Population dynamics of the malaria vector *Anopheles arabiensis* from northern KwaZulu Natal, South Africa (2014-2019)

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Epidemiology (Infectious Disease Epidemiology).

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Declaration

I, Sinalo Gqunu, declare that this research report is my work. It is being submitted in partial fulfilment for the degree of Master of Science in Epidemiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature: 

Date: 04 November 2022

Dedication

This one is for my mother, Thelma Ngeziwe Gqunu; she fought tooth and nail to ensure I pursued this degree. And to my sponsors, the Tiso Foundation, who have been with me since my second year of my undergraduate, this too is for you.

Abstract

Background

Over the years, South Africa has reported a significant decrease in indigenous cases but malaria cases have remained stagnant despite continued efforts to eliminate the disease. The failure to completely eliminate malaria transmission has been ascribed to several factors, including a limited understanding of the intricate bionomics of vectors driving the ongoing residual malaria transmission. *Anopheles arabiensis* (the primary malaria vector in South Africa) and several potential secondary vectors have been implicated in malaria transmission. However, the population dynamics of these vectors and their individual roles in the ongoing residual malaria transmission are not well described. Several factors affect mosquito population dynamics, these include population density and environmental and ecological factors. This study aims to describe and determine the factors that influence the population dynamics of the *Anopheles arabiensis* population from Jozini, KwaZulu Natal, in South Africa.

Methods

The data used in this study were collected from sections 2, 8, and 9 in Mamfene (KwaZulu-Natal) and stored in the SIT database housed at the National Institute for Communicable Diseases (NICD). For purposes of this work, data collected between 2014 and 2019 were retrieved from the SIT database and summarised using descriptive statistics. Furthermore, multiple linear and logistic regression models were used to determine factors that influenced or were associated with two distinct outcomes. The outcomes were *An. arabiensis* density for the multiple linear regression model and the occurrence of *An. arabiensis* for the multiple logistic regression model. For both regression models, these factors included the section, season, and year of collection; the average temperature, humidity, and wind speed observed during the collection period; and the distance between households and traps. To model the effect of sex, the multiple linear regression model used the number of females in the *An. Arabiensis* population, while the logistic regression model used both sexes and the males served as a reference group.

Results

Out of 7838 mosquitoes collected, 4234 (53.0%) were members of the *Anopheles gambiae* complex, and 1198 (15.3%) were members of the *Anopheles funestus* group. *Anopheles arabiensis* was the most abundant species from the *An. Gambiae* complex contributing 49.0% of the total collection, while *An. Parensis* dominated the *An. Funes*tus group contributing 8.4%

of the total collection. The *An. Arabiensis* population density peaked during autumn and was at its lowest in winter. The multiple linear model showed that factors that influenced *An. arabiensis* density were section, year, and season of collection; the temperature and humidity observed during the collection period; and the number of *An. arabiensis* female mosquitoes collected. Multiple logistic regression modeling showed an association between the occurrence of *An. arabiensis* and section and season of the collection, the sex collected, and the temperature and humidity observed during collection. The logistic regression model also showed that the was an interaction between average rainfall and season of collection which positively influenced the occurrence of *An. Arabiensis*.

Conclusion

This study showed that *Anopheles arabiensis* occur in sympatry with various other anophelines. Most of the other anophelines sampled were previously implicated as potential malaria vectors in South Africa. These findings confirm that Mamfene is receptive to malaria transmission, with more than one vector probably driving the ongoing residual malaria transmission. This presents challenges to current vector control strategies that are mainly focused on the primary malaria vector, *An. arabiensis*. Overall, it can be concluded that the most efficient time to conduct supplementary vector control activities is during winter when conditions are least favourable for the primary vector, *An. arabiensis*. It is recommended that malaria control programmes should enhance winter larviciding as a supplementary vector control strategy.

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Finally, thank you to me, I did it!

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Abbreviations

An. – Anopheles

aOR – Adjusted odds ratio

ANOVA – Analysis of variance

AREC – Animal Research Ethics Committee

CI – Confidence Interval

DDT – Dichlorodiphenyltrichloroethane

DNA – Deoxyribonucleic acid

IRS – Indoor Residual Spraying

IQR – Interquartile Range

EVI – Enhanced Vegetation Index

KPN – Kruger National Park

KZN – KwaZulu Natal

MS – Microsoft

NICD – National Institute for Communicable Diseases

P. – Plasmodium

PCR – Polymerase chain reaction

Pf EIR – *Plasmodium falciparum* entomological inoculation rate

RH – Relative humidity

SA – South Africa

SDI – Species Diversity Index

SIT – Sterile Insect Technique

SSA – Sub-Saharan Africa

s.s – sensu stricto

WHO – World Health Organization

Chapter 1: Introduction

This chapter introduces the study through background information, briefly explaining why this study was carried out. Following the background is a review of relevant literature which probes further into essential aspects of the study by appraising literature concerning the following topics: the diversity of the *Anopheles* mosquito species that cause malaria; the spatiotemporal distribution of the primary malaria vector (*Anopheles arabiensis*) in South Africa; and the factors that influence the population dynamics of *Anopheles* mosquitoes. After the literature review is the problem statement and the justification, the chapter concludes with the research question, aim, and objectives.

1.1. Background

Malaria background

Malaria is a disease that is caused by several protozoan species belonging to the genus *Plasmodium*: *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*. It is transmitted from human to human through the bite of the female *Anopheles* mosquito [1]. Other rare modes of malaria transmission include mother-to-child transmission (transplacentally or during labour) [2], blood transfusion transmission [3–5] and needle stick injury transmission [3,4,6]. Over 90% of malaria infections in Africa are caused by *P. falciparum*. Though the transmission levels vary across Africa, the public health impact of *P. falciparum* infections has been felt immensely on this continent [7]. This is because Africa has the most effective and efficient vector species of human malaria, namely, *An. funestus*, *An. coluzzii* and *An. gambiae*. These species are associated with a high *P. falciparum* entomological inoculation rate (*Pf* EIR).

Malaria incidence, prevalence, and control in South Africa

Sub-Saharan Africa (SSA) is riddled with malaria, and South Africa is one of the few SSA countries experiencing very low malaria transmission levels. Very low malaria transmission areas are defined as areas that have an annual parasite incidence below a hundred cases per thousand population, and a prevalence of *P. falciparum*/*P. vivax* malaria between zero and less than one percent [8]. The burden of malaria in South Africa is mainly limited to three endemic provinces, the north-eastern part of KwaZulu Natal (KZN), Mpumalanga and Limpopo [9,10]. The remarkable achievements in reducing the malaria burden are primarily credited to a well-organised malaria control programme premised on effective control of malaria vectors using indoor residual spraying (IRS) of insecticides to wall surfaces and roofs of houses in affected areas [11].

Despite the effectiveness of current control strategies, malaria transmission persists in South African endemic provinces [12,13]. The failure to further lower the malaria burden is due to several factors, including that the IRS is not fully effective as a standalone strategy. IRS mainly targets mosquitoes that feed and rest indoors making it ineffective against mosquito populations that feed and rest outdoors. Such mosquito populations have been implicated as the drivers of the ongoing residual malaria transmission in South Africa [9,12,14]. Furthermore, the continual use of insecticides during annual spraying programmes results in the evolution of insecticide resistance. This occurs by selecting malaria vector species and strains resistant to insecticides, thus leading to malaria vector control failure. High pyrethroids resistance and low DDT resistance have been reported in South Africa [11,15].

Most importantly, there is a need to strengthen the current surveillance systems and adapt the system to address the ongoing malaria transmission dynamics — the WHO classified malaria surveillance as one of the three malaria control pillars [16]. The South African malaria surveillance system is a dual system consisting of epidemiological surveillance (detection of malaria cases) and entomological surveillance (i.e., vector surveillance). [10] While significant progress has been made in improving malaria case detection (epidemiological surveillance), limited progress has been made in improving the vector surveillance component. The challenge of the current vector surveillance system is that it is not fully addressing the requirements of surveillance under low malaria transmission settings. Mainly, information on the bionomics of vector species implicated in the ongoing residual malaria is unknown. In addition, information on the malaria vectors' monthly and seasonal population dynamics and the details of the factors that influence their density changes is limited.

Malaria vectors in South Africa

The major vectors implicated in malaria transmission in South Africa belong to the *Anopheles gambiae* complex and the *Anopheles funestus* group [9,10]. The malaria vector species from the *An. gambiae* complex in South Africa is *An. gambiae sensu stricto* (*s.s*), *An. arabiensis,* and *An. merus* [9,10]. Of these *An. arabiensis* and *An. merus,* are presumed to be responsible for malaria transmission after the successful eradication of *An. gambiae* s.s [9,10]. From the *An. funestus* group, *An. funestus s.s* has since been nearly eradicated after the reintroduction of dichlorodiphenyltrichloroethane (DDT) [12,13]. Recently a member of the *An. funestus* group (*An. vaneedeni*) has been implicated as possible malaria vector species responsible for the ongoing malaria transmission [12].

Since *An. arabiensis* is currently the known primary vector in South Africa, this study aims to describe and determine the factors that influence the population dynamics of this species. These findings will likely assist in designing evidence-based vector control programmes that will strengthen the current interventions.

1.2. Literature review

1.2.1. Diversity of the *Anopheles* species in sub-Saharan Africa

There are over 500 known species of *Anopheles*, and only 70 are proficient vectors of human malaria [17]. Sub-Saharan Africa has ecological conditions suitable for the proliferation of the most dominant and efficient vectors, including members from the *An. gambiae* complex and the *An. funestus* group [18].

The *An. gambiae* complex consists of eight species: *An. gambiae, An. coluzzii*, *An. arabiensis, An. melas, An. bwambae, An. merus, An. quadriannulatus* and *An. amharicus* [19]. Among these eight species, two are zoophilic (*An. quadriannulatus* and *An. amharicus*) and have never been implicated in transmitting malaria, while the other six are malaria vectors [19]. Of the six species, three — *An. gambiae, An. coluzzii*, and *An. arabiensis* — are proficient dominant malaria vector species. They occupy a similar ecological environment and are often found in overlapping geographical stretches across sub-Saharan Africa [19]. The other vector species are *An. melas* and *An. merus*, coastal saltwater tolerant species breeding in brackish waters [7,19]. They are classified as minor vectors; however, they can become dominant vector species in localised areas [7]. The last species of the *An. gambiae (*s.*l.)* complex is *An. bwambae*. This is a minor vector species that is also saltwater tolerant and is highly restricted in its distribution, having been identified only in the geothermal springs of western Uganda [7].

