Population composition and seasonal dynamics of mosquito communities across landscape gradients in southern Africa, with emphasis on selected arbovirus vector species and their role in disease transmission

By

TODD JOHNSON

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Supervisors: Professors   L.E.O. Braack

A.P.G. Almeida

DECLARATION

I, the undersigned, declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy in Medical Virology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Todd Johnson

ETHICS STATEMENT

This PhD protocol was part of the active surveillance program for zoonotic arboviruses across South Africa, which was initiated by the Zoonoses Research Unit (now Centre for Viral Zoonoses). The author, whose name appears on the title page of this thesis, has obtained, for the research described in this work, the applicable research ethics approval from the Animal Ethics Committee protocol number H012-16. Subsequent Ethical clearance was granted through the "One Health approach to detect zoonotic arboviruses through surveillance in animals and vectors, and development of molecular and serological assays to define their epidemiology in South Africa" protocol number 155/2019. The author declares that he has observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the Policy guidelines for responsible research.

ACKNOWLEDGEMENTS

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Todd Johnson

Supervisor: Prof. L.E.O. Braack
Co-supervisor: Prof. A.P.G. Almeida
Department of Medical Virology
University of Pretoria

For the degree PhD (Medical Virology)

SUMMARY

Mosquito-borne arboviruses are of considerable public health importance as they cause some of the most important emerging and re-emerging infectious diseases affecting humans and animals in many parts of the world including southern Africa. The threat of large epidemics of mosquito-borne arboviruses are often associated with climatic conditions, global warming, animal migrations, surface water, wind, topography, harbourage, vegetation, food supply and abundance of competent mosquito vectors. The goal of this project is to provide an in depth understanding of mosquito community dynamics and the importance of mosquito vector populations in the maintenance and transmission of mosquito-borne diseases in southern Africa.

Firstly, a review of past and current literature was conducted to highlight: (a) the current state of knowledge regarding the most important mosquito-borne viruses of medical significance in southern Africa (b) lesser known mosquito-borne arboviruses with the potential of causing zoonotic health threats for humans in southern Africa. (c) key aspects of the ecology of mosquito vectors of medically significant mosquito-borne viruses in southern Africa. d) gaps in knowledge regarding southern African arbovirus mosquito vectors. Most of the studies on mosquito-borne viruses in southern Africa can be clustered into specific programmes led by

Kokernot and Smithburn in the 1950s, McIntosh in the 1970s and 1980s, Swanepoel in the 1970s, Venter and others in more recent years, and have largely been restricted to South Africa, Mozambique and Zimbabwe. Twenty-six (26) arboviruses have been isolated from mosquitoes in southern Africa. Of these, Chikungunya (CHIK), Sindbis (SIN), West Nile (WN), Wesselsbron (WES), Spondweni (SPO), Banzi (BAN), Dengue (DEN), Bunyamwera (BUN), Germiston (GER) and Rift Valley fever (RVF) viruses are known to cause human illness. Middelburg (MID) and Shuni (SHUN) viruses are also important, causing neurological symptoms in animals with zoonotic potential for humans in South Africa. There are eight mosquito-borne arboviral infections most likely to impact humans in southern Africa (CHIK, MID, SIN, DEN, WES, WN, SHUN and RVF viruses). Mosquitoes in the subfamily Culicinae (mostly *Aedes* and *Culex* mosquitoes) are the most frequently associated with arbovirus transmission (115 and 105 types of arbovirus, respectively). Understanding the role of mosquito vector species in arbovirus transmission is vital for the development of new strategies to control the spread of arboviral diseases. In southern Africa, a few species in the genera *Anopheles*, *Coquillettidia* and *Mansonia* have also been implicated as vectors of arboviruses. Surveys over multiple decades across southern Africa have provided an insight regarding which species of mosquitoes are involved in the transmission of at least the most common of the mosquito-borne zoonotic arboviruses. These cluster within the genera *Aedes* and *Culex*, each representing a different transmission strategy. *Aedes*-borne viruses such as CHIK, DEN and WES tend to have primate or human reservoir hosts (McIntosh, 1986), while *Culex*-borne viruses often use birds as reservoir hosts, and these factors influence the distribution and epidemiology of the diseases they cause in humans and animals. *Aedes* and *Culex* have different breeding strategies and preferences which also represent fundamental differences. These mosquitoes are *Aedes aegypti*, *Aedes furcifer/cordellieri*, *Aedes circumluteolus*, *Aedes unidentatus*, *Aedes mcintoshi*, *Aedes caballus*, *Aedes juppi*, *Culex theileri*, *Culex zombaensis*, *Culex univittatus*, *Culex neavei* and *Culex rubinotus*.

To determine mosquito community dynamics and mosquito vector distributions, sampling mosquito vectors at six sentinel sites in three provinces in the northern part of South Africa where recent cases had been detected in animals. Adult mosquitoes were collected from two horse properties in Gauteng Province; two wildlife reserves in Limpopo Province and at Orpen Gate in Kruger National Park and Mnisi Area in Mpumalanga Province between 2014–2017,

using carbon dioxide-baited light and tent traps. *Culex poicilipes*, was the most abundant species caught during the study period. Highest diversity and species richness were found at Lapalala Wilderness Reserve, while the lowest diversity and abundances were at Orpen in Kruger National Park. *Aedes aegypti, Ae. mcintoshi, Ae. metallicus, Ae. vittatus, Cx. pipiens* sensu lato, *Cx. theileri* and *Cx. univittatus,* which are potential arbovirus vectors, had the widest geographical distribution in northern South Africa. Also collected were *Anopheles arabiensis* and *An. vaneedeni*, both known malaria vectors in South Africa. Therefore, arbovirus surveillance and vector control programs should be augmented in peri-urban and mixed rural settings where there is greater risk for arbovirus transmission to humans and domestic stock.

Since climate has reportedly been associated with disease transmission, it's important to understand the extent of its influence on mosquito abundance and distribution in northern South Africa. Thus, population composition, abundance and diversity of mosquitoes collected over a three-year period were determined and correlated to diverse climatic conditions during those years in order to determine seasonal trends in occurrence, abundance and distribution. Marked differences in the temporal distribution and seasonal abundances of the seven medically important mosquito vectors encountered from the two distinct geographic regions and climates. Statistical models have shown that climatic factors play a crucial role in shaping the population dynamics of *Ae. mcintoshi, Ae. vittatus, An. arabiensis*, Cx. pipiens *s.l., Cx. poicilipes, Cx. theileri* and *Cx. univittatus* both in Highveld Grassland and Middleveld Bushveld regions of northern South Africa. High summer temperatures and rainfall lead to increased vector density which might trigger outbreaks of RVF, SIN and WN viruses on the inland plateau of South Africa. This study also showed that abundances of RVF and WN virus vectors are related to elevation. These findings will be important in predicting the timing of onset and spread of future epidemics such as WN and RVF viruses, in southern Africa and other geographical settings with similar climates.

PUBLICATIONS AND COMMUNICATIONS

Publications:

Anthony J. Cornel, Yoosook Lee, António Paulo Gouveia Almeida, **Todd Johnson**, Joel Mouatcho, Marietjie Venter, Christiaan de Jager and Leo Braack (2018) Mosquito community composition in South Africa and some neighboring countries. *Parasites & Vectors* (11)331: pp 12.

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International Communications:

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Milehna M. Guarido*, **Todd Johnson**, Mpho Rakgotho, Elizabeth M. Botha, Antonio Paulo Gouveia Almeida, Marietjie Venter. Investigation of *Aedes* mosquitoes in selected rural and urban sites in Gauteng, Limpopo and Mpumalanga provinces, South Africa and associated zoonotic arboviruses in 2016. [Oral] Keystone Symposia: Vectors, Pathogens and diseases: Current Trends and Emerging Challenges. 10–15 September 2017, Durban, South Africa (Presenter*)

Todd Johnson, Takalani Nelufule, Milehna M. Guarido, Marietjie Venter, Antonio Paulo Gouveia Almeida, Lawrence Edward Oliver Braack. Diversity, abundance and distribution of mosquitoes associated with vectorborne disease in three ecologically distinct zones of northern South Africa. [Poster] Keystone Symposia: Vectors, Pathogens and diseases: Current Trends and Emerging Challenges. 10–15 September 2017, Durban, South Africa

DEDICATION

I would like to thank God Almighty for granting me His Grace and strength to complete my studies.

May I also take this opportunity to express my sincere appreciation to my wife, Alinesi and our daughters, Amy, Laura and Nes for the encouragement and support you provided during the course of this study.

LIST OF ABBREVIATIONS

Aed	*Aedeomyia*
Ae	*Aedes*
AMTV	Arumowot virus
An	*Anopheles*
BAGV	Bagaza virus
BANV	Banzi virus
BBKV	Babanki virus
BUNV	Bunyamwera virus
BWAV	Bwamba virus
CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya virus
Coq	*Coquillettidia*
Cs	*Culiseta*
Cx	*Culex*
CYV	Chaoyang virus
DENV1-4	Dengue virus serotypes 1 to 4
D	Simpson dominance index
ENS	Effective Number of Species
Fi	*Ficalbia*
GERV	Germiston virus
H'	Shannon Index
IDH	Intermediate Disturbance Hypothesis
J	Equitability index
KNP	Kruger National Park
Ma	*Mansonia*
MBD	Mosquito-borne diseases
MIDV	Middelburg virus
Mi	*Mimomyia*
Mosq	Mosquitoes
MOSV	Mossuril virus
MOZ	Mozambique

NDUV	Ndumu virus
NRIV	Ngari virus
OLIV	Olifantsvlei virus
ONNV	O'Nyong-Nyong virus
PGAV	Pongola virus
QBV	Quang Binh virus
RVFV	Rift Valley fever virus
S	Species richness
RSA	South Africa
SAWS	South African Weather Service
SFV	Semliki Forest virus
SHOKV	Shokwe virus
SHUV	Shuni virus
SINV	Sindbis virus
SPOV	Spondweni virus
UGSV	Uganda S virus
Ur	*Uranotaenia*
USUV	Usutu virus
WESV	Wesselsbron virus
WITV	Witwatersrand virus
WNV	West Nile virus
YFV	Yellow fever virus
ZIKV	Zika virus

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CHAPTER ONE

Overview of medically-important mosquito-borne arboviruses and their vectors in southern Africa

1.0 Introduction

Arboviruses or arthropod borne viruses are a group of viruses that are maintained in nature mainly through biological transmission between susceptible vertebrate hosts by blood-feeding arthropods such as mosquitoes, ticks, midges and sandflies (WHO, 1967). These viruses are of considerable public health importance as they cause some of the most important emerging and re-emerging infectious diseases affecting humans and animals in many parts of the world (Gubler, 2002) including southern Africa (Jupp, 2001, Venter and Swanepoel, 2010). Examples include dengue virus, West Nile virus, yellow fever virus and chikungunya virus. They frequently cause clinical disorders of varying severity in both humans and animals, ranging from mild, or more severe febrile illness to meningo/encephalitis and/or haemorrhagic fever, that can lead to coma and death (Ochieng et al., 2013).

Taxonomically, arboviruses are comprised of different families such as *Flaviviridae* (genus *Flavivirus*), *Togaviridae* (genus *Alphavirus*), *Bunyaviridae* (genus *Nairovirus, Orthobunyavirus, Phlebovirus* and *Tospovirus*), *Rhabdoviridae* (genus *Vesiculovirus*), *Reoviridae* (genus *Orbivirus* and *Coltivirus*) and *Orthomyxoviridae* (genus *Thogotovirus*), as partly reflected in Table 1. Most of the zoonotic arboviruses affecting humans are in the families *Flaviviridae* and *Togaviridae*. However, there are several other important human and animal arboviruses belonging to the *Bunyaviridae*, such as Rift Valley fever virus (mosquito-borne), Crimean-Congo hemorrhagic fever virus (tick-borne) and Toscana virus (sandfly-borne) (Go et al., 2014).

Table 1: Arthropod-borne viruses isolated from mosquitoes and other sources in southern Africa

ARBOVIRUS	GLOBAL DISTRIBUTION	OCCURRENCE IN SOUTHERN AFRICA	VECTORS	HOST	PLACE OF DISCOVERY	REFERENCES
FAMILY: **GENUS:** *FLAVIVIRUS*						
BANZI	East & South Africa	Botswana Namibia Mozambique South Africa Zimbabwe	*Culex rubinotus*	Rodents Humans Domestic animals	Ndumu, RSA	(Jupp, 1996, McIntosh, 1986, Jupp, 2004b, Jupp et al., 1976, McIntosh et al., 1976b, Venter, 2018)
DENGUE	Worldwide in the tropics	Occasional outbreaks in Mozambique, RSA & Namibia	*Aedes aegypti*	Primates Humans	Durban, RSA	(McIntosh, 1986, Jupp, 1996, Rautenbach, 2011, Gubler, 2001, Guzman et al., 2010, Kemp and Jupp, 1991, Msimang et al., 2013)
SPONDWENI	South & West Africa	Mozambique South Africa	*Aedes circumluteolus* *Aedes fryeri/fowleri* *Aedes cumminsi* *Eretmapodites silvestris* *Mansonia uniformis* *Mansonia africana*	Humans?	Lake Simbu, RSA*	(McIntosh, 1980, Swanepoel, 2003, Kokernot et al., 1962a, McIntosh et al., 1962, Venter, 2018)
UGANDA S	Central, East, West & South Africa	South Africa	*Anopheles coustani* *Anopheles brucei* *Culex* spp	Birds Primates	Port Shepstone, RSA	(Swanepoel, 2003, McIntosh, 1980, Venter, 2018)
USUTU	Africa & Europe	South Africa	*Culex neavei*	Birds Humans Domestic animals	Ndumu, RSA	(McIntosh, 1980, Swanepoel, 2003, Venter, 2018)

TABLE 1: CONTINUED **WESSELSBRON**	Central, East, South & West Africa, Asia	Mozambique South Africa Botswana Namibia Zimbabwe	*Aedes mcintoshi* *Aedes luridus* *Aedes unidentatus* *Aedes caballus* *Aedes juppi* *Aedes circumluteolus* *Aedes* (*Neomelaniconion*) spp *Culex univittatus* *Mansonia uniformis*	Domestic animals Rodents? Humans	Wesselsbron, RSA	(Jupp, 1996, Burt et al., 2014, Kokernot et al., 1958, Jupp, 2004b, McIntosh, 1980, Swanepoel, 2003, McIntosh, 1986, Venter, 2018)
WEST NILE	Some parts of Africa, Madagascar & Central Europe	Lineage 2: Southern Africa	*Aedes caballus sensu lato* (*s.l.*) *Aedes circumluteolus* *Coquilletidia microannulata* *Culex neavei* *Culex pipiens* *Culex theileri* *Culex univittatus*	Birds Humans Domestic animals	Ndumu, RSA	(Burt et al., 2014, Gubler, 2007, Hubalek and Halouzka, 1999, Jupp and NK, 1986, Jupp, 2005, Jupp, 1996, Jupp, 2001, Kulasekera et al., 2001, McIntosh et al., 1976a, McIntosh, 1980, Murgue et al., 2001, Petersen and Roehrig, 2001, Sejvar, 2003, Uyar, 2013, Swanepoel and Cruickshank, 1974, Zehender et al., 2017, Swanepoel, 2003, McIntosh, 1986, Venter, 2018)

TABLE 1: CONTINUED

TOGAVIRIDAE						
ALPHAVIRUS **CHIKUNGUNYA**	Africa, Europe, Middle East, Asia, North, Central & South America, Oceania	Southern Africa	*Aedes furcifer/cordellieri* *Aedes aegypti* *Aedes fulgens* *Aedes vittatus*	Primates Humans	Letaba, RSA	(Jupp, 2005, Jupp, 1996, Gudo et al., 2016, Anonymous, 2017a, Burt et al., 2014, Gubler, 2001, Jain et al., 2008, Jupp and McIntosh, 1990, Prinsloo, 2006, Swanepoel and Cruickshank, 1974, Swanepoel, 2003, McIntosh, 1980, McIntosh, 1975, McIntosh, 1986)
MIDDELBURG	East, West & South Africa	South Africa Zimbabwe	*Aedes juppi* *Aedes caballus* *Aedes* (*Neomelaniconion*) spp *Aedes circumluteolus* *Aedes albocephalus* *Aedes marshalli* *Aedes dentatus-leesoni* *Aedes cumminsii* *Aedes tarsalis* *Aedes subdentatus* *Aedes lineatopennis (Ae. mcintoshi)* *Culex theileri*	Birds Domestic animals Wildlife Humans	Middelburg, RSA	(McIntosh, 1980, Burt et al., 2014, van Niekerk et al., 2015a, Jupp PG et al., 1987, Venter, 2018)
NDUMU	Central, South, East & West Africa	Southern Africa	*Aedes circumluteolus* *Mansonia uniformis*	Domestic animals Humans	Ndumu, RSA	(McIntosh, 1980, Swanepoel, 2003, Kokernot et al., 1961, Venter, 2018)

TABLE 1: CONTINUED TOGAVIRIDAE *ALPHAVIRUS*						
SEMLIKI FOREST	East, West & South Africa	Mozambique South Africa	*Aedes argenteopunctatus* *Aedes circumluteolus* *Aedes (Aedimorphus)* spp	Primates Humans	Namacurra, MOZ	(McIntosh, 1980, Swanepoel, 2003, Smithburn and Haddow, 1944, Venter, 2018)
SINDBIS	East, North, South & West Africa, Middle East, Europe, Australia, South East Asia	Southern Africa	*Aedes circumluteolus* *Aedes cumminsii* *Culex neavei* *Culex tigripes* *Culex univittatus* *Mansonia africana*	Birds Domestic animals Humans	Springs, RSA	(McIntosh, 1980, Jupp, 1996, Burt et al., 2014, Jupp, 2005, Jupp and NK, 1986, Swanepoel and Cruickshank, 1974, Venter, 2018)
OLIFANTSVLEI	East, North & South Africa	South Africa	*Culex pipiens*	Unknown	Johannesburg, RSA	(Swanepoel, 2003, McIntosh, 1980, McIntosh, 1978b)
BUNYAVIRIDAE						
PHLEBOVIRUS						
RIFT VALLEY FEVER	Africa, Madagascar & Middle East	Southern Africa	*Aedes aegypti* *Aedes dentatus* *Aedes circumluteolus* *Aedes mcintoshi* *Aedes juppi* *Aedes caballus* *Anopheles coustani* *Culex neavei* *Culex pipiens* *Culex theileri* *Culex zombaensis* *Eretmapodites quinquevittatus*	Domestic animals Wildlife Rodents Humans	Johannesburg, RSA	(Alexander, 1951, Anonymous, 2017b, Blomström et al., 2016, Davies, 2010, Fafetine et al., 2016, Gubler, 2001, McIntosh, 1972, McIntosh, 1986, Pienaar and Thompson, 2013, Prinsloo, 2006, Rautenbach, 2011, Swanepoel and Coetzer, 2004, Swanepoel, 2003, Jupp, 1996, Swanepoel and Cruickshank, 1974, Venter, 2018)

TABLE 1: CONTINUED						
BUNYAVIRIDAE *PHLEBOVIRUS* **ARUMOWOT**	East, Central & West Africa	South Africa	*Culex rubinotus*	Rodents?	Port Shepstone, RSA	(Swanepoel, 2003, McIntosh, 1980, McIntosh et al., 1976b, Kemp et al., 1974)
ORTHOBUNYAVIRUS **BUNYAMWERA**	Central, East, South & West Africa	Mozambique South Africa Zimbabwe	*Aedes circumluteolus* *Aedes pembaensis* *Aedes bevisi* *Aedes (Stegomyia)* spp *Culex rubinotus*	Domestic animals Rodents Humans Primates	Ndumu, RSA	(McIntosh, 1980, Swanepoel, 2003, Smithburn, 1958, Venter, 2018)
GERMISTON	East & South Africa	Mozambique South Africa Zimbabwe	*Culex rubinotus* *Culex theileri*	Rodents Domestic animals Humans	Germiston, RSA	(McIntosh, 1986, McIntosh, 1980, Kokernot et al., 1960, Jupp et al., 1976, McIntosh et al., 1976b, Venter, 2018)
LUMBO	Southern Africa	Mozambique South Africa	*Aedes pembaensis*	Rodents? Primates?	Lumbo, MOZ*	(Kokernot et al., 1962a, McIntosh, 1980)
PONGOLA	East, Central, South & West Africa	Mozambique South Africa	*Aedes mcintoshi* *Aedes circumluteolus*	Humans	Lake Simbu, RSA	(McIntosh, 1980, Swanepoel, 2003, Kokernot et al., 1957b, Venter, 2018)
SHUNI	West & South Africa, Middle East	Mozambique South Africa Zimbabwe	*Culex theileri*	Domestic animals Humans Wildlife	Onderstepoort, RSA	(van Eeden et al., 2012a, Swanepoel, 2003, Causey et al., 1972, Golender et al., 2015, Venter, 2018)
SIMBU	Central, South & West Africa	South Africa	*Aedes circumluteolus*	Domestic animals	Lake Simbu, RSA	(Swanepoel, 2003, McIntosh, 1980, Weinbren et al., 1957, Causey et al., 1972)

TABLE 1 CONTINUED						
ORTHOBUNYAVIRUS						
TAHYNA	Southern Africa, Europe, Asia	Mozambique	*Aedes pembaensis*	Unknown	Lumbo, MOZ	(Bardos and Danielova, 1959, McIntosh, 1980, Swanepoel, 2003, Hannoun et al., 1966, Lu et al., 2009)
SHOKWE	East, South & West Africa	Mozambique South Africa Zimbabwe	*Aedes circumluteolus Aedes cumminsii Mansonia africana*	Humans	Ndumu, RSA	(Swanepoel, 2003, McIntosh, 1980, McIntosh et al., 1972, Venter, 2018)
WITWATERSRAND	East & South Africa	Mozambique South Africa Zimbabwe	*Culex rubinotus*	Rodents	Germiston, RSA	(Swanepoel, 2003, Jupp et al., 1976, McIntosh et al., 1976b)
RHABDOVIRIDAE						
MOSSURIL	Southern Africa	Mozambique South Africa	*Culex sitiens Culex neavei*	Unknown	Lumbo, Mozambique	(Kokernot et al., 1962b, Swanepoel, 2003, McIntosh, 1980)
REOVIRIDAE						
LEBOMBO	Southern Africa	South Africa	*Aedes circumluteolus*	Unknown	Ndumu, RSA	(Brown et al., 1991, McIntosh, 1980)

RSA* = South Africa
MOZ* = Mozambique

Of the over 600 recognized arboviruses worldwide, approximately 150 are important human/animal pathogens (Cleton et al., 2012, Burt et al., 2014). Most arboviruses are known to primarily circulate among wildlife while some can be maintained in mosquito eggs which remain dormant until rain or flooding triggers hatching of infected larvae (Liang et al., 2015). These can lead to mosquito-borne disease outbreaks especially in urban environments without the need for animal reservoir hosts. The urban transmission cycle plays an important role in perpetuating some of these viruses. Large epidemics are often associated with climatic extremes such as episodes of heavy rainfall, but also migrations of animals or viruses into new areas (Chevalier et al., 2010, Cleton et al., 2012), temperature, humidity, surface water, wind, topography, harbourage, vegetation and food supply. Mosquito-borne viruses are mostly confined to specific habitats and become seasonally abundant with transmission corresponding to the abundance of competent mosquito vector species (Day and Shaman, 2011).

The purpose of this review is to highlight: a) the current state of knowledge regarding the most important mosquito-borne viruses of medical significance in southern Africa b) lesser known mosquito-borne arboviruses with the potential of causing zoonotic health threats for humans in southern Africa. c) key aspects of the ecology of mosquito vectors of medically significant mosquito-borne viruses in southern Africa. d) gaps in knowledge regarding southern African arbovirus mosquito vectors.

1.1 Mosquito-borne zoonotic arboviruses of known or potential importance in southern Africa

Most of the studies on mosquito-borne viruses in southern Africa, largely restricted to South Africa, Mozambique and Zimbabwe, can be clustered into specific programmes led by Kokernot and Smithburn in the 1950s, McIntosh in the 1970s and 1980s, Swanepoel in the 1970s and in more recent years Venter and colleagues (Venter, 2018). This review will therefore reflect this geographic emphasis, but other findings relating to neighbouring southern African nations will be provided as available. The northern geographic boundary of southern Africa corresponds with the upper limits of Namibia, Botswana, Zimbabwe and the Zambezi River where it flows through Central Mozambique (Branch, 1998, Apps et al., 2008).

A literature search suggests that 26 arboviruses have been isolated from mosquitoes in southern Africa as summarized in Table 1. Of these, Chikungunya (CHIK), Sindbis (SIN), West Nile (WN), Wesselsbron (WES), Spondweni (SPO), Banzi (BAN), Dengue (DEN), Bunyamwera (BUN), Germiston (GER) and Rift Valley fever (RVF) viruses are known to cause human illness (Swanepoel and Cruickshank, 1974, McIntosh, 1986, Prinsloo, 2006, Burt et al., 2014, Rautenbach, 2011). Recent reports by Venter and others (Venter et al., 2014) have shown that Middelburg (MID), and Shuni (SHUN) viruses are also important, causing neurological symptoms in animals with zoonotic potential for humans in South Africa. The discussion below focuses on eight mosquito-borne arboviral infections most likely to impact humans in southern Africa, based on historic and recent epidemiological findings (McIntosh, 1986, Swanepoel and Cruickshank, 1974, Jupp, 1996, Jupp, 2005, Prinsloo, 2006, Rautenbach, 2011, Venter, 2018).

1.2 Togaviridae: *Alphavirus*

The family Togaviridae is classified into two genera: Rubivirus and Alphavirus. Alphaviruses are comprised of 29 virus species and are mostly mosquito-borne (Korsman et al., 2012).

1.2.1   Chikungunya virus (CHIKV)

Chikungunya virus (Table 1) was first isolated from a febrile patient in Tanzania in 1953 (Jain et al., 2008). The virus was subsequently described from South Africa by Gear (Gear and Reid, 1957). Prior to 2013, CHIKV outbreaks occurred in tropical areas of Africa, Indian and Pacific Ocean Islands, southern and south-eastern Asia. However, by late 2013, the virus had spread to the Caribbean and South America (Burt et al., 2014). Since then local transmission has been detected in 17 countries or territories throughout the Americas where more than 1.7 million suspected cases have been recorded (Fischer and Staples, 2014). In South Africa, CHIKV occurs in the tropical and subtropical eastern lowlands of Mpumalanga and Limpopo Provinces as well as coastal northern KwaZulu-Natal (Jupp and McIntosh, 1990). Other outbreaks of the disease were also reported in Zimbabwe in 1961/1962 and 1971. Since the last outbreak in 1977, only periodic cases have been confirmed in travellers returning to South

Africa from endemic areas (Burt et al., 2014). However, recent serological evidence has revealed virus circulation among febrile patients in southern Mozambique (Gudo et al., 2015, Gudo et al., 2016). CHIKV is presently spreading in North and South America, with over 1 million suspected cases and at least 22,000 laboratory-confirmed cases (Anonymous, 2017a). The major vectors of CHIKV in southern Africa are *Aedes furcifer* and the closely related *Ae. cordellieri*. Vervet monkeys and baboons are the primary vertebrate hosts for the virus (Jupp, 1996, McIntosh, 1986). The disease is associated with joint pain, headache and muscular pain and maculo-erythematous rash (Burt et al., 2014).

