MODERATE GENETIC VARIATION AND POPULATION FRAGILITY IN BLACK RHINO (*Diceros bicornis michaelli*) OF LAKE NAKURU NATIONAL PARK

By

DAVID NJOROGE THUO

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN ANIMAL GENETICS AND BREEDING IN THE FACULTY OF VETERINARY MEDICINE DEPARMENT OF ANIMAL PRODUCTION

UNIVERSITY OF NAIROBI

2015

DECLARATION

I David Njoroge Thuo declare that this thesis is my own work and has not been submitted before for any other degree or examination at any other institution of tertiary education.

Signature……. Date……

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

Signature…… Date……..

DR. J.O JUNGA (PhD)

Department of Animal Production

University of Nairobi

Signature…… Date……..

DR J.M Kamau (PhD)

Department of Biochemistry

University of Nairobi

Signature…… Date…….....

Dr K. E. Githui (PhD)

Molecular Genetics Laboratory

National Museums of Kenya

ACKNOWLEGEMENTS

I am grateful to the National Museums of Kenya Molecular Genetics Laboratory staff especially Eunice and Muriithi for their invaluable support and guidance during my laboratory work.

I am thankful to my supervisors, Dr Junga, Dr Kamau and Dr Kem for their support, guidance and extensive reviews of the preliminary and final draft of this thesis. Dr Amimo and Kenya Wildlife Service also deserve special mentions and thanks for their input in sample collection.

I extend profound thanks to Prof Tom Gilbert of the University of Copenhagen for providing financial assistance, without which this study would not have been a success.

To my family I will forever be indebted for your unending support and patience with this degree and helping progress my career aspirations. Special thanks to my classmates Evans and Cynthia who made the course interesting and enjoyable.

Lastly, but most important, I thank the Lord my God for granting me both the patience and resolve to push on when I felt that it was a hopeless scenario-it is only the Lord who can fully appreciate the trial that this has been.

DEDICATION

This thesis is dedicated to my beloved parents Mr. and Mrs. Johnson Thuo Wairiri, who are ‘my number one fans’. They have relentlessly pursued the best educational opportunities for me. Without their personal sacrifices for my education, I am convinced I would not be where i am today.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDICES

LIST OF ABBREVIATIONS

µM Micro molar

AFLP Amplified fragment length polymorphism

AfRSG African rhino specialist group

AMOVA Analysis of molecular variance

BINJ Neighbor joining

BLAST Basic local alignment search tool

Bp Base pairs

D.b Dicerosbicornis

D-Loop Mitochondrial Displacement loop

DNA Deoxyribonucleic acid

DNAsp DNA sequence polymorphism

EDTA Ethylenediaminetetraacetic Acid

H Haplotype diversity

IRF International rhino foundation

IUCN International union foor the conservation of nature

KWS Kenya wildlife service

LNNP Lake Nakuru national park

MtDNA	Mitochondrial deoxyribonucleic acid
Mya	Million years ago
NCBI	National centre for biotechnology information
Ne	Effective population size
PCR	Polymerase chain reaction
PVA	Population viability analysis
RAPD	Random amplified polymorphism DNA
rRNA	Ribosomal ribonucleic acid
SSC	Species survival commission
TAE	Tris Acetate EDTA
tRNA	Transfer ribonucleic acid
WWF	World wildlife fund
II	Nucleotide diversity

ABSTRACT

The black rhino (*Diceros bicornis*) is critically endangered. Consequently, the species is managed in parks and is often translocated to expand their range into areas where they have been extirpated. Management of genetic variation has been identified as an important consideration in long-term conservation strategies for many wild species including black rhino. In this study I aimed at determining the extinction risk for the black rhino in Lake Nakuru National Park, Kenya so as to provide information that can be incorporated into management decisions to improve the long-term viability and persistence of the population.

Sixteen individuals from Lake Nakuru National Park subpopulations were randomly sampled for this study. General standard molecular methods were employed. Genetic information was obtained from 572 base pair mitochondrial D-loop sequences; a population viability analysis was also conducted using quantitative data of black rhino from Lake Nakuru National Park.

The Mitochondrial DNA marker (572bp) revealed 13 polymorphic sites and 6 haplotypes. Only two haplotypes (Hap1 and Hap 2) were shared by the sampled individuals, the marker detected a moderate genetic diversity (h=0.742±0.084) and a relatively lower nucleotide diversity (π= 0.0079 ± 0.0008).

The population viability analysis baseline simulations showed that Lake Nakuru National Park black rhino has a 0.00 probability of extinction during the next 75 years. However, continuing threats make this subpopulation highly vulnerable to any change. Sensitivity simulations of anthropogenic impacts showed that small increases in habitat loss (2%) and population harvesting (3%) had drastic effects on population decline with a 100% probability of extinction. Findings from this study suggest that black rhino in Lake Nakuru National park maintain a moderate level of genetic diversity and is currently not under risk of extinction or population

decline. However, the need for conservation actions focused on preventing poaching, modulating translocation program and promoting the conservation of available habitat is imperative.

CHAPTER ONE

INTRODUCTION

1.1 Background

Wildlife based tourism is a key economic activity and a major foreign exchange earner in Kenya. However, anthropogenic instigated factors like poaching, land degradation and fragmentation have drastically reduced the population size of numerous large herbivores over the past two centuries thereby risking the potential of this industry.

The black rhinoceros (*Diceros bicornis*), hereafter referred to as black rhino, has suffered one of the most dramatic decline of all mammals in the recent history (Gamier *et al*, 2001). The species currently categorized as critically endangered under the criteria of the International Union of Conservation for the Nature Red List (IUCN, 2012) is believed to have thrived in excess of hundreds of thousands only a century ago (IUCN SSC AfRSG, 2008, CITES, 2011). By 1992 the number were globally decimated to a low population size of approximately 2300 individuals (Walpole *et al*, 2001, International Rhino Foundation, 2006). In Kenya alone black rhino numbers decreased catastrophically from an estimated 20,000 individuals in 1970 to 550 in 1984, 381 in 1987 and, 398 in 1991 to about 631 in 2013 (Kenya Wildlife Service rhino strategic plan 2012-2016, 2012). The decline in population has or is associated with smaller herds than those recorded historically.

It is well established that when populations become small and isolated they are more likely to decrease in the level of genetic variation due to the effects of inbreeding depression and/or genetic drift thus, reducing their evolutionary potential and increasing the risk of extinction (Frankham,2005, Garner *et al,* 2005). Inbred offspring have low total fitness and tend to die young further affecting the demography of the small population and thus making it smaller..

It is therefore imperative that the level of endangerment of these small isolated populations of black rhino be assessed and informed management recommendation made so as to reduce the chances of extinction. In this study, mitochondrial DNA D-Loop region which is the most variable region in the mtDNA was used to determine the level of variation within the *Diceros bicornis michaeli* source population at Lake Nakuru National Park. In addition, population viability analysis (PVA) simulations were done using individual-based program (VORTEX 9.50), to assess the anthropogenic impacts that are essential for the successful development of conservation actions for the long-term survival of this species.

1.2 Statement of problem

Tourism is one of the largest sectors of Kenya's economy accounting for about 11% of the country's gross domestic product (Kenya National Bureau of Statistics, 2013), with wildlife tourism forming the major backbone of the industry.

Black rhino has suffered one of the major declines in the recent history due to both human-mediated and natural factors. Consequently, comprehensive management strategies have been adopted; translocation, corporal protection and ear tagging. Nonetheless, the recovery rate has been insignificant thus rendering the population to an extinction risk which will adversely affect the country's economy. The question the rhino management team is asking; could the reduced

recovery rate (increase in number) due to ecological, human-wildlife interaction, increased tourism number in the park, genetics and or a combination of one of the above?

1.3 Justification

Species decline may be caused by many factors which include: habitat loss, poaching, climate change and loss of genetic variation. Indeed, variation is the raw material for evolutionary forces hence its loss reduces animal's adaptability to changing environmental conditions.

The black rhino in Lake Nakuru National park is a breeding nucleus population thus its significant value in restocking other subpopulations in the country. The loss of genetic variation of this population will directly pose danger to the survival of the whole Kenya's population. This study aim at assessing the level of genetic variation within the Lake Nakuru population using mitochondrial DNA and also conduct a population viability analysis. The information thereof will help conservation managers make informed decisions on the sustainable management of this species that will ultimately lessen their risk of extinction.

1.4 Overall objective

To determine the extinction risk for the black rhino in Lake Nakuru National Park

1.4.1 Specific objective

1. To quantify the level of genetic variation of black rhino within Lake Nakuru National Park.
2. To determine how anthropogenic factors such as habitat loss and harvesting would affect the population decline of the black rhino in Lake Nakuru National Park.

CHAPTER TWO

LITERATURE REVIEW

2.1 Role of wildlife to Kenyan economy

Wildlife managed by the Kenya Wildlife Service (KWS) forms the backbone of the tourism industry, since most visitors come first and foremost to view wildlife (Udoto, 2012). KWS accounts for 90% of safari tourism and about 75% of the total tourism earnings. Indeed, wildlife tourism is the proverbial goose that lays the golden egg in Kenyan economy. Tourism is the second largest sector of Kenya's economy, accounting for about 11% of the gross domestic product (GDP) making it the third largest contributor after agriculture and manufacturing (Kenya National Bureau of Statistics, 2013). It is also the Kenya's leading foreign exchange earner, generating about 96 billion shillings in 2012 which was a 1.92% drop from the 97.5 billion shillings obtained in 2011 (Kenya National Bureau of Statistics, 2013).

In addition, tourism and travel directly and/or indirectly supports approximately 313,500 jobs which accounts for about 4.8% of the total employment in Kenya (WTTC, 2012) Therefore, wildlife conservation is inextricably linked to Kenya's economic development and the livelihood of its people (Udoto,2012).

2.2 Study species

The black rhino is the third largest mammal in Africa after the African elephant (*Laxodonta africana*) and the white rhino (*Ceratotherium simum*). It has a total body length of approximately 3.5 meters and an average body weight of 1400 kilograms (Emslie and Brook, 1999). Like the white rhino the black rhino has two prominent horns with the front one growing as long as 1.4

meters. Unlike their Asian counterpart (Sumatran, Javan and Indian rhino) the African black rhino lack the incisors and canine teeth (Emslie and Brook,1999), according to Lacombat (2005) their low crown (brachyodont teeth) enable them to browse on course plant material like long grass, twigs and leaves.

Four extant subspecies are currently recognized based on slight morphological differences and geographic distribution (Du Toit, 1987, Kim *et al*, 2011); *Diceros bicornis bicornis* (South-western), *D.b. minor* (South-eastern), *D.b. michaeli* (Eastern) and *D.b. longipes* (Western). Distinguishing the sex of a rhino in the field can be difficult because males have undescended testes and, therefore, lack a scrotum (Kingdon, 1997). The genitalia of both sexes face backwards and they are capable of projecting urine up to three to four meters (Schenkel and Schenkel-Hulliger, 1969). Black rhino tend to be a social and while female home ranges overlap, males tend to live in mutually exclusive home ranges (Owen-Smith 1988; Conway and Goodman 1989).

In the wild, males attain sexual maturity at 7-9 years with females reaching at between 4-6 years. The first parturition of females in the wild is estimated at around 5 years, but in some population it might take up to 12 years.(Adcock and Amin, 2006),while the average age of male at first reproduction is 10 years and 1 month (EAZA,2009).

