ASSESSING HABITAT, DISTRIBUTION, AND CHARACTERIZATION OF CRICKETS (ORTHOPTERA: GRYLLIDAE)

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A Thesis Submitted to the Board of Post Graduate Studies in Partial Fulfillment of the Requirements for the Award of the Degree of Doctor of Philosophy in Food Security and Sustainable Agriculture of Jaramogi Oginga Odinga University of Science and Technology

DECLARATION AND APPROVAL

Declaration

I declare that this thesis is my original work and has not been submitted wholly or in part for any award of degree in this or any other institution of learning.

Signed ……..…….. Date: ……..…….

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Approval

This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

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ABSTRACT

Edible crickets can act as an alternative source of food and feed when production from conventional plants and animals is disrupted. Natural habitats for the crickets continue to shrink and fragment due to climate change as well as anthropogenic pressures. Although habitat loss has been reported as the main cause of species extinction, knowledge on the habitat requirements of edible insects is scanty. The objectives of this study were to: (i) assess the distribution of crickets based on habitat preference (ii) determine the effects of temperature on development and survival of crickets (iii) characterize the cuticular hydrocarbons that generate desiccation resistance in the crickets and (iv) characterize the morphological diversity of haemocytes associated with cellular immunity in the crickets. A survey was conducted in Western Kenya to assess the distribution of crickets based on habitat preference. Thirteen descriptive variables were used to create a habitat distribution model. Akaike information criteria (AIC) was applied to estimate the habitat preference for each cricket species. The effects of temperature on the development, and survival of crickets were determined at six constant temperatures (18, 22, 26, 30, 34 and 38 ^{0}C). The cuticular hydrocarbon profiles of cricket species were identified and quantified by gas chromatograph – mass spectrometry (GC-MS). To assess the morphological diversity of haemocytes, hemolymph smears were prepared, and microscopic examinations made. The results indicated that the cricket species can be classified into three groups, Group I (*Acheta domesticus* and *Diestrammena asynamora*) which preferred areas near settlement, Group II (*Scapsipedus icipe*, *Gryllus bimaculatus*, and *Brachytrupes membranaceus*) that preferred fields and grasses, and Group III (*Gryllotalpa africana*) that preferred wet lands. The optimum temperature estimated for egg-to-adult development ranged from 26 ˚C to 34 ˚C. Further, a homologous series of n- alkanes, alkenes, and methyl branched alkanes were identified. Haemocytes were classified into six distinct types with prohaemocytes, plasmatocytes and granulocytes being the most numerous cells in the hemolymph of the crickets.

This study concludes that the most preferred habitats for crickets are natural vegetation, areas near water bodies, having high shelter density and away from human settlement. In addition, the results suggest that the long chain cuticular hydrocarbons increase with increase in temperature of the cricket's habitat and provide greatest protection against desiccation. Understanding how insects adapt and survive under stress and identifying the physiological processes that occur during that time may allow us to better conserve their habitat and prevent species extinction.

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LIST OF ACRONYMS

AHP	Anti Hunger Programme
AIC	Akaike Information Criterion
AMPs	Anti Microbial Peptides
ANOVA	Analysis of Variance
CBD	Convention for Biodiversity
CC	Climate Change
CFS	Committee on World Food Security
CHC	Cuticular Hydrocarbons
DHC	Differential Haemocyte Count
EMR	Effective Multiplex Ratio
FAO	Food and Agriculture Organization of the United Nations
GC- MS	Gas Chromatograph – Mass Spectrometry
GIS	Geographical Information System
GLM	General Linear Model
GLMM	General Linear Mixed Models
HC	Hydrocarbons
HSD	Honesty Significant Difference
IFAD	International Fund for Agricultural Development
JOOUST	Jaramogi Oginga Odinga University of Science and Technology
LDA	Linear Discriminant Analysis
LME	Linear Mixed Effects
MI	Mitotic Index
NRM	Nuclear Magnetic Resonance
NSCs	Neuro Secretory Cells
PCA	Principal Component Analysis
PCs	Principal Components
PO	Phenol Oxidase
PRI	Photochemical Reflectance Index
PTG	Prothoracic Gland
PTTH	Prothoracico Tropic Hormone
RCBD	Randomized Complete Block Design
RCMRD	Regional Centre for Mapping of Resources for Development

RH	Relative Humidity
RP	Resolution Power
RPM	Revolutions per Minute
RS	Remote Sensing
SAS	Statistical Analysis System
SD	Standard Deviation
SDG	Sustainable Development Goals
SDW	Sterile Distilled Water
SQRT	Square Root Transformation
THC	Total Haemocyte Count
UNCCD	United Nations Secretariat of the Convention to Combat Desertification
UNDP	United Nations Development Programme
UNEP	United Nations Environmental Program
UNICEF	United Nations Children's Fund
WFP	World Food Programme
WFP	World Food Summit
WHO	World Health Organizatio

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Climate change, rapidly increasing population and regional conflict has created massive food shortages across multiple countries in Africa (Cargill, 2014; Angela, 2006). While the causes vary from country to country, the needs are largely the same – immediate provision of food and water, as well as long –term solutions that address the root causes (Tilman and Clark, 2014). An important achievement is the consumption of insects that has helped in reducing cases of malnutrition among the rural poor indicating the significance of edible insects in bridging the food insecurity gap (Lang and Barling, 2013; FAO, 2010a; Rahman, 2001).

Crickets*, Orthoptera; Gryllidae* are some of the under-utilized insect species that can provide an alternative source of food and animal feed, reducing the over exploitation of fisheries to sustain agricultural and aquaculture production (Ayieko *et al*., 2016; Rana *et al*., 2009).

Africa boasts a rich biodiversity comprising several endemic and endangered species (Sultana *et al*., 2013). This species abundance and diversity is on the decline due to climate change that has continually threatened biodiversity and ecosystem services in the region (Banjo *et al.*, 2006). A number of anthropogenic factors coupled with climate change threaten insect populations when, these insects are a delicacy among several communities in the world and a nutrient power house for human, livestock, poultry and fish (Ayieko *et al*., 2012; Banjo *et al*, 2006). These anthropogenic factors have a profound effect on distribution, colonization, survival, abundance, behavior, fitness, and the life history traits of these insects (Sultana *et al*., 2013; Milson *et al*., 2006). Extremes of these factors limit the insect's geographic range, either by causing direct natural mortality or by limiting the range of host plants or animals (Ayieko *et al*., 2010; Breshears *et al*., 2008). Habitat loss, fragmentation and the introduction of exotic species are the most significant causes of species loss (Kavishe, 2016; Ward and Lariviere, 2004).

Crickets are distributed throughout tropical and temperate regions, except at latitudes above 55 ^{o}, with the greatest diversity being in the tropics (Martins, 2014; Jaganmohan *et al*., 2013; FAO 2010b). They occur in varied habitats from grasslands, bushes,

forests, mashes, beaches and caves (Resh and Carde 2009). They are omnivorous generalists that prefer tall grassland habitats (Chapman *et al*., 2013; Hardy *et al*., 1983). Crickets are mainly nocturnal and are known for loud persistent chirping song of males trying to attract females, although, some species are mute (Cigliano *et al*., 2020; Resh and Carde, 2009). The singing species have good hearing ability via the tympana on the tibiae of the front legs (Otte, 2007).

Crickets play an important role in maintaining the balance of ecosystems (Umpold and Schulter, 2013). They break down plant material, renewing soil minerals and are an important source of protein for many households, reducing the pressure on fish resources that have been used to formulate poultry feeds (FAO 2010b; Rana *et al*., 2009). The adult cricket is composed of 47 % crude protein, 10 % carbon, and 25 % fat; food nutrients on dry weight basis (Ayieko *et al*., 2016; Banjo *et al*., 2006). In addition, the insect contains a variety of minerals and vitamins (Banjo *et al*., 2006). When the diet is enriched with fish offal, the adults are rich in omega-3 and essential unsaturated fatty acids (Umpold and Schluter, 2013; Banjo *et al*., 2006). When dried for use as feedstuff, the adults have an estimated value comparable to soybean or meat and bone meal (FAO 2010b). Their value as a product might be higher if they are used live as a special feed type (Rana *et al*., 2009).

Global warming, a gradual increase in the overall temperature of the earth's atmosphere results to habitat loss, shifts in climatic conditions and in habitats that surpass migration capabilities (Reznik and Vaghina, 2011). Phenotypic plasticity, the capacity of a single genotype to exhibit variable phenotypes in different environments, is common in insects and is often highly adaptive (Tomberlin and Sheppard, 2002). Most studies have concluded that insects would become more abundant as temperatures increase, through a number of interrelated processes, including range extensions and phenological changes (Reznik and Vaghina, 2011). Insects are poikilotherms; significantly affected by climatic factors, with temperature being the most prominent environmental factor with marked influence on insect biology and behavior (Aksit *et al*., 2007; Infante, 2000). However, individual species responses vary when exposed to stressful conditions (Mori *et al*., 2005; Miller and Paustial, 1992). Insects respond either through change in behavior to avoid stress by migration or through changed activity patterns (Kutcherov, 2016; Hagstrum and Miliken 1988). These insects can continuously adapt to the stress conditions through selection or by plastic responses; by changes in morphology, life history, or physiology (Manrique *et al*., 2012; Lopatina *et al*., 2007).

The exoskeleton of insects is covered by a thin wax composed of cuticular hydrocarbons (CHCs) that evolved to protect them from water loss and help in recognition (Blomquist and Bagneres, 2010; Martin and Drijfhout, 2009c). The CHCs restrict the amount of water lost from the insects' body to the surrounding environment by controlling the trans-cuticular water flux (Martin and Drijfhout, 2009a; Rantala *et al*., 2003). This is particularly important in arthropods due to their high surface area to volume ratio (Rantala and Kortet, 2004; Tregenza and Wedell, 1997). In the Orthoptera, these chemicals further, help in kin recognition, mate recognition and act as indicators of both immune competence and dominance (Kortet and Hedrick 2005, Rantala *et al,*. 2003).

The ability of insects to survive in all geographical regions of the world indicates their strong innate immune response to pathogens and stress (Berger and Slavickova 2008; Miranpuri *et al*., 1991). Insect blood cells or haemocytes are the main components of cellular immune responses (Berger and Slavickova 2008; Silina, 2003). The counts of haemocytes, total haemocyte count (THC) and differential haemocyte count (DHC) are influenced by environmental conditions such as relative humidity, temperature, photoperiod and disease (Sokolova *et al*., 2000; Pandey *et al*., 2010).

This study evaluated the habitat preference and distribution of cricket using species presence data from field surveys. The study then investigated the effects of temperature on development and survival of crickets. In addition, the study characterized the cuticular hydrocarbons that generate dessication resistance in crickets and further assessed the morphological diversity of the haemocytes associated with cellular immunity in crickets.

1.2 Statement of the Problem

The major problem facing Africa is food insecurity and malnutrition (FAO, 2010b). Crickets, Orthoptera; Gryllidae are some of the under-utilized insect species that can act as a source of food and feed, reducing the pressure on fish resources used to formulate animal feeds (Van Huis, *et al.*, 2013; Ayieko *et al*., 2012). These edible insect species continue to become extinct from habitats worldwide (Miller and Paustian, 1992). They are in critical demographic crisis from habitat loss because of climate change, global warming and expanding human populations (Ayieko *et al*., 2010; Collinge *et al*., 2003). Although habitat loss has been reported as the main cause of species extinction, knowledge on the habitat requirements of edible insects is sparse

Climate variability has imposed large fitness costs on insects showing diapause and other life cycle responses, threatening population persistence (Gilloolly *et al.,* 2002; Lopatina *et al*., 2014). Temperature is the most prominent environmental factor having marked influence on phenotypic plasticity of insects (Aksit *et al*., 2007; Garcia- Barros, 2000). Maximum and minimum temperatures are projected to increase by 3.5 ^{0}C to 4 ^{0}C in more than half of Western Kenya by 2030. Several studies have documented the optimum temperature for development of various life stages of insects (Lopatina *et al*., 2014). However, data on thermal biology of edible crickets are so limited that they are inadequate to predict population dynamics in the changing environment.

Dessication stress is a significant threat to survival of terrestrial animals, with insects developing adaptation strategies to cope with the harsh environment (Krupp *et al*., 2019). A very thin hydrocarbon-based wax coating called the epicuticular layer covers the insects' skin that controls transcuticular water flux (Buellesbach *et al*., 2013; Rantala *et al.,* 2003). Studies have linked cuticular hydrocarbons to desiccation resistance in terrestrial insects, although there is little information on the specific hydrocarbons that mediate dessication resistance in crickets in the study area.

The insect has one of the largest germplasm collections in the world, with several species that occur in varied habitats (Jagamohan *et al*., 2013; Resh and Carde*,* 2009). These insects are versatile in as far as their adaptation to the environment is concerned indicating their strong innate immune response (Pandey *et al*., 2010). One of the key components in insects' capacity to endure harsh environments is their innate immune system (Sokolova *et al*., 2000; Jalali and Salehi, 2008). Although some studies have examined the cellular defense mechanism of insects, haemocyte morphology of edible crickets has not been widely examined.

1.3 General Objective

To elucidate knowledge and information on spatial distribution, habitat preference and characteristics of crickets (Orthoptera, Gryllidae) for food and nutritional security.

1.3.1 Specific Objectives

1. To assess the habitat preference and distribution of crickets in Western Kenya.
2. To determine the effects of temperature on the development and survival of two cricket species, *Acheta domesticus* and *Gryllus bimaculatus.*

3. To chatacterize the cuticular hydrocarbons that generate dessication resistance in two cricket species, *Acheta domesticus* and *Gryllus bimaculatus*

4. To characterize the morphological diversity of haemocytes associated with cellular immunity in two cricket species, *Acheta domesticus* and *Gryllus bimaculatus.*

1.3.2 Research Hypotheses

H_0: 1. There is no significant effect of habitat variables on the distribution of cricket species in Western Kenya.

H_0: 2. Temperature has no significant effect on the development and survival of crickets, *Acheta domesticus* and *Gryllus bimaculatus.*

H_0:3. There is no significant difference in the the cuticular hydrocarbons that generate dessication resistance in two cricket species, *Acheta domesticus* and *Gryllus bimaculatus.*

H_0: 4. There is no significant difference in the haemocyte diversity associated with cellular immunity in two cricket species, *Acheta domesticus* and *Gryllus bimaculatus*

1.4 Significance of the Study

Insects have improved the livelihoods of many families in Africa (FAO, 2010a). They form an important part of human diet and a source of animal feed playing an important role in improving food security (Ayieko *et al*., 2012). The insects have a protein content closely related to that of meat, chicken and fish and therefore could act as alternative sources for both food and feed (Banjo et al., 2006). They are cheaper and relatively more nutritious compared to the conventional sources of protein (Van Huis, 2013).

Cricket remains an insect species with high demand mainly in the aquaculture and agricultural production to replace fishmeal (Barwa, 2009). The dire need for more sustainable alternative sources of protein for food and feed is fueling the enthusiasm to rear these organisms on an industrial scale (Nijdam *et al*., 2012).

Mapping of suitable habitats and linking with relevant species through geographical information system (GIS) and Remote sensing (RS) can significantly improve species conservation and preservation methods (Lui, 2009; De Leeuw and Albritch, 1996). The habitat models are important tools for understanding the habitat characteristics of different organisms, evaluating habitat quality and developing species management strategies (De Leeuw and Albritch, 1996; Pereira and Itami, 1991). Modeling the relationship between habitat variables and cricket occurrence would help in identifying

optimal conditions, and conditions of stress or intolerance for the species (Jagamohan *et al*., 2013). Habitat suitability models can provide a tool for conservationists to predict areas of species occurrence and develop plans to further, protect these habitats.

Potential changes in abiotic factors associated with climate change have a dramatic influence on insect's phenotype (Astuti *et al.,* 2013; Aksit *et al*., 2007). Understanding the effects of temperature on development and survival of crickets would provide information on how climate change shapes insect ecologies and enable the development of forecasting models for this edible insect.

Cuticular hydrocarbons evolved to protect the insects from desiccation (Ablard *et al*., 2012; Blomquist and Bagneres, 2010). Understanding the quantitative differences in cuticular hydrocarbon (CHC) profiles in populations of crickets may explain whether species would be able to the cope with the stresses, they encounter in the face of climate change. The hematological investigations may provide diagnostic keys to stresses and act as valuable indicators of the physiological status of the insects.

1.5 Scope

This study evaluated the habitat preference and distribution of cricket species across four agroecological zones (Lower Midlands 1, 2, 3 and 4) in Siaya and Busia Counties (Swallo *et al*., 2002). Field surveys were conducted and data collected based on in-situ presence - absence data. Topographic and other environmental data used were gotten from digital photographic maps and the United Nations Environmental programme (UNEP) Western Kenya Environmental Outlook. In addition, the study investigated the effects of temperature on the development and survival and further characterized the cuticular hydrocarbons and haemocyte diversity in two cricket species *Acheta domesticus* and *Gryllus bimaculatus.*

1.6 Definition of Terms

Anthropogenic factors – Are factors that result from the effects of human beings on the environment. They refer to environmental changes caused directly or indirectly by people.

Biodiversity - Biological diversity is the change among living organisms from all ecosystems and ecological complexes of which they are part. They include different levels of biodiversity including genetic diversity, species, ecosystem and the complexities of biotic and abiotic interactions.

Climate change - A change in global and regional climate patterns, caused largely by increased levels of atmospheric carbon dioxide and other inert gases produced by the use of fossil fuels.

Cuticular hydrocarbons - consist of multiplex blend of straight chain, unsaturated, and methyl-branched constituents. The cuticles of essentially all insects are enclosed by the hydrocarbons, which act as a waterproofing and communication signal agents.

Food access – access by people to enough resources to acquire sufficient meals for a nutritious diet

Food availability – the availability of sufficient amounts of food of the required quality

Food utilization – Utilization of food by the supply of proper diet, clean water, sanitation and healthcare in order to achieve good health and nourishment.

Food stability – sufficient quantities of nutritious food should be available at all times in order for a community to be food secure.

Habitat preference - Is the habitat with the highest potential of being selected by a species given the opportunity.

Haemocytes - A cell of the haemolymph of several invertebrates, mostly arthropods. They denote the major cellular constituent of an insect's immune system.'

Life history traits - are events that make up an organism's life such as birth, weaning, adult, and death, their occurrence at a specific age, stage, order and time.

Phenotypic plasticity - Refers to alterations in an organism's behavior, morphology and physiology as affected by a new environment. It significantly affects the way organisms cope with changes in environment. Phenotypic plasticity includes different forms of permanent or temporary environmentally induced morphological, physiological, behavioural, and phenological changes.

Spatial distribution. Refers to the geographical arrangement of individual entities, a habitat, phenomenon in space and the geographic association among them.

1.7 Conceptual Framework

Figure 1.1, shows the conceptual framework of the study. Entomophagy has the potential of providing a cheap source of food and feed to help reduce food insecurity and malnutrition in Africa. However, climate variability and anthropogenic pressures subject insects to stresses they have never encountered, threatening their existence. This study assessed the habitat preference and distribution of crickets, and investigated the effects of temperature on the development and survival of crickets. Further, the study

characterized the cuticular hydrocarbons that generate desiccation resistance in the crickets and characterized the haemocytes that mediate innate immune response on the crickets when faced with stress. Data and information generated from the study on distribution and habitat preference of crickets will be disseminated and used to identify biodiversity hotspots, better preserve habitats to ensure sustainable supply of edible insects from our natural systems. Effects of temperature on the development and survival of crickets would provide information on the behavior of these insects in the face of a projected 3 ºC global warming. Identification of cuticular hydrocarbons and haemocytes will provide information on the large fitness costs incurred by these insects in order to adapt to the adverse environmental conditions they encounter. The information from this study may be used to better conserve biodiversity, ensure continuous supply of edible insects and other ecosystem services. The overall result would be improved food security and livelihoods, especially to the rural poor, majority of who depend directly on biodiversity and ecosystem services for their survival.

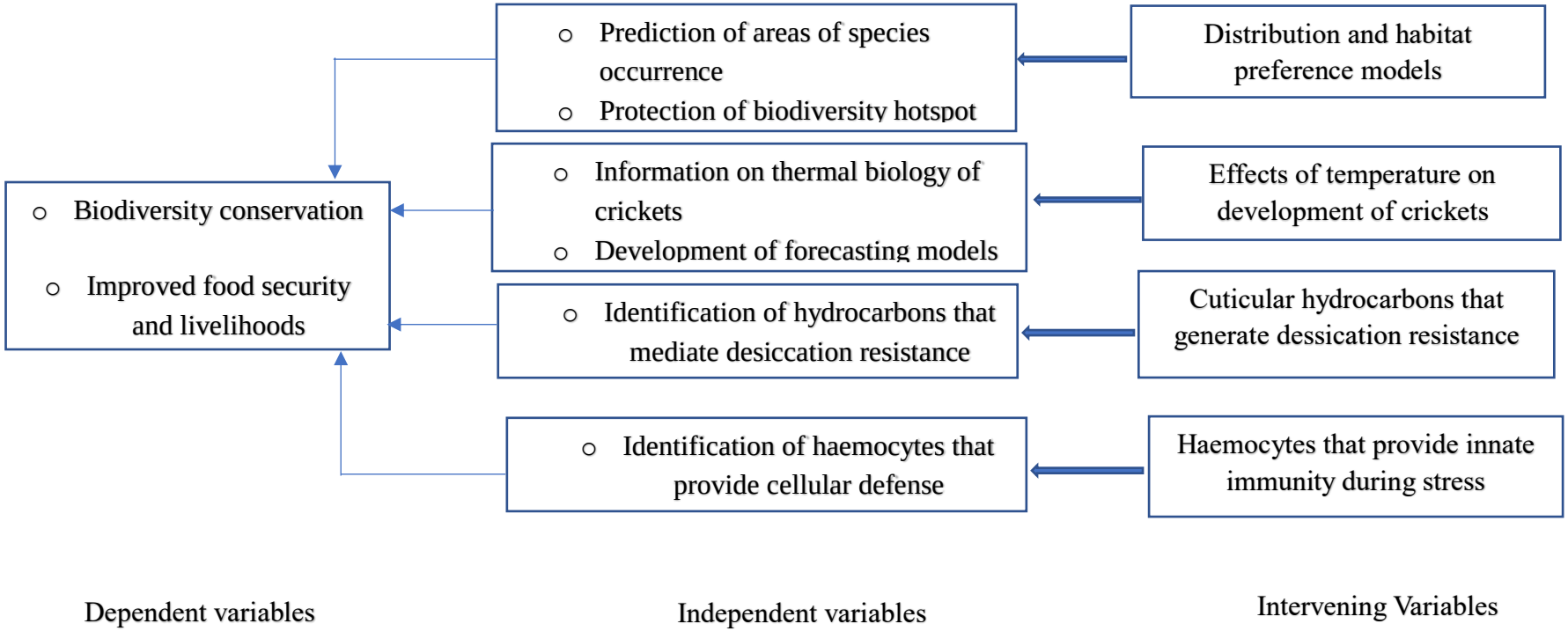


Figure.1.1: Conceptual Framework

CHAPTER TWO
LITERATURE REVIEW

2.1 Food Security

Food security is achieved when everyone, at all times, has physical and financial access to enough, safe, and nourishing food to match his or her dietary needs and food preferences for an active and healthy life. (World Food Summit, 1996). This definition emphasizes four dimensions of food security: First, food availability, which emphasizes the availability of sufficient quantities of food of appropriate quality (Committee on World Food Security (CFS), 2005). Second is food access that focuses on acquisition of enough materials by individuals to get proper nourishment (Clay, 2002). Third is food utilization, which focuses on utilization of food through adequate diet, clean water, sanitation and health care to reach a state of nutritional well-being where all physiological needs are met (McClain-Nhlapo, 2004). Fourth dimension emphasizes on the availability and accessibility of food at all times (FAO, Anti Hunger Programme, 2002). This multidimensional human rights-based approach to food security focuses on the promotion and recovery of livelihoods, emphasizing on the concepts of vulnerability, risk coping and risk management (McClain-Nhlapo, 2004; Clay, 2002). Food insecurity therefore is analysed as a social and political construct, a departure from the traditional method of linking food security to starvation and crop failures (Heidhues *et al*., 2004).

Around 8.9 percent of the world's population—more than 690 million people—are undernourished. According to estimates, 2 billion people worldwide did not have regular access to healthy, sufficient food in 2019 (FAO, IFAD, UNICEF, WFP and WHO, 2020). Diet quality and malnutrition is worsened by food insecurity, leading to undernutrition, overweight and obesity. Approximately, one quarter of the world population experience moderate to severe food insecurity, majority of them live in Africa and Asia (FAO, IFAD, UNICEF, WFP and WHO, 2020). Table 2.1 shows the distribution of food insecurity by severity across world regions.

Table 2. 1: Distribution of food insecurity by severity across World regions

Region	Total population (Millions of people)	Moderate food insecurity (millons of people)	Severe food insecurity (millions of people)	% severe food insecurity
Africa	1308	653.6	237.2	18.13
Latin America &Caribbean	648	203.7	60.1	9.27
Asia	4601	996.5	392.2	8.53
N. America & Europe	1113	88.9	12.0	1.08

Source: FAO, IFAD, UNICEF, WFP and WHO, 2020: United Nations, Department of Economic and Social Affairs, Population Division. 2019.

