DIVERSITY OF GRASSHOPPERS AND LOCUSTS, LIFE PARAMETERS, FAT AND PROTEIN CONTENT OF *Acanthacris ruficornis* IN NAKURU COUNTY, KENYA

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree. Where other peoples' work has been used, this has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

This thesis is dedicated to my mum, Irene Wanjiru and my brothers; Daniel Ndirangu and Martin Ngari.

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ABSTRACT

Locusts and grasshoppers in order Orthoptera and suborder Caelifera are indicators of ecosystem quality and an important source of nutrients in the food chain. However, there is limited information on the diversity and distribution of various species in the country. This study evaluated the diversity and distribution of grasshoppers and locust species in the ecological zones of Nakuru County, Kenya. Further, the study assessed life history parameters, fat and protein content of *Acanthacris ruficornis*, the dominant locust species. Specimens collected were identified to species level. Life history parameters study was done on colony of *Acanthacris ruficornis* reared in aluminium cages at 30ºC temperature and 30% ±2 relative humidity in the insects' room, Egerton University. Crude fat was estimated using Soxhlet extraction method while crude proteins were estimated using Kjeldahl's digestion method.

A total of 456 individuals were collected and were found to belong to Acrididae family (93.4%) and Pyrgomorphidae family (6.6%). *Aiolopus thalassinus* was the most abundant (27.4%) and distributed species. Abundance was highest in zone II (47%) and lowest in zone IV (24.3%). Overall Shannon-Wiener diversity index (H′) was 2.38 while zone II, III and IV had H′=2.44, H′=1.37 and H′=1.3 respectively. Overall Simpson's dominance index (D^{-1}) was 0.125 while zone II, III and IV had D^{-1}= 0.095, D^{-1}=0.313 and D^{-1}=0.254 respectively. Poisson regression showed that diversity and abundance was not significantly different ($P > 0.05$) among the ecological zones. Percentage similarity index was highest between zone III and IV (3.30) and lowest between zone II and zone IV (1.23).

Acanthacris ruficornis laid an average of 158 ± 2.65 eggs per pod which took an average of 35 ± 1.33 days to hatch with a hatching percentage of 91.78 ± 1.00%. 73.97 ± 1.88% of the total nymphs survived to adulthood taking an average of 36.88 ± 2.48 days to reach pharate stage, 54

± 3.61 days to mate and 62.67 ±1.45 days to lay eggs. In terms of crude protein, second nymphs, third nymphs, fourth nymphs and fifth nymphs had 55.2%, 58.14%, 64.09% and 60.38% respectively with 14.53%, 15.07%, 14.84% and 7.71% of crude fat in the same order. Poisson's regression showed both crude fat and crude protein were significantly different ($P< 0.05$) between the different nymphal stages. Some of the grasshoppers and locusts in Nakuru County were shown while the amount of crude fat and protein recorded in the instars shows that *Acanthacris ruficornis* has potential as a source of protein and fat for use as food and animal feed when reared in large numbers.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Locusts and grasshoppers are insects found in grassland ecosystems. They recycle plant nutrients by passing plant tissues through their digestive system where enzymes and microorganisms break them down into smaller pieces which hasten the degradation of cellulose and other materials. These are then released into the soil as fecal matter which decomposes releasing nutrients which favours new plant growth (Coleman and Hendrix, 2000). They are herbivorous in nature where they convert plant tissues into animal tissues. As a result, they provide food for organisms of higher food webs where they contribute to those organisms' biomass and therefore influence their survival, reproductive efficiency and abundance diversity (Lewinsohn and Price, 1996).

Grasshoppers and locusts are one of the insects involved in human entomophagy. They are preferred because of their big size which provides a lot of biomass. They also have high protein, crude fat, dry matter, vitamins and minerals content which makes them a complete meal. Their consumers have therefore derived various ways of capturing and cooking them. *Oxya yezoensis*, a grasshopper species consumed in Japan is collected during the rice harvesting period. Collection is done early in the morning when it's easy to capture them as they are wet from the morning dew (Nonaka, 2009). In Oaxaca south Mexico harvesting of edible grasshoppers, *Sphenarium purpurascens*, begins with the arrival of the rainy season in late spring and continues through early winter. Their collection takes place early in the morning between 4 – 5am when they are cool and dormant because they are too active and difficult to catch during the hotter part of the day. They are scooped using conical nets of 80cm diameter and 90cm depth

without a handle or handpicked and then transferred into a cardboard box for transport (Cohen *et al*, 2009). In Thailand, grasshoppers are collected and consumed after the rainy season where the adult females with eggs are preferred as they are tastier and richer in proteins and fats than the male grasshoppers and nymphs (Paul *et al*, 2016).

Proteins and fats are among the major nutrients needed in human bodies. These nutrients are provided by both plants, with the conventional source being grains, and animals especially livestock. However, livestock proteins are preferred to plants' as it contains all essential amino acids (Hoffman and Falvo, 2004). For livestock, supplements especially fish meal, are provided in order to generate enough proteins and fats to sustain mans' demands which raises the cost of production especially feed costs. With the ever increasing human population, the demand for production of beef, pork and chicken meat increases. This necessitates research efforts towards evaluating alternative, good quality, reliable and cheap to produce sources which can substitute those currently in use (Van Huis *et. al*., 2013).

As documented above, grasshoppers and locusts have a lot of potential in terms of fat and protein content. Their numbers in grassland ecosystems influence survival of other organisms and therefore indicate ecosystem quality. However, more information about their diversity and distribution is needed. In addition, most of the literature available on life history parameters document grasshopper and locust species of major economic importance like *Schistocerca gregaria* and therefore more literature is needed on the little known species. The information provided by this study on the diversity, distribution, life history parameters, fat and protein content of the nutritively important species of grasshoppers and locusts in Nakuru County can be used as a basis to multiply this species for use as alternative sources of aforementioned nutrients for humans and animals.

1.2 Problem statement and justification

Locusts and grasshoppers are very useful in ecosystems and to humans. Little information exists about their diversity, relative abundance and distribution in Nakuru County. Studying their diversity will provide information about the species that exist in the ecological zones of this study area, their relative abundance as well as the distribution of individuals. This knowledge would serve as an indicator of ecosystem quality as the number of Caeliferans relate positively to other organisms present especially those preying on them.

In addition, humans are facing challenges in terms of attaining enough fat and protein sources for food and feed for domestic animals. Grasshoppers and locusts' high reproductive potential and a wide range of feeding habits makes them suitable sources of animals' proteins and fats because their diet can easily be manipulated to obtain the desired levels of the aforementioned nutrients. This potential won't be exploited unless they are reared in mass numbers and their nutrient composition determined which will make their source reliable.

This study aimed to gather data on the diversity, the distribution and relative abundance of locusts and grasshoppers in the three ecological zones of Nakuru County. In addition, it will provide information on rearing which will serve as the basis of production, consumption and their commercialization. The knowledge about the fat and protein contained in the different growth stages will help to determine the best harvesting stage.

1.3 Objectives of the study

The broad objective was to determine diversity and distribution of grasshoppers and locusts species in Nakuru County, study the life parameters, fat and protein content of the instars of the dominant locust species, *Acanthacris ruficornis*.

1.4 Specific Objectives

i). To determine diversity and distribution of grasshopper and locust species in Nakuru County.

ii). To determine the life history parameters of *Acanthacris ruficornis* from Nakuru County.

iii). To determine fat and protein content of *Acanthacris ruficornis* from Nakuru county.

1.5: Research hypothesis

There is a variation in diversity and distribution of grasshoppers and locusts species across different zones in Nakuru County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Locusts and grasshoppers (Acrididae family)

Short horned locusts and grasshoppers belong to order Orthoptera, suborder Caelifera and superfamily Acridoidea which includes family Pamphagidae, Chrotogonidae, Pyrgomorphidae, Catantopidae, Oedipodidae, Arcypteridae, Gomphoceridae and Acrididae (Liu *et al*, 2008). However, Acrididae family has the most common grasshoppers and locusts' species worldwide. For instance, in Pampas, Argentina, Acrididae family had five subfamilies including Melanoplinae, Gomphocerinae, Acridinae, Copiocerinae, and Leptysminae which had fourteen, nine, three, two and one species respectively. Romaleidae family had one subfamily with five species (Cigliano *et al*, 2002). In the Uttar Pradesh state of India, Acrididae family had five subfamilies including Acridinae, Oedipodinae, Spathosterminae, Hemiacridinae, Eyprepocnemidinae, Oxyinae and Catantopinae which had two, seven, one, three, one, six and two species respectively. Pyrgomorphidae family had one subfamily, Pyrgomorphinae which had four species (Akhtar *et al*, 2012) while in Rajshahi district, eight species were recorded belonging to Acrididae and Tetrigidae families (Mahdi *et al*, 2018). In the Fraser's hill, Peninsular Malaysia, Acrididae family had five subfamilies including Acridinae, Catantopinae, Cyrtacanthacridinae, Oedipodinae and Oxyinae which had two, nine, one, three and two species respectively. Chorotypidae family had Chorotypinae and Eruciinae subfamilies with one and four species respectively; Pyrgomorphidae had Pyrgomorphinae family with one species; Trigonopterygidae family with Trigonopteryginae subfamily and one species while Tetrigidae family had subfamilies Metrordorinae, Scelimeninae and Tetriginae which had three, one and four species respectively (Tan and Kamaruddin, 2014). In Eurasian Steppe, three species

belonging to Acrididae family were recorded (Sergeev, 2021). In Palestinian territories, Acrididae family had six sub families which included Acridinae, Calliptaminae, Cyrtacanthacridinae, Eyprepocnemidinae, Gomphocerinae and Oedipodinae (Abusarhan *et. al.*, 2017). A study in the upper reaches of Heihe River in China recorded 3194 individuals which belonged to three families, ten genera and thirteen species (Li *et. al.*, 2021). In Africa, in a study in the higher mountains in west Cameroon, Acrididae, Pyrgomorphidae, Tetrigidae and Thericleidae family had eighteen, four, three and one species respectively (Kekeunou *et. al.*, 2017). In Brazil, Acridoidea superfamily had four families, five subfamilies and twenty five species (Almeida and Camara, 2008). In South Africa, five species belonging to Acrididae family were recorded in Kwazulu Natal in Zululand sugarcane (Bam, 2014), while in the succulent Karoo, Pamphagidae and Lentulidae families' had one species each while Acrididae family had sixteen species (Gebeyehu and Samways, 2006). In southern Nigeria, Tettigonidae, Pyrgomorphidae and Acrididae family had three, two and six species respectively (Oku *et al*, 2011).

In Kenya, Mungai *et. al.*, 1995, studied Acridoidea superfamily from Lake Baringo area where he recorded 70 species belonging to four families. Eumastacidae and Pamphagidae had one species each while Pyrgomorphidae had six. Acrididae was also found to contain sub-families which include Acridinae with 17 species, Oedipodinae with 18, Catantopinae with seven, Tropidopolinae with one, Hemiacridinae with three, Calliptaminae with 3, Cyrtacanthacridinae with seven, Gomphocerinae with three and Truxalinae with three.

2.2: Diversity, Distribution and Abundance of locusts and grasshoppers

Diversity of species is the number of different species in an ecosystem. It also includes number of individuals within each species in a given ecosystem. Distribution is the pattern in which members of a given species are arranged across a number of habitats and is determined by the presence or the absence of a species in a given area (Fowler *et al*, 2013). Species abundance is the number of individuals while species distribution is the description of the number of individuals observed for each different species encountered within a community. Abundant species are those that will be found in large numbers in a given habitat at a time (McGill *et al*, 2007). Studies about diversity, distribution and abundance of grasshoppers and locusts provide information about their preferred habitats which can be used for their conservation and control. They also provide information about population structure and extinction status (Wheeler, 1990).

Abiotic factors influences diversity, distribution and abundance of locusts and grasshoppers in various ways (Savopoulou *et al*, 2012). They influence the increase of body size which in turn influence the rate at which individuals grow, acquire resources and how they will reproduce (Bidau *et al*, 2012). Temperature influences their physiological functioning like feeding which in turn influences cell differentiation, development and growth. They will therefore adjust to temperature variations and preferences to those that will maximize these functions. Low temperatures slow down development while high temperatures hastens their development (Miller *et al*, 2009). Rainfall influences their breeding by providing soils with moisture for oviposition. Varying degrees of moisture allows eggs to develop and hatch. With less soil moisture, eggs enter diapause or quiescence which prevents successful breeding (Crooks and Cheke, 2014). Rainfall also provides precipitation which promotes plants' growth and the plant diversity generated provides a variety of food which promotes growth rate, fecundity and survival.