The gestation period is approximately 15 months (Bertschinger,1994) with the cow giving birth to a single calf weighing approximately 35-50 kilograms. Weaning occurs at around 2 years of age for the offspring. The mother and calf remain together post-weaning for an additional 2-3 years until the next calf is born. In the wild both sexes can reach an age of 40 years. However, in captivity a male black rhino has reached an age of 49 years (Felts, 2007, MacDonald, 2004

2.3 Origin and distribution of black rhino

Black rhino is believed to have diverged from the Asiatic two horned rhino approximately 14 million years ago (Hooyer, 1976), it is considered more primitive and older than its African counterpart (African white rhino) which diverged from the latter between 2 and 5 million years ago (Lacombat, 2005).

Historic distribution records show that hundreds of thousands of black rhino ranged across the sub-Saharan Africa only a century ago (Figure 2.1). Many countries like Ghana. Nigeria, Togo, Ivory Coast, Benin, Mozambique, Chad, Burkina Faso and Sudan have since lost their rhino populations altogether due to poaching and habitat loss (IUCN, 2008).

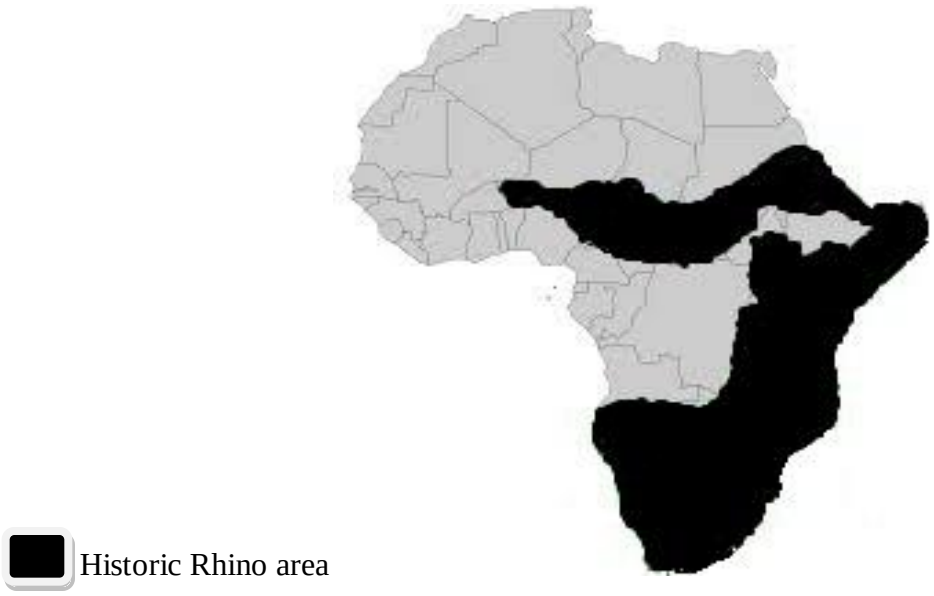

Figure 2.1.Map showing the historic distribution of black rhino (*Diceros bicornis*) in Africa (Adopted from International Rhino Foundations, 2008)

According to the IUCN (2008) there are five extant subspecies of black rhino living in different parts of Africa. However, the existence of one of the five surviving sub species (*Diceros bicornis bruceii*) is still not confirmed with data presented at the IUCN-SSC-AfRSG meeting (2004) showing that four individuals might be surviving in Ethiopia. These four recognized subspecies exhibit different ecological partitioning in exploiting different habits but they overlap in some areas.

The Eastern subspecies (*Diceros bicornis michaeli*) which is the focus in this study have its stronghold in Kenya (80.3%) with few numbers found in Northern Tanzania (Okita-Ouma *et al,* 2007). In the beginning of the 20th century Kenyan *D.b michaeli* were so abundant that they were viewed as agricultural pest that potentially impaled human land use (Brett, 1993) according to IUCN SSC AfRSG (2008), in 1970's the species numbered over 20,000 and had a wide distribution in Kenya. However, accelerated poaching and human settlement led to a dramatic decline of black rhino to less than 400 individuals in 1990's (Okita-Ouma *et al,* 2007). This population collapse resulted in small, isolated, demographically inviable subpopulations scattered across fragmented regions in Kenya with many facing local extinctions (Muya *et al,* 2011). Consequently, Kenyan population has been categorized into 16 sub populations (Figure 2.2) distributed on State, private, county council and community lands (Figure 2.3) across the country.

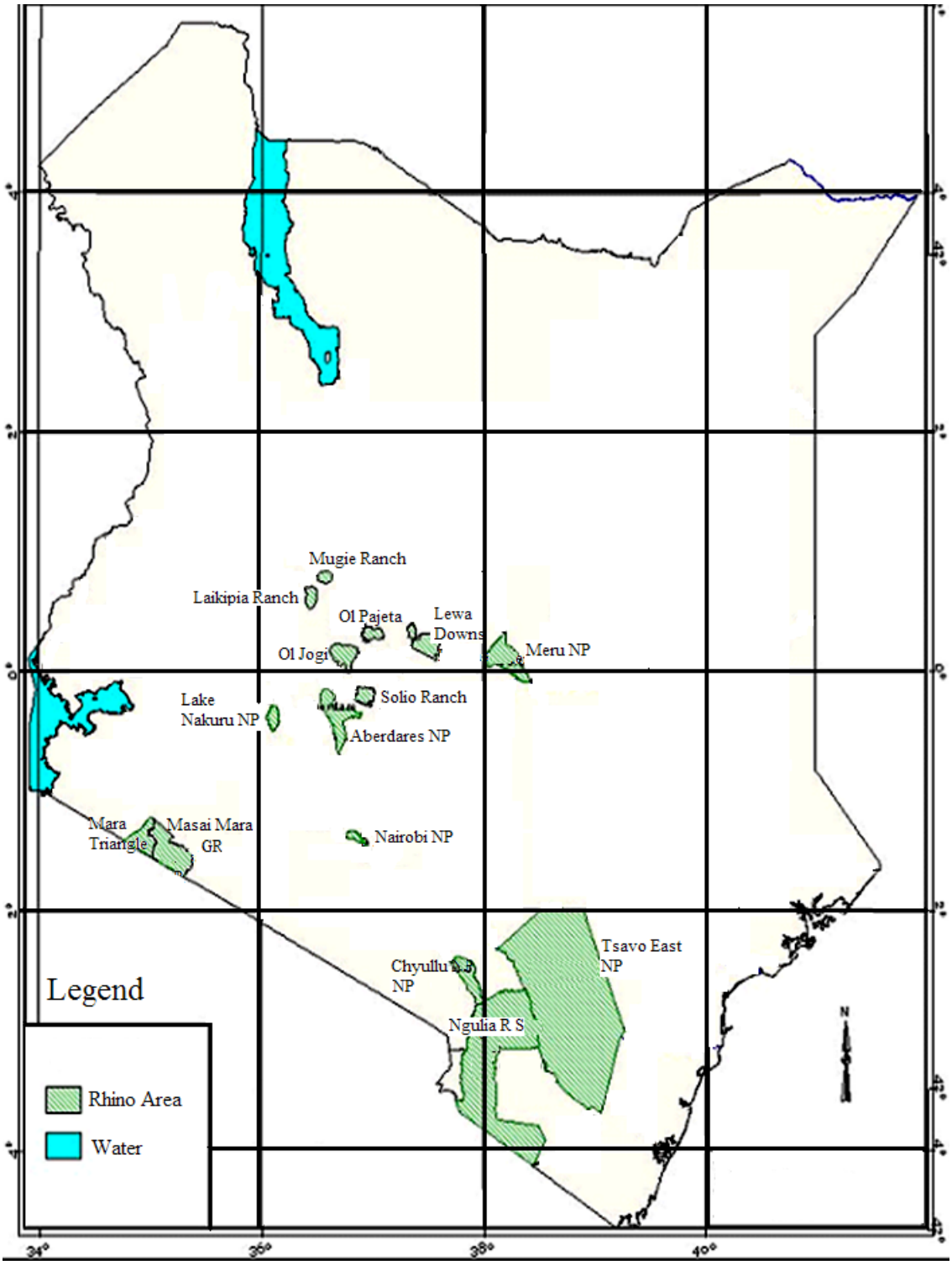

Figure 2.2 Map showing the location of the extant 16 subpopulation of black rhin in Kenya.Sourced from KWS GIS Department, 2014

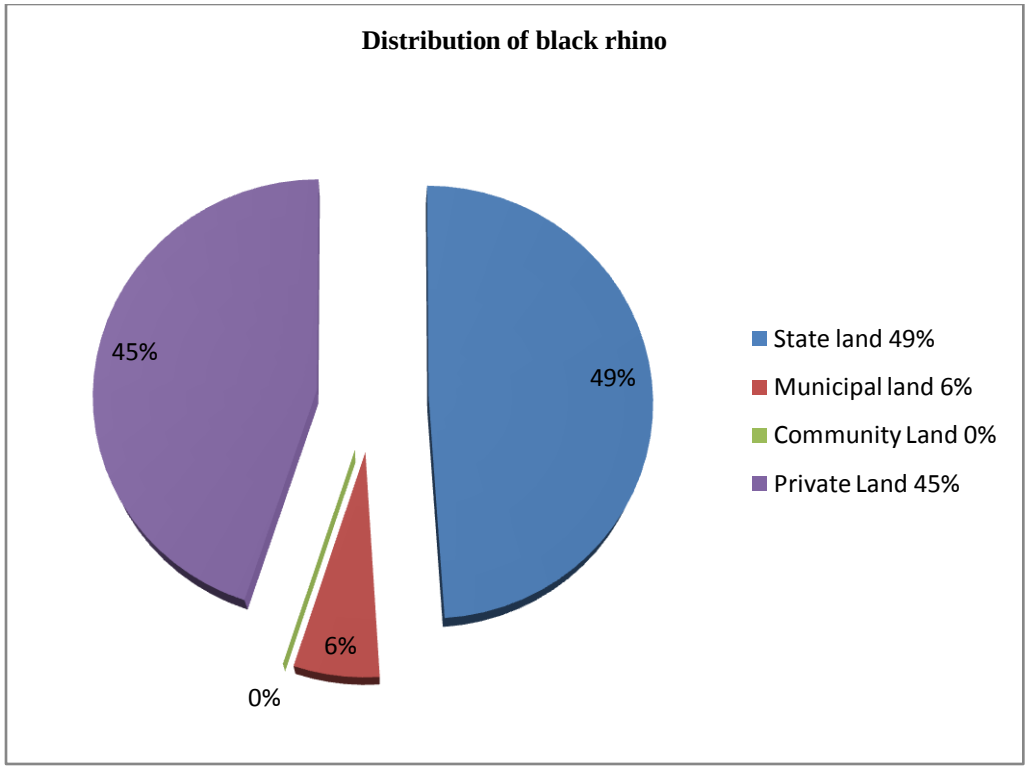

Figure 2.3 A Pie chart representation of the distribution of *D. b. michaelio*n different lands in Kenya. Sourced from Kenya Wildlife Service, 2015.

2.4 Threats to rhinos

The Black, Javan and Sumatran rhino has been classified as critically endangered while the Indian and the White rhino has been classified as endangered and near threatened respectively by The World Conservation Union (IUCN) hence all the rhino species has been considered as "at risk" of extinction (IUCN, 2007).