2.2 The State of Food Security in Africa

In Africa, food and nutrition security is off track demanding serious and urgent attention, with about 18.13 % of the population experiencing serious food emergencies that require external assistance (FAO, IFAD, UNICEF, WFP and WHO. 2020). In sub Saharan Africa, the rising food insecurity is alarming, almost four times higher than in any other region, with the highest prevalence of undernourishment estimated in 2016 to be up to 20 % of the population (Berry *et al*., 2015; FAO, 2010b). The population in Africa experiencing hunger is approximately 238 million people, and is expected to increase to 350 million people by 2050 partly due to the 3ºC warming trajectory that will disrupt Africa's food systems within the next 30 years (McClain-Nhlapo, 2004). A loss of upto 30 % growing areas for maize and banana and a further 60 % for beans will expose the population to serious food crisis (Heidhues *et al*., 2004). In Eastern Africa, the situation is dire, with about one third of the population undernourished by 2020, and 12.8 million children acutely malnourished (Garnett *et al*., 2002; FAO, *Anti Hunger Programme*, 2002). An additional number of people, approximately 7.2 million are threatened by starvation and a further 26.5 million people facing acute food insecurity (Van Huis, 2020; FAO, 2010b).

Food insecurity and hunger are caused by many factors often being intertwined with one another (Angela, 2006; Heidhues *et al*., 2004). Generally, the principal causes of

poverty and hunger are; conflict, climate change and weather, degrading natural resources, lack of investments in agriculture and unstable markets(Tilman and Clark, 2014; Adams *et al*., 2004); with the aftershocks of COVID-19 worsening the situation (Shafiur *et al*., 2020). Food situation in Africa therefore, demands a whole new system approach focusing on sustainable animal protein sources from natural systems that can feed the undernourished population (Van Huis, 2013; Rahman, 2001). Conservation of these natural systems is therefore fundamental in ensuring continuous supply of ecosystem services to the most vulnerable members of the population, to improve food security.

2.3 Biodiversity Conservation and Food Security

2.3.1 Biodiversity Conservation and Food Availability

Biodiversity is a major constituent of food security through which appropriate nutrition can be achieved. Most people at risk are dependent on food collected from natural ecosystems (FAO, 2019). An array of species from the wild sources are rich in dietary and nutritional diversity (FAO, IFAD, UNICEF, WFP and WHO, 2020). This wild collection of food includes fish, plants, insects, bush meat and fungi (CBD, FAO, World Bank, UNEP, and UNDP, 2016). Among wild species, insects are the most versatile in as far as adaptation to the environment is concerned (Garnett *et al*., 2002; FAO, 2014). A diversity of edible insects will increase the availability of cheap sources of protein, reducing malnutrition and food insecurity in general (Piling *et al.,* 2020) . Conservation of biodiversity is crucial in maintaining various ecosystem functions such as pollination, soil productivity and water quality, which are significant in agricultural productivity (Lowder *et al*., 2020). Further preserving genetic variety and ecosystem diversity in agricultural techniques (agro-biodiversity) can lessen farmers' vulnerability to climate change and market volatility (FAO, 2019; Garnett *et al*., 2002). Sustainable use of biodiversity in terrestrial ecosystems is essential to ensure that the species harvested for food and medicine remain vital for current and future generations (Ricciardi *et al.,* 2021).

2.3.2 Biodiversity Conservation and Food Utilization

A rich biodiversity will provide people with a variety of food products, which can be combined and processed to provide healthy diets (CBD, FAO, World Bank, UNEP, UNDP, 2016). The nutrient contents of these foods vary across plant and animal species.

Insects harvested from the natural ecosystems, have been recorded to be rich in proteins, minerals and other useful compounds superior to beef and chicken (FAO, 2014). Further, other services provided by biodiversity determines the safety and nutrition profile of the diets (FAO, 2019). For example, local ecosystems act as sources of water purification, fuel wood, medicine and source of micro-organisms used in many food-processing and food preservation enterprises (FAO, IFAD, UNICEF, WFP and WHO, 2020).

2.3.3. Biodiversity Conservation and Food Access

Majority of the rural poor depend specifically on biodiversity and ecosystem services for their income and subsistence (FAO, 2019). The rural poor and forest- dwelling households acquire up to 90 % of their livelihoods from ecosystem services and other non-marketed goods (Penuelas *et al*., 2020). Natural ecosystems provide affordable, locally available and well-distributed sources of food (FAO, 2019). Loss of biodiversity and ecosystem services expose women and children to long hours engaging in various activities such as food, fuel and water, further increasing gender inequalities (Kotir, 2011). The conservation of biodiversity and their sustainable use can result to increased productivity, higher resource use efficiency and enduring sustainability (CBD, FAO, World Bank, UNEP, UNDP, 2016). Biodiversity can provide people with a ability to congregate and collect food to eat and as a source of income, thus improving food access (Garnett *et al.*, 2002).

2.3.4 Biodiversity Conservation and Food Stability

Conservation of biodiversity ensures a continuous supply, acquisition and sustainable utilization of food, and offers resilience to natural hazards and disasters (FAO, IFAD, UNICEF, WFP and WHO, 2020; Watson *et al*., 2018). Food can be gotten from plants and animals at distinctive times of the year within changing natural conditions (Piling *et al*., 2020). Some animals such as insects are well adapted to extremes of climate as well as pests and disease threats (FAO, 2014). Wild biodiversity can therefore act as an alternative source of nourishment when generation from ordinary plants and animals is disturbed (Lowder *et al.,* 2021). Wetlands act as crucial habitat to endemic species, store surface, subsurface and ground water, and reduce the incidences of flooding (Committee on World Food Security (CFS) (2005). They also help to capture process and dilute pollutants (FAO, 2014). Natural grasslands and forests help in maintaining healthy watersheds (FAO, IFAD, UNICEF, WFP and WHO, 2020). It is more cost

effective to manage these ecosystems and maintain the services they provide than to rely on built technologies (Pingali *et al*., 2005). Conservation of biodiversity also helps to prolong the lifetime and productivity of water infrastructure such as reservoirs, water supply facilities, irrigation networks and dams (Committee on World Food Security, 2005). Significant carbon stores such as forests, wetlands and coastal ecosystems are crucial in avoiding dangerous changes to the earth's atmospheric temperatures, global warming and climate change (FAO, 2019). Attempts to shield these habitats from destruction is a cost effective and proven way to mitigate the negative impacts of climate change (Ricciardi *et al*., 2021). These ecosystems serve as natural buffers against extremes of climate, natural disasters and increase resilience to climate change (FAO, 2014). Maintaining a whole ecosystem will guarantee solidness of food supply by decreasing the adverse effects of natural hazards, providing an optimum habitat for wild species, therefore ensuring availability of ecosystem services for future generations.

2.4 Justification for Utilization of Insects for food and Feed

Entomophagy has helped in reducing cases of malnutrition among the rural poor indicating the significance of edible insects in bridging the food insecurity gap (FAO, 2010a; Ayieko *et al*., 2016). Humans worldwide consume about 2100 species of insects. These edible insects can breed and grow very fast which makes them a reliable, alternative source of protein for humans and livestock. A number of environmental, health and social benefits have been achieved from the use of insects as food and feed (Van Huis *et al*., 2013; Oonicx *et al*., 2010). Insects are cold – blooded, hence have a better feed conversion rates than other animals. Feed-to-meat conversion rates vary widely depending on the type of animal and production practice used with insects being extremely efficient (Nijdam *et al.,* 2012). Insects can convert 2 kg of feed into 1 kg of their mass, compared to conventional livestock that require 8 kg of feed to produce 1 kg of their body weight (Oonicx *et al*., 2010). The production of greenhouse gases by most insects is lower and they use less water compared to conventional livestock (Van Huis, 2013). The bio-waste that insects consume, including human and animal waste, compost, and animal slurry, can be converted into high-quality protein that can be utilized in animal feed (Nijdam *et al*., 2012). Conventional livestock farming requires large tracks of land for production, whereas insects require very little space (Oonincx *et al*., 2010). Due to their high fatty acid content, insects play a crucial role as a food

supplement for undernourished children because they offer high-quality protein and nutrients comparable to those found in meat or fish (Barwa, 2009). The probability of insects spreading zoonotic diseases is limited, while they contain an array of micronutrients and fiber (Ayieko *et al*., 2012; Banjo *et al*., 2006). Figure 2.1 shows the various ways and forms in which insects can be presented and used for food and feed.

Figure 2. 1: Insects for food and feed (Adapted from Food and Feed conference, 2022)

2.5 Crickets

The order orthoptera, are a expansive collection of "jumpers" counting crickets, locusts, grasshoppers, katydids and ground hoppers that can be found in most environments (Aslam, 2009; Resh and Carde, 2009). They are recognized mainly by their jumping hind legs, three tarsal segments, and long tactile cerci bearing clumps of knobbed hairs, mandibulate mouthparts and a large prothorax (Otte, 2007).

Crickets, a family of the Gryllidae (true crickets) are cold-blooded nocturnal insects related to bush crickets and distantly to grass hoppers (Otte, 2007). Their bodies are

cylindrical, having round heads with long antennae. Behind the head is a smooth robust protonum (Chapman *et al*., 2013). The abdomen ends in a pair of long cerci with females having a long *femora* providing power for jumping (Bidau, 2014; Magurran, 2004). The front wings are adapted as tough leathery elytra and males of some species chirp by rubbing parts together in order to attract mates (Hardy *et al.,* 1983; Chopard, 1961). The hind wings are membranous and folded when not in use for flight, however many species are flightless (Resh and Carde, 2009). Although over 800 species occur in Africa, crickets have not been taxonomically revised in this region and the true diversity of species may be significantly higher (Cigliano *et al*., 2020. Magurran, 2004). The cricet is a species of focus in Africa because they are edible, having feed conversion ratios superior to both termites and mealworms (Nijdam *et al*., 2012)

2.6 Cricket Biology and Life-cycle

The insect order, orthoptera, forms part of the hemi metabolic insect groups, characterized by developing nymphal instars, resembling the mature adult (Otte, 2007). Crickets undergo incomplete metamorphosis characterized by three stages in their life cycle: egg, nymph and adult (Chapman *et al*., 2013; Resh and Carde, 2009). They can live for over six weeks and their entire life cycle lasts two to three months depending on their surroundings (Chapman *et al*., 2013; Otte, 2007). To mate the male crickets chirp their wings together. The female cricket has a long needle like protrusion (ovipositor) used for laying eggs in addition to two cerci and can lay up to 200 eggs at a time in any available damp substrate (Cigliano *et al*., 2020).

A cricket begins its life as an egg then breaks the egg capsule and dig out of the substrate. After about 14 days, it will have developed into a nymph (Otte, 2007). Nymphs look like small versions of adult crickets with a few differences (Chapman *et al*., 2013). They are not yet well developed, so initially do not have wings and females do not have ovipositors. These young crickets often become prey for larger crickets and other insects (Chapman *et al*., 2013; Resh and Carde, 2009). In order to grow, a nymph has to shed its hard exoskeleton into a new one, which is soft and milky white but hardens within hours. This process is called molting and happens eight to ten times (Resh and Carde, 2009). A nymph will begin growing its wings after about one month (Otte, 2007). Once a cricket reaches maturity, its wings are fully developed and it only has two goals, eating and mating (Hardy *et al*., 1983; Chopard, 1961). A male will try to attract fertile females and once mating has occurred, a female will spend her time

finding suitable places to lay her eggs (Resh and Carde, 2009). Figure 2.2 shows the cricket lifecycle; eggs, nymphs and adults.

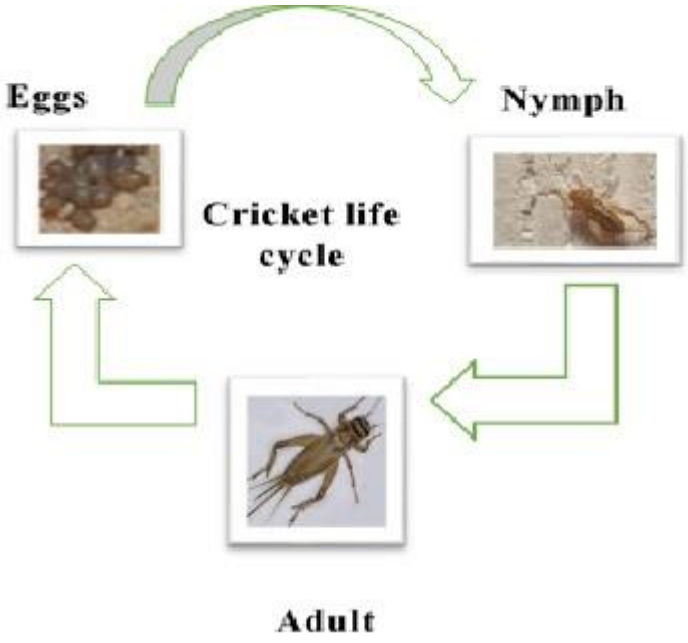


Figure 2. 2: Cricket Lifecycle (Adapted from Enchanted learning .com)

2.7 Climate Change and Insect Abundance

Climate change coupled with disturbance of insect natural habitat has led to serious disruptions of this important natural resource (FAO, 2010b; Collinge *et al*., 2003). The anthropogenic variables have a significant impact on conveyance, colonization, survival, behavior, wellness, and the life history characteristics of insects (Balamkar and Jadesh, 2012; De Leeuw and Albritch, 1996). Under extreme environmental stress, the geographical range of these insect populations are limited by natural mortality or range of host plants or animals (Khadija *et al*., 2013; Ayieko *et al*., 2010). United Nations framework convention on climate change (UNFCCC) and the convention for biodiversity (CBD) recognize that climate change is one of the greatest threats to biodiversity (FAO, 2010a). As the temperatures fluctuate, the number of insects may alter, due to interrelated forms, such as range expansions and phenological changes, as well as expanded rates of populace improvement, development, movement, and overwintering (Lassau *et al*., 2005; FAO, 2010a;). Increasing atmospheric carbon dioxide (CO_2) is also affecting insect species abundance (Chemura *et al*., 2018; Ahn et al., 2016). However, individual species responses vary, if exposed to stressful conditions, either behaviorally Maintaining a strategic distance from the stress by

getting away, by movement or changed action designs or ceaselessly adjust to the stretch condition (Khadija *et al*., 2013). They can adjust through choice or by plastic reactions, that's, by changes in morphology, life history, or physiology (Aslam, 2009). Some insects mature faster under warm temperatures resulting to a shorter lifecycle of the insect, and allowing seasonal breeding to start earlier than usual and last longer (Basset *et al*., 2012).

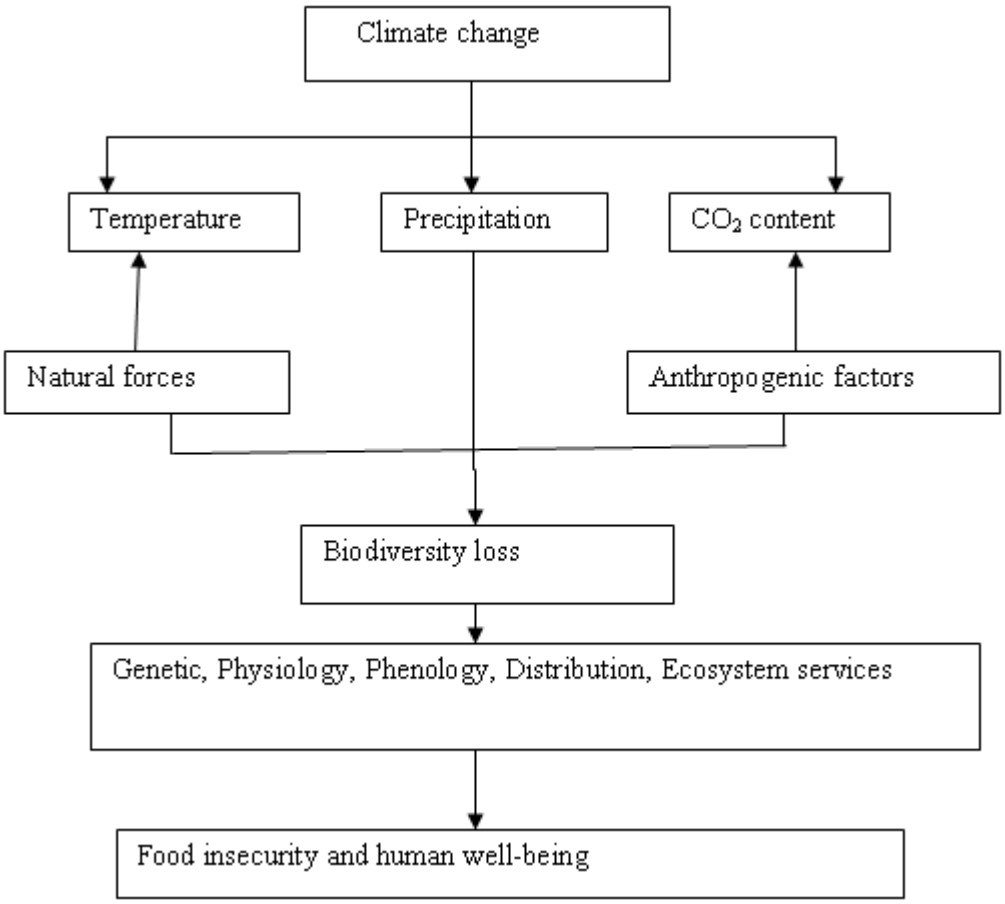


Figure 2.3: Climate change, biodiversity and food security nexus

2.8 Habitat Preference and Assessment

Habitat selection is an important part of an insect's response to climate change (Lui, 2009; Collinge *et al*., 2003). Various inferences illustrating how environmental factors control the distribution of species form the basis of habitat models (Kwon *et al.*, 2015). The presence of vegetation cover in forests, grasslands and shrub lands contain food, shelter and oviposition subtrates (Collinge *et al.*, 2003). Farmlands and areas adjacent to water sources equally have these habitat characteristics (Kwon *et al.*, 2015; Khadijah *et al*., 2013). Elevation, slope and aspect are topographic components, which are always incorporated since they indicate the magnitude of radiant energy that contributes to

habitat conditions for organisms (Lassau *et al*., 2005; Wolters, 2003). Crickets inhabit all habitat types and play major roles in the function and stability of terrestrial and aquatic ecosystems (Lui, 2009). The insect forms a major component of terrestrial biodiversity and plays a role as an indicator of environmental conditions (Collinge *et al*., 2003; Augustine *et al*., 1996). Therefore, their bio monitoring can aid efforts to conserve and restore biodiversity, evaluate the impacts of climate change, and to protect ecological services (Lui, 2009). These crickets occur in varied habitats from grasslands, bushes, forests, mashes, beaches and caves (Resh and Carde, 2009). Preservation of the species therefore requires a complete knowledge of their spatial requirements. Habitat evaluation is the assessment of the suitability of land as habitat for specific species (Julie *et al*., 2021; De Leeuw and Albritch, 1996). A species habitat suitability map displays the suitability of the place as a habitat for the specific species (Julie *et al*., 2021; Colinge *et al*., 2003). Habitat-suitability modeling using geo-spatial tools are important techniques used to assess global impacts of climate change (Collinge *et al*., 2003; Pereira and Itami, 1991). Remote sensing (RS) and Geographical information system (GIS) techniques are important in the management of natural resources, environmental monitoring and crucial in studies of organisms' habitats (Milson *et al*., 2001; De Leeuw and Albritch, 1996). They can be used to study species assemblages, scale-dependent habitat preferences and geographical fragmentation of populations, habitat heterogeneity, and ecological integrity (Milson *et al*., 2001; Pereira and Itami, 1991). For proper conservation planning, there is need for relevant, reliable, and timely geo information from remote sensing (RS) and geographical information systems (GIS) (Lui, 2009; De Leeuw and Albritch, 1996).

In this study, habitat components studied for the model were food, shelter and water. Topographic factors (elevation, slope and aspect) were gotten from digital topographic maps drawn to a scale of 1:5000. The digital elevation model was then converted into slope, relief and wetness (Kwon *et al.*, 2015). Anthropogenic factors threaten species existence through habitat loss and fragmentation (Wolters 2003; Margalef, 1958). Water is essential in the life of any living organism therefore the presence of a water body affects the distribution of species. In this study, the distance from existing water bodies was used to explain the choice of each species to distances from water.

2.9 Phenotypic Plasticity

The term plasticity refers to how much the environment modifies phenotypic expression (Lopatina *et al*., 2014). Phenotypic plasticity, the capacity of a single genotype to exhibit variable phenotypes in different environments is common in insects and is often highly adaptable (Gandolfo *et al*., 2008; Tomberlin and Sherpherd, 2002). Plasticity and climate change determine whether a population or species will be able to cope with the stresses, they encounter (Li and Park, 2020; Burkett *et al*., 2014). Plastic responses allow an individual to respond rapidly to new conditions (Lopatina *et al*., 2014; Gillooly *et al.,* 2002). These plastic responses affect insects resulting in modifications of many traits determining the capacity of adaptation of insects, including behaviour, physiology and morphology (Mori *et al.,* 2005; Garcia – Barros, 2000). Temperature is the most prominent environmental factor with marked influence on insect biology and behavior (Easterling *et al*., 2000; Gillooly *et al.,* 2002). Temperature determines seasonal cycles including various aspects of insect biology, such as sex ratio, adult life span, morphology, survival, and reproduction (Gandolfo *et al*., 2008; Lopatina *et al*., 2007). Temperature subsequently influences colonization, dispersion, plenitude, behavior, life history, and wellness of insects (Li and Park, 2020; Sultana *et al*., 2013). Temperature controls population development of insects, therefore data on thermal requirements of this edible insect is vital for conservationists (Burkett *et al*., 2014; Lopatina *et al*., 2014).

2.10 Insect Life History Traits

Life history traits are events that make up an organism's life such as birth, weaning, adult, and death, their occurrence at a specific age, stage, order and time (Walsh *et al*., 2019). These events, juvenile development, mature age, initial age of reproduction, number of eggs, senescence and death depends on the insects' environment (Creigton *et al.,* 2009). Life history traits determines how an insect's energy is dispensed to reproduction, growth, and survival. There are several life history traits in insects and an insect's fitness is affected by its changing life history traits (Flatt and Heyland, 2011). An insect's fitness is shaped by an important trait such as fecundity; one where its change constitutes a great difference to an insect's level of fitness (Creigton *et al*., 2009). Insects have life history traits that make them better objectives for conservation attempts (Walsh *et al*., 2019). Life history traits include: birth weight, growth sequence,

age and size when fully fledged, number, size, and sex ratio of juveniles, age- and size-specific reproductive devotions, oviparous or viviparous, semelparous or iteroparous, prolificacy, mortality, longevity and life span (Walsh *et al*., 2019).

Fecundity is a measure of the reproductive success of an animal, often stipulated as the number of eggs or offspring yielded by an animal and it is dependent on various factors, including environmental changes, food availability, length of breeding season, and frequencies (Berger *et al*., 2008; Etienne and Louis, 1982).

A number of trade-offs occur in the life history traits of insects, which are physiological and relate to energy regulation (Walsh *et al*., 2019; Ofomata *et al*., 2000). Energy allocated by a female to yield offsprings with a higher birth weight increases the chances of survival of the off springs but decreases fecundity (Gustafsson *et al*., 1995). The outcome of high fecundity is that a lot of energy is assigned to reproduction and with less left for maintenance (Gustafsson *et al*., 1995). Muscle mass is consumed for energy as fat reserves are depleted, and the state of the body deteriorates. Reduced longevity is caused by an organism's susceptibility to disease, parasites, and predators as a result of immune system and physical condition degeneration (Gustafsson *et al*., 1995). Semelparous species exhibit "big bang" reproduction, in which they expend all of their available energy in a brief period of time and then perish (Walsh *et al*., 2019). Iteroparous species are those that have long enough lifespans to have a good chance of reproducing more than once, and their life history traits typically balance the energy allocated to reproduction and maintenance supplies. Because of this, smaller adults are physically and energetically stifled and have fewer children than larger adults (Gustafsson *et al*., 1995). Adults who are smaller devote more energy to growth—improving posture and increasing body size—and less to reproduction (Calvo and Molina, 2005; Garcia –Barros, 2000). Larger adults are better able to allocate more energy to reproduction in order to increase the success of their reproduction (Jasienka, 2009). Even when a species is physiologically mature and capable of reproducing, there is an age-related scheme of energy devoted to reproduction that causes it to be delayed (Creigton *et al.,* 2009). This characteristic evolved to balance the energy used for upkeep, growth, reproduction, and survival (Jasienka, 2009).

Insects have high fecundity such that when environments are perfect, the number of eggs produced can be magnified by ten-fold or more over the course of a single generation (Creigton *et al*., 2009). Insect conservation endeavors sometimes report extraordinary population gains once key management strategies are understood and

enforced. They have very short development times, which when merged with their fecundity, give insects the prospect to acclimatize fast (Jasienka, 2009). Evolutionary adjustment to temperature fluctuations and different photoperiods occur swiftly in insects making them better adapted to their habitats (Walsh *et al*., 2019; Gustafsson *et al*., 1995).

An essential trait of hermaphroditic organisms is their sex ratio, which is influenced by both their internal genetics and their environment (Flatt and Heyland, 2011). The 1:1 sex ratio ensures that both the parents invest equally in their offspring's genetic makeup, making it genetically stable. However, due to biological diversity in nature, dioecious species have a significantly different sex ratio, which results in a clear male or female dominance of the community (Walsh *et al*., 2019). Previous research has demonstrated that a lower or greater sex ratio will impair the ability of mature male and female insects to reproduce and mate, which will negatively impact the population and reproduction of insects (Berger *et al*., 2008). The quantity of eggs laid by female fluctuates according on the sex ratio, although under specific sex ratios, increased fecundity can be maintained.