Precipitation also structures habitats and microhabitats which influences feeding preferences, flight capacity and reproductive potential. However, heavy rainfall physically damages larval and adult stages. It also provides difficulties in consuming enough nutrients from water laden plant tissues. Low rainfall results in less growth of vegetation and therefore lack of enough food (Yi *et al*, 2011).

Biotic factors also influence locusts and grasshoppers' diversity, distribution and abundance. Vertebrate predators like birds, small mammals, lizards and invertebrate ones like spiders directly feed on them. Their foraging and location in a habitat is influenced as they are made to move higher in the grasslands to escape ground predators and in the opposite direction to escape aerial predation (Wineland *et al*, 2015). Parasites like *Acridomyia canadensis* feeds on the body's fluid and penetrates the host's body through rasping. Females lay eggs in the body of the host through the hole made and once larvae emerge, they feed on the host (Hostetter, 2000). Larva of *Laius villosus* is a parasite that feeds on egg pods of Australian plague locust, *Chortoicetes terminifera* while *Scelio fulgidus* feeds on its eggs (Farrow, 1974). Parasitoids like *Scelio pembertoni* destroy the eggs of grasshopper *Oxya chinensis* preventing hatching and the establishment of a new generation (Lomer *et al*, 2001). Nematodes also influence population dynamics of locusts. Those that belong to Spirurida family lay their eggs on vegetation where they are consumed by locusts and once they get into the digestive tract, they hatch into juveniles. These pass through the gut wall and into the haemocoel where they develop into the adult stage. As they develop, they cause degeneration of tissues of the host, retard development, suppress oocyte growth and degenerate flight muscles which reduce flight ability in adult hosts (Rusconi *et al*, 2016). Microorganisms includes protozoans like *Nosema acridophagus*, *Nosema cuneatum* and *Nosema locustae* which infect locusts and grasshoppers when they consume spores on food

items and infected cadavers and once ingested, they attack the fat bodies and the pericardial neural tissue of the host resulting to death. When a fungal spore of *Metarhizium anisopliae* come into contact with the exoskeleton of a grasshopper, they develop a tube that penetrates the body wall where it grows rapidly inside the host killing the grasshopper. They then grow back through the body wall, form vegetative stalks that produce spores which are discharged into the atmosphere and infect more grasshoppers (Muller, 2006). Examples of bacteria that affect survival and distribution of grasshoppers and locusts includes *Coccobacillus acridiorum* which caused dysentery in locusts and grasshoppers cultures resulting to high mortality levels and *Bacillus prodigiosus* which killed desert locusts after two weeks of consumption (Zelazny *et al*, 1997**).** Examples of virus includes Entomopoxvirus and Crystalline allay virus strains which contributes to high mortality of locusts and grasshoppers (Streett *et al*, 1997). *Eutrombidium trigonum* is a parasitic mite which sucks blood of grasshoppers in nymphal and adult stage as well as preying on eggs in the adult stage. It attaches itself on wings which prevents normal flight which makes the grasshopper host easier to catch by predators or facilitates attack by parasitic enemies (Severin, 1944).

2.3: Economic importance of grasshoppers and locusts

Grasshoppers and locusts are known crop pests with desert locust, *Schistocerca gregaria, Calliptamus italicus, Dociostaurus maroccanus, Nomadacris septemfasciata* and *Chortoicetes terminifera* among examples of crop pests (Zhang *et al*, 2019). Examples of grasshopper pests includes *Melanoplus sanguinipes*, *Melanoplus bivittatus*, *Melanoplus differentialis* and *Melanoplus femurrubrum* (Dakhel *et al*, 2020). Some are more particular in the type of plants they feed on and therefore feed exclusively on monocots or dicots while others are mixed feeders (Bullen, 1966). Compared to grasshoppers, locusts' are more devastating crop pests because they can transform from a cryptic solitarious phase to a gregarious phase which destroy 80 – 100% of crops and pasture leaving bare ground (Egonyu *et al*, 2021). Their populations can grow quickly to large swarms of between 40 – 80 million individuals and therefore reach damaging levels very quickly. These swarms migrate hundreds of kilometers per day and therefore invade areas covering millions of square kilometers (Sharma, 2015). One female desert locust has been found to consume 1.5 grams of vegetation in a day. If the locusts formed a swarm, the consumed amounts would be in tonnes (Spinage, 2012). In addition, they have excellent survival strategies. For instance, *Oedaleus senegalensis* attacks grasslands and if this food source depletes due to desiccation, they invade millet fields which usually remains green for long periods and destroys it within a few days (Langewald *et al*, 1999). Australian plague locust, *Chortoicetes terminifera*, is multivotine with three to four generations in a year which occurs in all seasons which increases its survival chances (Hunter *et al*, 2001). Their outbreaks occur mostly in the desert and scrub regions of northern Africa, the Middle East and Southwest Asia. In Morocco, desert locust outbreak in 1954 – 1955 caused losses of more than $50 million and in Ethiopia, an outbreak that occurred in 1958 resulted to grain losses totaling 167,000 tonnes which were

enough to feed a million people for one year. In Guinea, African migratory locusts' outbreak caused 40% loss of rice crops in 1930 while 35% loss of banana crops were lost between 1931 and 1934 (Showler, 1995). In 1987, the tree locust, *Anacridium melanorhodon* attacked Hashab tree that produces gum. Initially, 20,000 hectares were infested which increased to 224,000 hectares the following season and reaching 1,366,762 hectares in 1989 (Haroon *et al*, 2018).

Locust outbreaks results into swarms which feed on many crops like vegetables, cereal crops and fruits like bananas and citrus where they cause crop damage resulting to crop failure. If the crop damage occurs on a large scale, the end result is famine and malnutrition and a rise food prices which aggravates poverty (Pandey *et al*, 2020). Australian plague locust swarms on the road can block car radiators if insect screens are not fitted and cause traffic hazards on roads due to poor visibility by the road users. Their hoppers have occasionally halted trains when they cause lack of adhesion on the rails as a result of their vast crushed numbers as well as causing temporary closure of runways due to poor visibilities by planes when touching down and taking off (Love and Riwoe, 2005). In Mali, the desert locust outbreak that occurred in 1987 to 1989 had an impact on education. There was a 25% reduction in the proportion of children enrolled in schools in the rural areas as compared to urban areas. The financial burden inflicted on families by that plague limited funds for school fees and increased the need for children to contribute to family incomes which reduced school attendance (Cease *et al*, 2015).

Before the invention of insecticides, farmers protected their crops from locusts and grasshoppers invasion through destruction of eggs, beating with sticks, burning and digging trenches (Eriksson, 2008). Other methods included making noise to prevent swarms from settling, burning roosting adults on trees, covering roosting sites with straw, trampling, burying them in trenches, chasing hoppers and ploughing egg oviposition beds. These methods did not require specialized

equipment, were low cost and didn't have an impact on the environment. They were however less effective, time consuming and labor intensive (Kietzka *et al*, 2021). Organochlorines were invented in the 1940s with DDT and dieldrin among the best at the time. These were later replaced by organophosphates. Use of chemical insecticides proved popular because they were effective when used a fast response in case of an outbreak (Bidochka *et al*, 1997). The most commonly used organophosphates include Fenitrothion, Fipronil, Sevin, Malathion, Diazinon, Dusrban, Dimethoate, Lindane, Endosulphan, Torbidan, Decis and Kafil super. They are however hazardous to operators, non-target organisms and the environment which has promoted the use of biological control. Use of botanicals extracted from plants especially from *Azadirachta indica* and *Melia volkensii* provide compounds like azadirachtin and salannin which are antifeedants, repellents and growth regulators (Safi *et al*, 2021). Bio pesticides like the Fungi *Metarhizium flavoviride* and oil formulations of other Fungi especially Beauveria *bassiana* and *Empusa grylli* and *Metarhizium anisopliae* spores together with protozoan *Nosema locustae* have proved a success especially when integrated with rational use of chemical pesticides (Latchininsky *et al*, 2011). Most entomopathogenic organisms reduce the targeted locusts and grasshoppers feeding which in turn reduces the size of the fat bodies at sexual maturation. This reduces their fertility and fecundity and ultimately their population (Arthur and Thomas, 2000). However, biological control has proven to be ineffective when compared to chemical insecticides. They take long to kill the target locusts after spraying, are expensive and effective doses are not yet identified (Fang *et al*, 2014).

2.4: Nutritional components of Grasshoppers and locusts

Humans in many parts of the world eat grasshoppers and locusts. Edible locusts includes the desert locust, *Schistocerca gregaria*; migratory locust, *Locusta migratoria*; red locust, *Nomadacris septemfasciata* and brown locust, L*ocustana pardalina (*Kim *et al*, 2019). These are collected from their natural environment or bought in local markets. For instance, *Schistocerca gregaria* is collected in mornings or at night when they are immobile due to low temperatures (Van Huis, 2021). In laboratories, they are reared in cages which were initially made of wood, fabric, metal or glass. Currently, cages made with aluminium wand a transparent screen are the most common (Hinks and Erlandson, 1994). Young locusts and females rich in eggs are preferred as they are tastier and rich in proteins (Nakagaki and De Foliart, 1991). In Madagascar, some of the consumed species include *Oxya hyla, Paracinema tricolor, Locusta migratoria* and *Cyrtacanthacris tatarica*. Children collect them as a common game when playing in the rice fields or walking home from school while adults mostly collect them during the peak periods. They are eaten as a snack or the main meal (Van Itterbeeck *et al*, 2019). In North east India, grasshopper species consumed include *Acrida gigantea, Agridium melanocorne, Agridium perigrinum, Lima corded, Holochlora albida* and *Thyrotropides ditymus* (Meyer-Rochow and Changkija, 1997); *Anacridium melanorhodon* is consumed in Sudan (Kinyuru *et al*, 2018) while cone headed grasshopper, *Ruspolia nitidula*, which is commonly referred to as nsenene is widely consumed in Uganda (Agea *et al*, 2008).

Grasshoppers and locusts provide proteins and fats to humans and domestic animals. Based on their growth stages, fats in nymphs range between 7.5% - 10.7% while in adults, they range between 7.5% - 14.7% (Makkar *et al*, 2014). For individuals, *Ruspolia nitidula* has 36-40% protein and 41-43% fat (Ssepuuya *et al*, 2017), *Melanoplus mexicanus* with 58.9% of proteins

and 11% fats (Elorduy *et at*, 2012); *Zonocerus variegatus* with 61.50% crude protein and 6.87% crude lipids (Alegbeleye *et al*, 2012*)*; *Oxya fuscovittata* with 63.96% crude protein and 6.49% crude lipid (Anand *et al*, 2008); *Oedaleus abruptus* with 60% crude protein and 10% crude fats (Ganguly *et al*, 2013) while *Sphenarium purpurascens* and *Sphenarium histrio* had 65% and 60% of crude protein respectively (Tang *et al*, 2019).

Examples of amino acids contained in locusts and grasshoppers proteins includes Aspartic acid, Methionine, Cysteine, Isoleucine, Leucine, Phenylalanine, Lysine, Tryptophan, Aspartate, Asparagine, Glutamine, Histidine, Methionine, Proline, Serine, Glycine, Arginine, Threonine, Alanine, Tyrosine and Valine (Ghosh *et al*, 2016). Fatty acids contained in their fats includes Caproic acid, Capric acid, Lauric acid, Tridecanoic acid, Myristic acid, Pentadecanoic acid, Palmitic acid, Palmitoleic acid, Heptadecanoic acid, Cis-10-heptadecanoic acid, Stearic acid, Elaidic acid, Oleic acid, Linolelaidic acid, Linoleic acid, Arachidic, Linolenic acid, Cis-eicosenoic acid, Linolenic acid, Cis-eicosadienoic acid, Behenic acid, Eicosatrienoic, Tricosanoic acid, Lignoceric acid and Docosahexaenoic (Mohamed Elagba, 2015).