The major threat to this species has been attributed to poaching for their highly valuable horn (Hsieh *et al.* 2003, Amin *et al.* 2003, Bollongino *et al.* 2003). The rhino horn powder is used for traditional medicine and has been superstitiously believed to cure a variety of ailments that range from impotence, snake poisoning, headache, cancer, fever to evil possession in the Far East

(Costa-Neto, 1999 and 2004, Martin and Vigne, 2003) with China, Hongkong and Taiwan nationals listed as the major Asian importers of African rhino horns (Martin and Vigne, 2003)

Rhino horn and skin are also prized for use in ornamental armor and weaponry in a variety of Asian cultures (Emilee, 2004; Rookmaaker, 2005), for example in Yemen rhino horn is used to make ornamental handles for Jambiyas (Daggers) which are associated with wealth and good luck, studies characterizing bush meat harvests (Bulte and Damania, 2005; Wato *et al.*,2006) identified rhino meat as a product. Though there is no scientific evidence that the rhino horn can cure any disease traditional use continues to draw demand and hence leading to the increase in poaching (Martin and Vigne, 2003).

Habitat loss and fragmentation as a result of the ongoing human growth and associated activities like land clearing for farming, logging and industrialization has also threatened the survival of rhino in a great way (Dierenfeld *et al.*, 2006; Dean and Foose, 2006; Dixon *et al*, 2007; International Rhino Foundation 2008).for sustainable conservation of the rhino species both genetic and ecologic factors should be put into consideration.

2.5 Causes of low genetic diversity in small population.

Primarily, larger populations are likely to harbor more individuals with variable alleles (Frankham *et al*, 2002; Freeland, 2005; Mills, 2007). Therefore, when they are reduced into smaller units by habitat fragmentation, catastrophe or poaching there is a possibility that the resultant subpopulation will only contain a subset of the total alleles and hence have a lower genetic diversity compared to the larger original population (Muya *et al*, 2011).

In small population, closely related individuals are likely to breed due to lack of alternative sources of mate hence increasing the chances of rare and injurious recessive alleles coming together and conferring homozygous disadvantage to the inbred offspring (Muya *et al*, 2011).

Frankham (1998, 2002) reported an increase in the rate of extinction in inbred populations of laboratory and domestic animals. Indeed, it was also noted that the population reduces further as they continue to inbreed. This phenomenon is supported by the extinction vortex which shows there is a feedback between reduced population, loss of genetic diversity and inbreeding (Frankham *et al*, 2002)

Genetic drift is the random change in allele frequency that occur because gametes transmitted from one generation to the next carry only a fraction of the allele present in the parental generation (Norman *et al*,1993) and it happens simply as by chance (Freeland,2005). This is more likely to happen where reproduction success within a population is variable, with some individuals producing more offspring than others. As a result, not all alleles will be reproduced at the same extent, and therefore allele frequency will fluctuate from one generation to the other in a non-adaptive random manner (Freeland, 2005). Every population experience genetic drift but it is more profound in small population (Norman *et al*,1993), and in absence of selection, drift will drive each allele to either fixation or allele extinction within a short period of time leading to an inverse overall effect of decreased genetic diversity.(Frankham *et al*, 2002; Freeland, 2005; Allendorf and Luikart, 2007).

However, small and isolated population of large mammal may retain a higher genetic diversity for a relatively long period of time, particularly if it has a history of a once large outbred population that has undergone a recent decline or if genetic drift is facing an opposing selection balance or by selection against inbred individuals (Chikhi and Bruford, 2005; Goossens *et al*, 2006). One way of reducing the effects of genetic drift in small populations is by maximizing their effective population size (Frankham and Ralls, 1998), through managing them as metapopulations. Although translocation could be the best method in achieving the objective it

may not always be advisable to translocate individuals between two genetically distinct subpopulations since this could lead to the break-up of allelic combinations that have been combined through local adaptation in the remaining subpopulations (Allendorf and Leary, 1986; Muya *et al*,2011) therefore the sustainable management of endangered species requires thorough knowledge on genetic variation of the extant individual.

2.6 Management and conservation strategies

After the realization that wildlife in Africa was declining at an alarming rate in the early 1990's British conservationists formed the society for the preservation of the Fauna of the Empire in 1903 (Akama, 1998). This played an instrumental role in conservation education and in sensitizing the public and the British government on the social and ecological value of conservation. The society advised the British government to start nature reserves in its colony so as to protect wildlife from the increasing habitat destruction. Consequently, the British government appointed a game committee which was to study and make a recommendation regarding the set up of game parks in Kenya (Nash, 1982). In 1945, the recommendations made by the game committee were approved by the British legislation and the pioneer national park (Nairobi-1946, Amboseli-1947 and Tsavo-1947) was created (Akama, 1998). Formation of these national parks has since led to adoption of several wildlife management strategies.

2.6.1 Management practices in respect to rhino

A translocation is a deliberate and mediated movement of wild individuals or populations from one part of their range to another (IUCN, 1995). This conservation tool has been used in the reintroduction of animals to areas where they have been suppressed by natural catastrophes

and/or anthropogenic factors (Singer *et al*, 2000), and in genetically augmenting existing population (Yamamoto *et al*. 2006).

Even though rhino species has undergone a population bottleneck which may imply that translocating individuals from this populations might cause a loss in genetic variation, establishing a founder population with individuals from different populations of the same subspecies or one's with high genetic variation may be more likely to be successful (Liberg, 1993).

The first successful black rhino translocation into Lake Nakuru National park was in 1986 where one black rhino was translocated from Lewa ranch and in 1988 one black rhino *was* successfully translocated from Lake Nakuru National park to Lewa ranch, followed by another 10 individuals to Mugie ranch in 2004 and 9 individuals to Meru National park in 2006, (KWS rhino programme census data, 2010). This management technique has been recommended as one of the guidelines in the conservation of rhino by the IUCN (Emslie *et al*, 2009).

Physical protection of individual population through corporal protection, rhino dehorning and remote monitoring of individuals with satellite and radio collar have been adopted in the conservation and management of the black rhino. Despite the risk and dangers associated with corporal protection, this technique has yielded successful impact on rhino conservation to date (Linklater, 2003; Hilborn *et al.* 2008). Most of the success in the recovery of the southern white rhino has been attributed to this protection (Amin *et al*, 2006). Conversely, this strategy is difficult and expensive because rhinos are often dispersed over wide geographical areas making individual monitoring and protection impossible (Hilborn *et al.*, 2006; Talukdar *et al*, 2007).

Rhino dehorning has set a platform for criticism with some conservationists supporting the idea while others disagreeing. Support for the rhino dehorning is based on the argument that it

prevents poaching as well as reducing intra-specific mortality from horn wound incurred during fighting (Berger and Cunningham1994). On the other hand, dehorning was thought to expose the calves of the dehorned mother at more risk to predators than their horned counterpart but this has not been conclusively supported (Brain *et al*,1999). Contrary to the study done by Lindeque and Erb (1996) that showed rhino horns have a wide-range and therefore are unlikely to be a measure of evolutionary significance, Garnier (2001) suggested that horn size is a measure of sexual selection or social dominance and dehorning the rhino would negatively affect the overall survival.

Even though radio-collars have been successfully used in tracking a variety of animal species like fish (Jepsen *et al,* 2001) this approach has yielded limited success in rhinos (Linklater, 2003; White *et al.* 2007) with the setback attributed to false transmission rate, injury to the rhino by the collar and ineffective design thus allowing the collar to slip off (Alibhai *et al.* 2001; Dinerstein *et al,* 2001). Moreover, this technique is considered invasive since its attachment requires tranquilization, which can lead to reduced fertility rate and in some cases death of the rhino (Dinerstein *et al.* 2001; Alibhai *et al.* 2001; Linklater, 2006).

As black rhino recovery continues the focus on the population growth (number of black rhino) need to be combined with that of population quality (population size and genetic variation) so as to produce a population with long-term capacity to respond to changes in environment.

2.7.1 Rhino conservation genetics

Conservation genetics encompasses both basic and applied approaches and uses a combination of several disciplines; molecular biology, population genetics, mathematical modeling, ecology, and evolutionary systematic. These provide comprehensive information on the level of genetic diversity within a species of conservation concern from the relationship among individuals

within single populations to consideration of the evolutionary affinities among related species (Frankham *et al.* 2004). If genetics is not incorporated in conservation, efforts may be directed to the wrong population or waste of valuable resources on a population that is not at risk of losing its biological diversity despite a population decline.

Habitat fragmentation and overexploitation are the primary reason for species decline and extinction (Allendorf and Luikart, 2007). Current decline of populations have prompted the conservation managers to adopt new management strategies among them reintroduction of species to enhance recovery rate (Nielsen *et al*, 2002; Gibbs *et al*, 2008) If the remaining number of individuals in a population of an endangered species is extremely small or in recovery, genetic considerations may be overlooked. Consequently, making it to be prone to loss of genetic diversity and inbreeding which may affect their evolutionary potential (Frankham, 2005).This limits a population's suitability or adaptability to its environment (Allendorf and Leary, 1986). Management of black rhino has mainly focused on protecting existing populations and creating new populations through the means of reintroductions, translocations and supplementations, and through captive breeding programs (Emslie *et al.*, 2007).

As the black rhino species recovery progresses, it is now necessary for metapopulation management to shift in emphasis from size to population quality indicators such as level of genetic variations. Various genetic studies have been conducted on the black rhino and although they have contributed in one way or the other in the understanding of rhino genetics, no conclusive report have been obtained due to contradictory results which have been attributed to small number of sample size and use of different genetic markers and thus necessitated need for further studies.

2.8 Molecular marker

2.8.1 Mitochondrial DNA markers (mtDNA)

Mitochondrial DNA consists of a haploid circular molecule found in the cellular mitochondria of most eukaryotes. It is typically maternally inherited in mammals and lacks recombination due to the nature of its replication process. it contains 37 genes of which 13 genes are involved in oxidative phosphoration. The remaining genes are transfer ribonucleic acids (tRNA) and ribosomal ribonucleic acid (rRNA) genes. The MtDNA genome also contains a non-coding region which is the most variable region of the mammalian mtDNA genome characterized by rapid change in sequence and length (Saccone *et al*, 1991).

MtDNA is more sensitive to changes in population demography because it has a quarter the population size compared with nuclear loci. MtDNA has a relatively high mutation rate and shows higher levels of polymorphism compared to many nuclear genes making it useful when looking for patterns of genetic differentiation (Moritz *et al*. 1987). Due to the non-recombining, uniparenteral inheritance and high rate of mutation, mtDNA markers has been utilized in the short-term and long-term management of populations, more specifically to: define evolutionary significant units (Moritz, 1994), ascertain phylogenetic conservation value of population and to measure genetic variation in recently declining populations.(Muya *et al*,2011).

Utility of MtDNA assay has been useful for studies that involve hair, fossils, bone or heavily degraded tissues because most mammal cell have one nuclear material but may have trace amount of mitochondria from which MtDNA can be isolated. (Hsieh *et al*, 2003; Broquet *et al.*, 2007).

2.9 Population viability analysis (PVA)

PVA is defined as the use of quantitative methods to predict the likely future status of a population or a collection of populations. This process identifies the viability requirements of, and threats faced by a species. It then assesses the rate of population decline and the risk of extinction over a defined time horizon for the population of a concern (Gilpin and Soule, 1986; Morris and Doak, 2002).