2.11 Cuticular Hydrocarbons

Insects have a layer of wax on their cuticle made up of lipids, phospholipids and glycolipids (Buellesbach *et al*., 2018; Schwander *et al*., 2013). Hydrocarbons form the major compound of the epicuticular layer in insects (Wagner *et al*., 2001; Savarit and Ferveur, 2002). Several glands on an insect's cuticle secrete hydrocarbons, which are organic molecules made exclusively of carbon and hydrogen (Bello *et al*., 2015; Martin and Drijfhout, 2009b). The hydrocarbons found in insects share the same fundamental structure, which is a lengthy carbon chain made up of somewhere between 5 and 50 carbon atoms (Buellesbach *et al*., 2018; Morgan, 2004). Most insects produce linear n-alkanes with more than 20 carbons in the chain, which melt at 35 °C, and the melting points of hydrocarbons rise with the length of the chain within each group (Buellesbach *et al*., 2018; Bello *et al*., 2015). These cuticular hydrocarbons are biologically stable and play an important role in preventing desiccation, controlling of trans-cuticular water loss and in insect communication (Jackson *et al*., 2007; Bonavita *et al*., 1997). These cuticular hydrocarbons are present in arthropods at every stage in their life and their production is affected by species, reproductive status, temperature, diet and sex (Monnin, 2006; Greene and Gordon, 2003). The cuticular hydrocarbons vary

proportionally among different insect species from only 3% to 95% (Buellesbach *et al*., 2018; Rouault *et al*., 2004). All of the carbon atoms in a saturated hydrocarbon, or n-alkane, are connected by a single bond (Buellesbach *et al*., 2018; Bonavita *et al*., 1997). They can also be found as unsaturated olefins, which are compounds with one (alkenes), two (alkadiene), or three (alkatrienes) double bonds at different points in the chain (Akino, 2006; Kaal and Janssen, 2008). Despite having one or more methyl groups (CH3) linked to one or more of the carbon atoms in the chain, either near the end or in the middle of the chain, methyl-branched hydrocarbons are nevertheless saturated molecules (Straub *et al*., 2022; Martin and Drijfhout, 2009b). The *n*-alkanes are usually mixed with alkenes and methyl-branched alkanes in order to keep the cuticle flexible, (Liang and Silverman, 2000; Gibbs, 1998). This mixture of hydrocarbons lowers their melting point on the cuticle allowing the waxy layer to remain pliable over broad thermal range necessary to adjust the permeability of the cuticle in a continuously changing environment (Kaal and Janssen, 2008; Morgan, 2004). Gas chromatography coupled with mass spectrometry (GC-MS) enables an easy and accurate analysis of cuticular hydrocarbons (Monnin, *et al*., 1998; Francis *et al*., 1989).

2.12 Alkanes

The cuticular hydrocarbon profile of insects is often dominated by linear alkanes, also known as n-alkanes, which are straight chain saturated hydrocarbons (Buellesbach *et al*., 2013; Benelli *et al*., 2012). In Arenivaga investigata, they exist in a continuous homologous sequence in the narrow size range C 27–C 31, while in Locusta migratoria cinerascens, they cover a greater size range from C 21–C 37 (Straub *et al*., 2022; Li *et al*., 2021). Even and odd chain length hydrocarbons are both present, however the odd-numbered chain length n alkanes are always more common (Li *et al*., 2021). Longer chain hydrocarbons offer the best water barrier due to their straight structure, which allows for close packing of the molecules to restrict transcuticular water flow (Bonavita *et al*., 1997). Insufficient n- alkane production is a problem for some Drosophila species, including Drosophila pseudoobscura and Drosophila mojavensis (Benelli *et al*., 2012; Toolson *et al*., 1990). As a result, they experience significant cuticular permeability and are vulnerable to desiccation stress (Martin and Drijfhout, 2009a). In warm conditions, insects generate more n-alkanes, and in cold environments, they generate more unsaturated hydrocarbons (Fezza *et al*., 2022; Wagner *et al*., 2001). Foraging insects have cuticles that receive more solar exposure than non-foraging

insects, such as the seed-eating desert harvester ant (Pogonomyrmex barbatus), which results in higher n-alkane concentrations (Martin and Drijfhout, 2009a).

2.13 Haemocytes

Insects do not have an acquired immune system, but have a well-developed innate response (Lavine and Strand, 2002). Haemocytes are biochemically active and sensitive cellular components, important for the defense mechanism of insects (Wu *et al*., 2016). Insects have two categories of immune responses; the cellular immune response, conveyed by the insect blood cells and humoral immune response, which is linked by diverse effector molecules, such as antimicrobial peptides (AMPs) and phenol oxidase (PO) cascade (Wu *et al*., 2016). The health of insects is affected by exposures to extremes of temperatures (Lavine and Strand, 2002; Lawrence, 2008). As poikilotherms, insects are affected by major temperature variations in a variety of ways, including cellular and immunological alterations, tissue deterioration, low survival rates, and decreased vigor (Duressa and Huybrechts, 2016). When exposed to dry circumstances at 25 ^{0}C, prodenia larvae showed an increase in haemocyte numbers (Jalali and Salehi, 2008; Silina, 2003). Desiccation-related fluid loss is what's causing the rise in the total hemoglobin count (THC) (Hong *et al*., 2018). This occurs after a rise in the production and release of pro thoracicotropic hormone (PTTH) from the brain and ecdyson from the pro thoracic glands, which is followed by an increase in the mitotic index (MI) of the cells (PTG) (Lavine and Strand, 2002). Ecdyson plays a significant role in the production, multiplication and differentiation of haemocytes (Kwon *et al*., 2014; Silina, 2003). The reduction of THC when exposed to very low temperatures is due to the clumping of cells that render the haemocytes unavailable from circulating hemolymph for counting (Wu *et al*., 2016; Berger and Slavickova, 2008). A decrease in haemocyte number has been reported with starvation in Papilio demoleus with an increase in THC following resumed feeding (Hong *et al*., 2018; Berger and Slavickova, 2008). Total haemocyte count reduces following starvation and this is because of changes in blood volume due to cell degeneration (Lavine and Strand, 2002; Schmidt *et al*., 2001). Prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipocytes, and oenocytoids are the typical haemocyte types in most insects (Miranpuri *et al*., 1991). Circulating haemocytes (sometimes called “blood cells”) play important roles in defense mechanisms against microorganisms in the hemocoel (Hong *et al*., 2018). Cellular defenses refer to haemocyte-mediated responses

such as phagocytosis, nodulation, and encapsulation (Lawrence, 2008; Schmidt *et al*., 2001).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Habitat Preference and Spatial Distribution of Crickets

3.1.1 Study Area

The study was carried out in Western Kenya, which extends between 34° 00E and 36° 30E as well as 0° N and 30° S (Jaetzold *et al*., 2006). Figure 3.1 shows the study area in Western Kenya comprising two counties of Siaya and Busia. The study area has four agro ecological zones (AEZ), Lower midland 1 (LM1), Lower midland 2 (LM2), lower midland 3 (LM3) and lower midland 4 (LM4). The region is a biodiversity sanctuary and one of the prime habitats of edible cricket species in its distribution range in Africa, but developments in agriculture, industry and urban centers has affected its distribution in the region (UNCCD, 2001). The study area has a mean annual rainfall of 1280 mm usually occurring from March to November (Okungu *et al*., 2005). The highlands however, receive more than 2400 mm of rainfall, from March to May and from July to September (Adger, 2006). Temperature ranges from a minimum of 10 ^{0}C and a maximum of 40 ^{0}C, with an expected increase of 2 ^{0}C to 2.5 ^{0}C in maximum and minimum temperatures by 2020 (Adger, 2006; UNFCC, 2006). With increasing climate vulnerabilities, the maximum and minimum temperatures are projected to further increase by 3.5 ^{0}C to 4 ^{0}C in more than half of the area by 2030 (UNEP, 2009; UNFCC, 2006).

The landscape in this region consist of a mosaic of farmland land (36 %), grassland (26 %), forest (24 %), scattered settlement and small towns (6 %), lakes and water ways (5 %), and a small proportion of other land use types (3 %) (Swallo *et al*., 2002; UNCCD, 2001). The study was conducted based on various climatic drivers that influence species diversity and abundance.

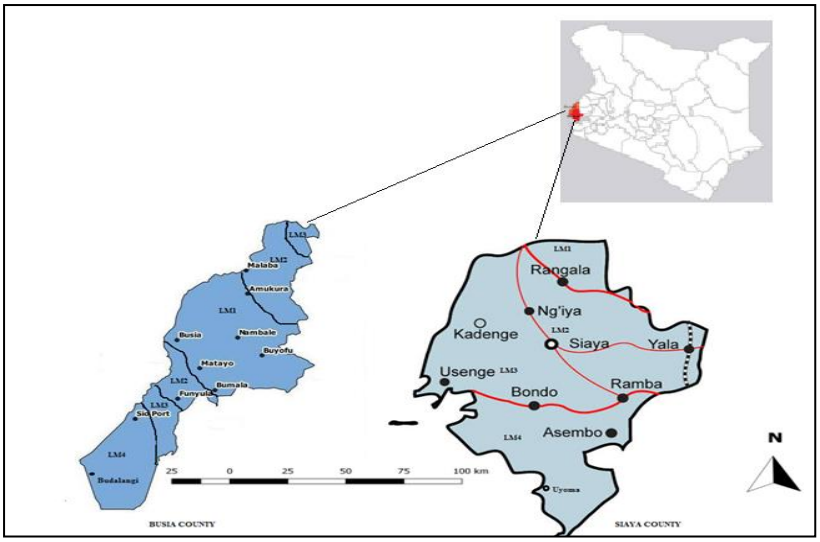


Figure 3. 1: Map of study area in Western Kenya

3.1.2 Factors Defining the Habitat Choice of Crickets

In this study, various environmental factors were examined for habitat modeling (Grillet *et al*., 2010). Table 3.1 shows thirteen environmental variables used in the study to determine their effects on habitat selection by cricket.

Table 3. 1: The variables used to predict habitat preference and distribution of crickets

S/No.	Variables	Description of each variable	Data type
1	AEZ	Agro ecological zones (LM1, LM2, LM3, LM4)	Continous
2	Land use	Forests, grasslands, farmlands, wetland, settlement	Continous
3	Ground cover	Presence of shelter	Continous
4	Slope	% Slope	Continous
5	Northness	Northness	Continous
6	Vegetation height	Height of the vegetation in m	Continous
7	Canopy closure	Measure of ground vegetation cover	Continous
8	Normalized difference vegetation index (NDVI)	Using Landsat enhanced thematic mapper (ETM) to measure different levels of greenness of vegetation	Continous
9	Distance from water body	Distance from water in m	Continous
10	Distance from roads	Distance from roads in m	Continous
11	Distance from settlement	Distance from settlement in m	Continous
12	Distance from farms	Distance from farms in m	Continous
13	Wetness	Degree of wetness	Continous

3.1.3 Cricket Sampling and Identification

3.1.3.1 Species Sampling

The population, which refers to the area from where collection of crickets was done, was stratified based on a cluster random sampling method. The population was divided into smaller groups known as clusters and samples randomly selected amongst these clusters. A sample size of the area surveyed was arrived at using a sample size table and the standardization equation (Appendix II)

Based on orthopteran occurrence of 1 % from biodiversity atlas, Kenya, the minimum sample size was determined according to the statistical formula:

$$N = \frac{\mathrm{Z}2P(1-P)}{D2}$$

N = Minimum sample size required

Z = z –value found in the Z- table – standard error

P = postulated prevalence rate of 1 % (0.01)

D = 0.05 = Inverse of 95 % confidence limit

Data on cricket abundance and distribution was collected through insect sampling and diversity analyses (Adetundan and Olusola, 2013). A field survey was conducted in the region to assess the diversity and abundance of cricket species. The sampling was carried out after every two weeks for six months using pitfall traps, sweep nets and hand collection (Ward and Lariviere, 2004; Sorensen, 1948). The pitfall traps were set in 3 replicates, 50 m apart in 3 different locations having similar environmental characteristics (Winder *et al*., 2001).

A dual arrangement pitfall trap with a length of 11cm and 10 cm wide was used in which a ditch is excavated and two vessels positioned in the pit and a brimful of soil stuffed up to the brink of the inner vessel (Sabu and Shiju, 2010). The trap, containing granulated sugar and bread crumbs in a 1 x 1 km grid across the study area, was set up from which a sum of adult crickets were recovered. The inner cup is a removable container that allow for setting and servicing of the trap (Nyundo and Yarro, 2007). The outer cup keeps the hole from back filling with soil. An elevated wooden tripod stand (5 cm above the ground level) was placed over the pitfall to keep off water, falling debris and small rodents (Nyundo and Yarro, 2007). House crickets were collected using sweep nets.

Sampling was done in 12 locations selected randomly as representatives of the 4 different AEZ in the county of Busia and 12 locations in Siaya county (Islam, 2018). For analysis of habitat preference, each location was clustered based on land use characteristics (Natural vegetation, agricultural land, wet land and settlement) totaling 96 sites and data on insect population recorded along the diagonals of each selected field. After identifying natural vegetation in lower midlands 1 (LM1) as the areas with the highest insect populations. The six locations of LM1, were further clustered based on nine environmental variables (Normalized difference vegetation index, distances from water, road, settlement and farms, vegetation height, presence of shelter, canopy closure and slope) (Breshears *et al*., 2008; Chapin *et al*., 2000) and data on insect population recorded.

Table 3.2 shows 24 surveyed sites (12 in Busia County and 12 in Siaya County) with their GPS coordinates.

Table 3. 2: Survey sites in Busia and Siaya Counties and their GPS coordinates

Busia county				Coordinates	
	Code	**AEZ**	**Location**	**Latitudes**	**Longitudes**
1	BS1	LM1	Alupe KALRO	0.4959	34.1331
2	BS2	LM1	Busia Youth Polytechnic	0.4518	34.1217
3	BS3	LM1	Butula Boys	0.3426	34.3341
4	BS4	LM2	Amukura health centre	0.5706	34.2719
5	BS5	LM2	Malaba town	0.6346	34.2756
6	BS6	LM2	Lukolis Dispensary	0.6086	34.2084
7	BS7	LM3	Kolanya Boys	0.7099	34.4004
8	BS8	LM3	Nangina mission hospital	0.2763	34.1017
9	BS9	LM3	Angurai Chiefs Camp	0.7123	34.3477
10	BS10	LM4	Bunyala Catholic	0.0939	33.9756
11	BS11	LM4	Bumbe Technical	0.1721	33.9955
12	BS12	LM4	Port Victoria forest station	0.0961	33.9781
Siaya County					
				Coordinates	
	Code	**AEZ**	**Location**	**Latitude**	**Longitude**
13	SY1	LM1	Yala St. Marys	0.0967	34.5314
14	SY2	LM1	Rangala School	0.1526	34.3296
15	SY3	LM1	Sigomere School	0.2018	34.3546
16	SY4	LM2	Ukwala Boys	0.1954	34.1894
17	SY5	LM2	Siaya ATC	0.0626	34.2878
18	SY6	LM2	Boro Trading Centre	0.0860	34.235
19	SY7	LM3	Kadenge Yala Swamp	0.0270	34.1810
20	SY8	LM3	JOOUST	0.0939	34.2586
21	SY9	LM3	Ajigo Dispensary	0.3538	34.5652
22	SY10	LM4	Usigu Health Centre	0.0605	34.0929
23	SY11	LM4	Naya Dispensary	0.3837	34.2834
24	SY12	LM4	Nyamonye School	0.0483	34.1385

Insects recovered were wet preserved in 70% ethanol mixed with a few drops of glycerin inside glass vials. Representative samples were taken to the Insect laboratory of Egerton university (EU), Kenya for identification. The genera of the specimens were identified using the available keys (Appendix v) and confirmed by recognized specialists (Sharkey, 2007; Choate, 2011). A habitat suitability model for crickets was prepared using the primary and secondary sources of data (Aslam, 2009; Wolters, 2003).

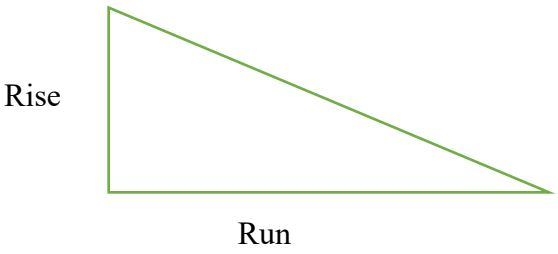
Table 3.3 shows the characteristics of the four agro ecological zones based on altitude in (m), mean annual temperature in ^{0}C and average annual rainfall in mm

Table 3. 3: Characteristics of the four Agro ecological Zones (AEZ)

AEZ	Altitude in (m)	Mean annual Temperature in ^{0}C	Average annual rainfall in mm
LM1	122-1440	21.0 - 22.2	1650 -2000
LM2	1200-1350	21.4 -22.3	1420 – 1650
LM3	1140-1500	21.0 – 22.7	1100 – 1420
LM4	1135-1200	22.3 -22.7	900 – 1200

3.1.3.2 Slope and Slope Orientation

To calculate the percent slope, the difference between the elevations of two points was divided by the distance between them, and the quotient multiplied by 100.



Percent slope = (Rise/Run) x 100

Where :

Rise = Difference in elevation between points

Run = Distance between the points

3.1.3.3 Normalized Difference Vegetation Index (NDVI)

The NDVI values were measured by a hand held device known as Plant Pen NDVI and PRI (photochemical reflectance index). The NDVI values were further assesed using the formula:

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

Where: NDVI = Normalized difference vegetation index

NIR = Near Infra red

3.1.3.4 Variables for the Model and Model Validation

Akaike Information Criterion (AIC) was used to examine the variables by means of backward stepwise selection at every step. Table 3.4 shows the AIC values of the thirteen environmental variables used in the study.

Table 3.4: Akaike Information Criterion (AIC) values of the different Environmental Variables

Steps	Gryllus bimaculatus		Scapsipedus Icipe		Acheta domesticus		Gryllotalpa africana		Diestrammena asynamora		Brachytrupes membranaceus	
	Var	**AIC**	**Var**	**AIC**	**Var**	**AIC**	**Var**	**AIC**	**Var**	**AIC**	**var**	**AIC**
1	AEZ	103	AEZ	106	AEZ	113	AEZ	102	AEZ	109	AEZ	93
2	Land use	98	Land use	99	Land use	97	Land use	98	Land use	97	Land use	86
3	Ground cover	98	shelter density	94	shelter density	97	shelter density	87	shelter density	103	shelter density	84
4	D. water	109	D. water	110	D. water	123	D water	67	D. water	122	D. water	79
5	D. farms	101	D. farms	105	D. farms	122	D. farms	98	D. farms	116	D. farms	127
6	D. roads	125	D. roads	126	D. roads	131	D. roads	112	D. roads	126	D. roads	112
7	D. settlement	122	D. settlement	130	D. settlement	96	D. settlement	114	D. settlement	93	D. settlement	123
8	Wetness	106	Wetness	122	Wetness	116	wetness	86	Wetness	108	Wetness	102
9	Slope orientation	135	Slope orientation	133	Slope orientation	147	Slope orientation	141	Slope orientation	143	Slope orientation	144
10	Slope	125	Slope	122	Slope	143	Slope	143	Slope	148	Slope	144
11	NDVI	99	NDVI	97	NDVI	98	NDVI	95	NDVI	99	NDVI	87
12	Canopy cover	133	Veg. height	138	Veg. height	141	Veg. height	137	Veg. height	142	Veg. height	140
13	Litter depth	121	Litter depth	105	Litter depth	112	Litter depth	121	Litter depth	119	Litter depth	131

D.water = distance from water; D. farms = distance from farms; D. roads = distance from roads; D. settlement = distance from settlement

3.2 Effects of Temperature on Development and Survival of Crickets

3.2.1 Colony Establishment

The insect colony was established at the Insect Farm of Jaramogi Oginga Odinga University of Science and Technology (JOOUST). The collected insects were fed on poultry growers mash and reared in the insect-rearing unit at JOOUST. Wild adults (2000 individuals, determined by weight) were collected from the selected habitats in Western Kenya. The crickets were reared in 60 L plastic buckets each stocked with about 100 crickets. The buckets were covered with mosquito nets to prevent entry of predators or escape of crickets (Mellisa, 2014; Clifford and Woodring, 1990). Drinking water was provided in a saucer of 16 cm diameter with a moist cotton wool at libitum. Egg trays measuring 29 cm x 29.5 cm were placed vertically in the buckets to act as hideouts (Ayieko et al., 2016; Mellisa, 2014).

3.2.2 Experimental Design

The experiment was arranged in a split plot design with two cricket species as the main plot, while six temperature regimes as subplots replicated three times. Newly laid cricket eggs, were collected randomly from the laboratory colonies to create the sets of cricket colonies from each species; *Acheta domesticus* and *Gryllus bimaculatus* and reared in an incubator under constant conditions. For this experiment, relative humidity, light intensity, and photoperiod were set at 60-90 %, 500 ± 25 Lux, and 12:12 respectively.

The temperature treatments were as follows:

T1 = 18 ºC, T2 = 22 ºC, T3 = 26 ºC, T4 = 30 ºC, T5 =34 ºC, T6 = 38 ºC.

The two cricket species, *Acheta domesticus* and *Gryllus bimaculatus* were selected because they are highly prolific, capable of surviving in captivity and well adapted to the climatic conditions of Western Kenya.

3.2.3 Data Collection

3.2.3.1 Fecundity

Adults of *Acheta domesticus and Gryllus bimaculatus* were selected and coupled in well ventilated transparent containers (20 cm x 20 cm x 15 cm) and treated to varying temperature regimes (Calvo & Molina, 2005). A total of ten pairings were chosen and an oviposition substrate consisting of a moistened cotton ball was placed within a sterile

petri dish in each container for egg laying. The number of eggs in each vial was counted under a stereomicroscope using a fine camel's hair brush (Otieno *et al*., 2019).

3.2.3.2 Weight and Fat Content

To remove any contaminants, the crickets were cleaned. Fresh body weight was measured at birth and once a week until death with an electronic scale (OHAUS Pioneer TM) set to the nearest 0.1 mg (Zebino *et al*., 2016).Adult males, virgin females and mated females were freeze killed for dry weights, and the specimens were maintained thermo-resistant glass vials and microwaved at 45^0C for 72 hours (Mellisa, 2014). The dry weights were calculated by using the dried specimens. Five grams of each specimen were put into Eppednorf ® tube (1.5 mL) in the soxhlet extractor for fat extraction. This was immersed in a 1:1 (vol:vol) petroleum ether solution and heated for 6 hours. The condensing unit was removed from the extraction unit and the sample was allowed to cool down. The samples were then dried once more for 48 hours and weighed after that (lean dry weight) (Wigglesworth, 1972; Mellisa, 2014). To a precision of 10^{-5} g, every weight was measured using analytical Sartorius ® balance (Infante, 2000). Fat content was calculated using the equation:

$$\text{Crude fat} = \frac{\text{weight of tube with sample} - \text{weight of tube}}{\text{weight of specimen}}$$

3.2.3.3 Structural Body Size and Sex Ratio

The length of the elytron, which is the distance between the apex and base, the width of the protonum at its widest point, and the hind leg's femur were used to estimate the structural body size. A digital calliper was used to measure each proportion with an accuracy of 0.01mm (Honnek, 1993). The sex ratio of adults was recorded.

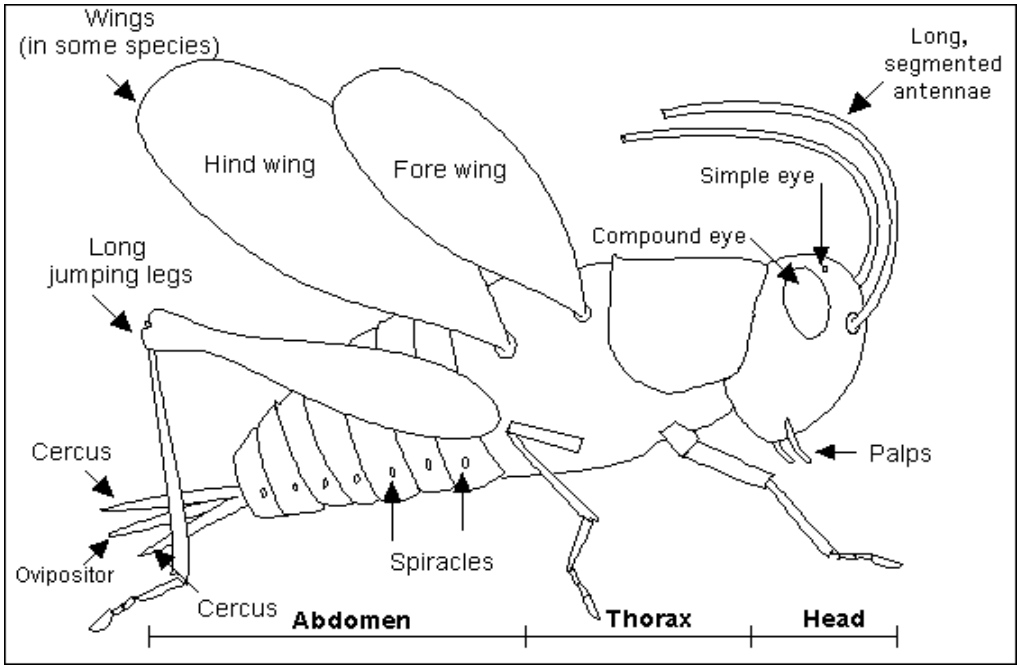


Figure 3.2: External anatomy of a cricket (Adapted from Enchanted learning.com)

3.2.3.4 Development Time and Longevity

The time to complete each life stage (egg, nymph, and adult) of the crickets was determined. The newly eclosed nymphs were marked with a fine-tipped brush with a small dot of ink in the protonum using non–toxic permanent ink pens (EDDING 751 band) due to its excellent adhesion, quick drying, and good visibility to estimate cricket longevity (Das *et al*., 2012). Different colours were used for each day of emergence (Infante, 2000; Bowling, 1955). Marked nymphs were returned to their respective buckets, daily observations made, and the waste removed and observed under a stereomicroscope to quantify the number of marked nymphs that had died (Bowling, 1955).