1.2.2   Middelburg virus (MIDV)

Middelburg virus was first isolated from *Culex theileri*, *Ae. caballus* and pools of mixed species of *Aedes* in South Africa in 1957 (McIntosh, 1980). Mosquito surveys conducted in the Free State Province suggested that *Ae. juppi* might be a reservoir vector for MIDV (Jupp PG et al., 1987). Almost twenty years later, the virus was isolated from the spleen of a horse which died in Zimbabwe after it showed symptoms resembling African horse sickness (Attoui et al., 2007). Middelburg and Shuni nucleic acids were also detected in a horse with symptoms of fever and anorexia in South Africa (van Eeden et al., 2012a). Although the significance of such coinfection and illness remains unclear, it does indicate that lesser known arboviruses should not be ignored as they may have potential significant health impacts on humans and animals. Recent disease and vector surveillance studies have shown that Middelburg (and also Shuni) virus is a potential zoonotic virus which can cause severe and fatal neurological diseases in horses and several wildlife species in South Africa (van Niekerk et al., 2015a, Venter et al., 2014, van Eeden et al., 2012a).

1.2.3   Sindbis virus (SINV)

Sindbis virus was first isolated from *Cx. univittatus* mosquitoes in 1952 at Sindbis in the Nile Delta in Egypt (Taylor et al., 1955). Three subtypes of the virus were initially isolated in South Africa in 1954 (Weinbren et al., 1956) from pools of mosquitoes comprising *Culex pipiens*, *Culex quinquefasciatus*, *Cx. univittatus*, *Cx. theileri*, *Culex tigripes* and *Culex annulioris* collected from near Johannesburg (Burt et al., 2014). SINV has also been detected in other

species of mosquitoes in many countries in Africa, Europe, South-East Asia and Australia, *Culex bitaeniorhynchus* in the Philipines, *Culex annulirostris* and *Coquilletidia fuscopennata* in Uganda (McIntosh, 1980). SINV infection in humans is widespread in southern Africa but in South Africa the virus is most prevalent in the northwestern Highveld regions of the country. *Cx. univittatus* is the principal vector of SINV in the inland plateau regions of southern Africa, while *Culex neavei* is the main vector in the coastal lowlands of KwaZulu-Natal Province in South Africa (Jupp, 1996, McIntosh, 1986). SINV is maintained in transmission cycles between various species of birds and the mosquito vectors *Cx. univittatus* (Jupp and NK, 1986) and *Cx. neavei* (Jupp, 1996). The virus is often associated with fever, headache, malaise, maculopapular rash, joint and tendon pains (Burt et al., 2014).

1.3 Flaviviridae: *Flavivirus*

Flaviviruses are a highly diverse group of cosmopolitan arboviruses, in some cases causing significant human disease. Flaviviruses are mainly tick-borne, although some members are mosquito-borne (*Culex* or *Aedes*-transmitted), or have unknown vectors while some viruses only infect mosquitoes and are incapable of infecting vertebrates (Coffey et al., 2013).

1.3.1 Dengue virus (DENV)

First reports of epidemics thought to be dengue emanated from three continents (Asia, Africa and North America) in 1779 (Gubler, 1998). However, the first confirmed outbreak of dengue haemorrhagic fever (DHF) occurred in the Philippines between 1953 and 1954 and became the leading cause of hospitalizations and deaths throughout Southeast Asia 20 years later. Dengue fever (DF) and DHF are potentially fatal diseases caused by any of 4 dengue viruses strains (DENV1–4) (Sharp et al., 2015). By 1998, DEN was the 2nd most important tropical infectious disease after malaria, with an estimated 100 million cases of dengue fever, 500,000 dengue haemorrhagic fever and approximately 25,000 deaths annually (Gubler, 1998). DENV is currently spreading at an alarming rate across the globe and is causing major epidemics in urban settings (Msimang et al., 2013). DENV has caused major outbreaks in many countries in tropical and subtropical Africa, Asia and South America in the recent past (Guzman et al., 2010). DENV has been infrequently recorded in southern Africa, but is probably under-

recorded and misdiagnosed where it has readily been introduced to infection-free areas (Sharp et al., 2015). In 1984, Mozambique experienced the first outbreak of DENV 3 while Angola experienced a large epidemic in 2013 (Sharp et al., 2015, Gubler et al., 1986, Sessions et al., 2013). In 2014, Mozambique experienced yet another outbreak, thirty years after the first reported outbreak , involving 193 cases (Kampango and Abílio, 2016, Massangaie et al., 2016). Several DENV outbreaks have occurred in what is now the KwaZulu-Natal Province of South Africa in 1897, 1901 and again between 1926 and 1927, when DENV 1 caused 50,000 cases and 60 deaths along the coast in and around Durban (Jupp, 2005). DENV is readily transmitted between humans by the urban form of *Aedes aegypti* (McIntosh, 1986). Unlike other mosquito-borne viruses, DENV has a limited vertebrate host range, which although initially were nonhuman primates now affects mainly humans (Coffey et al., 2013, Braack et al., 2018). The symptoms of dengue are by fever, frontal headache, retro-orbital pain, body pains, nausea and vomiting, joint pains, weakness and rash. In some cases, patients may experience anorexia, altered taste sensation and mild sore throat. However, DHF is characterized by skin haemorrhages, gum bleeding, epistaxis, menorrhagia and gastrointestinal haemorrhage (Gubler, 1998).

1.3.2 Wesselsbron virus (WESV)

Wesselsbron virus was first discovered and described from *Aedes circumluteolus* at Simbu (South Africa) in 1955. The virus was later isolated from *Aedes caballus* and pools of *Aedes* (*Neomelaniconion*) spp., which were probably *Aedes lineatopennis* (now referred to as *Aedes mcintoshi*) and *Aedes luridus* during a disease outbreak in sheep in the Middelburg area of the Eastern Cape Province of South Africa (McIntosh, 1980, Kokernot et al., 1958). WESV has also been isolated from *Aedes mcintoshi* in Zimbabwe. Aside from southern Africa, the virus has been detected in camels, humans and mosquitoes in three West African countries, namely Nigeria, Senegal and Cameroun respectively. WESV has also been isolated from mosquitoes in Madagascar and *Aedes* spp. in the Central African Republic (McIntosh, 1980, Burt et al., 2014).

The major vectors of WESV on the temperate inland plateau of South Africa are *Aedes caballus-juppi* and the *Aedes mcintoshi-luridus-unidentatus* group, while *Aedes*

circumluteoulus is the likely vector in the coastal lowlands of northern KwaZulu-Natal Province and Mozambique (Jupp, 1996). *Aedes mcintoshi* is the major vector of WESV on the Zimbabwean Highlands (Jupp, 2004b). The primary modern-day vertebrate hosts are most likely domestic animals, with epizootic cycles involving sheep, cattle and flood water *Aedes* mosquitoes (Kokernot et al., 1958). One virus isolation has been recorded from the gerbil *Desmodillus auriculatus*, thus implicating rodents as possible hosts (Jupp, 1996). In sheep, the virus causes fever, hepatitis, icterus, haemorrhage and abortion. WESV is associated with congenital defects in both sheep and cattle. Human infection is characterized by fever, headache, body pains, insomnia and rash (McIntosh, 1986).

1.3.3 West Nile virus (WNV)

West Nile virus was first discovered in the blood of a febrile woman from north-western Uganda in 1937 (Hubalek and Halouzka, 1999, Smithburn et al., 1940) but has since been isolated from birds, humans and mosquitoes in many countries around the world (Sejvar, 2003, Kokernot et al., 1956, Petersen and Roehrig, 2001, Kulasekera et al., 2001). However, the first outbreak of neuroinvasive disease linked to WNV was reported in Israel in 1957. WNV has also been isolated from horses with confirmation of zoonotic transmission to humans (Venter et al., 2014). Since humans and horses are poorly vireamic, they are considered incidental or dead end hosts in the transmission cycle (Jupp, 2001, Jupp, 2005). Although infection has occurred in domestic stock and wildlife, recurrent isolations from wild birds has incriminated them as primary hosts of the virus. WNV is an emerging infection which is spread by birds through migrations. Bird migrations create a mechanism for the dispersal and establishment of WNV into new regions often very distant from where the infection was originally acquired (Rappole et al., 2000, Dusek et al., 2009, Hagman et al., 2014). The virus is now recognized as widespread and is an important causative agent of viral encephalitis worldwide, with a geographic distribution that covers much of Africa, Europe, Asia, Australia, North, Central and South America (Chancey et al., 2015, Kramer et al., 2008, Gubler, 2007, Murgue et al., 2001, Jupp and NK, 1986).

In southern Africa, the largest recorded epidemic occurred in the Karoo and Northern Cape Province of South Africa in 1974, affecting tens of thousands of people over a 2500km^2 area

(McIntosh et al., 1976a). Another outbreak occurred between 1983 and 1984 in the Witwatersrand-Pretoria region of South Africa (Jupp and NK, 1986). Periodic outbreaks of WNV have also been recorded from Limpopo, Mpumalanga and Free State Provinces while serological evidence exists from cattle in Zimbabwe (Blackburn and Swanepoel, 1980, Jupp and NK, 1986, Burt et al., 2014). The virus utilises a wide range of bird hosts and its primary vectors are the ornithophilic mosquitoes *Culex univittatus* and *Culex* neavei (Jupp and NK, 1986). *Culex univittatus* is the major vector in the temperate inland plateau areas of southern Africa (Jupp, 2001, Jupp, 1996). WNV has also been isolated from *Culex pipiens*, *Aedes caballus*, *Aedes circumluteolus* and *Coq. microannulata* (McIntosh, 1980). Although primarily ornithophilic, *Culex univittatus* also feeds on humans and occasionally enters houses. In humans, WNV infection is mostly associated with fever, headache, fatigue, malaise, muscle pain, and weakness (Hayes et al., 2005) while horses are associated with neurological disease (Venter et al., 2010).

1.4 Bunyaviridae: *Phlebovirus*

The *Bunyaviridae* family is the largest and most diverse group of RNA viruses and has a worldwide distribution. The *Phlebovirus* genus contains 70 viruses, which comprise 9 species and 33 tentative species. These viruses infect vertebrates, invertebrates and plants but most of are transmitted by arthropods (Elliott and Brennan, 2014).

1.4.1 Rift Valley fever virus (RVFV)

Rift Valley fever is a zoonotic disease of wildlife, domesticated livestock (especially sheep and cattle) and humans throughout Africa as well as the Arabian Peninsula (Pienaar and Thompson, 2013, Swanepoel and Coetzer, 2004, Bird et al., 2009). Although RVF is infectious to a variety of wild and domestic animals, sheep are the most affected (Swanepoel and Coetzer, 2004). The disease was first diagnosed in the Rift Valley of Kenya in the 1930s (Davies, 2010). In southern Africa, severe outbreaks of RVF became evident in countries such as South Africa, Namibia and Zimbabwe in the 1950s (Pienaar and Thompson, 2013). A major outbreak of RVF in South Africa caused the deaths of an estimated 100,000 sheep and 500,000

abortions among ewes between 1950 and 1951 (Swanepoel and Coetzer, 2004, Alexander, 1951). This major epidemic also affected cattle but unlike the sheep only a few cattle losses were recorded (Swanepoel and Coetzer, 2004). Smaller outbreaks of the disease have been reported in South Africa in 1952-53, 1955-59, 1969-71, 1981, 1996 (Swanepoel and Coetzer, 2004, Pienaar and Thompson, 2013). The most recent outbreak which lasted from 2008 to 2011 spread to all provinces in South Africa resulting in over 19,000 cases in livestock and 26 fatal cases in humans (Pienaar and Thompson, 2013, Glancey et al., 2015). RVF has subsequently given rise to several smaller periodic epizootics in livestock and wildlife, as well as epidemics in humans in South Africa (Pienaar and Thompson, 2013) and other countries in southern Africa such as Namibia and Zimbabwe (Nanyingi et al., 2015). These outbreaks have often been triggered by heavy rains which favour the breeding of mosquito vectors, affecting mainly cattle, sheep, goats and wildlife (Nanyingi et al., 2015, Pienaar and Thompson, 2013, Swanepoel and Coetzer, 2004). RVF is now endemic to all countries in southern Africa except Lesotho and Swaziland (Anonymous, 2017b). However, no epizootics or epidemics of RVFV have been reported in Botswana and Mozambique (Nanyingi et al., 2015, Blomström et al., 2016, Fafetine et al., 2016). In 2000, a major outbreak of RVF affecting sheep and goats was reported in Saudi Arabia and Yemen. This outbreak lasted from late 2000 to early 2001 and resulted in the deaths of 245 humans and the loss of thousands of sheep and goats (Swanepoel and Coetzer, 2004).

In southern Africa, several species of mosquitoes from different genera have been implicated in the transmission of Rift Valley Fever virus. Current evidence suggests that *Aedes mcintoshi* (formerly known as *Ae. lineatopennis*) and *Ae. dentatus* are the most important vectors of RVFV in Zimbabwe whereas *Anopheles coustani* and *Cx. theileri* have been implicated as epidemic vectors through virus isolations in field collected mosquitoes in both South Africa and Zimbabwe (Swanepoel and Coetzer, 2004, McIntosh, 1972). In South Africa, *Ae. mcintoshi, Ae. dentatus, Ae. unidentatus, Ae. juppi, Ae. caballus* and *Cx. theileri* are the most important mosquito vector species on the inland plateau while *Ae. circumluteolus* and *Cx. zombaensis* are the main vectors in the KwaZulu-Natal coastal lowlands. *Aedes argenteopuntatus* appears to be an important potential maintenance vector on the coastal lowlands but is less common (Jupp and Cornel, 1988). *Culex poicilipes* has also been implicated as a potential epidemic vector occurring within the same area (Jupp and Cornel,

1988, Swanepoel and Coetzer, 2004). *Eretmapodites quinquevittatus* has been implicated as a vector of RVFV in the coastal lowlands around Port Shepstone in KwaZulu-Natal Province (McIntosh, 1972).

1.4.2   Shuni virus (SHUV)

In southern Africa, Shuni virus was first isolated from *Cx. theileri* mosquitoes caught at Olifantsvlei near Johannesburg and from cattle in Pretoria in 1967, and cattle and a goat in the KwaZulu-Natal Province of South Africa (McIntosh, 1980). This detection took place during the same period the virus was discovered in *Culicoides* midges and livestock in Nigeria (West Africa) as part of arbovirus surveys conducted between 1964 and 1970 (Causey et al., 1972, Lee, 1979, Moore et al., 1975). Shuni virus was later isolated from the brains of 2 horses that died of neurological diseases, 1 in South Africa and the other in Zimbabwe (van Eeden et al., 2012a). The role of SHUV as a possible cause of unexplained neurological diseases in humans and other animals in Africa is potentially underestimated (van Eeden et al., 2012a). Very little additional published information appears to be available regarding this virus.

1.2   Ecology of mosquito species known to transmit medically significant arboviruses in southern Africa

Arboviruses have developed the capability to infect both mosquito and vertebrate hosts, thereby creating the platform for optimized spread of human and animal ailments. A substantial number of mosquito species are known vectors of arboviruses in the wild, while many others are capable vectors under laboratory settings (Conway et al., 2014). Approximately 300 mosquito species are known to transmit arboviruses (Liang et al., 2015). Mosquitoes in the subfamily Culicinae (mostly *Aedes* and *Culex* mosquitoes) are the most frequently associated with arbovirus transmission (115 and 105 types of arbovirus, respectively). Understanding the role of mosquito vector species in arbovirus transmission is vital for the development of new strategies to control the spread of arboviral diseases. In southern Africa, a few species in the genera *Anopheles*, *Coquillettidia* and *Mansonia* have also been implicated as vectors of arboviruses (McIntosh, 1980, McIntosh, 1975).

Historic and recent epidemiological studies have established that CHIK, SIN, WN, WES, BAN, DEN and RVF viruses are the most important mosquito-borne viruses in southern Africa (Rautenbach, 2011, McIntosh, 1986, Jupp, 1996, Swanepoel and Cruickshank, 1974). A review by Burt (Burt et al., 2014) addresses some of the medically important mosquito-borne viruses, but focuses more on lesser known arboviruses in South Africa.

Surveys over multiple decades across southern Africa (Jupp and NK, 1986, Kokernot et al., 1962a, Kokernot et al., 1962b, McIntosch et al., 1972, McIntosh et al., 1983, McIntosh et al., 1976b, Worth et al., 1961, Kokernot et al., 1961, Gear and Reid, 1957) have provided good insight regarding which species of mosquitoes are involved in the transmission of at least the most common of the mosquito-borne zoonotic arboviruses. These cluster within the genera *Aedes* and *Culex*, each representing a different transmission strategy. *Aedes*-borne viruses such as CHIK, DEN and WES tend to have primate or human reservoir hosts (McIntosh, 1986), while *Culex*-borne viruses often use birds as reservoir hosts, and these factors influence the distribution and epidemiology of the diseases they cause in humans and animals. *Aedes* and *Culex* have different breeding strategies and preferences which also represent fundamental differences. In the discussion below emphasis is given to the 7 most important *Aedes* species, followed by the 4 most important *Culex* species, summarizing the known ecology of each. These mosquitoes are *Aedes aegypti, Aedes furcifer/cordellieri, Aedes circumluteolus, Aedes unidentatus, Aedes mcintoshi, Aedes caballus, Aedes juppi, Culex theileri, Culex zombaensis, Culex univittatus, Culex neavei* and *Culex rubinotus*.

1.2.1 *Aedes* (*Stg.*) *aegypti*

Aedes aegypti is the key vector of yellow fever, DEN and CHIK viruses. This mosquito is widely distributed in most tropical and subtropical regions of the world (Service, 1992, Brown et al., 2014) between latitudes 35°N and 35°S below an elevation of 1000m (Muktar et al., 2016). *Ae. aegypti* is originally from Africa where its ancestral form *Ae. aegypti formosus* is a zoophilic treehole breeding mosquito (Kraemer et al., 2015). This species was initially introduced into new areas, especially seaports, by watercraft before spreading inland (Cheong et al., 1986). Likely, ships transporting slaves and goods carried eggs and adults of these mosquitoes to the Caribbean islands and other tropical and subtropical parts of the world. *Ae. aegypti* is a

complex species which can exist in two or three forms: domestic, peridomestic and sylvatic (Tabachnick et al., 1979). Domestic forms live and breed in small containers such as discarded tins, bottles, tyres, plant-pots or similar holders in urban settings, which are often in and around houses. The peridomestic form is often found in altered environments (often located between urban and rural zones) and farms while the sylvatic forms inhabit tree holes, tree stumps, pools in river beds and natural containers in forests (Tabachnick et al., 1979). Embryonic development can take two days in the tropics until hatching, while in temperate regions eggs can take up to a week to hatch (Nelson, 1986). Once embryonic development is completed, eggs can withstand long periods of desiccation before hatching when submerged in water (Toma et al., 2011). *Ae. aegypti* is a competent vector because it is highly anthropophilic and has adapted to breeding in containers in or around households and adults often rest in houses (Service, 1992). The multiple blood feeding within each ovarian cycles, on humans and high survival rates (Reiter, 2007b) jointly contribute to *Ae. aegypti* causing serious epidemics of disease in urban areas. *Ae. aegypti* also has the ability to distribute a few eggs at different sites, a practice called skip oviposition which serves as a driver for dispersal in this species (Reiter, 2007a).

Ae. aegypti has been widely recorded across southern Africa. In South Africa, it is commonly encountered in the tropical and subtropical areas of the country (Muspratt, 1956). The distribution of this species is probably governed by climate and its geographical range lies around the 20˚C isotherm (Muspratt, 1956, Kemp and Jupp, 1991) with the south-western part of the Western Cape Province falling outside the isothermal zone (Edwards, 1941). This species has established disjunct populations at different altitudes and locations in South Africa and is mainly associated with wooded savannah, forest and urban environments (Muspratt, 1956). As in other parts of the world, several mosquito surveys conducted in the Eastern Cape Province and southern KwaZulu-Natal coastal areas have confirmed that local *Ae. aegypti* populations are highly anthropophilic and have adapted to the peridomestic environment (Kemp and Jupp, 1991, Edwards, 1941, Mattingly, 1953). *Ae. aegypti* populations found in the Free State Province and the north-eastern parts of Limpopo and Mpumalanga Provinces are sylvatic and have become adapted to a wide range of larval habitats including natural tree holes and leaf axils (Kemp and Jupp, 1991). Ground and rock pools are occasionally exploited when they contain dead leaves (Muspratt, 1956). Although

females of the domestic or urban forms of *Ae. aegypti* exhibit a strong preference for human blood, they also feed on other animals, mostly mammals (Edwards, 1941). Sylvatic or rural forms appear not to be as strongly attracted to humans as urban forms (Kemp and Jupp, 1991). *Aedes aegypti* bites mostly during daylight hours, typically with a preference for the period about two hours after sunrise and several hours before sunset, but will also bite at night (Anonymous, 2018b). *Aedes aegypti aegypti* was the most dominant vector in the northern towns of Mozambique during an outbreak of dengue in 2014 while *Ae. aegypti formosus* was the most dominant in the capital, Maputo (Higa et al., 2015).

1.2.2   *Aedes* (*Dic.*) *furcifer/cordillieri*

The *Aedes furcifer* group in the Afrotropical Region comprises of three species: *Ae. furcifer*, *Ae. taylori*, and *Ae. cordellieri* (Huang, 1986)*. Ae. furcifer* and *Ae. cordellieri* are indistinguishable morphologically except by the confirmation of differences in male genitalia (Jupp, 1998). *Ae. furcifer* and *Ae. cordellieri* are key vectors of CHIKV in southern Africa (Jupp, 1996) and have both allopatric and sympatric distributions in the sub-continent (Jupp, 1998). Studies by Jupp (Jupp, 1998) show that *Ae. cordellieri* is found primarily within the tropical region (east of 18˚C mean midwinter isotherm), while *Ae. furcifer* is more common in the subtropical areas (west of this isotherm line). In South Africa, the two species are usually found in northern KwaZulu-Natal coastal lowlands, Kruger National Park, and the moist eastern part of Mpumalanga and Limpopo Provinces (Jupp, 1996, Jupp and McIntosh, 1990).

Peak population numbers follow periods of heavy rainfall, and such population peaks are often linked with outbreaks of CHIKV in humans. *Ae. furcifer* and *Ae. cordelieri* prefer to breed in tree holes usually in wooded savanna or evergreen forest (Huang, 1986). Both species feed mostly at night when primates are resting in trees or rocky outcrops but will readily bite humans they encounter on the ground (Jupp, 2005).

1.2.3   *Aedes* (*Neo.*) *circumluteolus*

In southern Africa, *Ae. circumluteolus* is one of the dominant mosquito species and most abundant in the tropical/sub-tropical coastal lowlands of northern KwaZulu-Natal northwards

into Mozambique (Jupp, 2004b, McIntosch, 1971). Like other congeners, *Ae. circumluteolus* is associated with floodwater and often oviposits at the edge of rivers and pans located within flood plains. Overwintering transovarially infected eggs then hatch when inundated with water and may give rise to disease outbreaks [111]. Larvae have often been associated with temporary shallow grassy pools of water on bush fringes (De Meillon et al., 1957). *Ae. circumluteolus* prefers to feed at ground level where it mostly targets humans, dogs, cattle, sheep and antelope (McIntosch et al., 1972, Paterson et al., 1964, Jupp, 2004a).

1.2.4 *Aedes* (*Neo.*) *unidentatus*

Aedes unidentatus is the primary vector of WESV and can become abundant at times in the higher rainfall areas of the Highveld and Middleveld of the Free State Province of South Africa (McIntosch, 1971). Prior to the revision of the subgenus *Neomelaniconion* by McIntosh (McIntosch, 1971), a few collections of this species in the Johannesburg area of South Africa were identified as *Aedes lineatopennis* (McIntosch, 1971, Jupp, 2004b, McIntosh, 1980). *Ae. unidentatus* is also one of several maintenance vectors of RVFV on the inland plateau of South Africa (Swanepoel and Coetzer, 2004). It is probably the most dominant species of the subgenus *Neomelaniconion* in these two areas where it breeds in temporary pools. *Ae. unidentatus* is a diurnal and nocturnal feeder with peak biting commencing shortly before sunset. The host preferences for this species includes goats, cattle, sheep and humans (Jupp, 2004b, McIntosch, 1971).

1.2.5 *Aedes* (*Neo.*) *mcintoshi*

Aedes mcintoshi is morphologically nearly indistinguishable from *Ae. lineatopennis* and closely resembles other African members of the subgenus *Neomelaniconion*, including *circumluteolus*, *luridus*, *luteolateralis*, and *unidentatus* (Huang, 1985a). *Aedes mcintoshi* is known to have a wide distribution across Africa, including Central, East, southern (Botswana, South Africa and Zimbabwe), North and West Africa. The species appears to be adapted to areas with intermediate temperatures (Jupp, 2004b). *Ae. mcintoshi* is widely distributed in areas with varying climate such as the Karoo, north-eastern Highveld, Lowveld and the coastal region of KwaZulu-Natal of South Africa and the Zimbabwean Highlands. Field studies on *Ae*

mcintoshi during an outbreak of RVF among cattle in Zimbabwe in 1969 revealed two strains of RVFV, 17 of WESV and two of MIDV (McIntosch, 1971, Huang, 1985a). *Ae. mcintoshi* is acknowledged as an important maintenance vector of RVFV in Zimbabwe and possibly also on the inland plateau in South Africa (Swanepoel and Coetzer, 2004). Although evidence for transovarial transmission of RVFV in *Ae. mcintoshi* remains elusive in South Africa (Jupp, 2005), isolates from field collected larvae and laboratory studies conducted in Kenya did confirm transovarial capacity and therefore potential for maintaining or initiating outbreaks in subsequent seasons (Linthicum et al., 1985, Romoser et al., 2011, Jupp, 2005). The peak biting period for *Ae. mcintoshi* is late afternoon until sunset with lesser peaks around dawn (Jupp, 2004a). Field observations showed a clear link between adult emergence of *Ae. mcintoshi* and wet season flooding of dambos (Linthicum et al., 1985, Jupp, 2004b). *Ae. mcintoshi* seems to occupy similar habitats with *Ae. unidentatus* in Gauteng Province and with *Ae. luteolateralis* along the northern coastal area of Kwa-Zulu Natal Province (Huang, 1985a). In South Africa, larvae of this species are encountered mostly in vegetated (especially sedge-grasses) flooded lakes, ground pools and to a lesser extent in dambos (Jupp, 2004b, Huang, 1985a). Preferred bloodmeal hosts for this species include goats, cattle, sheep and humans (McIntosch, 1971, Jupp, 2004a, McIntosh, 1972).

