work needs to be done to determine the relationship between vector intensity and incidence of infection with *T. evansi* in countries where the disease is endemic. Use of GIS maps allowing both the quantification of vector intensity and a spatial awareness of high vector numbers, will help clarify the role and timing of high vector intensity with increased disease incidence and allow control measures to be used judiciously. It is possible that environmental proxies could be developed that are related directly to disease prevalence.

CHAPTER TEN
CLASSIFICATION AND REGRESSION TREE ANALYSIS: ENVIRONMENTAL AND SPATIAL ASSOCIATIONS WITH *TABANUS* PREVALENCE

10.1 Introduction

Classification and Regression Trees (CART) (Breiman *et al.*, 1984) are constructed by repeatedly splitting data, defined by a simple rule based on a single explanatory variable (recursive binary splitting). At each split the data are partitioned into two mutually exclusive groups, each of which is as homogeneous as possible. The splitting procedure is then applied to each group separately. The objective is to partition the response into homogeneous groups, but also to keep the tree reasonably small (De'ath and Fabricius, 2000; O'Connor and Wagner, 2004).

CART analysis has been used in a wide variety of applications, including highway safety analyses (Stewart, 2007), avalanche activity indices (Davis and Elder, 2007) financial applications e.g. credit scoring (Grossman and Poor, 1996) and health applications such as low birth weight analysis (Fu, 2004), food safety (Mokhtari *et al.*, 2006) and evaluation of chest pain (Buntinx *et al.*, 1992).

CART analysis is well suited to ecological data where it can be used to describe and predict patterns and processes. The benefits of using CART include: ability to handle a broad range of data types including numeric, categorical, ratings and survival data; ease and robustness of construction; ease of interpretation and ability to handle missing values. Ecological data are frequently complex, and relationships between variables may be non-linear and involve interactions. Commonly used statistical modeling techniques often fail to find meaningful ecological patterns from such data (De'ath and Fabricius, 2000).

CART analysis of abundance of soft coral taxa in the Great Barrier Reef in relation to physical and spatial environmental information revealed habitat definitions that were

consistent with known experimental findings (De'ath and Fabricius, 2000). CART analysis has also been used successfully to predict species richness of birds in the United States, the predictor variables of which were a number of climate and land-cover variables (O'Connor and Wagner, 2004). The use of CART to reliably predict the level of tree mortality from the trap catches of spruce beetles, and to correlate trap catch patterns with endemic and epidemic conditions (Hansen *et al.*, 2006) is an application of potential interest to surra modeling.

This chapter explores the use of CART analysis in examining the relationships of *Tabanus* and the six most numerous *Tabanus* species with spatial, environmental and meteorological variables, in an attempt to identify factors associated with the presence of the genus and the individual species.

10.2 Materials and Methods

The data set contained abundance (catch per day) for each calendar month at each site for all species of *Tabanus* combined and for the six most abundant species of *Tabanus* (i.e. monthly summaries of data). The variables that were included in the analysis are shown in Appendix 8, Table A8.01. Normalised Difference Vegetation Index (NDVI) is defined in Chapter Nine, Section 9.2.2.1. Vapour pressure is defined in Chapter Nine, Section 9.2.2.3.

Classification and Regression Tree (CART) analyses were used to analyse the data (CART, Salford Systems, California USA). The term prevalence is used to indicate the proportion of months that a species was present for all sites monitored (12 sites x 17 months = 204 site-months).

10.3 Results

The frequency distribution of the abundance data showed a large number of zero values and was highly skewed, which indicated that the data were not suitable for use in parametric analyses. When ranked data were analysed, only a quarter (25.8 %) of the variability could be explained (adjusted R^2) using all the available variables. Therefore the analysis focused only on associations between prevalence and environmental factors, as opposed to predictive modeling of abundance.

The variables associated with higher prevalence of each of the six most abundant *Tabanus* species, and with total catches of *Tabanus* are shown in Table 10.01.

Table 10.01: Dichotomous associations for individual species. N.B. Abbreviations used: vapour pressure (VP), minimum and maximum temperature (T min, T max), Normalised Difference Vegetation Index (NDVI).

Genus/ Species	**Order of splits/ nodes**	**Higher prevalence when:**
Tabanus	1. VP	> 22.6
	2. Latitude (for VP>22.6)	> -12.3 (north of 12.3˚S)
	3. VP (for Lat <= -12.3)	> 29.9
	4. Evaporation (for VP<=29.9)	> 5.5
T. ceylonicus	1. Latitude	> - 11.3 (north of 11.3˚S)
	2. T min (for Lat > -11.3)	<= 24.8
T. dorsobimaculatus	1. VP	> 30.1
	2. T min (for VP > 30.1)	> 24.3
T. innotabilis	1. T max	> 32.0
	2. NDVI (for T max >32.0)	<= 0.13
	3. Rain (for NDVI >0.13)	> 2.5
	4. T max (for Rain > 2.5)	<= 33.2
T. notatus	1. VP	> 27.9
	2. T max (for VP> 27.9)	> 30.6
T. pallipennis	1. Latitude	> -16.41 (north of 16.4˚S)
	2. Solar radiation (for lat > 16.4˚S)	> 17.4
T. strangmannii	1. VP	> 27.5
	2. NDVI (for VP > 27.5)	> 0.20
	3. VP (for VP < 27.5)	> 24.4
	4. NDVI (for VP > 24.4)	> 0.20

CART analyses indicate that vapour pressure was an important variable for *Tabanus* overall, and for a number of individual species.

The overall prevalence (over all site-months) of each species was: *T. ceylonicus* 12.3%, *T. dorsobimaculatus* 15.4%, *T. innotabilis* 9.6%, *T. notatus* 22.4%, *T. pallipennis* 22.4% and *T. strangmannii* 18.0%

For *Tabanus*, the most important variable was vapour pressure, with values greater than 22.6 hPa being associated with higher prevalence (75% versus 33%). When vapour pressure was high, latitude north of 12.3°S was also associated with higher numbers (95% versus 66% at lower latitudes). For latitudes south of 12.3°S, high vapour pressure (>29.9 hPa) was again associated with greater abundance (83% versus 59%), but when vapour pressure was below this figure, evaporation > 5.5 mm was related to higher *Tabanus* numbers (68% versus 38%) (Figure 10.01).

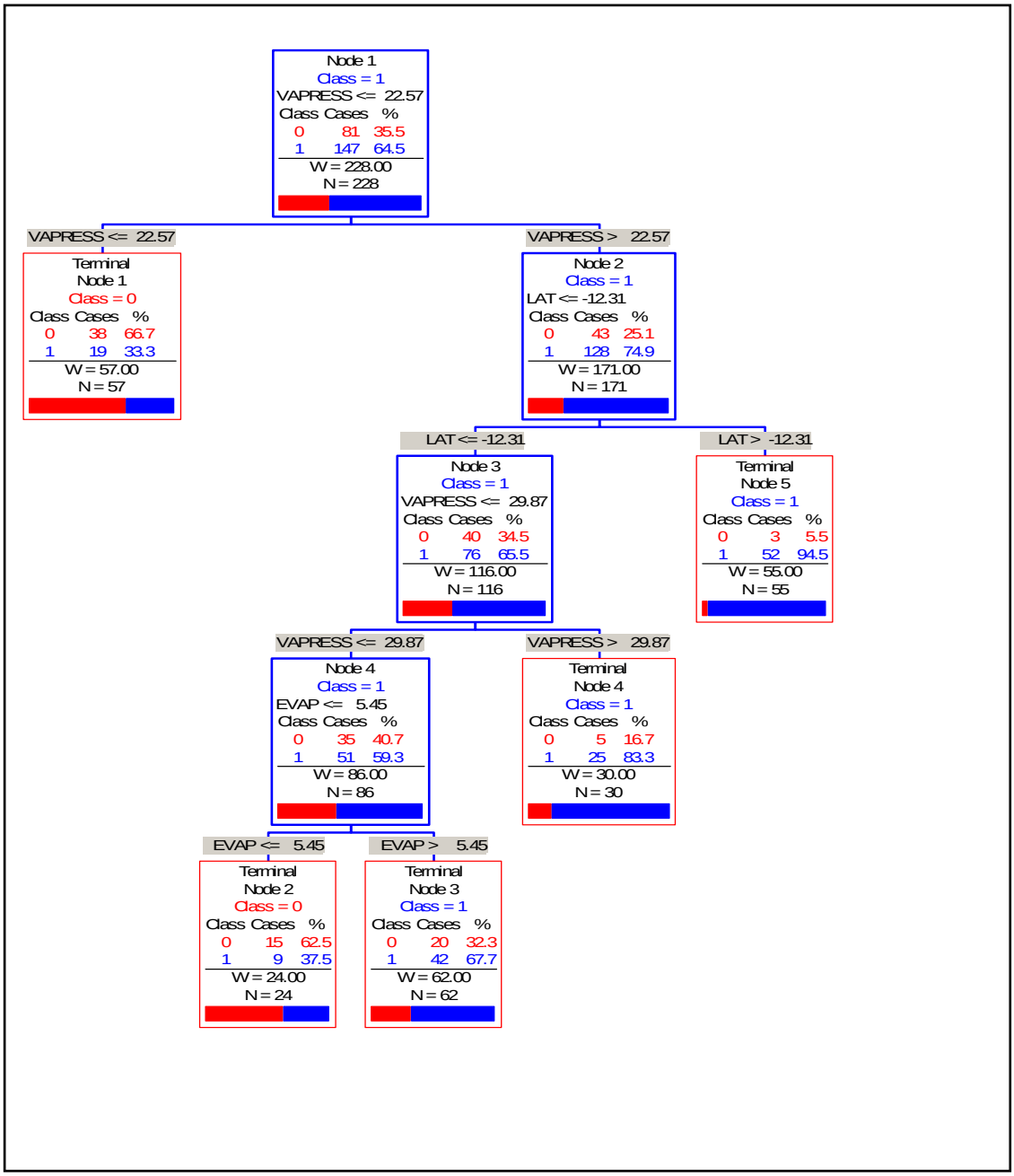


Figure 10.01: CART analysis for *Tabanus*

For sites north of latitude 12.3°S, mean vapour pressure above 22.6 was recorded during the months from October to June (Figure 10.02). For sites south of latitude 12.3°S (Weipa and below), vapour pressure exceeded 29.9 hPa only during the months from January to March (inclusive) (Figure 10.03). Vapour pressure is highly correlated with

rain (0.71, p<0.0005) and maximum relative humidity (0.79, p<0.0005). Vapour pressure during the study ranged from 14.1- 33.1 hPa with a mean of 25.8 hPa.

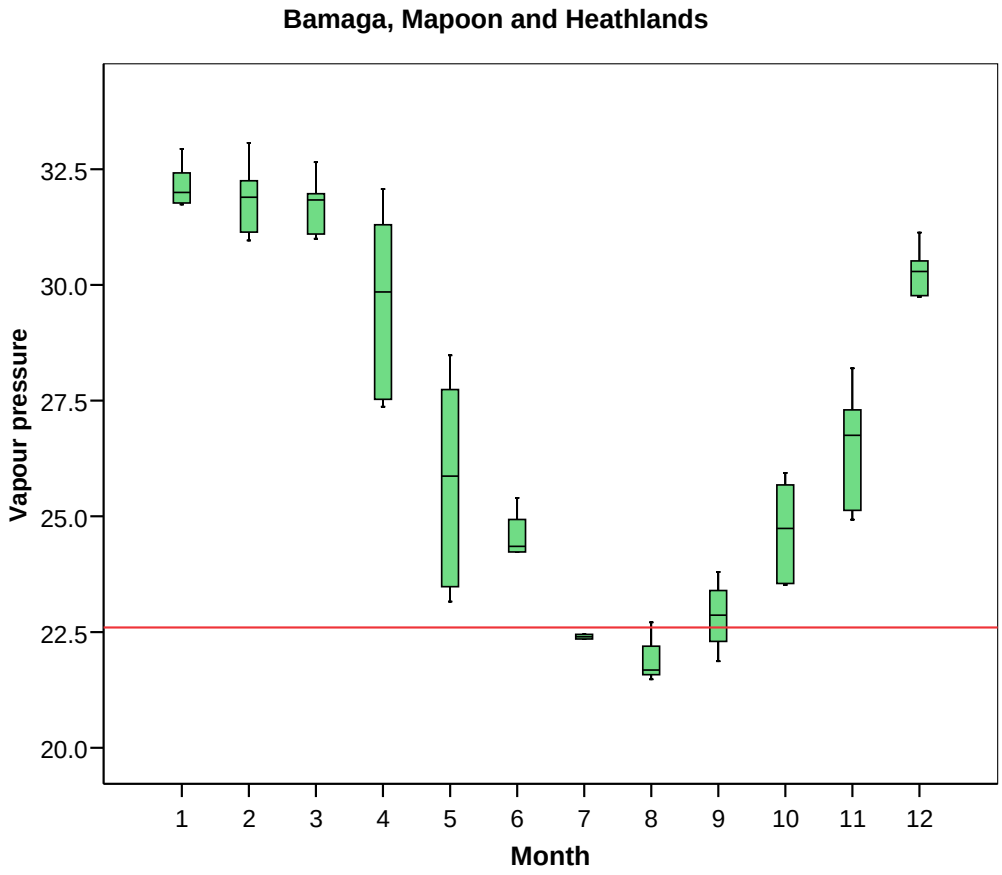


Figure 10.02: Boxplots of vapour pressure during calendar months of the study, for sites north of Weipa (reference line at 22.6 hPa)