Kjeldahl is the most common method for determining protein content. Total amount of nitrogen in a sample is multiplied by a protein factor, commonly 6.25 in meat, where the final figure amounts to total crude protein. It assumes an even distribution of amino acids which may lead to a slight overestimation of the total proteins. In insects, the use of 6.25 as nitrogen conversion factor results to overestimation because it doesn't consider the amount of non- protein nitrogen contained in the polysaccharide chitin of the exoskeleton which produces a false protein content (Jonas-Levi and Martinez, 2017). This overestimation can be around 8.5% to 22% in most insects. For accurate protein estimate, non-protein nitrogen should be subtracted from the total nitrogen or a 4.76 conversion factor should be used (Clarkson *et al*, 2019). When extracting fats,

the food sample can be dried to obtain dry weight while wet weight can also be used. Fat is extracted using a non – polar solvent like Diethyl ether, hexane or petroleum spirit which condenses in the condenser of the Soxhlet extraction unit leaching out oils or fats from the food sample in the thimble and into the extraction flask. Before extraction starts, the weight of the flasks is taken. At the end of the extraction process, the total weight of the oils or fats in the extraction flasks is determined by subtracting the weight of the fat extracted from the extraction flask. The obtained weight is then divided by weight of the food sample and then multiplied by hundred to obtain fat percentage (Oonincx and Finke, 2021). In grasshopper and locusts, the fat content varies largely due to their differences in species, diet, metamorphic stage and the extraction method (Ghosh *et al*, 2017).

2.5: Life parameters of Grasshoppers and Locusts

Grasshoppers and locusts' eggs are laid in moist soils, grasses, wood, cracks in barks and other plant tissues. Female accessory glands secrete a substance which adheres eggs to the soil particles. This forms a capsule of cemented soil around oothecae or egg cases (Shah *et al*, 1998). Some of the eggs may hatch while the rest may enter a period of diapause. For eggs laid in soil, vermiform larvae wriggles to the soil surface through the soft foam that covers the eggs and they are referred as the first nymphs (Maeno *et al*, 2013). Most of them start feeding on the same day while others will start in the second day. They then form groups of hoppers called bands which start to move about (Nevo, 1996). Compared to adults, nymphs have less developed pronotum, reduced number of segments in the antennae and reduced development of reproductive organs and wings (Ingrisch and Rentz, 2009). Different species undergo different number of instars. These instars also vary between males and females and under different environmental conditions. Adult females usually have a one to two weeks pre oviposition period during which they are courted by males, copulate and eggs develop internally (Shrestha *et al*, 2021).

Various studies have recorded the number of eggs laid by locusts and grasshoppers. Locusts include *Atractomorpha sinensis with* 100 (Li *et al*, 2020), *Nomadacris septemfasciata* with 20 – 100 eggs for gregarious locust and 20 – 195 for solitary locusts (Lecoq *et al*, 2011), *Schistocerca gregaria* with 49 (Schmidt and Albutz, 1994) and *Locusta migratoria migratorioides* with 52 (Tu *et al*, 2012). Examples of grasshoppers include *Melanoplus sanguinipes* and *Melanoplus bivittatus* with an average of 21.8 and 43.3 eggs per pod respectively (Smith, 1966). *Kosciuscola cognatus* and *Kosciuscola usitatus* had an average of 17.65 and 22.93 respectively (Dearn, 1977). Another grasshopper *Sphenarium purpurascens* has one generation per year with five nymphal instars which require 5 – 6 weeks to complete development. Females produce one to

two ootheca with each having 15 to 38 eggs. (Cerritos and Cano- Santana, 2008). *Oedaleus senegalensis* lay an average of twenty five eggs per pod. They oviposit twice with a duration of five or six days. The emerging nymphs have five instars which take three weeks to reach pharate adulthood and this stage take an average of ten days to become capable of laying eggs (Jago *et al*, 1990).

In terms of time taken to hatch, *Locusta spp eggs* took between 11 to 25 days, *Schistocerca gregaria* 14 – 32 days while *Nomadacris septemfasciata* took between 24 - 36 days to hatch. (Ackonor, 1989). In a study to determine the instars' number, morphometry and duration of *Phymateus leprosus*, it was established to have ten juvenile instars. Each had a development duration of 2-8 weeks each while their overall development period amounted to one year. Adult lifespan amounted to 2-8 months with mating occurring after three months (Kohler *et al*, 2007). *Ronderosia bergi* had a total of five nymphal instars with embryonic development time averaging 40.6 days and the mean life span of cohorts was 22.6 weeks (Mariottini *et al*, 2010).

Dociostaurus maroccanus nymphs took between 36-38 days to complete post embryonic development with 60% survival rate. The first, second, third, fourth and fifth instars duration was 6-8; 4-6; 7-9, 4-6 and 9-12 days respectively (Quesada-Moraga and Santiago-Alvarez, 2001). South American locust, *Schistocerca cancellata*, eggs took 15 days while the first, second, third, fourth, fifth and sixth instars took four, six, three, four; seven, and nine days respectively to complete their development. Nymphs took an average of 33.24 days to develop into adults. The average lifespan of adults was 135.84 and 138.04 days for females and males respectively. Female individuals laid an average of 73 eggs per egg pod. (Sanchez *et al*, 1997). *Arcyptera brevipennis vicheti* has five nymphal developmental stages. The first and second instars moult after three days while third and fourth instars moult after three days (Schultner *et al*, 2012)

Taeniopoda eques has five instars. Eggs take 39 days to hatch while hatched nymphs take 56 days to mate, 74 days to oviposit and have a life span of 105 days (Whitman and Orsak, 1985). *Romalea microptera* laid 3.5 to 4.5 egg pods and the interval between the pods was 17 days. Each egg pod contained an average of 57.9 to 69.8 eggs. Longevity of adults was 103.8 to 113.5 days (Hunter – Jones, 1967).

2.6: Insect Life Tables

Life tables help to increase the understanding of population dynamics of an insect population. They summarize the number of survivors, deaths, fecundity, and the life expectation i.e. the time to be lived by the individuals in the various growth stages in the life cycles of insects (Kakde *et al*, 2014). They reveal the growth stage with great mortalities and survival. (Harcourt, 1969). They give a detailed description of the growth, development, reproduction and the predation rate of target populations which is important for biological control of insect pests. (Chi and Su, 2006). Life tables drawn from field data can be used to study how biotic and abiotic factors affects the fitness of a population. The only problem that arises is the difficulty in tracing population survival and reproduction under variable environmental conditions. However, in a laboratory, conditions are controlled which helps reveal maximum growth potential of a population (Gabre *et al*, 2005).

Horizontal or the cohort life table is the one commonly drawn for insects study. It involves following a group of individuals from the time they are hatched until they all die where the number of deaths are recorded daily in each growth stage (Portilla *et al*, 2014). It only considers the female age specific populations but ignores the male populations (Ning *et al*, 2017).

However, this kind of table can't describe correctly the growth and stage differentiation of insect populations. It doesn't consider the variation in the development stages among individuals in both males and females because development rate is different in sexes and among individuals. Such omissions may cause errors in calculating the increase of individuals, their reproductive rate and the generational time (Huang and Chi, 2013). This weakness is overcome by drawing a two sex life table which includes the developmental rates of the male individuals (Chi and Huang, 2012).

CHAPTER THREE

DIVERSITY, DISTRIBUTION AND ABUNDANCE OF LOCUSTS AND GRASSHOPPERS IN NAKURU COUNTY, KENYA

3.1 Introduction

Locusts and grasshoppers with a short antennae belong to order Orthoptera and sub order Caelifera. They have tegmina wings, long hind legs with an enlarged femur and spines, short antennae, short ovipositor and a tympanum that is located on abdominal segment (Kumar and Usmani, 2014). They are one of the most widely distributed fauna and are found in grasslands, tropical rain forests, shrub lands, deserts, wetlands and mountain regions around the world. Their specific microhabitat preferences, abundance, ease with which they can sampled, sensitivity to the modification of biotic and abiotic factors in their habitats and their mobility make them important indicators of specific environmental conditions (Ngoute *et al*, 2020). They are a source of nutrients for both invertebrates like parasitoids and small vertebrates and therefore serve as important links in food chains. Their scarcity would disturb the trophic levels in an ecosystem (Akwanjoh & Tita, 2020).

Grasshoppers and locusts are herbivores though a few practice cannibalism which makes them omnivores (Hansen *et al*, 2011). Grasshoppers feed mainly on grasses while locusts feed on a variety of vegetation which makes them devastating crop pests. Locusts' populations can grow quickly with the right ecological conditions and are able to aggregate under high population densities. They consume their own body weight daily depending on species and stage of development. Their nymphs form collective marching groups which pass through vegetation consuming them. These populations result to outbreaks that are unpredictable and highly mobile which makes management and control difficult (Song, 2018). For instance, a 2003 – 2005

outbreak of the desert locust in North Africa, locusts control operations costs increased from $1 million to $75 million a year later (Lecoq, 2005).

They feed on leaves which reduces plants' biomass, interfere with allocation of carbon to various parts, raise production of defensive compounds and trigger abscission (Chapman *et al*, 2003). A reduction of plant leaves also impacts the structure of plant canopies thus altering the cover and light availability to underlying communities which in turn influence the soil microclimate, temperature, moisture and also increases leaching of nutrients from the forest floor due to the passage of light energy. They therefore influence the abundance and richness of plant species by limiting their fitness in both outbreak and non-outbreak scenarios (Andersson, 2016).

Grasshoppers and locusts influence terrestrial cycling of plant nutrients and therefore change the quantity, quality and timing of nutrient input to soils or sediments. When foliage materials are passed through their guts, they are broken down which increases the surface area available for microbial attack as well as increasing the microbial communities. The vegetation rich in nutrients are then deposited to the forests floor as excrement, frass, green fall and insect biomass in form of carcasses. Dead insect bodies that are deposited on the ground contain more easily decomposed nutrients than leaf litter. These substances stimulates bacterial degradation which rapidly decompose them to release nutrients mainly nitrogen, phosphorus and carbon for plant uptake (Belovsky and Slade, 2002). In addition, the ingested nutrients may not be fully digested and assimilated and some is excreted (Lovett *et al*, 2002). These have distinctive chemical properties that alter rates of organic matter decomposition and nutrient release within an ecosystem which increases plant abundance and therefore alters the plant community composition which enhances ecosystem productivity (Montemayor *et al*, 2018).

Grasshoppers and locusts diversity studies identifies species in a particular ecosystem, their distribution and relative abundance estimate. Data obtained allows study of poorly known sites for their richness, structure, distribution and endemism. Those sites that are intensively studied can be mapped and the population dynamics followed for some time which would help to understand the extent and causes of the diversity and the processes through which it is generated and maintained. The information obtained can be used for pest management related to plants as well as conservation and environmental management. Regarding grasshoppers and locusts, they are important as they influence the way in which ecosystem functions because their numbers relate positively to other organisms present especially their predators, parasitoids and scavengers. There exists little information about them as the emphasis is on their negative impacts in most biological research areas. Their diversity, distribution and relative abundance has also received little attention due to time, financial, personnel especially availability of taxonomists and funds constraints. According to Nakuru County integrated development plan, 2013 – 2017, there exists three ecological zones but there is limited knowledge regarding number of species, their distribution, relative abundance and rarity. The data collected partly filled this gap and also formed a basis for future and further studies.