The main malaria vector species in South Africa belong to the *An. gambiae* complex species and three of them; *An. gambiae s.s, An. arabiensis* and *An. merus* have been historically implicated in malaria transmission in SA [10]. However, *An. gambiae s.s* was eradicated in the '60s. *Anopheles merus* was confirmed as a malaria vector in South Africa in 2016 [20], making two vectors from this complex directly involved in the ongoing residual malaria transmission: *An. arabiensis* and *An. merus*, however, the latter plays a minor role in transmission [9,20].

The *An. funestus* group consists of five subgroups, with three of these five subgroups consisting of at least 13 African species [21,22]. The African *An. funestus* species subgroups are *An. funestus, An. minimus and An. rivulorum* [21,22]. The *An. funestus* subgroup consists of *An. funestus s.s, An. funestus-like, An. aruni, An. confusus, An. parensis, An. vaneedeni* and *An. longipalpi*s type

C [21,22]. The *An. minimus* subgroup comprises *An. leesoni* and *An. longipalpis* type A [21,22]. Lastly, the *An. rivulorum* subgroup consists of *An. rivulorum s.s, An. rivulorum-like, An. brucei* and *An. fuscivenosus* [21,22].

Among the 13 *An. funestus* group vector species, the most dominant and efficient malaria vector is *An. funestus s.s*. Other members of this group have only been implicated as malaria vectors using circumstantial evidence. These are *An. rivulorum*, *An. rivulorum*-like, *An. parensis, An. leesoni,* and *An. vaneedeni* [12,13]. *Anopheles rivulorum* has been implicated in malaria transmission in Zambia [23]. Similarly, studies conducted in Kenya and Tanzania also found this species infected with *P. falciparum* sporozoites; however, the species have not been directly implicated as a vector [24,25]. *Anopheles vaneedeni* has been recently implicated in malaria transmission in SA [12].

The *An. rivulorum*-like is another species from the *An. funestus* group that is potentially a malaria vector. It was recently sampled in Kruger National Park (KNP), SA; however, it tested negative for the presence of *P. falciparum* sporozoites (5). Mouatcho and colleagues [13] attributed the negative test result to the vector being collected inside the Kruger National Park (KNP), a strictly managed game reserve. They also added that the KNP attracts middle to high-income tourists who are less likely to carry infective gametocytes. Furthermore, the authors argued that the situation in the KNP is not representative of malaria-endemic areas immediately adjoining this nature reserve on both the South African and Mozambican sides. They proposed that further research is needed to implicate or rule out this species in malaria transmission.

Anopheles parensis is another member of the *An. funestus* group that has been recently implicated as a potential malaria vector in South Africa [14]. However, its contribution to malaria transmission is likely minimal because of its zoophilic tendencies [14]. *Anopheles leesoni* is the last member of the *An. funestus* group that has been suspected as a secondary/minor malaria vector. Only one study in Tanzania found this species naturally infected with *P. falciparum* [25]. However, not many studies have been done in SA to implicate it in local malaria transmission. The rest of the *Anopheles funestus* group members are non-vectors, and there are no records of their involvement in the transmission of malaria.

In conclusion, the only species in South Africa implicated as potential vectors in the ongoing residual malaria transmission are *An. arabiensis, An. parensis, An. vaneedeni,* and *An. merus*.

1.2.2. Factors that influence *Anopheles arabiensis* population assemblage

Various ecological and environmental factors influence mosquito population densities. In a study carried out in eastern Uganda, it was found that rainfall and enhanced vegetation index (EVI) were associated with high mosquito densities [26]. On the contrary, increasing distance to water sources was associated with low levels of mosquito density [26]. Similar results were obtained in a study conducted in western Kenya by Amek *et al*. [27]. Another study in northern Sudan further stressed the importance of water sources [28]. In this study, many mosquito breeding sites were associated with riverside habitats. The same survey found mosquito breeding sites in leaking underground pipes and brickworks. Other sites that have been found to provide suitable mosquito breeding habitats are tyre tracks, stagnant water pools due to poor sanitation, agricultural fields, construction sites and swimming pools [29,30].

Another parameter that influences mosquito survivorship is temperature. Temperature plays a significant role in the development and survival of mosquitoes [31]. Furthermore, it also plays a vital role during the sporogonic cycle of *Plasmodium* [32]. Temperatures above 22°C are usually suitable for malarial transmission [27]. In a laboratory study conducted in South Africa to compare the development and survival of *An. arabiensis* and *An. quadriannulatus* in light of varied ecological conditions, including temperature — the authors found that *An. arabiensis* larvae were more tolerant to higher temperatures [33]. Additionally, they found that this species' larvae survived better when competing against *An. quadriannulatus* at the 20 – 30°C temperature range and the 18 – 35°C temperature range [33]. Another South African study conducted by Abiodun *et al*. — to develop a deterministic mosquito model that explained the population dynamics of *An. arabiensis* under varying climatic conditions — showed that temperatures below 15°C and above 30°C negatively influenced the dynamics of *An. arabiensis* mosquitoes [31].

The maximum temperature of 30°C is slightly contradictory to the findings of Davies *et al* as they showed that *An. arabiensis* larvae could survive at temperatures of up to 35°C. However, it is important to note that in the study conducted by Davies *et al*., *An. arabiensis* larvae were specifically competing against *An. quadriannulatus* larvae and it was observed that when the two species were mixed, the survivourship of *An. arabiensis* larvae was higher. On the contrary, the model built by Abiodun *et al*. modelled the dynamics of *An. arabiensis* alone. Furthermore, Davies *et al*. only studied the aquatic stages of *An. arabiensis* while Abiodun *et al*. built a model that included both the aquatic and adult stages of the mosquito. Though the temperature tolerance of larvae influences the temperature tolerance of adult mosquitoes, it is

established that mosquito larvae are more tolerant to higher temperatures than their adult counterparts [31].

Humidity is another factor that plays a role in mosquito survival [34]. Low levels of relative humidity (RH) have been shown to have a negative impact on mosquito survival as they decrease their lifespan [34,35]. There is a significant relationship between relative humidity and temperature [35]. Lyons *et al*. showed that, at 100% RH levels, *An. arabiensis* mosquitos had higher survival across varying temperatures (20 – 30°C) compared to RH levels of 55% and 5% [35]. The studies conducted by both Abiodun *et al.* [36] and Lyons *et al*. [35] show that levels of RH greater or equal to 60% have no adverse effects on adult *An. arabiensis* mosquitoes. Increasing temperatures and low humidity levels trigger desiccation in mosquitoes [35]. Studies on the effects of low RH on mosquito survival indicate that low RH induces physiological changes in mosquitoes due to water stress [34,35]. These physiological changes then induce a reduction in water loss which allow the mosquito to conserve its water [34,35]. Additionally, the effect of humidity and temperature variation differs by sex. Female *An. arabiensis* mosquitos survive longer under varying temperature and humidity levels compared to males — a difference that was attributed to the larger size and thicker cuticles of females which contribute to decreased water loss rate [35].

The last and often neglected factor influencing vector population density is wind [37]. Wind speeds greater than 2.5 m/s were shown by Braak *et al*. to decrease the biting activity of *An. arabiensis* [38]. Also, Kaiser *et al*. showed that wind has a negative impact on the swarming and flight activity of *An. arabiensis* [39]. Additionally, another factor that has an effect on mosquitoes through wind is carbon dioxide (CO_2) attraction. Carbon dioxide attraction allows mosquitoes to locate hosts by detecting human settlements and flying towards them [40]. At higher wind speeds — speeds greater than 3.5 m/s according to Kaiser *et al*. — mosquitoes would be unable to fly towards hosts as they would be swept in the direction of the wind currents [39]. Therefore CO_2 attraction indirectly controls the growth of the vector population by allowing the vectors to find the hosts, feed on them and get the blood they need to nourish their eggs [40]. Since the vectors need blood to sustain their reproduction, the presence or absence of hosts also becomes a crucial aspect [41].

In summary, the *Anopheles arabiensis* mosquito population dynamics are influenced by rainfall, vegetation, distance to water sources, availability of breeding sites, temperature, relative humidity, presence or absence of hosts and wind. The abundance of mosquitoes is

likely to be very high in areas with high rainfall, EVI and relative humidity, warm to high temperatures, many water sources and breeding sites, and areas with human settlements north of reservoirs.

1.2.3. Spatiotemporal distribution of *Anopheles arabiensis* in sub-Saharan Africa

There have not been many studies conducted on the spatiotemporal analysis of *An. arabiensis* or any of the vectors that are endemic to South Africa. However, there is vast literature on this subject from other sub-Saharan African countries [26–28,42–44]. Though these studies have been done in SSA, they were for different purposes, such as explaining the genetic structure of the vector (*An. arabiensis*) [44], its host feeding preferences [43], its habitat preferences [42] or explaining the dynamics of transmission of malaria [27,28].

In a four-year (2010-2013) national mosquito survey that exclusively focussed on the spatiotemporal distribution of *An. arabiensis* over seven sites (Busoro, Karambi, Rukara, Bukora, Meshesha, Bungwe, and Kicukiro) in Rwanda, members from the *An. gambiae* complex were the most dominant anopheline species [27]. Furthermore, the study showed that 84.4% of *An. gambiae* complex sampled were *An. arabiensis*. In this study, two localities contributed the highest number of *An. arabiensis*, with its population peaking in August. These findings were surprising as August is one of Rwanda's coldest and driest months. The authors explained the anomaly to one of the localities, Meshesha, in the country's Western province, where irrigation rice farming is practised. These conditions ensure perennial availability of breeding sites to support *An. arabiensis* proliferation. There are two annual rice irrigation cycles during rice production that could have provided suitable conditions for mosquito breeding. On the contrary, the highest mosquito density in other sites like Busoro was recorded in March, which falls under a wet, warm-season [45].