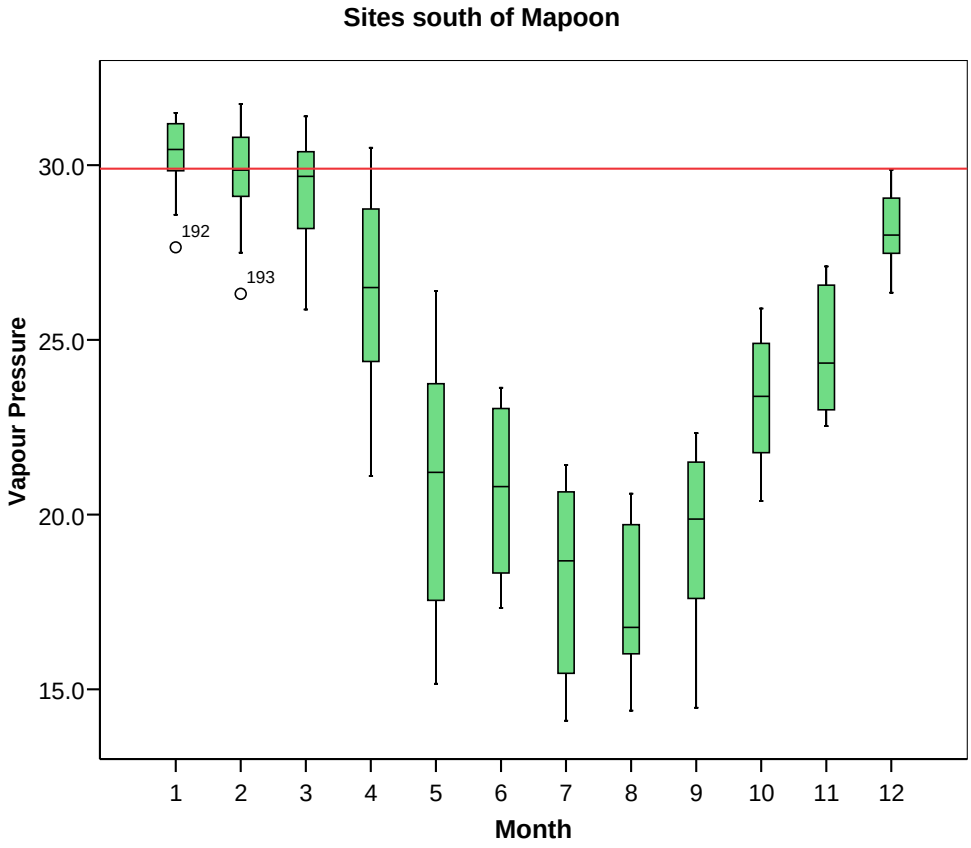


Figure 10.03: Boxplots of vapour pressure during calendar months of the study, for sites south of Mapoon (reference line at 29.9 hPa)

For *T. ceylonicus*, the most important variable was latitude, with highest prevalence of this species found north of 11.3°S (70% versus 7%). In these northern latitudes, minimum temperatures below 24.8°C were also associated with higher numbers (100% versus 40% at temperatures above this level) (Figure 10.04).

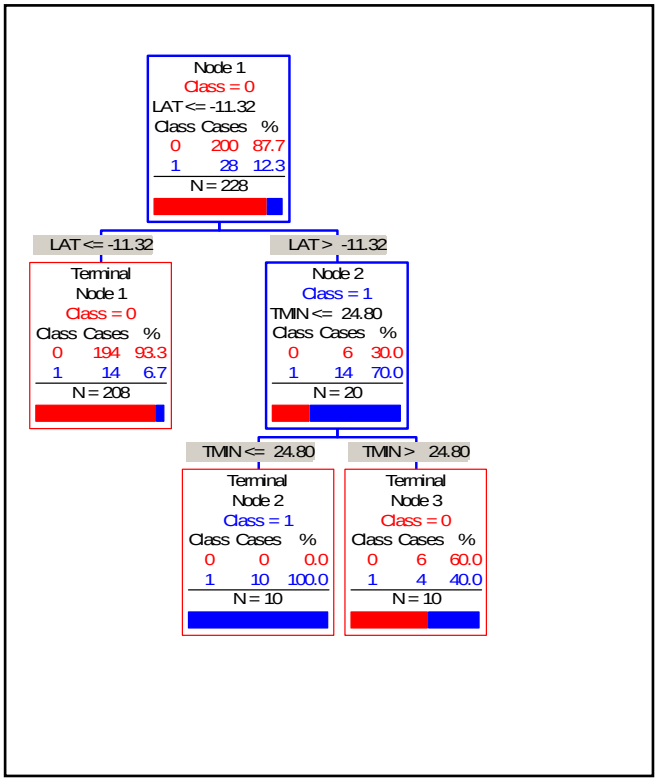


Figure 10.04: CART analysis for *T. ceylonicus*

Tabanus dorsobimaculatus had higher prevalence at vapour pressures greater than 30.1 hPa (35% versus 10%) and at high vapour pressures, was more numerous when minimum temperatures exceeded 24.3°C (54% versus 16%) (Figure 10.05).

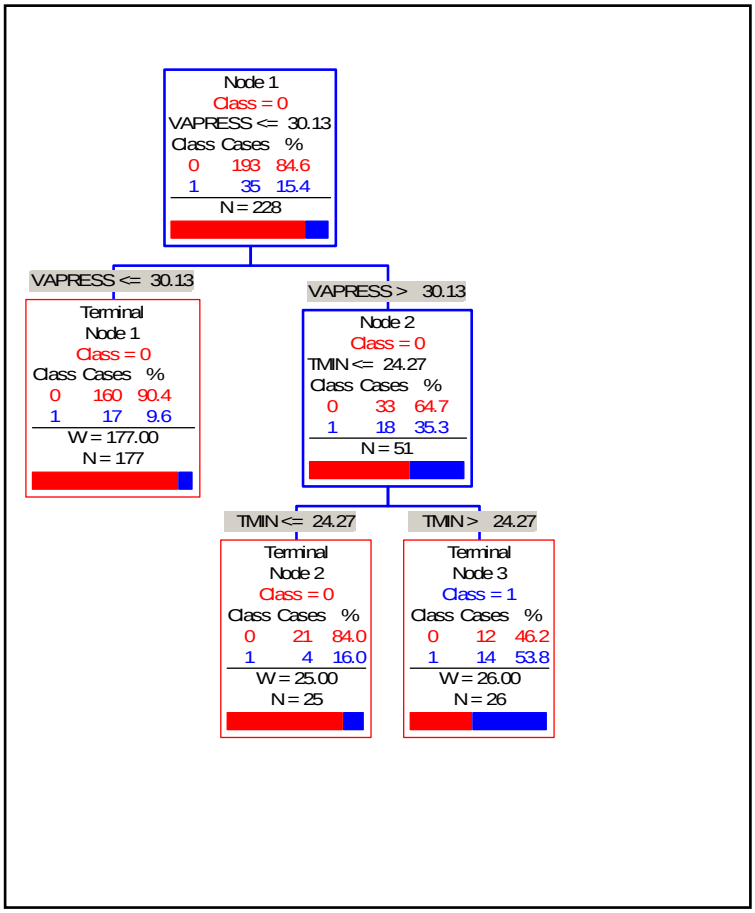


Figure 10.05: CART analysis for *T. dorsobimaculatus*

The variable of greatest association with *T. innotabilis* numbers was maximum temperature, at values greater than 32.0°C (18% versus 2%). When maximum temperatures were above this level, NDVI less than or equal to 0.13 was associated with higher abundance of this species (41% versus 6%). When NDVI values exceeded 0.13, higher numbers of this species were present when rainfall was above 2.5mm (13% versus 0%). When rainfall was above 2.5 mm, greater abundance of *T. innotabilis* occurred when maximum temperatures were less than 33.2°C (20% versus 6%) (Figure 10.06). However, none of the splits resulted in a grouping with *T. innotabilis* present in a majority of samples.

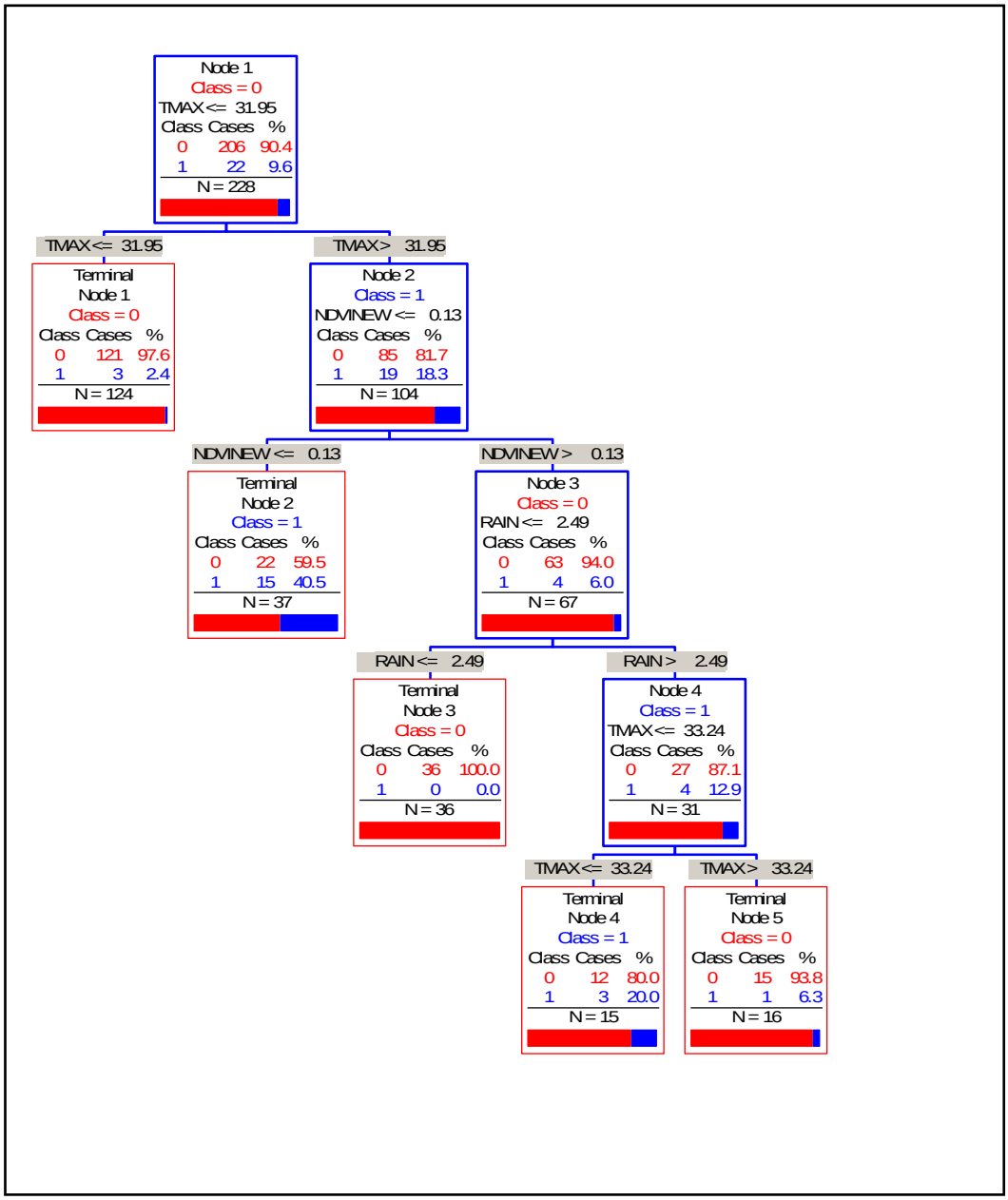


Figure 10.06: CART analysis for *T. innotabilis*

Higher prevalence of *T. notatus* was associated with vapour pressure above 27.9 hPa (54% versus 2%). When vapour pressures exceeded this level, higher numbers of this species also occurred with maximum temperatures above 30.6°C (63% versus 13%) (Figure 10.07).

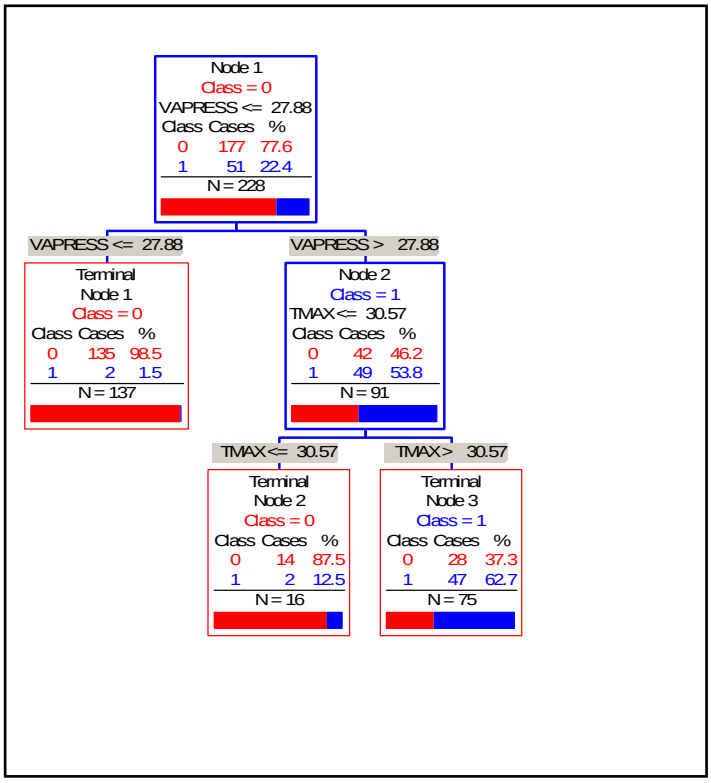


Figure 10.07: CART analysis for *T. notatus*

For *T. pallipennis*, higher numbers were associated with latitudes north of 16.4°S (28% versus 5%). At more northern latitudes, higher prevalence was also associated with solar radiation values higher than 17.4 MJ/m^2 (39% versus 12%) (Figure 10.08).