3.3 Characterization of the Cuticular Hydrocarbons

Individual crickets were submerged for 5 minutes in 4 mL of hexane that included an internal standard (n-pentadecane) at a concentration of 10 ppm in order to measure the amount of cuticular hydrocarbons. This extract was tested using a GC-MS (Shimadzu GCMS QP2010 with Stabilwax column) in split mode and equipped with a Stabil wax column with an internal diameter of 3.0 to 0.25 mm. Helium was used as the carrier gas (Monnin *et al*., 1998; Carlson *et al*., 1989). A column temperature profile was designed in which the analysis started at a temperature of 50 ^{0}C for 1 min and climbed to 250 ^{0}C

for 20 minutes in order to optimize the separation of the extract. Transfer line between GC and MS set to 250 ^{0}C (Thomas and Simmons, 2008; Monnin *et al*., 1998). According to the technique suggested by Carlson *et al*., (1989) the double bond locations of alkenes were determined by derivatizing hexane extracts from chosen individuals of each cricket species. The extract was dried with nitrogen before being magnetically stirred into 200 mL of hexane. Then, 100 mL of iodine solution (dissolved in diethyl ether, 6% m/v) and 200 mL of dimethyl disulfide (DMDS) (both from Sigma-Aldrich) were added to the mixture. The mixture was extracted using a 5% sodium thiosulfate solution after being diluted in hexane (Thomas and Simmons, 2008; Carlson *et al.,* 1998). Following the method suggested by Carlson *et al*., (1989), the organic phase was then dried with sodium sulfate and analyzed by GC-MS. The oven temperature was initially set to 80 °C for 2 minutes, increased by 30 °C per minute up to 180 °C, then increased by 3 °C every minute until 300 °C, where it was maintained for 80 minutes (Monnin *et al*., 1998; Carlson *et al*., 1989)

3.4 Characterization of Haemocytes

3.4.1 Insect Specimens

The insect colony was established at the insect farm of Egerton University (EU). The cricket species *Acheta domesticus* and *Gryllus bimaculatus* were reared in buckets at a temperature of 26 º C, and 34 º C respectively, at a relative humidity of approximately 65 %, under a 12h light/12 h dark cycle (Ayieko *et al*., 2016; Clifford and Woodring, 1990). The crickets were fed on poultry growers mash and drinking water provided in a saucer of 16 cm diameter with a moist cotton wool ad libidum. Egg trays measuring 29 cm x 29.5 cm were placed vertically in the buckets to act as hideouts (Ayieko et al., 2016; Mellisa, 2014). Adult females of each species were selected and used for the experiment.

3.4.2 Haemocyte Isolation and Identification

Hemolymph smear preparations was made to determine the haemocyte types of *Acheta domesticus* and *Gryllus bimaculatus*. Each sample was cold anasthesized at -20 ºC for five minutes, surface sterilized with 70 % ethanol and then rinsed with distilled water (Berger and Slavickova, 2008). Hemolymph samples of all insects was obtained from the junction of the femur and thorax of living specimens via heparinized hematocrit

capillary tubes (Lawrence, 2008). The collected hemolymph was diluted (100x dilution in an anticoagulant buffer. Ten slides were prepared from the hemolymph of each species. Smears was immediately made, air-dried, and then stained using Wright's – Geimsa stain (Sokolova *et al*., 2000; Miranpuri *et al*., 1991). Photomicrographs of haemocytes were taken with an Axio Scope.A1 photomicroscope. Haemocytes were placed in a sterile disposable haemocytometer slide. The haemocyte concentration was counted using a Burker chamber under a CX 31 microscope (Olympus, Japan) with a magnification of 400X

3.5. Data Analysis

3.5.1 Assessing Habitat Preference and Distribution of Crickets

The following biodiversity indices were computed using Past3 software; (Hill *et al*., 2005).

1. Shannon weiner index. (H) (Shannon and weiner, 1963; Margalef, 1958).
2. Pielou's evenness index (E) (Diserud and Ø degaard, 2007, Pielou, 1966).
3. Simpson index (D) (Crane and Baker, 2011; Simpson, 1949).

Difference in species occurrence was tested using analysis of variance (ANOVA). A generalized linear model with a binomial distribution of the response variable and logit link function was used for analysis (Pinheiro *et al*., 2018; Box *et al*., 2005). Linear mixed effect (LME) models were used to determine the environmental variables that best explained the changes in population density of crickets along an altitudinal gradient in the study area (Nazir *et al*., 2014; Morris, 1963. All possible models were constructed based on sets of sampled environmental variables, and evaluation was done using Akaike Information Criterion (AIC) (Akaike, 1974). The best model was selected, and its statistical significance determined. All the analyses were performed in the R environment (R - Core Team 2017; R - Studio team, 2016*).*

3.5.2 Effects of Temperature on the Development and Survival of Two Cricket Species *Acheta domesticus* and *Gryllus bimaculatus.*

Differences in fecundity, weight and fat content, structural body size and sex ratio, development time, and longevity among temperature treatments were tested using analysis of variance (ANOVA). The significance of pair-wise correlations amongst the measured parameters was tested using Pearson's correlation coefficient (r). The significance of the correlation of the phenotypic factors in relation to changes in

temperature was assessed using Spearman's (ρ) correlation coefficient (Pinheiro *et al*., 2018). All the analyses were performed in the R environment (R - Core Team 2017; R - Studio team, 2016).

3.5.3 Characterization of Cuticular Hydrocarbons

Retention times were represented by the peak numbers and various hydrocarbons identified. Relative peak sizes were normalized with a log transformation and PCA run. Principal Components with eigen vectors greater than 1 were extracted using correlation matrix (Martin and Drijfhout, 2009b). The study used MANOVA (multivariate analysis of variance) to test for species and sex differences in the cuticular hydrocarbon expressions of the two crickets (R core team, 2013; Martin and Drijfhout, 2009b).

3.5.4 Characterization of Haemocytes

Raw data of the haemocyte types was compared among the species with two-sampled t-tests for both species separately. All data was analysed for normality of distribution and variance tested for homogeneity. Differences were considered significant at $p < 0.05$. The data analysis was done in the R- environment (R core Team, 2017).

CHAPTER FOUR
RESULTS

4.1 Assessing Habitat Preference and Distribution of Crickets

4.1.1 Occurrence of Cricket Species across Four Agro Ecological Zones in Western Kenya

Table 4.1 shows the total number of individual cricket species recovered from the four agro ecological zones in Western Kenya. A total of 3535 insects were recorded, comprising 3335 insects belonging to 6 identified crickets species and 200 others. The mean number of cricket species showed significant differences (p- value= 0.00055, R^2 = 0.9327) among the four agro ecological zones (Table 4.1). Highest mean ranking was recorded in lower midland 1 (162.00), followed by lower midland 2 with mean ranking of 138.00, lower midland 3 with mean ranking of 120.71 and lowest in lower midland 4 with a mean ranking of 84.28. The presence of more species in lower midland 1 was due to favourable conditions for cricket survival such as the presence of more numbers of plant species as cricket's diet, shelter and breeding substrate. Lowest numbers in lower midland 4, regions bordering Lake Victoria were recorded in sites far off from the water body. Across the habitats, *Gryllus bimaculatus* was the most abundant species (1005) followed by *Scapsipedus icipe*. (909) and *Gryllotalpa africana* (583) which was confined only to wetlands. *Diestrammena asynamora* recorded (367), *Acheta domesticus (*306) and the least number of species was recorded in *Brachytrupes membranaceus (165)*. Additional 200 species with very low frequency of occurrence across the habitats were unidentified and recorded as others.

Table 4.1: Occurrence of cricket species across four agro ecological zones in Western Kenya

		Agro ecological zones(AEZ)				
Species	**English name**	**LM1**	**LM2**	**LM3**	**LM4**	**Total**
Gryllus bimaculatus	Two spotted cricket	315	276	239	175	1005
Scapsipedus Icipe	Scapsipedus icipe	292	251	223	143	909
Diestrammena asynamora	Spider cricket	112	104	89	62	367
Acheta domesticus	House cricket	101	82	70	53	306
Gryllotalpa africana	African mole cricket	179	163	145	96	583
Brachytrupes membranaceus	Giant cricket	56	43	37	29	165
Others		79	47	42	32	200
TOTAL		1134	966	845	590	**3535**
Mean		162.00	138.00	120.71	84.28	

p-value = 0.00055, $R^2 = 0.9327$

4.1.2: Relative Abundance and Diversity Indices for Cricket Species Across Four Land Use Types

Table 4.2 shows the relative abundance and diversity indices for cricket species across four land use types. Natural vegetation had the highest percentage relative abundance of (44.07 %) followed by wetlands with (27.51%), agricultural lands with 18.39 % and the lowest in areas near settlement at 10.03 %. Both natural vegetation and wetlands recorded higher diversity indices with natural vegetation recording higher Simpson's index (D' = 0.194) and Shannon's index (H' = 0.361) than wetlands with Simpsons index (D' = 0.076) and Shannon's index (H' = 0.355). Wetlands recorded higher evenness index (E' = 0.052) than natural vegetation with an evenness index of E' = 0.049. Agricultural lands recorded lower diversity indices (D' = 0.034, H' = 0.311, E' = 0.048) but were superior to areas near settlement which recorded the least diversity indices (D' = 0.010, H' = 0.231, E' = 0.039).

Table 4.2: Relative abundance and diversity indices for cricket species across four land use types.

	Relative abundance (%)	Simpsons index (D)	Shannon Index (H^l)	Evenness (E^1)
Natural vegetation	44.07	0.194	0.361	0.049
Wetlands	27.51	0.076	0.355	0.052
Agricultural lands	18.39	0.034	0.311	0.048
Settlement	10.03	0.010	0.231	0.039

4.1.3: Identification of the Cricket Species Sampled from Habitats in the Study Area

Figure 4.1 shows six major cricket species identified with letters (A) – (F) sampled from different habitats within the study area. The following cricket species were the most abundant across habitats in Western Kenya.

(A)Diestrammena asynamora (Brunner Von Watten nyl, 1988)

English: Camel cricket; Spider crickets

These are brown crickets with a humpback appearance. They are flightless having a long body of about 5 cm long, with long and legs about 10 cm long.

They have a drum shaped femora, long slender antennae, large hind legs with a long thin tibiae. Majorly found in houses, caves and beneath logs.

(B)Gryllotalpa Africana

English – African mole cricket

The crickets have cylindrical bodies about 3 – 5 cm long covered with fine dense hair. They have small eyes and fore limbs that resemble a shovel highly adapted to burrowing. They spend most of their lives underground but adults develop wings and disperse during breeding. Males have a conspicuous loud sound often made from an open burrow.

(C) Acheta domesticus (Linnaeus, 1958)

English: House cricket

The house cricket is grey or brown, about 15 – 20 cm long. Males and females are similar in appearance with females having a brown or black ovipositor about 12 cm long, sorounded by two appendages at the rear.

(D)Scapsipedus icipe

These are dark brown crickets with males having an elongated and excavated face and mandibles, where as females posses a normal face and mandibles. The females have a narrow ovipositor with smooth margins

(E) Brachytrupes membrenaceous

English-Giant cricket

This is the largest of true crickets. The insect is robust having a feebly pubescent body and legs. The male protonum is widened strongly in front while the female ovipositor is disproportionately short compared to the body size.

(F) Gryllus bimaculatus De Geer

English- Two spotted cricket

Two spotted crickets vary greatly in colouration, ranging from black to reddish brown and yellow tegmina. They are medium to large sized crickets with rounded head and convex face. Females have dark brown, slender and straightened ovipositor.

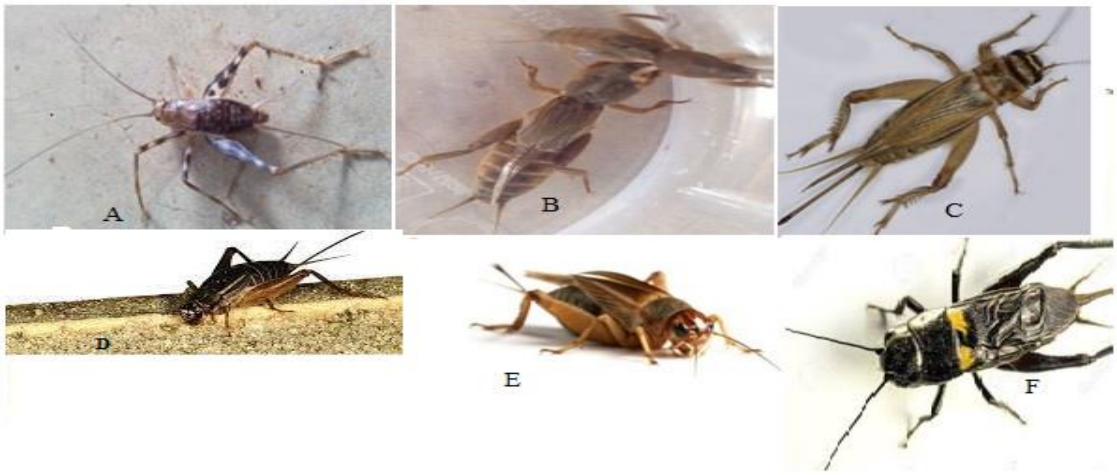


Figure 4.1: Cricket species sampled during the study

A= *Diestrammena asynamora, B= Gryllotalpa africana, C= Acheta domesticus, D= Scapsipedus icipe, E = Brachytrupes membranaceus, F= Gryllus bimaculatus.*

4.1.4: Effects of Land Use Types on the Number of Individual Cricket Species

Table 4.3 shows the number of individual cricket species recovered across four land use types. Number of cricket species were significantly influenced by land use (p – value < 0.0001, $R^2 = 0.971$) (Table 4.3). Natural vegetation recorded the highest mean number of species (251.17) followed by wetlands with a mean number of 153.67, agricultural lands (100.00) and the least (51.00) was recorded in settlements. Wetlands showed higher number of *Gryllotalpa* species with fewer representations of the other species. *Gryllus bimaculatus* recorded the highest (1101) individual numbers, followed by *Scapsipedus icipe* (1023), *Gryllotalpa africana* (644), *Diestrammena asynamora* (214), *Acheta domesticus* (190) and the least (163) individual cricket population was recorded with *Brachytrupes membrenaceus*. Natural vegetation recorded the highest number of *Gryllus bimaculatus* (613) followed by *Scapsipedus icipe* (555), *Gryllotalpa africana* (127), *Brachytrupes membraneceous* (113) and *Diestramena asynamora* (56) and the lowest in *Acheta domesticus* (43). Agricultural lands recorded the highest *Gryllus bimaculatus* (225). This was followed by *Scapsipedus icipe* (217), *Gryllotalpa africana* (70), *Diestrammena asynamora* (42), *Acheta domesticus* (36) and the least was *Brachytrupes membrenaceus* (10). Settlement recorded the highest numbers of *Acheta domesticus* (111). This was followed by *Diestramena asynamora* (96), *Gryllus*

bimaculatus (35), *Scapsipedus icipe* (33), *Gryllotalpa africana* (23) and the lowest was *Brachytrupes membrenaceus* (8).

Wetlands recorded the highest numbers of *Gryllotalpa africana* (424), followed by *Gryllus bimaculatus* (228), *Scapsipedus icipe* (218), *Brachytrupes membrenaceous* (32), *Diestrammena asynamora* (20) with no representations of *Acheta domesticus*.

Table 4.3: Occurrence of cricket species cross four land use types

	Land use types				
Species	**Natural vegetation**	**Agricultural land**	**Settlement**	**Wetlands**	**Total**
Gryllus bimaculatus	613	225	35	228	1101
Scapsipedus icipe	555	217	33	218	1023
Diestrammena asynamora	56	42	96	20	214
Acheta domesticus	43	36	111	00	190
Gryllotalpa africana	127	70	23	424	644
Brachytrupes membranaceus	113	10	08	32	163
Total	1507	600	306	922	**3335**
Mean	251.17	100	51	153.67	
p-value < 0.0001, $R^2 = 0.971$					

4.1.5: The Effects of Slope and Slope Orientation on the Occurence of Six Cricket Species in Western Kenya

Table 4.4 shows the effect of slope and slope orientation on the occurrence of six cricket species. There was no significant difference in slope and slope orientation with cricket species, although more species (48) were recorded at a slope angle of 10.1 to 15% and more (71) when the slope orientation was to the west (Table 4.4). This indicated that the crickets prefer lower altitude but mostly in mid elevation. The cricket diversity decreases with increase in elevation.

Table 4. 4: The effects of slope and slope orientation on the occurence of six cricket species in Western Kenya

Species	*Gryllus bimaculatus*	*Scapsipedus icipe*	*Diestrammena asynamora*	*Acheta domesticus*	*Gryllotalpa africana*	*Brachytrupes membranaceus*	*Total*
% Slope							
0 - 5	10	10	4	7	9	4	44
5.1 – 10	13	13	5	4	8	4	47
10.1 – 15	17	12	4	4	7	4	48
>15	10	9	4	4	7	4	38
Slope orientation							
Flat	15	13	7	8	6	5	54
North	9	8	7	8	4	4	40
East	10	11	6	5	7	6	45
South	11	12	4	4	7	4	42
West	23	19	9	8	6	6	71

4.1.6 Effects of Normalized Difference Vegetation Index (NDVI) on the Occurrence of Six Cricket Species in Western Kenya.

Table 4.5 shows the effects of normalized difference vegetation index (NDVI) Significant differences were recorded in places with different NDVI indices (p- value = 0.000147, R^2 = 0.9762). The number of crickets and increase in NDVI showed a positive correlation. Higher species numbers (128) were recorded with NDVI ranging from 0.5 to 1. This was followed by a total of 105 species with NDVI value ranging from 0.25 to 0.5. The lowest species numbers (59) were recovered with NDVI values ranging from -1 to 0.25 (Table 4.5).

Table 4.5: Effects of normalized difference vegetation index (NDVI) on the occurence of six cricket species in Western Kenya

	NDVI		
Species	**-1 to 0.25**	**0.25– 0.5**	**0.5 - 1**
Gryllus bimaculatus	19	36	41
Scapsipedus icipe	18	30	37
Diestrammena asynamora	7	12	17
Acheta domesticus	6	10	12
Gryllotalpa robusta	5	10	13
Brachytrupes membranaceus	4	7	8
Total	**59**	**105**	**128**
Mean	**9.83**	**17.5**	**21.33**
p-value < 0.000147, R^2 = 0.9762			

4.1.7 Effects of Canopy Closure on the Occurrence of Crickets

Table 4.6 shows the effects of canopy cover on the occurrence of cricket species. Significant differences were observed on species occurrence with increased canopy cover (p- value < 0.0008443, R^2 = 0.9056). The results of the analysis of the canopy cover on distribution of crickets showed higher species within the canopy cover ranging from 51% - 75% and 26 – 50%. The species numbers were lower in canopy cover of 0 - 25% than within the canopy cover ranging from 76% - 100%. Statistically, the correlation between the canopy cover and the number of crickets showed a positive relationship, *w*hich indicates that numbers of cricket species increases when crown cover increases up to 80%. Crickets mostly preferred within the crown cover between 51 – 75%, compared to the canopy cover of 25 – 50%. This is mainly because of the presence of adequate shelter within the canopy cover of 51 – 75%, with a relatively un-

decomposed leaf litter layer and with a greater amount of dry dead sticks and branches (Table 4.6). However, the soil in the crown canopy between 76 -100% were observed to be moist with a thick layer of decomposing leaf litter where very little number of crickets were recovered.

Table 4.6: Effects of canopy closure on the occurrence of six cricket species in Western Kenya

	% Canopy Closure		
Species	**25 – 50**	**51-75**	**76 – 100**
Gryllus bimaculatus	17	21	15
Scapsipedus icipe	15	19	13
Diestrammena asynamora	6	10	6
Acheta domesticus	5	11	5
Gryllotalpa africana	5	11	15
Brachytrupes membranaceus	4	11	9
Total	**52**	**83**	**63**
Mean	**8.67**	**13.83**	**10.5**
p- value < 0.0008443, R^2 = 0.9056			

4.1.8: Effects of Ground Cover on the Occurrence of Crickets

Table 4.7 shows the effects of ground shelter on the occurrence of six cricket species. There were significant differences species occurrence with shelter density (p – value < 0.000197, R^2 = 0.9632) (Table 4.7). Higher species numbers (108) were recovered at ground cover ranging from 51-75 % with lowest (36) at ground cover ranging from 0 - 25 %. Higher shelter density in forests, and other cricket habitats contributed to high species numbers because of the presence of dead logs, branches, and wood stump, a potential hideout for the crickets.

Table 4.7: Effects of ground cover on the occurence of six cricket species in Western Kenya.

	% Ground Cover			
Species	**0 – 25**	**26– 50**	**51 - 75**	**76 -100**
Gryllus bimaculatus	10	19	32	29
Scapsipedus Icipe	8	17	29	25
Diestrammena asynamora	4	8	12	10
Acheta domesticus	4	6	10	8
Gryllotalpa africana	6	11	18	16
Brachytrupes membranaceus	4	4	7	5
Total	**36**	**65**	**108**	**93**
Mean	**6.00**	**10.83**	**18.00**	**15.50**
p-value < 0.000197, R^2=0.9632				

4.1.9 Effects of Distance from Water Bodies, Farms, Roads and Settlement

Areas near water bodies recorded significantly high species number compared to areas further away from water bodies. Significant differences were recorded in places far away from human activity (Roads, p-value = 0.00000366, R^2= 0.9849; water, p-value = 0.0000349, R^2 = 0.9678; farm; p-value = 0.0005, R^2 = 0.9176; settlement, p-value = 0.0004664, R^2 = 0.9245) (Table 4.8). Places far away from human activities recorded more species than areas near farms, settlement and roads. The results show that crickets preferred maximum inter-water distances of 0- 150 m with a mean of 36.50, followed by distance between 151- 500 m with a mean of 27.50, and lowest preference in habitats at a distance more than 500 m with a mean of 16.50 (Table 4.8). Areas near roads (0 – 150 m) recorded the lowest mean number of cricket species (9.83) where as sites at distances greater than 500m recorded the highest mean number of species (19.67). No significant differences were recorded with distances from farms, although higher mean species numbers (18.67) were recorded at distances far away from farms (> 500m) and lowest (16.33) at a distance of less than 150m. Crickets preferred areas far away from human settlement. Highest mean (25) were recorded at a distance > 500m and lowest (10) at a distance < 150m. Table 4.8 shows the Effects of distance from water, roads, farms and settlement on the occurrence of six cricket species in Western Kenya.

Table 4.8: Effects of distance from water, roads, farms and settlement on the occurence of six cricket species in Western Kenya.

	Distance (m)		
	<150	**151 - 500**	**>500**
Species		**Distance from water**	
Gryllus bimaculatus	66	50	27
Scapsipedus icipe	59	47	25
Diestrammena asynamora	25	19	14
Acheta domesticus	19	16	16
Gryllotalpa africana	37	23	6
Brachytrupes membranaceus	13	10	11
Total	**219**	**165**	**99**
Mean	**36.50**	**27.50**	**16.50**
		Distance from roads	
Gryllus bimaculatus	19	29	39
Scapsipedus Icipe	18	25	35
Diestrammena asynamora	7	10	13
Acheta domesticus	6	8	12
Gryllotalpa africana	5	8	12
Brachytrupes membranaceus	4	6	7
Total	**59**	**86**	**118**
Mean	**9.83**	**14.33**	**19.67**
		Distance from farms	
Gryllus bimaculatus	32	35	38
Scapsipedus icipe	29	32	34
Diestrammena asynamora	12	13	13
Acheta domesticus	10	11	12
Gryllotalpa africana	8	8	8
Brachytrupes membranaceus	7	7	7
Total	**98**	**106**	**112**
Mean	**16.33**	**17.67**	**18.67**
		Distance from settlement	
Gryllus bimaculatus	20	32	42
Scapsipedus icipe	17	23	37
Diestrammena asynamora	8	12	14
Acheta domesticus	8	13	14
Gryllotalpa Africana	4	7	10
Brachytrupes membranaceus	3	5	6
Total	**60**	**92**	**123**
Mean	**10.00**	**15.33**	**20.5**

4.1.10 Variables for Model to Predict Presence of Each Cricket Species

Variables were removed step by step by finding out which model with remaining variables could be best explained by the lower AIC (Table 4.9). A smaller AIC indicates a better model; therefore, there is greater deviance explained for each environmental variable. The best-combined variables were recorded. Variables that remained after being removed by the backward stepwise selection were mostly related to habitat components (shelter density, AEZ, distance from bodies of water, NDVI and distance from farmland and human disturbances). These findings indicate that crickets responded positively to environmental factors associated with habitat components, while they shunned harsh conditions and disturbances resulting from anthropogenic factors.