PVA is majorly oriented towards the management of rare and threatened species with short-term and long-term objectives, focused on promoting the persistence of the species.(Beissinger and McCullough, 2001). The technique requires information on the demography, ecology and habitat requirement of the species (Miller and Lacy, 2003). More accurate information on these parameters offers the researchers a wide scope to more realistically simulate alternative future population scenarios (Ellner *et al*, 2002).

PVA has been widely incorporated in conservation planning of various animal species ranging from: Loggerhead sea turtle, (Couse *et al*, 1987), Lead-beater's Possums (Lindemeyer and Possingham 1994), Black rhino (Soka *et al*, 2014; Lederer *et al*, 2011), Rare darter (Hartup *et al*, 2007), Southern elephant seal (Galimberti *et al*, 2001) to Blue-throated macaw (Rosa and Juan, 2012) yielding positive results which ultimately lessens the probabilities of extinction.

CHAPTER THREE

MATERIALS AND METHODS)

3.1.1 Study site location

Lake Nakuru National Park (LNNP) is situated approximately 150km North-West of Nairobi in Kenya's central Rift Valley on grid reference point 0° 15'S and 36° 7'E and covers an area of 140 km^2**.**The mean altitude is 1759 m and average annual rainfall is 876 mm.The park surrounds Lake Nakuru, which is the low point in a catchment basin of about 1800 km^2. To the west of the Park occur the great complex fault scarps of the Mau Escarpment, whereas to the east lies the Lion Hill range whose scenic view reveals a shimmering pink glow across the lake created by a population of over a million flamingoes.

Three seasonal rivers: Njoro, Enderit, and Makalia drains into the lake, rising from the southern and western catchment areas of the Park. The main entrance to the Park lies to the north and is linked to the town center by a tarmac road. Traffic on this road is protected from animal movements by a fence that encloses an area covering about 50ha.This area is covered by a variety of vegetation dominated by Euphorbia, tall cactus like trees and acacia woodland. In addition, the area acts as a link for limited animal movements between the western and eastern sectors of the Park.

Lake Nakuru National Park was selected as a priority area for the development of a rhino sanctuary in 1983, and received top priority for funding and development in 1985 (Jenkins, 1985, KRRP, 1985). Onto the two resident adults which existed in the park before the perimeter was ringed with an electric fence additional stocking was commenced in 1987, with one large adult

male being introduced from Kitengela area outside Nairobi National Park, and one adult male (originating from the Nyeri Forest) was introduced from Lewa downs. Then, in a successful operation which was carried out in four phases over three months, 15 rhinos were translocated into the park from Solio ranch. In 1990, stocking was completed with a further four rhino from Nairobi National Park, widening the genetic base of the founder population (Rhino Conservation Program, 1993). The population has since increased to about 60 individuals. And due to increase in numbers and general health of the introduced rhinos, LNNP has become a major source population of black rhino for close to two decades, with a translocation capability of 5 rhinos every 2 years. The park is also a home to many other wildlife species, including some of the 'Big five' (Lion, leopard and the Cape buffalo)

3.1.2 Sample size determination

The bottleneck model developed by Franken and Soule (1981) that suggest, retention of heterozygosity in a population is approximately equal to (1-1/2N) where N is the population size after the bottleneck, was used in sample size determination. The model predicts 10 individuals as able to retain 95% of the genetic variation of the original population after a bottleneck. Since Kenyan black rhino have undergone a recent bottleneck (Muya *et al,* 2011). this study targeted a minimum of 10% of the Lake Nakuru National Park census subpopulation size which was considered to give sufficient genetic variation. Sixteen individuals which were more than the calculated figure were randomly sampled..

3.1.3 Sampling

Samples of blood and ear pinna were collected from individuals of *Diceros bicornis michaeli* in LNNP subpopulation (n=16) in Kenya. The samples were acquired opportunistically during the

routine Kenya Wildlife Service (KWS) rhino management program that includes ear notching, disease surveillance and translocation. The samples were collected and stored in cryovials containing 1ml EDTA, to avoid extreme temperatures and direct sunlight samples were placed in a cool box and transported to the National Museums of Kenya, Molecular Genetics laboratory where they were stored at -20"C until further analyses.

3.1.4 Laboratory analyses

3.1.4.1 DNA Extraction

Total genomic DNA from blood and tissue samples was extracted using respective genomic isolation kit, Qiagen QIAamp DNA Blood Mini Kit was used for blood samples while DNeasy® Tissue Kit was used for tissue samples following the manufacturers protocol (Qiagen Inc. Valencia, CA, USA, 2014)

3.1.4.2 Polymerase chain reaction and Purification

Fragment of the mitochondrial DNA control region was amplified using the primers mt15996L (5'-TCCACCATCAGCACCCAA-AGC-3') and mt16502H (5'- TTTG-ATGGCCCTGAAGTAAGAACCA- 3') (Brown and Houlden, 2000). The primer were first reconstituted and diluted using sterile double distilled water (ddH_2O) to a concentration of 100uM and stored at -20"C. and before the start of the PCR procedure, 400ul of each forward and reverse primer was diluted into a final concentration of 20uM using ddH_2O and stored at -4"C.

PCR reactions were performed in a final volume of 25 µl containing 1 µl of DNA extract, and 24µl of reaction mix that contained 10 µl of master mix from QIAGEN multiplex kit, 2 µl of primer mix (to make 0.2 µM primer mix concentration from stocks of 20µM concentration of

both forward and reverse primers), 3 µl of Qsolution and 9 µl of water. Thermal cycling was carried out using an Eppendorf Mastercycler for; 94℃ 4 minutes (Initial denaturation), 94℃ 30 seconds (Denaturation) , 52-56℃ 30 seconds (Annealing), 72℃ 2 minutes (Extension), repeated for 35 cycles, followed by a final step of 72℃ 5 minutes. 2ul of PCR products were loaded on a 1% agarose gel stained with a 1% Ethidium Bromide labeled with a 100bp ladder, the gel was then run at 100 volts for 1 hour in 1x TAE buffer. The gel was visualized and photograph taken.

PCR products were purified using The Thermo Scientific GeneJETPCR Purification Kit (Thermo scientific Inc.) according to the manufacturer's instructions. PCR product purification was done in their original tubes thus minimizing any chance of contaminant.

A mixture containing 20ul of PCR product and 20ul of binding buffer (ratio 1:1) was prepared for each sample in a clean 50ul PCR tube. The change in color to yellow signified an optimal PH for DNA binding The resultant solution was then transferred to the GeneJET purification columns and Centrifuged for 1 minute at 10,000 x g, the flow-through in the collection tube was discarded and purification column placed back into it. 700 µL of Wash Buffer (diluted with 100% ethanol in a ratio of 1:5) was added to the GeneJET purification column centrifuged for 1 minute at 10,000x g, the flow-through in the collecting tube was once again discarded. The empty GeneJET purification column was centrifuged for an additional 1 min to completely remove any residual wash buffer. The GeneJET purification column was transferred to a clean 1.5mL microcentrifuge tube, then Added 50 µL of Elution Buffer to the center of the column membrane and centrifuged for 1 min at 12,000 x g. The GeneJET purification column was then discarded. Purified DNA stored at -20°C awaiting further analyses.

3.1.4.3 DNA Sequencing

Purified products of all the samples in this study were sequenced to obtain the precise nucleotide sequences. 5ul for each sample was sequenced using the same primers used for PCR amplification on both the forward and reverse direction, the Big dye termination technique ABI Capillary system (genetic Analyzer 3730-48) was used at MacrogenInc, South Korea.

3.2 Population viability analysis (PVA)

3.2.1 Data collection

To attain a projected black rhino increase by at least 5% per annum which will see Kenya reach a total of 1000 rhinos by 2020. Kenya Wildlife Service (KWS) initiated a consistent monitoring of the black rhino population in the country providing the information for improving the conservation of threatened populations. Consequently, demographic data (births, deaths, translocation, density, sex and age structure) have been collected to improve the management of this species.

Available life-history data of the Lake Nakuru National Park *D.b michaeli* were incorporated into the PVA model, if specific data of LNNP was unavailable, information from other black rhino subpopulations were used from published and unpublished literature or records. Model parameters were set as follows for baseline simulation;

Duration of year

Inorder to satisfy 'number of young per year' which must be a whole number, a year was adjusted from 365 days (default) to 490 days. The gestation period of a rhino is 15.33 months(460) days (Linklater, 2007) so as to enter a whole number (i.e. 1) instead of a fraction

(i.e. 0.8), a 'year' was adjusted to reflect 490 days to accommodate one birth per year, plus an additional 30 days which is the minimum time required to become pregnant again. This was done to avoid over-estimating the number of births in the simulations. The adjusted year is referred to as the 'gestational year'.

Number of iterations

Although, 100 iterations is usually adequate to uncover tendencies (Lacy, 1993) a higher number of between 500 and 1000 iterations are recommended to provide more rigorous results (Miller and Lacy, 2005). Model in this study used 1000 iterations.

Inbreeding depression

This study agrees with previous study of Kenyan *D.b michaeli* population (Muya *et al*, 2011) that the level of genetic variation was not low enough to be of concern. However, scenarios with and without inbreeding were modeled to compare how inbreeding depression influences the outcome of the simulated population.

In the inbreeding depression scenario a median lethal equivalent value of 3.14 was selected. The value is based on a survey of 40 mammal populations by Ralls *et al* (1988). According to Miller and Lacy (2005) inbreeding depression reduced the survival of offspring only in the first year hence the result of inbreeding depression were conservative.

Age of first reproduction for males (Yr)

VORTEX considers the age of the first reproduction as the age of first parturition, not simply the onset of sexual maturity. According to Lent and Fike (2003) rhino males reproduce at approximately nine years old. Based on the gestational year, age was adjusted from nine years to seven years (9 years x 365.25 days/490 days =7.14 years that is ~7 years..

Age of first reproduction for females (Yr)

Females black rhino normally gives their first birth between ages 6.5-8.5 (Eaza, 2009). Based on the gestational year the average (7.5) was adjusted to 6 years (7.5 years x 365.25/490 days = 5.59 ~ 6 years.

Sex ratio of young

Based on KWS personnel observations at LNNP and discussions with other rhino experts, sex ratio in this study was estimated at 1:1.

Catastrophe

Catastrophes are remarkable environmental events that are outside the limit of the normal environmental variation affecting both or either reproduction and survival (Miller and Lacy, 2005). Since, catastrophic events by their very nature are unpredictable, usually with devastating consequences, making managing for a catastrophe almost impossible No catastrophe was modeled in these scenarios.

Environmental variation

Since Kenya Wildlife Service had no data on environmental variation, 10% of variation was set set in mortality rate, fecundity rate and carrying capacity. Considering the seasonal variation in rainfall pattern from year to year this value seems appropriate.

Female breeding pool (%)

The percentage of adult females that was considered for breeding each year was calculated from the data obtained from KWS. The park has a total of 27 females of which 18 are adults, 6, sub-adults and 5 calves. Therefore, 67% was used for baseline simulation.

Initial population size

Current population size of the black rhino in LNNP was averaged from the census records, 50 was entered as the initial population size and the default 'stable age distribution' was chosen.