3.2 Materials and Methods

3.2.1 Study area

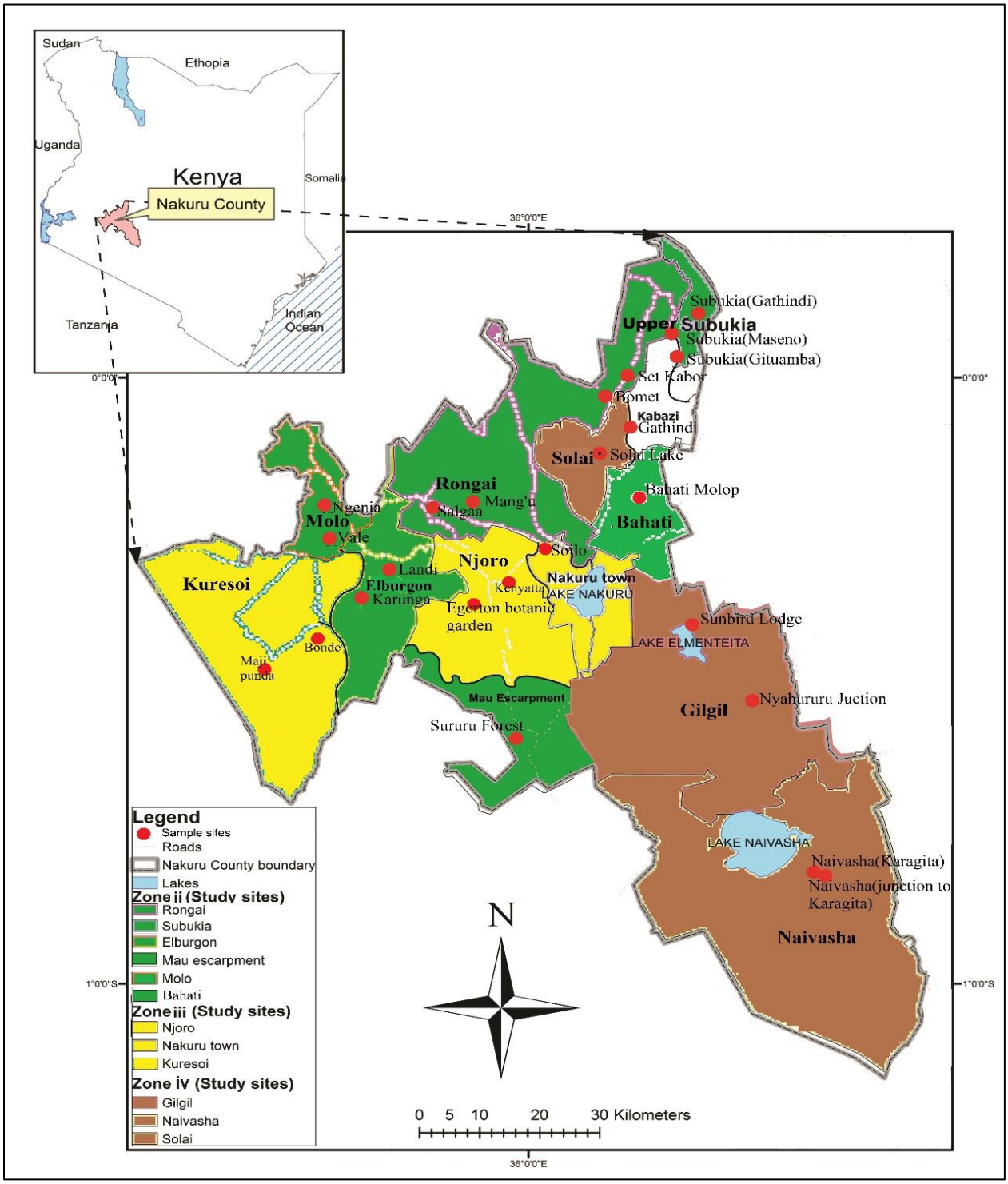

Figure 1: Map showing randomly sampling sites in the Ecological Zones.

The study was carried out in Nakuru County which lies in the Great Rift Valley covering an area of 7,495.1km^2. It is located between longitude $35^0$28'W and $35^0$36'E; latitude $0^0$13'N and $1^0$10'S. Short rains fall between October to December while long rains fall between March and May. Hottest months range between December and March with temperatures averaging 29.3^{0}C while coldest month ranges between June and July with temperatures averaging 12^0C.

The three study zones include Zone II which has upper Subukia, Rongai, Molo, Elburgon and Mau. It is elevated between 1980-2700 metres above sea level. Its rainfall ranges between 1200 - 2600mm per annum. It has deep and well drained volcanic and Latosolic soils whose high nutrient content supports forests, thickets of bamboo and grasslands.

Zone III includes Nakuru town, Njoro and Kuresoi which is elevated between 900-1800 metres above sea level. Its rainfall ranges between 950 to 1500 mm per annum. It has Latosolic soils which supports forbs, herbs, shrubs, grasses, trees, climbers and epiphytes.

Zone IV includes Gilgil, Naivasha and Solai and is elevated between 900-1800 metres above sea level. Its rainfall amount ranges between 500-1000mm per annum. Its soils includes Alluvial and Locustine deposits which supports grasses often 3-6 feet tall at maturity, drought and browse resistant trees and open shrub layer usually 3 – 15 feet high.

3.2.2 Sample collection

Grasshoppers and locusts were collected between 9 am and 4 pm as this is the duration in which they are easily captured. This is because, before 9 am, they are less active and therefore hard to find them and after 4 pm, they start looking for resting sites and therefore disappear. Sites were sampled based on vegetation as the more vegetation there are, the higher the chances of finding grasshoppers and locusts. Sampling methods included flushing them from vegetation using the handle of the sweep net or searching in the grasses, shrubs and trees as some would escape into them. Capturing involved a sweep net or using hands where necessary. Both the nymphs and adults were collected. Longitude and latitude coordinates were recorded to facilitate plotting of the points of the study area.

The collected specimens were killed by placing them in a 500ml Kilner jar which contained cotton wool soaked with 6.9% Diethyl ether concentration. They were sorted according to morphological features, labelled, counted and their photos taken. Sample representatives were pinned in an insect's box of 30cm width, 30cm length and 3 inches' height using size three entomological pins. In the four corners of the box were fitted naphthalene crystals for preservation. Identification was done at the National Museums of Kenya, Nairobi to species, genus, subfamily and family level.

Figure 2: A grasshopper pinned using a size three entomological pin and pinned grasshoppers and locusts in an insects' box.

3.2.3 Data Analysis

An overall measure of locust and grasshopper abundance at each site was estimated by summing the counts of all species. Species dominance (D) was determined according to a method by Buschini and Woiski (2008): D = (abundance of a species/total abundances recorded) × 100. If D was > 5%, the species was considered dominant, if 2.5% < D < 5%, the species was considered an accessory species/ species of intermediate abundance, and if D < 2.5%, the species was considered an incidental species. Rare species were the ones that had less than 5 individual grasshoppers or locusts and/ or sampled from only one ecological zone. The unique species were the ones occurring with one individual: singleton, or with two individuals: doubleton). Species diversity and distribution in different ecological zones that included Shannon-Wiener Diversity Index (H′) and Simpson's Dominance Index (D^{-1}) were calculated using PAST software. Poisson's regression was used to test the significance differences among ecological zones for both indices. The Sørensen, Jaccard and percentage similarity indices were used to compare similarities among the zones and were calculated according to Kiernan, 2014 using Microsoft excel 2013:

Jaccard's index:

$$SJ = \frac{c}{a + b + c}$$

Where c is the number of common species among the study sites; a and b are the unique species in each site (i.e. the number of species present in site a but absent in site b and vice versa). Sorensen's index

$$CC = \frac{2c}{a + b + 2c}$$

For both indices, the higher the value, the more ecologically similar two sites are.

If quantitative data are available, a percentage similarity index can be computed. These indices compare number of similar and dissimilar species present between two sites but also incorporate abundance. In this case, Gaucho similarity percentage index was calculated in the following way:

$$PS_{ij} = \frac{200 \sum min\,(y_{ki} y_{kj})}{\sum y_{ki} + y_{kj}})$$

Where y is the number of common species among the study sites being compared, while i (number of sites like 1, 2 and 3), i is site 1 while k is site 2. Σki and Σkj is the total number of abundances of the species in each study site (e.g. total abundance in upper zone plus lower zone).

3.4. Results and Discussion

3.4.1 Results

The families, subfamilies, species and dominance of the grasshoppers and locusts collected are shown in Table 1. A total of 456 grasshopper and locust individuals were collected in the ecological zones. Of the total individuals collected, 93.4% belonged to Acrididae family which constituted to 6 subfamilies, 15 genera and 16 species. The rest 6.6% belonged to Pyrgomorphidae family and comprised of one subfamily, two genera and two species. There were 5 dominant species, *Aiolopus thalassinus, Acrotylus blondeli, Acrotylus patruelis, Parasphena ngongensis and Coryphosima stenoptera* ; 6 species with intermediate dominance; *Acanthacris ruficornis, Trilophidia conturbata, Sphingonotus turkanae , Rhaphotittha nyuki, Pezocantantops impotens* and *Chrotogonus hemipterus* and 7 incidental species *Cyrtacanthacris tatarica, Ornithacris pictula, Gastrimagus verticalis, Paracinema tricolor, Heteropternis couloniana, Tylotropidius gracilipes Pyrgomorpha conica*. In the ecological zones, abundance was highest in zone II (47%), followed by zone III (28.7%), while zone IV had the least number of individuals (24.3%). The most abundant, dominant and highly distributed species was *Aiolopus thalassinus* (D = 27.4) while the least dominant species were *Ornithacris pictula, Gastrimagus verticalis* and *Heteropternis couloniana* which were found in zone II only.

Table 1: Distribution of locusts and grasshoppers within the study area at family, sub-family, species and dominance level.

Family	Sub-Family	Species	Zone II	Zone III	Zone IV	Total individuals	Dominance (D) (%)
Acrididae	Cyrtacanthacridinae	*Acanthacris ruficornis*	6	3	12	21	4.6
	Cyrtacanthacridinae	*Cyrtacanthacris tatarica*	0	9	0	9	2.0
	Cyrtacanthacridinae	*Ornithacris pictula*	3	0	0	3	0.66
	Oedipodinae	*Aiolopus thalassinus*	36	65	24	125	27.4
	Oedipodinae	*Acrotylus patruelis*	9	0	42	51	11.18
	Oedipodinae	*Acrotylus blondeli*	27	0	33	60	13.16
	Oedipodinae	*Trilophidia conturbata*	21	0	0	21	4.6
	Oedipodinae	*Gastrimagus verticalis*	3	0	0	3	0.66
	Oedipodinae	*Sphingonotus turkanae*	12	0	0	12	2.60
	Oedipodinae	*Paracinema tricolor*	0	6	0	6	1.30
	Oedipodinae	*Heteropternis couloniana*	3	0	0	3	0.66
	Gomphocerinae	*Rhaphotittha nyuki*	18	0	0	18	3.93
	Eyprepocnemidinae	*Tylotropidius gracilipes*	7	0	0	7	1.52
	Acridinae	*Coryphosima stenoptera*	21	12	0	33	7.23
	Catantopinae	*Pezocantantops impotens*	15	0	0	15	3.3
	Catantopinae	*Parasphena ngongensis*	3	36	0	39	8.6
Pyrgomorphidae	Pyrgomorphinae	*Pyrgomorpha conica*	9	0	0	9	2.0
	Pyrgomorphinae	*Chrotogonus hemipterus*	21	0	0	21	4.6
TOTAL			214	131	111	456	100

Diversity and abundance indices for locusts and grasshoppers in different areas within ecological zones of Nakuru County are shown in Table 2. Upper Subukia with H′ =1.77 and D^{-1}=0.160 had the highest diversity indexes. Mau escarpment, Nakuru town and Gilgil with H′ = 0.00 and D^{-1}=1.00 had the lowest diversity indexes. Diversity in the ecological zones was not significantly different as shown in Appendix 3.