1.3. Problem statement:

South Africa is one of 21 countries that initially pledged to achieve zero local malaria transmission by 2020 [46]. Over the years, South Africa has reported a significant decrease in indigenous cases but fails to further lower malaria to meet its malaria elimination objectives [46]. The failure to meet the elimination target has been ascribed to several factors, including a limited understanding of the intricate bionomics of vectors driving the ongoing residual malaria transmission. *Anopheles arabiensis* and several potential secondary vectors have been implicated in malaria transmission in South Africa [1,9,12,13]; however, their population dynamics and role in the continuing residual malaria transmission are not well described. Particularly,

details on factors that influence the population dynamics of the primary vector *An. arabiensis* is mainly unknown.

1.4. Justification:

The success of any vector control programme is hinged on knowledge of the population dynamics of the targeted species. Therefore, it is critical to understand the distribution of vectors and how their densities change with time. This information is valuable for malaria control programmes as it determines when and where to target vector control interventions. Furthermore, understanding factors that determine the dynamics and distribution will allow the malaria control programme to adapt and tailor its vector control strategies. Against this background, this study was conducted to gather information on the population dynamics of the primary malaria vector *An. arabiensis* from a malaria-endemic area and the factors that influence its distribution. The data will provide information on the entomological drivers responsible for the continuing residual malaria transmission in South Africa and accelerate its malaria elimination initiatives.

1.5. Research Question

What factors influence the population dynamics of the *Anopheles arabiensis* population in Jozini, northern KZN?

1.6. Aim

To describe and determine factors that influence the population dynamics of *Anopheles arabiensis* population from Mamfene (Jozini district), northern KZN.

1.7. Objectives

1. To describe the diversity of *Anopheles* mosquitoes from Mamfene, northern KZN.
2. To describe the monthly population density of *An. arabiensis* among three different sampling areas in Mamfene.
3. To identify factors associated with *An. arabiensis* density in Mamfene.
4. To identify factors that influence the presence of *An. arabiensis* vector mosquito in Mamfene.

Chapter 2: Methods

This chapter details the methodology utilised to address this study's objectives and answer the research question. In this chapter, there are details of the study design, study site; study population; data collection; variables to be analysed; the statistical analysis plan (which includes both the data management and analyses processes), and ethical considerations for the study. This chapter is presented as follows; sections 2.2 – 2.4 are mainly a description of the primary study with the last line in section 2.4 introducing the secondary study, and hence the rest of the chapter is a description of the secondary study.

2.1 Study design

This was a cross-sectional study that used secondary data collected between 2014 and 2019 in Mamfene, northern KZN.

2.2 Study site

The study site where the primary data were collected is Mamfene (Jozini municipality), north of KwaZulu/Natal province in South Africa. Ongoing entomological surveillance is underway at the site as part of a project investigating the applicability of the sterile insect technique (SIT) as a complementary vector control tool. The study area (see figure 1) is divided into three main sampling sites; section 2 (control site), section 8 (control site), and section 9 (treatment site). The coordinates of the sections are as follows: Section 2 (S 27°24′14.2″; E 032° 12′41.8″), Section 8 (S 27°27′34.3″; E 032° 10′43.7″) and Section 9 (S 27°23′50.5″; E 032° 12′20.1″).

***Figure 1*: Map showing Mamfene, northern KZN, South Africa, where the primary data used in this study was collected.** The red pin depicts the Mamfene area, and the three mosquito sampling sites, i.e., sections 2, 8 and 9, are represented by the blue stars. The map was obtained from the following source: Map data (c) 2021 AfriGIS (Pty) Ltd, Google (https://www.google.co.za/maps/place/South+Africa/).

2.3 Study population

The population consists of all mosquito specimens that were collected from Mamfene, Jozini, northern KwaZulu Natal, South Africa.

2.4 Data collection

The mosquito specimens were collected approximately eight times every month from permanently stationed clay pots over six years (February 2014 – December 2019). However, it is important to note that in September 2018, the traps were repositioned and more traps were added. Over the study period, climatic data (average daily values) were concurrently collected from the Makhathini Research Station (27°23′42.45″S; 32°10′48.48″E) which is located about 3.2 km from Section 9. Ecological data were obtained during routine mosquito sampling. These data were then stored in the SIT database housed at the National Institute for Communicable Diseases (NICD). Hence all the data analysed in this study were retrieved from the NICD SIT database.

2.5 Variables

To address the first objective, the variables that were analysed were species type (categorical), Simpson's diversity index (continuous), section (categorical), month (categorical), year (categorical), and season (categorical). The species type variable consisted of 12 *Anopheline* species: *An. arabiensis*, *An. coustani*, *An. demeilloni*, *An. leesoni*, *An. marshallii* group, *An. merus*, *An. parensis*, *An. pretoriensis*, *An. quadriannulatus*, *An. rivulorum*, *An. rufipes*, and *An. vaneedeni* and all anopheline specimens that could not be identified to species level were classified as others. The Simpson's diversity index computation was done as shown in equation 1 below. It also included anophelines classified as others. This consisted of specimens that could not be accurately identified morphologically. The section variable consists of sections 2, 8 and 9. The month, season and year variables comprised 12 months and four seasons and the six years under review (2014-2019), respectively.

To address the second objective, the variables that were analysed were the population density of *An. arabiensis* (continuous); section (categorical); month (categorical); year (categorical); and season (categorical). The computation for population density is shown in equation 2.

To address the third objective, the outcome variable for fitting the model was *An. arabiensis* population density (continuous), and the exposure variables were average humidity (categorical); average temperature (categorical); average wind speed (categorical); average rainfall (continuous); female count (continuous); section (categorical); and season (categorical). Lastly, the outcome variable for fitting the model that addresses the last objective was whether the species collected was *An. arabiensis* or not (binary). The explanatory variables were the same as those in objective 3 except for female count which was substituted for the variable sex (binary). The average temperature was adjusted for as the uncategorised (continuous) variable, and we also adjusted for distance from a trap to a household (continuous).

2.6 Data management and statistical analysis

2.6.1 Data management

The data were extracted from the NICD database in comma-separated values format and or Microsoft (MS) Excel, and they were cleaned in both MS Excel and Stata version 16.0. A new variable, "season," was introduced during data cleaning. This variable was created such that the starting and end dates for each season were as follows:

- Summer: 01 December – 29 February

- Autumn: 01 March – 31 May
- Winter: 01 June – 31 August
- Spring: 01 September – 30 November

The retrieved data provided the counts of 12 different species and included data on mosquitoes whose species type (others) could not be determined. These were all used to compute the Simpson's diversity index. The primary interest of this study was the major vector, *An. arabiensis,* therefore, computation for population density was limited to this species.

***Equation 1*: Formula for calculating Simpson's diversity index**

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

Where:

D = Simpson's diversity index

N = total number of organisms, i.e., all species summed up

n = total number of organisms of each species

Σ = Sum

***Equation 2*: Formula for calculating *An. arabiensis* density**

$$Population\ density = \frac{n}{n_{traps}}$$

Where:

n = total number of *An. arabiensis* mosquitoes that were collected in that section per given month.

n_{traps} = total number of traps available in that section per given month; this included both active and inactive traps, inactive traps were those that did not trap any mosquitoes.

The data on the distance from a trap to the nearest household, used in logistic regression analysis, were generated from Google maps. This was done by measuring the distance between the trap (using the trap's GPS coordinates) and the nearest household structure. The climatic variables were averaged for each month and year. In line with literature, the variables average humidity, temperature, and wind speed were further categorised as shown below.

The categories for average humidity were:

- Category 1: humidity less than 60%, coded as one.

- Category 2: humidity greater or equal to 60%, coded as two.

The categories for average temperature were:

- Category 1: temperatures less than 20°C, coded as one.
- Category 2: temperatures between 20 – 30°C, coded as two.
- Category 3: temperatures more than 30°C, coded as three.

The categories for average wind speed were:

- Category 1: wind speed less than 2 m/s, coded as one.
- Category 2: wind speed greater or equal to 2 m/s, coded as two.

2.6.2 Statistical analysis

All statistical analyses carried out in this study were performed in Stata version 16.0, and all graphs were generated in Microsoft Excel. For all analyses, the significance level was set at 5% i.e., $p< 0.05$ indicated a statistically significant result.

Mosquito summary and comparisons

The descriptive statistics for table 1 were generated in Stata. The number of mosquitoes collected in each section through the six years was summarised as the relative frequencies and stratified by year, season, sex, and year. The species richness was determined using Simpson's diversity index (see equation 1). Median differences in the diversity index among different sections, years and seasons were determined using the Kruskal-Wallis test. Where there were significant differences, Dunn's test was used to conduct pairwise comparisons and separate the medians. The Kruskal-Wallis test was also used to compare *An. arabiensis* density across the different sections as the Shapiro-Wilk test showed that the density was not normally distributed.

Multivariable linear regression

To determine the factors that influence the changes in the population density of *An. Arabiensis*, a multivariable linear regression model was fitted. The modelling was carried out in three main steps: firstly, the model was fit using the population density of *An. arabiensis* as the outcome variable and adjusting for the explanatory variables (see section 2.5, paragraph 2 for the variables).

Secondly: the assumptions of linear regression models were checked using various tests. To check for normality, a normal probability plot was plotted. The Ramsey RESET test and the

Breusch-Pagan/Cook-Weisberg test for heteroskedasticity were used to check for omitted variables and homogeneity of variances. To check for multicollinearity, the variance inflation factor (VIF) was assessed. In the final model, the LOWESS was used to check for linearity.

A natural log transformation was performed since the outcome variable violated these assumptions. The prediction error of the models was assessed using the Akaike Information Criterion (AIC). Before settling on a final model, fractional polynomials were used to evaluate whether or not the continuous explanatory variables needed to be transformed and Cook's distance was used to assess outliers. The stepwise variable selection approach was explored; however, its exploration ceased because it eliminated climatic factors (rainfall and wind speed), which were deemed necessary from the literature review [26,37,40] and which we wanted to control for in the model.

Logistic regression

To identify the factors that influenced the presence of *An. arabiensis*, a multivariable logistic regression model was fit. The outcome variable (species) was coded 1 if the mosquito species was *An. arabiensis* and 0 if it was any other species or the species could not be identified. To assess effect modification between average rainfall and the different seasons, interactions were assessed. The prediction error of the models was evaluated using both the likelihood ratio test and the Akaike Information Criterion (AIC). The adjusted odds ratios (aOR) and the 95% confidence intervals (CI) were used to determine the association between each covariate and the outcome.