1.2.6   *Aedes* (*Och.*) *caballus*

Ae. caballus is a key vector of WESV and RVFV (Jupp, 2004b, Jupp, 1996). *Ae. caballus* populations have been encountered in the Karoo and southern Free State Province of South Africa, but their relative abundances require further investigation. This species is adapted to higher temperatures and lower rainfall but appears to be absent from the coastal belt of KwaZulu-Natal Province and Mozambique (Jupp, 2004b, McIntosh, 1973). *Ae. caballus* was the most prevalent mosquito encountered during an outbreak of WESV in the Eastern Cape Province (Kokernot et al., 1958). The preferred breeding habitat for this species is characterized by sedge or grassland in small or large temporary depressions after heavy rainfall (Jupp, 2004b). *Ae. caballus* tends to be a diurnal species, which feeds shortly before and after sunrise, mostly on goats, sheep, cattle and humans (Jupp, 2004b, McIntosh, 1973).

1.2.7 *Aedes* (*Och.*) *juppi*

Aedes juppi is one of the key vectors of WES and RVF viruses and has similar ecological requirements to *Ae. caballus* (Jupp, 1996, McIntosh, 1980, Jupp, 2004b, Swanepoel and Coetzer, 2004, McIntosh et al., 1980)*.* This species is probably the largest and easiest to recognize in the *Ae. caballus* group. *Ae. juppi* is endemic to temperate regions of southern Africa and probably the most abundant member of the *caballus* group in the Highveld of South Africa (McIntosh, 1973). *Aedes juppi* is present in the Western Cape Province of South Africa, a region not known to host *Ae. caballus.* Studies in the Free State Province of South Africa suggested that the sedge, *Mariscus congestus*, is an excellent indicator of *Ae. juppi* oviposition sites (Gargan et al., 1988). Like *Ae. caballus*, *Ae. juppi* prefers feeding during the day, especially before and after sunrise, on goats, sheep, cattle and humans (McIntosh, 1973, Jupp, 2004b).

1.2.8 *Culex* (*Cux.*) *theileri*

Culex theileri is broadly distributed in Africa, Europe, Asia and the Middle-East (Becker et al., 2003). In southern Africa, this species occurs in Namibia, South Africa and Zimbabwe (Anonymous, 2018a). *Culex theileri* is one of the main epidemic vectors of RVFV in domestic animals in South Africa and Zimbabwe (Swanepoel and Coetzer, 2004, McIntosh, 1980, McIntosh, 1986). Field studies and viral transmission experiments have implicated this species as the most efficient mosquito vector of RVFV on the inland plateau of South Africa (McIntosh et al., 1980). Not only is *Cx. theileri* the most highly susceptible to RVFV during natural infection and experimental transmission, but it is also the most important vector as it occurs in large numbers throughout the epizootic area and adults remain active over an extensive period from early summer to midwinter (McIntosh et al., 1980). This species is rare in the KwaZulu-Natal coastal lowlands and in the Limpopo-Mpumalanga Lowveld (Jupp, 2004b, Jupp et al., 1980). *Culex theileri* breeds in a wide range of pools from clear water and vegetation to polluted water with animal waste (Jupp, 1967). It is relatively ornithophilic but also feeds on goats, cattle, sheep and humans, with a preference for biting during the first few hours after sunset (Jupp, 2004a).

1.2.9 *Culex (Cux.) zombaensis*

Culex zombaensis is a sub-tropical species absent from the temperate inland plateau of South Africa. This species is moderately common and widely distributed in the northern KwaZulu-Natal coastal lowlands but rare in the south (McIntosh et al., 1983, McIntosch et al., 1972). *Culex zombaensis* was the major vector of RVFV during an epizootic in the KwaZulu-Natal coastal lowlands in 1981(McIntosh et al., 1983), being the only mosquito species of high abundance and yielding multiple isolations of RVFV during the outbreak. *Culex zombaensis* prefers rivers and permanent pans with clean water and emergent vegetation. Although it is predominantly a ground-hunting species which feeds on cattle, sheep, goats, antelope and humans at night, *Cx. zombaensis* also feeds on monkeys in the canopy of trees (Jupp, 2004b, McIntosch et al., 1972).

1.3.0 *Culex (Cux.) univittatus*

This species is widely distributed in the temperate highlands of the Afrotropical region, straddling countries across southern and East Africa and also reaching Madagascar and Yemen (Mixão et al., 2016). The *univittatus* group is comprised of two species: *univittatus* and *neavei* (Jupp and Harbach, 1990, Jupp, Mixão et al., 2016). *Culex univittatus* is common in South Africa especially in the Highveld and Karoo regions where it is a key vector of WN and SIN viruses (Jupp, 1971). In addition to being the maintenance vector of WN and SIN viruses, *Cx. univittatus* is considered a mosquito of public health importance in South Africa for its role as a competent vector in the transmission of WN, SIN and Usutu viruses (Jupp and NK, 1986, Braack et al., 2018). Epidemics of West Nile fever are possibly triggered by increased *Cx. univittatus* feeding on humans during periods of high vector densities (Causey et al., 1972). *Culex univittatus* reaches maximum abundance during periods of high rainfall coinciding with high temperatures (van Der Linde, 1982, Jupp, 1973). Adult females are known to overwinter in a nulliparous rather than parous state (Jupp, 2004b). Laboratory studies confirmed that *Cx. univittatus* females can survive, in a quiescent state in relatively low temperatures of up to 114 days at 14˚C (Cornel et al., 1993). *Culex univittatus* utilizes breeding habitats ranging from seasonal to permanent pools with clear water and vegetation. *Culex univittatus* primarily

feeds on birds but may also use mammals and humans during periods of peak abundance (Jupp et al., 1980).

1.3.1 *Culex* (*Cux.*) *neavei*

Culex neavei was initially described as a sibling species of *Cx. univittatus* but subsequently elevated to distinct species after discovery of subtle morphological differences and also reproductive isolation (Jupp). The two species are mostly allopatric with *Cx. neavei* occurring in the subtropical coastal lowlands of eastern and northeastern KwaZulu-Natal regions of South Africa but with some overlap occurring between the two species in the Lebombo mountains (Jupp, 1971). Unlike *Cx. univittatus* which is largely ornithophilic, *Cx. neavei* is both anthropophilic and ornithophilic (Jupp, 1971, McIntosch et al., 1972). This species is an important vector of RVF and SIN viruses (McIntosh, 1980, McIntosh et al., 1980, Jupp, 1996).

1.6 Gaps in knowledge about southern African arbovirus mosquito vectors

Ever since the introduction of Yellow fever from Africa to the Caribbean Islands and South America in the 1600's, mosquito-borne arboviruses have increased their spread and impact globally. Brief successes were achieved in vector control in the mid-20th century which brought some relief, but proved of temporary nature. Recent decades have seen a strong upsurge in the scale of public health threat by an increasing number of mosquito-borne viruses spreading across the globe, examples being Chikungunya, West Nile virus, and Zika. The ability to counter these challenges has proven inadequate and is attributable to a number of factors. These include:

I.   Improved vector identification
II. Vector biology
III. Anthropological behaviour
IV. Climate change

I.   Improved vector identification

Entomological expertise is essential for guiding surveillance and vector control programs, but there is currently a serious shortage of entomologists, more especially taxonomists. There are

relatively few African countries promoting entomology programmes at undergraduate level while some countries only have a few practicing expert entomologists (WHO, 2014). The scarcity of expert taxonomists can eventually lead to a pool of many wrongly identified mosquito vector species in the region. For example, the identification of some cryptic species such as the *Culex pipiens* complex has to some extent been successful with male mosquitoes, it has in most cases been problematic with females. A better understanding of cryptic species and diagnostic tools to identify such species is required. Thus, correct identification of known and potential mosquito vectors is especially important for any mosquito-based arbovirus surveillance program to be successful.

II. Vector biology

The spatial spread of mosquito-borne diseases (MBDs) is generally dependent on the distribution of suitable vector species. Factors affecting the species diversity, abundance and seasonal distribution of vector mosquitoes is in large measure related to climate and various environmental parameters. However, the impact and extent of these factors on mosquito distribution and abundance in southern Africa remains poorly understood. Thus, an understanding of the diversity and abundance of mosquito vectors across different landscapes and season is essential in determining the risk for MBDs and their dispersal across different geographic regions. Furthermore, mapping the distribution of changing mosquito populations in response to climatic variation and other factors may provide valuable information for evaluating risk potential for the transmission of arboviruses in southern Africa.

III. Anthropological behaviour

Large-scale land transformation due to anthropogenic activities are resulting in rapidly escalating changes in previously existing ecosystems, the organisms they support, the diseases associated with such land systems, and the consequences on human populations. Mosquitoes are reportedly among the most sensitive to human-induced environmental changes such as temperature, humidity, favourable breeding sites which ultimately affect their survival, density and distribution (Uneke, 2009). Habitat disturbance can therefore greatly affect the abundance and richness of mosquitoes (Junglen et al., 2009). Deforestation was shown to influence the biting rate of the malaria vector, *Anopheles darlingi*, in South

America (Vittor et al., 2006), whereas human-driven land use changes are associated with both altered abundance of arbovirus vector mosquitoes for West Nile virus and malaria mosquitoes in Europe (Ponçon et al., 2007b, Ponçon et al., 2007a). Global movement of people and goods through international trade and travel may also promote the spread of mosquito-borne viruses to areas where potential vectors exist (Tatem et al., 2006b, Tatem et al., 2006a, Wilson, 1995, De La Rocque et al., 2011). This tendency is believed to be worsened by climate change, which is increasing the suitability for vector-disease transmission in previously non-endemic areas (De La Rocque et al., 2011, Githeko et al., 2000).

IV.   Climate change

Mosquitoes are sensitive to changes in climate because temperature and humidity affect their survival rates (Uneke, 2009). Temperature is a major determinant of viral replication rates in mosquitoes and vector population growth rates (Dar and Wani, 2010, Kilpatrick et al., 2008). The period between one blood meal and the next is also influenced by temperature, thus increasing the chances of disease transmission (Martens et al., 1995, Scott et al., 2000, Morin et al., 2013). As to whether climate change influences mosquito populations in southern Africa, remains unclear. It is also currently not known if novel mosquito-borne viruses may emerge and spread and whether climate change will make the region more suitable to vectors and pathogens. There is speculation that changes in climate might result in epidemics of mosquito-borne diseases because of varying mosquito vector lifecycles and species' range shifts but this has not been proven in southern Africa (Dar and Wani, 2010, Mellor and Leake, 2000, Davis and Vincent, 2017, Campbell et al., 2015, Elbers et al., 2015, Reiter, 2001).

1.7   Aims and objectives

The PhD was designed to answer the questions listed in the objectives. Although the PhD candidate was involved in preparing the protocols, collection and identification of mosquitoes, the candidate was not involved in identifying and setting up of the sentinel sites.

1.7.1   Aims

1. To gain an understanding of the diversity and abundance of mosquito species in a range of climate and landscape zones in southern Africa.

2. To evaluate current knowledge of mosquito vector/arbovirus interactions in southern Africa.

1.7.1.1   Secondary objectives

1. To determine the mosquito species diversity and relative abundance in key landscape zones known to be subject to periodic arbovirus outbreaks.

2. To investigate the influence of environmental variables on mosquito population dynamics under field conditions.

3. To identify potential mosquito vectors and their association with arboviruses in southern Africa.

1.7.2   Hypothesis

The population composition of arbovirus vector mosquitoes varies with season and landscape gradients in southern Africa.

CHAPTER TWO

Mosquito community composition and abundance at contrasting sites in northern South Africa, 2014–2017*

Abstract

Most data on species associations and vector potential of mosquitoes in relation to arboviral infections in South Africa date back from the 1940s to late 1990s. Contextual information crucial for disease risk management and control such as the sampling effort, diversity, abundance and distribution of mosquitoes in large parts of South Africa still remains limited. This paper investigates the spatial variation in community composition, diversity, distribution, and abundance as well as the sampling effort on mosquito vectors at six sentinel sites in three provinces in the northern part of South Africa where recent arboviral cases had been detected in animals. Adult mosquitoes were collected from two horse properties in Gauteng Province; two wildlife reserves in Limpopo Province and at Orpen Gate in Kruger National Park and Mnisi Area in Mpumalanga Province between 2014–2017, using carbon dioxide-baited light and tent traps. *Culex poicilipes*, was the most abundant species caught during the study period. Highest diversity and species richness were found at Lapalala Wilderness Reserve, while the lowest diversity and abundances were at Orpen in Kruger National Park. *Aedes aegypti, Ae. mcintoshi*, *Ae. metallicus*, *Ae. vittatus*, *Cx. pipiens* sensu lato, *Cx. theileri* and *Cx. univittatus,* which are potential arbovirus vectors, had the widest geographical distribution in northern South Africa. Also collected were *Anopheles arabiensis* and *An. vaneedeni*, both known malaria vectors in South Africa. Therefore, arbovirus surveillance and vector control programs should be augmented in peri-urban and mixed rural settings where there is greater risk for arbovirus transmission to humans and domestic stock.

Keywords

Mosquitoes, Abundance, Arboviruses, Malaria, Distribution, Diversity, Richness, Mosquito vectors, Sampling effort, Landscape

2.0   Introduction

In southern Africa, the species associations and vector potential of mosquitoes in relation to arboviral infections received particular attention during the period early 1940s to late 1990s (Edwards, 1941, Gear et al., 1955, Jupp et al., 1980, Kokernot et al., 1957a, Worth et al., 1961, Jupp and Phillips, 1998). South Africa is a known hotspot for the occurrence of arboviruses of medical importance (Venter et al., 2014, Venter, 2018, Braack et al., 2018). Despite the research of earlier decades and the increasing recognition of the importance of mosquitoes as agents of public health concern, there is little or no information on the diversity, distribution, and relative abundance of mosquito vectors in large parts of South Africa and the sub-region (van Der Linde, 1982). A recent published survey (Cornel et al., 2018) did attempt to partially address this information gap by describing the species composition, diversity, distribution, and relative abundance of mosquitoes at selected broadly distributed localities within the country, but the study was very limited in time and space.

Several landscape attributes have been shown to be important in predicting mosquito abundance and composition (Reisen, 2010). Land cover/vegetation, canopy cover, soil properties, elevation, and hydrology are but a few of the known environmental factors that have been associated with abundance of mosquitoes (Burkett-Cadena et al., 2013). An understanding of the abundance and spatial distribution of mosquito vectors across different landscapes is essential in determining the risk for mosquito-borne diseases (Ostfeld et al., 2005, Roche et al., 2012). In mosquito borne disease systems, pathogen transmission may vary with changes in host and mosquito species diversity, abundance, and distribution across a landscape (Smith et al., 2004, Roiz et al., 2015, Burkett-Cadena et al., 2013). Since vectors are not evenly distributed within their geographical range, aggregation occurs within the landscape (Smith et al., 2004, Zhou et al., 2004, Ellis, 2008). Similarly, the spatial pattern of disease risk is patchy, with highest risk being in areas with consistent or periodic high numbers

of prime vector species combined with patches of pathogen reservoir (Ostfeld et al., 2005, Eisen and Eisen, 2008). Thus, vector diversity, density and distribution of species are important elements of the spatial dynamics of mosquito borne diseases (Borg et al., 2007).

The present study was undertaken to provide greater detail and granularity regarding spatial variation in community composition, diversity, distribution, and abundance of mosquito vectors in sentinel monitoring areas of northern South Africa known to be prone to regular occurrence of zoonotic arbovirus outbreaks in humans or animals, and assessment of the effect of sampling effort. Collected mosquitoes were also subjected to virus assays for vector incrimination to confirm or improve on current understanding regarding vector status.

2.1   Materials and Methods

2.1.1 Study sites

Monthly mosquito collections were carried out from 2014–2017 at four core sites: two nature reserves (Marakele National Park and Lapalala Wilderness Reserve) in Limpopo Province and two horse farms at Boschkop (East of Pretoria) and Kyalami (Midrand/Johannesburg) in Gauteng Province in South Africa. Opportunistic sampling was also conducted at two other sites: Orpen Gate in Kruger National Park and Mnisi in the Ehlanzeni District Municipality in Mpumalanga Province (Figure 2.1). The ecological features associated with these sites include Highveld grassland (Boschkop and Kyalami), Middleveld Bushveld (Lapalala and Marakele) and Lowveld Bushveld (Orpen and Mnisi) ecosystems (Rutherford et al., 2006). The Lowveld Bushveld is a region which varies between 150 and 600m in elevation while the Middleveld Bushveld lies between 600 and 1200m above sea level. The largest subregion is the plateau which makes up a 1200–1800m high central area known as the Highveld. Physical characteristics associated with the sampling localities are shown in Figure 2.2. The sites also occur in different climatic zones ranging from warm semi-arid, subtropical oceanic highland to humid subtropical climates (Peel et al., 2007). Some of these aspects are discussed in greater detail later in this paper.

Figure 2. 1: Map of South Africa showing the six vector surveillance sites. Red stars show the 4 core sites with regular monthly sampling 2014 to 2017 while blue stars show opportunistic sites periodically from 2015.
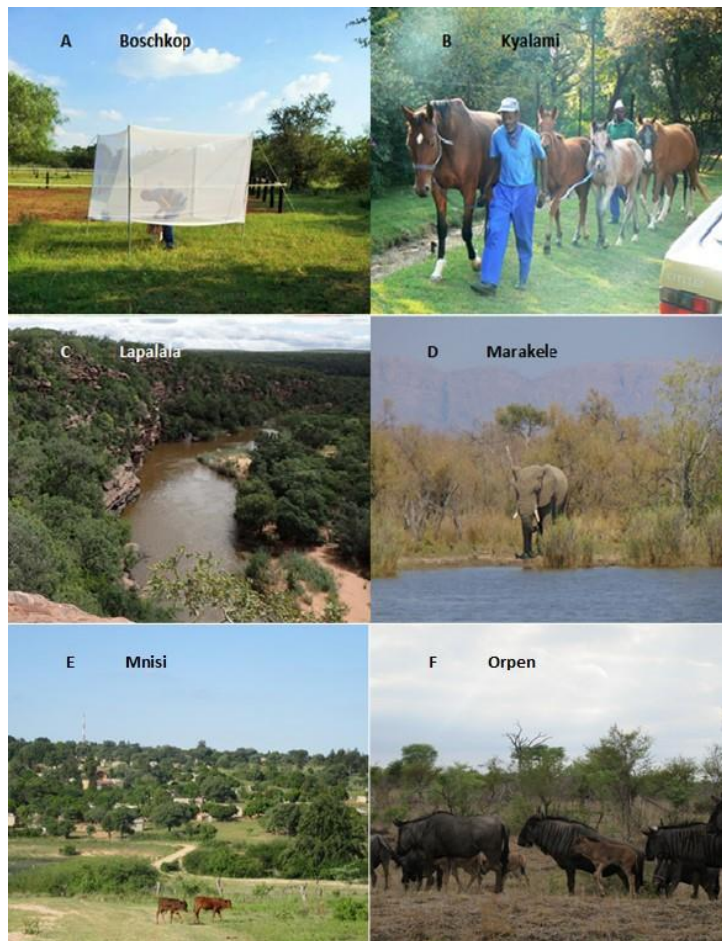
Figure 2. 2: Some of the physical features found at the sampling locations in northern South Africa. A: Boschkop. B: Kyalami. C: Lapalala. D: Marakele. E: Mnisi. F: Orpen.

2.1.2 Geographic and climatic attributes of sampling sites

The key geographic and climatic attributes of the various sampling areas are summarized in Table 2.1. These attributes are critically important in enabling interpretation and understanding of mosquito diversity and abundance at the various collection sites. Also important to note is that the regular monthly sampling sessions at Boschkop, Kyalami, Lapalala, and Marakele meant that collections were made in both dry and wet seasons, over multiple years. This also means that data distortions due to infrequent collections at Orpen and Mnisi during predominantly dry periods are compensated for and enable a more accurate reflection of true diversity and abundance.

Table 2. 1: Geographic and climatic features associated with mosquito sampling sites in northern South Africa, 2014–2017.

	Lapalala	Marakele	Boschkop	Kyalami	Orpen	Mnisi
Location (GPS coordinates)	S23°53'59.8" E28°17'50.1"	S24°17'37.4 E27°30'11.7"	S25°49'40.2" E28°25'12.4"	S25°59'22.3" E28°01'56.1"	S24°28'55.8" E31°23'08.9"	S24°40'41.2" E31°16'15.5"
Province	Limpopo	Limpopo	Gauteng	Gauteng	Mpumalanga	Mpumalanga
Land-use	Wildlife Reserve	Wildlife Reserve	Horse farm	Horse farm	Wildlife Reserve	Mixed rural cattle/wildlife/human use
Landscape description	Mountains and hills with fairly dense savanna woodland, several river-courses	Open plain with moderate to sparse savanna woodland, one narrow river	Peri-urban grassland	Peri-urban grassland	Flat, natural fairly dense savanna woodland	Flat, significantly transformed open savanna woodland
Ecological zone	Middleveld Bushveld	Middleveld Bushveld	Highveld Grassland	Highveld Grassland	Lowveld Bushveld	Lowveld Bushveld
Annual rainfall (mm)	400–900 (Ruwanza and Mulaudzi, 2018, Hulsman et al., 2010, Ben-Shahar, 1987)*	556–630 (van Staden and Bredenkamp, 2005)*	677–697**	723–790**	550–600 (Gertenbach, 1980)*	600–700 (Gertenbach, 1980, Gaudex, 2014)*
Annual mean maximum temperature (°C)	30.9**	30.2**	27.7**	25.6**	29.6**	29.4**
Annual mean minimum temperature (°C)	16.1***	14.5***	12.5***	12.6***	17.1***	17.1***
Altitude (m)	1163	969	1372	1415	452	468
Blood-meal availability	Abundant and diverse range of birds and mammals	Abundant and diverse range of birds and mammals	Horses, humans and limited range of other mainly domestic animals and some wild birds	Horses, humans and limited range of other mainly domestic animals and some wild birds	Abundant and diverse range of birds and mammals	Plentiful mix of cattle, humans, birds and low abundance of wildlife species.

*References
**Source: Climate-Data.org
***Based on 3 year (2014–2016) climatic data provided by the South African Weather Service (SAWS). Means were computed on data from the SAWS Station close to the study site.

2.1.3 Mosquito collection

Mosquito sampling for this study commenced in January 2014 and continued until May 2017. Sampling took place once every month for three consecutive nights at the nature reserves and for two nights at the horse farms, with each sampling period running from 4:00pm to 6:00am. Mosquitoes were collected using mouth aspirators, polystyrene cups and two types of CO_2-baited traps: Centers for Disease Control (CDC) (Newhouse et al., 1966) miniature light traps and Mosquito Tent traps (MTT). Two light (ultraviolet and incandescent) and three to five tent traps were erected each night at the nature reserves while two light and three tent traps were placed per night at the horse farms. Traps were emptied at or very soon after dawn, all collection containers placed in cooler boxes with dry ice at -80˚C until microscopically sorted and identified on cold plates (to ensure virus preservation) a few hours later, and immediately returned to cold storage.

2.1.4 Mosquito identification

A stereomicroscope was used to separate mosquitoes placed on an ice brick. Mosquitoes were sorted by locality, trap type, and date of collection, and then morphologically identified to species level using regional keys and descriptions (Edwards, 1941, Gillies and Coetzee, 1987, Gillies and De Meillon, 1968, Jupp, 1996). Damaged specimens lacking key morphological identification characteristics were identified to genus level and recorded as such. Specimens destined for PCR assay (*An*. *funestus* group and *An*. *gambiae* complex) were placed in silica gel tubes, while other species were pooled maximum 50 specimens per tube, each species was separated, for virus assays. Although *Cx. quinquefasciatus* and *Cx. pipiens* are sympatric in the highveld region, no attempt was made to identify the two to species level using taxonomic keys. In this particular case, individual specimens from both species were referred to as *Cx. pipiens s.l.*

2.1.5 Data Analysis

Kolmogorov-Smirnov (Lilliefors modification) and Shapiro-Wilk tests were used to analyse mosquito count data for normality, while Levene's test was used to test for homogeneity of variance with Statistica 13.3 (Drezner and Turel, 2011, Royston, 1982, Shapiro and Wilk, 1965,

Gastwirth et al., 2009). Due to the lack of normality of the data, large standard deviations and lack of homogeneity of variance, non-parametric tests were used to analyse mosquito densities. Since mosquito abundance data were not normally distributed, the non-parametric test, Kruskal-Wallis (> two samples) test was used to assess differences in mosquito density/trap-night across localities and ecological zones. Chi-square tests were applied to investigate whether there were any differences in abundance of known or suspected mosquito vector species between sites. Species diversity, evenness, and equitability were calculated for all localities in the three Provinces, localities compared by category; peri-urban (Boschkop and Kyalami), mixed rural (Mnisi) and sylvatic (Lapalala, Marakele, and Orpen). As the performance of diversity estimators varies among data, the following well-known species diversity estimators were assessed using PAST software (Hammer et al., 2001): Shannon and Simpson diversity indices. Shannon *H'* is a measure of the diversity itself, shown in a comparative way. This calculated as $H' = -\sum_{i=1}^{R} p_i \ln(p_i)$, where $p_i = n_i/N$. The Shannon Index is based on the uncertainty that an individual taken at random from the dataset is predicted correctly as a certain species. Larger values represent larger uncertainty, thus greater diversity. Simpson's Index of Diversity was calculated; $1-D = 1-\sum(n_i(n_i-1)/N(N-1))$, where n_i is the number of the ith species and N is the total number of specimens in the study area or habitat. Simpson's Index of Diversity reflects the probability that two individuals taken at random from the dataset are not the same species. Values for Simpson's Index of Diversity range between 0 and 1, with larger values representing greater diversity. This method is sensitive to sample size, whereas the Simpson's Index puts more weight on dominant species and is hardly influenced by a few rare species. The Shannon Evenness (*E*) is a measure of how much the species are equal in the sense of number of specimens, for each time of collection. It is a similar measure to equitability and is given by *E = e^H'/S* where *H'* is the observed diversity index, and *e* is the base of the natural logarithms. The quantity *e^H'* is the minimum number of equally common species which could yield the observed diversity *H'*. The equitability (J) concept assumes that J = *S'/S*, where *S'* is the theoretical number of species which would yield the observed diversity *H'* if their relative abundances followed the broken-stick model of MacArthur. In order to ensure that estimates of *S* were reliable, estimates of total species number based on extrapolations from species accumulation curves were computed using the Chao1 richness estimator, for each organism in PAST and compared them with the observed total species. The Chao1 was used because individual counts of species in

samples usually underestimate species richness and greatly depend on sampling effort and sample completeness (Chao and Chiu, 2001). To verify whether sufficient trapping effort was made during the mosquito surveys to achieve a statistically sound estimate of species diversity, rarefaction curves of the species and the number of collected mosquitoes were generated using PAST software. Rarefaction is an interpolation of a biodiversity sample to a smaller number of individuals for comparison among samples. For species incidence data, rarefaction interpolates between a specific sample and a smaller number of sampling units.