Number of years

To visualize a lengthy time period, simulations were run for 75 gestational years which translates to 101 calendar years. Since black rhino generations are not documented for LNNP. A published literature shows the black rhino generation (BRG) is c 10 years (Foose *et al*, 1983) therefore, 75 gestational years tested approximately 10 black rhino generations.

Maximum age of reproduction, number of progeny per year

Black rhinos maximum age of reproduction is approximately 37 years (Owen, 1988; Eaza, 2009), based on gestational years, age was adjusted from 37 years to 28 years (37 years x 365.25/490=27.8 ~28years). Female black rhino gives birth to one offspring per gestational year.

Mortality rate

Mortality rates of males and females aged between 0-3, 3-6 and 7 and above were calculated from the unpublished records obtained from Kenya Wildlife service, as shown in table 3.2 below.

Carrying capacity (K)

This is the number of individuals a population can sustain (relying on resources in the area) and it remains constant due to births cancelling out deaths (Owen-smith, 2001). LNNP management cannot increase the parks carrying capacity unless the physical size of the park is increased through acquisition of land. Soke *et al* (2014) estimated the carrying capacity of LNNP black rhino at 71 individuals. Therefore, carrying capacity of the simulated population was set at 71 for baseline scenario.

Table 3.1 Input data used for the Population Viability Analysis of the LNNP black rhino subpopulation. Values and annual average

Parameters	Values
Number of populations	1
Number of iterations	1,000
Number of years	75
Carrying capacity	71
Initial abundance	50
Reproductive system	Polygynous
Breeding age	7years(males) 6 years(females)
Maximum breeding age	28 years
Environmental variation	10%
Sex ratio	50:50
Maximum number of progeny	1
% Adults breeding	Females=67% Males=47%
Mean number of offspring	1

Anthropogenic simulations

Poaching (Modeled as harvesting) which has been and still is considered the most serious threats to the black rhino species (Garnier *et al*, 2001) due to their highly valued horn was simulated, harvesting of individuals 3 years and above (sub-adult and adults) over a consecutive period of 50 years to assess the effects of poaching on the population decline. Probabilities of extinction were assessed under different harvesting quotas including 0.5, 1, 2, 3 and 5% during 50 and 75 years, respectively

In addition, Mau forest which is the major source of rivers draining into Lake Nakuru has in the past been overexploited for its rich diversity of trees subsequently reducing the water level of the lake. However, in the past few years, the government and private environmental agencies have devoted in the rehabilitation of the forest which translates to more water flowing into the lake. This increased water flowing into the lake has increased the lakes water level thus reducing the browsing area of the herbivores in the park. Also, the growth of an invasive species (*Solanum incunum*) has also contributed in increasing the rates of habitat loss. A recent estimate of water level increase by the Kenya Wildlife Service was 0.13% (unpublished).To assess the effects of habitat loss, a series of simulations modeling water level increase and invasive species as a decrease in carrying capacity (K) over time. These simulations included 0.5, 1. 2, 3 and 5% decreases in carrying capacity each year.

3.3 Data analysis

3.3.1 Mitochondrial DNA analysis

BioEdit version 5.0.6 (Hall, 2001) was used to edit the DNA sequences after visually inspecting the chromatograms of every sequence data. Sequence data obtained from the forward and reverse primers were crosschecked to confirm the polymorphic sites detected. The background noise of the first and last nucleotides was deleted and the sequence chromatogram was scanned for manual calling of inconsistent base calling and then the sequences were aligned by Clustal Omega (Larkin *et al*, 2012). Consensus sequences were trimmed into 572 base pair with BioEdit Version 7.2.5 (Hall. T.A, 1999) and saved in FASTA format for further analysis compatibility. The generated query sequences were then compared with the already published nucleotide sequences in the GenBank using the Basic Local Alignment Search Tool (BLAST). This relationship was based on the E-value percentage coverage and percentage identity.

Genetic diversity within the Lake Nakuru National Park subpopulation was determined using haplotype diversity (*h*) (the probability that two randomly chosen haplotypes in a population are different) and nucleotide diversity (π) (Probability that two randomly chosen homologous nucleotides are different) the procedure was effected by a computer program DnaSP Version 5.10.1 (Rozas *et al*. 2009). To compute for nucleotide composition, molecular evolution genetics analysis software (Tamura *et al*, 2013) was used.

A mismatch distribution analysis of the number of pairwise differences between haplotypes was performed in ARLEQUIN Version 3.5 (Eschofier and Licher, 2009) to test for population size changes.

3.3.2 Population viability analysis

A baseline simulation as well as alternative simulation scenarios was modeled assuming that conditions for each simulation were going to persist during the 75-year period. VORTEX 9.99 (Lacy *et al*, 2009) was used. The VORTEX model takes into account a description of the species reproduction system and reproductive rates, a specified age structure, age-specific mortality rates, catastrophic events, demographic and environmental stochasticity, density dependence and options for the harvest and augmentation of the population. It uses mortality rate and calculates fertility based on the number of males and females in the breeding pool and the mean number of progeny per year. Two specific results including differences in the probability of extinction or risk of decline and population growth rate (Lambda) were obtained.

3.4 Assumptions and limitations in the methodology

The main assumption made in the methodology was that all the quantitative data obtained from Kenya Wildlife Service rhino database was accurate and reliable and that the genetic samples (n=16) collected from individual rhinos in this study was representative of the whole Lake Nakuru National Park rhino sub population and would be applied to other black rhino subpopulations in Kenya.

Collection of biological samples from the individual rhinos was done opportunistically during disease surveillance and translocation which are not often done hence this study used used a small sample size..

CHAPTER FOUR

RESULTS

4.1 DNA Sequencing

Sequences of approximately 579 base pairs long were obtained for all the 16 samples from LNNP subpopulation.

The 16 sequences were aligned and trimmed to 572bp long. BLAST (basic local alignment search tool) (Zheng *et al*., 2000) that employs nucleotide collection (nr/nt) was conducted; resulting in to 200 sequence hits with significant alignments with our query MtDNA D-Loop sequences. Among these, the largest sequence identity of 100% was observed with one hit AF187834.1 corresponding to *Diceros bicornis* mitochondrial D-Loop partial sequence (Figure 4.1) Nucleotide composition of the whole population was also calculated and averaged as shown in table 4.1 (relative values) . The fragments were AT-rich (A 28.9%, T31.8%,).

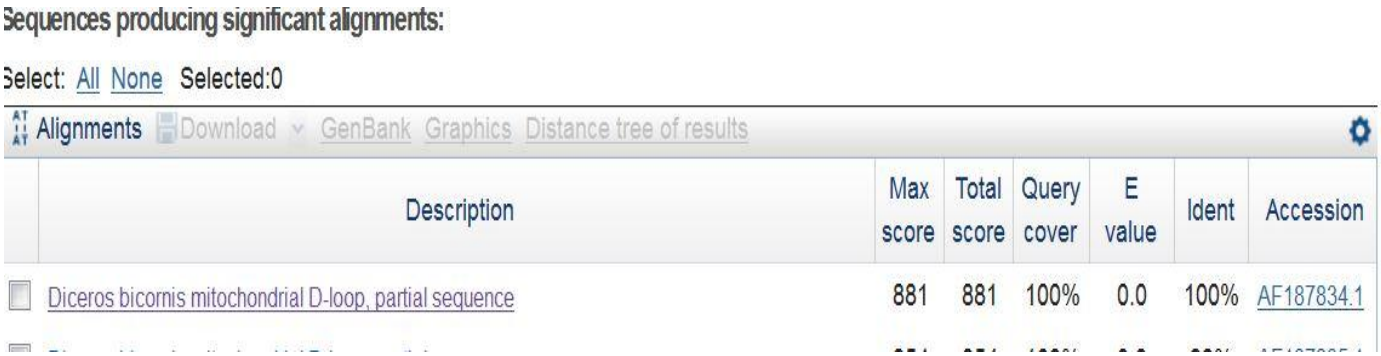

Figure 4.1 Basic Local Alignment Search Tool results showing the largest sequence identity. Sourced from National Centre for Biotechnology information.

.

	T(U)	C	A	G	Total
BRNak1	32.0	24.8	28.8	14.3	572.0
BRNak2	32.0	24.8	28.8	14.3	572.0
BRNak3	31.8	25.0	28.8	14.3	572.0
BRNak4	31.8	25.0	28.8	14.3	572.0
BRNak5	31.8	25.0	28.8	14.3	572.0
BRNak6	31.8	25.0	28.8	14.3	572.0
BRNak7	31.8	25.0	28.8	14.3	572.0
BRNak8	31.8	25.0	28.8	14.3	572.0
BRNak9	32.0	24.8	28.8	14.3	572.0
BRNak10	32.0	24.8	28.8	14.3	572.0
BRNak11	31.8	25.0	28.8	14.3	572.0
BRNak12	31.8	25.0	29.2	14.0	572.0
BRNak13	31.5	25.3	29.2	14.0	572.0
BRNak14	32.0	24.8	28.8	14.3	572.0
BRNak15	31.5	25.3	28.8	14.3	572.0
BRNak16	31.5	25.3	29.0	14.2	572.0
Avg.	31.8	25.0	28.9	14.3	572.0

4.2 Mitochondrial DNA variation within LNNP

The 572bp aligned sequences (n=16) included 13 polymorphic sites at positions; 76, 100, 104, 108, 220. 222. 257, 272, 309, 401, 410. 484 and 537 and there were no insertions or deletions.

The sequences also revealed 6 haplotypes in the LNNP subpopulation. BRNak12, BRNak13, BRNak15, BRNak16 each had a unique haplotype while BRNak1, BRNak2, BRNak9, BRNak10, BRNak14 shared a common MtDNA haplotype, as did BRNak3, BRNak4 BRNak5, BRNak6, BRNak7, BRNak8 and BRNak11] (Table 4.2). Thus, the *D.b michaeli* colony in LNNP contains atleast 6 maternal lineages.

Table 4.2 Distribution of 13 observed polymorphic sites and 6 D-loop haplotypes found in the LNNP subpopulation. Only variable position are shown dot indicate identity with the sequence of Hap 1. N in the last column shows the number of individual sharing a haplotype.

Haplotypes	**Variable sites**													**N**
	76	100	104	108	220	222	257	272	309	401	410	484	527	
Hap1	G	T	T	A	G	T	A	G	C	T	T	G	G	**5**
Hap2	.	C	C	G	A	.	G	A	T	.	.	.	.	**7**
Hap3	.	C	C	G	A	.	G	A	T	.	.	.	.	**1**
Hap4	A	C	.	.	A	.	G	A	.	C	C	.	.	**1**
Hap5	.	C		.	.	C	G	A	.	.	C	A	A	**1**
Hap6	.	C	.	.	A	C	G	A	.	.	C	.	.	**1**

The average haplotype diversity of the entire LNNP subpopulation (n-16) was moderate (0.742±0.084) and also showed relatively low nucleotide diversity (0.00793± 0.00085)

4.3 Population size changes

The expected mismatch for the LNNP subpopulation control region data set was described by parameters estimated from the sudden expansion model (τ = 5.812, Θ_0 = 0.002, Θ_1 =6.404,).

Where τ is the, Θ_0 is the initial theta while Θ_1 is the final theta. The population did not show a unimodal pattern of mismatch distribution curve, as expected in case of population expansion (Fig 4.2). The sum of squared deviations (SSD) of mismatch distribution was not significant, indicating that the curve did not fit the sudden expansion model tested. Neutrality tests applied to search for additional demographic signs of population expansion were also in concordance with the above pattern.