2.7 Ethical Considerations

This study was reviewed and approved by the Animal Research Ethics Committee (AREC) of the University of the Witwatersrand, Johannesburg (see appendix 2). Since this was secondary data analysis, permission to use the dataset was granted by the National Institute of Communicable Diseases (see appendix 3).

Chapter 3: Results

This chapter is presented in light of the objectives; section 3.1. describes the diversity of the Anophelines collected from Mafene (Table 1 – 2 and Figure 2). Section 3.2. describes the density of the *An. arabiensis* in Mamfene (Figure 3). Section 3.3. describes factors that influence the population density of *An. arabiensis* and its occurrence — Tables 3 and 4, respectively.

3.1 Description of the Anopheline diversity

3.1.1. Mosquito composition and distribution

In total, 7991 mosquitoes were collected from outdoor-placed traps, but 150 were dropped from the analysis because they were not allocated an identification number from the field. Two mosquitoes were dropped because they had no indication of which section they were collected. Lastly, a single mosquito was morphologically identified as *An. gambiae* but was dropped from analysis due to uncertainty of its identification. There were attempts to amplify the DNA of the specimen for sequencing; however, they were unsuccessful. If this specimen was indeed *An. gambiae*, it would have serious implications on malaria control as *An. gambiae* was one of the major vectors in South Africa but was eradicated in the 1960s. Therefore a total 7838 mosquitoes — belonging to 17 different species — collected over the six years under review were used in the analysis (table 1).

Overall, the majority 43.8% (n = 3435) were collected from section 9 and the least number of mosquitoes 19.6% (n = 1 535) were collected from section 8. Mosquito sampling productivity was high in 2019, contributing 39.7% (n = 3110) of the total collections. Stratifying mosquitoes collected by gender showed that 64.3% (n = 5 037) were females, with each section being dominated by females. The largest collection of mosquitoes 33.1% (n = 2598) was obtained during autumn while the winter recorded the least 15.4% (n = 1 206). Of the 17 species that were collected, 49.0% (n = 3839) were *An. arabiensis* and only one *An. maculipalpis* was collected during the entire 6-year sampling period. A proportion of 5.8% (n = 455) mosquitoes, could not be identified to species level by the identification methods used.

***Table 1*: Summary of anopheline mosquitoes collected using clay pots from Mamfene, KwaZulu-Natal between January 2014 – and December 2019 stratified by section, year, gender, season, and species.**

Variable		Number of mosquitoes collected per section (%)			Total
		Section 2	Section 8	Section 9	
Overall number collected		2868 (100)	1535 (100)	3435 (100)	7838
Year					
	2014	161 (5.61)	58 (3.78)	60 (1.75)	279
	2015	223 (7.78)	300 (19.54)	155 (4.51)	678
	2016	775 (27.02)	261 (17.00)	376 (10.95)	1412
	2017	576 (20.08)	87 (5.67)	355 (10.33)	1018
	2018	459 (16.00)	264 (17.20)	618 (17.99)	1341
	2019	674 (23.50)	565 (36.81)	1871 (54.47)	3110
Sex					
	Female	1923 (67.05)	1062 (69.19)	2052 (59.74)	5037
	Male	943 (32.88)	471 (30.68)	1383 (40.26)	2797
	Missing	2	2	0	4
Season					
	Summer	694 (24.20)	408 (26.58)	1 137 (33.16)	2241
	Autumn	987 (34.41)	410 (26.71)	1 201 (34.96)	2598
	Winter	508 (17.71)	230 (14.98)	468 (13.62)	1206
	Spring	679 (23.68)	487 (31.73)	627 (18.25)	1793
Species					
	An. arabiensis	1488 (51.88)	674 (43.91)	1677 (48.82)	3839
	An. coustani	9 (0.31)	17 (1.11)	11 (0.32)	37
	An. demeilloni	3 (0.10)	0	0	3
	An. leesoni	30 (1.05)	23 (1.50)	47 (1.37)	100
	An. marshallii group	70 (2.44)	63 (4.10)	252 (7.34)	385
	An. merus	256 (8.93)	36 (2.35)	47 (1.37)	339
	An. parensis	222 (7.74)	78 (5.08)	355 (10.33)	655
	An. pretoriensis	6 (0.21)	19 (1.24)	6 (0.17)	31
	An. quadriannulatus	34 (1.19)	7 (0.46)	15 (0.44)	56
	An. rivulorum	51 (1.78)	25 (1.63)	122 (3.55)	198
	An. rufipes	3 (0.10)	16 (1.04)	7 (0.20)	26
	An. vaneedeni	30 (1.05)	168 (10.94)	47 (1.37)	245
	An. maculipalpis	0	1 (0.07)	0	1
	An. pharoensis	2 (0.07)	3 (0.20)	0	5
	An. squamous	0	2 (0.13)	0	2
	[£]*An. gambiae* complex	254 (8.86)	74 (4.82)	289 (8.41)	617
	[¥]*An. funestus* group	243 (8.47)	208 (13.55)	393 (11.44)	844
	Other	167 (5.82)	121 (7.88)	167 (4.86)	455

[£]Mosquitoes morphologically identified as belonging to the *An. gambiae* complex but failed to be determined to species level using PCR.

[¥]Mosquitoes morphologically identified as belonging to the *An. funestus* group but was unable to be determined to species level using PCR.

Analysis of mosquito collections by sampling area shows that more mosquitoes were collected from section 2 than any other section during 2014, 2016 and 2017. On the contrary, more mosquitoes were sampled from section 9 during 2018 and 2019. Section 8 only dominated

collections during 2015. In sections 2 and 9, the highest number of mosquitoes was collected in autumn, 34.4% (n = 987) and 35.0% (n = 1201) respectively. Section 8 collected its highest number in spring 31.7% (n = 487). The least number of mosquitoes was captured in the winter season across all the sections. All three sections recorded very high proportions of *An. arabiensis*.

3.1.1. Species diversity

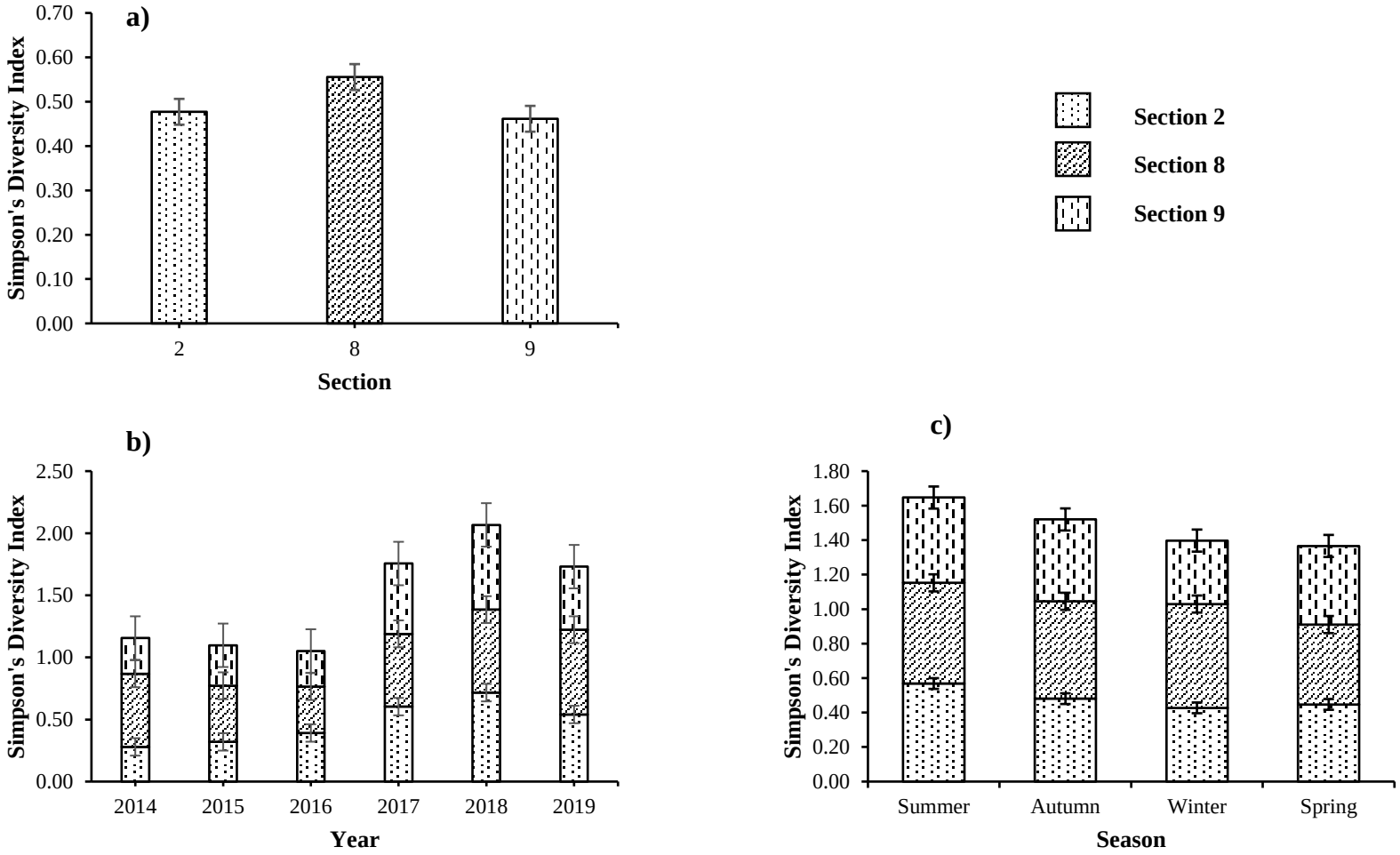

***Figure 2*: Simpson's diversity index of *Anopheles* mosquitoes collected using clay pots from Mamfene, KwaZulu-Natal between January 2014 – and December 2019 stratified by the sections across different years (b) and seasons (c).**

In total, fifteen different anopheline species were collected over the six-year sampling period. These included three members from the *An. gambiae* complex (*An. arabiensis*, *An. merus* and *An. quadriannulatus*), two members from the *An. funestus* subgroup (*An. vaneedeni* and *An. parensis*) and one member each from the *An. minimus* subgroup (*An. leesoni*) and *An. rivulorum* subgroup (*An. rivulorum s.s*). Stratification of species by section showed that section 8 had the highest species richness over the collection period with an overall species diversity index of 0.56, followed by sections 2 and 9, with a diversity index of 0.48 and 0.46, respectively (figure 2a). The overall difference in species diversity between the sections was not statistically significant (Kruskal-Wallis, $\chi^2 = 4.30$, p = 0.1165).