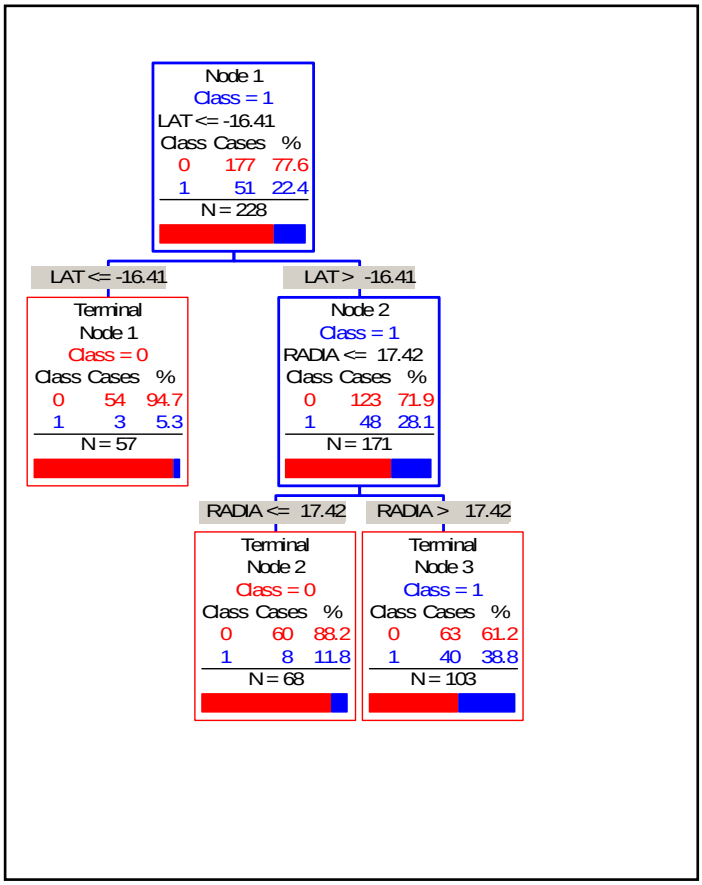


Figure 10.08: CART analysis for *T. pallipennis*

Greater prevalence of *T. strangmannii* was related to vapour pressure in excess of 27.5 hPa (38% versus 4%). At high vapour pressure levels, higher *T. strangmannii* numbers also occurred where NDVI was greater than 0.20 (53% versus 3%). When vapour pressure was below 27.5 hPa, higher abundance of this species occurred with vapour pressures greater than 24.4 hPa (11% versus 0%). When vapour pressure was greater than 24.4 hPa, higher numbers again occurred when NDVI was greater than 0.20 (21% versus 0%) (Figure 10.09).

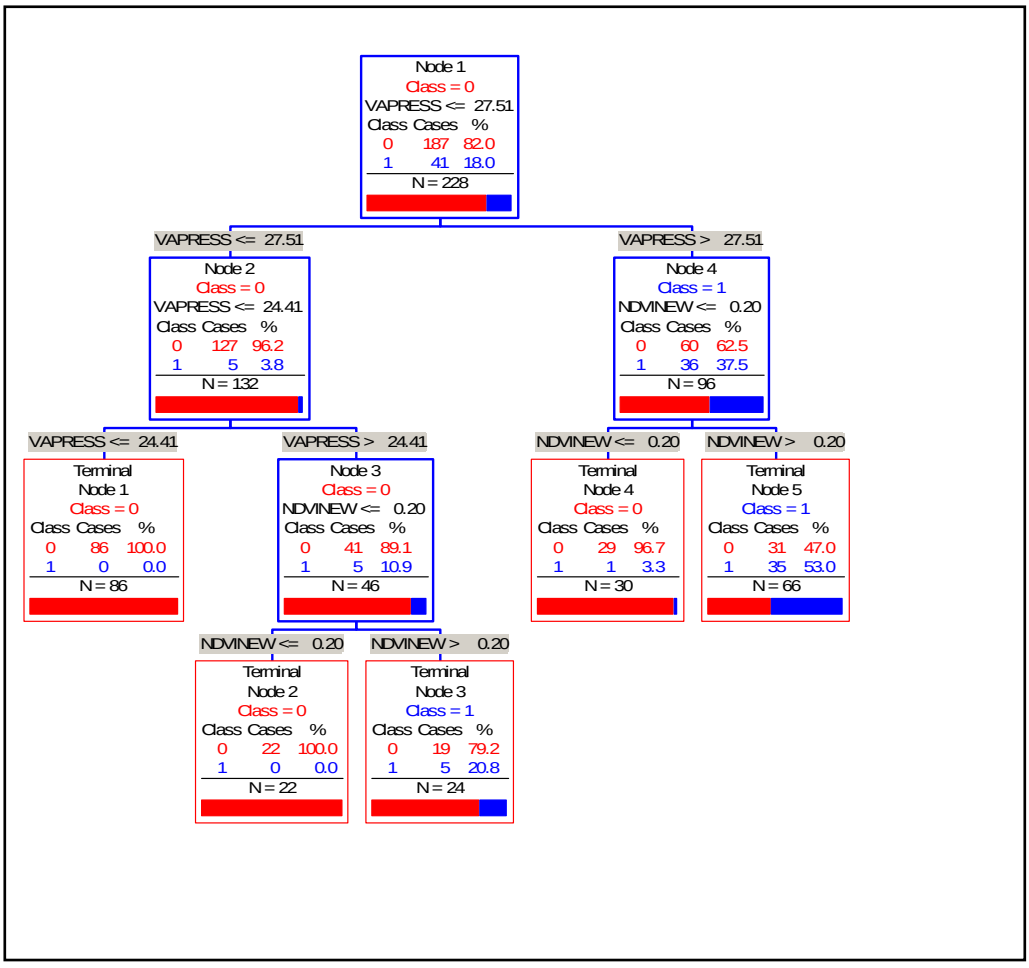


Figure 10.09: CART analysis for *T. strangmannii*

10.4 Discussion

The genus *Tabanus* as a whole is of most interest to the development of a risk model for surra, since it is likely that all members of the genus are vectors of the disease (Luckins, 1998). The CART analysis indicates that the highest prevalence of the genus occurs where vapour pressure exceeds 22.6 hPa, and when vapour pressure was above this level, areas north of latitude 12.3˚S (above Weipa) were also associated with higher prevalence. This means *Tabanus* is most often present during the months from October to June in latitudes north of Weipa. Next highest prevalence occurs south of Weipa, during the months of January, February and March (when vapour pressure exceeds 30 hPa). This distribution is consistent with the results of the GIS mapping of *Tabanus* spatial abundance patterns (Chapter Nine).

Vapour pressure was often associated with greater prevalence of *Tabanus* and individual species in this analysis. The association between vapour pressure and rainfall indicates that the presence of adult tabanids is associated with increased moisture in the environment. The aquatic breeding environment favoured by immature stages of Tabanidae has been elaborated on previously: increased prevalence at times when oviposition sites are available to the adult females has obvious adaptive value to members of this genus.

The variables that were significantly related to higher *Tabanus* prevalence were vapour pressure, latitude and evaporation. However, each of the six individual *Tabanus* species was associated with a different combination of variables. *Tabanus ceylonicus* was associated with latitude and minimum temperature, while *T. pallipennis* was associated with latitude and solar radiation. *Tabanus dorsobimaculatus* was associated with vapour pressure and minimum temperature, while *T. notatus* was associated with vapour pressure and maximum temperature. *T. innotabilis* was associated with maximum temperature, NDVI and rain while *T. strangmannii* was associated with vapour pressure and NDVI.

Latitude is an important explanatory variable for the genus *Tabanus*, as well as *T. ceylonicus* and *T. pallipennis*. The reasons for this are not currently clear, and further studies are needed to clarify why this is the case.

Variables that were included in the analysis that were not associated with either the genus *Tabanus* or the six individual species included: year, month, relative humidity at maximum temperature, relative humidity at minimum temperature, evaporation SP, broadscale vegetation, longitude and presence of cattle. It is interesting to note that there were no broadscale vegetation or month effects, after the effect of weather variables had been included. NDVI or "vegetation greenness" is produced from a combination of previous rainfall and temperature. This indicates that almost all the prevalence splits, other than latitude, are due to temperature and moisture effects.

This CART analysis could be used in a predictive GIS model that is simpler and more generic than the model used in Chapter Nine. This may enable mapping that is less time period specific i.e the use of average annual climate variables within a GIS based on this CART analysis would predict the average annual spatial risk, on a monthly basis.

CHAPTER ELEVEN
GENERAL DISCUSSION

This thesis aimed to characterize the spatial and temporal patterns of tabanid activity in northern Queensland and thereby to enable an evaluation of the spatial and temporal patterns of transmission risk of surra, if an incursion should occur in Australia. Information on opportunistic collections of different tabanid species in Australia was compiled by Mackerras (1956; 1970) but no previous longitudinal surveys linking environmental conditions and tabanid presence and abundance had been performed.

11.1 Overall patterns of variation in tabanid activity

Tabanid activity showed substantial and systematic variation in both space and time, related to climatic patterns.

Spatial variation in tabanid activity was evaluated by monitoring trap-caught tabanids from 12 sites in northern Queensland, over 21 months (two wet and one dry season). Relationships were identified between the number of individuals of different species caught and the climatic attributes of each site. These relationships enabled prediction of spatial patterns of tabanid abundance and diversity across the broader region of northern Queensland. In general, tabanids were more abundant and more diverse in wetter and more northern locations.

Temporal variation in tabanid activity was evaluated at four different scales: between years (annual), between months within years (seasonal), between days (daily) and at different times of day (three-hourly).

On the annual scale, the longitudinal survey in northern Queensland enabled year to year variation to be quantified and relationships with meteorological variables from the year previous to tabanid collection to be identified. In general, cloudier, wetter years resulted in higher tabanid abundance the following year.

On the seasonal scale, the study established that the highest levels of tabanid activity overall occurred in the wet season from December to March, which was consistent with experience in south-east Queensland (Spratt, 1974a) and the African tropics (Okiwelu, 1975; Lehane, 2005). However, different species exhibited different seasonal patterns, so that the wetter and more tropical locations with more species also had more prolonged periods of tabanid activity.

On the daily and three-hourly scales tabanid activity was related to short-term fluctuations in meteorological variables and the patterns found were species-specific. Both daily and three-hourly variations in activity patterns occurred over a short time period and were probably caused primarily by variation in host-seeking-behaviour rather than in tabanid abundance.

Therefore climatic conditions were important predictors of three-hourly, daily, seasonal and yearly activity and abundance patterns in disparate areas of northern Queensland. This was a major finding of the study and is discussed in more detail below.

11.2 Components of the study

11.2.1 Measuring activity via trapping

At the start of this study it was important to establish efficient trapping methods that were appropriate for north Queensland species of tabanids and were robust enough to remain functional throughout the experimental period. Previous studies comparing the efficiency of different trap types and attractants for local species of tabanids had not previously been reported in Australia, and differences between species had been noted in other countries (Roberts, 1972; 1976). Comparisons of the Nzi and canopy traps with different attractant types indicated that the Nzi trap with attractants CO_2 and octenol was the combination of choice for capturing high numbers of tabanids with the greatest range of species. This combination was limited to areas where transport of CO_2 and monitoring

of attractant emissions was logistically feasible. It was used for intensive tabanid studies in Townsville in the daily activity study which yielded live tabanids for use in the tabanid feeding behaviour study. For remote region collections, such as for the 21-month survey in Cape York Peninsula and Townsville, where sampling periods were as long as 28 days, the Nzi trap without any attractant was the most useful and practical option. These results were consistent with the results of trap comparisons conducted by Mihok and co-workers (2006) who found that Nzi traps caught five times more tabanids, on average, than canopy traps. The attractant comparisons also supported previous findings that reported the combination of octenol and CO_2 was the best combination, followed by CO_2 alone, followed by octenol alone and then no attractant (French and Kline, 1989; Hayes *et al.*, 1993).

11.2.2 Temporal variation: daily and three-hourly variation in tabanid activity

Meteorological influences on tabanid daily activity patterns had not been previously studied in Australia and there was previously no firm data on activity patterns of different species of Australian tabanids. In this study, results of the daily activity study revealed that the three common species of tabanid in Townsville, *T. pallipennis*, *T. townsvilli* and *P. silvester* exhibited variation in their patterns of activity throughout the day and between days. These differences in activity patterns within and between days corresponded to relationships with different weather variables. These results are in keeping with those observed by Burnett and Hayes (1974) and Dale and Axtell (1975) who similarly found species differences in the within-day variation in activity patterns, that were related to different responses to meteorological variables. The results of this study suggested that the timing of feeding behaviour studies should accommodate the peak activity patterns of the tabanid species involved, since it is likely that these within day activity patterns are closely related to peak biting activity on hosts (Spratt, 1974a; Hollander and Wright, 1980). In addition, this study provides information relevant to the attenuation of risk of transmission in an endemic disease situation. For example, stabling horses during the late afternoon when biting activity is highest may help reduce the possibility of infection in such a situation.

In the daily activity study, there was no effect of either time of day or day number on the proportion of *T. townsvilli* or *T. pallipennis* with visible developing eggs. This indicates that there was no significant difference in the reproductive status of these species within or between days, so that feeding behaviour experiments are unlikely to be biased by differences in reproductive status throughout the sampling period, for a particular tabanid species. However, between species, there were differences in the proportion of tabanids with visible developing eggs, which may have been due to differences in the time since emergence from the pupal state, or variation in the autogenous ability of species. Also, there was no evidence from the body size data that different classes of flies belonging to the four main tabanid species in Townsville, were active at different times of day. *Tabanus townsvilli* size did vary significantly throughout the study period, indicating that there was some turnover in the population of this species. It is difficult to judge the effect that this population turnover, and the age structure in general, might have had on the behaviour of tabanids used in studies and whether it could introduce any potential bias if not taken into account. However it is worth keeping in mind for future studies on feeding behaviour, that changes in age structure may occur during the course of the sampling period, and that this may affect the feeding behaviour of tabanids.

The tabanid feeding behaviour study had low landing and feeding percentages. The lack of feeding was attributed to problems with the methodology of observation which used trap-caught tabanids released into a screened enclosure with a host animal, monitored by a nearby observer. It is recommended that future studies on landing and feeding behaviour utilise tabanids that are naturally attracted to host animals in a field environment and employ the use of either electric nets to intercept flies (Vale, 1974) or observations made by a person either in a ventilated pit or on a tower (Schofield and Torr, 2002), to remove the effect that the observer may have on feeding behaviour (Hargrove, 1976). Information on the position and duration of tabanid feeding on host animals will help to clarify where application of insecticide should be focussed in order to ensure maximum efficacy, if control measures are required (Torr and Hargrove, 1998). In addition, feeding persistence, or likelihood of host switching, should give an

indication as to the potential for disease transmission for that species. Increased rates of host switching increase the likelihood that pathogen transmission will occur, although literature on tabanids in Indonesia indicates it is likely that all members of the genus *Tabanus* are likely to share a predilection for high rates of host switching, especially in circumstances where the distance between hosts is not excessive (Dieleman, 1986; Foil, 1989).