Table 4.9: Summary of the best combination of variables for model to predict presence of each cricket species

Species	**Selected Environmental Variables (order)**	**AUC**	**CvAUC**
Gryllus bimaculatus	Land use, shelter density, NDVI, AEZ, distance from water, distance from farm	0.887±0.044	0.766±0.023
Scapsipedus icipe	Land use, shelter density, NDVI, AEZ, distance from water, distance from farm	0.845±0.048	0.737±0.016
Acheta domesticus	Land use, shelter density, NDVI, distance from settlement	0.772±0.032	0.689±0.028
Gryllotalpa africana	Land use, distance from water, distance from farms, wetness, shelter density, NDVI, AEZ.	0.885±0.054	0.761±0.043
Brachytrupes membranaceus	Land use, shelter density, NDVI, AEZ, distance from water, distance from farm, distance from road, distance from settlement	0.712±0.04	0.657±0.027
Diestrammena asynamora	Land use, shelter density, NDVI, AEZ, distance from water,	0.764±0.036	0.665±0.014

4.1.11 Categories of Crickets Recovered Based on Habitat Preference

Group I (*Acheta domesticus and Diestrammena asynamora)* showed higher preference for all locations on settlements and had very different preferences for several environmental conditions (Figure 4.2). They live anywhere in the house irrespective of the environmental conditions. Thay rea not sensitive to human disturbance.

Group II (*Scapsipedus icipe, Gryllus bimaculatus* and *Brachytrupes membranaceus*) preferred fields with tall grasses. *Gryllus bimaculatus and Scapsipedus icipe* simultaneously preferred lower elevation and mid elevation areas within the grasslands, they are sporadically distributed in the fields. The large sized *Brachytrupes membranaceus* had a different survival tactics since it had its wide home range in the forests and other tall trees from where it could feed but assured of its security away from human disturbance.

Gropu III *Gryllotalpa africana* preferred locations within the wetlands (Figure 4.2). Presence of water and general wetness were important and critical factors describing the preference of *Gryllotalpa africana* to its habitat. The wetness protects this species from desiccation and at the same time providing ready access to food and breeding sites. This species was more cautious and sensitive to anthropogenic land use than the other species.

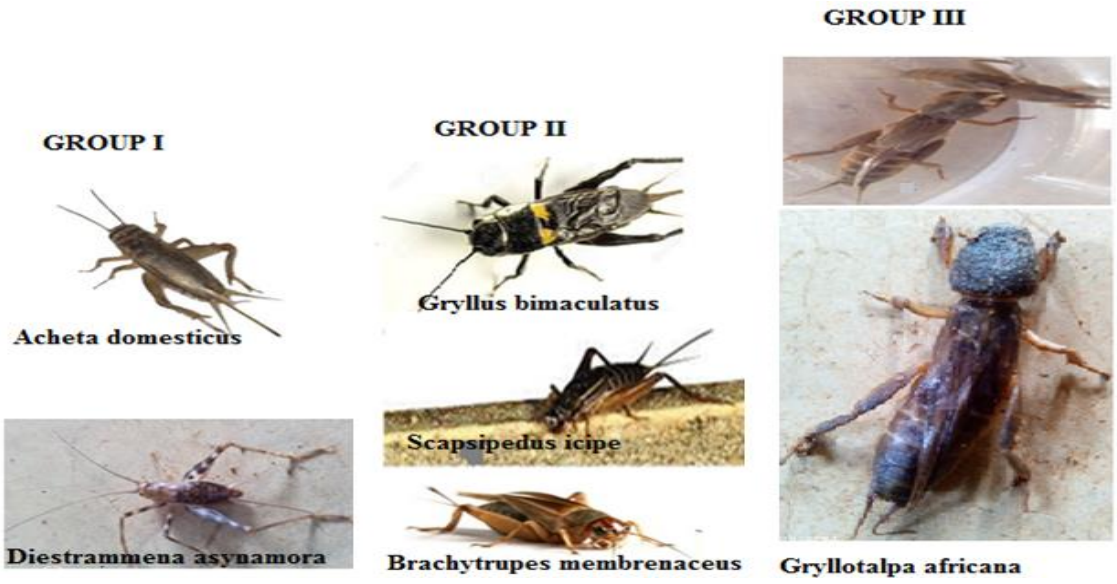


Figure 4.2: Categories of crickets based on habitat preference

4.2 Effects of Temperature on the Development and Survival of Cricket Species, Acheta Domesticus and Gryllus Bimaculatus.

4.2.1 Effects of Temperature on Fecundity, Adult Longevity and Sex Ratio

4.2.1.1 Fecundity

The effects of six constant temperatures on fecundity of two cricket species (*Acheta domesticus* and *Gryllus bimaculatus*) differed significantly ($F_{5, 205} = 272$; $p < 0.001$) (Table 4.10). *Acheta domesticus* reared at 26 ºC recorded the highest fecundity of 1360 eggs per female. This was followed by those reared at 30 ºC at 1342 eggs per female. The lowest fecundity (101 eggs per female was recorded on *Acheta domesticus* reared at 18 ºC. Results indicated that the fecundity of crickets reared at 26 ^{0}C and 30 ^{0}C were not significantly different but the two were all different from those reared at 18 ºC, 22 ºC, 34 ºC and 38 ºC. For *Gryllus bimaculatus*, the highest fecundity (1722 eggs per female) was recorded in species reared at 30 ºC. This was followed by those reared at 26 ºC (680 eggs per female). The lowest fecundity (123 eggs per female) was recorded at 18 ºC. The interaction effects of fecundity and species was significant, with the highest fecundity (1722 eggs) recorded in *Gryllus bimaculatus* reared at 30 ºC whereas the lowest (101 eggs) was recorded in *Acheta domesticus* reared at 18 ºC (Table 4.10). Most of the eggs were laid in groups or batches in the soaked cotton wool.

4.2.1.2 Adult Longevity

Adult longevity of the two cricket species differed significantly ($p \leq 0.05$) among the temperature treatments. Longevity of both females and males was significantly higher (female: $F_{5, 401} = 7.5$, $P < 0.001$; male: $F_{5, 401} = 6.4$, $P < 0.001$) at 18 ºC than at other temperatures, with the shortest recorded for *Acheta domesticus* (female: 20.19 days; male: 26.67 days) and *Gryllus bimaculatus* (Females: 25.56 days: Males: 27.49 days) at 38 ºC (Table 4.10).

Acheta domesticus reared at 18 ºC recorded the highest adult longevity of 92.35 days for the males and 74.26 days for the females. Those reared at 38 ºC recorded the lowest adult longevity of 26.67 days for the males and 20.19 days for the females. *Gryllus bimaculatus* had the highest adult longevity of 93.43 days for males and 75.26 days for females at a temperature of 18 ºC. No significant differences were observed by combined effects of

species and temperature although, the highest (93. 43 days) adult longevity was reported in *Gryllus bimaculatus* at 18 ºC while the shortest (20.19 days) in *Acheta domesticus* at 38 ºC (Table 4.10). The longevity of males was higher than that of females at all temperatures, but no significant differences were observed between them.

4.2.1.3 Sex Ratio

There was a significant difference (p - value ≤ 0.05) on the analysis of sex ratio between the two cricket species (Table 4.11). Sex ratio was female biased at lower temperatures (18 ºC, 22 ºC, and 26 ºC) but male biased at 34 ºC and 38 ºC. Crickets reared at 18 ºC had the highest female to male ratio of 2.03 and 2.23 for *Acheta domesticus* and *Gryllus bimaculatus* respectively. The sex ratio was almost equal at a temperature of 30 ºC.

Table 4.10: Effects of temperature on fecundity, adult longevity and sex ratio of two cricket species; *Acheta domesticus* and *Gryllus bimaculatus*

Temperature (^{0}C)	Fecundity (Eggs/ female/ generation)	Adult Longevity (Days) Mean ± SE		Sex ratio (Female: Male)
		Females	Males	
Acheta domesticus				
18	101a	74.25 ±0.03c	92.35 ±0.08f	2.03
22	376b	65.71 ±1.02b	90.28 ±0.06e	1.33
26	1360c	65.40 ±1.14b	88.23 ±0.03d	1.13
30	1342c	61.05 ±0.15b	73.09 ±0.25c	1.04
34	306b	58.15 ±0.07b	62.19 ±0.78b	0.85
38	106a	20.19 ±0.01a	26.67 ±2.14a	0.61
Gryllus bimaculatus				
18	123a	75.26 ±0.13d	93.43 ±1.81f	2.23
22	463b	67.80 ±0.29c	90.22 ±1.05e	1.86
26	680b	68.42 ±0.71c	88.13 ±0.34d	1.27
30	1722c	62.13 ±1.21c	72.12 ±0.22c	1.02
34	660b	54.21 ±0.48b	60.17 ±1.36b	0.67
38	132a	25.56 ±0.55a	27.49 ±0.09a	0.52

SE - Standard error. Means in the same column followed by different letters were significantly different (Student – Keul's test. P< 0.05)

4.2.2 Effects of Temperature on Adult Weight, and Fat Content

4.2.2.1 Adult Weight

The effects of temperature on adult weight of the crickets differed significantly ($F_{5, 54}$ = 2.1; p < 0.001). The highest body weight (22.04 g and 20.47 g) for *Acheta domesticus* was

recorded at 26˚C for both females and males respectively (Table 4.11). In *Gryllus bimaculatus*, the highest adult weight of 23.42 g and 21.61 g was recorded at 30 ºC for females and males respectively.

4.2.2.2 Fat Content

At the significance level of $p \leq 0.05$, the temperature treatment yielded significantly different fat contents between the two cricket species (Table 4.11). *Acheta domesticus* reared at 22 ºC recorded the highest fat content (19.43 and 17.71 g /100g dry weight) for females and males respectively. *Gryllus bimaculatus* recorded the highest fat content (11.78 and 9.51 g/100g dry weight) at 30^0C for females and males respectively. The lowest fat content was recorded in crickets reared at 38 ºC for both species. Significant ($p < 0.05$) temperature x species interaction effects on fat content were observed, although the magnitude of the interaction was relatively small, which suggested that fat content was, influenced more by the temperature treatment than species.

Table 4.11: Effects of temperature on adult weight and fat content of two cricket species; *Acheta domesticus* and *Gryllus bimaculatus*

	Temp (^{0}C)	Fat content (g/100g dry weight)		Adult weight	
		Mean ± SE			
		Females	Males	Females	Males
Acheta domesticus	18	18.78e	17.50c	21.5c	18.22c
	22	19.43de	17.71c	21.5c	19.88d
	26	17.66d	15.47b	22.04c	20.47d
	30	15.13c	14.22b	21.43c	20.15d
	34	10.95b	10.40a	18.75b	16.73b
	38	9.71a	9.08a	18.19a	13.19a
Gryllus bimaculatus	18	11.45a	10.45d	18.40a	16.37a
	22	12.11b	10.15c	19.53a	16.84a
	26	11.76a	9.44ab	22.17b	19.86b
	30	11.78a	9.51bc	23.42b	21.61b
	34	10.88a	9.37ab	19.12a	21.53b
	38	10.12a	8.45a	19.04a	17.19a

SE - Standard error. Means in the same column followed by different letters were significantly different (Student – Keul's test. P< 0.05)

4.2.3 Effect of Temperature on Structural Body Length

Both temperature and cricket species had significant effects on structural body length (p value = 0.0008 and p-value <.0001 respectively). *Acheta domesticus* reared at 26 ºC recorded higher lengths: body length 18.3 mm and 18.4 mm for males and females respectively. Length of tegmina; 9.2 mm and 13.4 mm; Length of femur of hind leg: 11.0 mm and 12.0 mm for males and females respectively. *Gryllus bimaculatus* had a higher (24.1 mm and 24.8 mm) body length at 30 ºC for males and females respectively. The lowest lengths were recorded in crickets reared at 18 ºC and 38 ºC (Table 4.12). The interaction effect of temperature and species on structural body length was significant. Crickets with the highest body length (24.8 mm) was obtained from *Gryllus bimaculatus* reared at 30 ºC with the shortest (16.0mm) lengths recorded in *Acheta domesticus* reared at 18 ºC (Table 4.12).

Table 4.12: Effects of temperature on the structural body size of two cricket species, *Acheta domesticus* and *Gryllus bimaculatus*

Temp	Body length (mm)		Length of tegmina (mm)		Length of femur of hind leg	
	Mean ± SE					
	Male	Female	Male	Female	Male	Female
Acheta domesticus						
18	16.0 ±0.06b	18.2 ±0.06c	8.5 ±0.13a	8.7 ±1.48a	8.3 ±0.09a	9.4 ±0.07a
22	17.2 ±1.14bc	21.1 ±0.08d	8.5 ±0.09a	8.6 ±1.23a	10.3 ±0.05a	9.8 ±0.34a
26	18.3 ±0.17c	18.4 ±0.12c	9.2 ±0.03a	13.4 ±0.18c	11.0 ±0.02b	12.0 ±2.56c
30	15.7 ±0.04a	16.2 ±0.11b	9.0 ±0.04a	13.0 ±0.03c	10.6 ±0.54b	11.8 ±1.43b
34	15.2 ±0.47a	16.1 ±0.31b	9.0 ±0.07a	12.8 ±0.27c	11.0 ±0.14b	11.8 ±0.76b
38	14.3 ±0.32a	14.4 ±0.72a	8.5 ±1.31a	10.3 ±0.41b	10.7 ±0.06b	11.5 ±0.44b
Gryllus bimaculatus						
18	18.7 ±0.08a	19.6 ±1.35a	15.0 ±0.19a	13.0 ±0.04a	11.0 ±0.04a	10.7 ±1.23a
22	19.4 ±0.23a	20.5 ±0.46a	14.7 ±0.07a	14.2 ±1.32a	11.8 ±1.43a	11.5 ±0.66a
26	22.5 ±0.32b	23.6 ±0.06b	17.2 ±0.01b	17.0 ±2.05b	12.0 ±1.33a	12.2 ±0.08a
30	24.1 ±0.86c	24.8 ±1.22c	18.3 ±0.35b	17.2 ±1.09b	13.6 ±1.28b	13.1 ±1.09b
34	23.8 ±0.71b	24.7 ±1.65c	19.5 ±0.87c	18.2 ±0.08b	14.0 ±0.61b	13.5 ±0.07b
38	23.1 ±0.12b	23.4 ±0.53b	17.5 ±0.06b	17.9 ±0.67b	13.6 ±0.55b	13.6 ±1.14b

SE - Standard error. Means in the same column followed by different letters were significantly different (Student – Keul's test. P< 0.05)

4.2.4 Effect of Temperature on Development of the Different Growth Stages of Crickets

The developmental times for each stage of the two cricket species, *Acheta domesticus* and *Gryllus bimaculatus* at six constant temperatures are presented in Table 4.13. There were significant differences (p-value ≤ 0.05) in the analysis of development amongst the temperature treatments. The average developmental time for each stage was significantly shortened as the temperature increased. The highest number of moults (10 moults) were recorded in *Gryllus bimaculatus* reared at 18˚C and eight moults from 26˚C to 38˚C. The average egg incubation time reduced from 44.06 days at 18˚C to 9.86 days at 38˚C and that of nymphs decreased from 187.76 days at 18˚C to 57.71 days at 38˚C. Few eggs hatched at 18˚C, and nymphs failed to complete development at 38˚C. *Acheta domesticus* recorded nine moults at 18˚C, followed by eight moults from 22˚C to 38˚C. On average, the overall period from egg to adult was highest at 231.82 days for *Gryllus bimaculatus* and 200.50 days for *Acheta domesticus* at 18˚C. The shortest duration (62.22 days) was recorded at 34˚C for *Gryllus bimaculatus* and 66.26 days for *Acheta domesticus* reared at 30°C.

Table 4.13: ***Effects of temperature on development times of the different stages of two cricket species, Acheta domesticus and Gryllus bimaculatus***

	Temp (0C)	Development time (Days) Mean ± SE												
		Egg incubation	**1st instar**	**2nd instar**	**3rd instar**	**4th instar**	**5th instar**	**6th instar**	**7th instar**	**8th instar**	**9th instar**	**10th instar**	**Total nymph**	**Egg – adult**
Acheta domesticus	18	38.20 ±0.04d	14.35 ±0.06d	16.63 ±0.06d	18.68 ±0.04c	18.59 ±0.32d	17.86 ±0.31c	18.17 ±0.18c	18.84 ±0.03d	19.93 ±0.07e	19.25 ±0.09a	-	162.30 ±0.34e	200.50 ±0.89e
	22	35.41 ±0.06c	12.27 ±0.15c	13.65 ±0.06c	16.07 ±0.05b	15.27 ±0.12c	15.55 ±0.06b	15.52 ±0.23b	16.04 ±0.14c	16.31 ±0.05d	-	-	120.68 ±1.23d	156.09 ±0.56d
	26	19.34 ±0.55b	6.61 ±0.22a	6.46 ±0.03ab	6.28 ±0.05a	6.54 ±0.35a	6.91 ±0.46a	6.74 ±0.08a	7.03 ±0.25a	7.43 ±0a.03	-	-	54.00 ±0.76a	73.34 ±0.87b
	30	10.21 ±0.03a	5.76 ±0.03a	5.89 ±0.01a	6.74 ±0.05a	6.58 ±0.09a	6.82 ±0.07a	6.82 ±0.05a	7.83 ±0.42ab	9.61 ±0.25b	-	-	56.05 ±0.54a	66.26 ±0.13a
	34	9.60 ±0.14a	8.32 ±0.01b	7.74 ±0.04b	7.43 ±0.04a	7.56 ±0.14a	6.79 ±0.21a	6.64 ±0.04a	7.81 ±0.29ab	9.53 ±0.36bc	-	-	61.82 ±0.45b	71.42 ±0.08b
	38	9.90 ±0.07a	8.21 ±0.04b	8.44 ±0.03b	7.87 ±0.08a	8.98 ±0.28b	7.14 ±0.11a	8.35 ±0.17a	9.88 ±0.04b	11.04 ±0.35c	-	-	69.91 ±0.67c	79.81 ±0.71c
Gryllus bimaculatus	18	44.06 ±0.62c	16.71 ±0.91c	17.33 ±0.73c	18.22 ±0.27c	19.36 ±0.37c	18.91 ±0.27c	18.02 ±0.08c	19.27 ±0.28c	19.73 ±0.19c	20.03 ±0.45b	20.18 ±0.76b	187.76 ±0.08e	231.82 ±0.07d
	22	37.22 ±0.07b	16.09 ±0.06b	15.47 ±0.05b	15.51 ±0.62b	15.00 ±0.47b	16.29 ±0.15b	16.47 ±0.16b	17.18 ±0.65b	18.48 ±0.26b	16.62 ±0.36a	17.69 ±0.16a	164.8 ±0.07d	202.02 ±0.13c
	26	10.71 ±0.33a	8.37 ±0.49a	8.51 ±0.08a	8.33 ±0.02a	8.61 ±0.43a	8.84 ±0.06a	8.06 ±0.27a	7.11 ±0.72a	7.32 ±0.22a	-	-	65.15 ±0.34c	75.86 ±0.56b
	30	10.56 ±0.51a	7.06 ±0.98a	7.43 ±0.14a	7.82 ±0.35a	7.49 ±0.71a	7.77 ±0.04a	5.98 ±0.18a	5.24 ±0.70a	5.33 ±0.34a	-	-	54.12 ±0.09a	64.68 ±0.65a
	34	9.45 ±0.11a	7.11 ±0.17a	7.57 ±0.14a	7.08 ±0.56a	7.45 ±0.11a	7.77 ±0.07a	5.17 ±0.07a	5.48 ±0.41a	5.14 ±0.72a	-	-	52.77 ±0.32a	62.22 ±0.17a
	38	9.86 ±0.82a	7.00 ±0.48a	7.04 ±0.07a	7.03 ±0.86a	7.27 ±0.21a	7.03 ±0.13a	8.00 ±0.03a	7.11 ±0.55a	7.23 ±0.19a	-	-	57.71 ±0.86b	67.57 ±0.18a

SE - Standard error. Means in the same column followed by different letters were significantly different (Student – Keul's test. P< 0.05)

4.2.5 Effects of Temperature on Survivorship of Different Growth Stages of Crickets

Different temperature treatments significantly influenced the survial of the developmental satges of crickets (Table 4.14). Crickets reared under all temperature treatments between the two species *Acheta domesticus* and *Gryllus bimaculatus* had significant survival rates. The study revealed that *Acheta domesticus* reared at 26 ºC were similar to those reared at 30 ºC and recorded significantly higher survival rates (86.30 % and 78.75 % respectively). In *Gryllus bimaculatus*, the highest percentage (86.51 %) egg to adult survival rates was recorded at 30 ˚C with the lowest (65.74 %) percentage survival rate recorded at 18 ˚C. The interaction between temperature and species was not significant although, the highest survival rates (86.51 %) at all temperatures were recorded in *Gryllus bimaculatus* reared at 30 ºC and the lowest (64.17 %) in *Acheta domesticus* reared at 38 ºC.

Table 4.14: Effects of temperature on egg - adult survival of two cricket species, *Acheta domesticus* and *Gryllus bimaculatus*

Temperature	**% Egg – Adult survival**											
	Egg hatchability	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar	7th instar	8th instar	9th instar	10th instar	Average
Acheta domesticus												
18	20.65	63.71	66.53	62.45	71.02	76.42	79.03	78.14	77.24	78.76	77.37	64.19
22	57.78	62.05	63.58	70.35	72.14	75.33	74.44	79.45	85.32	83.53	84.84	69.23
26	61.06	76 46	75.80	81.13	85.39	83.54	90.55	95.01	96.16	00	00	86.30
30	61.00	76.37	81.62	83.78	82.28	91.51	96.22	96.47	89.21	00	00	78.75
34	63.32	76.64	77.11	83.09	89.46	91.06	97.01	90.18	94.43	00	00	76.73
38	58.49	68.34	72.29	81.12	87.04	90.15	71.23	66.60	73.85	00	00	64.17
Gryllus bimaculatus												
18	19.58	65.87	68.65	64.57	73.29	78.63	81.39	80.27	78.43	79.65	79.55	65.74
22	59.96	64.16	64.49	73.58	73.38	76.65	76.57	80.54	87.50	85.71	86.52	70.92
26	62.23	78 54	78.98	80.34	85.56	85.61	91.73	96.12	96.50	00	00	80.48
30	62.20	71.44	85.54	87.87	87.12	92.69	98.44	97.23	91.54	00	00	86.51
34	63.21	78.78	79.03	86.34	91.67	94.23	98.25	91.24	97.64	00	00	81.24
38	59.56	70.29	74.34	83.35	89.28	91.34	70.34	65.98	76.98	00	00	71.95

4.2.6 Correlations among the Variables

The correlations between different characters of cricket development and survival are shown in Table 4.15. Both positive and negative association amongst the variables were identified. Development time was positively correlated with longevity at 30ºC (r=0.4546). Adult weight and fat content are positively and closely correlated (r = 0.8692, p-value = 0.004) while fecundity and adult longevity were negatively correlated (r = -0.0953). Increased female longevity has been recorded as a result of reduced egg production in *Drosophila* species. The decrease in female longevity may be due to higher energy diverted towards the reproductive machinery. Significant positive correlations between fecundity and adult weight (r = 0.8424, p-value < 0.0001) were recorded (Table 4.15). Body size and fecundity are a function of genetics and the environment. Large females have higher fecundity; therefore, selection should favour increased body size.

A significant negative correlation was observed between development times (r = 0.7316, p-value = 0.003) and adult sizes, suggesting that an increase in development rate resulted in reduced body size and weight. Correlations between development times and adult weights shows that there is a lot of potential for using them to assess the calibre of insects reared. This is because insects with lower development rates have high adult weights and sizes and accumulate more fat content. A decline in size was followed by an increase in inhibition of reproductive maturation, as reflected by the decline in fecundity. The increased inhibition of the reproductive cells has been found to be followed by a decrease in size and weight in several insects. Reduced reproductive development associated with a decline in fecundity under low temperatures is caused by poorly developed ovaries.