However, mismatch distribution showed two major peaks at position 4 and 8 respectively. The pattern possibly signifies two population expansion events that occurred about 4 and 8 mutational time units ago.

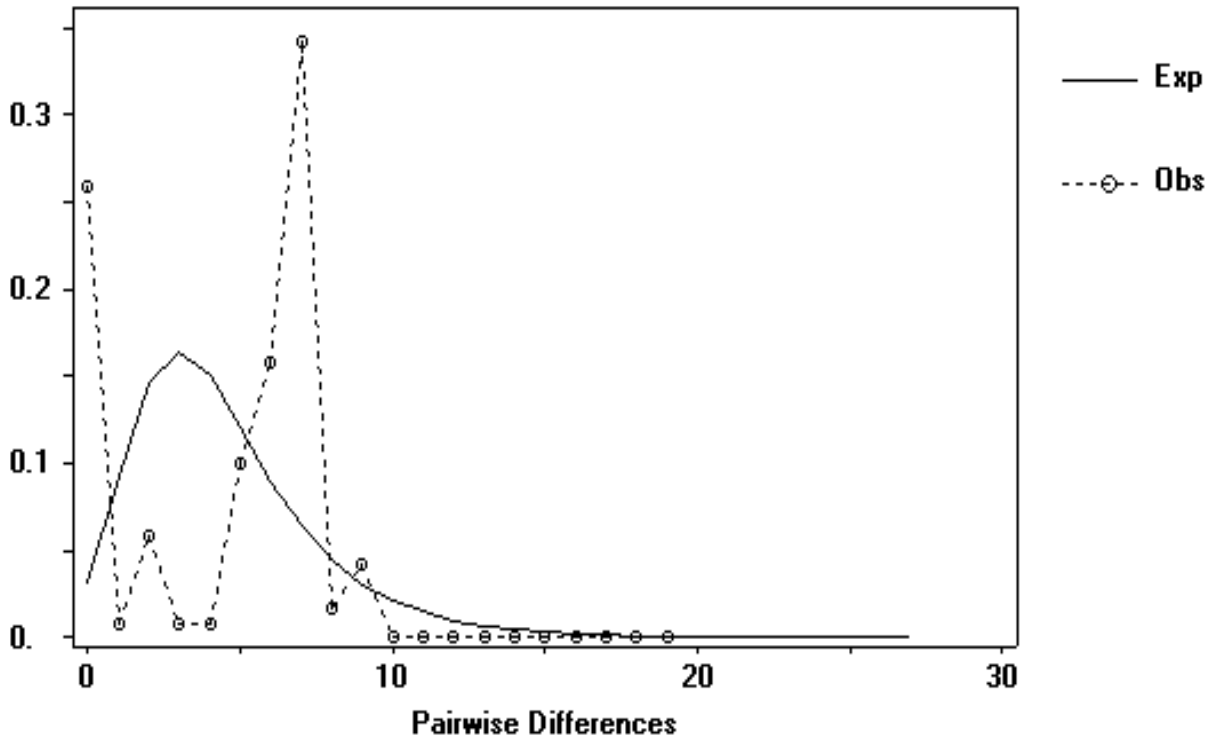

Figure 4.2 Observed and expected mismatch distribution under population expansion model for LNNP black rhino mtDNA control region sequences.

4.4 Population viability analysis simulations

The baseline simulation using all individuals resulted in a probability of extinction of 0.00 for the subpopulation over the 75 gestational years (101 calendar years) with a growth rate (**λ**) of 1.254 (Table 4.3). The subpopulation increased by 30% for the first 10 years of simulation and then stabilized for the remaining years of simulations.

As expected, population performance of black rhino in terms of decline and extinction probabilities showed varying fluctuating patterns under different percentages of habitat loss (modeled as decrease in carrying capacity) and harvesting, thus, increasing the probability of extinction (Table 4.3 and Figure 4.5). Simulation with 0.5% of habitat loss had a little effect on the subpopulation (Table 4.3 and Figure 4.4). Although, the difference caused was not statistically significant the final population decreased by 20% from the initial population size at the end of the simulation.

Table 4.3 Habitat Loss and Harvesting PVA Simulations.Population Growth Rate (λ) and Probabilities of Extinction (PE).

Percentage changes		**Habitat loss**	**Harvesting**
	Λ	PE	PE
Baseline	1.254	0.00	0.00
0.5%	1.254	0.01	0.04
1%	1.254	0.11	0.13
2%	1.254	1.00	0.76
3%	1.254	1.00	1.00
5%	1.254	1.00	1.00

A 1% loss of habitat each year had a significant impact (i.e. 0.11) on the subpopulation decline, reducing the subpopulation size by 46% during the first 40 years and 66.67% after 50 years.

As expected, 2% had an even greater impact on subpopulation extinction, decreasing by 90% the number of individuals during the first 40 years of simulation and wiping out the whole population before the end of the 75th year.

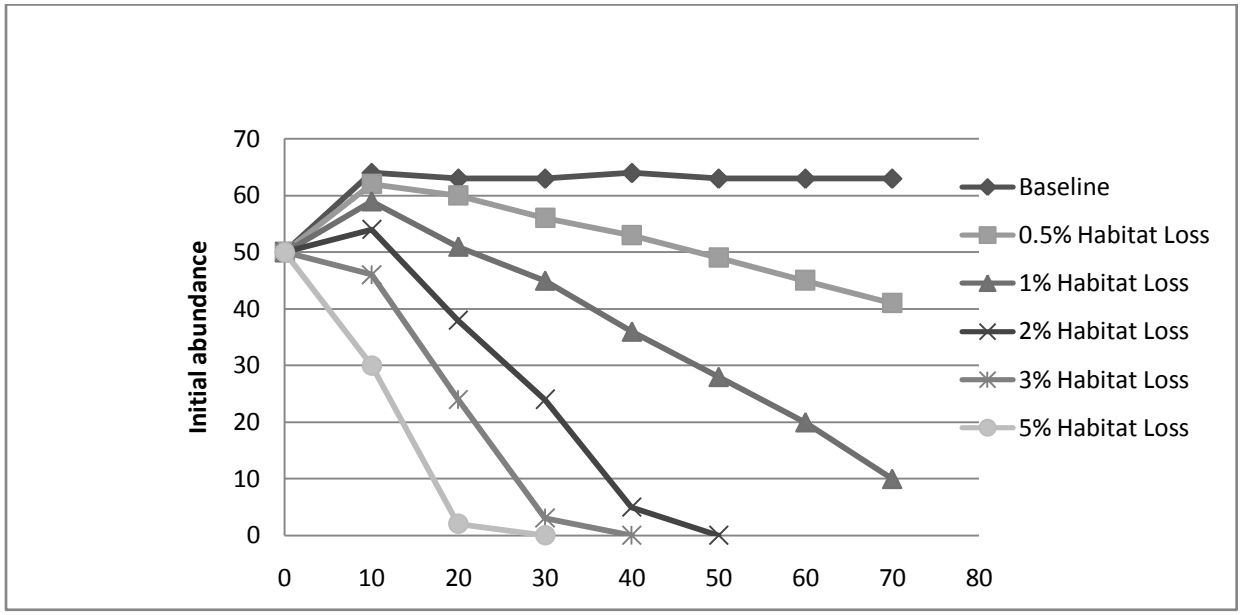

Figure 4.3 Habitat loss simulations. Different lines represent the mean final abundance of Lake Nakuru National Park populations in simulations ran with different percentages of habitat loss

Simulation with 3% and 5% of habitat loss had a more drastic effect causing the population to run extinct at 40 and 30 years respectively.(Figure 4.3)

Harvesting different percentages of individuals during the first 10 years over a 75 year period resulted in slight population increase (at 0.5% and 1% harvest) while reducing the population of the other percentages of harvesting (Figure 4.4).

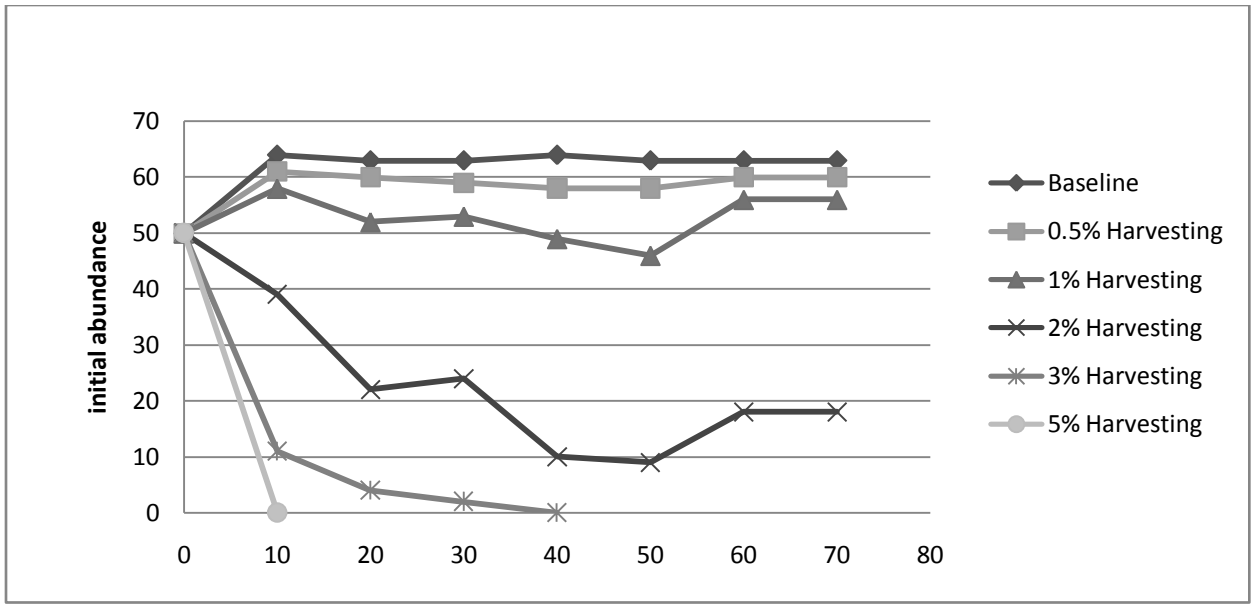

Figure 4.4 Harvesting simulations. Different lines represent the mean final abundance of the Lake Nakuru National Park subpopulations in simulations ran with different percentages of harvesting.

Simulation showed that starting at 0.5% of harvesting, probability of extinction became significantly different from that of the baseline simulation (Table 4.4). The harvesting at 5% on yearly basis showed a greatest impact on the population decline and extinction probabilities

CHAPTER FIVE

DISCUSSION

Despite, the dire need for conservation of the Kenyan black rhino few conservation research have been conducted. These studies have majorly focused on; management (Okita-Ouma, 2004) ecology (Muya and Oguge, 2000, Patton and Jones, 2007), behavior (Morinte and Keter, 2000), security (Martin and Vigne, 2003) and diseases (Obanda *et al.*, 2008). However, genetics studies which is of vital concern in small population has received relatively little attention (Brown and Houlden, 2000, Scott, 2008.Muya *et al*. 2011).