11.2.3 Spatial and temporal variation tabanid activity across North Queensland

The longitudinal survey of tabanids in Cape York Peninsula and Townsville captured a total of 38 species of Tabanidae, which included 15 species of *Tabanus*. One of the species caught in substantial numbers, *T. ceylonicus*, is a known transmitter of surra in endemic countries (Luckins, 1998). These results indicate that species likely to transmit *T. evansi* are present in reasonable numbers in an area of possible incursion. It was also evident that different species were most abundant at different times of the wet season, and that the wet season was the time of highest overall abundance. However, unexpectedly two species, *T. ceylonicus* and *T. pallipennis,* had peaks of activity during the dry season as well. Species diversity was related to long-term annual average climate variables, particularly those associated with increased moisture in the environment, such as rainfall and humidity. Abundance was not related to long-term weather, but was related to weather conditions from the previous year.

The months of the year during which *Tabanus* activity occurred indicated the temporal periods of likely transmission risk. These periods of activity at a site were heavily influenced by which species of tabanid predominated, as each species typically had its flight period at certain times of the year depending on the weather. A wider variety of *Tabanus* species at a site increased the likelihood that there was activity through more months of the year, because of the additive effects of the overlapping flight periods of different species. If *T. ceylonicus* or *T. pallipennis* was present in significant numbers, it was likely that dry season activity would occur. Bamaga and Mapoon both had *Tabanus* activity for an average of 11 months of the year. Weipa, Rutland Plains and Lakefield

had *Tabanus* activity for 8 – 10 months of the year, indicating that transmission of surra could occur during most of the year in these locations.

Annual abundance of tabanids in northern Queensland, was related to weather variables, mostly from the previous year. This relationship was extrapolated over the north of the Queensland region to produce GIS maps of abundance of the six most common species of *Tabanus* and the genus *Tabanus* overall. These maps represent a hypothesis arising from this study about regional patterns of abundance. That is, they represent the likely abundance if the species was present and all other explanatory variables remained constant, rather than distribution maps, as climatic relationships only explained part of the variation in tabanid abundance. More survey work, together with examination of historical records, would be required to produce reliable distribution maps.

Similarly, the CART analysis does not provide confident predictions of species distributions, however it gives an indication of factors associated with increased likelihood of tabanid presence month by month, and its results are generally consistent with the study of annual variation. The CART analysis also suggests greatest prevalence of the genus *Tabanus* in the northern part of Cape York Peninsula, above Weipa, since these areas have favourable environmental combinations for more prolonged periods.

11.2.4 Variation in tabanid size

Individuals of most species of tabanid varied in size among the locations where they were captured. In general, larger individuals were captured at wetter and more northerly sites. These individuals are likely to be more fecund and possibly more effective disease vectors.

11.3 Variation in the risk of disease transmission

The outputs described in 11.2 above give an indication of when and where species of *Tabanus* occur in abundance and allow an assessment of the temporal and spatial risk of disease transmission associated with their presence.

The key findings of this thesis are that high rainfall areas in the most northern part of Cape York pose a substantially higher risk of incursion and establishment of surra than other areas in northern Queensland and should be the particular focus of surveillance activities for this disease. Northern sample sites, particularly Bamaga, have high abundance of a diverse range of *Tabanus* species, so there is high vector intensity in this region and *Tabanus* activity is present for 11 months of the year on average, which indicates a relatively higher risk of temporal and spatial risk of disease transmission. In addition, tabanids in this area are relatively large, and would be expected to have large mouthparts capable of transmitting a substantial amount of blood, increasing the likelihood of infectious pathogen transfer. All of these factors, coupled with the geographic proximity of this area to Indonesia and Papua New Guinea, mean that Bamaga and adjacent areas in the very north of Cape York present the greatest risk of incursion and establishment of surra in northern Queensland.

However, coastal areas with high rates of landing of illegal fishing vessels that may contain surra-infected animals are also areas of possible geographic risk of incursion. If these landing sites co-incide with areas and times of high vector intensity, there may be substantial associated risk of incursion and establishment of surra. These results indicate that surveillance activities should be focussed on the most northern part of Cape York, and that the livestock exclusion zone already in place in northern Cape York should help prevent establishment of surra in livestock in this region (Anon., 2007a). However, the ubiquity of macropods and presence of other possible host animals such as dogs and pigs means that establishment of surra could still occur there. While Northern Cape York had previously been identified as high risk, because of its proximity to PNG, this is the first report to demonstrate that high relative vector abundance and activity for much of the

year compounds this risk and increases the importance of current surveillance measures. It also provides a rational basis for further focussing of resources for disease detection in this area, particularly if pre-border surveillance for surra indicates increased or novel sero-prevalence in adjacent countries.

11.4 Limitations of the study

The most important limitation of this study is that measurements made throughout are of tabanids caught in traps, rather than a direct measurement of tabanids biting animals, or of total abundance. The number of tabanids caught in a trap will vary according to daily activity patterns, abundance and "trappability" i.e. the qualities of tabanids that cause them to be attracted to a trap and caught in it, which may or may not be a true reflection of the tabanid population that bites animals. While existing information supports the assumption that trap-caught tabanids do in fact reflect the species composition and biting rate on nearby animals (Spratt, 1974a; Hollander and Wright, 1980), further experimental work to confirm this is required for north Queensland species of tabanids. Information on the relationship between trap catch and biting rate, in addition to correlations between biting rate and disease incidence, would allow the likelihood of disease transmission to be calibrated to trap catch results.

A second important limitation of this study was that only one trap was set up at each site. We do not know the extent to which tabanids exhibit spatial heterogeneity (both in terms of numbers and species composition) within a local area. It is likely that micro-habitat does have a significant effect on trap catch and it is important to evaluate and characterise its effects, so that this can be taken into account when modelling fine-scale vector distribution.

11.5 Future directions

The CART analysis for *Tabanus* could be used to produce a generic GIS spatial and temporal risk model, on a monthly basis, using average annual weather variables,

providing all the variables are available in raster format. This GIS map could then be translated into a predictive risk map using actual weather data to produce predictive abundance maps by month.

As mentioned previously, it is important to evaluate the effect of micro-habitat on trap catch of tabanids. This might enable local effects of factors such as vegetation to be taken into account when transposing trap catch results into spatial abundance models. In addition, correlating trap catch results with the biting rate of species on nearby host animals would provide valuable information useful to translating trap catch results into likelihood of disease transmission. Further feeding behaviour studies would be worthwhile to evaluate the effect these behaviours might have on the potential for successful mechanical transmission of pathogens.

The combination of feeding behaviour and the amount of blood transferred between hosts (which may be a function of mouthpart size) are likely to be important determinants of vectorial capacity. While individual mechanistic trials would be ideal for proof of vector competence, it is unlikely that studies on all possible vector species could be adequately performed. It would be preferable therefore to determine if there are minimum requirements for pathogen transmission, and to grade the likelihood of vector competence on the basis of these requirements, giving a scale of vector competence, that could be combined with vector intensity to produce a risk model for transmission of surra by vectors. Such methods may help to clarify the potential role that non-*Tabanus* species of Tabanidae might play in the potential for surra transmission. If non-*Tabanus* species were also found to be possible vectors, this might increase the areas and times of possible transmission risk substantially in northern Australia.

Additional information that is required to clarify the relationship between vector numbers and disease incidence, is a longitudinal study of sero-prevalence of surra and corresponding vector intensity in a surra-endemic area. Vector incrimination studies could also be performed in endemic areas i.e. studying the species that occur in highest numbers when high levels of sero-conversion occur; identifying the presence of

T. evansi in these species of tabanids; and use of mechanistic studies to ensure that they are indeed physically capable of transmitting surra to uninfected animals. When vector species are incriminated in the transmission process, they can be studied in further detail to clarify their ecology (particularly the phenology) and identify methods of vector control that are appropriate for that species.

This concept of linking disease epidemiology (surra sero-prevalence) with high numbers of competent vectors biting animals is important to distinguish from merely the presence of tabanids that are highly competent. As Foil discussed in his studies on EIAV transmission: very high numbers of vectors that transmit small amounts of blood can be more important to disease transmission than low numbers of tabanids that transmit a large amount of blood (Foil, 1989).

The use of GIS mapping of annual and spatial abundance of incriminated surra vectors in endemic countries could be implemented when the relationships of tabanid species with weather and other environmental variables has been ascertained in these countries. Establishing direct relationships of disease prevalence with environmental and meteorological variables would result in an even simpler model for producing GIS disease risk maps, although they may be less accurate and flexible. It may be easier and more efficient to access raster- based satellite meteorological and environmental data for developing countries, that may not have reliable or numerous meteorology stations, which are required to develop raster-based meteorology variables for use in spatial modelling. This would enable prioritising of times and places of highest risk of transmission, without the need for extensive disease surveys. This would enable better use of strategic treatment regimes and other management practices that attenuate risk.

In the case of Australia, where surra is currently exotic, the GIS maps of *Tabanus* abundance could be extrapolated over the entire region of northern Australia and combined with layers of density and distribution of possible mammalian host species and areas of high geographical risk to produce a GIS based model of the risk of surra incursion and establishment in northern Australia. This risk model could further be

enhanced by the addition of a state-transition model of disease spread between animals. An animated model on a monthly time period basis would enable a multi-faceted output that would show:

a) where and when the risk of transmission is highest if incursion occurred (allowing establishment)

b) if incursion occurred in a certain geographic area at a given time of year, what the likely rate and extent of disease spread would be.

Addition of information on disease intelligence, especially data on when and where landing of illegal international vessels carrying animals from surra-endemic countries is most likely, would add further value to this modelling exercise.

In addition, determination of the host preferences of tabanid species from Cape York Peninsula would also help provide a basis for evaluating the likely outcome of a surra incursion in Australia. It is unknown at this stage whether much useful information on blood-meals can be gained from trap-caught tabanids, since they are likely to be host-seeking when caught.

This present study has allowed the spatial and temporal patterns of surra transmission risk in Cape York to be characterized, and for some strategies to minimize the likelihood of surra establishment to be identified. Much more work will be needed to establish appropriate response strategies to be put in place should an incursion occur.

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APPENDIX ONE: SPECIES CAUGHT IN TRAP AND ATTRACTANT COMPARISON STUDY

Table A1.01: Complete list of species caught and numbers collected. Includes 2 males (*T. townsvilli* and *Psuedotabanus spp.)* Also includes a distinct but unidentified Tabanus species (*Tabanus sp. A*).

Species	**Total number collected**	**Percent-age of total**	**Number collected in Nzi trap**				**Number collected in canopy trap**			
			Attractant				**Attractant**			
			Nil	**Oct**	**CO_2**	**CO_2+ oct**	**Nil**	**Oct**	**CO_2**	**CO_2+ oct**
Tabanus townsvilli Ricardo	382	21.4	16	53	107	169 (1 male)	0	4	16	17
Tabanus pallipennis Macquart	348	19.6	4	30	51	115	2	36	44	66
Tabanus strangmannii Ricardo	268	15.1	24	48	43	119	0	5	17	12
Tabanus dorsobimaculatus Macquart	92	5.2	1	5	21	31	0	6	6	22
Tabanus notatus Ricardo	38	2.1	1	6	6	24	0	0	0	1
Tabanus parvicallosus Ricardo	32	1.8	0	0	5	10	0	1	10	6
Tabanus obscurilineatus Taylor	18	1.0	0	0	3	0	0	2	6	7
Tabanus Unknown A	3	0.2	0	0	2	1	0	0	0	0
Tabanus concolor Walker	2	0.1	0	0	0	2	0	0	0	0
Tabanus particaecus Hardy	1	0.1	0	0	0	1	0	0	0	0
Dasybasis clavicallosa group	269	15.1	10	43	73	115	0	0	7	21
Dasybasis trilineatus sp.	1	0.1	0	0	0	1	0	0	0	0
Cydistomyia ?brevior (Walker)	2	0.1	0	1	0	1	0	0	0	0
Cydistomyia musgravii	2	0.1	0	0	1	1	0	0	0	0
Cydistomyia sp. indeterminate*	1	0.1	0	0	0	1	0	0	0	0
Psuedotabanus silvester (Bergroth)	275	15.4	40	95	41	91	0	3	2	3
Psuedotabanus distinctus Ricardo	24	1.3	4	2	4	12	0	0	0	2
Lilaea fuliginosa (Taylor)	16	0.9	2	2	5	6	0	0	1	0
Mesomyia spp. (male)	1	0.1								
Unidentified	5	0.3	1	1	0	2	0	0	0	1
Total number caught	**1780**									

APPENDIX TWO: DAILY ACTIVITY STUDY ANALYSES

Table A2.01: Overall numbers and percentages of each species in each collection period.

Species	**Collection period**				**Total**
	1	**2**	**3**	**4**	
T. pallipennis					
Count	29	46	99	293	467
Expected count	53.0	106.4	126.5	181.2	467.0
Percentage	6.2%	9.9%	21.2%	62.7%	100%
T. townsvilli					
Count	46	154	150	22	372
Expected count	42.2	84.7	100.7	144.4	372.0
Percentage	12.4%	41.4%	40.3%	5.9%	100.0%
P. silvester					
Count	32	19	23	80	154
Expected count	17.5	35.1	41.7	59.8	154.0
Percentage	20.8%	12.3%	14.9%	51.9%	100.0%
T. dorsobimaculatus					
Count	9	14	5	2	30
Expected count	3.4	6.8	8.1	11.6	30.0
Percentage	30.0%	46.7%	16.7%	6.7%	100.0%
Total					
Count	116	233	277	397	1023
Expected count	116.0	233.0	277.0	397.0	1023.0
Percentage	11.3%	22.8%	27.1%	38.8%	100.0%

Table A2.02: Activity in different collection periods on different days for *T. pallipennis*.

	Collection period				
Day number	**1**	**2**	**3**	**4**	**Total**
1	5 9.3%	2 3.7%	11 20.4%	36 66.7%	54 100%
2	1 2.0%	2 3.9%	15 29.4%	33 64.7%	51 100%
3	3 6.4%	7 14.9%	5 10.6%	32 68.1%	47 100%
4	2 7.4%	3 11.1%	5 18.5%	17 63.0%	27 100%
7	2 6.5%	3 9.7%	14 45.2%	12 38.7%	31 100%
9	2 6.5%	7 22.6%	7 22.6%	15 48.4%	31 100%
13	0 0%	0 0%	4 14.3%	24 85.7%	28 100%
15	3 7.5%	1 2.5%	7 17.5%	29 72.5%	40 100%
Total	18 5.8%	25 8.1%	68 22.0%	198 64.1%	309 100%

Table A2.03: Activity in different collection periods on different days for *P. silvester*.