2.2   Results

2.2.1 Interpolating species richness and sampling effort with rarefaction

Using two different trap types at core and opportunistic sites in northern South Africa, a total of 42,286 mosquitoes were collected from 1,654 trap-nights between January 2014 and May 2017. Rarefaction curves suggest that species richness is higher at Lapalala followed by Marakele while it was similar at Kyalami and Boschkop but much lower than what was recorded at the two nature reserves (Figure 2.3). Mosquito abundance data from core sites are shown in Table 2.2. Estimates of total species number based on trap type showed that observed richness values likely underestimated total richness as the rarefaction plots for Marakele and Lapalala are almost beyond their exponential curves and thus, begin to level off just beyond sixty species. This suggests that there is a likelihood of discovering more species with further sampling and increased effort as estimated by the Chao1 in Table 2.3.
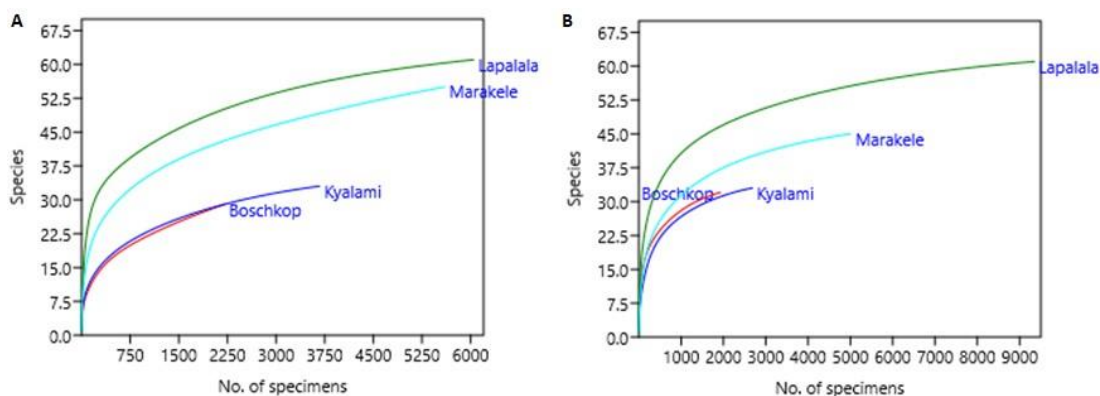
Figure 2. 3: Sampling effort. Species based rarefaction curves generated from tent (A) and light (B) trap collection data showing number of species against number of specimens recorded at core sampling sites within South Africa. A: Tent traps. B: Light traps.

Table 2. 2: Mosquito sampling effort of two trapping techniques and their associated yield at six localities in northern South Africa, 2014–2017.

Locality	Number of catch efforts / trap-nights	Mean number of catches		Total number of catches	
		Light trap	Tent trap	Light trap	Tent trap
Boschkop	308	19.6	10.5	1922	2208
Kyalami	299	27.3	18.2	2678	3664
Lapalala	507	51.1	16.9	9345	6042
Marakele	432	37,0	17.7	4994	5599
Mnisi	64	8,0	73.6	20	4610
Orpen	44	11.5	36.4	185	1019

2.2.2 Diversity indices

Mosquito species richness was highest at Lapalala Wilderness Reserve where a total of 74 species was documented over a period of 3 years (507 trap nights); the lowest richness of 29 species was recorded in a period of less than two years at Orpen (44 trap nights; Table 2.3). Biodiversity estimates on total collections of both core and opportunistic catches were calculated per locality (Table 3). A comparison of diversity using the Shannon and Simpson indices showed that Lapalala (H'=2.842, λ=0.9066) had the highest diversity while Orpen had the least (H'=0.8914, λ=0.314). The Simpson dominance index confirmed the presence of dominant species at Orpen (D=0.686), which is less diverse than the other sites. Species evenness (E) ranged from 0.08409 in Orpen to 0.2317 in Lapalala (Table 2.3). In this study, the mosquito community was somewhat evenly distributed at Lapalala but less so at Orpen.

2.2.3 Species composition and relative abundance of mosquitoes

Ninety-five species representing 10 genera: *Aedeomyia*, *Aedes*, *Anopheles*, *Coquillettidia*, *Culiseta*, *Culex*, *Ficalbia*, *Mansonia*, *Mimomyia*, and *Uranotaenia* were collected in either or both the tent and light traps (Figure 2.4 and Table 2.4). The most abundant genera were *Culex* (38.4%), *Anopheles* (32.2%) and *Aedes* (21.2%) (Table 2.4 and Figure 2.4). This is a simplistic and generalized representation which neglects the underlying distortions of greater sampling intensity and habitat diversity at specific sites, but nevertheless does facilitate some general overall impression of mosquito composition. *Culex poicilipes*, represented by 5,793 specimens was the most abundant species caught in this survey, comprising 13.7% of the total (Table

2.4), but most individuals were collected from Marakele (where it represented 35.8% of the catches) and Mnisi (where it represented 38% of the catches) (Figure 2.5). However, it was not among the seven most widely distributed species. *Aedes aegypti, Ae. mcintoshi*, *Ae. metallicus*, *Ae. vittatus*, *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* had the widest geographical distribution in northern South Africa (Table 2.5). In the savanna regions (Middleveld and Lowveld) *An. coustani* (Lapalala), *Cx. poicilipes* (Marakele, Mnisi) and *Ae. vittatus* (Orpen) were the most dominant mosquito species. In the Central Highlands, at Boschkop and Kyalami, *Cx. theileri* and *Cx. univittatus* were the most abundant species. A sizeable population of *Cx. pipiens s.l.* accounting for approximately 20% was among the three most dominant species at Kyalami. Species with relative abundances of less than 2.5% at a given habitat were considered as 'rare or other', with eighty-three taxa falling into this category (Table 2.4), which represents 15.4% of total species recorded.
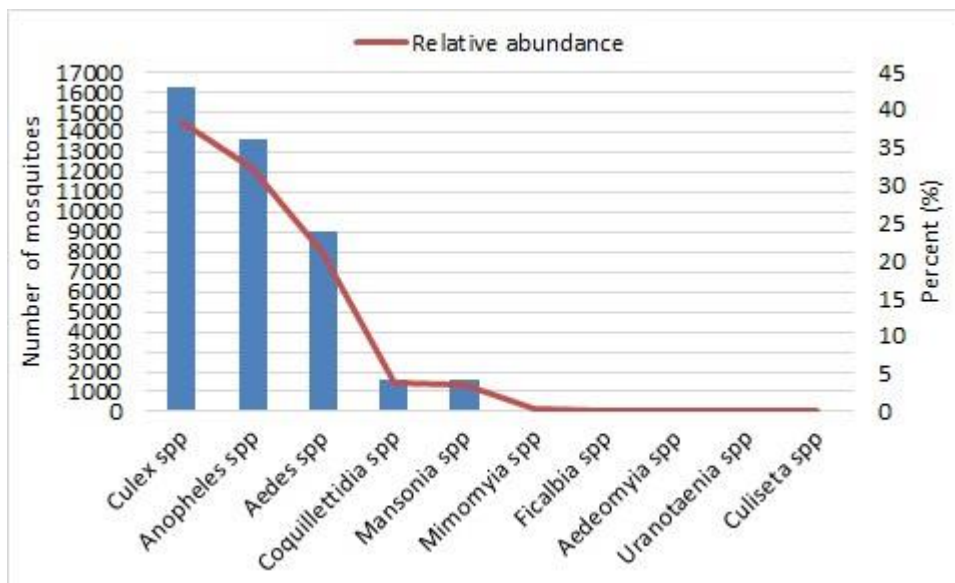
Figure 2. 4: Total abundance. Mosquito numbers grouped by genera including the total number of adults collected from all six sampling localities.
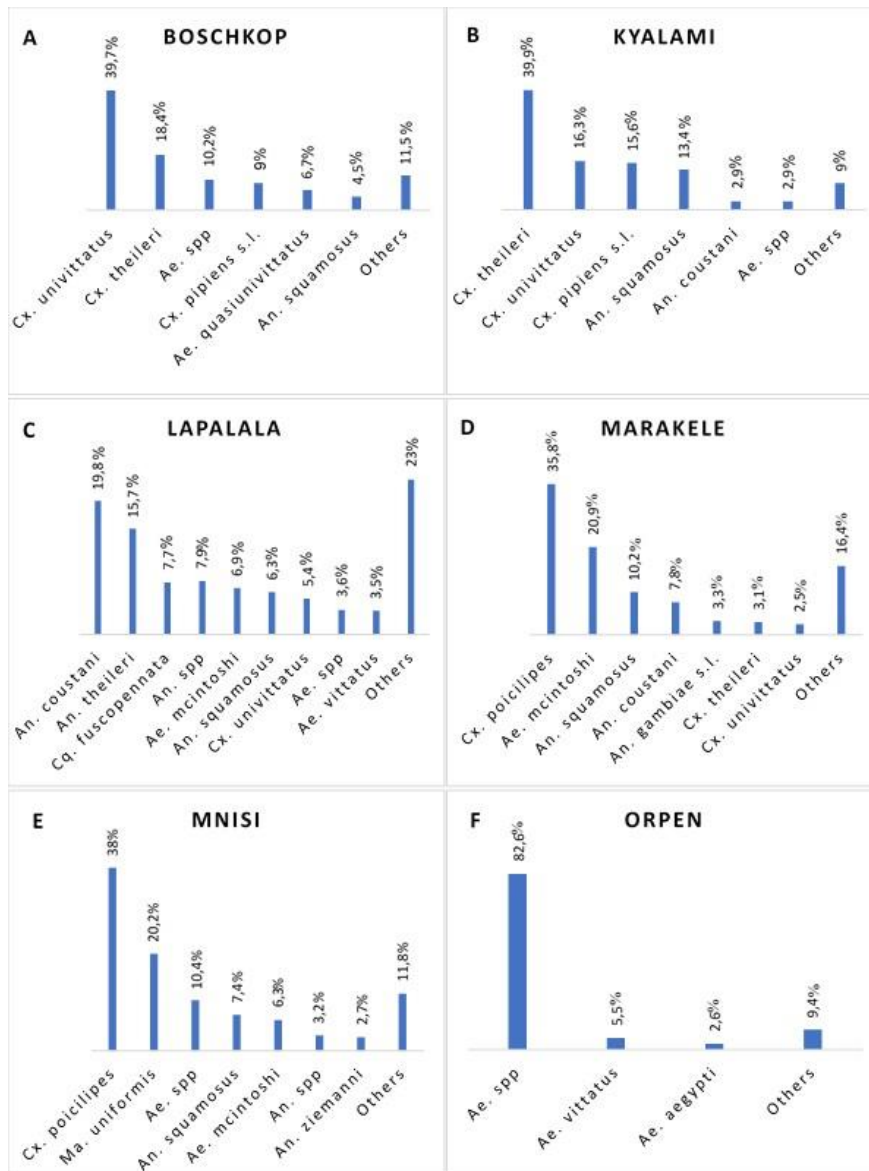
Figure 2. 5: Species composition and relative abundance of mosquito vectors collected from the six localities irrespective of trap type. A: Boschkop. B: Kyalami. C: Lapalala. D: Marakele. E: Mnisi. F: Orpen.

When the landscape of northern South Africa is differentiated into peri-urban, mixed rural, and sylvatic locales, there are detectable differences in the species composition and relative abundances of the most dominant species (Figure 2.6). In rural settings such as the Mnisi area, the two most abundant species were *Cx. poicilipes* and *Ma. uniformis* while in peri-urban areas east of Pretoria and north of Johannesburg, *Cx. theileri*, *Cx. univittatus* and *Cx. pipiens* were the most dominant. However, in sylvatic areas which are all wildlife sanctuaries, the most dominant species were *Cx. poicilipes*, *An. coustani* and *Ae. mcintoshi*.
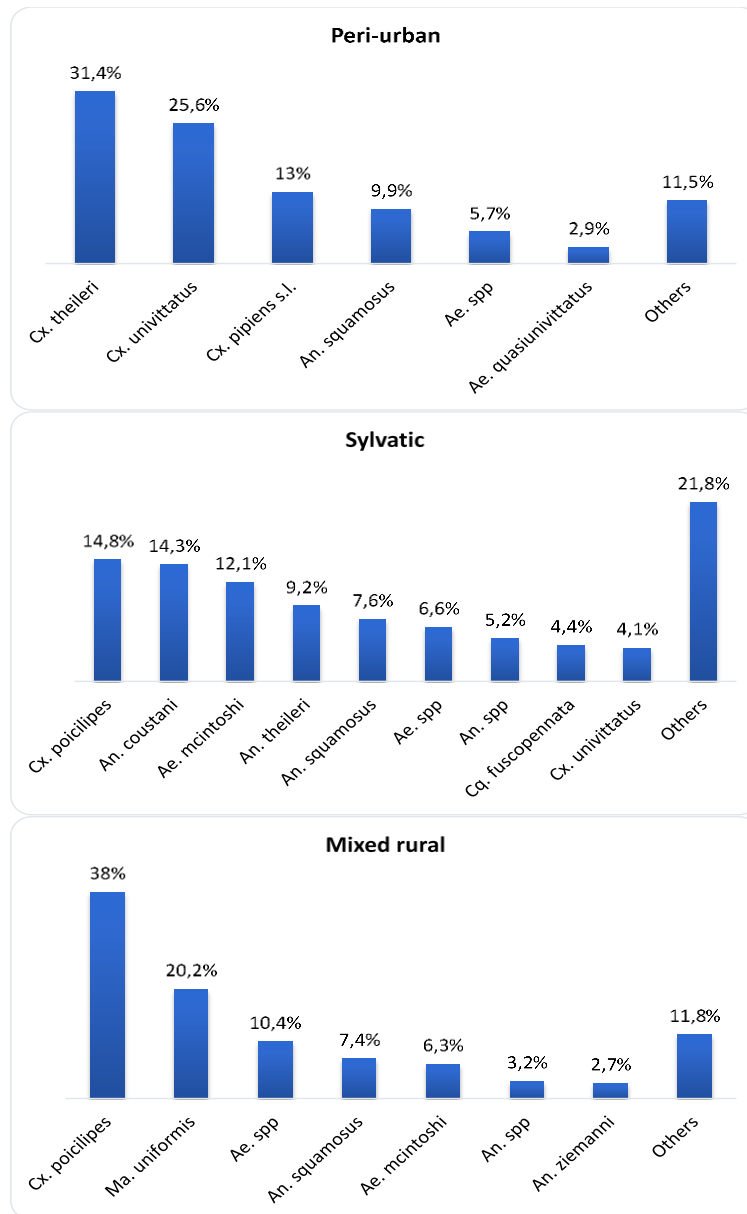
Figure 2. 6: Composition and relative abundance of dominant mosquito species. Mosquitoes were collected from both tent and light traps in three landscape settings within northern South Africa. A: Peri-urban. B: Mixed rural. C: Sylvatic.

2.2.4 Geographic distribution of mosquito species across localities and landscapes

The mean abundance of the most dominant mosquito species caught in both trap types at core sites in northern South Africa is shown in Figure 2.7. The species are arranged alphabetically by genus from *Aedes* to *Culex*. Although present at all core sites, the mean abundance for *Ae. mcintoshi* was almost zero at Boschkop, Kyalami and Orpen but averages ranged between three and five mosquitoes/trap-night at Lapalala, Mnisi and Marakele respectively (Kruskal-Wallis = 115, *P*<0.001).
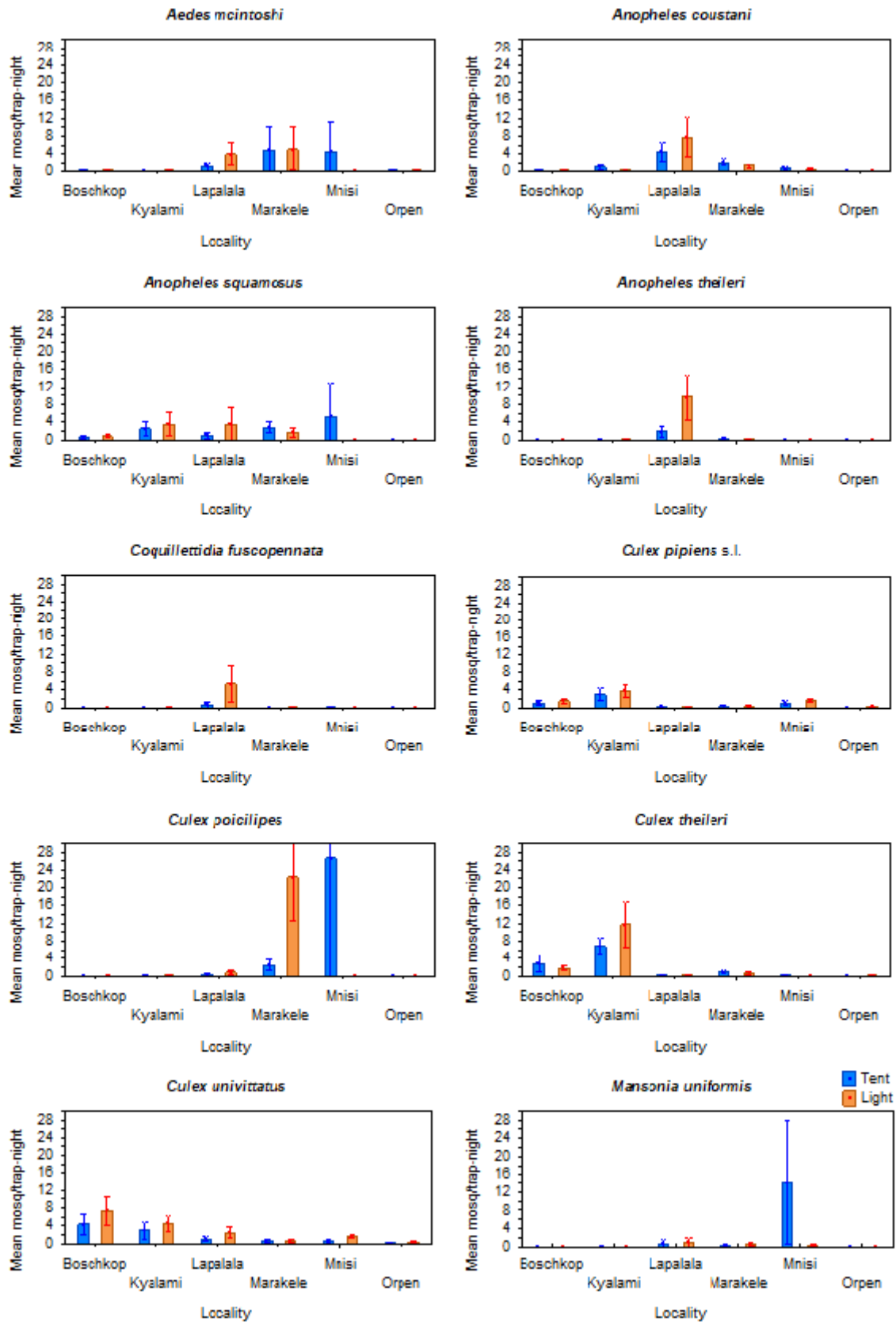
Figure 2. 7: Mean abundance of most dominant mosquito species. These species were collected from core sites within South Africa between January 2014 and May 2017. The whiskers and boxes on the bars represent SDs and SEs of the mean of mosquitoes/trap-night for tent and light trap collections.

Anopheles coustani, although present at all sites except Orpen, attained higher abundances at Lapalala where the mean was almost six mosquitoes/trap-night (Kruskal-Wallis = 282, *P*<0.001. In the remaining three regions, this species was less abundant with the mean being ≤2 mosquitoes/trap-night. The average abundance of *Anopheles squamosus* was low at all sites with the highest mean value recorded being slightly above five mosquitoes/trap-night in tent traps at Mnisi (Kruskal-Wallis = 126, *P*<0.001). This species was completely absent in both tent and light traps at Orpen. *Anopheles theileri* though only recorded at Boschkop, Kyalami, Lapalala and Marakele, only reached high numbers from light traps at Lapalala. The mean abundance of this species from light traps was nine mosquitoes/trap-night compared to less than two mosquitoes/trap-night in tent traps.

Coquillettidia fuscopennata, was recorded only at Kyalami, Lapalala, Marakele and Mnisi, with a mean of five mosquitoes/trap-night at Lapalala and almost zero at Kyalami, Marakele and Mnisi (Kruskal-Wallis = 183, *P*<0.001).

Although present at all localities, *Cx. theileri* was the most abundant species at Kyalami with average catches reaching six and 11 mosquitoes/trap-night in tent and light traps respectively. *Culex univittatus,* though present at all sites, was only common at Boschkop and Kyalami.

The abundance for *Culex pipiens s.l.* was generally low at all localities except Kyalami where the mean was above three mosquitoes/trap-night. *Culex poicilipes* was the most abundant at Mnisi where it attained an average of 26 mosquitoes/trap-night from tent traps. However, at Marakele, mean was highest in light traps. Although there was a significant difference in mosquito density between sites for each of the species (Kruskal-Wallis test *P*-value for all species was <0.001), high standard deviations from the mean were registered for most species (Figure 2.7).

In the Lowveld, *Culex poicilipes* reached a peak density of 13 mosquitoes per trap from <1 mosquito per trap in the Highveld region. The mean density for *An. squamosus* increased from <2 mosquitoes per trap in the Highveld to more than two mosquitoes per trap in the Lowveld region. *Mansonia uniformis* reached a high of seven mosquitoes per trap in the Lowveld from

<1 in the Highveld. The mean density for *Culex pipiens s.l.* decreased from the Highveld (>2 mosquitoes/trap) to the Middleveld (almost zero mosquitoes/trap) but then a slight increase was noticed in the Lowveld (<1 mosquitoes/trap). A clear decline in mosquito density was noticed for *Cx. univittatus* and *Cx. theileri* from the Highveld to the Lowveld. As for *Ae. mcintoshi*, there was a tendency for the mean density to increase towards the centre of the distribution range for this study which is the Middleveld. Similar tendencies were also observed for *An. coustani* and *Cq. fuscopennata*.

2.2.5 Abundance and distribution of mosquitoes of known medical importance

From this survey, two species complexes known to contain vectors of malaria, *Anopheles gambiae s.l.* and *Anopheles funestus s.l.*, were collected. Of the *Anopheles gambiae* complex, *An. arabiensis* was collected from Lapalala, Marakele, Orpen and Mnisi, albeit in very low numbers except for Marakele were it was among the most dominant vectors. Of the *Anopheles funestus* group, the species identified by PCR assays was *An. vaneedeni,* a secondary vector for malaria in the region (Burke et al., 2017, Mouatcho et al., 2018). There was a significant difference between locality and mosquito abundance for the seven widely distributed potential vectors of arboviruses with the Chi-Square test *P*-value for all species being <0.001). *Aedes dentatus* which is a vector for Middelburg (MID) and Rift Valley fever (RVF) viruses while *Ae. unidentatus* is a vector for RVF virus are quite common in the Highveld Grassland region (McIntosch, 1971, McIntosh, 1980, Braack et al., 2018, Venter, 2018, McIntosh, 1972). This also applies to *Ae. juppi* which is a known vector for MID, Wesselsbron (WES) and RVF viruses in the same region (Jupp PG et al., 1987, McIntosh, 1980, McIntosh et al., 1980, Braack et al., 2018, Venter, 2018). While *Ae. aegypti* was present at all the six localities, it occurred in low numbers. *Ae. vittatus*, a potential vector for Babanki (BBK), Chikungunya (CHIK), Ngari (NRI), Pongola (PGA), WES, Yellow fever (YF) and Zika (ZIK) viruses though present at all localities was recorded in higher numbers at Lapalala with catches exceeding 500 mosquitoes for the entire duration of the study (Braack et al., 2018, McIntosh, 1980, Jupp and McIntosh, 1990, Venter, 2018, Diallo et al., 2014, Diagne et al., 2015). The presence of such a diversity of arbovirus vectors at the various collection sites explains the periodic outbreaks of arbovirus cases recorded from these sites and emphasizes the risk of continued outbreaks in future. These aspects are discussed in a subsequent chapter.

2.3   Discussion

The main limitation with species richness estimates is their dependence on sampling effort which is seldom documented (Gaston and Biodiversity, 1996). This creates a dilemma of determining the absolute species richness of a taxonomic group or geographic area because the frequency at which new species are being noted is an important variable (May, 1990, Simon, 1983). The inadequacy of information on sampling effort hinders the comparison of the richness of different regions (Gaston and Biodiversity, 1996). Nevertheless, the use of new estimators such as the individual-based and sample-based assessment protocols help to correct the situation. In this survey, estimates of species richness from the individual-based rarefaction curves reveal that the spatial distribution of mosquitoes in the selected rural, semi-urban and wildlife sites in the Northern part of South Africa is random (Gotelli and Colwell, 2011). If mosquito species were spatially aggregated, individual-based rarefaction would have overestimated species richness when all trap type catches are combined. The sample size for the rarefaction models was large and the curves were flattened towards the right suggesting high species turn-over. A steep rise in the curves denotes a quick increase in the number of species obtained during sampling from each of the core sites. The flattening of the graph at a later stage signifies repetition of similar species at Lapalala and Marakele. In addition, flattening of the graph probably implies that a good representative coverage of individual species has been measured. This shows that sampling effort was enough for obtaining a representative number of species in the two localities. However, more intensive sampling is needed to yield the maximum number of species at other sites. Despite reasonable sampling being achieved, it is possible that additional intensive sampling may continue to yield new species as suggested by the Chao1 estimate (Table 2.3), if larger sampling habitats are explored. Estimates of species richness will continue to increase as more sites are thoroughly examined over an extended period. For effective sampling to occur, special attention should be given to the species abundance distribution as it needs greater sampling effort where evenness is low (Lande et al., 2000, Yoccoz et al., 2001). Overall, estimates of species richness based on rarefaction predicted higher species numbers from tent than light traps at both Lapalala and Marakele for the same number of individuals while the opposite was true at Boschkop (Figure 2.3). Therefore, due to the significant differences in species yields between tent and light traps, there is a need to use both trap types for

ecological, arbovirus and malaria vector surveillance (Cornel et al., 2018). Although there is variation in the degree to which different mosquito species are attracted to light traps, this method is among the most widely used tools for vector surveillance. However, one of the disadvantages of using light traps, is that they are usually biased in ways that may affect data interpretation for epidemiological studies. If used alone, light traps often fail to collect important or infected vectors, and can be unproductive when competing ambient light is present (McDermott and Mullens, 2017).