Table 2: **Shannon-Wiener Diversity Indices and Simpsons' Dominance Indices for locusts and grasshoppers in different areas within ecological zones in Nakuru, Kenya**

Ecological zone	Area	Shannon-Wiener index	Simpsons' index
Zone II	Upper Subukia	1.77	0.160
	Rongai	1.64	0.219
	Molo	0.95	0.301
	Elburgon	1.24	0.400
	Mau Escarpment	0.00	1.000
Zone III	Njoro	1.24	0.301
	Kuresoi	0.99	0.389
	Nakuru Town	0.00	1.000
Zone IV	Naivasha	1.02	0.371
	Gilgil	0.00	1.000
	Solai	0.54	0.720

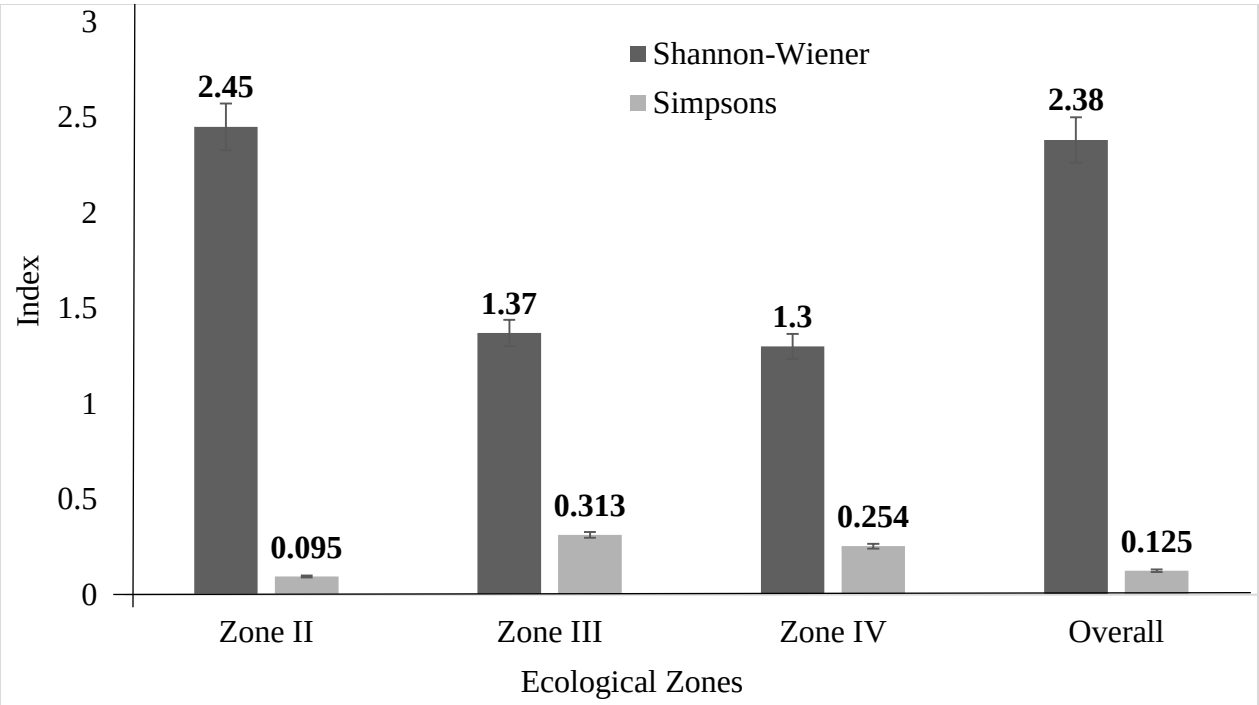

Figure 3: Shannon-Wiener diversity index and Simpsons' dominance index for locusts and grasshoppers in the ecological zones.

Diversity and abundance indices for locusts and grasshoppers in the ecological zones of Nakuru County are shown in Figure 2. Overall Shannon-Wiener diversity index was found to be H′=2.38 while Simpson's dominance index was D^{-1}= 0.125. Zone II had H′= 2.45 and D^{-1}= 0.095, zone III with H′=1.37 and D^{-1}=0313 and zone IV with H′ = 1.3 and D^{-1}= 0.254.

Shared species, Jaccard's index, Sorensen's index and percentage similarity index are shown in table 3. Four species were shared between zone II and zone III, two species were shared between zone III and IV while four species were shared between zone II and zone IV. Jaccard's similarity index was 0.22 between zone II and zone III, 0.25 between zone II and Zone IV and 0.25 between zone III and zone IV. Sorensen's index was 0.36 between zone II and zone III, 0.4 between zone II and IV and 0.4 between zone III and IV. Percentage similarity index was 2.32% between zone II and III, 1.65% between zone III and IV and 2.46% between zone II and IV.

Table 3: Shared species between the ecological zones

	Zone II	Zone III	Zone IV
Zone II (S_J)		0.222	0.25
(CC)		0.36	0.40
(SP)		4	4
% similarity index		2.319	2.462
Zone III(S_J)	-	-	0.25
(CC)			0.4
(SP)			2
% similarity index			1.653

S_J, Jaccard similarity coefficient; CC, Sörensens similarity coefficient; SP, shared species

3.4.2 Discussion

Across the three ecological zones, 456 individuals of grasshoppers and locusts were collected. Abundance was highest in zone II and lowest in zone IV. In a habitat, variables like temperature, humidity, light intensity, food availability, sites for oviposition, hiding places and the presence of predators influences species abundance and distribution. Food choices affects a species fecundity, development and survivorship and therefore its relative abundance will increase with an increase in plant community structure (Falcone, 2010). The type of vegetation found in the three zones included small forests, thickets of bamboo, forbs, herbs, shrubs, grasses, tress, climbers, epiphytes, browse resistant trees, cactus, aloe vera and open shrub layer. These provides a variety of food for the phytophagous, oligophagous and monophagous species and a hideout from predators and harsh environmental conditions like sun and rain which facilitates their survival (Hussain *et al*, 2017).

Of the total individuals collected, they belonged to eighteen species which belonged to two families, Acrididae and Pyrgomorphidae. Two species belonged to Pyrgomorphidae family while the rest sixteen belonged to Acrididae. Acrididae members were distributed in the three ecological zones while Pyrgomorphidae members were found in zone II only. This distribution

differences can be attributed to food choices which influences components of fitness especially fecundity, development and survivorship (Joern, 1979). Members of the Pyrgomorphidae family are monophagous in nature where their choices of plants is influenced by secondary chemicals, nutrient contents, concentration and distribution and therefore feed almost exclusively on a single plant species. They are aposematic and obtain their defensive compounds against enemies from their diets (Sword *et al*, 2000). From the prefential toxic plants they feed on, they obtain substances which they utilize and convert into toxins which are stored in their bodies, others isolate toxins from the plants they feed on depending on the plants' chemical concentrations or they can synthesize their toxins by detoxifying the plants secondary compounds which makes them distasteful to predators which deters or kills them. They have well developed gut barriers to plant secondary compounds (Joron, 2009). The plants they feed on are located in a certain ecosystem due to soil conditions which explains why the family was found in one locality. However, Acrididae family are phytophagous, where they feed on both forbs and grasses and therefore inhabit habitats with monocot and dicot vegetation (Jerath, 1968). They breed in short grasses and lay eggs in moist soil. Nymphs and adults migrate to the long grasses which contributes to their wide range of diversity and distribution (Paulraj *et al*, 2009).

Aiolopus thalassinus was the most abundant and also equally distributed across the ecological zones. They belong to subfamily Oedipodinae whose members primarily feed on grass, both wild and cultivated grasses, wet and dry meadows and secondarily on forbs (Kristin *et al*, 2007). In the ecological zones studied, the named vegetation was distributed across which ensured their presence. They breed in all seasons which ensures continuous reproduction and hatching and therefore nymphs have no specific season for emergence, growth and dispersal (Heifetz and Applebaum, 1995). Their females do not oviposit in dry soil and therefore frequently migrate to

other suitable habitats from their usual meadows for egg laying and the nymphs that emerge migrate through walking or flying for short distances to prevent depletion of the suitable food ensuring their even distribution (Soomro *et al*, 2016).

Few numbers of grasshoppers and locusts were recorded in this study in comparison to other studies near the study area. For instance, Mungai *et al*, 1995, in Lake Baringo area, recorded seventy species, ten subfamilies and two families while this study recorded eighteen species, seven subfamilies and two families. This difference is attributed to the difference in the sample collection methods, size of study area and the time lapse between the two studies.

However, during this study, it was noted that there exists few grassland habitats and massive habitat degradation due to environmental degradation caused by soil erosion, overgrazing and human actions especially massive development activities which include construction of roads and buildings, population expansion, filling up the wetlands and agricultural intensification which use broad spectrum pesticides, herbicides and insecticides.

Habitat loss greatly interferes with invertebrates' species diversity because they are less mobile when compared to vertebrates, their lifecycles are short and their specificity to host plants makes them more specialized in microhabitats. This has resulted to death of small fauna and plants. This has broken down the equilibrium of the ecosystem which has symbiotic relationships with the grasshoppers and locusts which have reduced their population and therefore the little diversity, abundance and distribution reported.

3.5 Conclusion and Recommendation

This study documented some of the grasshoppers and locusts species that occupy the ecological zones of Nakuru County but many may remain undocumented. Sample collection was done once due to resources and time limitation. It is therefore recommended that diversity and distribution studies of Caeliferans be done in all seasons which will show the species that exist and also portray how different seasons affect diversity, abundance and distribution.

CHAPTER FOUR

LIFE PARAMETERS, FAT AND PROTEIN CONTENT OF THE LOCUST *Acanthacris ruficornis*

4.1 Introduction

Consumption of insects is practised by at least 2 billion people across the globe in at least 113 countries though this practice is more common in the tropical countries than temperate ones. They are a delicacy to some people while they provide the bulk of animal protein in others' diet (Oonincx, 2015). They are a healthy source of food compared to non - processed animal protein products because they have a fewer number of pathogens in their guts which have no active replication and this risk can be mitigated through effective processing (Mezes, 2018). They also have a high reproductive capacity because of short generation times as well as a high efficiency in food use which promotes faster growth (Herrera *et. al.*, 2020).

Grasshoppers and locusts comprise 13% of the total consumed insects and consumption involves both nymphs and adults. Their big size makes them conspicuous and therefore easily located and collected in their natural environment. Their large number of eggs ensures high fecundity (Doberman *et al*, 2017). There exist about 35 genera of edible short horned grasshoppers (DeFoliart, 1995). Their protein content varies from 52.1% to 77.1% (Das *et al*, 2009) while crude fat ranges from 4.3% to 22.9% (Bukkens, 1997). They require simple infrastructure and care for their rearing and also gain a lot of mass in a short time (Khusro *et al*, 2012).

Grasshoppers and locusts' consumers collect them from their natural environment which is threatened by various factors. These includes over exploitation, which occurs when the number of mature and immature collected exceeds regeneration capacity which happens when mature insects are collected before their first mating or before they lay eggs. In addition, habitat

degradation caused by deforestation, overgrazing and pollution reduces their abundance and distribution. Their availability is also seasonal which requires timing for collection, like when locusts form swarms (Rumpold and Schluter, 2015).

These limitations create the need for an efficient rearing system. Such a rearing system would ensure large amounts of locusts are produced in a continuous basis and aid in the provision of important nutrients especially proteins and fats. It would also relieve pressure on the wild population, allow for more controlled product regarding quality and quantity and help provide the suitable environmental conditions for growth and development (Ochiai *et al*, 2020).

They would also help identify areas from which contamination would arise especially chemicals, feeds, cleaning agents and the processing steps. When feed companies use insects as ingredients, large, reliable, high quality and continuous supplies are needed which can only be provide when insects are reared in large rearing facilities (Jansson and Berggren, 2015). The established rearing systems would provide new opportunities for mass rearing, processing and sale of insect products.

Insect life tables provide information on development, number of eggs laid and nymphs that hatch, survival and mortality of a species (Lee and Gillespie, 2011). They help to predict population levels and therefore serve as an efficient tool of studying population dynamics (Sun *et al*, 2015). They give the most comprehensive description of the growth, survival and fecundity. They analyze survival or mortality in different stages of life and also estimate reproductive and population parameters. This provides deeper knowledge of lifecycle, reproductive and population parameters which is necessary for mass rearing under laboratory conditions (Rueda *et al*, 2010).

However, there requires information on the life parameters of locusts and grasshoppers to help in establishment of a rearing system while the determination of the amounts of nutrients like protein

and fat of the nymphs in the different instars would provide information on the stage with the highest amount of nutrients. Construction of life tables would help in the understanding of the survival and mortality rates of the different growth stages. This study evaluated the life parameters and life tables of the locust, *Acanthacris ruficornis,* in Nakuru County with the aim that the information collected would provide the much needed information about their lifecycle which would form the basis for mass rearing. Fat and protein percentages of the different growth stages was also established to help determine the best harvesting stage which would be the stage with the highest percentages of the aforementioned nutrients.

4.2 Materials and methods

4.2.1 Study of life parameters

Adult locusts were collected from Njoro (Botanical garden of Egerton University), upper Subukia (Maseno) and Naivasha (Karagita) in Nakuru County, Kenya in January 2017. They were transported to Egerton University, Njoro where they were reared in the insects' laboratory located in the Biotechnology block. *Acanthacris ruficornis* collected from the field were reared in one cage. The rearing cages were made of aluminium with 50 cm length, 50cm width and 60cm height. At the top, there was a fine mesh for ventilation while another mesh was positioned on the lower side with a movable slide below it for excreta dropping and removal. On the right side, there was an opening for feeding and cleaning purposes. A wire stand (60 cm length) was installed vertically inside for relaxing, moulting and heat access purposes. Above the wire stand was installed a power bulb (75 watts) for providing heat. The front side was fixed with a transparent glass pane to provide a view of the locusts in the cages.

Figure 4: Rearing cage

Temperature in the cages was maintained at 30ºC using 75 watts bulbs while relative humidity was maintained at 30% ±2. They were fed on ten days old wheat seedlings of the Njoro BW2 variety. The wheat were grown in one-kilogram plastic pots and were watered daily and then fed to the locusts ten days post emergence. These were replaced daily irrespective of whether the seedlings have been fully consumed and were supplemented with wheat bran.