	Collection period				
Day number	**1**	**2**	**3**	**4**	**Total**
1	18 42.9%	8 19.0%	4 9.5%	12 28.6%	42 100%
7	12 14.8%	8 9.9%	12 14.8%	49 60.5%	81 100%
8	2 13.3%	2 13.3%	3 20.0%	8 53.3%	15 100%
Total	32 23.2%	18 13.0%	19 13.8%	69 50.0%	138 100%

Table A2.04: Proportion of daily catch of *T. townsvilli* in each collection period for different group sizes

Collection period	**1**	**2**	**3**	**4**	**Total**
Group 1 (<10)	16 (22.9%)	27 (38.6%)	22 (31.4%)	5 (7.1%)	70
Group 2 (11-30)	18 (10.1%)	65 (36.3%)	83 (46.4%)	13 (7.3%)	179
Group 3 (>31)	12 (9.8%)	62 (50.4%)	45 (36.6%)	4 (3.3%)	123
Total	46	154	150	22	372

Table A2.05: Proportion of daily catch of *T. pallipennis* in each collection period for different group sizes

Collection period	**1**	**2**	**3**	**4**	**Total**
Group 1 (<10)	6 (6.4%)	9 (9.6%)	19 (20.2%)	60 (63.8%)	94
Group 2 (11-30)	14 (6.3%)	26 (11.8%)	49 (22.2%)	132 (59.7%)	221
Group 3 (>31)	9 (5.9%)	11 (7.2%)	31 (20.4%)	101 (66.4%)	152
Total	29	46	99	293	467

Table A2.06: Proportion of daily catch of *P. silvester* in each collection period for different group sizes

Collection period	**1**	**2**	**3**	**4**	**Total**
Group 1 (≤ 30)	2 (6.5%)	3 (9.7%)	7 (22.6%)	19 (61.3)	31
Group 2 (> 31)	30 (24.4%)	16 (13.0%)	16 (13.0%)	61 (49.6%)	123
Total	32	19	23	80	154

Table A2.07: Proportion of each species with visible developing eggs

Species	**Ovarian activity**		**Total**
	No (inactive)	**Yes (active)**	
P. silvester	37 (37.4%)	62 (62.6%)	99 (100%)
T. dorsobimaculatus	7 (63.6%)	4 (36.4%)	11 (100%)
T. pallipennis	110 (53.9%)	94 46.1%)	204 (100%)
T. strangmannii	13 (72.2%)	5 (27.8%)	18 (100%)
T. townsvilli	115 (79.3%)	30 (20.7%)	145 (100%)
Total	282 (59.1%)	195 (40.9%)	477 (100%)

Table A2.08: Ovarian activity of *P. silvester* by day

Day number	**Ovarian activity**		**Total**
	No	**Yes**	
1	20 47.6%	22 52.4%	42 100%
5	0	3 100%	3 100%
6	4 57.1%	3 42.9%	7 100%
7	8 25%	24 75%	32 100%
8	5 33.3%	10 66.7%	15 100%
Total	37 37.4%	62 62.6%	99 100%

Table A2.09: Variation in daily mean wing size by day

Day number	*T. townsvilli*	*T. pallipennis*	*P. silvester*
	Mean +/- S.D.	**Mean +/- S.D.**	**Mean +/- S.D.**
1	9.1 +/- 0.2 (n=4)	7.5 +/- 0.6 (n= 53)	5.9 +/- 0.3 (n= 42)
2	8.8 +/- 0.5 (n=40)	7.6 +/- 0.5 (n=30)	-
3	8.6 +/- 0.6 (n=29)	7.4 +/- 0.6 (n=33)	-
4	8.4 (n=1)	7.5 +/- 0.4 (n=19)	-
5	8.2 +/- 0.7 (n=4)	7.3 +/- 0.5 (n=6)	6.2 +/- 0.1 (n=3)
6	8.2 +/- 0.1 (n=5)	7.2 +/- 0.5 (n=15)	5.8 +/- 0.2 (n= 6)
7	8.7 +/- 0.6 (n=26)	7.1 +/- 0.4 (n=15)	5.9 +/- 0.3 (n= 78)
8	8.7 +/- 0.6 (n= 3)	7.5 +/- 0.4 (n= 9)	5.7 +/- 0.3 (n=15)
12	7.7 +/- 0.3 (n= 3)	7.3 +/- 0.3 (n= 10)	-
19	8.6 +/- 0.6 (n= 24)	7.3 +/- 0.6 (n= 9)	-
20	8.3 +/- 1.1 (n= 2)	7.5 +/- 0.2 (n= 10)	-
Total	8.6 +/- 0.6 (n= 141)	7.4 +/- 0.5 (n= 209)	5.8 +/- 0.3 (n= 144)

Table A2.10: Weather variables given in half hourly increments. Variables derived from the values provided by BOM are indicated with an asterisk (*). Method of calculation used to provide data used in analysis is provided. Collection periods were 3 hourly.

Rain since 9am (mm)	**Rain during collection period (mm and Y/N)***	**Air temperature (˚C)**	**Relative humidity (%)**	**Wind speed (km/hr)**	**Wind direction (degrees true)**	**Wind quadrant***	**Station level pressure (hPa)**
Maximum rainfall in collection period since 9am	Rain during collection period calculated from rain since 9am (in mm) & also categorised as Yes/ No	Average during collection period	Average during collection period	Average during collection period	Average during collection period	Quadrant 1: 0-90° 2: 90-180° 3:180-270° 4:270-360°	Average during collection period

Table A2.11: Daily weather variables. Variables derived from the values provided by BOM are indicated with an asterisk (*).

Weather variable	**Calculation used**
Minimum temperature (°C)	None
Maximum temperature (°C)	None
Rainfall (mm)	None
Rainfall 7 days before *	Sum of rainfall during the 7 days preceding the collection day
Rain 30 days before *	Sum of rainfall during the 30 days preceding the collection day
Evaporation (mm)	None
Sunshine (hours)	None
Direction of maximum wind gust	Not used
Speed of maximum wind gust (km/h)	Not used
Time of maximum wind gust	Not used
9am Temperature (°C)	None
9am relative humidity (%)	None
9am cloud amount (oktas)	Not used
9am wind direction	None
9am wind speed (km/h)	None
9am MSL pressure (hPa)	None
3pm Temperature (°C)	None
3pm relative humidity (%)	None
3pm cloud amount (oktas)	Not used
3pm wind direction	None
3pm wind speed (km/h)	None
3pm MSL pressure (hPa)	None

Table A2.12: Descriptive statistics of weather variables during collection period (n=124).

	Range	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Rain since 9am (mm)	27.40	.00	27.40	1.6435	.40844	4.54817
Air temperature (˚C)	7.70	23.90	31.60	28.0032	.14525	1.61738
Relative humidity (%)	46.80	46.20	93.00	73.0089	.86417	9.62296
Wind speed (km/hr)	28.70	5.00	33.70	18.7476	.54692	6.09025
Wind direction (degrees)	191.00	15.00	206.00	102.8048	3.55017	39.53304
Pressure (mmHg)	8.90	1004.60	1013.50	1010.3508	.19570	2.17926
Wind quadrant	2.00	1.00	3.00	1.6613	.05107	.56865
Rain in collection period	27.20	.00	27.20	1.1274	.37659	4.19348

APPENDIX THREE: FEEDING BEHAVIOUR STUDY ANALYSES

Table A3.01: Summary of all behaviour studies

Date/ Time	**Host**	**Tabanids Released**	**Species Breakdown**	**Time Given To Feed**	**Outcome**
1. 8.30-10.30am 7th March 2006	Heifer (Red Brahman, tag #5123)	48	Released 48 of the following: 24 *T. concolor* 24 *Cydistomyia A* 5 *C. doddi* 1 *T. pallipennis*	2 hours	4 landed, 3 fed (3 CA)
2. 4.35-6.20pm 30th March 2006	Pig (entire male, Large White, ear tag # 02)	34	11 *T. townsvilli* 21 *T. pallipennis* 1 *Dasybasis* 1 *P. silvester*	1 hour 45 mins	17 landed, 2 fed (2TP)
3. 5-6.20pm 4th April 2006	Pig (entire male, Large White, ear tag # 03)	61	27 *T. townsvilli* 30 *T. pallipennis* 1 unidentified 2 *T. dorsobimaculatus* 1 *L. fuliginosa*	1 hour 20 mins	18 landed, 2 fed (2TP)
4. 4.30-5.50pm 5th April 2006	Pig (entire male, Large White, ear tag # 03)	36	19 *T. townsvilli* 13 *T. pallipennis* 2 *T. dorsobimaculatus* 1 *L. fuliginosa* 1 *T. strangmannii*	1 hour 20 mins	17 landed, 6 fed (2TP, 4TT)
5. 3.55-5pm 6th April 2006	Pig (entire male, Large White, ear tag # 03)	45	6 *T. townsvilli* 36 *T. pallipennis* 1 *T. dorsobimaculatus* 2 *L. fuliginosa*	1 hour 5 mins	10 landed, 1 fed (TP)
6. 4.30-5.45pm 11th April 2006	Sheep (wether, ear tag # 6026)	49	32 *T. townsvilli* 11 *T. pallipennis* 3 *T. dorsobimaculatus* 2 *L. fuliginosa* 1 unidentified	1 hour 15 mins	4 landed, 2 fed (LF, TP)
7. 4.20-5.50pm 12th April 2006	Sheep (wether, ear tag # 6026)	68	47 *T. townsvilli* 19 *T. pallipennis* 2 *T. dorsobimaculatus*	1 hour 30 mins	12 landed, 1 fed (TT)

8. 4.30-5.30pm 13th April 2006	Sheep (wether, ear tag # 6026)	68	53 *T. townsvilli* 10 *T. pallipennis* 1 *L. fuliginosa* 4 *T. dorsobimaculatus*	1 hour	12 landed, 0 fed
9. 4.15-5.15pm 19th April 2006	Heifer (Red Brahman, tag #5123)	25	13 *T. townsvilli* 10 *T. pallipennis* 2 *T. dorsobimaculatus*	1 hour	1 landed, 1 fed
10. 4-5.15pm 24th April 2006	Heifer (Red Brahman, tag #5123)	46	7 *T. townsvilli* 38 *T. pallipennis* 1 *T. strangmannii*	1 hour 15 mins	0 landed, 0 fed
11. 3.35-4.35pm 26th April 2006	Heifer (Red Brahman, tag #5123)	27	3 *T. townsvilli* 20 *T. pallipennis* 4 *T. strangmannii*	1 hour	0 landed, 0 fed
12. 10-11am 28th April 2006	Heifer (Red Brahman, tag #5123)	30	7 *T. townsvilli* 18 *T. pallipennis* 3 *T. strangmannii* 2 *T. concolor*	1 hour	0 landed, 0 fed
13. 4.10pm-5.20pm 2nd May 2006	Wallaby (Agile, male, immature)	37	16 *T. townsvilli* 18 *T. pallipennis* 2 *T. strangmannii* 1 *L. fuliginosa*	1 hour 10 mins	3 landed, 2 fed (2TP)
14. 4.40-5.40pm 3rd May 2006	Wallaby (Agile, male, immature)	49	34 *T. townsvilli* 10 *T. pallipennis* 2 *T. dorsobimaculatus* 1 *L. fuliginosa* 2 *T. parvicallosus*	1 hour	0 landed, 0 fed
15. 4.45-5.45pm 4th May 2006	Wallaby (Agile, male, immature)	29	12 *T. townsvilli* 15 *T. pallipennis* 2 *L. fuliginosa*	1 hour	1 landed, 0 fed
Totals	15 studies (5 heifer, 3 wallaby, 3 sheep, 4 pig)	652		18 hours 40 mins	99 landed, 20 fed (11TP, 5TT, 1LF, 3BB)

Table A3.02: Table of tabanid species names and abbreviations used

Species Name	**Abbreviation**
Tabanus concolor	TC
Cydistomyia A (unidentified species of *Cydistomyia*)	CA
Cydistomyia doddi	CD
Tabanus townsvilli	TT
Tabanus pallipennis	TP
Lilaea fuliginosa	LF
Tabanus dorsobimaculatus	TD
Tabanus strangmannii	TS
Pseudotabanus silvester	ps
Dasybasis clavicallosus	dasy
Unidentified species	unid
Tabanus parvicallosus	Tparvi

APPENDIX FOUR: SEASONAL AND SPATIAL VARIATION ANALYSES

Appendix A4.01: Numbers of species of Tabanidae, *Tabanus* species and non-*Tabanus* species, Total Tabanidae and Range of species abundance for 11 sites on Cape York and one at Townsville

Site	**Number of species of Tabanidae**	**Number of *Tabanus* species**	**Number of non-*Tabanus* species**	**Total Tabanidae (Abundance)**	**Range of abundance for species**
Bamaga	18	9	9	438	1- 108
Coen	9	4	5	130	1-92
Cooktown	10	4	6	69	1-55
Heathland National Park	15	5	10	302	1- 149
Karumba	4	3	1	187	1-98
Lakefield National Park	10	5	5	447	1- 123
Lockhart River	15	7	8	525	1-190
Mapoon	14	9	5	121	1-45
"Rutland Plains"	11	6	5	218	1-79
"Stirling"	8	5	3	26	1- 6
Townsville	10	6	4	120	1- 31
Weipa	11	8	3	56	1- 9
Total	39	16	23	2639	1- 628

This table does not count "indeterminate" species in number of species, but does include abundance data grouped under genera. It does include those that are unknown (but distinct) species.
(Data to end May 2006)

Appendix A4.02 Summary of Tabanidae, *Tabanus* spp. and non-*Tabanus* spp. for all sites

Taxon	**Number of species (Species richness)**	**Abundance (Mean ± SE) (By site)**	**Range of abundance (by site)**
Tabanidae	39	213.3 ± 47.7	26-525
Tabanus spp.	16	116.5 ± 24.6	3-256
Non-*Tabanus* spp.	23	80.4 ± 24.8	0-283

Table A4.03: Results of T- test comparing ambient UV readings on days when new and used Laserlite UV measurements were made

t-Test: Two-Sample Assuming Equal Variances		
T- test (ambient UV readings) using average MED/hr:		
	new Laserlite	*old Laserlite*
Mean	1.835	2.039
Variance	0.739	1.062
Observations	4.000	4.000
t Stat	-0.304	
P(T<=t) two-tail	0.771	

Table A4.04: Results of T- test comparing UV readings from the two Safesun® meters