Mosquito species richness and diversity were higher in protected areas within the savanna such as Lapalala Wilderness Reserve and Marakele National Park. The number of equally abundant species needed to obtain the same mean proportional *species* abundance or effective number of species (ENS) based on Shannon indices was almost double that of Marakele and Mnisi at Lapalala. The Shannon index along with the Simpson diversity index are widely used for the estimation of richness and diversity in most ecological studies (Chiarucci et al., 2011, Spellerberg and Fedor, 2003). However, for disproportionate relative abundance distributions, evenness depends greatly on species richness rather than diversity indices (Gosselin, 2006). In a similar study, Cornel and colleagues (Cornel et al., 2018) also found high levels of diversity at Lapalala and other wildlife reserves which are located within the savanna such as Kruger National Park and the Okavango Delta in Botswana. These patterns of diversity are probably related to the availability of diverse breeding habitats, ecological integrity, abundant wildlife as sources of bloodmeals, abundance of vegetation/canopy cover, landscape/topographic heterogeneity, an array of microclimates, favourable climate and plenty of water in these areas (Cornel et al., 2018, Chaves et al., 2011, Moncayo AC et al., 2000, Rueda et al., 1990, Gillies, 1953). Seasonal patterns of rainfall play a significant role for many *Aedes* species whose drought-resistant eggs only hatch after occasional flooding leading to peaks in mosquito abundance (Braack et al., 2018, Swanepoel and Coetzer, 2004). The arrangement and diversity of mosquito fauna in these woodlands can also be affected by the variation in habitat traits. For example, light, temperature, and humidity vertical gradients exist in tropical broadleaf forests while larval habitats, hosts, and resting sites may differ across forest layers (Bates, 1949, Yanoviak, 1999). The rich structural complexity at Lapalala and Marakele has led to fine partitioning of habitat space. As a result, species abundance has become more uniform with differences in the abundance of many

species that coexist in untransformed areas being more gradual or evenly distributed (Verberk, 2012). These results seem to suggest that mosquito species richness decreased along landscape-level drivers such as climate, elevation, vegetation and host availability from the Middleveld savanna portion of Limpopo Province to Lowveld savanna areas. Conversely, the decrease in species richness from Middleveld to Highveld Grassland areas in Gauteng Province reveals the presence of more cold adapted species such as *Ae. dentatus, Ae. juppi, Cx. theileri and Cx. univittatus.* These are mosquitoes associated with temperate or high altitude areas receiving significant amounts of rainfall (McIntosh et al., 1980, McIntosh, 1973, Jupp, 1971, McIntosch, 1971, Jupp, 1996).

As part of a major wildlife reserve, Kruger National Park, Orpen has constant presence of wildlife including non-human primates but is located in a dry woodland setting with low availability of surface water for much of the year. In this study, species homogeneity and equitability were lowest at Orpen. The level of diversity was also much lower than in the two other wildlife sanctuaries at Lapalala and Marakele. The Chao1 index also shows that there was a considerable underestimation of species richness at Orpen (Table 3). The Chao1 index unlike the Jaccard and Sorensen classic indices which are negatively biased if under sampling occurs, is better suited for estimating richness between samples of different sizes, are known or suspected to be under sampled, or contain many rare species (Chao et al., 2005). Reasons for this low diversity may well have been related to relatively few collection events and the generally dry conditions which prevailed preceding all these Orpen collection periods. The importance of taking such local context into account is reflected in the findings of Cornel and colleagues (Cornel et al., 2018), where certain localities such as Shingwedzi, Lower Sabie and Tshokwane had high but different diversity indices, in contrast to the low diversity findings at Orpen in this current study, despite all these locations being within the same wildlife reserve (Kruger National Park). The same need for an understanding of local contextual differences in habitat are essential to understand the low richness at nature reserves such as Rooipoort (Northern Cape, SA), Kogelberg (Western Cape, SA), Tswalu (Northern Cape, SA) and Vilankulo district in southern Mozambique.

Although there is a clear difference in the physiognomy of the urban or peri-urban landscape from that of natural habitats on the globe including South Africa, few studies have focused on

the species diversity of mosquitoes in urban ecosystems. Several studies suggest that urban communities can alter the environment in many ways that impact on the diversity of mosquitoes by either reducing or increasing abundances and even causing extinction of some local species (McKinney, 2008, Gibson and Russell, 2006, Robert, 2009, Grimm et al., 2008, Newbold et al., 2015, Kark et al., 2007, Lowe et al., 2016, Beck et al., 2002, Costa and Magnusson, 2002, Klein et al., 2002). Nevertheless, peri-urban and urban areas still consist of different types of habitats (housing, green areas, roads) giving rise to highly organized areas with many interfaces between them (Grimm et al., 2008). While the features used to describe 'urban' sites differ greatly among studies, this study found lower species diversity in peri-urban areas compared with sylvatic habitats but not mixed rural settings. This level of diversity recorded at Boschkop and Kyalami can be attributed to the availability of diverse breeding habitats both natural and artificial, plenty of surface water (ponds, dams, rivers) and animal hosts such as domestic stock and birds.

Several theories have been proposed to explain variations in species richness and diversity. One of them is the niche diversification hypothesis, which states that diversity is a function of the range of habitats and of the degree of specialization of resident species. In this hypothesis stable ecosystems such as forests or woodlands are more likely to have higher species diversities. The intermediate disturbance hypothesis (IDH) states that regular *disturbances* create nonequilibrium conditions that lessen the likelihood of competitive exclusion between coexisting species, subsequently promoting high species richness and diversity (Connell, 1978, Sousa, 1979). The IDH was advanced to explain species diversity patterns in species rich ecosystems. However, tests of the IDH in tropical settings are scarce, limited and controversial (Wilkinson, 1999, Bongers et al., 2009). This study did not find major differences in species diversity between Lapalala and Marakele, both of which are in the savanna ecosystem. Nevertheless, this work supports the findings by Cornel and colleagues (Cornel et al., 2018) and other workers (Marí and Jiménez-Peydró, 2011, Reiskind et al., 2017) suggesting that mosquito diversity is highest in untransformed natural areas such as nature preserves.

This study, which was mainly focused on adult mosquitoes, found *Culex* and *Anopheles* as the most prevalent genera across all surveyed sites in the northern part of South Africa, however, differences between sites were observed. Other studies carried out in close proximity to the

study area in the past decades have revealed different findings. Steyn and colleagues (Steyn and JJ, 1955) collected 538 mosquitoes comprising 21 species in three genera in the upper Limpopo River Valley not far from Lapalala. In a similar study conducted over a short period, Cornel and colleagues (Cornel et al., 2018) found *Anopheles* and *Coquillettidia* to be the most dominant at Lapalala whereas, in this study conducted over a period of 38 months in the same area, recorded a total of 15,387 mosquitoes composed of 74 species from nine genera with a predominance of *Anopheles* and *Aedes*. However, in Kruger National Park, Cornel and colleagues (Cornel et al., 2018) catches were predominantly made up of *Mansonia* species followed by *Culex* and *Anopheles*. In their survey, Schultz and colleagues (Schulz et al., 1958) collected four genera comprising 25 species with *Aedes* and *Culex* being the most abundant at multiple collection sites including Orpen. Contrastingly, we caught a total of 1,204 adult mosquitoes at Orpen which were composed of 29 species from three genera, with a predominance of *Aedes* (95%) and *Culex* (3.5%) species in a period of 15 months. Despite the limited number of breeding sites available for sampling at Orpen, the number of aedine species caught was slightly more than what Schultz and colleagues (Schulz et al., 1958) collected from Kruger National Park. The use of tent and light traps was effective in sampling a variety of species over an extended period in a confined area despite the hot and dry weather conditions experienced during the survey.

The general goal of biogeography and population ecology is to understand the distribution of species abundances across spatial scales (Gaston and Blackburn, 2003, Vandermeer and Goldberg, 2013). For instance, there is a tendency for species to be most abundant in the centre of their geographic ranges (Hengeveld and Haeck, 1982, Brown, 1984, Holt et al., 1997, McGill and Collins, 2003). In this study, the abundances for *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* were greatest in the Highveld Grassland region while A*e. mcintoshi*, *An. coustani*, *An. theileri* and *Cq. fuscopennata* populations were higher in the Middleveld Bushveld region. As for *An. squamosus*, *Cx. poicilipes* and *Ma. uniformis*, they were most abundant in the Lowveld Bushveld region. *Aedes mcintoshi*, *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* are the most widely distributed species in the study area (Figure 8).
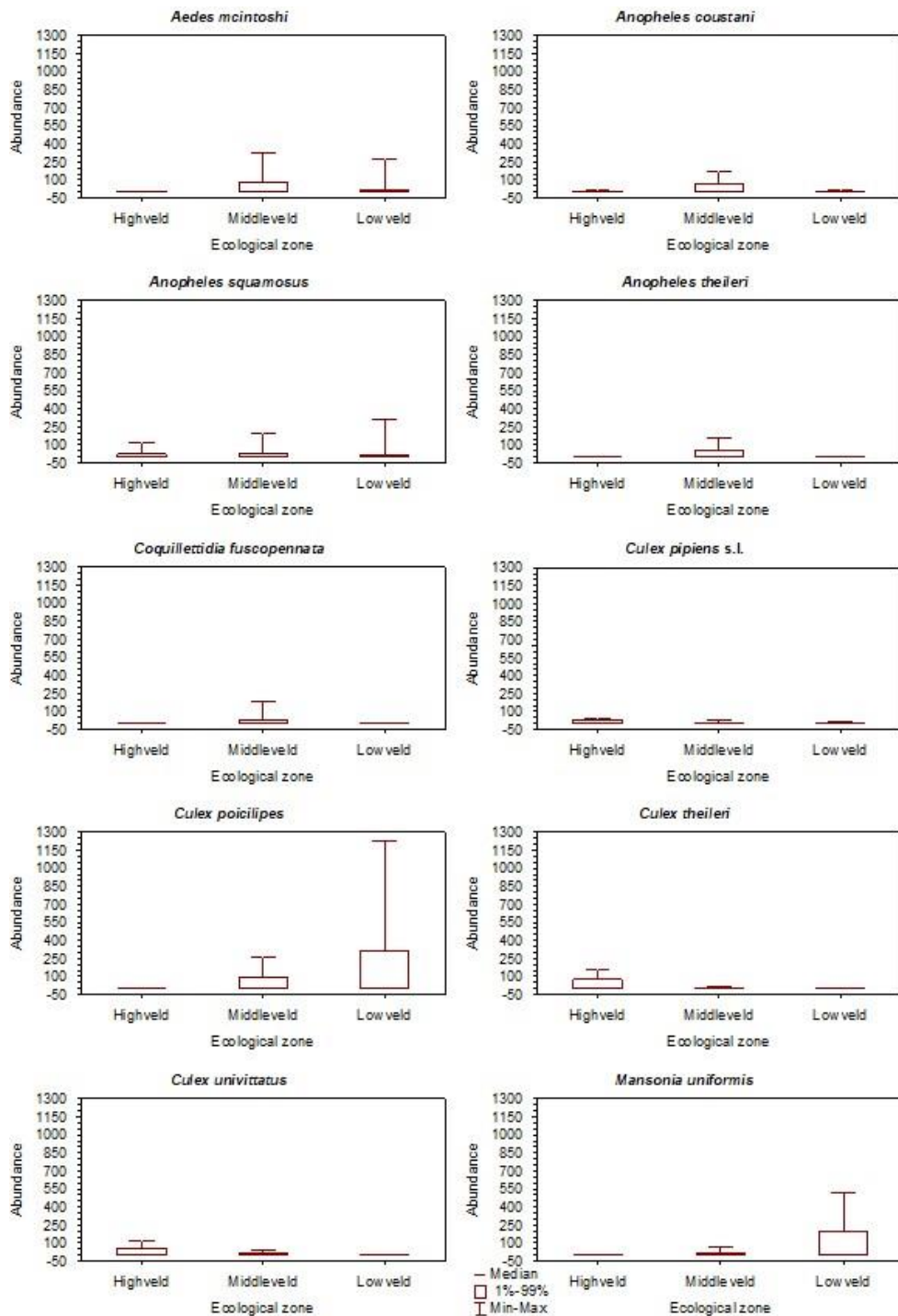
Figure 2. 8: Geographic distribution of the most abundant species. Mosquitoes were caught in tent and light traps from the three ecological zones. Median values are represented by the lines within each box, while the box ends show the interquartile range (1 – 99%). The whiskers on each box indicate minimum and maximum data point values, and do not include outliers.

These species seem to have a strong ecological plasticity that allows them to adapt to a wide range of habitats. Populations of widespread species are known to adapt to variable climates and landscapes, from equatorial to temperate climates (Verberk, 2012). As with earlier

findings from other studies, this study confirms that *Cx. theileri* is the most abundant mosquito potential vector in the Highveld Grassland region (Jupp, 2004b, McIntosh et al., 1980). This analysis also suggests that *Cx. theileri* is rare in the Limpopo-Mpumalanga Lowveld (Jupp, 2004b) while *Cx. univitattus* is widely distributed in the temperate highlands of southern Africa (Mixão et al., 2016, Jupp, 1971).

Aedes mcintoshi appears to be adapted to areas with intermediate temperatures and is widely distributed in the Karoo, north-eastern Highveld, Lowveld and the coastal region of KwaZulu-Natal of South Africa and the Zimbabwean Highlands (McIntosch, 1971, Jupp, 2004b). This species occurs in high densities at sites with aquatic/floating vegetation in the Middleveld and Lowveld regions. A positive association between *Cx. poicilipes* and habitats with floating vegetation has been confirmed in Kenya (Muturi et al., 2007). *Culex poicilipes* though not as widely distributed as *Ae. mcintoshi*, *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus,* does seem to have a strong ecological plasticity that allows for its adaptation in the three ecological zones. In a similar study, Cornel and colleagues (Cornel et al., 2018) also found *Cx. poicilipes* and *Cx. pipiens* to be among the most widespread species though *Ma. uniformis* was equitably abundant over the subregion. In this study, *Ma. uniformis* was one of the most dominant species collected from Mnisi, a site in the Lowveld region not far from one of Cornel and colleagues (Cornel et al., 2018) collection sites.

It is important to note that of the 95 species of mosquito observed in this work, eight have never been recorded previously in the study area. This study allowed the capture of species poorly and irregularly found in northern South Africa such as *Ae. luteolateralis*, *Ae. pachyurus*, *Cx. sitiens*, *Ficalbia. circumtestacea*, *Fi. uniformis*, *Mimomyia lacustris* and *Mi. pallida*. In Gauteng Province, several specimens were identified as *Ae. pachyurus*, a species previously known to occur in KwaZulu-Natal and the Cape Provinces (Jupp, 1996, Worth and Paterson, 1961, Muspratt, 1955). *Aedes luteolateralis* previously believed to only occur in Free State (Jupp, 1996), KwaZulu-Natal (Jupp, 1996, Huang, 1985b) and Cape Provinces (Jupp, 1996) was discovered in Limpopo Province. *Culex sitiens*, initially known to occur in Mozambique (Jupp, 1996, Worth and Meillon, 1960), was also found in Limpopo. *Ficalbia circumtestacea*, a species only found in KwaZulu-Natal in the early 1960s (Worth and Paterson, 1961, Jupp, 1996) has probably extended its geographical range into Limpopo while *Fi. uniformis*

previously detected in Mozambique and Zimbabwe between the mid-1950s and early 1960s is now also found in Limpopo Province which is adjacent to the two countries (Leeson, 1958, Worth and Meillon, 1960). In most cases, species with restricted geographic boundaries tend to be scarce (Verberk, 2012). There is a possibility that *Mi. lacustris* and *Mi. pallida* have extended their known geographical limit from KwaZulu-Natal to Limpopo. From this study and many others, it is not easy to explain the complete absence of some species from certain localities at times (i.e. periods of many years between collection events) and their occurrence within the same environs at other times (i.e. different times of the year) in large numbers. There is, however, a likelihood that they continue to survive in refugia or localities from which they are assumed to have disappeared, albeit in small numbers that they are most often disregarded. In addition, spatial distribution of hosts might also have a profound effect on the distribution, composition and abundance of mosquito vectors in the study areas.

Thirty species known or suspected vectors of arthropod-borne viruses (arboviruses) in southern Africa and elsewhere (Table 5) were collected during this survey (McIntosh, 1980, Braack et al., 2018, Venter, 2018). Each of the surveyed localities had more than nine species and at least three genera that are suspected vectors. The highest diversity of potential vectors was recorded at Lapalala and Marakele with the least being from Orpen. The presence of such a diversity of arbovirus vectors at the various collection sites explains the periodic outbreaks of arbovirus cases recorded from these sites and emphasizes the risk of continued outbreaks in future. Mosquito species considered as medically important for their role in arboviral transmission in northern South Africa based on their abundance are *Ae. mcintoshi*, *Cx. pipiens s.l.*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus.* They are all known or potential vectors of arboviruses in southern Africa. *Aedes mcintoshi* is major vector of Wesselbron virus (WESV) on the temperate inland plateau of South Africa and on the Zimbabwean Highlands (Jupp, 1996, Jupp, 2004b). It is also acknowledged as an important maintenance vector of Rift Valley fever virus (RVFV) in Zimbabwe and possibly also on the inland plateau in South Africa (Swanepoel and Coetzer, 2004). *Culex pipiens s.l.* because of its high degree of anthropophily was more prevalent in the urban areas in Gauteng Province and to a lesser extent in touristic wildlife regions such as Marakele National Park. This species is a potential vector for West Nile (WN) and (RVF) viruses including the lesser known Olifantsvlei (OLI), Semliki Forest (SF), Sindbis (SIN) and Usutu (USU) viruses (McIntosh, 1978b, McIntosh, 1980, Swanepoel, 2003,

Swanepoel and Coetzer, 2004, McIntosh, 1986, Venter, 2018). *Culex poicilipes* is an epidemic vector for RVF in the Lowveld (Swanepoel and Coetzer, 2004), thus presenting the possibility of triggering zoonotic outbreaks of the disease in an area which has a wildlife/livestock/human interface. *Culex theileri* is one of the main epidemic vectors of RVFV in domestic animals in South Africa and Zimbabwe (Swanepoel and Coetzer, 2004, McIntosh, 1980, McIntosh, 1986). *Culex univittatus* is a competent vector in the transmission of WN, SIN and USU viruses (Jupp, 1971, Jupp, 1973, Mixão et al., 2016) in the Highveld and Karoo regions. Outbreaks of WN (Venter et al., 2017), SIN, MID (van Niekerk et al., 2015b) and Shuni (van Eeden et al., 2012b) viruses which have recently been recorded in animals around our 6 surveillance spots contributed to these sites being selected for this study. Future investigations should focus on identifying virus in the mosquito collections. Widespread distribution of *Aedes aegypti* was recorded in this study although at low levels. This should be monitored for its epidemic potential of emerging or imported viruses.

In the context of malaria vectors, the eastern regions of Limpopo and Mpumalanga Provinces (as well as KwaZulu-Natal, but the latter is well outside the current study area) are malaria endemic regions where the main vector is considered to be *An. arabiensis* (a member of the *An. gambiae* complex) with *An. vaneedeni* (a member of the *An. funestus* group) playing a secondary role (Burke et al., 2017). Nevertheless, this information is of importance and value from a public health and tourism perspective in creating awareness among health department officials and tourism operators regarding the potential for malaria transmission. Munhenga *et al*. (Munhenga et al., 2014) recorded high numbers of *An. gambiae* complex in the northern Kruger National Park (part of Limpopo Province), while La Grange & Coetzee (La Grange and Coetzee, 1997) found 85% of mosquito catches in Thomo Village (also north-eastern Limpopo Province) to comprise *An. funestus* complex. These results contrast with the findings of this study, yet again emphasizing locational differences and the dangers associated with extrapolating findings from one area to likely mosquito composition and abundance in localities even within the same geographic Province. Further evidence is that Mbokazi *et al.* (McIntosh et al., 1980) recorded the malaria vector *An. merus* (also a member of the *An. gambiae* complex) to be abundant in the southern region of Mpumalanga Province, geographically not very distant from the Orpen and Mnisi collections sites where no presence of *An. merus* was found.

2.4   Conclusion

These findings suggest that mosquito diversity and richness are greater in untransformed natural areas especially the Middleveld Bushveld region and mixed rural settings compared to peri-urban areas in the northern part of South Africa. Landscape-related factors such as land use, vegetation, host presence and hydrological characteristics appear related to mosquito abundance, distribution and mosquito composition. The most widely distributed known and potential arbovirus vector species detected in this study were *Ae. aegypti*, *Ae. mcintoshi*, *Ae. metallicus*, *Ae. vittatus*, *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus.* The most important malaria vector in northern South Africa is *An. arabiensis* while *An. vaneedeni* plays a lesser role in malaria transmission in the region, and this project confirmed its presence at Orpen and Mnisi. Arbovirus surveillance and vector control programs should be augmented in urban and mixed rural settings where there is greater risk for arbovirus transmission to humans and domestic stock.

Table 2. 3: A comparison of various diversity indices based on total mosquito collections at both core and opportunistic sites sampled in South Africa, 2014–2017.

	Boschkop	Kyalami	Lapalala	Marakele	Mnisi	Orpen
Taxa_*S*	38	39	74	60	41	29
Total number of individuals sampled	4130	6342	15387	10593	4630	1204
Simpson dominance (*D*)	0.2179	0.2305	0.09339	0.193	0.2082	0.686
Simpson diversity (1-*D*)	0.7821	0.7695	0.9066	0.807	0.7918	0.314
Shannon *H'*	2	1.882	2.842	2.219	2.079	0.8914
Shannon Evenness (*E*)_e^H/*S*	0.1945	0.1684	0.2317	0.1533	0.1949	0.08409
Equitability (*J*)	0.5499	0.5138	0.6603	0.542	0.5597	0.2647
Chao1	40.63	40.11	79.63	62.55	45.2	55

Table 2. 4: Relative abundance of mosquito species collected from all sampling localities in the northern part of South Africa, 2014–2017.

Genus	Species	Light trap	Tent trap	Light + tent trap	Relative abundance (%)
Aedeomyia	*Aed. furfurea*	27	20	47	0,111
Aedes	*Ae. aegypti*	71	86	157	0,371
	Ae. albothorax	5	2	7	0,017
	Ae. argenteopunctatus	6	13	19	0,045
	Ae. caballus	2	0	2	0,005
	Ae. cumminsii	10	15	25	0,059
	Ae. dentatus	10	70	80	0,189
	Ae. dentatus group	94	18	112	0,265
	Ae. dentatus/leesoni group	47	48	95	0,225
	Ae. durbanensis	0	1	1	0,002
	Ae. fascipalpis	1	2	3	0,007
	Ae. filicis?	5	2	7	0,017
	Ae. fowleri	83	49	132	0,312
	Ae. furcifer/cordellieri	5	2	7	0,017
	Ae. haworthi	0	2	2	0,005
	Ae. heishi	1	0	1	0,002
	Ae. hirsutus	88	94	182	0,430
	Ae. juppi	40	13	53	0,125
	Ae. ledgeri	13	0	13	0,031
	Ae. leesoni group	19	3	22	0,052
	Ae. luteolateralis	0	1	1	0,002
	Ae. marshalli	2	0	2	0,005
	Ae. mcintoshi	1430	2178	3608	8,532
	Ae. metallicus	24	21	45	0,106
	Ae. microstictus	32	38	70	0,166
	Ae. ochraceus	1	18	19	0,045
	Ae. pachyurus	53	12	65	0,154
	Ae. quasiunivittatus	404	239	643	1,521
	Ae. simpsoni	6	0	6	0,014
	Ae. species	850	2026	2876	6,801
	Ae. sudanensis	5	15	20	0,047
	Ae. unidentatus	10	5	15	0,035
	Ae. unilineatus	32	9	41	0,097
	Ae. vexans	0	2	2	0,005
	Ae. vexans group	5	0	5	0,012
	Ae. vittatus	350	284	634	1,499
Anopheles	*An. coustani*	1622	2486	4108	9,715
	An. funestus s.l.	237	204	441	1,043
	An. gambiae s.l.	75	397	472	1,116
	An. longipalpis	0	1	1	0,002
	An. maculipalpis	0	4	4	0,009
	An. marshalli	119	66	185	0,437
	An. natalensis	4	2	6	0,014
	An. pharoensis	20	44	64	0,151
	An. pretoriensis	72	86	158	0,374
	An. rhodesiensis	2	0	2	0,005
	An. rufipes	158	235	393	0,929
	An. species	1034	573	1607	3,800
	An. squamosus	1275	2157	3432	8,116
	An. theileri	1789	722	2511	5,938
	An. ziemanni	18	222	240	0,568
Coquillettidia	*Cq. cristata*	48	120	168	0,397
	Cq. fuscopennata	986	219	1205	2,850
	Cq. maculipennis	15	4	19	0,045
	Cq. species	7	196	203	0,480
Culiseta	*Cs. longiareolata*	1	0	1	0,002
Culex	*Cx theileri*	1686	2140	3826	9,048
	Cx. annulioris	62	110	172	0,407
	Cx. antennatus	6	55	61	0,144
	Cx. argenteopunctatus	2	0	2	0,005
	Cx. bitaeniorhynchus	0	4	4	0,009
	Cx. duttoni	3	19	22	0,052
Genus	Species	Light trap	Tent trap	Light + tent trap	Relative abundance (%)

Table 2.4: Continued					
	Cx. ethiopicus	54	107	161	0,381
	Cx. horridus	0	2	2	0,005
	Cx. neavei	2	71	73	0,173
	Cx. nebulosus	1	0	1	0,002
	Cx. pipiens s.l.	597	1081	1678	3,968
	Cx. poicilipes	3160	2633	5793	13,700
	Cx. rubinotus	0	30	30	0,071
	Cx. simpliciforceps	1	1	2	0,005
	Cx. simpsoni	3	4	7	0,017
	Cx. sitiens	1	4	5	0,012
	Cx. species	477	171	648	1,532
	Cx. terzii	8	16	24	0,057
	Cx. thalassius	0	2	2	0,005
	Cx. tigripes	4	9	13	0,031
	Cx. trifilatus	0	2	2	0,005
	Cx. trifoliatus	5	19	24	0,057
	Cx. tritaeniorhynchus	0	4	4	0,009
	Cx. univittatus	1427	2272	3699	8,748
	Cx. zombaensis	0	1	1	0,002
Ficalbia	*Fi. circuntestacea*	1	0	1	0,002
	Fi. species	9	4	13	0,031
	Fi. uniformis	39	14	53	0,125
Mansonia	*Ma. africana*	0	41	41	0,097
	Ma. species	8	66	74	0,175
	Ma. uniformis	235	1213	1448	3,424
Mimomyia	*Mi. hispida*	17	3	20	0,047
	Mi. lacustris	68	4	72	0,170
	Mi. mediolineata	4	0	4	0,009
	Mi. mimomyiaformis	2	6	8	0,019
	Mi. pallida	15	0	15	0,035
	Mi. species	12	3	15	0,035
Uranotaenia	*Ur.* species	22	5	27	0,064

Table 2. 5: Known or suspected disease vector mosquitoes and their abundances at six localities in northern South Africa.