Figure 5: Egg tube, potted wheat plants and locust feeding on wheat seedlings.

Three glass laying tubes of 10cm length and 4cm diameter were filled with sand and placed under the laying openings at the bottom of the cage. This sand had been sieved to obtain the fine one which was then sterilized in an oven at 180^0C for 24 hours to kill microorganisms and then moistened using distilled water. On laying, they were covered with a transparent polythene paper, fastened with rubber bands or cell-tape in order to maintain humidity while small perforations were made on the upper side using a needle to provide aeration and also prevent water accumulation on the polythene. These tubes were incubated at 30^0C and monitored from the 20^{th} day to check for the emergence of nymphs. Once the nymphs emerged, they were reared to adulthood and their eggs collected. These were incubated and once they hatched, the nymphs were reared in one cage and when they matured, their eggs were collected and incubated and these formed the first generation that was studied. Eggs from one locust were counted together with the emerging nymphs. These were put in one cage and one cage represented a replicate. Locusts were reared in six cages which amounted to six replicates. Life parameters recorded included time taken by the eggs to hatch, number of eggs laid, the number of hatchlings, number of eggs that hatched and those that didn't, number of nymphs that survived and those that didn't, days taken to mate and the number of days taken to lay eggs.

4.2.2 Determination of Fat and protein content

Twenty locusts for determining fat and protein content were obtained from each cage. These included the second, third, fourth and fifth nymphal instars and were each obtained two days after moulting. They were killed through deep freezing and the inedible parts which included legs, wings and antennae removed. They were then oven-dried at 105°C for 24 hours and afterwards milled using a food blender. The resulting powder was divided into three samples and each sample formed a replicate.

4.2.2.1 Determination of crude fat content

Soxhlet method; AOAC (2012) 989.05; 933.05 was used for percentage fat estimation. 5 grams of the sample was weighed into the extraction thimbles and the initial weights of the extraction flasks taken. Fat extraction was done using petroleum spirit in apparatus for eight hours. The extraction solvents were poured and the extracted fat dried in a hot air oven for about 15 minutes before the final weights of the flasks with the extracted fat was taken. The following formula was used to calculate the Crude fat:

$$crude\ fat\ (\%) = \frac{weight\ of\ fat\ extracted}{weight\ of\ sample} * 100$$

4.2.2.2 Determination of crude protein content

AOAC Kjeldahl procedure 955.04 (AOAC, 1995) was used to determine percentage crude protein. 0.265 grams of the sample was weighed into a digestion flask together with a catalyst (5g of K_2SO_4 and 0.5g $CuSO_4$ and 15 mL concentrated H_2SO_4). The mixture was heated in a fume hood till the digest turned blue, signifying the end of the digestion process. The digest was then cooled, transferred to a 100mL volumetric flask and topped up to the mark with distilled water. A digestion blank with catalysts and acid was used as a control. Ten (10) mL of the diluted digest was transferred into the distilling flask and washed with about 2mL distilled water. Thereafter, 15 mL of 40% NaOH was added and washed with about 2mL of distilled water. Distillation was done to a volume of about 60mL distillate.

The distillate was titrated using 0.1 NHCl to an orange color of the mixed indicator which signified the end point. The nitrogen content was calculated as follows:

$$\%\ \text{nitrogen} = N(HCl) * \frac{(V_1 - V_2)}{S} * 0.014 * 100$$

Where:

V_1 = Titer for sample (mL); V_2 = titer for blank (mL)

N = normality of standard HCl solution (0.1);

S = weight of the sample taken (g)

Protein % = nitrogen x protein factor (6.25)

4.2.3 Life tables

Life tables were calculated according to Portilla *et al*, 2014. Where x = life stages; N_x = number of individuals alive at the beginning of the life stage; d_x= number of individuals dying in each life stage; l_x= total number of individuals surviving in each life stage; q_x= fraction of individuals dying in each life stage; p_x= fraction of individuals surviving in each life stage; L_x= number of days lived in each life stage; T_x= total number of days to be lived beyond age x where $T_x = L_x + (L_x + 1) + (L_x + 2)$ ……; ex = life expectancy (average time in days yet to be lived) of each life stage and is calculated as $e_x = T_x / p_x$

4.4 Data Analysis

Data from the six cages was summarized. Statistical analysis was performed in SAS® software version 9.1 where mean, standard error, coefficient of variation, minimum and maximum of the dependent variables were estimated to 2 decimal points. Protein, fat and life table data was summarized using Microsoft excel 2013. Poisson's regression was used to test the significance difference between instars for both protein and fat content.

4.5 Results and Discussion

4.5.1 Results

Life parameters of *Acanthacris ruficornis* are shown in Table 4. Eggs laid by individual locust was 158 ±2.65 with a minimum of 153 and a maximum of 162 and these took an average of 35.33 ±1.33 days to hatch. There emerged between 141 to 150 nymphs which translated to 91.78 ±0.62 hatching percentage with 8.22 ±0.62 percentage failing to hatch. The first locust reported to reach pharate adult took 33.33 ±2.33 days with all the locusts in the cage taking 32.12 to 40.49 days to reach the same stage. There was a survival rate of 73.97 ±1.88 % which ranged between 70.80 – 77.30%. They took 54.00 ±3.61 days to mate and 62.67 ±1.45 to lay eggs.

Table 4: Descriptive statistics for Life parameters of *Acanthacris ruficornis*

Variable	Mean ± SE	CV	Min	Max
Days eggs took to hatch	35.33 ±1.33	6.54	34.00	38.00
Number of eggs laid	158.00 ±2.65	2.90	153.00	162.00
Number of nymphs hatched	145.00 ±2.65	3.16	141.00	150.00
Eggs that didn't hatch	13.00 ±1.00	13.32	12.00	15.00
Percentage of eggs that hatched	91.78 ±0.62	1.16	90.57	92.60
Percentage of eggs that didn't hatch	8.22 ±0.62	12.99	7.40	9.43
Average days to reach pharate adult	36.88 ±2.48	11.66	32.12	40.49
Number of nymphs that survived	107.33 ±4.37	7.05	102.00	116.00
Percentage of nymphs that survived	73.97 ±1.88	4.40	70.80	77.30
Days taken to mate	54.00 ±3.61	11.56	47.00	59.00
Days taken to lay eggs	62.67 ±1.45	4.02	60.00	65.00

Key: SE= Standard error; CV=Coefficient of variation; Min= Minimum; Max =Maximum

The life table of *Acanthacris ruficornis* is shown in table 5. In each life stage, eggs, first nymphal instars, second nymphal instars, third nymphal instars, fourth nymphal instars, pharate adults and adults lived for 35.33, 5.22, 7.22, 10.22, 14.22, 47.34 and 30.22 days respectively. The life expectancy for eggs, first nymphal instars, second nymphal instars, third nymphal instars, fourth nymphal instars, pharate adults and adults were 38.49, 44.22, 14.14, 18.22, 24.89, 63.29 and 78.26 days respectively.

Table 5: Life tables of *Acanthacris ruficornis*

X (Life stages)	N_x	d_x	l_x	q_x	p_x	L_x	T_x	e_x
Eggs	158	13	145	0.082	0.918	35.33	35.33	38.49
First nymphal instars	145	12	133	0.083	0.917	5.22	40.55	44.22
Second nymphal instars	133	16	117	0.120	0.880	7.22	12.44	14.14
Third nymphal instars	117	5	112	0.043	0.957	10.22	17.44	18.22
Fourth nymphal instars	112	2	110	0.018	0.982	14.22	24.44	24.89
Pharate adults	110	2	108	0.018	0.973	47.34	61.56	63.27
Adults	108	1	107	0.009	0.991	30.22	77.56	78.26

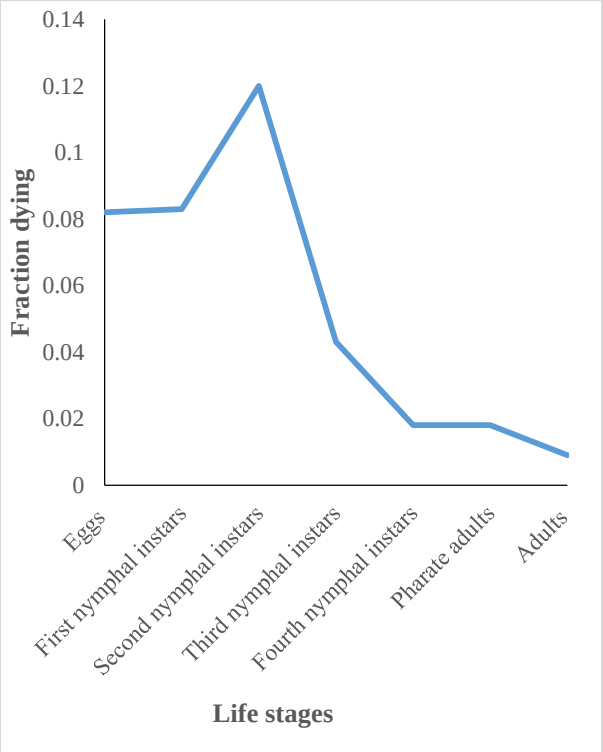

Figure 5a: Fraction of *Acanthacris ruficornis* dying in each nymphal instars

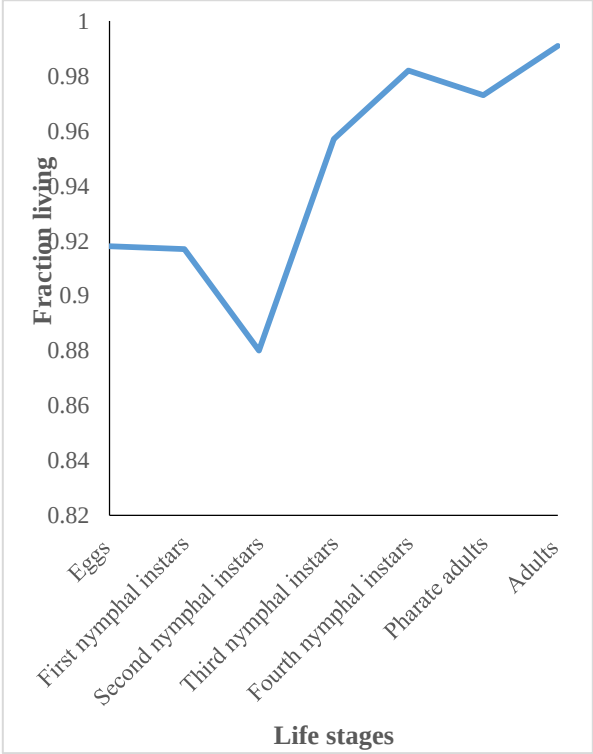

Figure 5b: Fraction of *Acanthacris ruficornis* surviving in each nymphal instars

Figure 6: Dying and surviving fractions of *Acanthacris ruficornis*

The fraction mortality rate of *Acanthacris ruficornis* is shown in Figure 5a. The second nymphal instars had the highest mortality fraction with 0.120 while adults had the lowest with 0.009. The fraction of individuals surviving in each life stage are shown in Figure 5b. Adults had the highest surviving fraction with 0.991 while second nymphal had the lowest with 0.880.