***Table 2*: Dunn's pairwise comparison (between the six years under review) of the species diversity of *Anopheles* mosquitoes collected using clay pots from Mamfene, KwaZulu-Natal (2014 –2019).**

2014	2015	2016	2017	2018	2019
2014	z = -0.54 p = 1.0000	z = -0.15 p = 1.0000	***z = -3.44** **p = 0.0044**	***z = -4.80** **p < 0.0001**	***z = -2.86** **p = 0.0322**
	2015	z = 0.46 p = 1.0000	***z = -3.48** **p = 0.0038**	***z = -5.12** **p < 0.0001**	***z = -2.79** **p = 0.396**
		2016	***z = -3.91** **p = 0.0007**	***z = -5.54** **p < 0.0001**	***z = -3.24** **p = 0.0090**
			2017	z = -1.63 p = 0.7804	z = 0.76 p = 1.0000
				2018	z = 2.42 p = 0.1163
					2019

The bold cells with asterisks (*) depict statistically significant differences.

Over the years, the species diversity index fluctuated (figure 2b), between 2015 and 2016, the species diversity index (SDI) showed a downward trend, but from 2016 to 2018, it showed an upward trend and dropped slightly in 2019. The highest diversity index was observed in 2018. There was a statistically significant difference in the species diversity across the years (Kruskal-Wallis, $\chi^2 = 52.03$, p = 0.0001). Subsequent pairwise comparisons showed significant species diversity across the years (table 2). Overall, there was no significant difference in species diversity between the seasons (Kruskal-Wallis, $\chi^2 = 4.44$, p = 0. 0.2176).

3.2. Description of the population density of *Anopheles arabiensis*

The population density of *An. arabiensis* (number caught per trap per month) shows a seasonal pattern (figure 3). There was a gradual increase in *An. arabienisis* density from October onwards and decline from April. The *An. arabienisis* density peaked around November (late spring) and February (summer); however, there were other peaks around March, April, June, and August. There were various shifts in density between the three sections over the study period. In 2014, 2016 and 2017, the density was higher in section 2 compared to all the other sections. In the later part of 2014 and 2015, the density was higher in section 8. In 2019, the density was higher in section 9; in 2018, it was high in sections 2 and 9.

Over the study period, the population density of *An. arabiensis* recorded was a minimum of 0 mosquitoes/trap/month and a maximum of 10.8 mosquitoes/trap/month. The overall median density recorded was 0.6 (IQR: 1.2) mosquitoes/trap/month. Section 2 had the highest *An. arabiensis* density with a median density of 0.8 (IQR: 1.6) mosquitoes/trap/month. Section 8 recorded the lowest *An. arabiensis* density with a median density of 0.4 (IQR: 0.9) mosquitoes/trap/month. Lastly, section 9 recorded a median density of 0.5 (IQR: 1.2) mosquitoes/trap/month. The difference observed in the population density of *An. arabiensis* across the sections was statistically significant (Kruskal-Wallis, $\chi^2 = 14.50$, $p = 0.0007$).

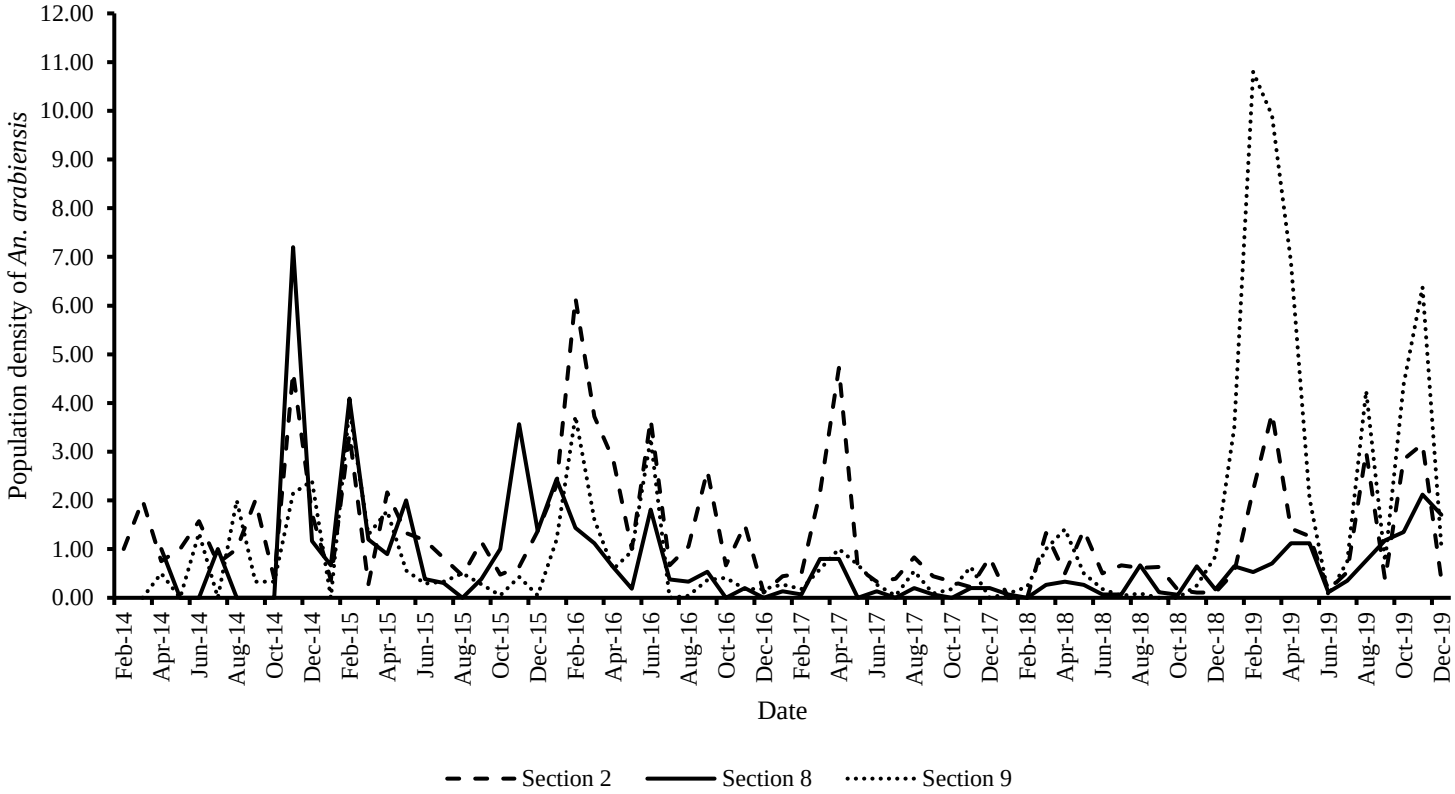

***Figure 3*: Population density of *Anopheles arabiensis* mosquitoes collected using clay pots from Mamfene, KwaZulu-Natal between January 2014 – and December 2019 presented by section.**

3.3. Factors that influence the density and collections of *Anopheles arabiensis*

3.3.1. Density-level results

***Table 3*: Summary of factors that influence the density of *Anopheles arabiensis* based on collections from the three sections in Mamfene, northern KwaZulu-Natal, South Africa (2014–2019).**

Covariate		Multivariable results	
		Coefficient	**p-value**
Section	2	Ref.	–
	8	-0. 28	0.035
	9	-0.44	0.001
Year	2014	Ref.	–
	2015	-0.76	0.001
	2016	-0.99	<0.001
	2017	-1.51	<0.001
	2018	-1.91	<0.001
	2019	-1.08	<0.001
Season	Summer	Ref.	–
	Autumn	0.45	0.005
	Winter	0.29	0.141
	Spring	0.07	0.644
Average temperature (°C)	< 20	Ref.	–
	20 – 30	0.65	0.002
	> 30	1.14	0.157
Average humidity (%)	< 60	Ref.	–
	≥ 60	0.78	0.002
Average wind speed (m/s)	< 2	Ref.	–
	≥ 2	-0.10	0.453
Average rainfall (mm)		-0.02	0.167
Female count		0.02	<0.001

Ref. = Reference point for each variable.

The factors that significantly influenced the *An. arabiensis* population densities were the section from which the mosquitoes were collected, the year in which they were collected, the

summer and autumn seasons, average temperature, average humidity, and the number of females available in the population i.e., female count.

Adjusting for all other factors, the *An. arabiensis* density in sections 8 and 9 was 25% (coef = -0.28, p = 0.035) and 36% (coef = -0.44, p = 0.001) lower than that in section 2, respectively. The year 2014 had the highest *An. arabiensis* population density compared to all the other years. The density of *An. arabiensis* was at its lowest in 2018. Adjusting for all other factors, the year is associated with an 85% decrease in *An. arabiensis* compared to 2014 (coef = -1.91, p <0.001). During the autumn season, the population density of *An. arabiensis* was 57% (coef = 0.45, p = 0.005) higher than the summer density. Average temperatures between 20 – 30°C were most suitable for *An. arabiensis* as they were associated with increased population density (coef = 0.65, p = 0.002). Similarly, average humidity levels greater than 60% were associated with increased population density (coef = 0.78; p = 0.002), that is, for humidity levels more than 60%, the population density increased by over 100% adjusting for other factors. Increases in the number of female *An. arabiensis* mosquitoes accounted for a 2% (coef = 0.02, p<0.001) increase in the population density.