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Table 4.15: Correlation coefficients for fecundity, fat content, adult longevity, adult weight, body length, length of tegmina, and length of femur of hind limbs of two cricket species; *Acheta domesticus* and *Gryllus bimaculatus.*

	Adult longevity	**Fat content**	**Fecundity**	**Adult weight**	**Body length**	**Length of tegmina**	**Length of femur of hind leg**	**Development times**
Adult longevity	1.0000	0.6546 0.0014	-0.0953 0.0002	0.7338 <.0001	0.2820 <.0001	0.2571 0.2901	0.1640 <.0001	0.4546 <.0001
Fat content	0.6546 0.0014	1.0000	0.7745 0.0003	0.8962 0.0004	-0.2316 0.0016	0.4707 <.0001	0.5183 0.0014	-0.0487 0.0005
Fecundity	-0.0953 0.0002	0.7745 0.0003	1.0000	0.8424 <.0001	0.4715 0.0004	0.0642 <.0001	0.0353 0.0005	-0.2748 <.0001
Adult weight	07338 <.0001	0.8962 0.0004	0.8424 0.0001	1.0000	0.3041 0.2522	0.4892 0.0003	0.2738 <.0001	-0.7316 0.0003
Body length	0.2820 <.0001	-0.2316 0.0016	0.4715 0.0004	0.3041 0.2522	1.0000	0.4318 0.0013	0.3748 <.0001	-0.3187 0.1515
Length of tegmina	0.2571 0.2901	0.4707 <.0001	0.0642 <.0001	0.4892 0.0003	0.4318 0.0013	1.0000	0.3172 <.0001	-0.3019 0.1727
Length of femur of hind leg	0.1640 <.0001	0.5183 0.0014	0.0353 0.0005	0.2738 <.0001	0.3748 <.0001	0.3172 0.0001	1.0000	-0.0596 0.8263
Development times	0.4546 <.0001	-0.0487 0.8263	-0.2748 <.0001	0.7316 0.0003	-0.3187 0.1515	-0.3019 0.1727	-0.0596 0.8263	1.0000

4.2.7 Regression Model

Employing a regression model where the dependent variables are development and survival and all the other factors are treated as independent, relationships between cricket development and survival and the other variables were further investigated. When using backward selection, non-significant variables were gradually eliminated. At a 0.05 percent significance level, any parameter still included in the model is significant. The model had a high level of significance and was able to explain 91.3 percent of the data's variability, according to the results shown in Table 4.16, which summarizes the model. According to the model's output, fecundity, adult lifespan, and body weight were the important factors that accounted for the variation in cricket development and survival. This suggests that there is a lot of promise for utilizing them to monitor the growth of crickets

Table 4.16: The regression model

Coefficients	Parameter Estimate	Standard Error	t Value	Pr (> |t|)
Intercept	6.003e-14	2.484e-13	2.420e-01	0.817
Fecundity	1.219e-17	7.290e-17	1.670e-01	<2e-16
Adult longevity	2.000e+00	7.821e-15	2.557e+14	<2e-16
Body weight	1.000e+00	3.931e-13	2.544e+12	<2e-16

All parameters left in the model are significant at 0.05 significance level.

4.3 Identification and Characterization of Cuticular Hydrocarbons from Two Species of Cricket: *Acheta domesticus* and *Gryllus bimaculatus.*

4.3.1 Identification of Cuticular Hydrocarbons

Extracts of adult crickets Acheta domesticus and Gryllus bimaculatus contained 44 cuticular hydrocarbons in detectable quantities (Table 4.17). Different amounts of straight-chain n-alkanes, branched alkanes with one or more methyl groups (methyl-branched alkanes), and unsaturated alkenes with chain lengths ranging from 25 to 31 carbons were all included in these cuticular hydrocarbons (CHCs). A homologous series of n- alkanes (pentacosane, hexacosane, heptacosane, octacosane, nonacosane and hentriacosane), alkenes (heptacosene, nonacosene, and hentriacosene), methyl alkanes (3-,5-,6-, 7-, 8-, 9-,11-, 12-, 13-, and 14-methyl), and dimethyl alkanes (3,7-, 3,9-, 5,9-

, 6,14-, 8,12-, 9,15- and 11,15-) with a carbon number range of C25 - C31) were identified. In the insects, hydrocarbons with odd numbered chain lengths were more than the even numbered chain lengths.

Table 4.17: Chemical compounds detected from the cuticular extracts of adult crickets of *Acheta domesticus* and *Gryllus bimaculatus*

peak	compound	Name	Molecular weight
1	C25:1	Pentacosane	350
2	n – C25	n-pentacosane	352
3	11-Me C25	11-methyl pentacosane	168,225
4	7-,5-Me C25	7-, 5- methyl pentacosane	112,280
5	11,15,-diMeC25	11,15-dimethyl pentacosane	168,239
6	3-MeC25	3 methyl pentacosane	308
7	5,9-diMeC25	5,9 –dimethyl pentacosane	155
8	n –C26	n- hexacosane	366
9	3,7-diMeC25	3,7-dimethyl pentacosane	127
10	13-, 12-, 11-MeC26	13-,12-,11- methyl hexacosane	197, 211, 182
11	C27:2	Heptacosene	376
12	C27:1	Heptacosane	378
13	C27:1	Heptacosane	378
14	n – C27	n- heptacosane	380
15	13-,11-, 9- Me C27	13-,11-,9- methyl heptacosane	196, 224, 169
16	7-Me C27	7-methyl heptacosane	309
17	5- Me C27	5- methyl heptacosane	336
18	11, 15-diMeC27	11,15 -dimethyl heptacosane	238
19	3-MeC27	3-methyl heptacosane	336
20	C28:1	octacosane	392
21	n – C28	n- octacosane	394
22	3,9-, 3,7- – diMeC27	3,-9, 3,7- dimethyl heptacosane	280, 309
23	14-,12-,10- MeC28	14-,12-,10- methyl octacosane	225, 183, 253
24	8- MeC28	8-methyl octacosane	308

25	6-MeC28	6- methyl octacosane	337
26	C29:2	Nonacosene	404
27	C29:2	Nonacosene	404
28	C29:1	Nonacosane	406
29	C29:1	nonacosane	406
30	n- C29	n-nonacosane	408
31	9-,7- Me C29	9-,7 – methyl nonacosane	309, 336
32	9,15-diMeC29	9,15- dimethyl nonacosane	224
33	Unknown	Unknown	393
34	3-MeC29	3-methyl nonacosane	364
35	5,9-diMeC29	5,9- dimethyl nonacosane	252
36	n-C30	n- triacontane	422
37	14-,12-,10-,8-MeC30	14-,12-,10-,18-methyl triacontane	211, 252, 308, 336
38	8,12-diMeC30	8,12- dimethyl triacontane	196
39	6,14,-diMeC30	6,14 –dimethyl triacontane	224
40	C31:2	Hentriacontene	432
41	C31:2	Hentriacontene	432
42	C31:1	Hentriacontane	434
43	n- C31	n- hentriacontane	434
44	9-MeC31	9- methyl hentriacontane	336

4.3.2 Cuticular Hydrocarbon Profiles of the Crickets

Similar to *Gryllus bimaculatus*, *Acheta domesticus* species showed same patterns of hydrocarbon diversity, but with a considerable reduction in hydrocarbon abundance. The two species varied in the relative abundance of the long chain hydrocarbons, each showing a drastic reduction in both the number of hydrocarbon-chain lengths and unsaturated alkenes. *Acheta domesticus* recorded higher quantities of unsaturated hydrocarbons (Figure 4.3) than *Gryllus bimaculatus* (Figure 4.4). Significant variability was recorded between the species with *Gryllus bimaculatus* recording greater quantities of long chain hydrocarbons, n –alkanes and decreased methyl branched alkanes. A significant difference was observed where the position of the first methyl branch was

shifted by two carbon units between the species, that is from positions 5 and 11 in *Gryllus bimaculatus* to positions 7 and 13 in *Acheta domesticus* respectively.

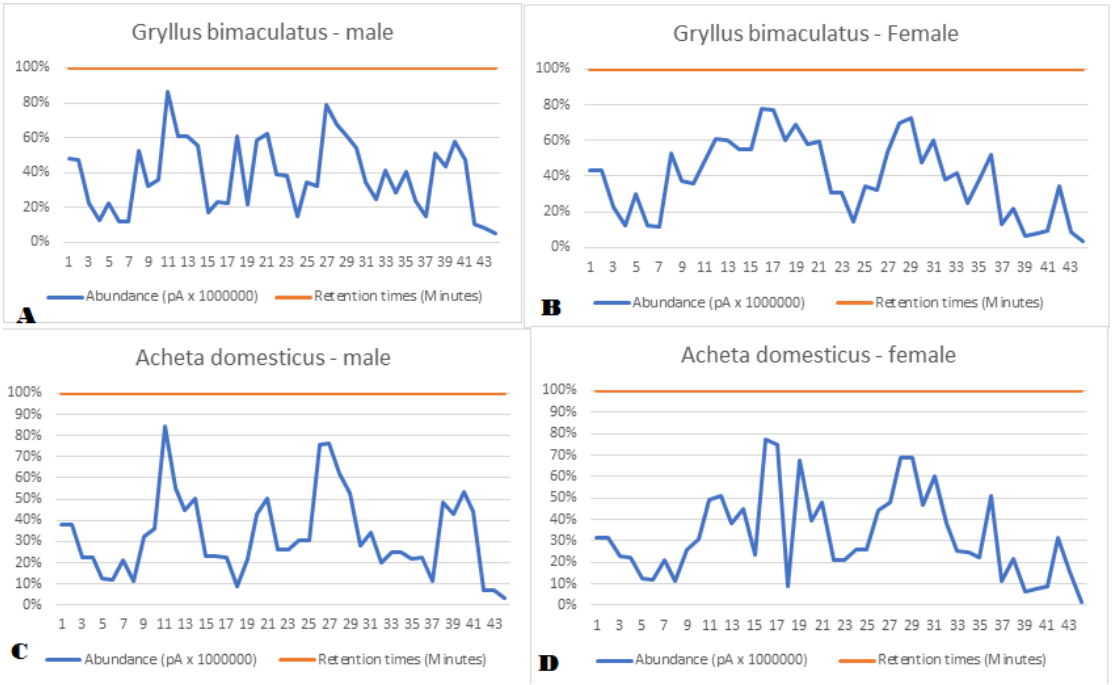

Figure 4.3: GC-MS spectrum showing the cuticular hydrocarbon (CHC) peak abundance of (A) Male, Gryllus *bimaculatus* (B) Female, *Gryllus bimaculatus* (C) Male, *Acheta domesticus* (D) Female, *Acheta domesticus*

Significant gender differences existed between the two species sampled. Males and females of the two species appeared to differ in the relative ratios of C27, C29, and C31 alkenes (Figure 4.4 and 4.5) albeit some females possessing male like profiles. Males possessed more heptacosene, nonacosene, hentriacosene and di methyl triacontanes eluting at 9.25, 13.16, 47.12 and 44.85 minutes. The remaining longer chain hydrocarbons that make up each cricket's species-specific profile were present in relatively low quantities in the male profiles. Females of both species were distinguished from those of males by increased relative abundance of 3-methyl heptacosane, 5- methyl heptacosane, 11,15- dimethyl heptacosane and 13-, 11-, 9-methyl heptacosane. This suggests that the patterns of relative compound abundances that distinguish male profiles from female profiles may be conserved across species. The hydrocarbons with increased concentration on females were among the compounds were more indicative of sex and less of species. While there were gender specific

differences in the cuticular hydrocarbons of the species, some results indicated no gender difference with females recording the same profile as the males.

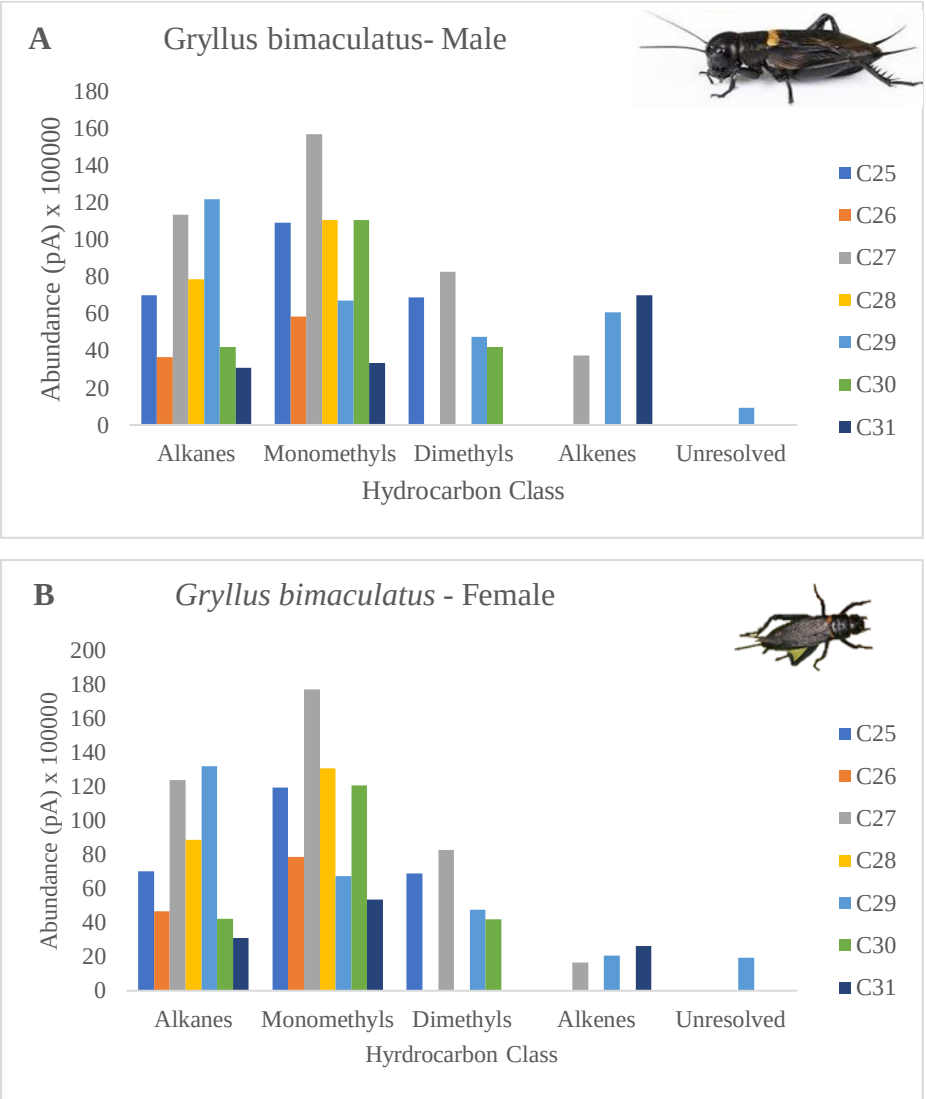

Figure 4.4: Cuticular hydrocarbon profile of *Gryllus bimaculatus*, pooled according to class and chain length: A (Males) and B (Females).

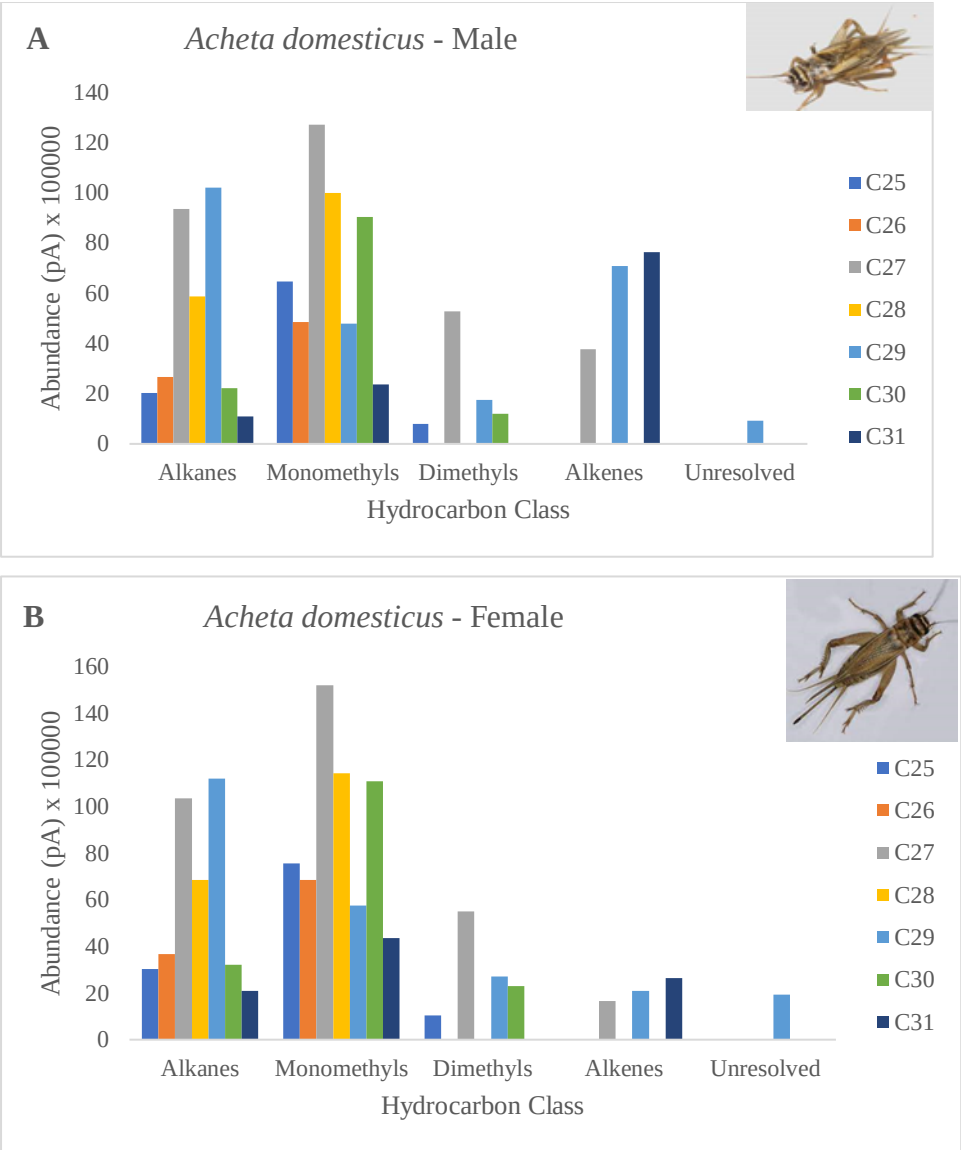

Figure 4.5: Cuticular hydrocarbon profile of *Acheta domesticus* pooled according to class and chain length: A (Males) and B (Females).

4.3.3 Principal Component Analysis

Principal component analysis returned 23 components. The study considered only those PCs where the eigen value was greater than 1 in the multivariate data set. Five components had an eigen value greater than 1 and collectively explained 77.4 % of the variance in cuticular hydrocarbon compounds (Table 4.18). The percentage of variance explained was 42.5 %, 15.5 %, 9.6%, 5.8 % and 3.8 % for PCs 1-5 respectively. PC1 separated the crickets based on species Gryllus bimaculatus and Acheta domesticus, while PC2 separated the two crickets based on sex. PC1 was positively loaded with 8

peaks (peaks 3, 4, 6, 7, 14, 21, 30, and 43). PC 2 was positively loaded to 5 peaks (peak 18, 19, 22, 26, and 41) while PC 3 was positively loaded to 3 peaks (peaks 26, 27, and 41). PC4 was positively loaded to peak 6 and negatively loaded to peak 39 while PC 5 was negatively loaded to peaks 2 and 32 and positively loaded to peak 44 (Table 4.18). The compounds that contributed most to PC1 were the long chain n-alkanes with positive factor loading indicative of Gryllus bimaculatus and dimethyl branched alkanes indicating the shift in position of first branching between the species. The cuticular hydrocarbon profiles between the sexes differed mainly in the relative amounts of unsaturated compounds (peaks 26, 27 and 41) for males, while females exhibited greater proportions of methyl branched alkanes (peaks 18, 19 and 22). PC 4 and 5 represent the relative abundances of a range of the hydrocarbons.

Table 4.18: Factor loadings of each cuticular hydrocarbon peaks on each of the five principal components (peaks with absolute values >0.2 are in bold)

Hydrocarbon	PC1	PC2	PC3	PC4	PC5
C25:1	-0.17	0.19	0.14	0.13	0.04
n – C25	0.12	-0.12	-0.12	-0.02	**-0.35**
11, 15-diMeC25	**0.24**	0.15	0.15	-0.07	-0.02
7, 9- diMeC25	**0.29**	-0.06	0.02	-0.12	-0.19
3, 7- diMeC25	**0.25**	-0.09	0.14	**0.24**	0.14
C27:1	0.19	-0.04	0.09	-0.01	0.15
n – C27	**0.32**	-0.09	-0.15	-0.12	-0.01
13-,11-, 9-MeC27	-0.12	-0.14	0.06	-0.01	-0.08
11-,15- diMeC27	0.17	**0.31**	0.18	-0.15	-0.04
3- MeC27	0.15	**0.23**	0.18	-0.11	-0.14
C28:1	0.18	0.11	0.16	-0.14	-0.17
n-C28	**0.44**	-0.15	-0.16	-0.19	-0.13
3, 9-, 3,7-diMeC27	**0.21**	**0.27**	-0.13	-0.09	-0.01
C29:2	0.12	**0.26**	**0.24**	-0.08	-0.03
C29:2	0.11	-0.16	**0.23**	-0.17	-0.01
C29:1	0.11	0.13	0.08	-0.16	-0.01

n-C29	**0.27**	0.17	0.13	-0.20	-0.12
9-,15- diMeC29	0.13	0.17	0.07	-0.11	**-0.23**
6-,14- diMeC30	0.15	0.05	-0.17	**-0.26**	-0.16
C31:2	0.08	**0.22**	**0.29**	-0.02	-0.05
C31:1	-0.03	-0.13	0.11	0.17	0.17
n-C31	**0.26**	0.19	0.17	-0.08	0.15
9- MeC25	0.18	-0.20	-0.07	-0.03	**0.25**

Table 4.19 shows the ANOVA table of the five components having eigen value greater than 1. The values were obtained from the principal scores

Table 4.19: ANOVAs of the five components with eigen values greater than 1 using the principal component scores.

		Model	**species**	**sex**	**Species x sex**
PC1	F	252.1	13.0	4.11	8.57
	P	<0.001	0.002	0.057	0.008
PC2	P	18.4	3.03	7.72	4.98
	F	<0.001	0.09	0.0123	0.038
PC3	P	8.7	1.21	0.16	4.84
	F	<0.001	0.28	0.70	0.041
PC4	P	0.003	0.06	0.19	0.038
	F	3.8	0.08	4.11	2.37
PC5	P	3.8	0.08	4.11	2.37
	F	0.005	0.77	0.057	0.14

4.4 Characterization of the Haemocytes Associated with Cellular Immunity in Two Cricket Species, *Acheta domesticus* and *Gryllus bimaculatus*

4.4.1 Identification of the Haemocyte Types in the Cricket Species

The two cricket species, *Acheta domesticus* and *Gryllus bimaculatus* shared similarity in their haemocyte types.

Figure 4.6 shows the Wright - Giemsa stained photomicrographs of the haemocytes of two cricket species *Acheta domesticus* and *Gryllus bimaculatus*. The haemocyte types were dentified in the haemolymph of both crickets include; Prohaemocytes, Plasmatocytes, Granulocytes, Adipohaemocytes, Oenocytoids and Spherulocytes.

Prohaemocytes

These polymorphic haemocytes occur in groups and are the smallest. They have a cell diameter ranging from 7.14 µm to 15.21 µm and a nucleus diameter ranging from 1.53 µm to 8.79 µm (Table 4.19). The cytoplasm covers a narrow area around the nucleus, which is located at the center, almost filling the cell (Figure 4.6).

Plasmatocytes

These are oval, highly polymorphic cells with a diameter ranging from a minimum of 11.91 µm to a maximum of 24.61 µm (Table 4.19). They have a large spindle shaped nucleus at the centre having a pointed end. The nucleus range in size from 3.45 µm to 15.82 µm. They are significantly larger than the prohaemocytes (Figure 4.6).

Granulocytes

These haemocytes have vacuoles in their cytoplasm, a small nucleus (6.07 µm to 10.98 µm) and several basophilic granules (Table 4.19). Their characteristics are intermediate between those of prohaemocytes and plasmatocytes. They can be categorized as large granulocytes having a diameter of 21.17 µm and small granulocytes with a diameter of 13.66 µm. They can be oval or spherical (Figure 4.6).

Oenocytoids

The nucleus is eccentrically located and the cell vary in shape from oval to spherical (Figure 4.6). They have no pseudopods but with a homogenous cytoplasm. The cell size range from 21.81 µm to 25.42 µm and a nucleus diameter ranging from 4.31 µm to 4.50 µm (Table 4.19)

Adipohaemocytes

These cells are relatively few compared to the rest having an average cell size from a minimum of 13.76 µm to 15.63 µm (Table 4.19). They occur in varied shapes from oval

to irregular shapes. The nucleus is small, concave or bi convex and centrally placed (Figure 4.6).

Spherulocytes

These are polymorphic cells that are characterized by highly basophilic spherules with small spherical vacuoles. The nucleus is eccentrically located, small and surrounded by a little cytoplasm. The average size of these cells range from 14.62 µm to 18.15 µm with a nucleus diameter ranging from 4.15 µm to 4.93 µm (Figure 4.6; Table 4.19).

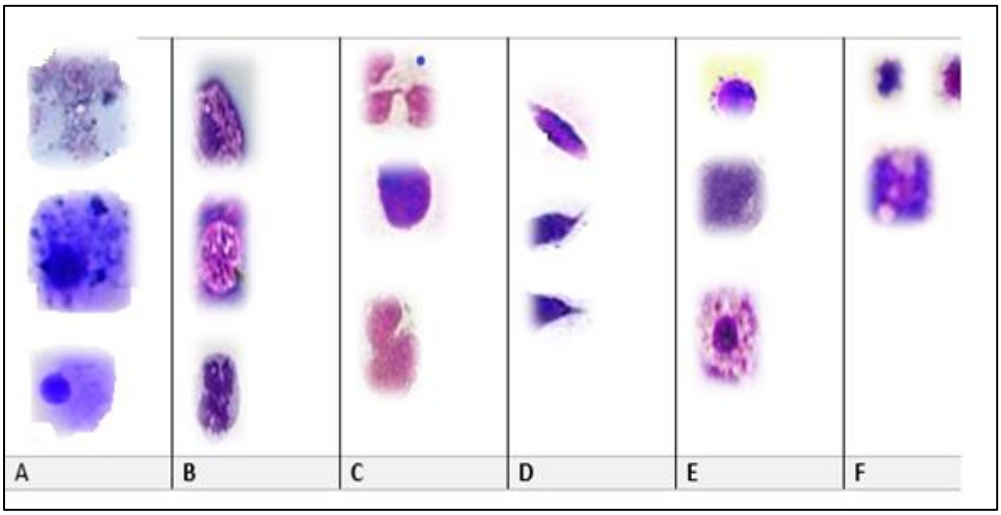

Figure 4.6: Wright - Giemsa stained photomicrographs of the haemocytes of two cricket species *Acheta domesticus* and *Gryllus bimaculatus*.