T-Test: Paired Two Sample for Means		
Meter comparison (MED/hr)		
	Meter One	*Meter Two*
Mean	2.21	2.12
Variance	0.18	0.15
Observations	3.00	3.00
t Stat	2.98	
P(T<=t) two-tail	0.10	

APPENDIX FIVE: *TABANUS* PHENOLOGY ANALYSES

Table A5.01 Summary of dominant species and their abundance patterns at each site

Site	Dominant species at site (Tabanus only)	Is peak time consistent b/t seasons?	Does emergence coincide with rain?	Does peak coincide with rain?	Does emergence lag after rain?	How long after?	Dry season activity	Species with dry season activity	Long-term avg rain > 1200mm WET or <1200mm DRY
BA	T. not > T. str, T. cey	Similar Dec-Feb Dec	 No No	 Yes No	 No No	-	 May-Jul Sept-Oct	 T. cey, pal, str T. cey, pal	> 1200mm WET
CN	T. not	No Jan April	 No Yes	 Yes Yes	 Same time as rain	1 mth before rain	No		<1200mm DRY
CK	T. pal = T. str	1 season only Jan	 No	 Yes	 Yes	2 mths	No		>1200mm WET
HE	T. str > T. not	Similar Feb-Mar Jan-Feb	 Yes No-just after	 Yes Yes	 No S2- yes	- 1 mth	Slight Oct-Nov May Oct-Nov	 T. pal, T. str T. cey	>1200mm WET
KA	T. inn	Similar Nov-Jan Dec-Jan	 No slight	 No No	 No No	Peaks 1-2 mths before rain	No		<1200mm DRY
LA	S1: T. not S2: T. not > T. str, T.pal later	Similar Jan-Mar Feb-Apr	 No Yes	 Yes Yes	 No No	1 mth before rain, Same time	 Jun (slight) Sep-Nov 04	 T. pal	>1200mm WET
LR	S1: T. not > T. str S2: T. not	Similar Jan-Feb Jan	 Yes Yes	 Yes Yes	 No No	-	No		>1200mm WET
MA	T. dor > T. not	Yes Jan-Feb Jan-Feb	 No No	(Closely) Yes Yes	 Yes	 3 mths 2 mths	Yes May-Jun Aug, Oct May06	 T. dor >T. cey T. inn T. dor (slight)	>1200mm WET

Site	**Dominant species at site (Tabanus only)**	**Is peak time consistent b/t seasons?**	**Does emergence coincide with rain?**	**Does peak coincide with rain?**	**Does emergence lag after rain?**	**How long after?**	**Dry season activity**	**Species with dry season activity**	**Long-term avg rain > 1200mm WET or <1200mm DRY**
RP	T. not> T. pal	Similar Jan-Feb Feb-Mar	 No Yes (slight)	(Closely) Yes Yes	No- doesn't for T. pal, does for T. not	 S1: 1 mth S2: 2 mths	Yes Oct-Nov May, Nov	 T. pal T. pal	Borderline 1225mm
ST	S1: T. pal= T. str S2: T. str> T. not	No Mar-Apr, May Jan-Feb	 No No	Not consistently, but is during rainy period	Yes	 4 mths 3 mths	Yes May	??	<1200mm DRY
TV	S1: T. pal> T. dor S2: T. dor	Similar Feb-Mar Mar-Apr	 No No	 No (after) Yes	Yes	 2 mths 3 mths	Yes Sept	??	<1200mm DRY
WE	T. inn = T. not	Similar Sep, Dec-Jan, Apr-May Sep-Oct, Jan	 No No	Not consistently 2 smaller peaks in Jan do	Summer peaks do	 2 mths 3 mths	Yes Sept Sept-Oct	 T. inn T. cey>T. inn	>1200mm WET

Table A5.02: Summary of the abundance patterns of each species overall

Species	Site	Peak Time Consistent b/t Seasons?	Peak Time	Peak Coincide With Peak Rain?	Emergence Lag After Rain?	How Long After? (Months)	Dry Season Activity?	Duration Of Activity
T. notatus	BA	Similar	S1: Dec-Feb S2: Dec-Jan	Y N (rain peaked before)	N N	Before rain Same time	N	S1: Dec-Mar S2: Nov-Feb
	CN	No	S1: Dec-Feb S2: Apr to Apr-May	Y Y	N N	Before rain Same time	N	S1: Dec-Mar S2: Dec to Apr-May
	HE	Yes	S1: Jan-Feb S2: Jan-Feb	Y Y	Y Y	2 1	N	S1: Jan-Apr S2: Dec-Mar
	LA	Similar	S1: Dec-Feb S2: Feb-Mar	Y Y	Y N	2 Same time	N	S1: Dec-Apr S2: Dec-Apr
	LR	Yes	S1: Jan-Feb S2: Jan-Feb	Y Y	N N	Same time Same time	N	S1: Jan-Mar S2: Jan-Feb
	MA	Yes	S1: Jan-Feb S2: Jan-Feb	Y Y	Y Y	2 2	N	S1: Jan-Mar S2: Jan-Feb
	RP	Similar	S1: Jan-Feb S2: Feb-Mar	Y Y	Y Y	1 1	N	S1: Dec-Mar S2: Feb-Apr
T. dorsobimacul-atus	BA	1st season only	S1: Feb-Mar	Y	Y	1	Y Sep	S1: Sep, Feb-Mar
	LA	Similar	S1: Jan-Feb S2: Feb-Mar	Y Y	Y Y	3 2	N	S1: Jan-Mar S2: Feb-Mar
	MA	Similar	S1: Jan to Apr-May S2: Jan-Feb	Y Y persisted 1 mth after rain ended	Y Y	2 2	N	S1: Jan-May S2: Dec-May
	RP	Yes	S1: Jan-Feb S2: Jan-Feb	Y Y	Y Y	2 2	N	S1: Jan-Feb S2: Jan-May
	TV	Similar	S1: Feb-Mar S2: Mar-Apr	N after rain peak Y	Y Y	3 3	N	S1: Feb to Apr-May S2: Mar-May
	WE	Similar	S1: Jan, Apr S2: Jan	Y (Jan) Y	Y Y	4, 7 3	N	S1: Jan, Apr S2: Jan

Species	Site	Peak Time Consistent Between Seasons?	Peak Time	Peak/ Emergence Coincide With Rain?	Emergence Lag After Rain?	How Long After? (Months)	Dry Season Activity?	Duration of Activity
T. pallipennis	BA	No	S1: Sep, May-Jun S2: Sep, Nov, Mar	N N	Not consistently	-	Y May-Jun Sep	S1: Sep, May-Jun S2: Sep, Nov, Mar
	LA	Similar	S1: Sep, Nov, Feb S2: Jan, Mar-Apr	Y (not early peaks) Y	Not consistently	1, 3 1, 3-4	Y Sep	S1: Sep-Dec, Feb to Apr-May S2: Dec-Feb, Mar to Apr-May
	LR	1st season only	S1: Sep, Jan	Y (in Jan)	Not consistently	1 (in Jan)	Y Sep	S1: Sep, Jan-Feb
	RP	No	S1: Oct-Nov, May S2: Nov, Mar	N N	Not consistently	Early peaks in Oct-Nov occur before rain	Y Apr-May Jun	S1: Sep-Jan, Apr-May to Jun S2:Oct-Jan, Mar-Apr
	TV	1st season only	S1: Feb-Mar	N	Y	2	N	S1: Feb-Apr
T. ceylonicus	BA	Yes	S1: Sep, Dec, May S2: Sep, Dec, May	N Dec peak does	Not consistently	-	Y Sep, May-Jun Sep-Oct, May	S1: Sep, Dec-Feb, Apr-May to Jul S2: Sep-Oct, Dec-Jan, Apr-May
	HE	No	S1: May S2: Dec	N N	Not consistently	7 1	Y May-Jun	S1: May-Jun S2: Dec
	LR	No	S1: Jan S2: Nov	Y N	Not consistently	1 -	N	S1: Jan S2: Nov
	MA	2nd season only	S2: Jun, Aug	N	No	-	Y Jun, Aug	S1: Jun S2: Aug
	WE	Yes	S1: Sep S2: Sep	No	No	-	Y Sep Sep	S1: Sep S2: Sep-Oct

Species	Site	Peak Time Consistent Between Seasons?	Peak Time	Peak/ Emergence Coincide With Rain?	Emergence Lag After Rain?	How Long After?	Dry Season Activity?	Duration of activity
T. innotabilis	KA	Yes	S1: Dec S2: Dec	Before rain peak (both seasons)	N	Before rain Same time	N	S1: Oct, Dec-Feb S2: Nov-Mar
	WE	Similar	S1: Sep, Mar, Apr-May S2: Sep	No (does in Mar)	Not consistently	5 months -	Y Sep Sep	S1: Sep, Mar, Apr-May S2: Sep
T. strangmannii	BA	No	S1: Feb-Mar S2: Apr-May	Yes (not May) Yes	Y Y	2 3	Y May	S1: Dec-Apr, Apr-May to Jun S2: Feb-May
	HE	Similar	S1: Feb to Apr S2: Feb-Mar	Yes Yes	Y Y	2 2	N	S1: Feb-May S2: Feb-May
	LA	Similar	S1: Feb-Mar S2: Mar-Apr	Yes Yes	Y Y	3 4	N	S1: Feb-Apr S2: Mar-May
	LR	Yes	S1: Feb S2: Feb (small)	Yes Yes	Y Y	2 2	N	S1: Jan-Mar S2: Feb
	ST	Similar	S1: Feb S2: Jan- Feb	Yes Yes	Y Y	4 3	N	S1: Feb S2: Dec-Mar

APPENDIX SIX: ANNUAL ABUNDANCE AND GIS PREDICTIVE MAPPING ANALYSES

Table A6.01: Results of linear regression for species diversity

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.758(a)	.574	.532	2.57850

a Predictors: (Constant), rainttl

ANOVA(b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	89.763	1	89.763	13.501	.004(a)
	Residual	66.487	10	6.649		
	Total	156.250	11			

a Predictors: (Constant), rainttl
b Dependent Variable: diversity

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.274	2.554		.890	.394
	rainttl	.006	.002	.758	3.674	.004

a Dependent Variable: diversity

Equation: Total number of species = 2.274 + 0.006 x total annual rainfall (interpolated- for period averaged from 1990-2005).

Table A6.02: Variables used for linear regression analysis of yearly tabanid data (same year and previous year for each variable). "Drill" data is interpolated weather data.

Variable	Units	Source
Vegetation	None	Environmental Protection Agency - as GIS shapefile. Site information extracted by overlaying site information
Temperature - maximum	˚C	BOM drill data - daily values averaged over "years"
Temperature - minimum	˚C	BOM drill data - daily values averaged over "years"
(Tmax + Tmin)/2	˚C	BOM drill data - daily values averaged over "years"
Rain- monthly	mm	BOM drill data - daily values averaged over "years"
Log 10 (Rain- monthly)	mm	BOM drill data - daily values averaged over "seasons"
Average annual rain	mm	BOM drill data - daily values averaged over "years"
Log 10 (average annual rain)	mm	BOM drill data - daily values averaged over "years"
Evaporation	mm	BOM drill data - daily values averaged over "years"
Solar radiation (ground measurement)	MJ/m^2	BOM drill data - daily values averaged over "years"
Solar radiation (satellite measurement)	MJ/m^2	BOM - Grid obtained from BOM had already been averaged over "seasons" Site data extracted by overlaying site information.
Vapour pressure	hPa	BOM drill data - daily values averaged over "years"
RH at Tmax	%	"BOM drill data - daily values averaged over "years"
RH at Tmin	%	BOM drill data - daily values averaged over "years"
(RH at Tmax + RH at Tmin)/ 2	%	BOM drill data - daily values averaged over "years"
NDVI	none	NOAA AVHRR 1km resolution (from CSIRO) - monthly composite grids of mean maximum values. Site values extracted by overlaying site information. NDVI values then averaged over "years"

Table A6.03: Description of GIS input files

Weather/ environmental element	**Resolution (grid cell size)**	**File type/ format**	**Source**
Vegetation-broadscale	1: 250 000 (polygons)	ArcInfo shapefile, GDA 94	Qld Herbarium, EPA
NDVI	0.01 degrees (1km approx.)	32 bit floating point format, WGS 84	CSIRO
Solar radiation (satellite measurements)	0.05 degrees (5km approx.)	ArcInfo grid interchange (e00), GDA 94	BOM
Rainfall (longterm)	0.025 deg (2.5 km approx.)	ArcInfo grid interchange (e00), GDA 94	BOM
Rainfall (nine monthly)	0.25 degrees (25km approx.)	ArcInfo grid interchange (e00), GDA 94	BOM
Minimum temperature	0.25 degrees (25km approx.)	ArcInfo grid interchange (e00), GDA 94	BOM

Table A6.04: Annual weather data averages for years of the study and long-term weather averages (highlighted box) for each sample site (Source: BOM).