Species	Known or suspected vector for	Boschkop	Kyalami	Lapalala	Marakele	Mnisi	Orpen	References
An. coustani	Malaria; Viruses (Bwamba, PGA, Uganda S & RVF)	16	185	3053	822	32	0	(ACAV, 2019, Nepomichene et al., 2015, Lutwama et al., 2002, McIntosh, 1980)
An. funestus s.l.	Malaria; Viruses (Bwamba; O'Nyong-Nyong)	2	2	295	113	29	0	(Gillies and Coetzee, 1987, Braack et al., 2018)
An. gambiae s.l.	Malaria; Viruses (Bwamba; O'Nyong-Nyong)	0	0	106	353	4	9	(Gillies and Coetzee, 1987, Braack et al., 2018)
Ae. aegypti	CHIK, Chaoyang, Dengue (1-4), RVF, Uganda S, YF and ZIK viruses	35	42	15	14	20	31	(Ochieng et al., 2013, ACAV, 2019, Braack et al., 2018)
Ae. argenteopunctatus	RVF and SF viruses	0	0	7	12	0	0	(Swanepoel and Coetzer, 2004, McIntosh et al., 1961)
Ae. caballus	MID, RVF and WES viruses	0	0	0	2	0	0	(ACAV, 2019)
Ae. cumminsii	RVF, Spondweni and Shokwe viruses	5	1	5	14	0	0	(ACAV, 2019, Braack et al., 2018)
Ae. dentatus	MID and RVF viruses	0	80	0	0	0	0	(McIntosh, 1972, McIntosh, 1980)
Ae. fowleri	RVF, Spondweni and WES viruses	1	4	99	28	0	0	(ACAV, 2019, Venter, 2018, Tantely et al., 2015)
Ae. furcifer/cordellieri	CHIK, Dengue and YF viruses	0	0	5	1	0	1	(Jupp and McIntosh, 1990, Jupp, 1998)
Ae. juppi	MID, WES and RVF viruses	33	18	0	2	0	0	(Jupp PG et al., 1987, ACAV, 2019)
Ae. mcintoshi	BBK, Bunyamwera, MID, Ndumu, NRI, Pongola, RVF and WES viruses	29	6	1063	2212	294	4	(Jupp, 1996, Ochieng et al., 2013, Swanepoel and Coetzer, 2004, Jupp and Kemp, 1998)
Ae. metallicus	YF virus	2	1	21	7	1	13	(Fontenille et al., 1997)
Ae. ochraceus	Ndumu and RVF viruses	0	0	4	15	0	0	(Braack et al., 2018)
Ae. simpsoni	BBK, NRI and YF viruses	4	2	0	0	0	0	(ACAV, 2019)
Ae. unidentatus	WES and RVF viruses	2	11	2	0	0	0	(Jupp, 1996, Jupp and Cornel, 1988)
Ae. vittatus	BBK, CHIK, NRI, PGA, YF and ZIK viruses	2	2	544	17	3	66	(Braack et al., 2018, Jupp and McIntosh, 1990, Diallo et al., 2014, ACAV, 2019)
Cq. fuscopennata	SIN virus	0	2	1192	6	5	0	(McIntosh, 1980)
Cx. annulioris	SIN virus	5	3	160	0	4	0	(Burt et al., 2014)
Cx. antennatus	RVF, SIN and WN viruses	0	2	47	8	2	2	(ACAV, 2019, Taylor et al., 1956)
Cx. neavei	Bagaza, Mossuril, SIN, Spondweni, USU, WES and WN viruses	0	0	5	3	67	0	(ACAV, 2019, Traore-Lamizana et al., 1994, Jupp, 2004b, Braack et al., 2018)
Cx. pipiens s.l.	OLI, RVF, SF, SIN, Uganda S, USU and WN viruses	372	992	97	138	75	4	(Swanepoel and Coetzer, 2004, ACAV, 2019, Braack et al., 2018, McIntosh, 1978a)
Cx. poicilipes	RVF, Bagaza, BBK and WN viruses	1	5	234	3794	1759	0	(Jupp and Cornel, 1988, Traore-Lamizana et al., 1994)
Cx. rubinotus	Arumowot, Bunyamwera, Banzi, Germiston, Ndumu and Witwatersrand viruses	0	0	29	1	0	0	(McIntosh et al., 1976b, Braack et al., 2018)
Cx. sitiens	Mossuril virus	0	0	3	1	0	1	(ACAV, 2019)

Species	Known or suspected vector for	Boschkop	Kyalami	Lapalala	Marakele	Mnisi	Orpen	References
Cx. thalassius	Bagaza virus	0	0	0	2	0	0	(ACAV, 2019)
Cx. theileri	Germiston, RVF, Shuni and WN viruses	759	2528	70	329	12	1	(McIntosh, 1972, Braack et al., 2018)
Cx. tigripes	BBK, Mossuril and SIN viruses	0	2	6	0	5	0	(ACAV, 2019, Burt et al., 2014)
Cx. univittatus	Bagaza, SIN, WES, USU and WN viruses	1641	1036	824	268	46	11	(Jupp and NK, 1986, Braack et al., 2018)
Cx. zombaensis	RVFV	0	0	0	1	0	0	(McIntosh et al., 1983, Jupp and Cornel, 1988)
Ma. africana	Bancroftian filariasis; Viruses (Bunyamwera, MID, Ndumu, RVF, Shokwe, SIN, Spondweni, USU)	0	0	0	0	41	0	(Braack et al., 2018, ACAV, 2019, Worth and Meillon, 1960)
Ma. uniformis	Bancroftian filariasis; Viruses (Bwamba, Ndumu, O'Nyong-Nyong, Spondweni, WES, ZIK)	0	2	366	147	933	0	(Lutwama et al., 2002, Braack et al., 2018, ACAV, 2019, Worth and Meillon, 1960, Ughasi et al., 2012)

Chapter 3

Seasonal dynamics of mosquito populations in relation to environmental factors in northern of South Africa

Abstract

Knowledge on the spatiotemporal dynamics of mosquito populations is necessary for implementing control measures including the risk of mosquito-borne disease incidence. Since climate has reportedly been associated with disease transmission, it's important to understand the extent of its influence on mosquito abundance and distribution in South Africa. This study attempts to investigate the role of environmental factors such as elevation, rainfall, temperature and humidity on the population dynamics and seasonality of potential vector mosquitoes of arboviruses and malaria in two ecosystems of northern South Africa. Mosquitoes were collected from two horse farms and two wildlife sanctuaries in Gauteng and Limpopo Provinces respectively from January 2014 to March 2017, using carbon dioxide-baited light and tent traps. There were vivid differences in the temporal distribution and seasonal abundances of the seven medically important mosquito vectors encountered from the two distinct geographic regions and climates. Statistical models have shown that climatic factors play a crucial role in shaping the population dynamics of *Ae. mcintoshi*, *Ae. vittatus*, *An. arabiensis*, *Cx. pipiens s.l.*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus* both in Highveld Grassland and Middleveld Bushveld regions of northern South Africa. High summer temperatures and rainfall lead to increased vector density which might trigger outbreaks of RVF, SIN and WN viruses on the inland plateau of South Africa. This study also showed that abundances of RVF and WN virus vectors are related to elevation. These findings will be important in predicting the timing of onset and spread of future epidemics such as WN and RVF viruses, in southern Africa and other geographical settings with similar climates.

3.1   Introduction

The existence of seasonal variations in tropical insect communities, including mosquitoes, is well recorded (Wolda and Wong, 1988, Wolda, 1989, Trueman and McIver, 1986, Helson et al., 1980, Hewitt and PH, 1982). Understanding the spatiotemporal dynamics of mosquito populations is important for implementing control measures as well as evaluating mosquito-borne disease incidence and risk (DeGroote et al., 2007, Godsey Jr et al., 2005), especially during this time when global warming is expected to result in climate change. Since the impact of climate change on mosquito-borne diseases is still controversial there is a sense of urgency to understand to what extent climate influences will impact on mosquito abundance, the latter one being one of the key factors involved in disease transmission (Roiz et al., 2014). If global warming is likely to result in significant changes in temperature and rainfall which in turn may influence mosquito-borne diseases, it is essential to study the impact of climate on mosquito vector density and seasonality. Relative humidity is also known to be an important factor affecting the life history patterns of mosquitoes (Wu et al., 2007). Landscape and climatic factors are known to influence the seasonality and distribution of mosquitoes (DeGroote et al., 2007).

Mosquitoes are sensitive to changes in temperature and humidity, these factors affecting their survival, density and distribution (Uneke, 2009). The link between climatic factors and seasonal abundance of mosquitoes can provide information that is crucial in determining parasite activity as well as disease risk and dispersal (Wegbreit and Reisen, 2000, Camargo et al., 1994, Rubio-Palis and Zimmerman, 1997, Conn et al., 2002, Vittor et al., 2006, Rosa-Freitas et al., 2007). Accurate data on the seasonal prevalence of mosquito species is also essential for the development of efficient vector control programs (Alten et al., 2000).

Landscape or environmental factors such as elevation can in some circumstances serve as better surrogates for mosquito species range prediction than time-dependent climatic factors because it is easier to measure or access relevant data (Watts et al., 2017). Elevation is currently used as an ecological proxy for *Aedes aegypti* range because it is linked to diverse environmental factors which are important for mosquito development such as temperature (Brady et al., 2014). Whereas elevation does not have a direct effect on arbovirus transmission

it could be used by policy makers to issue warnings to travellers to remain within certain elevational thresholds in endemic areas; as example, the risk of yellow fever virus (YFV) acquisition in parts of Africa and South America is limited to areas below 2,300m altitude (Jentes et al., 2011).

The occurrence, abundance and distribution of mosquitoes in southern Africa have been documented in a number of studies (Edwards, 1941, Steyn and JJ, 1955, Muspratt, 1955, Muspratt, 1956, Worth and Paterson, 1961, Gillies and De Meillon, 1968, Jupp, 1969, van Der Linde, 1982, Coetzee et al., 1993, Jupp, 1996, La Grange, 1995, Jupp, 2004b, Cornel et al., 2018, Munhenga et al., 2014, Leeson, 1958, Schulz et al., 1958, Sande et al., 2015, Kamwi et al., 2012). An attempt to understand the seasonality of specific arbovirus mosquito vectors and their association with certain environmental factors was conducted in the late 1970s in the Free State Province of South Africa (Hewitt and PH, 1982). However, none of these studies examined the linear relationship between potential arbovirus/malaria vectors and climatic factors affecting mosquito populations in southern Africa.

In this survey, the spatiotemporal patterns and seasonality of mosquito vector abundance were explored while the environmental variables (elevation, rainfall, temperature and humidity) and abundance of potential vectors of arboviruses (*Ae. mcintoshi*, *Ae. vittatus*, *Cx. pipiens s.l.*, *Cx. poicilipes*, *Cx. theileri*, *Cx. univittatus*) and malaria (*An. arabiensis*) in two ecological zones (Middleveld Bushveld and Highveld Grassland) of northern South Africa were also assessed.

3.2   Materials and methods

3.2.1. Study sites

This study was conducted at four sites which include two nature reserves. These sites are Marakele National Park (S24°17'37.4 E27°30'11.7") and Lapalala Wilderness Reserve (S23°53'59.8" E28°17'50.1") in Limpopo Province and two horse farms namely Liberty Stables at Boschkop (S25°49'40.2" E28°25'12.4") and Glenfox Stables at Kyalami (S25°59'22.3" E28°01'56.1") in Gauteng Province in South Africa. The sites are located in Middleveld

Bushveld (Lapalala and Marakele) and Highveld Grassland (Boschkop and Kyalami) ecosystems (Rutherford et al., 2006). The vegetation found at Lapalala is mostly woodland while Marakele is dominated by mixed grassland/sparse woodland. The sites also occur in two different climatic zones, namely semi-arid (Marakele and Lapalala) and subtropical oceanic or temperate highland (Boschkop and Kyalami) (Peel et al., 2007, Beck et al., 2018). The height above sea level is 1372m at Boschkop, Kyalami 1415m, Lapalala 1163m and 969m at Marakele.

Figure 3. 1: Map of South Africa showing the four vector surveillance sites where regular monthly sampling was conducted from January 2014 to May 2017.

3.2.2   Mosquito collection

Adult mosquitoes were collected for more than three years from January 2014 to May 2017 from the four study localities described above. Sampling took place once every month at the two nature reserves and at the two horse farms. Mosquitoes were collected using two types of CO_2-baited traps: CDC (Centers for Disease Control) miniature light traps and Mosquito Tent traps (MTT), with the aid of mouth aspirators. Two light (ultraviolet and incandescent)

and three to five tent traps were used for three consecutive days at each wildlife reserve while two light and three tent traps were placed for two days at each of the horse farms. Trapping of mosquitoes began around 4:00pm in the afternoon and lasted until 6:00am the following morning. After collection, adult mosquitoes in polystyrene cups were stored in cooler boxes with dry ice at -80˚C before and after identification.

3.2.3   Mosquito identification

A stereomicroscope was used to separate mosquitoes placed on an ice brick. Mosquitoes were sorted by locality, trap type, and date of collection, and then morphologically identified to species level using regional keys and descriptions (Edwards, 1941, Gillies and Coetzee, 1987, Gillies and De Meillon, 1968, Jupp, 1996). Damaged specimens lacking key morphological identification characteristics were identified to genus level and recorded as such. Specimens destined for PCR assay (*An. funestus* group and *An. gambiae* complex) were placed in silica gel tubes, while other species were pooled maximum 50 specimens per tube, each species pooled separately, for virus assays. Although *Cx. quinquefasciatus* and *Cx. pipiens* are sympatric in the highveld region, no attempt was made to identify the two to species level using taxonomic keys. In this particular case, individual specimens from both species were referred to as *Cx. pipiens s.l.*

3.2.4   Climatic data

Daily rainfall, humidity and temperature data for each of the four sites were obtained from the South African Weather Service (SAWS). Four SAWS weather stations in the study area were selected to be included in the analysis based on the following criteria:

- The weather station has daily rainfall, daily humidity, maximum temperature, and minimum temperature data.
- The weather station sampled consistently from January 2014 to May 2017.

3.2.5 Data analysis

Statistical analyses were performed on abundance data of the predominant species captured during this study to establish which environmental variables were linked to species distributions as well as abundance in the focus areas. The Kolmogorov-Smirnov (Lilliefors modification) and Shapiro-Wilk tests were used to analyse mosquito count data for normality, while Levene's test was used to test for homogeneity of variance with Statistica 13.3 (Drezner and Turel, 2011, Royston, 1982, Shapiro and Wilk, 1965, Gastwirth et al., 2009). Due to the lack of normality of the data, large standard deviations and lack of homogeneity of variance, non-parametric tests were used to analyse mosquito densities. Since mosquito abundance data were not normally distributed, the non-parametric test, Kruskal-Wallis (> two samples) test was used to assess differences in mosquito density/trap-night across localities and ecological zones. To characterize the seasonal distribution of female mosquito abundance, we calculated the mean pooled mosquito abundance per trap-night. Pearson correlation coefficients were computed for the dominant genera and species using Statistica 13.3 to study the correlations between mosquito abundance and environmental variables. Mosquito count data for the most prevalent species were fitted to general regression models using simple regression to analyse designs with a single continuous predictor variable (rainfall, temperature, humidity). Three mosquito species collected from both light and tent traps in the Highveld Grassland region and Lapalala Wilderness reserve and five from Marakele National Park were selected for use in statistical analyses based upon their relative abundances in sampling collection and/or their status as a potential arbovirus vector.

3.3 Results

3.3.1 General abundance and distribution of mosquitoes

During the three year investigation presented in this chapter, a total of 36,452 specimens belonging to 10 genera (*Aedes*, *Aedeomyia*, *Anopheles*, *Coquillettidia*, *Culiseta*, *Culex*, *Ficalbia*, *Mansonia*, *Mimomyia and Uranotaenia*) and 87 species were captured in 1546 trap-nights from the four sampling stations (Table 3.1). The most widely distributed species were *Ae. aegypti*, *Ae. cumminsii*, *Ae. fowleri*, *Ae. hirsutus*, *Ae. mcintoshi*, *Ae. metallicus*, *Ae.*

quasiunivittatus, Ae. vittatus, An. coustani, An. rufipes, An. squamosus, An. theileri, Cx. ethiopicus, Cx. pipiens, Cx. poicilipes, Cx. theileri and *Cx. univittatus* (Table 3.1). The most abundant species at Boschkop and Kyalami in the Highveld Grassland ecosystem were *Cx. pipiens* sensu lato, *Cx. theileri* and *Cx. univittatus*. In the Middleveld Bushveld, the situation was different from the temperate highlands with the most dominant species at Lapalala Wilderness reserve being *Ae. mcintoshi, Ae. vittatus, An. coustani, An. squamosus, An. theileri, Cq. fuscopennata and Cx. univittatus* and. However, at Marakele National Park, the most abundant species recorded in this study were *Ae. mcintoshi, An. coustani, An. gambiae s.l., An. squamosus, Cx. poicilipes, Cx. theileri* and *Cx. univittatus*. From the lesser known genera, *Ma. uniformis* was frequently encountered at Lapalala and Marakele. In terms of genera, *Aedes*, *Anopheles* and *Culex* were the most dominant at the four areas or localities sampled (Table 3.1).

Table 3.1: Total number of mosquitoes collected per species including trap-nights from all sampling localities in northern South Africa between January 2014 and May 2017.

Genera	Species	Boschkop (308 trap-nights)	Kyalami (299 trap-nights)	Lapalala 507 trap-nights)	Marakele (432 trap-nights)
Aedes	*Ae. aegypti*	35	42	15	14
	Ae. albothorax	0	0	5	2
	Ae. argenteopunctatus	0	0	7	12
	Ae. caballus	0	0	0	2
	Ae. cumminsii	5	1	5	14
	Ae. dentatus	0	80	0	0
	Ae. dentatus group	98	14	0	0
	Ae. dentatus/leesoni group	45	50	0	0
	Ae. durbanensis	1	0	0	0
	Ae. fascipalpis	0	0	1	1
	Ae. fowleri	1	4	99	28
	Ae. furcifer/cordellieri	0	0	5	1
	Ae. heishi	0	0	1	0
	Ae. hirsutus	3	5	60	108
	Ae. juppi	33	18	0	2
	Ae. ledgeri	0	0	13	0
	Ae. leesoni group	15	6	0	0
	Ae. marshalli	0	0	2	0
	Ae. mcintoshi	29	6	1063	2212
	Ae. metallicus	2	1	21	7
	Ae. microstictus	0	0	65	5
	Ae. ochraceus	0	0	4	15
	Ae. pachyurus	40	25	0	0
	Ae. quasiunivittatus	276	24	321	11
	Ae. simpsoni	4	2	0	0
	Ae. species	420	181	561	237
	Ae. sudanensis	1	0	6	11
	Ae. unidentatus	2	11	2	0
	Ae. unilineatus	0	0	26	3
	Ae. vexans	0	0	0	2
	Ae. vexans group	2	0	3	0
	Ae. vittatus	2	2	544	17
Aedeomyia	*Aed. furfurea*	0	44	0	3
Anopheles	*An. coustani*	16	185	3053	822
	An. funestus s.l.	2	2	295	113

Table 3.1: Continued					
	An. gambiae s.l.	0	0	106	353
	An. longipalpis	0	0	1	0
	An. marshalli	1	0	184	0
	An. natalensis	3	1	1	0
	An. pharoensis	0	0	2	58
	An. pretoriensis	3	0	121	19
	An. rhodesiensis	0	0	2	0
	An. rufipes	25	35	247	66
	An. species	29	13	1221	197
	An. squamosus	187	850	973	1080
	An. theileri	1	9	2415	86
	An. ziemanni	0	0	71	41
Coquillettidia	*Cq. cristata*	0	0	167	1
	Cq. fuscopennata	0	2	1192	6
	Cq. maculipennis	0	0	19	0
	Cq. species	1	0	201	1
Culiseta	*Cs. longiareolata*	0	1	0	0
Culex	*Cx. annulioris*	5	3	160	0
	Cx. antennatus	0	2	47	8
	Cx. argenteopunctatus	0	0	2	0
	Cx. bitaeniorhynchus	0	0	2	2
	Cx. duttoni	0	0	10	3
	Cx. ethiopicus	2	2	116	35
	Cx. neavei	0	0	3	3
	Cx. nebulosus	0	0	1	0
	Cx. pipiens s.l.	372	992	97	138
	Cx. poicilipes	1	5	234	3794
	Cx. rubinotus	0	0	29	1
	Cx. simpsoni	2	0	4	0
	Cx. sitiens	0	0	3	1
	Cx. species	63	136	163	201
	Cx. terzii	3	19	2	0
	Cx. thalassius	0	0	0	2
	Cx theileri	759	2528	70	329
	Cx. tigripes	0	2	6	0
	Cx. trifilatus	0	0	1	1
	Cx. trifoliatus	0	1	9	11
	Cx. tritaeniorhynchus	0	0	1	0
	Cx. univittatus	1641	1036	824	268
	Cx. zombaensis	0	0	0	1
Ficalbia	*Fi. circuntestacea*	0	0	1	0
	Fi. species	0	0	3	10
	Fi. uniformis	0	0	43	10
Mansonia	*Ma. uniformis*	0	2	366	147
	Ma. species	0	0	9	2
Mimomyia	*Mi. hispida*	0	0	20	0
	Mi. lacustris	0	0	15	57
	Mi. mediolineata	0	0	1	3
	Mi. mimomyiaformis	0	0	5	2
	Mi. pallida	0	0	13	2
	Mi. species	0	0	5	10
Uranotaenia	*Ur.* species	0	0	22	2

3.3.2 Seasonality and abundance of potential arbovirus vector mosquitoes in the Highveld Grassland region

Culex pipiens s.l., *Cx. theileri* and *Cx. univittatus* were the most abundantly captured mosquitoes suspected or known to transmit arboviruses in the Highveld Grassland region of

South Africa. *Culex pipiens s.l.* and *Cx. univittatus* are known or potential vectors of Sindbis (SIN) and West Nile (WN) viruses (ACAV, 2019, Jupp, 2001, McIntosh, 1986) whereas *Cx. theileri* is a suspected vector for Rift Valley fever (RVF) and WN viruses (ACAV, 2019, Jupp, 2001, McIntosh, 1972). The seasonal distributions of these species reveal population fluctuations in different months. Although there were variations in mean abundances for a greater part of the sampling period, mosquito catches were predominantly made up of *Cx. univittatus* at Boschkop (Figure 3.2). *Culex univittatus* attained the highest peak density of 26.2 mosquitoes per trap-night followed by *Cx. theileri* at 9.2 mosquitoes in the month of January. *Culex pipiens s.l.* only achieved a peak of 3.7 mosquitoes/trap-night in the month of November which is less than what was recorded for the two other species. The frequency of *Cx. theileri* in trap catches was continuous throughout 2015 and 2016 while *Cx. univittatus* was absent for three months (May, July, September) in 2015 and one month (June) in 2016. *Culex pipiens s.l.* was caught repeatedly throughout 2016 with the month of June being an exception. However, for all three species maximum population peaks were attained in the months of November and January, more especially in the 2014 and 2017 sampling season.

All three vector species *Culex pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* attained high mean abundances between November and April at Kyalami. *Culex theileri* and *Cx. univittatus* reached their highest population peaks of 23.9 and 10 mosquitoes/trap-night in the months of December and February respectively while a maximum abundance of 7.2 mosquitoes/trap-night was recorded for *Cx. pipiens* in December (Figure 3.1). *Culex theileri* was the most frequently encountered mosquito vector at this site for the entire study period. The seasonality of *Culex theileri* adults remains active over an extensive period including winter months (June and July) at Kyalami (Figures 3.2). The Kruskal-Wallis Test showed that the mean abundances of mosquitoes changed significantly over the study years in the Highveld (*Cx. theileri*: $\chi^2 = 11.36$, DF = 3, $P = 0.01$; *Cx. pipiens s.l.*: $\chi^2 = 32.35$, DF = 3, $P < 0.001$; *Cx. univittatus*: $\chi^2 = 16.46$, DF = 3, $P = 0.0009$) while the Mann-Whitney U Test indicated significant differences in mean abundances between sites for *Cx. theileri* (Mann-Whitney $U = 28{,}133.50$; $P < 0.001$) and *Cx. pipiens s.l.* (Mann-Whitney $U = 39{,}833.50$; $P = 0.004$).

3.3.3 Seasonality and abundance of potential arbovirus and malaria vector mosquitoes in the Middleveld Bushveld region

Mosquito species considered to be medically important for their role in arboviral transmission at Lapalala Wilderness reserve are *Ae. mcintoshi*, *Ae. vittatus* and *Cx. univittatus*. These mosquito species are important vectors of arboviral diseases such as dengue, Chikungunya, Middelburg, Yellow fever, RVF, SIN, WN and Zika viruses (McIntosh, 1980, Jupp, 1996, McIntosh, 1972, Sudeep and Shil, 2017). The mean densities per trap-night for malaria vectors was quite low at this location, so they were no considered in this analysis. While populations of both *Ae. mcintoshi* and *Ae. vittatus* are most common between November and February of each year at Lapalala, *Cx. univittatus* populations continue surviving in the field throughout the year. During this survey, *Ae. mcintoshi* reached a peak of 9.2 mosquitoes/trap-night in January while *Ae. vittatus* and *Cx. univittatus* attained maximum population peaks of 4.6 and 4.4 mosquitoes/trap-night in November and March respectively (Figure 3.3). Populations of *Ae. mcintoshi* and *Ae. vittatus* declined in 2015 and 2016. The Kruskal-Wallis Test showed that the mean abundances of mosquitoes changed significantly over the study years at Lapalala (*Ae. mcintoshi*: χ^2 = 99.58, DF = 3, *P* < 0.001; *Ae. vittatus*: χ^2 = 16.79, DF = 3, *P* = 0.0008; *Cx. univittatus*: χ^2 = 88.1, DF = 3, *P* < 0.001).