A= Pro haemocytes, B = Granulocytes, C = Oenocytoids, D = Plasmatocytes, E = Adipo haemocytes, F = Spherulocytes

Table 4.20 shows the morphology of the six different haemocytes in the haemolymph of crickets. Prohaemocytes had the largest maximum cell diameter (51,21 µm) while adipohaemocytes recorded the lowest maximum cell diameter (15.63 µm). Oenocytoids recorded the mean cell area of 1751.08 µm while the lowest mean cell area (98.03 µm) was recorded in prohaemocytes. Plasmatocytes had the largest (291.50 µm) mean area of nucleus while prohaemocytes recorded the lowest (20.90 µm) mean area of the nucleus.

Table 4.20: Morphology of the six different haemocytes found in the haemolymph of the crickets (Means +/- SE)

		Prohaemocytes	Plasmatocytes	Granulocytes	Spherulocytes	Adipohaemocytes	Oenocytoids
Cell diameter (µm)	Max.	51.21	24.61	21.17	18.15	15.63	25.42
	min	7.14	11.19	13.66	14.62	13.76	21.81
Nucleus diameter (µm)	Max.	8.79	15.82	10.98	4.93	5.19	4.50
	min	1.53	3.45	6.07	4.15	4.17	4.31
Mean Cell area(µm^2)		98.03	1046.96	952.31	842.99	678.06	1751.08
Mean Nucleus area (µm^2)		20.9	291.50	228.20	64.72	68.77	60.93

4.4.2 Quantification of the Haemocyte Counts in Two Cricket Species

4.4.2.1 Total and Differential Haemocyte Counts

Figure 4.4 shows the total count of the haemocytes of two cricket species *Acheta domesticus* and *Gryllus bimaculatus*. The average total haemocyte count for *Acheta domesticus* was 13228 ± 3.14 cells /mm^3. The haemacolor-stained cells were counted under light microscopy for differential haemocyte counts and were comprised of 40 % granulocytes, 24 % plasmatocytes, 10 % prohaemocytes, 14 % spherulocytes, 11 % adipocytes, 1 % oenocytoids. The mean total haemocyte count for *Gryllus bimaculatus* was 19801 ± 2.45 cells/mm^3. The haemacolor-stained cells were counted under light microscopy for differential haemocyte counts and were comprised of 30 % granulocytes, 26 % plasmatocytes, 15% prohaemocytes, 18 % spherulocytes, 9 % adipocytes, 2 % oenocytoids. The granulocytes are most frequent than other haemocyte types in the haemolymph. The mean number of total haemocyte counts of *Gryllus bimaculatus* was significantly different from *Acheta domesticus*. Higher numbers of prohaemocytes and plasmatocytes were recorded in *Gryllus bimaculatus* than in *Acheta domesticus*. *Acheta domesticus* recorded higher amounts of granulocytes and adipohaemocytes than *Gryllus bimaculatus* but no statistically significant difference was reported between these species in relation to the average number of plasmatocytes and granulocytes (Figure 4.7).

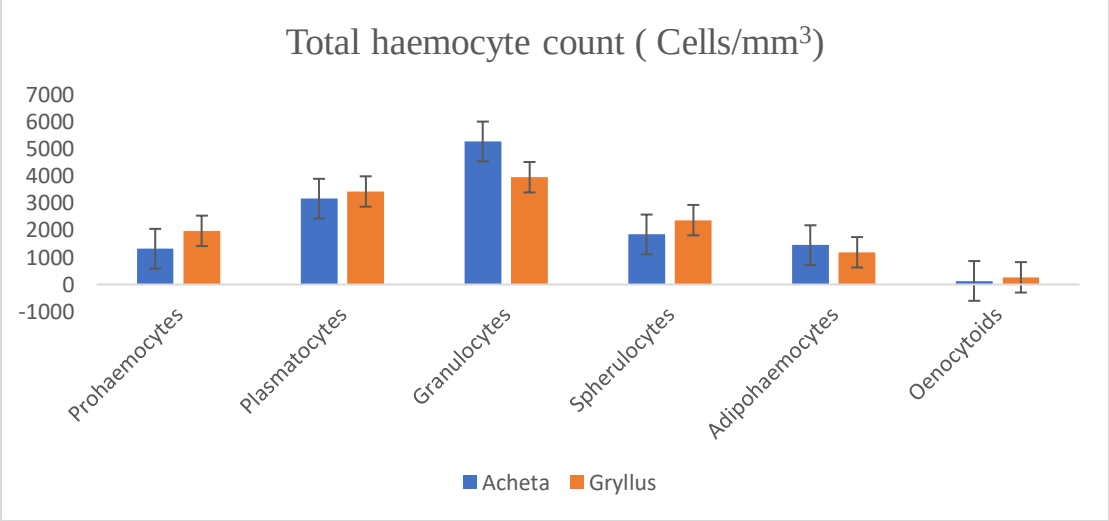

Figure 4.7: Total haemocyte count of two cricket species, *Acheta domesticus* and *Gryllus bimaculatus*

CHAPTER FIVE

DISCUSSION

5.1 Assessing Habitat Preference and Distribution of Crickets

The results showed that the cricket species in this study were primarily categorized into three groups. Group I (*Acheta domesticus and Diestrammena asynamora)* showed higher preference for all locations on settlements and had very different preferences for several environmental conditions. Jaganmohan *et al*., (2013) also indicated similar results in a previous research on urban domestic gardens in Bangalore. Specifically, these species did not appear to be sensitive to living close to buildings, and it showed a higher frequency in areas with higher building density (Bowling, 1955). Group II (*Scapsipedus icipe, Gryllus bimaculatus* and *Brachytrupes membranaceus*) preferred fields with tall grasses. *Gryllus bimaculatus and Scapsipedus icipe* simultaneously preferred lower elevation and mid elevation areas within the grasslands. The preference of *Gryllus bimaculatus and Scapsipedus icipe* for the grassland areas is due to cover resources for shelter. Chemura *et al*., (2018) reported similar results with *Henicus whellani chop* (Orthoptera: *Stenopelmatidae*) in South Eastern districts of Zimbabwe. G*ryllus bimaculatus* did not prefer to be close to buildings, although this species was not sensitive to higher densities of buildings (around 500 m^2/ha). Most species appeared to depend on farmland and bodies of water and were often found occurring at places close to farmlands (< 200) and bodies of water (< 150). Hermann *et al*., (2012), in a study on drivers of specialist diversity reported that multiple factors are responsible for shaping the diversity and abundance of species. Group III (Gryllotalpa africana) preferred locations within the wetlands. Presence of water and general wetness were important and critical factors describing the preference of *Gryllotalpa Africana* for its habitat (Hermann *et al.,* 2012). Specifically, this species preferred areas close to farmland (0- 150 m) and bodies of water (0 - 150). Moreover, the probability of its occurrence increased as the Normalized difference vegetation index (NDVI) value increased in wetlands. Bidau, (2014) and Sultana *et al*., (2013), reported similar results showing that orthoptera diversity followed a specific pattern determined by the presence of food and shelter. These findings indicate that regions with higher probability of occurrence for group I were distributed in almost all of the human settlement areas, while regions with higher probability of occurrence for Group II were sporadically distributed throughout the fields. Regions with higher probability for

Group III were distributed throughout the wetlands and areas near water bodies. Furthermore, regions with a higher occurrence probability for species included in Group I were likely to be coincident with settlement boundaries, whereas regions for Group II were likely to be distributed in the grasslands. Regions for Group III were likely to be distributed in the wetlands and near streams. The occurrence probability for Group II was relatively higher (> 0.6), while the probability for Group I did not exceed 0.3. Species in Group I tend to prefer to live anywhere in homes, regardless of environmental conditions. *Acheta* preferred fluctuant and concave topography for its living, and was often found at places not far from buildings and other artificial land uses. Normalized difference vegetation index (NDVI) also explained the preference of *Brachytrupes membranaceus* for its habitat, with a positive response to greenness. *Brachytrupes membranaceus* was also shown to stay within a distance less than 500 m of water, although it was found everywhere, indicating that it was not dependent on this aspect. Roads did not significantly influence the occurrence of most recovered cricket species, although most were not found close to roads and showed slightly higher preference for areas further away from the roads. *Brachytrupes membranaceus* appeared everywhere without any dependence on aspect, but it showed a slightly higher occurrence on the western side of the mountain.

Similar findings were recorded by Adetundan and Olusola, (2013) and Basset *et al*., (2012) which, showed that the diversity and abundance of arthropods were influenced by vegetation types. Although some cricket species were not sensitive to the disturbance, most tended to avoid such disturbances (Alignan *et al*., 2018; Chemura *et al*., (2018). *Acheta domesticus and Diestrammena asynamora* are general species that appeared not to be restricted by human disturbance (Bowling, 1955). Their requirements fluctuated but most preferred buildings as a cover resource to hide from predators. There were no clear data describing the preference of *Brachytrupes membranaceus* for its selected habitat. The results only indicated its preference for higher canopy cover and shelter. *Gryllotalpa africana* showed preference for wetness. Indeed, the results strongly indicated that *Gryllotalpa africana* preferred places near water and constantly moist and sufficient greenness. Such areas would be optimal for these organisms because they enable the species to protect itself from dessication while enabling easy access to food and oviposition sites (Belamkar and Jadesh, 2012; Allouche *et al*., 2006).

5.2. Effects of Temperature on the Development and Survival of Cricket Species, *Acheta domesticus* and *Gryllus bimaculatus*

5.2.1 Temperature and Fecundity

According to Colin and Spurgeon (2019), extreme heat could result in either temporary or permanent infertility or the inactivation of sperm stored in the spermatheca, reducing fertility. High temperatures are known to frequently hasten pre-imaginal development in insects that overwinter as adults, ensuring the timely development of the diapausing stage before the start of winter (Manrique *et al*., 2012). Nonetheless, the maturation of different species is restricted naturally and consequently, miniature and diaphanous adults are usually produced with accelerated development (Lamb *et al*., 2009). On the other hand, successful overwintering depends on sufficient fat and glycogen reserves, which are often positively correlated with body weight (Garcia-Barros, 2000). Several researchers have recognized the importance of larger weight in enhancing fat content in insects. High-temperature acceleration of pre-imaginal development combined with its inhibition of reproductive maturation is recorded for several species. Our investigation showed that cricket females stopped laying eggs at 18°C, indicating that low temperatures also caused sterility in these species (Calvo & Molina, 2005). Both extremes of temperatures resulted in moribund ovaries leading to very low or no egg production. In addition, large females have a greater potential for fecundity and some other selective advantages (Padmavathi *et al*., 2008; Aksit *et al*., 2007). Thus, an insect faces two seemingly opposite challenges: increase the adult weight or speed up pre-adult development. Fast development results in small adults, as in the cotton bollworm, *Helicoverpa armigera* (Hubner) and some other species of insects.

5.2.2 Temperature and Development

An essential aspect that has a significant impact on how insects develop is the temperature (Neven, 2000). Crickets are not an exception. The development of insects due to fluctuating temperatures differs among species (Padmavathi *et al*., 2008). A decrease in the speed of development with reduced temperatures is common, with a marked increase in the period taken in every stage (Ikemoto & Takai, 2000). The findings of this study show that when the temperature rose, the length of time that various stages of crickets took to develop decreased (Ahn *et al*., 2016). However, most eggs did not hatch when the temperature was 18^0C, and neither the eggs nor the nymphs

matured when the temperature was 38^0C. The outcomes from this study were consistent with Colin & Spurgeon's (2019) findings that insects could not finish their normal development at 18 or 38 degrees Celsius. Thus, both low and high temperatures were harmful to the growth of crickets. Under laboratory conditions, a temperature range of 26 to 34^0C proved acceptable for the development of crickets. The growth rate of an insect and temperature show a positive correlation when the temperature range is appropriate, becoming sigmoid over the complete temperature ranges through which insects are capable of developing.

5.2.3 Temperature and Survival

According to the study's findings, crickets are sensitive to the "severe temperatures" (18 °C and 38 °C) utilized here. As a result, eggs may not hatch or nymphs may not mature fully. As a result, both low and high temperatures hindered the survival and growth of crickets, and an ideal temperature range for the insect's development was between 26 and 30 °C. It is crucial to keep in mind that the high temperature varies during the day by roughly 10 C and is not always constant in nature. Further research is therefore necessary to determine whether the locations' high ambient temperatures affect the ability of crickets to establish. The results of our investigation showed that temperature had a significant impact on cricket development, influencing survival and reproduction. Similar results were reported by Fischer *et al*., 2003, in a study on the temperature requirements of butterflies. Laboratory studies provide information for modelling species performance under stressful environmental conditions and during optimization of rearing. These parameters estimated under laboratory conditions at constant temperature could pose a challenge when applied in the field since in nature; insects are exposed to fluctuating temperatures (Colin and Spurgeon, 2019; Infante,

5.3 Identification and Characterization of Cuticular Hydrocarbons that Generate Dessication Resistance in Two Cricket Species; *Acheta domesticus* and *Gryllus bimaculatus.*

The most prevalent hydrocarbons among those found were branched alkanes, linear alkanes, and a small number of alkenes. Hydrocarbons can occur in their saturated form (*n*-alkanes), in which all the carbon atoms are joined by single bonds (Bonavita *et al*., 1997). Hydrocarbons that are definite genetically, such as linear n- alkanes render the best resilience against desiccation since their cluttered molecules are closely knit (Gibbs and Rajpurohit, 2010). Methyl-branched hydrocarbons are also saturated compounds

but are bonded to one or more of the methyl groups (CH_3) in the chain, either near the end or in the middle of the chain (Martin and Drijfhout, 2009b). They participate in chemical communication, phenotypic signals for mate and kin recognition. The *n*-alkanes are usually mixed with alkenes and methyl-branched alkanes in order to keep the cuticle flexible, (Liang and Silverman, 2000; Gibbs, 1998). This mixture of hydrocarbons lowers their melting point on the cuticle allowing the waxy layer to remain pliable over broad thermal range necessary to adjust the cuticle's flexibility in a continuously changing environment (Kaal and Janssen 2008).

This study demonstrates that cuticular hydrocarbon expression differs significantly between species, and species identity may be established using the composistion of their cuticular hydrocarbons. The Cuticular hydrocarbons are plastic in respect to the insects environment. The increased temperature associated with the habitat of *Gryllus bimaculatus* increases the risk of dessication. When it comes to an insect's ability to withstand desiccation, there is a trade-off between short and long chained cuticular hydrocarbons, with the latter offering more desiccation resistance (Gibbs *et al*., 1997). Linear n-alkanes are more prevalent at higher temperatures; while methyl branched alkanes are less prevalent. (Sprenger *et al*., 2018; Woodrow *et al*., 2000). The closer packing of the molecules made possible by the n-alkanes' straight structure is perfect for regulating the flow of transcuticular water, with longer chain hydrocarbons acting as the best water barrier (Bazinet *et al*., 2010). Some Drosophila species, including *Drosophila pseudoobscura* and *Drosophila mojavensis,* are unable to synthesize adequate n- alkanes in sufficient amounts. Their high cuticular permeability makes them vulnerable to desiccation stress (Wagner *et al*., 2001; Blomquist *et al*., 1985). In warm conditions, insects create more n-alkanes, and in cold environments, they produce more unsaturated hydrocarbons (Martin and Drijfhout, 2009a). This study recorded an increased quantity of unsaturated hydrocarbons in *Acheta domesticus*. This may be due to its house habitat associated with room temperatures with lesser risks of dessication. Foraging insects, like the seed-eating desert harvester ant (*Pogonomyrmex barbatus*), have cuticles that are more exposed to sunlight and, as a result, contain more n-alkanes than non-foraging insects whose cuticles spend more time underground (Wagner *et al*., 2001). High temperatures and low relative humidity again caused Pogonomyrmex barbatus ant populations to produce more n-alkanes (Wagner *et al*., 2001). This suggests that environmental conditions have a significant impact on how n-alkanes are regulated in insects.

There was a shift in position of first methyl branching between the species. It's probable that the first divergence in cuticular hydrocarbon expression was brought on by environmental adaptation given that these species often inhabit different habitats. Many of the cuticular hydrocarbons produced by these crickets could be utilized to distinguish between species. This first divergence may have been triggered by non volatile and robust dimethyl and long chained alkanes that are crucial in lowering the risks of desiccation.

Female specific cuticular hydrocarbons stimulate male courtship behavior while male specific cuticular hydrocarbons influences a females propensity to mate (Clutton-Brock and Landley, 1997; Gennin *et al*., 1986). Males and females of both species had the same cuticular hydrocarbon profile but with differences in peak abundance. This sexual cuticular hydrocarbons dimorphism suggests that the cuticular hydrocarbons function as sex pheromones. The sex pheromones are a blend of cuticular hydrocarbons rather than a single compound and are important for courtship and mating (Tregenza and Wedell, 1997). The higher levels of monomethyl heptacosane and dimethylheptacosane in some females suggests that these compounds blends with other compounds to act as sex pheromones in females. Males display avoidance behavior to male cuticular hydrocarbons but courtship behavior to female hydrocarbons (Tregenza and Wedell, 1997).

Some results indicated no gender difference with females having cuticular hydrocarbon composition similar to that of males. This could be a male mimicry that has emerged as a sexual harassment avoidance tactic (Clutton - Brock and Langley, 1997). The disruption of female energy budgets and potential physical injury caused by male sexual harassment reduces fertility (Bots *et al*., 2009; Gay *et al*., 2009). Males will also try to seclude females inside burrows, mate guard (Bateman and Mc Fadyen, 1999), and may actively inhibit spermatophore detachment in crickets, according to Bateman *et al*., (2006), who also found that male harassment shortens female longevity. The excessive polymorphism seen in female cuticular hydrocarbon profiles has the potential to confuse males (Fincke, 2004) and reduce excessive male attention by interfering with the mechanisms used by males to identify potential mates, preventing them from effectively adapting to any particular female morph (Bussiere *et al*., 2006), and reducing harassment to the unusual female phenotypes. Additionally, the creation of cuticular hydrocarbons for communication depletes the body's supply of cuticular hydrocarbons, which serve as a desiccation defense (Chung and Carrol, 2015; Hardy *et*

al., 1983). There are tradeoffs between protective cuticular hydrocarbons and unsaturated cuticular hydrocarbons which leave crickets susceptible to desiccation while increasing their reproductive success.

5 4 Characterization of the Haemocytes Associated with Cellular Immunity in Two Cricket Species, *Acheta domesticus* and *Gryllus bimaculatus*.

Insects are physiologically affected by a variety of pressures, which manifest in their low vigor and survival rates (Alvarado *et al*., 2015; Johnson and Bennet, 1996). For insect growth and metamorphosis, haemocytes are crucial markers. It is discovered that they exhibit variations in their types, numbers, and configuration under various conditions, which ultimately have an impact on the health and loss of insects (Adamo, 2010; Inglis *et al*., 1996). Blood cells in circulation, or haemocytes, are essential to defense systems against pathogens, parasites in the hemocoel, and other abiotic stressors (Rantala and Roff, 2005; Rantala *et al*., 2002). After Giemsa staining, the haemolymph of the two species of cricket, Acheta domesticus and Gryllus bimaculatus, contained six distinct haemocyte types that were all easily distinguishable in smears, including prohaemocytes, plasmatocytes, granulocytes, adipohaemocytes, spherulocytes, oenocytoids, and coagulocytes.

The distinctive basophilic granules that fill the cytoplasm of granulocytes in Giemsa or methylene blue-stained smears make them easy to identify (Rantala and Roff, 2005). Granulocytes are also enormous in size. The majority of pathogen encapsulation, active nodulation, and phagocytosis are mediated by granulocytes (Lavine and Strand, 2002). Their plasma membrane is used to create a variety of sticky nets that they utilize to collect other hemoglobin-producing cells and carry out cellular immune response (Negri *et al.,* 2014).

As shown in the hemolymph smears of the crickets, plasmatocytes exhibit the greatest degree of cell shape variability. Due to cytoplasm expansions, they can have any shape, including spherical, fusiform, or entirely irregular. These cells release filamentous growths, which have been observed in numerous dipterans. Their intermediate forms, which exist between prohaemocytes and plasmatocytes, imply that the latter developed and differentiated from prohaemocytes. In a similar manner, the presence of multiple cells exhibiting intermediate forms and distinctive staining features in these species point to the development of granulocytes, adipohaemocytes, and spherulocytes from plasmatocytes. The tissues harmed by the stress the insects experienced may mend more

quickly if granulocytes and plasmatocytes predominate over other types of hemoglobin. The smallest, prohaemocytes, are distinguished by a uniform, dense, or thin basophilic coating of cytoplasm surrounding the nucleus. They appear to be juvenile, tiny plasmatocytes and are seen in groups. They have a focus on cell division (Mac Millan and Sinclair, 2011). In crickets, adipohaemocytes are haemocytes that occasionally have a big lipid-like vesicle that can distort the cell.. They share traits with other insect species that have been seen (Ribeiro and Brehelin, 2006).

According to certain research, adipohaemocytes resemble granulocyte subtypes. Oenocytoids are specialized for secretion and storage and can grow to be as big as granulocytes or even bigger (Castillo *et al*., 2006). In crickets, their fine granulation and the acidophilic cytoplasm seen following Giemsa staining can be used to identify them. Most insects do not have this sort of hemoglobinocyte. Small basophilic or acidophilic spherules scattered throughout the cytoplasm help differentiate spherulocytes from other hemoglobin-containing cells (Negri *et al*., 2014). Our research shows that the appearance of cells with intermediate morphological traits and staining following Giemsa treatment suggest that granulocytes have changed into oenocytoids. This is consistent with the notion put forth by Gupta (1979), which suggests that granulocytes have the capacity to differentiate into adipohaemocytes, spherulocytes, and oenocytoid cells. According to our findings, Gryllus bimaculatus had more hemoglobin-containing cells in its blood than Acheta domesticus did. The host defense system may be present in this (Pandey *et al*., 2010; Lazzaro and Little, 2009). Insects' usual response to stress is to produce more haemocytes in their hemolymph. The haemolymph is a useful buffer that offers tissues a stable environment in a constantly shifting and demanding external environment (Adamo *et al*., 2011; Elliot *et al*., 2002). When faced with challenging circumstances, many insect species concentrate their haemolymph by reducing the amount of water in their bodies (Freitak *et al*., 2003). This increases the osmolality of the insect's haemolymph and improves its capacity to endure a range of temperatures (Strand 2008; Lorenz, 2003). Given the above, a decrease in haemolymph volume is the expected response to high temperature stress in insects (Inglis *et al*., 1996).

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CHAPTER SIX
CONCLUSIONS AND RECCOMMENDATIONS

6.1 Conclusions

Acheta domesticus and *Diestrammena asynamora* were not sensitive to human disturbance; instead, they showed higher preference for the house habitat. Relatively large sized crickets such as *Brachytrupes membranaceus* had quite different strategies for their habitats. First, these large cricket species had the wider home range in forests and other tall trees so that they could get the flexibility to take their food within a safety according to the presence of their food and cover resources. Overall, these results indicate that *Gryllotalpa africana* was much more cautious and sensitive to anthropogenic land uses than other species.

The results provide important information regarding the thermal requirements of *Acheta domesticus* and *Gryllus bimaculatus*. The development of *Acheta domesticus* from egg to adult would occur between the thermal range 22ºC and 30ºC, while that of *Gryllus bimaculatus* would occur between the thermal range 26ºC, and 34ºC. The optimal growth rate was observed at 26ºC for *Acheta domesticus* and 30ºC for *Gryllus bimaculatus*. This study therefore shows that cricket development, survival, and distribution could be affected by future temperature increases.

This study concludes that cuticular hydrocarbons are plastic with respect to the insect's environment and hydrocarbon profiles are therefore shaped by natural selection, biotic and abiotic factors within the habitat of the insect. Long chained cuticular hydrocarbons increase with increase in temperature of the cricket's habitat and provide the greatest protection against desiccation. Understanding the fitness costs imposed by stress on tropical insects is important in predicting their adaptation and survival in the changing environment.

To survive at high temperatures and other stresses, different insect species may respond in different ways by changing their behavior or physiology to counteract the harmful effects of temperature stress. Since crickets are most abundant in the tropics, these species must develop various mechanisms to survive desiccation stress. The higher

haemocyte counts in *Gryllus bimaculatus* result in better survival strategy, a possible reason for the higher populations of this cricket species in most habitats.