3.3.2. Case-level results

***Table 4*: Adjusted odds ratios summarising the factors that are associated with collecting *An. arabiensis* based on collections from the three sections in Mamfene, northern KwaZulu-Natal, South Africa (2014-2019).**

Covariate		**Multivariable association results**	
		Adjusted odds ratio (95% CI)	**p-value**
Section	2	Ref.	–
	8	0.67 (0.59 – 0.77)	<0.001
	9	0.85 (0.76 – 0.95)	0.003
Season	Summer	Ref.	–
	Autumn	1.08 (0.95 – 1.23)	0.245
	Winter	2.04 (1.68 – 2.48)	<0.001
	Spring	1.46 (1.27 – 1.69)	<0.001
Sex	Male	Ref.	–
	Female	1.22 (1.11 – 1.34)	<0.001
Distance to the nearest household (m)*		1.002 (0.999 – 1.005)	0.118
Average temperature (°C)		1.08 (1.06 – 1.10)	<0.001

Average rainfall (mm)		0.99 (0.98 – 1.00)	0.194
Average humidity (%)	< 60	Ref.	–
	≥ 60	1.91 (1.53 – 2.40)	<0.001
Average wind speed (m/s)	< 2	Ref.	–
	≥ 2	0.99 (0.89 – 1.09)	0.787
Interaction terms			
Season and average rainfall (mm)	Autumn	1.02 (1.00 -1.03)	0.022
	Winter	0.86 (0.80 – 0.92)	<0.001
	Spring	0.94 (0.87– 1.01)	0.090

Ref. = Reference point for each variable. Effects of the distance from a trap to the nearest household are minimal but significant, hence using more decimal places.

The odds of collecting *An. arabiensis* from section 8 was 33% lower than the odds of collecting this species from section 2 (aOR = 0.67, p<0.001), adjusting for all other factors. Similarly, the odds of collecting *An. arabiensis* from section 9 were lower than those collecting this species from section 2 (aOR = 0.85, p = 0.003), adjusting for all other factors.

Adjusting for all other factors, the interaction between average rainfall and season showed that a unit increase in average rainfall was associated with increased odds of collecting *An. arabiensis* over the autumn season compared to the summer (aOR = 1.02, p = 0.022). On the contrary, as expected, unit increases in average rainfall were associated with decreased odds of collecting *An. arabiensis* over winter compared to summer (aOR = 0.86, p<0.001).

The odds of collecting female *An. arabiensis* mosquitoes were higher (aOR =1.22, p<0.001) than males, adjusting for all other factors. Adjusting for all other factors, a unit increase in average temperature was associated with increased odds of collecting *An. arabiensis* (aOR = 1.08, p<0.001). Lastly, there were greater odds of collecting *An. arabiensis* at humidity levels that are greater or equal to 60%. (aOR = 1.91, p<0.001) , adjusting for all other factors.

Chapter 4: Discussion

In this study, we described anopheline assemblage and determined factors that influence the population dynamics of *An. arabiensis* population from Jozini, northern KZN. This chapter explains the results of this study and explores how the results obtained address the aim and its subsequent objectives of the work. The chapter ends with the study's limitations, conclusion, and recommendations.

4.1. Study findings

This study presents a comprehensive survey of all anopheline mosquitoes in the northern KwaZulu/Natal Province. Previous entomological surveys have generally been limited to known malaria vector species, i.e., members of the *An. gambiae* complex and *An. funestus* group.

4.1.1. Description of the Anopheline diversity

A total of fifteen different *Anopheles* species were collected from outdoor-placed traps during the 6-year sampling period. *Anopheles arabiensis* dominated the *An. gambiae* complex and occurred in sympatry with other anophelines, while *An. parensis* dominated the *An. funestus* group collections. Generally, autumn was the most productive season, and winter was the least productive season. The year 2019 had the highest collection of anophelines, and the majority of the anophelines collected over the study period were females. Regarding diversity, the species diversity index varied across the years and between seasons peaking in 2018 and in summer, respectively. The diversity across sections was not statistically significant.

4.1.2. Population density of *Anopheles arabiensis*

The population density of *An. arabiensis* showed a seasonal pattern with a gradual increase from October onwards and a steady decline from April until July. Though not a yearly occurrence, the density of *An. arabiensis* was most abundant during November and February, with February 2019 having the most prominent peak ever observed during the six-year study period. There were also other minor peaks observed around March (2019), April (2017), June (2016), and August (2019).

4.1.3. Factors influencing *Anopheles arabiensis* density and collections

4.1.3.1. Factors influencing *Anopheles arabiensis* density

Our model showed that the factors that influenced *An. arabiensis* population density were section, year, season, average temperature, average humidity, and female count. The *An. arabiensis* population density was highest in section 2 compared to all the other sections. In terms of the year, the *An. arabiensis* density was highest in 2014 and lowest in 2018. The *An. arabiensis* density was most abundant over the rainy autumn season. High levels of humidity, temperature, and the number of females increased *An. arabiensis* population density.

4.1.3.2. Factors influencing *Anopheles arabiensis* collections

The factors that were associated with the collection of *An. arabiensis* were section, season, rainfall, distance from a trap to a household, sex, average temperature and average humidity. There were greater odds of collecting *An. arabiensis* from section 2 compared to the other two sections. Unit increases in rainfall were associated with increased odds of collecting *An. arabiensis* over autumn and decreased odds over winter. Increases in the distance from a trap to a household were associated with reduced odds of collecting *An. arabiensis*, however, the 2019 model showed a reverse association. Female *An. arabiensis* mosquitoes were collected more often than males. Increased temperatures and humidity levels greater than 60% were associated with increased odds of collecting *An. arabiensis*.

4.2. Description of the Anopheline diversity

The high level of diversity observed in this study was comparable to a similar cross-sectional anopheline survey conducted in the Kruger National Park, South Africa [47]. The perennial occurrence of the fifteen species (in sympatry) observed in this study has severe implications for vector control and malaria transmission, particularly because some of these species have been implicated as malaria vectors in South Africa [10,12,14,48]. Their outdoor-resting behaviour complicates the current vector control strategy which targets indoor resting populations [12,14]. Moreover, these vectors have the potential to sustain transmission after the main vector has been reduced through control measures such as IRS [49]. The high species diversity observed in Mamfene implies an abundance of resources and favourable conditions for the proliferation of species sampled. These conditions include perennial breeding sites, availability of blood meal sources (humans and or domestic animals), vegetation to provide nectar as an energy source, minimal predators, mud housing, and grass-thatched roofing [26,41,50].

The increased diversity observed in 2018 was likely due to increased competency in morphological identification. There was training administered to staff on species identification in 2018. The uniform species diversity index observed across the three sections suggests that they all provide suitable conditions for the species they harbour. Additionally, all three sections lie within a 5 km radius of section 9, and thus the closeness in the geographical distance indicates that they have similar ecological conditions. The varying diversity index observed across the seasons was likely due to varying climatic factors, particularly rainfall. There was a difference in the rain between the years and the driest year had the least number of species. The change in species diversity over the years implies a need for continuous entomological surveillance; since species occurrence is not static, vector control activities should be reviewed based on species diversity and their bionomics including their vectorial capacity.

The high density of *An. arabiensis* observed during the study period was expected and tallied with previous studies conducted in South Africa [9,14,47]. Furthermore, Dandalo *et al.* [9] directly implicated *An. arabiensis* as a primary malaria vector in South Africa; its abundance suggests that it is possibly still the primary vector responsible for the ongoing residual malaria transmission in Mamfene. In addition, our findings showed that *An. arabiensis* occurs perennially. This is supported by previous studies conducted in South Africa [9,47], which showed that *An. arabiensis* occurs perennially. The abundance and occurrence of *An. arabiensis* throughout the year in Mamfene provides circumstantial evidence which shows that — despite the ongoing indoor residual spraying programme — this area remains receptive to malaria [51] and probably explains the perennial residual malaria transmission. Moreover, though spraying (IRS) is done yearly, it still fails to target the outdoor populations of *An. arabiensis*. All *An. arabiensis* collected in this study were caught resting outdoors. Therefore, to target the entire population of *An. arabiensis*, current malaria control programmes must incorporate this dynamic feeding and resting behaviour of this species.

Data from this study showed that other members of the *An. gambiae* complex (*An. merus* and *An. quadriannulatus*) occur in sympatry with *An. arabiensis*. Both these species have been recorded in this area before by Dandalo and colleagues [9]. The occurrence of *An. merus* throughout the study period is concerning since this species has been implicated as a secondary vector in South Africa [10,48] and could contribute to the ongoing residual malaria transmission. Moreover, since current vector control methods only target indoor-resting mosquito

populations, the abundance of this species outdoors poses a serious challenge to malaria control efforts.

The occurrence of *An. merus* in Mamfene, though previously recorded, is unusual because this species is known as a saltwater breeder. The majority of the water bodies in Mamfene are freshwater. However, studies have shown that this species is adaptable. Despite its preference for brackish waters, *An. merus* can complete development in salinity levels ranging from 0-100% [52,53]. Therefore, the adaptability of this species shows that brackish waters are not a necessity for its survival; they merely increase its abundance. In addition, the presence of algae has been associated with *An. merus* larvae. Algae is known to serve as a food source for the larvae. *Anopheles merus* mainly prefers to breed in pools and ponds instead of swamps, animal hoofs, and wells [52]. All these factors are present in Mamfene providing a suitable habitat for the species.

The occurrence of members of the *An. funestus* group (*An. vaneedeni*, *An. rivulorum*, *An. leesoni*, and *An. parensis*) has also been previously recorded in this area [9]. In 2017, Burke *et al.* [12] raised concerns regarding the perennial occurrence of these members of the *An. funestus* group and suggested that their contribution to malaria transmission in SA be investigated. Subsequently, Burke and colleagues have since implicated two species — *An. vaneedeni* [12] and *An. parensis* [14] as vectors that could possibly be minimally contributing to the ongoing malaria transmission in SA. This raises concerns regarding the role in malaria transmission of the other *An.funestus* group members, *An. rivulorum* and *An. leesoni*, sampled during this study. These species have been implicated as vectors in other countries [23,25], and it could be possible that they might be contributing to the ongoing residual malaria transmission in Mamfene. Furthermore, the perennial occurrence of *An. vaneedeni* and *An. parensis* might pose a challenge to malaria control efforts since current vector control efforts are not tailored against them. However, their specific role in local malaria transmission has not been fully described.