The most important species in Marakele National Park are *Ae. mcintoshi*, *An. arabiensis*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus* based on relative abundance and their role as potential arbovirus/malaria vectors. *Anopheles arabiensis* is an important vector for malaria (Gillies and Coetzee, 1987) while *Ae. mcintoshi*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus* are suspected vectors for arboviral diseases such as Middelburg, RVF, SIN and WN viruses (McIntosh, 1980, Jupp, 1996, McIntosh, 1972). The seasonality of *Ae. mcintoshi* populations ranged from January to April but reached a maximum peak of 34.2 mosquitoes/trap-night in the month of March although population numbers were depressed in 2015 and 2016. The malaria vector, *An. arabiensis* (Gillies and Coetzee, 1987) emerged in September after being absent from trap catches in the months of July and August. Its seasonality ranges between December and June but the highest peak of 2.6 mosquitoes/trap-night was only recorded in April. From this study, *Cx. theileri* and *Cx. univittatus* were the only mosquito vectors found throughout the year with population peaks of 2.5 and 3.3 mosquitoes/trap-night being

achieved in April respectively. *Culex poicilipes* also occurs throughout the year except for the month of September. The average catch/trap-night *for Cx. poicilipes* and the other four species was highest in the period between January and May (Figure 3.3). The Kruskal-Wallis Test showed that the mean abundances of mosquitoes changed significantly over the study years at Marakele (*Ae. mcintoshi*: χ^2 = 109.91, DF = 3, *P* < 0.001; *An. arabiensis*: χ^2 = 99.79, DF = 3, *P* < 0.001; *Cx. poicilipes*: χ^2 = 81.25, DF = 3, *P* < 0.001; *Cx. theileri*: χ^2 = 17.47, DF = 3, *P* = 0.0006; *Cx. univittatus:* 25,08327, DF = 3, *P* < 0.001).
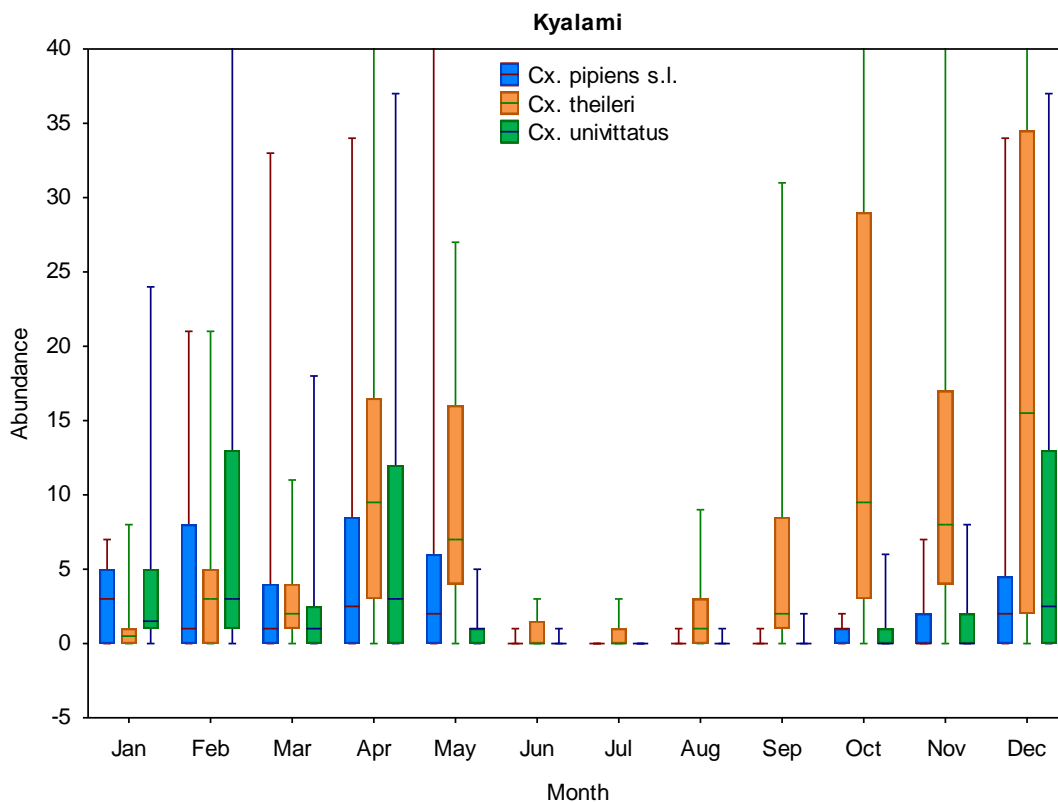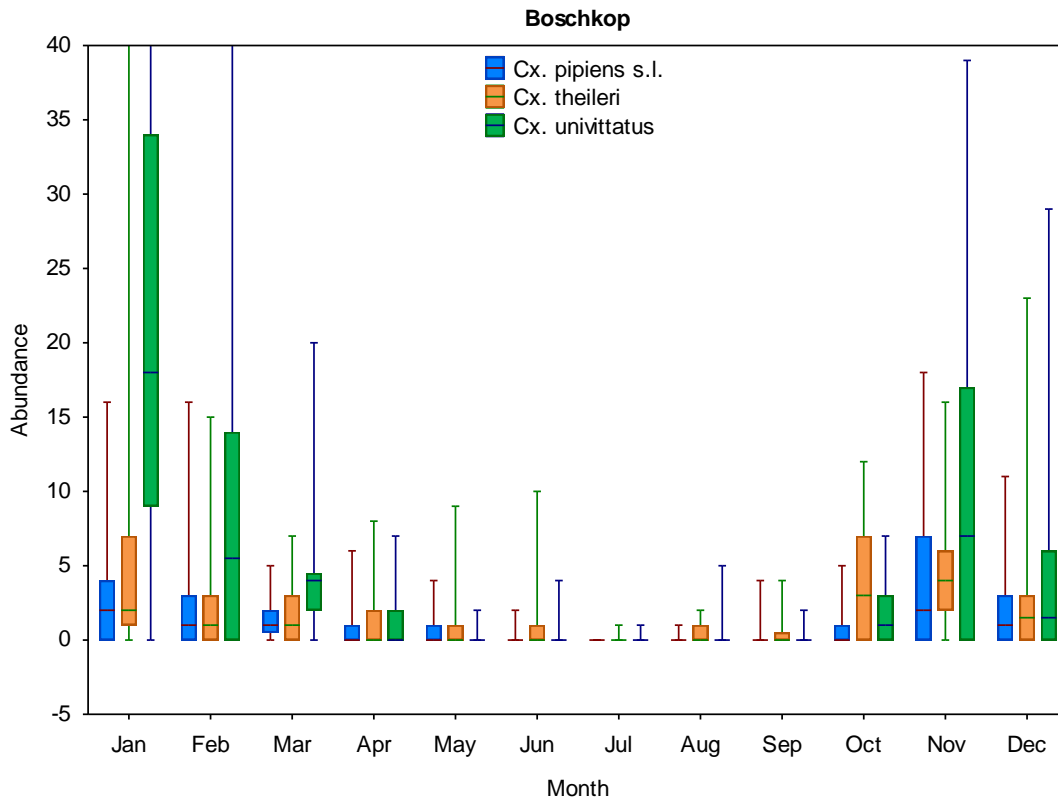
Figure 3.2: Monthly distribution and abundance of potential arbovirus vector mosquitoes caught per trap-night at Boschkop and Kyalami in the Highveld Grassland region of South Africa between January 2014 and May 2017. Median values are represented by the lines within each box, while the box ends show the interquartile range (25–75%). The whiskers on each box indicate minimum and maximum data point values, and do not include outliers.
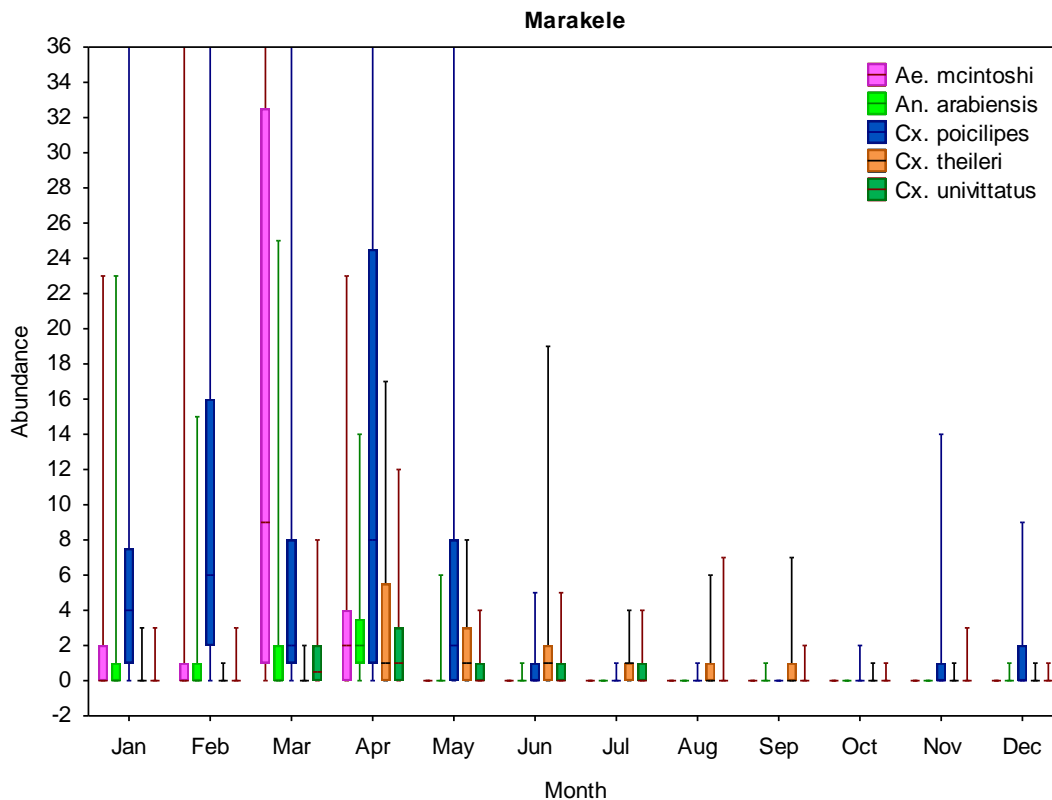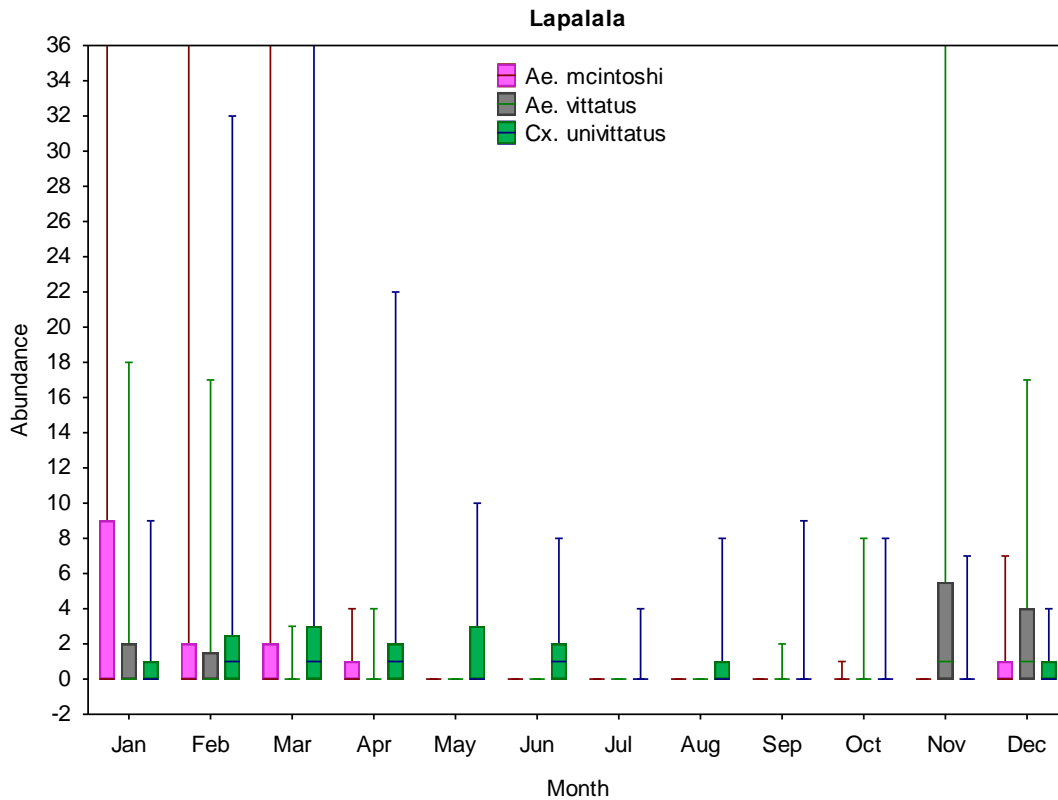
Figure 3.3: Monthly distribution and abundance of potential arbovirus vector mosquitoes caught per trap-night at Lapalala and Marakele in the Middleveld Bushveld region of South Africa between January 2014 and May 2017. Median values are represented by the lines within each box, while the box ends show the interquartile range (25–75%). The whiskers on each box indicate minimum and maximum data point values, and do not include outliers.

3.3.4   Influence of landscape variables on abundance of potential arbovirus vector mosquitoes in the Highveld Grassland and Middleveld Bushveld regions

Bivariate Spearman correlation analyses of most abundant and medically important species in relation to landscape variables at Marakele showed that the abundance of three species, *Ae. mcintoshi*, *Cx. poicilipes* and *Cx. theileri* had highly significant but weak correlations with elevation (Table 3.2). At Kyalami, there were no significant correlations between abundance of the two most dominant species, *Cx. theileri* and *Cx. univittatus* and elevation. There were also no significant correlations reported between elevation and dominant species at Boschkop and Kyalami in the Highveld. The Kruskal-Wallis Test showed that there were significant differences in the abundances of *Ae. mcintoshi* (Kruskal-Wallis = 104, *P* < 0.001), *Cx. poicilipes* (Kruskal-Wallis = 389, *P* < 0.001) and *Cx. theileri* (Kruskal-Wallis = 516, *P*< 0.001) with elevation (Figure 3.4).
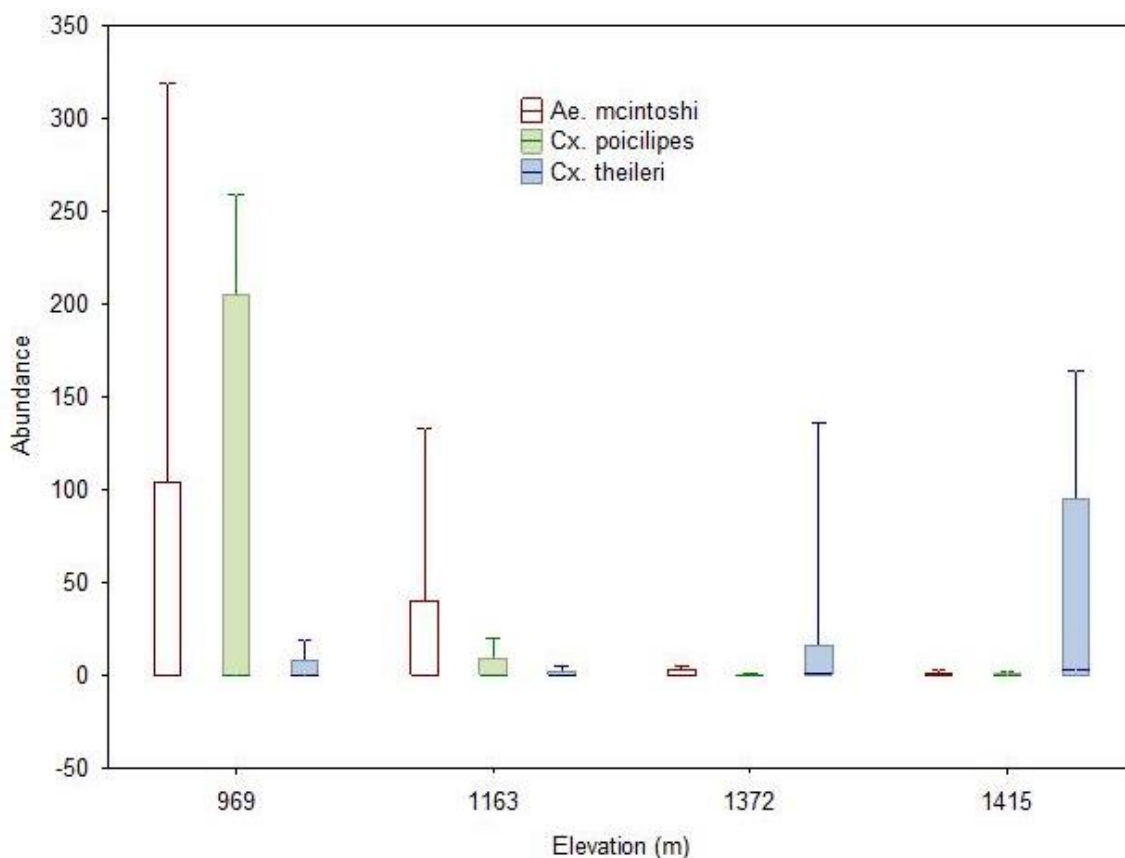
Figure 3.4: Abundance of potential arbovirus vector mosquitoes with increasing elevation in northern South Africa between January 2014 and May 2017. Median values are represented by the lines within each box, while the box ends show the interquartile range (1 – 99%). The whiskers on each box indicate minimum and maximum data point values, and do not include outliers.

Table 3.2: Analysis of correlation between Landscape parameters and mosquito density of dominant mosquito species in the Highveld and Middleveld regions. Marked correlations are significant at *P* < 0.05.

Pair of Variables	Locality	N	Spearman *r*	t(N-2)	p-value
Cx. theileri & Elevation (m)	Marakele	451	0,131470	2,81018	0,005168
Cx. poicilipes & Elevation (m)	Marakele	451	0,191169	4,12691	0,000044
Ae. mcintoshi & Elevation (m)	Marakele	451	0,099828	2,12593	0,034054

3.3.5 Influence of climatic variables on seasonal dynamics of potential arbovirus vector mosquitoes in the Highveld Grassland and Middleveld Bushveld regions

The seasonal dynamics of the most abundant species revealed population variations in different months at all the study sites. *Culex pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* were the dominant vectors present almost throughout the year with major peaks occurring during the rainy season (September–May) in the Highveld while smaller peaks were sometimes recorded in June and August at Boschkop and Kyalami respectively (Figures 3.5 and 3.6). In the 2017 sampling season the mean density/trap for *Cx. pipiens s.l.* was exceptionally high at Kyalami compared to *Cx. theileri* and *Cx. univittatus* in the months of February, March and May (Figure 3.6).

Although *Ae. mcintoshi*, *Ae. vittatus* and *Cx. univittatus* were the most prevalent at Lapalala, the seasonality of *Ae. mcintoshi* and *Ae. vittatus* was restricted to the rainy season, unlike *Cx. univittatus* which was present throughout the year (Figure 3.7). During the 2013/2014 and 2014/2015 rainy seasons, the mean abundances for *Ae. mcintoshi* and *Cx. univittatus* were much higher than for *Ae. vittatus*. In contrast, in the 2015/2016 rainy season the average catches/trap-night increased for *Ae. vittatus* compared to the other two species (Figure 3.7). The 2015/2016 and 2016 rainy season received moderately low to low rainfall compared to the other years with the highest rainfall of 95mm being recorded in February 2016. The 2017 sampling season was dominated by *Cx. univittatus* from February to May.

In Marakele National Park, the seasonality of *Ae. mcintoshi* and *An. arabiensis* was limited to the rainy season while the incidence of *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus* was more or less throughout the year with occasional breaks in some months. During the 2013/2014 rainy season, the trap catches were mostly dominated by *Ae. mcintoshi*, *Cx. poicilipes* and *Cx.*

univittatus while the end of the 2014/2015 rainy season favoured mostly *Cx. poicilipes*. Interestingly, in the 2016 and 2017 rainy season, there was an increase in the average catch/trap-night for all species (Figure 3.8).

In this study, fluctuations in rainfall, mean maximum and minimum temperatures were positively correlated with the occurrence of *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* in at Boschkop. At Kyalami, the relationship between *Cx. theileri* with mean maximum and minimum temperatures was insignificant and was thus not included in the analysis. The lack of correlation between relative humidity and *Cx. theileri* was observed at both Boschkop and Kyalami (Table 3.3). The correlations of rainfall (r_s = 0.6; P < 0.001) and minimum temperature (r_s = 0.61; P < 0.001) with the mean abundance of *Cx. univittatus* were moderately strong and positive at Boschkop.

There was positive correlation of average abundances for *Ae. mcintoshi, Ae. vittatus* and *Cx. univittatus* with all climatic variables except for minimum and maximum temperatures with *Cx. univittatus* at Lapalala. As for Marakele, mean abundances for *Ae. mcintoshi*, *Ae. arabiensis*, *Cx. poicilipes* and *Cx. univittatus* were positively correlated with rainfall while *Ae. mcintoshi*, *An. arabiensis* and *Cx. poicilipes* were positively correlated with minimum temperature. *Culex poicilipes* was the only species with a positive correlation with maximum temperature at Marakele. *Aedes mcintoshi*, *An. arabiensis*, *Cx. theileri* and *Cx. univittatus* were negatively correlated with maximum temperature while *Cx. theileri* and *Cx. univittatus* were both inversely correlated with rainfall and minimum temperature (Table 3.3).

General regression models (GRM) showed that all climatic factors have a significant effect on the abundance or presence/absence of all mosquito vectors with humidity being an exception for *Cx. theileri* at Boschkop. The most significant factors for *Cx. pipiens s.l.* abundance were rainfall and humidity while minimum and maximum temperature played a major role in the abundance of *Cx. theileri* at Kyalami. As for *Cx. univittatus*, all climatic variables except for maximum temperature were important for the occurrence and abundance of this species at Kyalami (Table 3.4). In the Middleveld, all four climatic variables had a notable influence on the abundance or presence of *Ae. mcintoshi, Ae. vittatus* and *Cx. univittatus* at Lapalala although minimum and maximum temperatures were an exception for *Cx. univittatus* and *Ae.*

mcintoshi respectively (Table 3.5). At Marakele, the presence or abundance of *Cx. theileri* was highly influenced by all climatic factors tested. Rainfall, minimum temperature and humidity had a significant influence on the presence/absence of *Ae. mcintoshi, An. arabiensis* and *Cx. poicilipes*. As for *Cx. univittatus*, humidity, minimum and maximum temperatures were the main climatic factors influencing the presence/absence of this species at Marakele (Table 3.4).

Table 3.3: Analysis of correlation between climatic variables and mosquito density of dominant mosquito species in the Highveld and Middleveld regions. Marked correlations are significant at *P* < 0.05.

Pair of Variables	Locality	N	Spearman *r*	t(N-2)	p-value
Cx. pipiens s.l. & Rainfall	Boschkop	308	0,406562	7,78430	0,000000
Cx. pipiens s.l. & Min temp	Boschkop	308	0,426398	8,24614	0,000000
Cx. pipiens s.l. & Max temp	Boschkop	308	0,372459	7,02050	0,000000
Cx. pipiens s.l. & Avg humidity	Boschkop	308	0,192034	3,42293	0,000704
Cx. theileri & Rainfall	Boschkop	308	0,443717	8,66119	0,000000
Cx. theileri & Min temp	Boschkop	308	0,368263	6,92893	0,000000
Cx. theileri & Max temp	Boschkop	308	0,342544	6,37793	0,000000
Cx. univittatus & Rainfall	Boschkop	308	0,603459	13,23842	0,000000
Cx. univittatus & Min temp	Boschkop	308	0,605851	13,32122	0,000000
Cx. univittatus & Max temp	Boschkop	308	0,485902	9,72505	0,000000
Cx. univittatus & Avg humidity	Boschkop	308	0,316808	5,84284	0,000000
Cx. pipiens s.l. & Rainfall	Kyalami	299	0,364182	6,738978	0,000000
Cx. pipiens s.l. & Min temp	Kyalami	299	0,283383	5,092500	0,000001
Cx. pipiens s.l. & Max temp	Kyalami	299	0,114298	1,982773	0,048314
Cx. pipiens s.l. & Avg humidity	Kyalami	299	0,421004	7,998884	0,000000
Cx. theileri & Rainfall	Kyalami	299	0,126277	2,193780	0,029026
Cx. univittatus & Rainfall	Kyalami	299	0,347716	6,391244	0,000000
Cx. univittatus & Min temp	Kyalami	299	0,343614	6,305677	0,000000
Cx. univittatus & Max temp	Kyalami	299	0,138978	2,418575	0,016183
Cx. univittatus & Avg humidity	Kyalami	299	0,486254	9,590040	0,000000
Ae. mcintoshi & Rainfall	Lapalala	541	0,301057	7,32949	0,000000
Ae. mcintoshi & Min temp	Lapalala	541	0,279764	6,76525	0,000000
Ae. mcintoshi & Avg humidity	Lapalala	541	0,378138	9,48311	0,000000
Ae. vittatus & Rainfall	Lapalala	541	0,241924	5,78854	0,000000
Ae. vittatus & Min temp	Lapalala	541	0,517540	14,04226	0,000000
Ae. vittatus & Max temp	Lapalala	541	0,470389	12,37535	0,000000
Ae. vittatus & Avg humidity	Lapalala	541	-0,170865	-4,02608	0,000065
Cx. univittatus & Min temp	Lapalala	541	-0,088561	-2,06418	0,039478
Cx. univittatus & Max temp	Lapalala	541	-0,258382	-6,20955	0,000000
Cx. univittatus & Avg humidity	Lapalala	541	0,353680	8,77854	0,000000
Ae. mcintoshi & Rainfall	Marakele	451	0,446149	10,5633	0,000000
Ae. mcintoshi & Min temp	Marakele	451	0,224836	4,8894	0,000001
Ae. mcintoshi & Avg humidity	Marakele	451	0,496656	12,1251	0,000000
An. arabiensis & Rainfall	Marakele	451	0,327753	7,3510	0,000000
An. arabiensis & Max temp	Marakele	451	-0,123918	-2,6462	0,008427
An. arabiensis & Avg humidity	Marakele	451	0,453182	10,7725	0,000000
Cx. poicilipes & Rainfall	Marakele	451	0,393444	9,0683	0,000000
Cx. poicilipes & Min temp	Marakele	451	0,303216	6,7424	0,000000
Cx. poicilipes & Avg humidity	Marakele	451	0,523175	13,0082	0,000000
Cx. theileri & Rainfall	Marakele	451	-0,291732	-6,4628	0,000000
Cx. theileri & Min temp	Marakele	451	-0,426013	-9,9778	0,000000
Cx. theileri & Max temp	Marakele	451	-0,452110	-10,7404	0,000000
Cx. theileri & Avg humidity	Marakele	451	0,266099	5,8494	0,000000
Cx. univittatus & Min temp	Marakele	451	-0,165172	-3,5487	0,000428
Cx. univittatus & Max temp	Marakele	451	-0,268288	-5,9013	0,000000
Cx. univittatus & Avg humidity	Marakele	451	0,316520	7,0704	0,000000

Figure 3.5: Seasonal changes in trap catches of *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* in relation to humidity, rainfall and temperatures at Boschkop from January 2014 to May 2017.