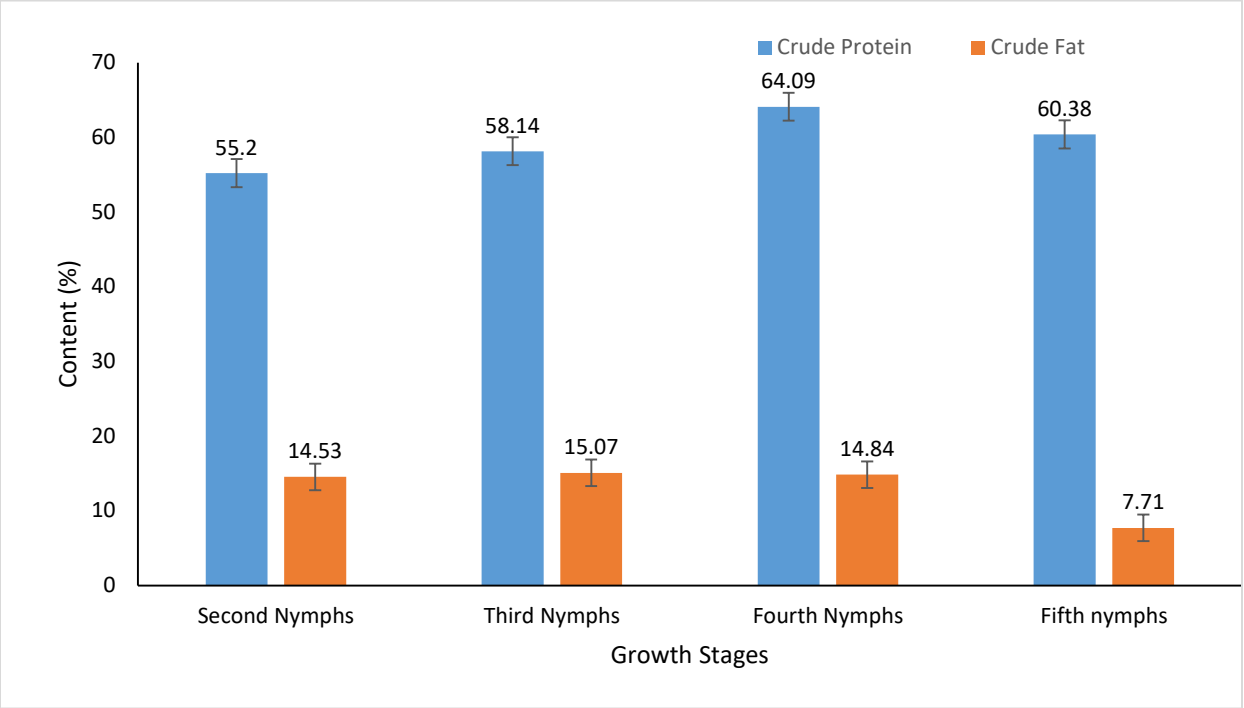

Figure 7: Crude fat and crude protein content of second, third, fourth and fifth nymphs.

Crude fat and crude protein content of the second, third, fourth and fifth instars are shown in Figure 6. Crude fat content was 14.53%, 15.07%, 14.84% and 7.71% in the second, third, fourth and fifth nymphs respectively. Crude protein content was 55.2%, 58.14%, 64.09% and 60.38% in the second, third, fourth and fifth nymphs respectively. The change between instars was significantly different for both protein and fat content as shown in Appendix 4. The fourth nymphal instar had the highest crude protein percentage while the fifth nymphal instar had the lowest percentage of crude fat.

4.5.2 Discussion

Acanthacris ruficornis laid an average of 158 eggs per pod. Other grasshoppers and locust species recorded include, *Dociostaurus maroccans* with an average of 34.83 eggs per pod (Baldacchino *et al*, 2012); Schistocerca *gregaria* with 49 (Schimdt and Albutz, 1994) and *Dichroplus maculipennis* which laid an average of 89 eggs (Mariottini *et. al.*, 2011). This species has a large body which provides a large space for packaging of a large number of ovarioles which contains a large number of developing oocytes and subsequent eggs (Jackson *et al*, 2011). Small grasshoppers may contain only four ovarioles in each ovary while big ones may have more than a hundred which explains more about the large number of eggs (Whitman, 2008). Fecundity is also thermal dependent and in the laboratory, insects produce more eggs as compared to those in the fields. This is because, the constant temperature provided in the laboratory allows for continuous activity especially feeding which results to substrate accumulation which is converted into eggs (Dempster, 1963).

Acanthacris ruficornis eggs took an average of 35.33 days to hatch and of the total number, 91.78 percent hatched while 8.22 percent didn't. Other studies reported that *Locusta migratoria* eggs took between 14 to 15 days to hatch (Tanaka, 2017), *Schistocerca gregaria* eggs took 72 days at 19^0c and 13 days at 13^0c (Hunter- Jones, 1964) and the *Chorthippus brunneus* eggs took 26 days (Cherrill, 2002). Egg hatching is affected by soil temperature, pod depth, pod orientation, soil moisture and biotic factors like pathogens in the soil (Guo *et al*, 2009). Pod depth and orientation influence heat accumulation inside the eggs and eggs at different depths have varying temperatures which results to different hatching times (Karpakakunjaram *et al*, 2002). It also influences moisture contents and therefore different rates of water absorption into the embryos and therefore different growth times (Symmons and Cressman, 2001). Pathogens feed on the eggs therefore destroying their structure (Woodman, 2015). In this study, egg pods

were provided with a constant temperature of 30^0C in the incubator and therefore the thermal unit was the same for the developing embryos at different depths in the egg pod. The sand used for laying was sterilized at 180ºC for 24 hours and then moistened with distilled water which eliminated pathogens. Since only one species was studied, it's therefore correct to assume all the factors were held constant for all the developing hatchlings therefore the high hatching percentage. However, this study noted that eggs at the bottom of the pod developed and matured to vermiform larvae but couldn't emerge through the soft foam due to compression in the sand. This also made rupturing of the chorion and wriggling out of the egg shell by the larvae difficult which explains the number of eggs that didn't hatch.

Survival rate was considered to be those nymphs that reached adulthood and was recorded as 73.97 ±1.88 %. In comparison with others, *Dociostaurus maroccanus* had a survival rate of 72.49 ± 0.82% (Quesada - Moraga and Santiago - Alvarez, 2001). There was a high mortality rate during the first ten days which later slowed down. The studied species lay eggs in moist soils and the saturation provides strong capillary forces which binds the soil particles together which makes it difficult for the hatchlings to wriggle to the soil surface. This results to fatigue during emergence from the eggs in the vermiform stage especially to those located deep in the egg batch which makes them weak. They were also poor in feeding and therefore deprived of nutrients due to weak mandibles and underdeveloped digestive system. In crowded populations, there results to cannibalism and increase in metabolic rate caused by lack of sufficient resting sites which results to limited access to food and therefore an increase in mortality rate.

The second, third, fourth and fifth nymphs had 55.5%, 58.14%, 64.09% and 60.38% of crude proteins respectively. Some of the listed species include *Schistocerca gregaria* whose adults had 56.97% of crude protein and 28.82% of crude fat while nymphs had 65.92% of crude protein and

15.15% crude fat (Wahed *et al*, 2019) and *Locusta migratoria* with 54.16% (Fombong *et al*, 2021). In insects, proteins increase in amount as the insect increase in size. A lot of protein synthesis occurs in larvae compared to adults in order to promote cell division (Gilbert and Schneiderman, 1961). These proteins form enzymes necessary for pigmentation, polymerization, molting and sclerotization (Chapman Reginald, 1982). These vary in amount and types between different instars of an insect and therefore the difference between the instars. In addition, proteins are used by adults to form adult structures which happens when larval stage is transforming into adults which reduces amount in the final instar (Andersen Svend, 2000).

The second, third, fourth and fifth nymphs had 14.53%, 15.07%, 14.84% and 7.71% of crude fat respectively. *Locusta migratoria* has 13% fat percentage (Kourimska and Adamkova, 2016). Lipids vary in quantity and composition depending on developmental stage of insects and their amounts are higher in the larval stages prior to metamorphosis. Holometabolous insect's larvae have a higher fat content than adults while hemimetabolous ones have less fat percentages compared to the adults. During metamorphosis, fat deposits are used as an energy source which results in lower fat content in adults (Whitman, 2008). In this study, the third and fourth nymphs had more fat amounts compared to the second and fifth nymphs which was the adult. This difference was caused by lipogenesis that involves anabolism of glucose into glycogen and the latter into lipids (Arrese and Soulages, 2010). A rise in lipids can also be caused by an increase in size which results to an increase in feeding and subsequently accumulation and increase in lipids, especially for those which can't be synthesized in their bodies (Gilbert and Chino, 1974). These two mechanisms indicate synthesis from the materials consumed. The species studied here were reared in cages and fed on wheat seedlings and wheat bran. The cages resulted in a high population density which increased contact and aggregation which resulted to increased activity

especially feeding which resulted to accumulation of lipids in the larval stages. However, in adults, there is a rapid increase in feeding during the first two weeks after becoming pharate adults which reduces afterwards (Hamadah, 2014). This reduction is maintained throughout the remaining life which means there is little accumulation of lipids and therefore the less amount as compared to the instars and adults. Utilisation of accumulated lipids in adults results to less lipids compared to the instars (Golebiowski *et al*, 2011). For instance, in vitellogenesis, the yolk deposited in eggs comprises of proteins, lipids and glycoprotein while courting and mating requires energy and fats being one of the respiratory substrate further reduces the amount of lipids in adults (Downer and Mathews, 1976). Large insects have relatively lower surface areas and hence less susceptible to surface losses like diffusion and heat transfer and therefore less prone to water loss (Beenakkers *et al*, 1985). They therefore require less fat in their bodies for oxidation and to prevent water loss which explains the difference between the large nymphs and the smaller nymphs.

4.4 Conclusion and Recommendation

Mortality rate was highest in the first nymphal instars and lowest in adults while survival rate was highest in the fourth instar and adults. This is consistent with the other locusts cited in this study. They also laid 158 eggs per pod which is ideal for mass production and therefore suitable for mass rearing. The third nymphal instars had the highest crude protein percentage followed by the fifth nymphs while the third nymphal instars had the highest crude fat percentage followed by the fourth nymphal instars and therefore these are the best harvesting stages in terms of the nutrients mentioned.

However, there were cases of cannibalism which contributed to mortality rate. Studying the causes of cannibalism in this study would help understand the cause of the behaviour and how it can be reduced. Growth rate should be determined at different temperatures, humidities and nutrition in order to determine whether the growth rate is hastened or slowed by a variation of the aforementioned factors.

Proximate analysis involved crude fat and proteins only at the different growth stages. However, amino acids and fatty acids profiling of the same would provide a more detailed source of proteins and lipids from the aforementioned locust species.

Insects collected from their natural environment and those reared in farms and laboratories may be infected with pathogenic microorganisms. Some of these are bacteria like Acinetobacter, Enterobactreiacea, Camphylobacter, Proteus, Staphylococcus, Pseudomonas, Bacillus and Escherichia. Others include Protozoa, Virus and Fungi which may be found on the insect cuticles' or in the gut. These may result to zoonosis and therefore pose zoonotic infections. Microbial analysis is necessary on the *Acanthacris ruficornis* studied in order to determine their microbial safety and eliminate any transmission risk posed to the consumers.

CHAPTER FIVE

GENERAL CONCLUSIONS, RECOMMENDATIONS AND FUTURE PROSPECTS

Nakuru County was chosen for this study because of the well outlined ecological zones which range from dry areas to wet areas. Some of the grasshoppers and locusts inhabiting these zones were identified. This information helped identify survival threats that these species face which would help draft conservation measures. This study also showed location and distribution of the locusts that possess potential for rearing.

Abundance was lowest in zone IV (24.3%) and highest in zone II (47%). This was attributed to the differences in ecological conditions with zone II having higher rainfall compared to zone IV which promotes growth of vegetation continuously and therefore availability of food and microhabitats which are crucial for survival, reproduction and distribution.

Aiolopus thalassinus was distributed across the three ecological zones. This species is phytophagous in nature, where it feeds on several families of plants which are spread across several areas. *Ornithacris pictula* (D = 0.66), *Gastrimagus verticalis* (D = 0.66) and *Heteropternis couloniana* (D = 0.66) were the least distributed and found in zone II only. Their monophagous nature, where they feed on a few species of plants, ensures they have limited food choices which also limits their distribution since these plants are found in certain ecological zones.

Zone II had the highest Shannon-Wiener diversity indices (H′) and Simpson's dominance index followed by zone III while zone IV had the least values. The higher the diversity the higher the H′ values. The lower the diversity the higher the dominance and high values will be obtained while the higher the diversity the lower the dominance and low D^{-1} will be obtained. Upper Subukia had the largest number of species with nine followed by Rongai with six. One species was found in Gilgil, Nakuru town and Mau escarpment and these were *Acrotylus patruelis*,

Aiolopus thalassinus and *Coryphosima stenoptera* respectively. It was hard to find a sampling site around Nakuru town due to lack of grasses caused by the massive construction while there was little vegetation in Gilgil which promoted the less diversity. In Mau escarpment, there existed lots of nymphs and was difficult to find adults. These were not sampled as it's usually hard to identify nymphs taxonomically.