This study showed that LNNP subpopulation has a relatively high number of haplotypes representing 38% of the total haplotypes (16) reported in the Kenyan metapopulation (Muya*et al*, 2011). Of the six haplotype represented in this subpopulation, haplotype 1 and haplotype 2 were extensively shared among the individuals while haplotype 3-6 were unique thus, represented by a single sequence. This occurrence is in concordance with other findings, as it is expected for a haplotype to be represented by one individual or shared by a portion of the population, because mitochondrial DNA variation is more frequent within species than between them (Naderi *et al,* 2007)

The subpopulation exhibited moderate haplotype diversity (0.742±0.084) which is consistent with other studies of *D. b michaeli* (*h*= 0.73, n=170, Muya *et al*, 2011) ,*D.b minor* (*h*=0.86, n=8, Brown and Houlden, 2000) signifying the subpopulation to be 'healthy' as a breeding population. However, these findings contradicted with other previous studies that reported low level of genetic variation in black rhino populations (Ashley *et al*. 1990; O'Ryan and Harley 1993; O'Ryan *et al*. 1994). Nonetheless, this low level of genetic variation could have been as a result of their choices of molecular marker.

LNNP rhino population was founded by several rhinos sourced from different locations in the country and therefore this could be the reason of this moderate haplotype diversity.

The population also showed a moderate nucleotide diversity (0.0071±0.000), which was in congruence with other previous studies (0.007±0.00, Muya *et al*, 2011; 0.074±0,001. Anderson-Lederer *et al*, 2012). Although, when compared to other African large mammals the levels showed to be relatively low for example; savanna elephants in Kenya and the endangered mountain zebra have nucleotide diversity of 0.016 and 0.015 respectively (Okello *et al*, 2008; Moodley and Harley, 2005). Observed nucleotide diversity clearly shows the impact of recent population reduction of black rhino.

Both neutrality test and mismatch distribution analysis of the Lake Nakuru National Park black rhino data does not support a recent population expansion, possibly because the sampled individuals might have been translocated from populations that were relatively abundant. The markedly ragged mismatch distribution does hint at a recent demographic fluctuation, possibly due to the population reductions of the 1970's – 1990's. The confinement of the black rhino into an enclosed sanctuary might have reduced the chances of the subpopulation expansion considering their earlier broader distribution range.

However, the mismatch distribution (Figure 4.3) showed two major peaks at position 3 and 8 respectively being similar to the peaks of 3 and 7 indentified previously (Muya *et al*, 2011). The pattern possibly signifies two population expansion events at about 4 and 8 mutational time units ago.

While genetics triggers a concern in black rhino declines, poaching and habitat loss poses the greatest threat to this species. Admittedly, there is no quantitative information about the impact of these activities on population extinction and population growth rate. Results of the PVA

baseline simulation suggest that, under current conditions, the LNNP black rhino has a 0.00 probability of extinction over the next 75 years. This scenario is particularly so considering the high level of protection and adequate security to keep off potential threats. However, growth rate estimates (1.254) did not reach the rate of replacement necessary to maintain the populations over a longer period of time, making the species more vulnerable to any change or threat. This may suggests that the park may be maintained as a nucleus breeding site for the potential translocation or reintroduction to other rhino sanctuaries in Kenya. In order to achieve this goal however, the KWS rhino team would be required to formulate a more effective management program which targets on maintaining an effective population size as well as managing other wildlife herbivores in the park.

Sensitivity analysis on the effects of poaching and habitat loss under different percentages proved to be important limiting factors for the LNNP black rhino. A 1% loss of habitat per year reduced the population abundance by more than half in the first 30 years of simulation As expected, 2% had an even greater impact on subpopulation extinction, decreasing by 90% the number of individuals during the first 40 years of simulation and wiping out the whole population before the end of the 75^{th} year. These results were particularly relatable given that increased water level in the lake (habitat loss) raised the competition pressure from other herbivores depending on similar browse resources. The results are consistent with those of Landman and Kerley (2014) that showed black rhino population sharing habitats with other browsers in Addo National Park, South Africa may be limited due to resource competition. Competition for the available resources may also result in calf mortality rate as well as extending inter-calving interval thereby reducing the population growth in black rhino population (Freeman *et al*, 2014)

The effects of poaching were tested through different harvesting quotas set during a consecutive 75 year period and indicated that a 3% rate of harvesting had a significant effect on the subpopulation over a short period of time. This result was also obtained by Soka *et al* (2014) in a study of black rhino where harvesting of 2 males and 2 females year after year showed a mean growth rate of 0.035 in the first five years, but the population declined considerably before becoming extinct after 45 years.

CHAPTER SIX

CONCLUSION AND RECCOMENDATION

From this study LNNP black rhino maintains moderate haplotype diversity which is a good level for a breeding population. However, while compared to other large African mammals this levels were considerably low. To maintain or probably raise the current level of genetic diversity a careful supplementation programme into this subpopulation should be constituted putting into consideration the pairwise genetic differences between the subpopulations in order to avoid mixing animals between genetically similar subpopulations.

As earlier mentioned, LNNP black rhino subpopulation is currently not under risk of extinction or population decline for the next 75 years if the prevailing conditions remain constant. Nonetheless, slight increase in habitat loss or population harvest had drastic effects on extinction risk over a short period of time. Therefore, there is need to implement effective measures to curb habitat loss as well as enforcing laws against poaching and illegal trade of rhino trophies.

Future genetic studies will be necessary possibly using nucleus DNA so as to get clear picture of both maternal and paternal information and perhaps sample the entire LNNP subpopulation. The study should also be extended to compare with other nucleus subpopulations. Furthermore, detailed genetic studies of this important subpopulation is necessary so as to come up with a conclusive management recommendation

REFERENCES

Akçakaya HR. Sjögren-Gulve P. (2000) Population viability analysis in conservation planning: an overview. *Ecological Bulletins* 48:9-21.

Alibhai S. Jewell K, ZC., Towindo, SS. (2001) Effects on immobilization on fertility in female black rhino. *Journal of Zoology* 253: 333-345.

xAllendorf FW, England PR, Luikart G, Ritchie PA, Ryman N (2008) Genetic effects of harvest on wild animal populations. Trends EcolEvol 23 (6):327-337

xAllendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell Publishing, 350 Main Street, Malden, MA U.S.A.

Amin R, Thomas K, Emslie, RH, Foose, TJ, Van Strien, N. (2006) 'An overview of the conservation status of and threats to rhino species in the wild', International Zoo Yearbook. 40, pp 96-117.

Amin R., Okita-Ouma B, Adcock K, Emslie RH, Mulama M, Pearce-Kelly P. (2006). An integrated management strategy for the conservation of eastern black rhinos(*Diceros bicornis michaeli*) in Kenya. International Zoo Yearbook 40: 118-129.

xAmin R, Bramer M, Emslie RH (2003) Intelligent data analysis for conservation:experiments with rhino horn fingerprint identification. Knowledge-Based Systems 16:329-336.

Amin R, Okita-Ouma B, Mulama M. (2001). Kenya implementing a new black rhino information management system.*Pachyderm* **30:** 96–97.

Amos B, Hoelzel AR. (1992) Applications of molecular genetic techniques to the conservation of small populations.*Biological Conservation* 61, p. 133–144.

Anderson-Lederer RM, Linklater WL, Ritchie PA (2012) Limited mitochondrial DNA variation within South Africa's black rhino (*D. b. minor*) population and implications for management. Afr J Ecol 50 (4):404-413.

Ashley MV, Melnick DJ, Western D (1990) Conservation genetics of the black rhino (*Dicerosbicornis*), I: Evidence from the mitochondrial DNA of three popuatlions. ConservBiol 4:71-77..

Beissinger SR, McCullough DR (2002) Population viability analysis.The University of Chicago Press.Chicago and London.

Bennun LA, Njoroge P (1999) Important Bird Areas in Kenya. East Africa Natural History Society, Nairobi, Kenya.

Bergl RA, Bradley JB., Nsubuga A. Vigilant L. (2008) Effects of habitat fragmentation, population size and demographic history on genetic diversity the cross river gorilla in a comparative context. *American Journal ofPrimatology* 70(9), pp. 848-859.

Boyce MS (1992) Population Viability Analysis.Annu Rev EcolSyst; 23: 481-506.

xBrett RA. (1993) Conservation strategy and management plan for the black rhino (Dicerosbicornis) in Kenya: Rhino conservation programme. Sponsored by the Zoological society of London, p. 69.

Brook BW, Lim L, Harden R, Frankham R. (1997) Does population viability analysis software predict the behavior of real populations? A retrospective study on The Lord Howe Island Woodhen*Tricholimnassylvestris*(Sclater).BiolConserv 82: 119-28.

Brook BW, O'Grady JJ, Chapman AP, Burgman MA, Akçakaya HR, Frankham R (2000) Predictive accuracy of population viability analysis in conservation biology. Nature; 404: 385-7.

xBrown SM, Houlden BA (2000) Conservation genetics of the black rhino (Dicerosbicornis). Cons Genet 1:365–370.

Burgman M, **Ferson S, Akçakaya HR, (1993)** Risk Assessment in Conser-vation Biology. New York: Chapman and Hall.

Cardillo M, Mace GM, Jones K, (2005) Multiple causes of highextinction risk in large mammal species. Science 309:1239–1241.

Clifford S, Anthony NM, Bawe-Johnson, M, (2004) Mitochondrial DNA phylogeography of western lowland gorillas (Gorilla gorilla gorilla).*Mol. Ecol.* 13(1551-1565).

Costa-Neto, (2004).Implications and Applications of Folk Zootherapy in the State of Bahia, Northeastern Brazil. Sustainable Development 12: 161-174.

Costa-Neto EM. (1999) Healing with animals in Feiro de Santana City, Bahia, Brazil.Journal of Ethnopharmacology 65: 225-230.

Coulson T, Mace GM (2001) Possingham H. The use and abuse of popu-lation viability analysis. Trends EcolEvol; 16(5): 219-21.

Craigie ID, Baillie JEM, Balmford A, Carbone C, Collen B (2010) Large mammal population declines in Africa's protected areas. Biological Conservation 143: 2221-2228.

Cromsigt J, Hearne J, Heitkonig I, Prins H (2002) Using models in the management of Black Rhino populations. Ecological Modelling149: 203-211.14.

Crouse D, Crowder L, Caswell H (1987). A stage-based population model for loggerhead seaturtles and implications for conservation. Ecology 68:1412-1423.

Dean C, Bos CA.(2006) The EAZA rhino campaign. International Zoo Yearbook 40: 210-217.

Dixon JD, Oli MK, Wooten MC, Eason TH, McCrown JW, Cunningham MW (2007). Genetic consequences of habitat fragmentation and loss: the case of the Florida black bear (*Ursusamericanusfloridanus*). Conservation Genetics 8: 455-464.

xDu Toit, Fosse RTJ, Cummings DHM (1987) Proceedings of African Rhino Workshop, Cincinnati, October 1986. *Pachyderm*.Special Issue no.9.

Ellner SP, Fieberg J, Ludwig D, Wilcox C. (2002) Precision of Population Viability Analysis. *Conservation Biology.*16:258-261.

Emslie R, (2011) *Diceros bicornis*.IUCN 2011.IUCN Red List of Threatened Species.Version 2011.2 <www.iucnredlist.org>, vol 2012.IUCN.

Emslie R, Brooks M (1999) African Rhino Status Survey and Conservation Action Plan, IUCN/SSC African Rhino Specialist Group.IUCN, Gland, Switzerland and Cambridge, UK.