Figure 3.6: Seasonal changes in trap catches of *Cx. pipiens* s.l., *Cx. theileri* and *Cx. univittatus* in relation to humidity, rainfall and temperatures at Kyalami from January 2014 to May 2017.
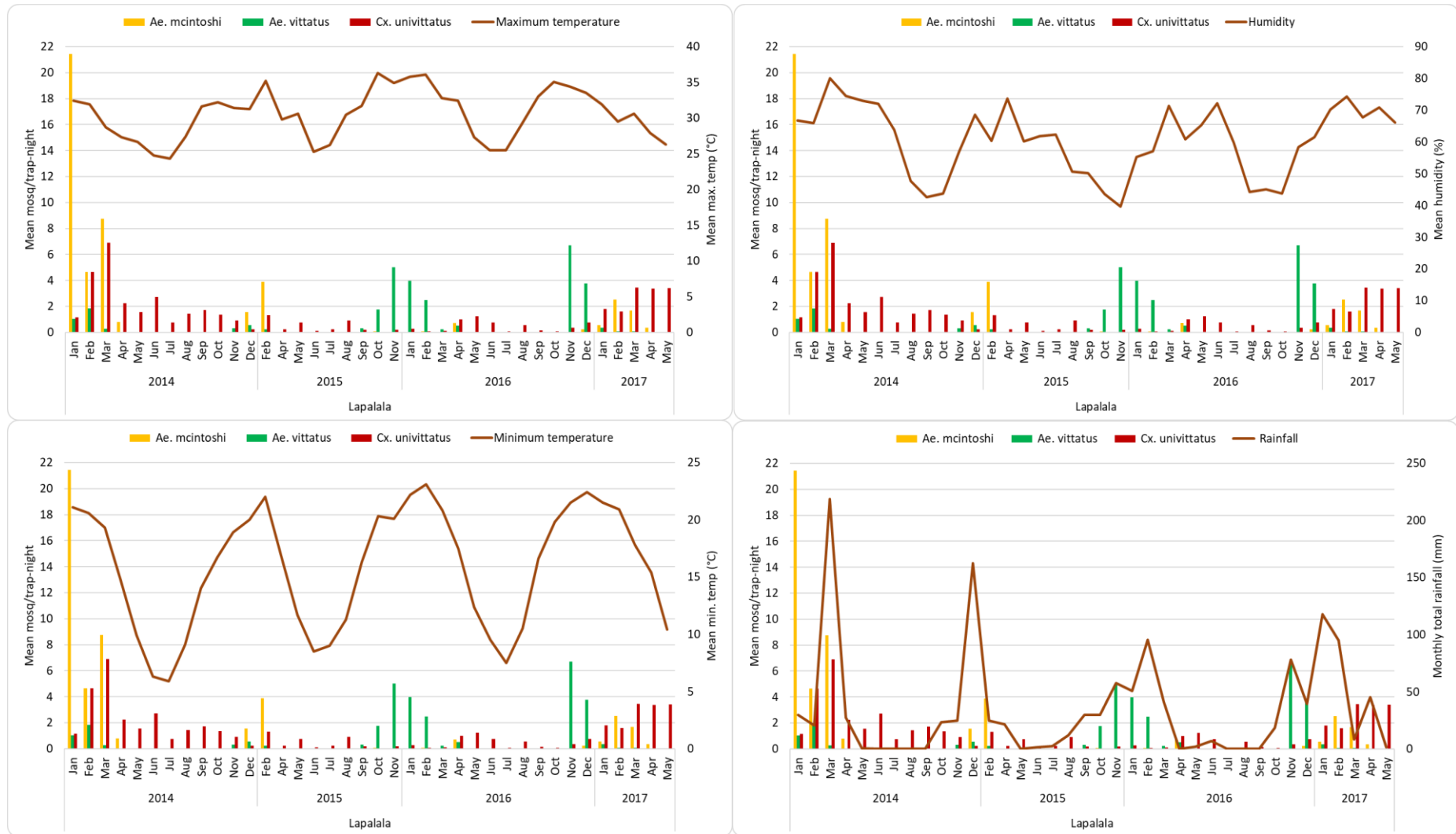
Figure 3.7: Seasonal changes in trap catches of *Ae. mcintoshi*, *Ae. vittatus* and *Cx. univittatus* in relation to humidity, rainfall and temperatures at Lapalala from January 2014 to May 2017.

Figure 3.8: Seasonal changes in trap catches of *Ae. mcintoshi*, *An. arabiensis*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus*, in relation to humidity, rainfall and temperatures at Marakele from January 2014 to May 2017.

Table 3.4: Best fitting General Regression (GRM) models explaining the effects of climatic variables on mosquito abundance or presence/absence for all species considered in the Highveld Grassland region. Marked correlations are significant at *P* < 0.05.

Boschkop	Rainfall	Min temp	Max temp	Avg humidity
Culex pipiens s.l.				
R	0,123	0,347	0,295	0,156
(Std. error)	0,006	0,032	0,030	0,014
(p-value)	0,033	0,000	0,000	0,006
(Confidence Interval -95%)	0,001	0,146	0,105	0,011
(Confidence Interval +95%)	0,024	0,274	0,224	0,067
Culex theileri				
R	0,187	0,179	0,163	0,056
(Std. error)	0,009	0,114	0,105	0,048
(p-value)	0,001	0,002	0,004	0,326
(Confidence Interval -95%)	0,012	0,139	0,096	-0,047
(Confidence Interval +95%)	0,047	0,589	0,511	0,141
Culex univittatus				
R	0,420	0,379	0,303	0,238
(Std. error)	0,013	0,169	0,159	0,073
(p-value)	0,000	0,000	0,000	0,000
(Confidence Interval -95%)	0,079	0,875	0,573	0,169
(Confidence Interval +95%)	0,129	1,538	1,200	0,455
Kyalami	**Rainfall**	**Min temp**	**Max temp**	**Avg humidity**
Culex pipiens				
R	0,123	0,093	0,034	0,207
(Std. error)	0,006	0,109	0,135	0,042
(p-value)	0,033	0,109	0,562	0,000
(Confidence Interval -95%)	0,001	-0,039	-0,188	0,071
(Confidence Interval +95%)	0,024	0,389	0,345	0,237
Culex theileri				
R	0,011	0,141	0,193	0,063
(Std. error)	0,013	0,241	0,296	0,096
(p-value)	0,852	0,014	0,001	0,281
(Confidence Interval -95%)	-0,023	0,119	0,419	-0,293
(Confidence Interval +95%)	0,028	1,069	1,586	0,085
Culex univittatus				
R	0,318	0,204	0,101	0,304
(Std. error)	0,007	0,131	0,165	0,051
(p-value)	0,000	0,000	0,081	0,000
(Confidence Interval -95%)	0,026	0,214	-0,036	0,178
(Confidence Interval +95%)	0,053	0,731	0,615	0,377

Table 3.5: Best fitting General Regression (GRM) models explaining the effects of climatic variables on mosquito abundance or presence/absence for all species considered in the Middleveld Bushveld region. Marked correlations are significant at *P* < 0.05.

Lapalala	Rainfall	Min temp	Max temp	Avg humidity
Aedes mcintoshi				
R	0,148	0,231	0,029	0,172
(Std. error)	0,005	0,058	0,116	0,035
(p-value)	0,001	0,000	0,506	0,000
(Confidence Interval -95%)	0,008	0,204	-0,151	0,073
(Confidence Interval +95%)	0,030	0,431	0,306	0,210
Aedes vittatus				
R	0,140	0,197	0,299	0,150
(Std. error)	0,002	0,022	0,042	0,013
(p-value)	0,001	0,000	0,000	0,000
(Confidence Interval -95%)	0,003	0,059	0,221	-0,072
(Confidence Interval +95%)	0,011	0,145	0,385	-0,020
Culex univittatus				
R	0,124	0,007	0,134	0,248
(Std. error)	0,003	0,028	0,054	0,016
(p-value)	0,004	0,872	0,002	0,000
(Confidence Interval -95%)	0,002	-0,050	-0,277	0,064
(Confidence Interval +95%)	0,013	0,059	-0,064	0,128
Marakele	**Rainfall**	**Min temp**	**Max temp**	**Avg humidity**
Aedes mcintoshi				
R	0,223	0,116	0,065	0,261
(Std. error)	0,017	0,199	0,327	0,084
(p-value)	0,000	0,014	0,168	0,000
(Confidence Interval -95%)	0,048	0,102	-1,093	0,317
(Confidence Interval +95%)	0,113	0,882	0,191	0,648
Anopheles arabiensis				
R	0,246	0,104	0,042	0,291
(Std. error)	0,002	0,020	0,033	0,008
(p-value)	0,000	0,027	0,370	0,000
(Confidence Interval -95%)	0,006	0,005	-0,094	0,038
(Confidence Interval +95%)	0,012	0,083	0,035	0,070
Culex poicilipes				
R	0,126	0,156	0,079	0,156
(Std. error)	0,019	0,223	0,369	0,097
(p-value)	0,007	0,001	0,092	0,001
(Confidence Interval -95%)	0,014	0,309	-0,102	0,135
(Confidence Interval +95%)	0,089	1,186	1,347	0,518
Culex theileri				
R	0,243	0,266	0,284	0,218
(Std. error)	0,001	0,014	0,024	0,006
(p-value)	0,000	0,000	0,000	0,000
(Confidence Interval -95%)	-0,009	-0,113	-0,194	0,018
(Confidence Interval +95%)	-0,004	-0,056	-0,101	0,043
Culex univittatus				
R	0,083	0,102	0,243	0,312
(Std. error)	0,001	0,012	0,019	0,005
(p-value)	0,079	0,031	0,000	0,000
(Confidence Interval -95%)	-0,004	-0,048	-0,135	0,024
(Confidence Interval +95%)	0,000	-0,002	-0,062	0,043

3.4   Discussion

This study showed a general increase in the abundance of *Ae. mcintoshi*, *Ae. vittatus*, *An. arabiensis*, *Cx. pipiens s.l.*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus* during the wet season compared with the dry season in both the Highveld and Middleveld regions. At all sites, high mosquito densities were observed during the September–May period each year. The most dominant species were *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* at Boschkop and Kyalami in the Highveld. These populations occurred at low densities throughout winter and early autumn, with a noticeable absence from April to July in 2014 for *Cx. theileri* and April to August for *Cx. univittatus* at Boschkop. It appears that both *Cx. theileri* and *Cx. univittatus* may overwinter as adults but this investigation did not include the larvae of these mosquitoes. Other studies conducted at the southern tip of the Highveld in the Free State Province of South Africa and at Olifantsvlei in Johannesburg found *Cx. pipiens, Cx. theileri and Cx. univittatus* to be the most prevalent even during dry summer months (Jupp et al., 1980, van Der Linde, 1982, Jupp, 1969) while *Cx. theileri* was the most active in winter (van Der Linde, 1982).

Seasonality and abundance of mosquitoes were related to climatic variables, yet the main factors did vary with the species and temporal scale used. This analysis revealed that the Highveld and Middleveld regions within northern South Africa may experience a single or double, concentrated peak in vector abundance during the rainy season between September and May, with distinct annual periodicity. These peaks probably represent distinct generations which seem to coincide with upper and lower thresholds of humidity, rainfall, temperature both in the rainy (most species) and dry season (non-hibernating species). This pattern is comparable to the seasonality of WN and RVF viruses in South Africa (Jupp, 2001, Pienaar and Thompson, 2013, Swanepoel and Coetzer, 2004).

Temperatures were significantly related to mosquito abundance and can have an effect on oviposition, survival rates, mortality, larval productivity and other population parameters (Reiter, 2001, Lafferty, 2009). It is worth noting that these temperature-dependencies at each stage are not the same, thus leading to the nonlinearities observed in population responses to temperature (Beck-Johnson et al., 2013). Monthly minimum temperatures were related to

seasonal abundance patterns in *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* at Boschkop while the same effect was observed for *Cx. theileri* and *Cx. univittatus* at Kyalami. As for Lapalala, *Ae. mcintoshi* and *Ae. vittatus* abundances were closely linked to minimum temperature. Minimum temperatures were also highly related to *Ae. mcintoshi*, *An. arabiensis*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus* abundances at Marakele. Seasonal abundance of *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* at Boschkop and *Cx. theileri* at Kyalami were also highly dependent on maximum temperatures.

As previously shown by the statistical relationships presented above, higher temperatures can lead to an increase in mosquito densities (Figures 4, 5, 6 and 7). Conversely, upper or lower mean temperatures during the season might cause a decrease in mosquito abundance (Paaijmans et al., 2010) (Figures 4, 5, 6 and 7) due to the balance between two opposite events. Earlier studies have also revealed that temperature variations have a profound effect on estimates of mosquito and malaria parasite development time (Paaijmans et al., 2009, Paaijmans et al., 2010). Higher temperatures reduce the survival rates (especially in adults) (Alto and Bettinardi, 2013) while lower temperatures can impede the developmental rates of immature stages (Paaijmans et al., 2010). *Culex univittatus* was absent from traps for one- or two-months during the winter months when minimum temperatures were in the range of 5.8–8.3°C at Kyalami in Johannesburg. Previous studies have also demonstrated the absence of Cx. *univittatus* adults from traps during the month of July possibly due to a transient form of hibernation (Jupp, 1969). The vast temperature tolerance of *Cx. theileri* and *Cx. univittatus* most likely made a remarkable contribution to their varied temporal distributions (Hewitt and PH, 1982). These environmental factors are thought to have triggered a rise in the density of *Cx. univittatus* leading to the incidence of SIN and WN viruses in the Highveld in 1984 (Jupp and NK, 1986). These results agree with previous findings suggesting that *Culex theileri* and *Cx. univittatus* are associated with temperate or high altitude areas receiving significant amounts of rainfall (Jupp, 1996, Jupp, 2004b, McIntosh et al., 1980, Jupp, 1971, Jupp, 2001, Jupp and NK, 1986).

The situation was different at Lapalala where *Aedes mcintoshi*, *Ae. vittatus* and *Cx. univittatus* were the most prevalent, while *Aedes mcintoshi*, *An. arabiensis*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus* were the most common vectors at Marakele. This study also found that the

abundance and onset of *Cx. univittatus* at Lapalala and Marakele was negatively correlated with average minimum temperatures (i.e., lower minimum temperatures delay the onset). The emergence of *Cx. theileri* during winter was also interrupted by lower minimum temperatures at Marakele. The observed year-round presence of *Cx. univittatus* at Lapalala suggests that both the rate of development and oviposition activity of *Cx. univittatus* are not restricted by the minimum temperatures occurring in this area. Most notably, these results are consistent with previous studies in South Africa, Russia and the USA that relate high summer temperatures to increased vector density and WN virus outbreaks (Jupp, 2001, Platonov et al., 2001, Reisen et al., 2014, Ruiz et al., 2010). In addition, regression models have also shown that temperature plays a crucial role in shaping the population dynamics of *Ae. mcintoshi*, *Ae. vittatus*, *An. arabiensis*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus*.

As expected, increasing monthly total rainfall was associated with an increase in the mean abundance of mosquito vectors at all sampling sites. Although mosquito vector populations tended to change gradually over the study period, maximum abundances were attained in the rainy season between October and May or during periods succeeding months with high rainfall activity. Climatic factors, especially rainfall, are known to influence mosquito abundance and distribution (Ahumada et al., 2004, Reisen et al., 2008). In this assessment, monthly rainfall was positively related to the mean abundances of *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* at Boschkop. As for Kyalami, rainfall had a significant impact on the seasonality and abundance of *Cx. pipiens s.l.* and *Cx. univittatus*. At both Lapalala and Marakele there was a positive and highly significant association between rainfall and mosquito abundance for all potential vectors except for *Cx. univittatus*. The absence or low numbers of *Ae. mcintoshi* at Lapalala and Marakele as well as *Ae. vittatus* at Lapalala during some months in the 2014/2015 and 2015/2016 rainy season, was clear proof that the seasonality of these two floodwater species is linked to rainfall as suggested by the regression models. Previous studies suggest that *Ae. mcintoshi* is probably adapted to areas with different climates and is widely distributed in the Karoo, north-eastern Highveld, Lowveld (Limpopo and Mpumalanga) and the coastal region of KwaZulu-Natal of South Africa and the Zimbabwean Highlands (McIntosch, 1971, Jupp, 2004b). However, heavy rainfall activity in some months caused a decline in the abundance of *Ae. mcintoshi* and *Ae. vittatus* at Lapalala and Marakele. In Mozambique, heavy rainfall has been observed to disrupt mosquito

breeding sites by washing away larvae or killing them (Charlwood and Braganca, 2012) and this is also true for puddle-breeding mosquitoes (Charlwood et al., 2011). Rainstorms have also been found to have a profound effect on mating and oviposition on gravid *Anopheles funestus* females in Mozambique (Charlwood and Braganca, 2012) while studies from Papua New Guinea found that heavy rainstorms did disrupt host-seeking behaviour in *Anopheles farauti* (Charlwood et al., 1988). Increased rainfall activity can also result in the death of mosquitoes due to flooding thus reducing ideal habitats required for larval development (LaPointe et al., 2012).

General regression models found mean humidity to be significantly associated with the abundance of *Cx. pipiens s.l.* and *Cx. univittatus* at both Boschkop and Kyalami in the Highveld. In the Middleveld Bushveld region, relative humidity was highly associated with the abundance of all medically important species (*Ae. mcintoshi, Ae. vittatus, An. arabiensis, Cx. poicilipes, Cx. theileri, Cx. univittatus*). Elevated levels of humidity can sustain basic survival rates of mosquitoes and promote excellent hatching rates (Nielsen and Nielsen, 1953, Costa et al., 2010). From this study, monthly mean humidity had a significant effect on the abundances of *Cx. univittatus* at Boschkop, *Cx. theileri* at Kyalami, *Ae. mcintoshi* at Lapalala and Marakele when humidity was above 71%, 64%, 66% and 80% respectively but this positive effect could also be due to other interactions between climatic factors. High humidity is known to increase longevity in mosquitoes (Wang et al., 2011) but can also be a warning of impending rainfall which might affect the development of larvae, mosquito dispersal and oviposition depending on the intensity of rainfall. In contrast, low humidity is likely to cause desiccation of eggs while adult mosquito longevity gets reduced (Day, 2016, Yamana and Eltahir, 2013, Sota and Mogi, 1992).

Mosquito abundance was highly correlated with landscape factors such as elevation for species such as *Ae. mcintoshi*, *Cx. poicilipes* and *Cx. theileri* at Marakele. *Aedes mcintoshi* and *Cx. poicilipes* were associated with areas of moderate to low elevations, especially the Marakele. This survey has shown that *Ae. mcintoshi* and *Cx. poicilipes* populations decline with increasing altitude from Marakele in the Middleveld to Kyalami in the Highveld while the opposite is true for *Cx. theileri*. *Culex theileri* is associated with temperate or high altitude areas receiving significant amounts of rainfall (Jupp, 1996, Jupp, 2004b, McIntosh et al., 1980,

Jupp, 1971, Jupp, 2001, Jupp and NK, 1986). These results suggest a high potential for mosquito-borne RVF (McIntosh, 1972, Swanepoel and Coetzer, 2004) and WN (Jupp, 2001, Jupp, 1996) virus transmission above 1,200m in South Africa. Since *Cx. theileri* is the most widespread and dominant mosquito in the Highveld with the ability to breed in pools with polluted organic waste from domestic animals and can feed on a wide range of hosts such as birds, domestic stock and humans, its chances of transmitting RVF or WN viruses are high on the inland plateau.

3.5   Conclusion

This study, based on seven medically important vector mosquitoes in northern South Africa, has revealed marked differences in temporal distribution and seasonal abundances between distinct geographic regions and climates. Differences in the number of rainy months, relative humidity and temperature may give rise to markedly different seasonal mosquito abundances and provide interesting perceptions on how possible climatic change can affect the future density of mosquito vectors in the Highveld, Middleveld and similar areas. Regression models have shown that climatic factors play a crucial role in shaping the population dynamics of *Ae. mcintoshi*, *Ae. vittatus*, *An. arabiensis*, Cx. pipiens *s.l.*, *Cx. poicilipes*, *Cx. theileri* and *Cx. univittatus*. High summer temperatures lead to increased vector density which can trigger outbreaks of RVF, SIN and WN viruses on the inland plateau of South Africa. This study also showed that abundances of RVF and WN virus vectors are related to elevation. Indeed, the mosquito abundance for two important potential vectors of RVF (*Ae. mcintoshi* and *Cx. poicilipes*) decreased with increasing elevation while that of *Cx. theileri*, a suspected vector for RVF and WN viruses increased with rising altitude. These findings will be important in predicting the timing of onset and spread of future epidemics such as WN and RVF viruses, in northern South Africa and other geographical settings with similar climates.

CHAPTER FOUR

4.0   General Conclusion

A study on the population composition and seasonal dynamics of mosquito communities in peri-urban, mixed rural and sylvatic areas was necessary due to increasing incidence of new and emerging zoonotic arboviruses being diagnosed from fatalities in wildlife and domestic animals, as well as from hospitalized human patients, in South Africa. The importance of several of these arboviral diseases, and the increasing threat of introduction of mosquito-borne pathogens such as Zika, there is a lack of recent collections and publications on distribution of specifically *Aedes* and *Culex* species. Despite the strong contributions made over many decades by medical entomologists (Jupp and McIntosh, 1967, McIntosh et al., 1967, McIntosh et al., 1978, Cornel et al., 1993, Uejio et al., 2012); existing knowledge tends to focus on certain species and particular geographic disease "Hotspots" such as endemic malaria areas or regular Rift Valley Fever outbreaks (Gear et al., 1955, Pienaar and Thompson, 2013). To address some of these knowledge gaps, a comprehensive assessment of mosquito vectors and the diseases that they transmit in southern Africa with a focus on arboviruses was adequately tackled in Chapter 1. An understanding of the mosquito vector/arbovirus interaction is an important milestone towards consolidating existing surveillance and mosquito-borne disease control programs.

This study also provided an understanding of the mosquito population diversity, distribution and abundance along landscape gradients in northern South Africa. Our findings, both field observations and extrapolations from sampling suggest that mosquito diversity and richness are greater in untransformed natural and mixed rural settings compared to peri-urban areas in the northern part of South Africa. Models based on the sampling effort seem to suggest that spatial distribution of mosquitoes in the selected mixed rural, peri-urban and wildlife sites is random. These results seem to suggest that mosquito species richness decreased along landscape-level drivers such as climate, elevation, vegetation and host availability from the Middleveld savanna portion of Limpopo Province to Lowveld savanna areas. Conversely,

the decrease in species richness from Middleveld to Highveld Grassland areas in Gauteng Province reveals the presence of more cold adapted species such as *Ae. dentatus, Ae. juppi, Cx. theileri and Cx. univittatus.* These are mosquitoes associated with temperate or high altitude areas receiving significant amounts of rainfall (McIntosh et al., 1980, McIntosh, 1973, Jupp, 1971, McIntosch, 1971, Jupp, 1996). However, peri urban and mixed rural areas with a high concentration of humans and domestic stock were associated with mosquito vectors known to have a greater potential to transmit diseases especially Rift Valley fever, Sindbis and West Nile viruses. This survey, which was mainly focused on capturing adult mosquitoes, recorded thirty species known or suspected vectors of arthropod-borne viruses (arboviruses) in southern Africa and elsewhere (McIntosh, 1980, Braack et al., 2018, Venter, 2018). The presence of such a diversity of arbovirus vectors at the various collection sites explains the periodic outbreaks of arbovirus cases recorded from these sites and emphasizes the risk of continued outbreaks in future. However, the most widely distributed known and potential arbovirus vector species detected in this study were *Ae. aegypti*, *Ae. mcintoshi*, *Ae. metallicus*, *Ae. vittatus*, *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus. Anopheles arabiensis*, which is a major vector of malaria in the northern part of South Africa was recorded while the role of *An. vaneedeni* as a secondary vector in malaria transmission in the region remains unclear. In addition, this analysis allowed the capture of species poorly and irregularly found in study area such as *Ae. luteolateralis*, *Ae. pachyurus*, *Cx. sitiens*, *Fi. circumtestacea*, *Fi. uniformis*, *Mi. lacustris* and *Mi. pallida* some of which have probably extended their geographical range.

In this survey, we managed to determine trends for mosquito abundance with landscape and climate factors, and also how potential arbovirus and malaria vector mosquitoes are strongly affected by environmental variables. The Highveld which is an area highly populated was associated with greater numbers of mosquitoes known to transmit arboviruses. The abundances for *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* were greatest in the peri-urban areas of the Highveld Grassland region while A*e. mcintoshi*, *An. coustani*, *An. theileri* and *Cq. fuscopennata* populations were higher in the sylvatic areas of the Middleveld Bushveld region. As for *An. squamosus*, *Cx. poicilipes* and *Ma. uniformis*, they were most abundant in mixed rural areas of the Lowveld Bushveld region. *Aedes mcintoshi*, *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus* are the most widely distributed species in the study area. As with earlier findings from other studies, this study confirms that *Cx. theileri* is the most abundant

mosquito potential vector in the Highveld Grassland region (Jupp, 2004b, McIntosh et al., 1980). This analysis also suggests that *Cx. theileri* is rare in the Limpopo-Mpumalanga Lowveld (Jupp, 2004b) while *Cx. univitattus* is widely distributed in the temperate highlands of southern Africa (Mixão et al., 2016, Jupp, 1971). *Culex poicilipes* though not as widely distributed as *Ae. mcintoshi*, *Cx. pipiens s.l.*, *Cx. theileri* and *Cx. univittatus,* does seem to have a strong ecological plasticity that allows for its adaptation in the three ecological zones. We were able to determine that mosquito populations increase with increased temperature and rainfall, however, heavy downpours may decrease the abundance. High summer temperatures lead to increased vector density which can trigger outbreaks of RVF, SIN and WN viruses on the inland plateau of South Africa (van Der Linde, 1982, Jupp, 1973, Swanepoel and Coetzer, 2004). This study also showed that abundances of RVF and WN virus vectors are related to elevation. Indeed, the mosquito abundance for two important potential vectors of RVF (*Ae. mcintoshi* and *Cx. poicilipes*) decreased with increasing elevation while that of *Cx. theileri*, a suspected vector for RVF and WN viruses increased with rising altitude. These findings will be important in predicting the timing of onset and spread of future epidemics such as WN and RVF viruses, in northern South Africa and other geographical settings with similar climates.

4.1 Suggestions for Future Research

Future investigations should focus on identifying viruses in mosquitoes while arbovirus surveillance and vector control programs should be augmented in urban and mixed rural settings where there is greater risk for arbovirus transmission to humans and domestic stock.

Widespread distribution of *Aedes aegypti,* a potential vector for Zika virus was recorded in this study although at low levels. This should be monitored for its epidemic potential to transmit emerging or imported viruses such as Zika. Aedes vittatus, was found in large numbers, therefore it should be tested for its ability to transmit CHIK, YF and Zika viruses.

Mosquito surveillance is important to understand how species composition and abundance change over time, with possible introduction of new species and the loss of resident species, as climate change may result in shifts in species distributions and possible disease spread.

Although this study was limited both geographically and in type of trap used, moderate to high diversity and species richness were found at all sites except Orpen in Kruger National Park. Future studies should consider setting up different types of traps to evaluate trap efficiency, mosquito abundance, breeding habitats, species composition as well as individual stages.

Since the potential for further outbreaks of SIN, WN and RVF viruses during summer months is high, surveillance of mosquito populations should continue to provide early detection and prevent disease outbreaks in "hotspot areas".

Our findings suggest that there is a decrease in mosquito abundance for *Ae. mcintoshi* and *Cx. poicilipes* with increasing altitude from the Middleveld Bushveld to the Highveld Grassland region while the opposite seems to be true for *Cx. theileri*. Does this mean species such as *Ae. mcintoshi* and *Cx. poicilipes* are adapted to warm climates and lower elevations while *Cx. theileri* is a cold adapted species found at higher elevations? If the hypothesis that elevation is an important driver in the distribution and abundance of mosquitoes, then further studies covering a wider geographical area should be conducted along an elevational gradient from the Middleveld Bushveld in Limpopo to the lower coastal areas in KwaZulu Natal, Eastern and Western Cape Provinces. Such studies would also help to dispel or support earlier studies which suggested that highest diversity occurred at lower altitudes.

Our climatic models did suggest that temperature and rainfall strongly affect mosquito seasonality and abundance. There is need for further surveillance in other parts of South Africa with different climatic conditions but presence of common Highveld species and similar species of flood water *Aedes* species such as the Karoo, Eastern Cape and Free State Provinces.

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