	Start Day for	Mean	Mean	Avg temp	Total	Total	Mean	Mean	Mean	Mean	Avg RH	Total
	Monthly Mean	T.Max	T.Min		Rain	Evap	Radn	VP	RHmaxT	RHminT		Evap_Sp
TSV	June 03- May04	30.20	20.42	25.31	**68.91**	193.77	20.24	21.79	50.03	86.58	68.31	193.77
					826.90							
	June04-May05	29.95	19.34	24.65	39.34	194.82	20.75	20.90	48.25	87.65	67.95	194.82
					472.10							
	June05-May06	29.37	21.01	25.19	92.15	188.82	19.59	22.83	55.08	88.91	71.99	188.82
					1105.80							
	TSV-LT	29.4	19.8	24.6	991.5	2264.0	20.1	21.6	51.8	88.6	70.2	2264.0
STG	June03-May04	34.05	21.96		**62.69**	216.85	21.26	20.89	39.03	76.55		216.85
					752.30							
	June04-May05	33.71	20.97	27.34	55.61	216.28	21.75	20.52	38.47	78.54	58.51	216.28
					667.30							
	June05-May06	33.57	22.31	27.94	89.20	201.53	20.16	22.83	43.93	82.33	63.13	201.53
					1070.40							
	STG-LT	33.6	21.4	27.5	850.8	2489.7	21.3	21.3	40.4	79.6	60.0	2489.7
RPL	Jun03-May04	33.9	21.6	27.7	81.7	201.1	19.3	23.1	43.8	86.8	65.3	201.1
					980.5							
	Jun04-May05	33.46	20.30	26.88	67.39	199.62	19.83	22.12	42.68	88.15	65.41	199.62
					808.70							
	Jun05-May06	33.59	21.93	27.76	141.25	186.32	17.80	24.29	47.08	89.51	68.29	186.32
					1695.00							
	RPL-LT	33.3	21.1	27.2	1225.5	2400.9	20.5	23.2	45.5	89.0	67.2	2400.9
MPN	Jun03-May04	32.0	23.0	27.5	166.3	188.4	18.9	26.6	56.2	93.1	74.7	188.4
					1996.1							
	Jun04-May05	31.76	22.21	26.99	119.89	180.90	19.63	25.84	55.14	93.76	74.45	180.90
					1438.70							
	Jun05-May06	31.77	23.22	27.49	169.10	173.62	17.88	27.25	58.39	93.57	75.98	173.62
					2029.20							
	MPN-LT	31.6	22.7	27.2	1797.3	2318.3	19.1	26.8	57.7	94.9	76.3	2318.3
LHR	Jun03-May04	29.9	22.0	26.0	154.9	184.2	17.5	26.2	62.3	94.6	78.4	184.2
					1858.6							
	Juno4-May05	29.56	21.03	25.30	153.86	178.70	18.98	25.30	60.78	94.80	77.79	178.70
					1846.30							
	Jun05-May06	29.68	22.35	26.02	192.93	169.03	17.12	26.85	64.26	94.33	79.29	169.03
					2315.20							
	LHR-LT	29.5	21.5	25.5	2020.7	2215.3	19.3	26.4	63.9	96.0	79.9	2215.3
LFD	Jun03-May04	32.2	20.9	26.6	82.8	177.5	18.7	24.4	50.9	94.6	72.7	177.5
					993.4							
	Jun04-May05	31.68	19.80	25.74	48.93	176.65	19.37	24.02	51.03	96.94	73.98	176.65
					587.10							

	Jun05-May06	32.04	21.25	26.64	129.55	175.38	17.87	26.22	55.11	97.66	76.38	175.38
					1554.60							
	LFD-LT	31.6	20.6	26.1	994.5	2108.9	19.8	24.8	53.1	96.2	74.6	2108.9
HLA	Jun03-May04	30.7	22.9	26.8	119.7	183.7	18.4	26.6	60.3	93.5	76.9	183.7
					1436.7							
	Jun04-May05	30.51	22.23	26.37	139.72	176.42	19.42	26.00	59.35	93.67	76.51	176.42
					1676.60							
	Jun05-May06	30.58	23.20	26.89	187.03	169.38	17.65	27.37	62.42	93.51	77.96	169.38
					2244.30							
	HLA-LT	30.4	22.6	26.5	1611.3	2241.8	19.0	26.9	62.1	95.3	78.7	2241.8
CKT	Jun03-May04	29.6	21.7	25.6	188.9	160.4	19.3	24.1	58.2	90.8	74.5	160.4
					2266.3							
	Jun04-May05	29.03	20.78	24.90	95.35	161.22	19.90	23.61	58.25	92.03	75.14	161.22
					1144.20							
	Jun05-May06	29.28	21.82	25.55	220.13	167.70	18.35	25.34	62.12	93.56	77.84	167.70
					2641.60							
	Ckt-LT	29.1	20.9	25.0	1568.7	1855.0	19.7	24.5	60.4	93.9	77.2	1855.0
COE	Jun03-May04	31.2	20.6	25.9	103.3	186.4	18.0	23.9	52.9	94.2	73.6	186.4
					1239.9							
	Jun04-May05	30.69	19.63	25.16	103.58	183.27	17.84	23.77	53.66	95.83	74.74	183.27
					1242.90							
	Jun05-May06	30.89	21.06	25.97	141.54	167.55	15.60	25.90	58.15	96.96	77.56	167.55
					1698.50							
	Coe-LT	30.7	20.4	25.5	1193.2	2209.0	19.4	24.1	54.6	95.1	74.8	2209.0
WPA	Jun03-May04	33.1	22.3	27.7	188.3	191.6	19.2	25.9	51.9	93.8	72.8	191.6
					2259.3							
	Jun04-May05	32.83	21.36	27.10	134.17	184.55	19.57	24.99	50.51	94.83	72.67	184.55
					1610.00							
	Jun05-May05	32.78	22.50	27.64	150.32	177.07	17.91	26.54	54.06	94.56	74.31	177.07
					1803.80							
	Wpa-LT	32.6	22.0	27.3	1973.8	2378.6	19.3	26.0	53.1	95.1	74.1	2378.6
BAM	Jun03-May04	30.6	24.1	27.3	200.6	183.0	18.5	27.3	62.4	90.5	76.5	183.0
					2407.1							
	Jun04-May05	30.38	23.61	26.99	121.17	174.75	19.62	26.99	62.08	90.21	76.14	174.75
					1454.00							
	Jun05-May06	30.50	24.30	27.40	199.02	168.52	17.82	28.24	64.69	91.16	77.93	168.52
					2388.20							
	Bam-LT	30.3	23.8	27.0	1916.7	2223.0	18.9	27.8	64.4	92.6	78.5	2223.0
KAR	Jun03-May04	33.6	22.4	28.0	67.6	218.5	21.5	20.6	39.2	72.8	56.0	218.5
					811.0							
	Jun04-May05	33.37	21.65	27.51	46.99	219.98	22.11	20.30	38.49	74.00	56.25	219.98
					563.90							
	Jun05-May06	33.08	22.69	27.89	85.33	200.15	20.45	22.70	44.71	79.45	62.08	200.15
					1023.90							
	Kar-LT	33.3	21.9	27.6	838.3	2586.0	21.2	21.1	40.6	76.1	58.3	2586.0

APPENDIX SEVEN: VARIATION IN TABANID BODY SIZE AMONG SITES AND FLIGHT SEASON ANALYSES

T. pallipennis:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BA	LA	.349	.2016	.425	-.221	.919
	LR	-.757*	.2639	.046	-1.503	-.010
	RP	-.210	.1979	.825	-.770	.350
	TV	.460	.2424	.332	-.226	1.146
LA	BA	-.349	.2016	.425	-.919	.221
	LR	-1.106*	.2409	.000	-1.787	-.424
	RP	-.559*	.1660	.012	-1.029	-.089
	TV	.111	.2171	.986	-.503	.726
LR	BA	.757*	.2639	.046	.010	1.503
	LA	1.106*	.2409	.000	.424	1.787
	RP	.547	.2379	.163	-.126	1.220
	TV	1.217*	.2760	.001	.436	1.998
RP	BA	.210	.1979	.825	-.350	.770
	LA	.559*	.1660	.012	.089	1.029
	LR	-.547	.2379	.163	-1.220	.126
	TV	.670*	.2138	.023	.065	1.275
TV	BA	-.460	.2424	.332	-1.146	.226
	LA	-.111	.2171	.986	-.726	.503
	LR	-1.217*	.2760	.001	-1.998	-.436
	RP	-.670*	.2138	.023	-1.275	-.065

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset		
		1	2	3
TV	8	6.850		
LA	18	6.961	6.961	
BA	10	7.310	7.310	
RP	20		7.520	7.520
LR	6			8.067
Sig.		.272	.118	.133

Means for groups in homogeneous subsets are displayed
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .261.

a. Uses Harmonic Mean Sample Size = 10.056.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

T. notatus:

Multiple Comparisons

Dependent Variable: wing

Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BA	CO	1.253*	.0923	.000	.979	1.526
	HE	.540*	.1067	.000	.224	.856
	LA	1.284*	.0954	.000	1.001	1.566
	LR	-.052	.0838	.996	-.300	.196
	MA	1.305*	.1754	.000	.786	1.825
	RP	.589*	.0929	.000	.314	.864
CO	BA	-1.253*	.0923	.000	-1.526	-.979
	HE	-.712*	.1043	.000	-1.022	-.403
	LA	.031	.0927	1.000	-.243	.306
	LR	-1.305*	.0807	.000	-1.544	-1.066
	MA	.053	.1739	1.000	-.463	.568
	RP	-.663*	.0902	.000	-.931	-.396
HE	BA	-.540*	.1067	.000	-.856	-.224
	CO	.712*	.1043	.000	.403	1.022
	LA	.744*	.1070	.000	.427	1.061
	LR	-.592*	.0969	.000	-.879	-.305
	MA	.765*	.1820	.001	.226	1.304
	RP	.049	.1049	.999	-.262	.360
LA	BA	-1.284*	.0954	.000	-1.566	-1.001
	CO	-.031	.0927	1.000	-.306	.243
	HE	-.744*	.1070	.000	-1.061	-.427
	LR	-1.336*	.0842	.000	-1.585	-1.086
	MA	.022	.1756	1.000	-.499	.542
	RP	-.695*	.0933	.000	-.971	-.418
LR	BA	.052	.0838	.996	-.196	.300
	CO	1.305*	.0807	.000	1.066	1.544
	HE	.592*	.0969	.000	.305	.879
	LA	1.336*	.0842	.000	1.086	1.585
	MA	1.357*	.1696	.000	.855	1.860
	RP	.641*	.0814	.000	.400	.883
MA	BA	-1.305*	.1754	.000	-1.825	-.786
	CO	-.053	.1739	1.000	-.568	.463
	HE	-.765*	.1820	.001	-1.304	-.226
	LA	-.022	.1756	1.000	-.542	.499
	LR	-1.357*	.1696	.000	-1.860	-.855
	RP	-.716*	.1743	.001	-1.232	-.200
RP	BA	-.589*	.0929	.000	-.864	-.314
	CO	.663*	.0902	.000	.396	.931
	HE	-.049	.1049	.999	-.360	.262
	LA	.695*	.0933	.000	.418	.971
	LR	-.641*	.0814	.000	-.883	-.400
	MA	.716*	.1743	.001	.200	1.232

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset 1	Subset 2	Subset 3
MA	11	7.318		
LA	63	7.340		
CO	72	7.371		
RP	70		8.034	
HE	42		8.083	
BA	64			8.623
LR	115			8.676
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .289.

a. Uses Harmonic Mean Sample Size = 38.233.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

T. dorsobimaculatus:

2. site

Dependent Variable: wing

site	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LA	8.980[a]	.413	8.149	9.811
MA	9.415	.126	9.162	9.668
TV	10.036[a]	.228	9.579	10.494

a. Based on modified population marginal mean.

T. innotabilis:

2. site

Dependent Variable: wing

site	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
KA	7.907	.078	7.753	8.062
WE	9.067[a]	.254	8.560	9.574

a. Based on modified population marginal mean.

T. strangmannii:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BA	HE	.600*	.1194	.000	.271	.929
	LA	.410	.2042	.267	-.153	.973
	LR	-.509*	.1714	.028	-.981	-.036
	ST	1.986*	.2914	.000	1.183	2.790
HE	BA	-.600*	.1194	.000	-.929	-.271
	LA	-.190	.1849	.842	-.700	.320
	LR	-1.109*	.1479	.000	-1.516	-.701
	ST	1.386*	.2781	.000	.619	2.153
LA	BA	-.410	.2042	.267	-.973	.153
	HE	.190	.1849	.842	-.320	.700
	LR	-.918*	.2221	.001	-1.531	-.306
	ST	1.577*	.3238	.000	.684	2.470
LR	BA	.509*	.1714	.028	.036	.981
	HE	1.109*	.1479	.000	.701	1.516
	LA	.918*	.2221	.001	.306	1.531
	ST	2.495*	.3041	.000	1.656	3.334
ST	BA	-1.986*	.2914	.000	-2.790	-1.183
	HE	-1.386*	.2781	.000	-2.153	-.619
	LA	-1.577*	.3238	.000	-2.470	-.684
	LR	-2.495*	.3041	.000	-3.334	-1.656

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset		
		1	2	3
ST	5	8.240		
HE	110		9.626	
LA	12		9.817	
BA	34		10.226	10.226
LR	20			10.735
Sig.		1.000	.083	.197

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .370.

a. Uses Harmonic Mean Sample Size = 13.447.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Figure A7.01 Post-hoc results of ANOVAs and/ or tables of means for *Tabanus* body size differences among sites

T. ceylonicus:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) flseas	(J) flseas	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.599*	.1360	.000	-.928	-.270
	4.00	-.326	.2176	.301	-.853	.201
2.00	1.00	.599*	.1360	.000	.270	.928
	4.00	.273	.1989	.363	-.208	.754
4.00	1.00	.326	.2176	.301	-.201	.853
	2.00	-.273	.1989	.363	-.754	.208

Based on observed means.

*. The mean difference is significant at the .05 level.

1. flseas

Dependent Variable: wing

flseas	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	5.854	.115	5.623	6.085
2.00	6.453	.073	6.306	6.600
4.00	6.180	.185	5.808	6.552

T. dorsobimaculatus:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) flseas	(J) flseas	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	1.083*	.2795	.001	.407	1.758
	3.00	-.985*	.2297	.000	-1.540	-.430
2.00	1.00	-1.083*	.2795	.001	-1.758	-.407
	3.00	-2.068*	.2977	.000	-2.787	-1.348
3.00	1.00	.985*	.2297	.000	.430	1.540
	2.00	2.068*	.2977	.000	1.348	2.787

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

flseas	N	Subset 1	Subset 2	Subset 3
2.00	10	8.410		
1.00	27		9.493	
3.00	18			10.478
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .570.

a. Uses Harmonic Mean Sample Size = 15.577.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

T. innotabilis:

1. flseas

Dependent Variable: w ing

flseas	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	8.376	.140	8.097	8.656
3.00	8.129[a]	.101	7.927	8.330

a. Based on modified population marginal mean.