6.2 Recommendations and Suggestions for Further Studies

6.2.1 Recommendations

The following are the recommendations:

- Since the crickets preferred high vegetation cover, cricket diversity in human occupied environment should be enhanced by continuous gardening and tree planting and wetlands conserved to preserve the endangered *Gryllotalpa africana.*
- Research institutions and extension officers should initiate a campaign drive to enlighten the public on the importance of biodiversity conservation to promote the sustainable use of insects for food and feed.
- Data on the effects of temperature be used to model development in the wild and estimate potential distribution limits. In addition, parameters such as adult weight, and fecundity, under different temperatures could be used to optimize production under mass rearing.
- Due to large fitness costs in terms of plasticity of the cuticular hydrocarbons and hemolymph volume, it would be necessary to protect biodiversity hotspots and reduce encroachment in to wetlands

6.2.2 Suggestions for Further Study

- There is need to develop biodiversity indicators that include both local and landscape requirements of different flora and fauna in Western Kenya as well as their interaction.
- Nature does not have a steady temperature; it can change by roughly 10^{o}C in a day. Therefore, further investigation is needed to determine whether the severe temperatures in this study location prevent some cricket species from spreading in the wild.

- Taxon specific combination of cuticular hudrocarbons quality and quantity may be required to allow better prediction of variation in water loss of crickets. Knowledge of the nature of this variation may have potential applications in enhancing existing conservation management strategies of this edible insect species.

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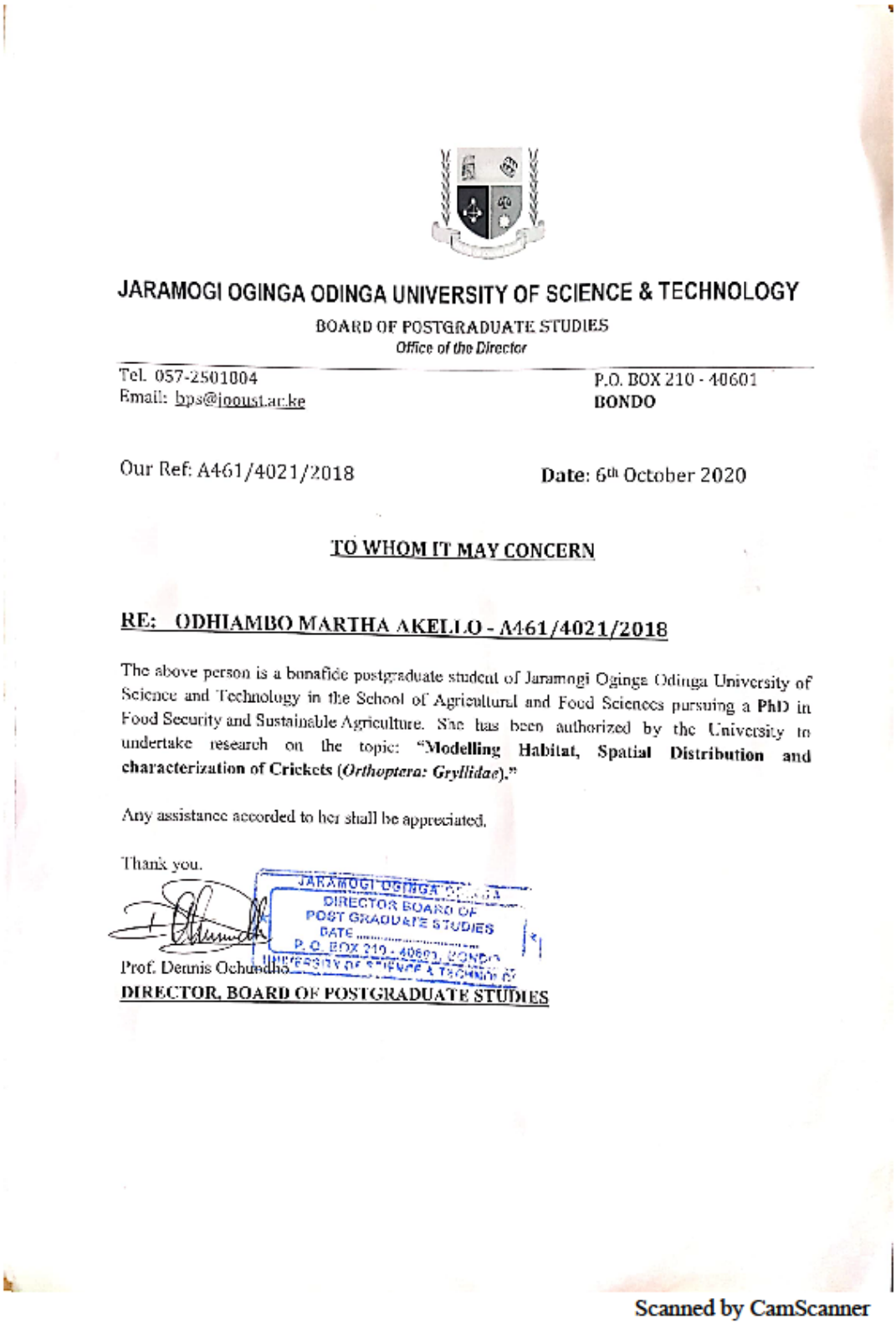
APPENDICES



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Our Ref: A461/4021/2018

Date: 6th October 2020

TO WHOM IT MAY CONCERN

RE: ODHIAMBO MARTHA AKELLO - A461/4021/2018

The above person is a bonafide postgraduate student of Jaramogi Oginga Odinga University of Science and Technology in the School of Agricultural and Food Sciences pursuing a **PhD** in Food Security and Sustainable Agriculture. She has been authorized by the University to undertake research on the topic: "**Modelling Habitat, Spatial Distribution and characterization of Crickets (*Orthoptera: Gryllidae*).**"

Any assistance accorded to her shall be appreciated.

Thank you.

JARAMOGI OGINGA ODINGA
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Prof. Dennis Ochuodho
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Appendix I: Authorization from the board of postgraduate studies to undertake research

Size of Population (N)	Sample Size (n) for Precision (E) of:			
	±3%	±5%	±7%	±10%
500	A	222	145	83
600	A	240	152	86
700	A	255	158	88
800	A	267	163	89
900	A	277	166	90
1,000	A	286	169	91
2,000	714	333	185	95
3,000	811	353	191	97
4,000	870	364	194	98
5,000	909	370	196	98
6,000	938	375	197	98
7,000	959	378	198	99
8,000	976	381	199	99
9,000	989	383	200	99
10,000	1,000	385	200	99
15,000	1,034	390	201	99
20,000	1,053	392	204	100
25,000	1,064	394	204	100
50,000	1,087	397	204	100
100,000	1,099	398	204	100
>100,000	1,111	400	204	100

A = Assumption of normal population is poor (Yamane, 1967). The er

Appendix II: Sample standardization table

```
martha_data3<-read.csv("martha_data3.csv")
> View(martha_data3)
> martha_data3
   temperature species fecundity Mlongevity sexratio MFatcontent Male
1            1  Acheta       121      51.35      1.28       0.0561    0
2            1 Gryllus       108      49.74      1.48       0.0585    0
3            2  Acheta       289      61.28      1.02       0.0553    0
4            2 Gryllus       277      50.28      1.13       0.0553    0
5            3  Acheta       378      58.23      1.04       0.0506    0
6            3 Gryllus       492      54.23      1.02       0.0553    0
7            4  Acheta      1313      49.09      1.39       0.0506    0
8            4 Gryllus      1722      41.09      1.00       0.0498    0
9            5  Acheta       268      40.19      0.74       0.0371    0
10           5 Gryllus       338      38.19      0.63       0.0435    0
11           6  Acheta        87      40.67      1.00       0.0300    0
12           6 Gryllus       128      36.67      0.98       0.0324    0
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> # question A
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> martha_data3$totalscore <-apply(martha_data3[,4:8],1,sum)
Error in `[.data.frame`(martha_data3, , 4:8) : undefined columns sele
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> # question A
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> martha_data3$totalscore <-apply(martha_data3[,4:5],1,sum)
> martha_data3$totalscore
 [1] 52.63 51.22 62.30 51.41 59.27 55.25 50.48 42.09 40.93 38.82 41.6
> boxplot(martha_data3$totalscore, col=c("magenta","green"),
+         main="Distribution of total score",
+         xlab="",
+         ylab="totalscore")
> mean<-tapply(martha_data2$totalscore,martha_data2$Treatment,"mean")
> summary(mean)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  75.59   81.50   95.10   92.10  102.07  105.47
> sd<-tapply(martha_data2$totalscore,martha_data2$Treatment,"sd")
> martha_data3$honors<-factor(ifelse(martha_data2$totalscore>=100,1,0
> martha_data3$honors
 [1] 1 1 1 1 0 0 0 0 0 0 0 0
Levels: 0 1
```

```
Call:
glm(formula = totalscore ~ fecundity + Flongevity + sexratio +
    FFatcontent + femalewgt, family = "gaussian", data = martha_data2)

Deviance Residuals:
       Min          1Q      Median          3Q         Max
-5.684e-14  -4.619e-14  -4.263e-14  -4.263e-14  -2.842e-14

Coefficients:
             Estimate Std. Error   t value Pr(>|t|)
(Intercept) 6.003e-14  2.484e-13 2.420e-01    0.817
fecundity   1.219e-17  7.290e-17 1.670e-01    0.873
Flongevity  2.000e+00  7.821e-15 2.557e+14   <2e-16 ***
```

Appendix III: Screen shot of part of the data used in the study

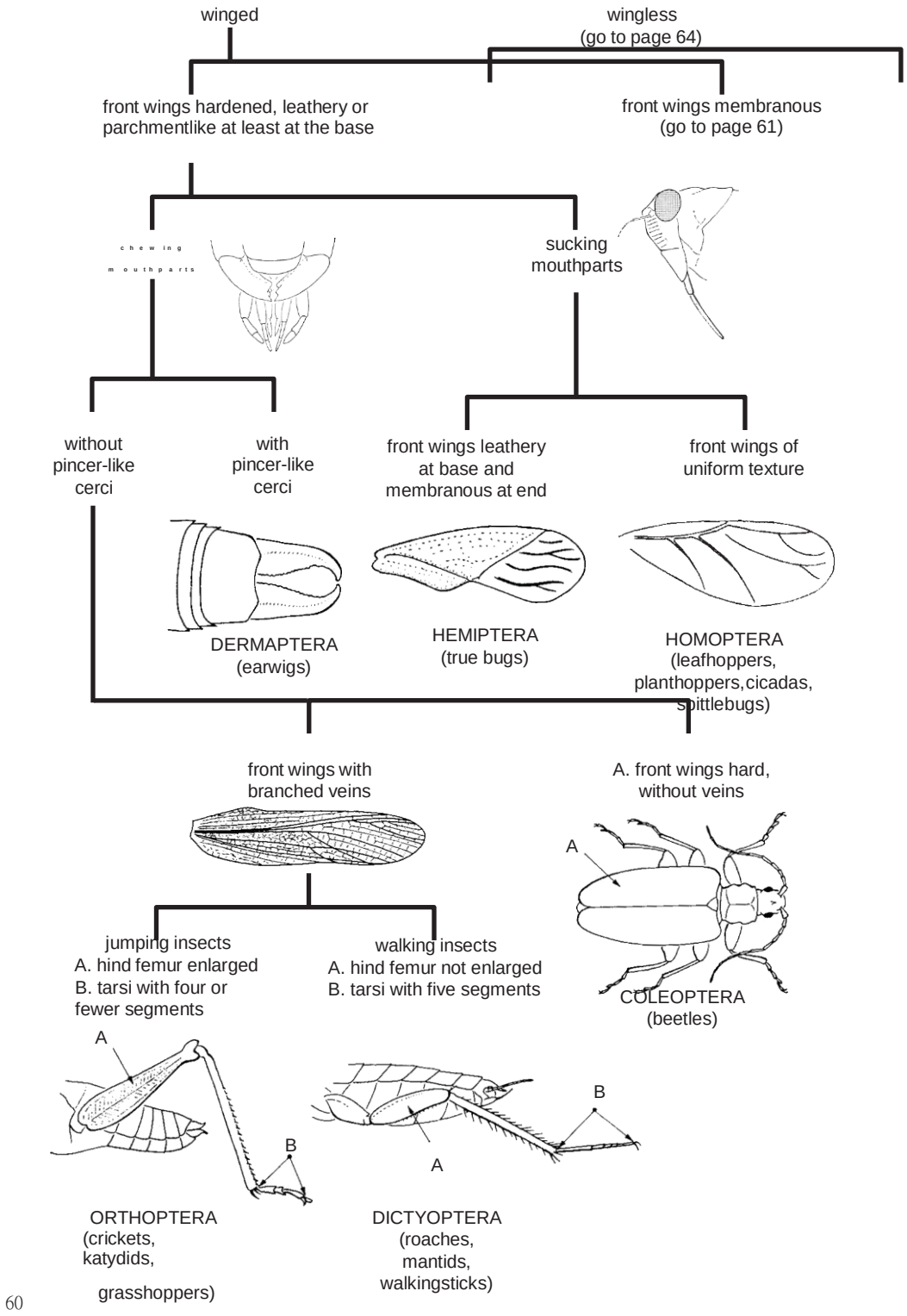
```
> martha_data1$totalscore
NULL
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> # question A
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> martha_data1$totalscore <-apply(martha_data1[,4:6],1,sum)
> boxplot(martha_data1$totalscore, col=c("magenta","green"),
+         main="Distribution of total score",
+         xlab="",
+         ylab="totalscore")
> mean<-tapply(martha_data1$totalscore,martha_data1$shelterdensity,"mean")
> summary(mean)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
   6.25    8.50   10.38   11.75   13.62   20.00
> sd<-tapply(martha_data1$totalscore,martha_data1$shelterdensity,"sd")
> martha_data1$honors<-factor(ifelse(martha_data1$totalscore>=16,1,0))
> martha_data1$honors
 [1] 0 0 1 0 0 0 1 0 0 0 1 0 0 0 0 0
Levels: 0 1
> martha_data1
   Treatment shelterdensity GB SI HR AD GR BM shelter.density totalscore honors
1          1         level1  1  1  1  2  1  2         level1          4      0
2          1         level2  7  7  3  2  3  3         level2         12      0
3          1         level3 16 15  4  5  9  5         level3         24      1
4          1         level4 11  6  4  3  6  5         level4         13      0
5          2         level1  3  2 10  1  2  1         level1         13      0
6          2         level2  7  6  2  2  4  1         level2         10      0
7          2         level3 14 13  4  5  5  5         level3         22      1
8          2         level4  9  8  3  3  5  2         level4         14      0
9          3         level1  2  2  1  1  1  1         level1          4      0
10         3         level2  6  5  2  2  3  1         level2          9      0
11         3         level3 12 11  4  5  4  5         level3         20      1
12         3         level4  8  7  2  3  4  3         level4         12      0
13         4         level1  2  2  1  1  1  1         level1          4      0
14         4         level2  4  4  1  1  2  1         level2          6      0
15         4         level3  9  8  3  3  5  2         level3         14      0
16         4         level4  5  4  1  2  3  3         level4          7      0
> mean<-tapply(martha_data1$totalscore,martha_data1$honors,"mean")
> sd<-tapply(martha_data1$totalscore,martha_data1$honors,"sd")
> xtabs(~martha_data1$shelterdensity+martha_data1$honors)
                          martha_data1$honors
martha_data1$shelterdensity 0 1
                    level1 4 0
                    level2 4 0
                    level3 1 3
                    level4 4 0
> crosstabs<-xtabs(~honors+shelterdensity,data=martha_data1)
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #question b
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #two-way ANOVA
> martha_data1$shelter.density=as.factor(martha_data1$shelterdensity)
> martha_data1$Treatment=as.factor(martha_data1$Treatment)
> model1<-aov(totalscore~Treatment+shelterdensity,martha_data1)
> summary(model1)
              Df Sum Sq Mean Sq F value   Pr(>F)
Treatment      3  110.0   36.67   6.055 0.015308 *
shelterdensity  3  418.5  139.50  23.037 0.000147 ***
Residuals      9   54.5    6.06
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> model1.tables(model1,type="mean")
> summary(model1)
              Df Sum Sq Mean Sq F value   Pr(>F)
Treatment      3  110.0   36.67   6.055 0.015308 *
shelterdensity  3  418.5  139.50  23.037 0.000147 ***
Residuals      9   54.5    6.06
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> model2<-aov(totalscore~Treatment+shelterdensity+Treatment:shelterdensity,data=m
_data1)
> summary(model2)
                       Df Sum Sq Mean Sq
Treatment               3  110.0   36.67
shelterdensity          3  418.5  139.50
Treatment:shelterdensity  9   54.5    6.06
> plot(model2)
Hit <Return> to see next plot: TukeyHSD(model1)
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #linear model
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> model3<-lm(totalscore~Treatment+shelterdensity+shelterdensity:Treatment,martha_
)
> anova(model3)
Analysis of Variance Table

Response: totalscore
                       Df Sum Sq Mean Sq F value Pr(>F)
Treatment               3  110.0  36.667
shelterdensity          3  418.5 139.500
Treatment:shelterdensity  9   54.5   6.056
Residuals               0    0.0
> summary(model3)

Call:
lm(formula = totalscore ~ Treatment + shelterdensity + shelterdensity:Treatment,
    data = martha_data1)

Residuals:
ALL 16 residuals are 0: no residual degrees of freedom!

Coefficients:
                                  Estimate Std. Error t value Pr(>|t|)
(Intercept)                      4.000e+00         NA      NA       NA
Treatment2                       9.000e+00         NA      NA       NA
Treatment3                       8.285e-15         NA      NA       NA
Treatment4                       9.543e-15         NA      NA       NA
shelterdensitylevel2             8.000e+00         NA      NA       NA
shelterdensitylevel3             2.000e+01         NA      NA       NA
shelterdensitylevel4             9.000e+00         NA      NA       NA
Treatment2:shelterdensitylevel2 -1.100e+01         NA      NA       NA
Treatment3:shelterdensitylevel2 -3.000e+00         NA      NA       NA
Treatment4:shelterdensitylevel2 -6.000e+00         NA      NA       NA
Treatment2:shelterdensitylevel3 -1.100e+01         NA      NA       NA
Treatment3:shelterdensitylevel3 -4.000e+00         NA      NA       NA
Treatment4:shelterdensitylevel3 -1.000e+01         NA      NA       NA
Treatment2:shelterdensitylevel4 -8.000e+00         NA      NA       NA
Treatment3:shelterdensitylevel4 -1.000e+00         NA      NA       NA
Treatment4:shelterdensitylevel4 -6.000e+00         NA      NA       NA

Residual standard error: NaN on 0 degrees of freedom
Multiple R-squared:      1,    Adjusted R-squared:    NaN
F-statistic:   NaN on 15 and 0 DF,  p-value: NA

> model.full<-glm(totalscore~GB+SI+HR+AD+GR+BM,family="gaussian",martha_data1)
> summary(model.full)

Call:
glm(formula = totalscore ~ GB + SI + HR + AD + GR + BM, family = "gaussian",
    data = martha_data1)

Deviance Residuals:
       Min          1Q      Median          3Q         Max
-7.105e-15  -3.553e-15   0.000e+00   1.110e-16   3.553e-15

Coefficients:
             Estimate Std. Error    t value Pr(>|t|)
(Intercept) -4.151e-16  2.434e-15 -1.710e-01    0.868
```

```
GB          -3.103e-16  1.583e-15 -1.960e-01    0.849
SI           1.000e+00  1.198e-15  8.345e+14   <2e-16 ***
HR           1.000e+00  4.623e-16  2.163e+15   <2e-16 ***
AD           1.000e+00  2.443e-15  4.094e+14   <2e-16 ***
GR           4.800e-16  1.404e-15  3.420e-01    0.740
BM           6.488e-16  1.436e-15  4.520e-01    0.662
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 1.415567e-29)

    Null deviance: 5.830e+02  on 15  degrees of freedom
Residual deviance: 1.274e-28  on  9  degrees of freedom
AIC: -1010.6

Number of Fisher Scoring iterations: 1

> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #forward variable selection
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> model.one<-lm(totalscore~1,martha_data1)
> step(model.one,scope=formula(model.full),criterion="AIC",direction="forward")
Start:  AIC=59.53
totalscore ~ 1

       Df Sum of Sq    RSS    AIC
+ SI    1    505.55  77.45 29.233
+ GB    1    505.07  77.93 29.331
+ AD    1    467.08 115.92 35.686
+ GR    1    399.41 183.59 43.042
+ BM    1    338.38 244.62 47.634
+ HR    1    167.08 415.92 56.126
<none>              583.00 59.530

Step:  AIC=29.23
totalscore ~ SI

       Df Sum of Sq    RSS     AIC
+ HR    1    73.066  4.388 -14.700
<none>              77.454  29.233
+ GB    1     8.679 68.775  29.332
+ AD    1     4.823 72.630  30.205
+ BM    1     4.116 73.337  30.360
+ GR    1     1.634 75.819  30.892

Step:  AIC=-14.7
totalscore ~ SI + HR

       Df Sum of Sq    RSS      AIC
+ AD    1    4.3880 0.0000 -1096.25
+ BM    1    1.9138 2.4742   -21.87
<none>              4.3880   -14.70
+ GB    1    0.4555 3.9325   -14.45
+ GR    1    0.0100 4.3780   -12.74

Step:  AIC=-1096.25
totalscore ~ SI + HR + AD

       Df  Sum of Sq        RSS     AIC
+ GR    1 2.0479e-30 1.4976e-29 -1096.3
<none>               1.7024e-29 -1096.2
+ GB    1 4.6508e-31 1.6559e-29 -1094.7
+ BM    1 2.3419e-31 1.6790e-29 -1094.5

Step:  AIC=-1096.3
totalscore ~ SI + HR + AD + GR

       Df  Sum of Sq        RSS     AIC
+ GB    1 6.8662e-30 8.1098e-30 -1104.1
```

```
<none>                1.4976e-29 -1096.3
+ BM    1 3.2900e-32 1.4943e-29 -1094.3

Step:  AIC=-1104.11
totalscore ~ SI + HR + AD + GR + GB

       Df  Sum of Sq        RSS     AIC
+ BM    1 2.8365e-30 5.2732e-30 -1109.0
<none>               8.1098e-30 -1104.1

Step:  AIC=-1109
totalscore ~ SI + HR + AD + GR + GB + BM


Call:
lm(formula = totalscore ~ SI + HR + AD + GR + GB + BM, data = martha_data1)

Coefficients:
(Intercept)           SI           HR           AD           GR           GB
  4.441e-15    1.000e+00    1.000e+00    1.000e+00   -1.205e-15    1.309e-15
        BM
 -6.429e-16

> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #question c
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #c)i.
> Model.full<-glm(totalscore~GB+SI+HR+AD+GR+BM,family="Gaussian",martha_data1)
> Summary(model.full)
> Model.full<-
+ #c)ii
+ Fit<-lm(totalscore~ GB,family ="Gaussian",martha_data1)
> #c) iv.
> cor(martha_data1[,5:8])
          HR        AD        GR        BM
HR 1.0000000 0.1916169 0.2547516 0.2022638
AD 0.1916169 1.0000000 0.7854780 0.8657995
GR 0.2547516 0.7854780 1.0000000 0.6992910
BM 0.2022638 0.8657995 0.6992910 1.0000000
> #c)v.
> Model3<-glm(totalscore~GB,family="gaussian",martha_data1)
> Summary(model3)
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #question d
> #>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
> #d) I,ii,iii
> Model_full
> glm(honors~Treatment+shelterdensity,family=binomial(link="log"),martha_data1)

Call:  glm(formula = honors ~ Treatment + shelterdensity, family = binomial(link
g"),
    data = martha_data1)

Coefficients:
         (Intercept)            Treatment2            Treatment3
          -1.400e+01             8.786e-25             7.033e-25
          Treatment4  shelterdensitylevel2  shelterdensitylevel3
          -2.243e+01            -9.996e+00             1.400e+01
shelterdensitylevel4
          -9.996e+00

Degrees of Freedom: 15 Total (i.e. Null);  9 Residual
Null Deviance:      15.44
Residual Deviance: 5.004e-06   AIC: 14
```

Appendix IV: Part of the data used in the study



Appendix V: A Pictorial Key to the Order of Adult Insects

PUBLICATIONS AND CONFERENCES

Publications

This thesis is based on the following published and draft research articles

1. Odhiambo M.A., Olweny C and Okuto E (2022): Habitat preference and distribution of crickets (Orthoptera; Gryllidae) in Western Kenya. Journal of Biology, Agriculture and Healthcare. ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) www.iiste.org. Vol.12, No.6, 2022. DOI: 10.7176/JBAH/12-6-04. Page 22-34.
2. Odhiambo, Martha Akello, Calleb Olweny Ochia & Erick Otieno Okuto. 2022. "Effects of Temperature on the Development and Survival of Cricket Species; Acheta domesticus and Gryllus bimaculatus (Orthoptera: Gryllidae)". *East African Journal of Agriculture and Biotechnology* 5 (1), 176-189. https://doi.org/10.37284/eajab.5.1.829
3. Odhiambo M.A., Olweny C and Okuto E (2022). Characterization of cuticular hydrocarbons that generate desiccation resistance in two cricket species Acheta domesticus and Gryllus bimaculatus. *Submitted to International Journal of Agricultural and biological sciences*
4. Odhiambo M.A., Olweny C and Okuto E (2022): Morphological diversity of haemocytes associated with cellular immunity in two cricket species, *Acheta domesticus* and *Gryllus bimaculatus* (Orthoptera; Gryllidae). Draft article

Conferences

1. The third international phytosanitary conference (virtual edition) held from 13th to 16th September 2020