The rest of the species sampled during this study (*An. coustani*, *An. demeilloni*, *An. marshallii* group, *An. pretoriensis, An. rufipes, An. maculipalpis, An. pharoensis,* and *An. squamous*) have all been previously collected in this study area and other parts of South Africa [51]. *Anopheles pretoriensis*, *An. rufipes* and *An. coustani* group were previously collected from Malahlapanga during an anopheline cross-sectional survey in northern Kruger National Park [47]. *Anopheles coustani* [54], *An. rufipes* [55,56], *marshallii* group [55], *An. pharoensis* [56], and *An. squamous* [57] have been identified as vectors in other sub-Saharan countries, while *An. demeilloni* [58], *An.*

pretoriensis [23] and *An. maculipalpis* [59] have only tested positive for *P. falciparum* but have not been implicated as vectors. Therefore, the role of these three potential vectors in malaria transmission in South Africa should be investigated, given their wide geographical distribution in the country.

The productive nature of the autumn season in this study is not surprising; this season offered the best collection conditions. Across the sampling period, the humidity levels averaged at no less than 68% during autumn, the wind speeds were constantly below 2 m/s, and the temperatures average at no less than 23°C. Similar conditions were found conducive for catching mosquitoes from the *An. gambiae* complex in a study done in KNP [47]. In conclusion, when vector control efforts are being prioritised, they should be done just before the peak season i.e., in winter to prevent the peak in density.

4.3. Description of the population density of *Anopheles arabiensis* and the factors associated with the density

The *An. arabiensis* density peak observed in this study corresponds to the South African peak malaria season which spans from November to April [51]. The seasonal occurrence of *An. arabiensis* observed in this survey is in line with the findings of Munhenga *et al*. [47] and Dandalo *et al*. [9], who reported a similar trend. Since the pattern of the density of *An. arabiensis* corresponds to the pattern of malaria cases, this provides further circumstantial evidence into *An. arabiensis* being the main driver of the ongoing malaria transmission in SA.

Our findings of the prominent peaks in density that occurred during February and November tallied with the findings of Munhenga *et al*. [47] and Dandalo *et al*. [9]. These peaks were expected as they fell within the height of rains — the month of February offered the highest rainfall hence the dominance of *An. arabiensis* caused by an abundance in breeding sites. Additionally, November borders the start of the summer rains hence an overlap in the start of the summer rains could explain the peak in density. The unexpected winter peak observed during June of 2016 could be attributed to the relatively warm temperature and high winter rains experienced that year. The August peak of 2019 could be explained by the additional added traps and repositioning of traps that was done in September 2018. The overall high mosquito density observed during 2019 might be defined by repositioning and new traps that were deployed.

This high density observed in 2014 compared to all other years as shown by the regression model cannot be attributed to environmental factors as 2014 had similar environmental factors

as the other years. The most likely explanation is that the addition of traps in subsequent years increased the number of traps that were not successful at trapping mosquitoes. Due to the inverse relationship between the number of mosquitoes collected and the number of traps available, additional traps that were not successful at trapping mosquitoes had the effect of lowering the apparent density levels.

The autumn density observed in this study contradicted the findings of previous studies, which observed the highest density in summer. The summer season generally carries higher mosquito density due to increased breeding sites due to the high warm summer rainfall [9,47]. However, during our study, the autumn season generally had a combination of high rainfall and warm temperatures; hence, this might explain the contradiction of this study with previous studies.

In line with the findings of other South African studies[35,36], our results showed that humidity levels that are equivalent to or exceed 60% are suitable for *An. arabiensis* as they were associated with increased *An. arabiensis* density. The finding that temperatures between 20-30°C are the most suitable for *An. arabiensis* tallied with the findings of another study conducted in KwaZulu-Natal [31]. Therefore, in light of these findings, it is essential to consider climatic conditions when designing vector control activities.

Lastly, our results showed that increases in the number of female *An. arabiensis* mosquitoes in the population were associated with increased levels of population density. This implies that if female mosquitoes were to be directly targeted by malaria control measures, the population of *An. arabiensis* would be reduced significantly. According to the model for every female *An. arabiensis* mosquito that is killed, the *An. arabiensis* density would be lowered by 2%.

4.4. Factors that influence the presence of *Anopheles arabiensis* population

Similar to the findings of Dandalo *et al.*[9], our results showed the odds of collecting *An. arabiensis* from section 8 were lower compared to the other 2 sections. In contrast, to our results, Dandalo *et al.*'s findings showed that the odds of collecting *An. arabiensis* from section 9 were higher compared to section 2. Another interesting observation was a continual shift of *An. arabiensis* collections dominance between sections 2 and 9; this occurrence was also observed in Dandalo *et al.*'s study. The change in density between sections 2 and 9 may be caused by mosquito migration between the two sections since they are only one kilometre apart. Section 8 had the lowest population density throughout the study period except in 2015, where

it dominated. The low population density in section 8 was probably due to how the section is structured; there are fewer household structures in section 8 compared to the other two sections.

In explaining mosquito productivity, our data showed an interdependence between season and rainfall. The logistic regression results for season and rainfall were obscure when the two variables were fitted in the model separately. However, when the two variables interacted, the model showed that increases in rainfall were associated with increased odds of collecting *An—arabiensis* over autumn and decreased odds over winter. The interdependency of rain and season showed that the impact of rain varied with season and that the interaction of these two variables influences the odds of collecting *An. arabiensis*. Our findings partially tallied with those of Dandalo and colleagues[9]. Mosquito densities peaked during summer and autumn for the two studies; however, in the Dandalo study, they observed the odds of collecting *An. arabiensis* (from the same study area) over autumn were lower than that of the summer season, contradicting this study's findings. In both instances, increased rainfall increased the number of available breeding sites; hence, the breeding sites would be higher during the rainy season and fewer during the dry season. Therefore, to control *An. arabiensis*, malaria control programmes can take advantage of the low winter population density through enhancing winter larviciding and the destruction of breeding sites.

Temperature increases are generally associated with increases in mosquito density [30,31,47,60]. As expected, our findings also showed that temperature increases were associated with greater odds of collecting *An. arabiensis*. However, the present results contradict those of two previous studies [26,61] that found temperature increases detrimental to mosquitoes and decreased survival. However, it is worth noting models in these 2 studies were constructed with a wider temperature range 16°C - 40°C. In this study minimum and maximum temperatures were 13.8°C and 32.8°C, respectively. It is well established that mosquitoes have a minimal and optimal permissive temperature range. Temperatures above 30°C are known to have adverse effects on adult mosquito survival [31,60]. It is not surprising that the models above showed the temperature inverse relationship with mosquito survival at higher temperatures. Lastly, humidity levels greater or equal to 60% were associated with increased odds of collection *An. arabiensis*; these findings tallied with those of Munhenga *et al.* [47] and Yamana and Eltahir [34]. The significance of climatic factors in determining mosquito density implies that these should be considered when planning malaria control programmes. Lastly, there were increased odds

of collecting female *An. arabiensis* mosquitoes compared to the males. However, this was expected as the sampling method used in this study was biased towards female collection.

In summary, the factors that influence *An. arabiensis* occurrence is increased levels of rainfall, temperature, humidity, and the autumn season. On the contrary, the winter season and geographical location, in this instance, section 8 negatively influenced the collection of *An. arabiensis*. The effects of the proximity of households were inconclusive. These findings thus indicate that it would be best to deploy malaria vector control measures in winter when it is cold and dry and all the factors that positively influence the *An. arabiensis* abundance is low.

4.5. Limitations

This study has limitations. We initially set out to perform a spatiotemporal analysis on the mosquito population from Mamfene. However, we were unable to do so due to a lack of shapefiles of the sections, and due to time constraints, we were unable to visit the study site and digitise them manually. Additionally, one of the factors that have been reported to influence mosquito population dynamics is the availability of — and the distance to — breeding sites. However, this was a factor that we could not explore in this study due to the insufficient data. The sampling between months and years was also non-standardised, i.e., although efforts were made to visit a trap twice every week, this was rarely the case. This presented challenges in analysing per year for the density model. The addition and repositioning of traps may have affected mosquito productivity and comparisons between years. Lastly, the distance between traps and households was measured using Google maps; therefore, the inaccuracy in those measurements needs to be considered when interpreting those results.

4.6. Conclusion and Recommendations

Anopheles arabiensis occur in sympatry with a variety of other anophelines. Some of the anophelines found in sympatry with *Anopheles arabiensis* have been implicated as malaria vectors making the study area receptive to malaria transmission, with more than one vector probably driving the ongoing residual malaria transmission. This presents a challenge to current vector control strategies that are mainly focused on the primary malaria vector, *An. arabiensis*. Therefore, it is recommended that an integrated vector control approach that targets all anophelines be implemented. The year-round occurrence of *An. arabiensis* highlights a need to conduct supplementary vector control activities when the density is low in order to prevent peak density when conditions become favourable. Against this background, enhanced winter

larviciding, house improvements, and community mobilisation should be considered among supplementary vector control strategies. Lastly, temperature and humidity are the factors that have the most significant influence on *An. arabiensis* density and should be taken care of when planning malaria control activities. This can also be best done by enhancing vector control during winter when temperature and humidity are at their lowest and thus conditions are least favourable for *An. arabiensis*.

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Chapter 6: Appendices

Appendix 1: Plagiarism Declaration

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Sinalo Gqunu (Student number: 2136197) am a

studentregistered for the degree of Master of Science (Epidemiology) in the academic year 3 .

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/orwithout acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided workexcept where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection)software indicating the level of plagiarism in my research document.