This study included various diversity indices because a single one can't completely describe the diversity, distribution, dominance, evenness and similarity of the locusts and grasshoppers sampled. These indices were also easy to calculate and interpret. Shannon-Wiener diversity indices for species richness works well when comparing two or more study sites simultaneously and also when dealing with random samples. Samples were collected randomly in this study while three ecological zones were studied simultaneously and therefore most appropriate in this study. However, it's biased against small samples though the effect on the results is not significant. Simpson's dominance index is used to show the dominant species in a study and gives more weight to dominant and abundant species. It's not affected by a few rare species and therefore the three rare species sampled didn't have any significance on the results. Sorensen's index, CC and Jaccard index, S_J are used to show the species similar in two or more study sites. Sorensen compares the species composition based on the number of common species in two ecosystems and the number of species specific to each ecosystem. Jaccard index is used to compare ecosystems based on the presence or absence of species and the more species both ecosystems have the more they have in common.

Of the eighteen species recorded, three had significant biomass. The first one was *Acanthacris ruficornis* which was distributed in the three ecological zones. In zone IV, it was found in Karagita, Naivasha; in zone III it was found in Botanical garden of Egerton University, Njoro

while in zone II, it was found in Maseno, Upper Subukia. The second one was *Cyrtacanthacris tatarica* which was found in Botanical garden of Egerton University, Njoro in zone III and *Ornithacris pictula* which was found in Maseno, Upper Subukia in zone II. These three species have great potential for rearing in future.

The life parameters, fat and protein content of the nymphs of *Acanthacris ruficornis*, were determined. Several materials are used for making the rearing cages but this study used aluminium as it is easier to clean due to its bright colour and any stains on it would easily be noticed. It also doesn't rust, get damp or break easily compared to iron, wood or glass respectively. This reduces contamination from metal oxides, fungal attack and ensures the safety of the person rearing them. Fat and protein content was determined for all the nymphs except the hatchlings as they are tiny and won't provide any significant biomass.

An average of 158 eggs per pod were laid which took an average of 35.33 days to hatch. Of the total eggs, 91.78% hatched while 8.22% didn't. Nymphs took an average of 36.88 days to reach pharate adult, 54 days to mate and 62.67 days to lay eggs. The number of nymphs that survived averaged 107 which translated to 73.97%. These results indicate a large number of eggs and therefore a high fecundity while it took approximately 98 days for eggs to hatch, nymphs to mature into adults and lay other eggs. This locust therefore possess a short generation time. Studying the number of times the females lay eggs would also increase the potential of the locust in terms of providing enough biomass.

Survival rate could have been high but for the cannibalism. Locusts practice cannibalism for many reasons. For instance, they may be lacking certain minerals and therefore feed on other members of their species to gain them. Sometimes, they practice it when they are under high population density. In this study, cannibalism was very prevalent especially in the third and

fourth nymphs which reduced the numbers of the nymphs in the cages. Studying the causes would help come up with measures to control it which would ensure that a large number survive. In terms of crude protein and fat, the nymphal instars had an average of 59.45% and 13.04% respectively. However, the fourth nymphal instars had the highest crude protein amount with 64.09% while third nymphal instars had the highest crude fat with 15.07%. These were the best harvesting stages in terms of these nutrients. This together with being general plant feeders proves it can provide high quality protein and fat for inclusion in human entomophagy as well as livestock feeds. Establishing the ash content would provide information about the mineral composition of this locust.

There are various types of consumers. There are those who would wish to rear the locust while others would wish to buy them. For those wishing to rear, they worry about the space required together with the power costs especially with the unstable power prices. Locusts are reared in cages in a room and the room can be structured in a way that will hold many cages. As for power there are many types of power installed based on consumption like domestic and commercial with the latter being cheaper than the former. In addition, solar panels are gaining a lot popularity due to their reliability especially when used together with car batteries. Therefore, when cost analysis is done, it would help in providing the power costs which would help in alleviating power cost fears.

For those consumers wishing to buy the locusts, their worries lies in the safety of the products. This locust was fed on wheat seedlings grown for ten days because locusts feed while hanging on plants and it's important to stimulate the natural conditions in a laboratory set up. These were grown in plastic containers with perforated bases. Soil used is obtained from uncultivated areas in order to reduce the risk of contamination especially from agrochemicals. It's then mixed with

well rotten manure in order to increase the amount of nutrients in the seedlings. The challenge however is to obtain wheat without agro chemicals like pesticides which can accumulate in the consumers. With chemicals used for agricultural purposes being linked with cancer, studying the level of accumulation in this locust would help ensure its safety.

Consumers would also be worried about the microorganisms present in this locust and their potential harm. Studying the microorganisms present would help alleviate this fear. Meanwhile, some simple procedures have been found to reduce these microorganisms to acceptable levels. Before harvesting, the locust should be starved for twenty four hours which removes fecal material from the gut which might be harboring microorganisms. Thermal treatment methods are aimed at reducing the bacteria, protozoa, viruses and parasites present. Methods that use high temperatures include soaking the locust in distilled hot water at temperatures above 90ºC for at least ten minutes and boiling for five minutes in distilled water. Methods that use low temperatures include freeze killing and freeze drying done in freeze dryers which reduces water amounts to below five percent in the final products reducing multiplication of microorganisms.

There are also fears about stomach upsets and constipation or injuries resulting from the spines. These together with wings and antennae should be removed especially in the adults. However, harvesting of the young larval instars should be encouraged because they have a soft cuticle which ensures digestibility. Their spines are also soft which reduces the task of removing the spines which some consumers would deem tedious.

Wheat seedlings were chosen as feed because they have a lot of nutrients, easily available, have fast growth and are succulent. They were supplemented with wheat bran which contained 61.13%, 16.01%, 8.50%, 11.49%, 2.87% and 9.33% of carbohydrate, crude protein, crude fat,

crude fibre, crude ash and moisture respectively. The amount of fat and protein in the locust can still be improved though developing feed formulas with high amounts of nutrients.

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APPENDICES

Appendix 1: Photos of Grasshoppers and Locusts found in the ecological zones

Tripholidia conturbata

Pezocantantops impotens

Parasphena ngongensis

Cyrtacanthacris tatarica

Aiolopus thalassinus

Heteropternis couloniana

Coryphosima stenoptera

Paracinema tricolor

Rhaphotittha nyuki

Tylotropidius gracilipes

Gastrimargus verticalis

Ornithacris pictula

Acrotylus blondeli

Acanthacris ruficornis

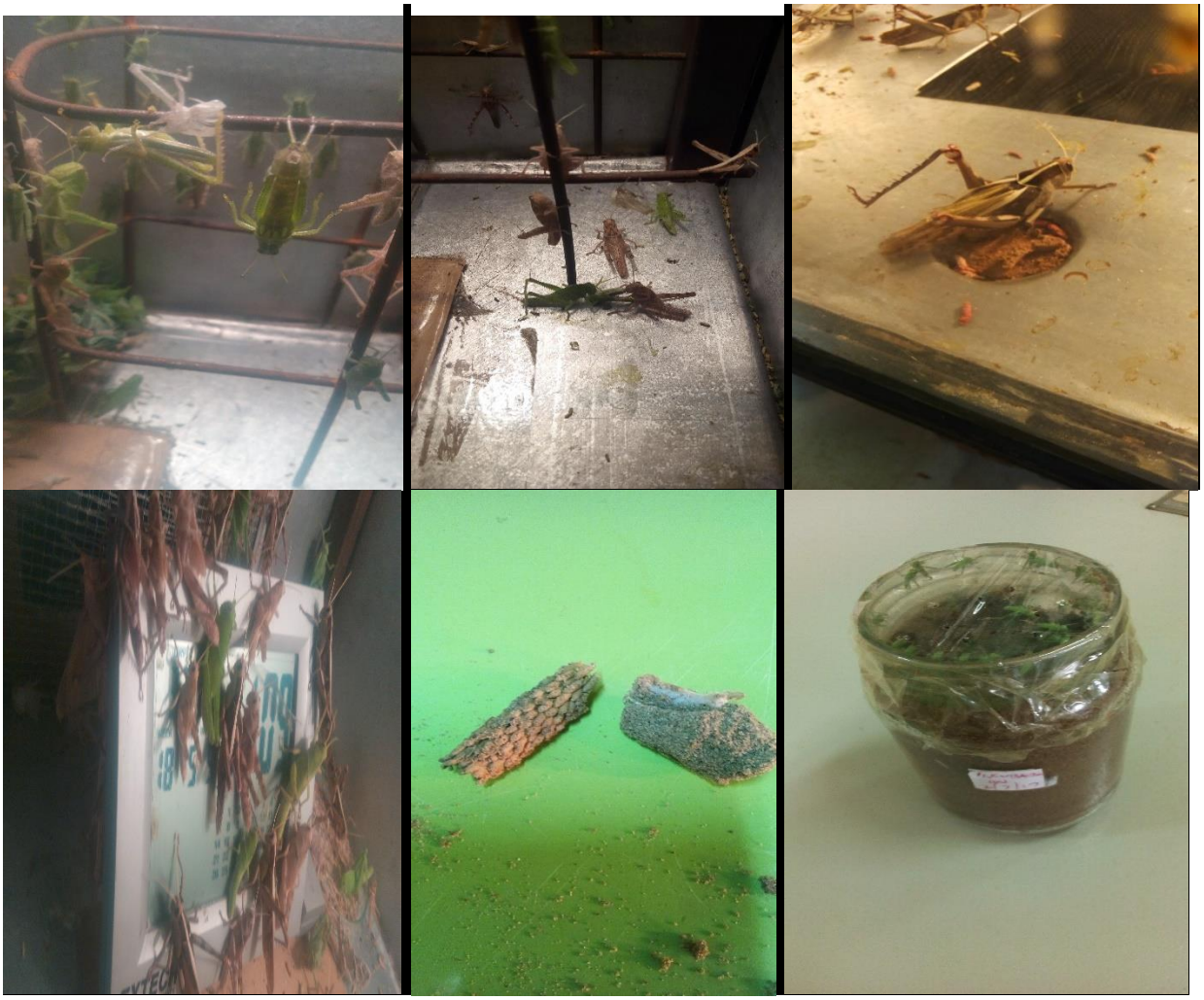
Acrotylus patruelis

Chrotogonus hemipterus

Pyrgomorpha conica

Sphingonotus turkanae

Appendix 2: Rearing photos for *Acanthacris ruficornis*



Appendix 3: Poisson's regression output of effect of ecological zone on indices

Least Squares Means for Shannon-Wiener indices

Effect	zone	Estimate	Standard Error	DF	Chi-Square	Pr > ChiSq	Alpha	Confidence Limits	
zone	four	0.2624	0.8771	1	0.09	0.7648	0.05	-1.4566	1.9814
zone	three	0.3148	0.8544	1	0.14	0.7125	0.05	-1.3597	1.9893
zone	two	0.8920	0.6402	1	1.94	0.1635	0.05	-0.3627	2.1467

Least Squares Means for Simpson's indices

Effect	zone	Estimate	Standard Error	DF	Chi-Square	Pr > ChiSq	Alpha	Confidence Limits	
zone	four	-1.3704	1.9842	1	0.48	0.4898	0.05	-5.2594	2.5185
zone	three	-1.1616	1.7874	1	0.42	0.5158	0.05	-4.6648	2.3417
zone	two	-2.3539	3.2444	1	0.53	0.4681	0.05	-8.7128	4.0051

Appendix 4: Poisson's regression output of effect of instar on protein and fat

Poisson's Least Squares Means for proteins

Effect	instar	Estimate	Standard Error	DF	Chi-Square	Pr > ChiSq	Alpha	Confidence Limits	
instar	FIRST	3.9375	0.0025	1	2.41E6	<.0001	0.05	3.9325	3.9425
instar	FOURTH	4.1012	0.0023	1	3.08E6	<.0001	0.05	4.0966	4.1057
instar	SECOND	4.0629	0.0024	1	2.91E6	<.0001	0.05	4.0582	4.0675
instar	THIRD	4.1602	0.0023	1	3.36E6	<.0001	0.05	4.1558	4.1647

Poisson's Least Squares Means for fat

Effect	instar	Estimate	Standard Error	DF	Chi-Square	Pr > ChiSq	Alpha	Confidence Limits	
instar	FIRST	2.6764	0.0238	1	12596	<.0001	0.05	2.6297	2.7232
instar	FOURTH	2.0425	0.0327	1	3891.6	<.0001	0.05	1.9783	2.1067
instar	SECOND	2.7125	0.0234	1	13412	<.0001	0.05	2.6666	2.7584
instar	THIRD	2.6971	0.0236	1	13058	<.0001	0.05	2.6508	2.7434