T. strangmannii:

Multiple Comparisons

Dependent Variable: wing

Tukey HSD

(I) flseas	(J) flseas	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.097	.1924	.958	-.596	.402
	3.00	-.465*	.1114	.000	-.755	-.176
	4.00	-.771*	.1480	.000	-1.155	-.387
2.00	1.00	.097	.1924	.958	-.402	.596
	3.00	-.369	.2065	.285	-.905	.167
	4.00	-.674*	.2283	.019	-1.266	-.081
3.00	1.00	.465*	.1114	.000	.176	.755
	2.00	.369	.2065	.285	-.167	.905
	4.00	-.305	.1659	.259	-.736	.125
4.00	1.00	.771*	.1480	.000	.387	1.155
	2.00	.674*	.2283	.019	.081	1.266
	3.00	.305	.1659	.259	-.125	.736

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

flseas	N	Subset 1	Subset 2
1.00	109	9.639	
2.00	11	9.736	
3.00	41	10.105	10.105
4.00	20		10.410
Sig.		.051	.328

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .370.

a. Uses Harmonic Mean Sample Size = 22.926.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Figure A7.02 Post-hoc results of ANOVAs and/ or tables of means for *Tabanus* body size differences among flight seasons

D. oculata:

Multiple Comparisons

Dependent Variable: wing

Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CO	HE	-.133	.2774	.989	-.896	.629
	LR	-.829*	.1901	.000	-1.351	-.307
	MA	-1.040*	.2235	.000	-1.655	-.426
	RP	-.682*	.1982	.006	-1.227	-.138
HE	CO	.133	.2774	.989	-.629	.896
	LR	-.696*	.2077	.008	-1.266	-.125
	MA	-.907*	.2387	.002	-1.563	-.251
	RP	-.549	.2151	.083	-1.140	.042
LR	CO	.829*	.1901	.000	.307	1.351
	HE	.696*	.2077	.008	.125	1.266
	MA	-.212	.1271	.458	-.561	.138
	RP	.147	.0737	.275	-.056	.349
MA	CO	1.040*	.2235	.000	.426	1.655
	HE	.907*	.2387	.002	.251	1.563
	LR	.212	.1271	.458	-.138	.561
	RP	.358	.1388	.077	-.023	.740
RP	CO	.682*	.1982	.006	.138	1.227
	HE	.549	.2151	.083	-.042	1.140
	LR	-.147	.0737	.275	-.349	.056
	MA	-.358	.1388	.077	-.740	.023

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset	
		1	2
CO	6	5.267	
HE	5	5.400	
RP	49		5.949
LR	182		6.096
MA	14		6.307
Sig.		.962	.367

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .210.

a. Uses Harmonic Mean Sample Size = 10.776.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

D. nemotuberculata:

1. flseas

Dependent Variable: w ing

flseas	Mean	Std. Error	95% Confidence Interval	
			Low er Bound	Upper Bound
1.00	6.602	.142	6.318	6.886
3.00	7.279	.205	6.868	7.690

L. fuliginosa:

Multiple Comparisons

Dependent Variable: w ing

Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Low er Bound	Upper Bound
BA	LA	.678	.3038	.230	-.200	1.556
	LR	.970	.3636	.088	-.080	2.020
	RP	.672	.3263	.315	-.271	1.614
	ST	.880	.4198	.295	-.333	2.093
	TV	-1.653*	.4019	.001	-2.815	-.492
LA	BA	-.678	.3038	.230	-1.556	.200
	LR	.292	.2197	.769	-.343	.927
	RP	-.006	.1502	1.000	-.440	.427
	ST	.202	.3038	.985	-.676	1.080
	TV	-2.331*	.2786	.000	-3.136	-1.526
LR	BA	-.970	.3636	.088	-2.020	.080
	LA	-.292	.2197	.769	-.927	.343
	RP	-.298	.2498	.839	-1.020	.423
	ST	-.090	.3636	1.000	-1.140	.960
	TV	-2.623*	.3428	.000	-3.614	-1.633
RP	BA	-.672	.3263	.315	-1.614	.271
	LA	.006	.1502	1.000	-.427	.440
	LR	.298	.2498	.839	-.423	1.020
	ST	.208	.3263	.988	-.734	1.151
	TV	-2.325*	.3030	.000	-3.200	-1.450
ST	BA	-.880	.4198	.295	-2.093	.333
	LA	-.202	.3038	.985	-1.080	.676
	LR	.090	.3636	1.000	-.960	1.140
	RP	-.208	.3263	.988	-1.151	.734
	TV	-2.533*	.4019	.000	-3.695	-1.372
TV	BA	1.653*	.4019	.001	.492	2.815
	LA	2.331*	.2786	.000	1.526	3.136
	LR	2.623*	.3428	.000	1.633	3.614
	RP	2.325*	.3030	.000	1.450	3.200
	ST	2.533*	.4019	.000	1.372	3.695

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset 1	2	3
LR	10	5.610		
ST	5	5.700	5.700	
LA	105	5.902	5.902	
RP	24	5.908	5.908	
BA	5		6.580	
TV	6			8.233
Sig.		.941	.079	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .441.

a. Uses Harmonic Mean Sample Size = 8.358.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

L. demeijerii:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
BA	HE	.879*	.1131	.000	.606	1.151
	LR	-.334*	.1161	.016	-.613	-.054
HE	BA	-.879*	.1131	.000	-1.151	-.606
	LR	-1.212*	.0925	.000	-1.435	-.990
LR	BA	.334*	.1161	.016	.054	.613
	HE	1.212*	.0925	.000	.990	1.435

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset 1	2	3
HE	27	4.696		
BA	12		5.575	
LR	23			5.909
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .106.

a. Uses Harmonic Mean Sample Size = 18.310.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

P. eyreana:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BA	LA	.274	.1228	.074	-.021	.568
	LR	-.210	.2052	.565	-.702	.282
LA	BA	-.274	.1228	.074	-.568	.021
	LR	-.484*	.1801	.024	-.915	-.052
LR	BA	.210	.2052	.565	-.282	.702
	LA	.484*	.1801	.024	.052	.915

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset 1	Subset 2
LA	55	5.076	
BA	12	5.350	5.350
LR	5		5.560
Sig.		.260	.449

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .149.

a. Uses Harmonic Mean Sample Size = 9.950.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

P. silvester:

Multiple Comparisons

Dependent Variable: w ing

Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Low er Bound	Upper Bound
BA	CO	.427*	.1012	.001	.136	.718
	HE	.325	.1292	.125	-.047	.696
	LA	.083	.0513	.589	-.065	.231
	LR	-.465*	.1012	.000	-.756	-.174
	TV	-.272*	.0770	.007	-.494	-.051
CO	BA	-.427*	.1012	.001	-.718	-.136
	HE	-.102	.1564	.987	-.552	.348
	LA	-.344*	.1021	.011	-.638	-.050
	LR	-.892*	.1343	.000	-1.278	-.505
	TV	-.699*	.1171	.000	-1.036	-.362
HE	BA	-.325	.1292	.125	-.696	.047
	CO	.102	.1564	.987	-.348	.552
	LA	-.242	.1299	.430	-.615	.132
	LR	-.789*	.1564	.000	-1.239	-.339
	TV	-.597*	.1420	.001	-1.005	-.188
LA	BA	-.083	.0513	.589	-.231	.065
	CO	.344*	.1021	.011	.050	.638
	HE	.242	.1299	.430	-.132	.615
	LR	-.548*	.1021	.000	-.841	-.254
	TV	-.355*	.0782	.000	-.580	-.130
LR	BA	.465*	.1012	.000	.174	.756
	CO	.892*	.1343	.000	.505	1.278
	HE	.789*	.1564	.000	.339	1.239
	LA	.548*	.1021	.000	.254	.841
	TV	.192	.1171	.571	-.145	.529
TV	BA	.272*	.0770	.007	.051	.494
	CO	.699*	.1171	.000	.362	1.036
	HE	.597*	.1420	.001	.188	1.005
	LA	.355*	.0782	.000	.130	.580
	LR	-.192	.1171	.571	-.529	.145

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

site	N	Subset 1	2	3	4
CO	12	4.883			
HE	7	4.986	4.986		
LA	77		5.227		
BA	88		5.310	5.310	
TV	23			5.583	5.583
LR	12				5.775
Sig.		.952	.064	.185	.567

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .108.

a. Uses Harmonic Mean Sample Size = 15.900.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Figure A7.03 Post-hoc results of ANOVAs and/ or tables of means for non-*Tabanus* body size differences among sites

D. oculata:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) flseas	(J) flseas	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
1.00	2.00	-.643*	.1758	.001	-1.057	-.228
	3.00	.196	.1094	.175	-.062	.454
2.00	1.00	.643*	.1758	.001	.228	1.057
	3.00	.838*	.2026	.000	.361	1.316
3.00	1.00	-.196	.1094	.175	-.454	.062
	2.00	-.838*	.2026	.000	-1.316	-.361

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

flseas	N	Subset 1	Subset 2
3.00	19	5.847	
1.00	230	6.043	
2.00	7		6.686
Sig.		.472	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .210.

a. Uses Harmonic Mean Sample Size = 15.012.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

D. nemotuberculata:

1. flseas

Dependent Variable: wing

flseas	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	6.602	.142	6.318	6.886
3.00	7.279	.205	6.868	7.690

L. fuliginosa:

Multiple Comparisons

Dependent Variable: wing
Tukey HSD

(I) flseas	(J) flseas	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	3.00	-.409*	.1219	.003	-.698	-.121
	4.00	-.064	.2590	.967	-.678	.549
3.00	1.00	.409*	.1219	.003	.121	.698
	4.00	.345	.2715	.414	-.298	.988
4.00	1.00	.064	.2590	.967	-.549	.678
	3.00	-.345	.2715	.414	-.988	.298

Based on observed means.

*. The mean difference is significant at the .05 level.

wing

Tukey HSD[a,b,c]

flseas	N	Subset 1
1.00	107	5.879
4.00	7	5.943
3.00	41	6.288
Sig.		.174

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .441.

a. Uses Harmonic Mean Sample Size = 16.988.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

L. demeijerii:

1. flseas

Dependent Variable: wing

flseas	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	5.318	.084	5.149	5.487
3.00	5.598	.084	5.429	5.766

P. eyreana:

1. flseas

Dependent Variable: wing

flseas	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	5.289	.077	5.134	5.443
3.00	5.694[a]	.195	5.305	6.083

a. Based on modified population marginal mean.

P. silvester:

1. flseas

Dependent Variable: wing

flseas	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	5.209	.045	5.119	5.298
2.00	4.780[a]	.147	4.490	5.070
3.00	5.438[a]	.055	5.329	5.546

a. Based on modified population marginal mean.

Multiple Comparisons

Dependent Variable: wing

Tukey HSD

(I) flseas	(J) flseas	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	.494*	.1524	.004	.134	.853
	3.00	-.059	.0483	.446	-.173	.055
2.00	1.00	-.494*	.1524	.004	-.853	-.134
	3.00	-.552*	.1496	.001	-.905	-.199
3.00	1.00	.059	.0483	.446	-.055	.173
	2.00	.552*	.1496	.001	.199	.905

Based on observed means.

*. The mean difference is significant at the .05 level.

Figure A7.04 Post-hoc results of ANOVAs and/ or tables of means for non-*Tabanus* body size differences among flight seasons

APPENDIX EIGHT: CART ANALYSES

Table A8.01: All variables used in analysis. NDVI and meteorological variables were averaged over calendar months, for same period as tabanid collections.

Variable	**Units**	**Source**
Year	None	-
Month	None	-
Temperature- maximum	°C	Bureau of Meteorology
Temperature- minimum	°C	Bureau of Meteorology
Rainfall	mm	Bureau of Meteorology
Evaporation	Mm	Bureau of Meteorology
Solar radiation (ground measurement)	MJ/m^2	Bureau of Meteorology
Vapour pressure	hPa	Bureau of Meteorology
Relative humidity at Tmax	%	Bureau of Meteorology
Relative humidity at Tmin	%	Bureau of Meteorology
Evaporation SP	mm	Bureau of Meteorology
Vegetation- broadscale	None	Qld Herbarium, EPA
NDVI	None	CSIRO
Latitude	Decimal degrees	GPS reading
Longitude	Decimal degrees	GPS reading
Presence of cattle	Yes/no	Site observations

Table A8.02: Site description (sorted from lowest to highest latitude)

Site	**Average annual rainfall (longterm average in mm)**	**Latitude/ longitude**	**Vegetation**	**Distance to permanent water/ tabanid breeding site**	**Presence of potential domestic hosts within 2km**
Bamaga	1917	10° 53.560 S/ 142° 23.939 E	Open woodland	<1km	No cattle
Heathland	1611	11° 45.028 S/ 142° 34.885 E	Grassland	<500m	Cattle/horses
Mapoon	1797	11° 58.046 S/ 141° 53.562 E	Dense woodland	<1km	No/few cattle
Weipa	1974	12° 39.636 S/ 141° 51.455 E	Open woodland	<5km	No/few cattle
Lockhart River	2021	12° 46.998 S/ 143° 18.314 E	Open woodland		No/few cattle
Coen	1193	13° 46.390 S/ 143° 07.664 E	Open woodland	<500m	Cattle/horses
Lakefield	995	14° 55.513 S/ 144° 12.000 E	Open woodland	<500m	Cattle/horses
Valley View	1569	15° 21.038 S/ 145° 01.811 E	Open woodland	<5km	Cattle/horses
Rutland Plains	1226	15° 38.327 S/ 141° 49.352 E	Open woodland	<500m	Cattle/horses
Stirling	850.8	17° 10.977 S/ 141° 42.617 E	Open woodland	<1km	Cattle/horses
Karumba	838	17° 29.555 S/ 140° 49.880 E	Grassland, right on coastline	<100m	Cattle
Townsville	991.5	19° 19’ 25.3 S/ 146° 45’ 52.3 E	Open woodland	<1km	Cattle/horses

Table A8.03: Wet and dry season numbers of each of the six most abundant species by site.

Site	T. ceylonicus		T. dorsobi-maculatus		T. innotabilis		T. notatus		T. pallipennis		T. strangmannii	
	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET
BA	17	36	1	2	1	1	3	105	4	6	0	52
HE	0	3	0	0	0	0	0	57	1	1	1	148
MA	2	0	0	45	2	0	0	11	1	0	0	2
WE	8	0	0	3	7	2	0	2	2	1	0	0
LR	1	3	0	0	0	2	0	190	1	5	0	33
CN	0	0	0	0	0	1	0	92	0	2	0	4
LA	0	0	0	6	0	0	0	84	5	16	0	12
CK	0	0	0	0	0	0	0	0	0	1	0	1
RP	0	0	0	9	0	0	0	79	15	7	0	0
ST	0	0	0	0	0	0	0	2	0	1	0	6
KA	0	0	0	0	4	94	0	1	0	0	0	0
TV	0	0	0	15	0	0	0	2	0	8	0	0

Wet = Dec 04- May 05 and Dec 05-May 06
Dry = Sep-Nov 04 and Jun 05-Nov 05
(i.e. two complete wet seasons and only 1.5 dry seasons)