Signature:  Date: 04 November 2022

Appendix 2: Supplementary results

1. Stratified analysis for the collection of *An. arabiensis*

***Table 5*: Adjusted odds ratios summarising the factors associated with collecting *An. arabiensis* stratified by year and based on collections from the three sections in Mamfene, northern KwaZulu-Natal, South Africa (2014-2019).**

Covariate		Multivariable association results					
		Adjusted odds ratio (p-value) (95% CI)					
		2014	**2015**	**2016**	**2017**	**2018**	**2019**
Section	2	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
	8	**0.35* (0.14 – 0.88)**	**0.56 * (0.34 – 0.91)**	0.79 (0.56– 1.12)	1.32 (0.80 – 2.20)	1.12 (0.69 – 1.82)	**0.44 *** (0.34 – 0.56)**
	9	1.25 (0.37 – 4.27)	1.32 (0.70 – 2.49)	0.96 (0.67 – 1.39)	0.73 (0.48 – 1.11)	**0.54 ** (0.36 – 0.80)**	1.12 (0.92 – 1.35)
Season	Summer	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
	Autumn	0.50 (0.13 – 2.01)	**0.39 ** (0.23 – 0.68)**	**3.59 *** (2.21 – 5.83)**	**3.49 *** (2.21 – 5.51)**	**5.33 *** (3.04 – 9.37)**	0.82 (0.67 – 1.01)
	Winter	1.30 (0.22 – 7.73)	**0.33 ** (0.15 – 0.73)**	**3.66 *** (1.80 – 7.45)**	**8.75 *** (4.38 – 17.49)**	**4.15 *** (2.08 – 8.28)**	0.89 (0.63 – 1.56)
	Spring	1.43 (0.46 – 4.50)	1.82 (0.91– 3.64)	0.89 (0.54 – 1.45)	0.75 (0.36 – 1.56)	0.81 (0.44 – 1.48)	**2.92 *** (2.28 – 4.75)**
Sex	Male	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
	Female	0.75	1.15	**1.90 *** **	**1.54 ** **	0.83	0.89

		(0.32 – 1.71)	(0.74 – 1.78)	**(1.45 – 2.48)**	**(1.14 – 2.09)**	(0.60 – 1.14)	(0.77 – 1.04)
Average humidity (%)	< 60	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
	≥ 60	1.19 (0.19 – 7.23)	0.43 (0.15 – 1.23)	1.68 (0.60 – 4.76)	1.44 (0.66 – 3.15)	1.35 (0.61 – 3.03)	**1.64 ** (1.07 – 2.53)**
Average wind speed (m/s)	< 2	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
	≥ 2	0.65 (0.28 – 1.51)	1.03 (0.59 – 1.81)	**2.38 *** (1.65 – 3.43)**	1.28 (0.91 – 1.80)	0.80 (0.57 – 1.14)	**0.80 * (0.67 – 0.94)**
Distance to the nearest household (m)‡		1.016 (0.990 – 1.042)	0.993 (0.983 – 1.003)	1.005 (0.997 – 1.013)	0.990 (0.980 – 1.001)	0.99* (0.979 – 0.999)	0.997 (0.991 – 1.002)
Average temperature (°C)		1.10 (0.92 – 1.33)	0.97 (0.88 – 1.07)	**0.92 * (0.86 – 0.98)**	**1.24 *** (1.17 – 1.32)**	**1.10 ** (1.03 – 1.16)**	0.98 (0.94 – 1.01)
Average rainfall (mm)		0.79 (0.48 – 1.31)	1.01 (0.98 – 1.03)	**3.54 *** (2.38 – 5.27)**	0.99 (0.95 – 1.03)	1.00 (0.96 – 1.04)	**0.87 *** (0.81 – 0.93)**
Interactions							
Season and average rainfall (mm)	Autumn	0.33 (0.09 – 1.23)	1.14 (0.73 – 1.80)	**0.25 *** (0.16 -0.37)**	omitted	0.95 (0.87 – 1.04)	**1.16 *** (1.08 -1.25)**
	Winter	omitted	0.73 (0.39 – 1.37)	**0.25 *** (0.17– 0.37)**	0.92 (0.43 – 1.94)	0.92 (0.80 – 1.06)	0.97 (0.31 – 3.00)
	Spring	omitted	1.84 (0.49 – 6.88)	**0.29 *** (0.19 – 0.45)**	**1.31 ** (1.10 – 1.55)**	0.92 (0.70 – 1.21)	0.93 (0.73 – 1.19)

Ref. = Reference point for each variable. ‡Effects of the distance from a trap to the nearest household are very minimal but significant hence the use of more decimal places. NB: The bolded cells depict statistically significant differences, and the p-values are depicted as follows: p<0.001 is denoted by ***, p<0.01 is denoted by **, and p<0.05 is denoted by *

2. Weather conditions averaged per season per year

***Table 6*: Average humidity – stratified by year and averaged per season – from the three sections in Mamfene, northern KwaZulu-Natal, South Africa.**

	Mean ± standard deviation (%)			
Year/Season	**Summer**	**Autumn**	**Winter**	**Spring**
2014	65.0 ± 2.8	75.5 ± 3.1	66.4 ± 7.0	69.1 ± 5.8
2015	70.5 ± 7.8	68.9 ± 6.4	70.9 ± 10.2	63.1 ± 5.1
2016	74.3 ± 4.7	75.6 ± 7.1	72.2 ± 6.4	73.1 ± 8.8
2017	74.1 ± 10.4	70.6 ± 3.0	68.1 ± 5.2	61.7 ± 8.6
2018	71.5 ± 5.6	77.3 ± 4.8	75.3 ± 7.9	66.8 ± 6.3
2019	70.7 ± 5.0	76.6 ± 4.3	70.0 ± 8.3	69.7 ± 6.1

***Table 7*: Average rainfall – stratified by year and averaged per season – from the three sections in Mamfene, northern KwaZulu-Natal, South Africa.**

	Mean ± standard deviation (mm)			
Year/Season	**Summer**	**Autumn**	**Winter**	**Spring**
2014	0 ± 0	0.4 ± 0.8	0 ± 0	0.8 ± 0.9
2015	6.2 ± 16.3	0.1 ± 0.8	0.2 ± 0.9	0.2 ± 0.6
2016	0.9 ±1.1	2.5 ± 3.9	1.0 ± 3.8	0.7 ± 1.0
2017	6.8 ± 9.6	0 ± 0	0.2 ± 0.5	1.2 ± 2.4
2018	1.7 ± 8.5	1.2 ± 3.0	1.5 ± 3.2	0.5 ± 1.8
2019	0.7 ± 2.4	5.4 ± 11.8	0.1 ± 0.2	0.3 ± 0.7

***Table 8*: Average temperature – stratified by year and averaged per season – from the three sections in Mamfene, northern KwaZulu-Natal, South Africa.**

	Mean ± standard deviation (°C)			
Year/Season	**Summer**	**Autumn**	**Winter**	**Spring**
2014	24.7 ± 1.2	23.1 ± 2.3	18.7 ± 2.4	23.9 ± 2.4
2015	26.8 ± 2.5	24.7 ± 2.0	21.1 ± 1.9	27.2 ± 3.2
2016	27.7 ± 1.6	26.1 ± 2.6	20.2 ± 1.7	24.3 ± 2.1
2017	26.1 ± 2.0	25.3 ± 2.9	20.1 ± 2.2	28.1 ± 2.3
2018	25.8 ± 1.9	24.2 ± 3.0	18.5 ± 2.6	22.6 ± 2.8
2019	26.9 ± 1.9	25.3 ± 2.5	20.4 ± 1.6	24.6 ± 2.4

***Table 9*: Average wind speed – stratified by year and averaged per season – from the three sections in Mamfene, northern KwaZulu-Natal, South Africa.**

	Mean ± standard deviation (m/s)			
Year/Season	**Summer**	**Autumn**	**Winter**	**Spring**
2014	2.2 ± 0.3	1.9 ± 0.6	1.6 ± 0.8	2.3 ± 0.7
2015	2.1 ± 0.6	1.7 ± 0.4	1.4 ± 0.7	2.6 ± 0.5
2016	2.0 ± 0.6	1.7 ± 0.6	1.5 ± 0.5	2.0 ± 0.7
2017	2.0 ± 0.9	1.2 ± 0.6	1.7 ± 0.6	2.7 ± 0.5
2018	2.0 ± 0.4	1.4 ± 0.6	1.6 ± 0.8	2.0 ± 0.4
2019	2.1 ± 0.5	1.6 ± 0.7	1.8 ± 0.6	2.1 ± 0.6

Appendix 3: Ethics waiver

ANIMAL RESEARCH ETHICS COMMITTEE
Registration number: AREC-101210-002

Date:18/02/2021

Certificate reference: Waiver 18-02-2021-O
Category: O
Applicant: Sinalo Gqunu
Department: National Institute for Communicable Diseases (NICD)
Tel: 0113866484; **Email**: 2136197@wits.ac.za

Re: Waiver from the Animal Ethics Research Committee of the University of the Witwatersrand

This letter is to confirm that Sinalo Gqunu, MSc student at NICD under the supervision Dr Drs Givemore Munhenga and Innocent Maposa (Wits), does not require full Animal Ethics Research Committee clearance to undertake the work titled "**Spatio-temporal analysis of malaria vector populations from northern KwaZulu Natal, South Africa (2014-2019)**".

Reason for waiver
This study will not be making use of animals and will use already collected data. The data used are from a surveillance program that investigated the use of the sterile insect technique in order to eradicate malaria in South Africa

Details of the study
The details of the primary study are well described by Dandalo and colleagues (Dandalo et al., 2017). Briefly data were collected from Mamfene (Jozini region) which is located in the north of the KwaZulu Natal province in South Africa between 2014 and 2019. The data were collected from permanently stationed sampling points twice a week every month from three different geographical sites. The data were collected as part of ongoing feasibility study investigating the applicability of the sterile insect technique (SIT) as a complementary vector control strategy under South African settings.

The individuals covered by the waiver are Sinalo Gqunu and Drs Givemore Munhenga and Innocent Maposa.

Please contact me should you require further information.

Yours sincerely

Prof Frederic Michel
Chair: Animal Research Ethics Committee
University of the Witwatersrand

Appendix 4: Turnitin Report

Turnitin-2.docx

ORIGINALITY REPORT

9%	7%	7%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	academic.oup.com Internet Source	3%
2	Leonard C Dandalo, Basil D Brooke, Givemore Munhenga, Leanne N Lobb et al. "Population Dynamics and Plasmodium falciparum (Haemosporida: Plasmodiidae) Infectivity Rates for the Malaria Vector Anopheles arabiensis (Diptera: Culicidae) at Mamfene, KwaZulu-Natal, South Africa", Journal of Medical Entomology, 2017 Publication	1%
3	www.tdhca.state.tx.us Internet Source	1%
4	www.nature.com Internet Source	<1%

Supervisors' signatures:

Supervisor's name: Dr Givemore Munhenga Signature: Date: 17/03/2022

Supervisor's name: Dr Innocent Maposa Signature: Date:17/03/2022