Emslie R, Milledge S, Brooks M, Van Strien N, Dublin T (2007) African and Asian Rhinoes - Status, Conservation and Trade (A report from the IUCN Survival Commission (SSC) African and Asian Rhino Specialist Groups and TRAFIC to the CITES Secretariat). vol CoP14 Doc. 54.

Emslie RH, Amin R, Kock RE (2009) Guidelines for the *in situ* re-introduction of African and Asian rhino, vol 1. Gland, Switzerland: ICUN.

Emslie. R, (2006) 'Rhino notes: Rhino population sizes and trends' Pachyderm 41, pp 100-105.

Emslie.R, (2004) Rhino populations and trends. Pachyderm 37: 107-110.

xEmslie R, Brooks, M (1999) African Rhino.Status Survey and Conservation Action Plan.IUCN/SSC African Rhino Specialist Group. IUCN: Gland,Switzerland and Cambridge, UK. p. ix + 92.

Emslie. Joubert, E(1971)The past and present distribution and status of the black rhino (*Dicerosbicornis*Linn. 1758) in South West Africa. *Madoqua*. 4:33-43 *Evolution* 19, pp. 153-170.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. EvolBioinform 1:47-50.

Felts A. (2007); Facility focus – Colombus Zoo; Rhino keeper association volume 1 Issue 4, pp 2-6.

Fernando P, Polet G, Foead NNL, Pastorini J, Melnick DJ. (2006) Genetic diversity, phylogeny and conservation of the Javan rhino(Rhino sondaicus). *Conservation Genetics* 7(3), pp. 439-448.

Foose TJ, Seal US.(1991).*Metapopulation Analysis Workshop Briefing Book* Nairobi, Kenya: IUCN/SSC/CBSG/KWS.

Foose TJ, Lacy RC, Brett R. Seal US. (**1992).**Kenya black rhino meta-population analysis-briefing book. Kenya Black Rhino Meta-population Workshop. KWS-Nairobi: IUCN/SSC/CBSG.

Foose TJ, Wiese RJ. (2006) Population management of rhino in captivity.International Zoo Yearbook 40: 174-196.

xFrankham R (2005) Genetics and extinction.Biol Cons 126 (2):131-140.

Frankham R. Ralls K. (1998) Inbreeding leads to extinction. *Nature* 392, pp. 441-442.

Frankham R. (1995) Effective population size/adult population size rations in wildlife:a review. *Genet Res* 66, p. 95–107.

Frankham R, Balloua JD. Briscoe DA. (2002) *Introduction to conservation genetics*. 1st ed. Cambridge University Press, pp. 72-174.

Freeland JR. (2005) *Molecular Ecology*. The Open University, Milton Keynes: John Wiley and Sons, LTD, p. Pp 388.

Friesen VL. Montevecchi WA, Gaston AJ, Barrett RT, Davidson WS (1996).Molecular Evidence for Kin Groups in the Absence of Large-Scale GeneticDifferentiation in a Migratory Bird. Evolution 50: 924-930.

FU YX. LI WH., (1993) Statistical tests on neutrality of mutations. *Genetics*, vol. 133, p. 693-709.

FU YX. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, vol. 147, p. 915-925.

Gaillard JM, Festa-Bianchet M, Yoccoz NG, Loison A, Toigo C (2000) Temporal variation in fitness components and population dynamics of large herbivores. Annu Rev EcolSyst 31:367-393.

xGarnier A, Rachlow JL, Hicks JF (2005) Patterns of genetic diversity and its loss in mammalian populations. ConservBiol 19 (4):1215-1221.

xGarnier A, Rachlow JL, Hicks JF (2005) Patterns of genetic diversity and its loss in mammalian populations. ConservBiol 19 (4):1215-1221.

xGarnier JN, Bruford MW, Goossens B. (2001) Mating system and reproductive skew in black rhino. *Molecular Ecology* 10, pp. 2031-2041...

Gibbs JP, Marquez C, Sterling EJ (2008) The role of endangered species reintroduction in ecosystem restoration: Tortoise-cactus interactions on Espanola Island, Galapagos. RestorEcol 16 (1):88-93.

Gilpin ME, Soule ME. (1986) Minimum viable populations of species extinction. In M.E. Soule (ed.). *Conservation biology: the science of scarcity and diversity*. Sinauer Associates, Inc., Sunderland, Massachusetts. 19-34,

Glenn TC, Stephan W, Braun MJ (1999) Effects of a populaton bottleneck on Whooping Crane mitochondrial DNA variation. ConservBiol 13:1097-1107.

Goddard J, (1966) Mating and courtship of the black rhino (Dicerosbicornis L.).*East African Wildlife Journal* 4, pp. 69-75.

Griffith B, Scott JM, Carpenter JW, Reed C, (1989) Translocation as a Species Conservation Tool - Status and Strategy. Science 245 (4917):477-480.

HALL TA., (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl. Acids Symp. Ser*., vol. 41, p. 95-98.

Harley EH, Baumgarten I, Cunningham J, O'Ryan C (2005) Genetic variation and population structure in remnant populations of black rhino, *Dicerosbicornis*, in Africa.MolEcol 14:2981-2990.

Hutchins M, Kreger MD (2006) Rhino behaviour: implications for captive management and conservation. International Zoo Yearbook40:150-173.

xInternational Rhino Foundation (2006) IRF Program Office, Yullee, Florida.http://www.rhinos-irf.org.

xIUCN SSC AfRSG.(2008) *Diceros bicornis. In: 2008 IUCN Red List of Threatened Species* IUCN 2008. Available at: <URL: www.iucnredlist.org

xIUCN Red List of Threatened Species.Version (2012).Downloaded June 15, 2014. Available from: www.iucnredlist.org

Jenkins PR (1985) Kenya black rhino management plan. Unpubl report WCMD, MoTW, GoK 15pp.

Johnstone OT, (2013) Kenya Forecasts Increase in Earnings, Tourist Arrivals This Year accessed Jun 24, 3:23 PM.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations.TrendsEcolEvol 17:230–241.

xKenya National Bureau of Statistics (2013),*Economic Survey*. Nairobi: Government Printer.Pierce, Benjamin A. Genetics: A Conceptual Approach. .

xKenya Wildlife Service, (2012) Conservation and management strategy for the black rhino (Diceros bicornis michaeli) in Kenya 2012-2016.Nairobi: Kenya Wildlife Service (KWS). Available at URL www.kws.go.ke.

xLacombat F (2005) The evolution of the rhino. . Save the Rhinos: EAZA Rhino Campaign 2005/6. European Association of Zoos and Aquaria, London.

Lacy RC, Borbat M, Pollak JP. (2012) VORTEX: A Stochastic Simulation of the Extinction Process. Version 9.99c. Chicago: Chicago Zoo-logical Society.

Lacy RC VORTEX: (1993) A computer simulation model for Population Viability Analysis. Wildlife 45-65.

Lacy RC.(1994) What is Population (and Habitat) Viability Analysis? Primate Conserv; 14/15: 27-33.

Lacy RC. (1997) Importance of genetic variation to the viability of mammalian populations. *Journal of Mammalogy*78, p. 320–335.

Lacy RC, Borbat M, Pollak JP (2005) *VORTEX: A Stochastic Simulation of the Extinction Process.Version9.50*. Brookfield, IL: Chicago Zoological Society.

Landman M, Kerley GI (2014) Elephant both Increase and Decrease Availability of Browse Resources for Black Rhino. Biotropica 46:42-49.

Lindenmeyer DB, Possingham. HP.(1994). The Risk of Extinction: Ranking managementoptions for Leadbeater's Possum using population viability analysis. Australian National University.

Ludwig D (1999) Is it meaningful to estimate a probability of extinction? Ecology 80: 298-310.

Mbwana J, (2006). Molecular characterization of *Haemophilusducreyi* isolates from different geographical locations. *J ClinMicrobiol.*.PMID: 16390960

Miller P. S, Lacy, R. (2005) *VORTEX: A Stochastic Simulation of the Extinction Process. Version 9.50 User's Manual.* Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, MN.

Miller PS., Lacy RC. (2003)*VORTEX: A Stochastic Simulation of the Extinction Process. Version 9.21 User's Manual.* Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, MN.

Moodley Y, Harley EH (2005) Population structuring in mountain zebras (*Equus zebra*): The molecular consequnces of divergent demographic histories. Con Genet 6:953-968.

Morales JC, Andau PM, Supriantna J, Zainuddin Z, Melnick D (1997) Mitochondrial DNA variability and conservation genetics of the Sumatran rhino. ConservBiol 11:539-543.

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it.SystBiol 51 (2):238-254.

Morkel P, Kennedy-Benson A. (2007).*Translocating black rhino: current techniques for capture, transport, boma care, release and post-release monitoring.*Report to AfRSG: pp85.

Morris WF, Doak DF. (2002)*Quantitative Conservation Biology, Theory and Practice of Population Viability Analysis.*Sunderland, Sinauar Associates Inc.

xMuya SM, Bruford JW, Muigai AWT, Osiemo ZB, Mwachiro E, Okita-Ouma B, Goossens B (2011) Substantial molecular variation and low genetic structure in Kenya's black rhino: implications for conservation. Conserv Genet 12:1575-1588.

xMuya SM. Oguge NO, (2000) Effects of browse availability and quality on black rhino (Dicerosbicornismichaeli Groves 1967) diet in Nairobi National park.

Nei M. (1987) Genetic distance and molecular phylogeny. In: Ryman, N., and Utter, Fed. *Population Genetics and Fishery Management*.University of Washington Press, pp. 193-223Seattle, USA.

Nei M., Tajima F, Tateno, Y. (1983) Accuracy of estimated phylogenetic trees from molecular data.II.Gene frequency data.*Journal of Molecular.*

Norman C. Ellstrand, Diane R Elam(1993) Population Genetic Consequences of Small Population Size: Implications for Plant ConservationPage 219 of 217-242.

Nyakaana S, Arctander P. Siegismund H. (2002) Population structure of the African savannah elephant inferred from mitochondrial control region sequences and nuclear microsatellite loci. *Heredity* 89, pp. 90-98.

O'Ryan C, Harley EH. (1993) Comparisons of mitochondrial DNA in black and white rhinoes.*Journal of Mammalogy*74, p. 343–346.

O'Ryan C, Flamand JRB, Harley EH. (1994) Mitochondrial DNA variation in black rhino (Dicerosbicornis): conservation management implications. *Conservation Biology* 8, p. 495–500.

Ogutu JO, Owen-Smith N, Piepho HP, Said MY (2011) Continuing wildlife population declines and range contraction in the Mara region of Kenya during 1977-2009. Journal of Zoology 285: 99-109.

Ogutu JO, Piepho HP, Dublin HT, Bhola N, Reid RS (2009) Dynamics of Mara-Serengeti ungulates in relation to land use changes.Jouranl of Zoology (London) 278: 1-14.

Ogutu ZA (2002) The impact of ecotourism on livelihood and natural resource management in Eselenkei, Amboseli ecosystem, Kenya.LandDegradDev 13:251–256.

Okello JBA., Masembe C, Rasmussen HB, Wittemyer G., Omondi P, Kahindi O, Muwanika VB, Arctander P, Douglas-Hamilton I, Nyakaana S. Siegismund HR. (2008) *Population Genetic Structureof Savannah Elephants in Kenya: Conservation and ManagementImplications*Journal of Heredity.

Okita-Ouma B. (2004). *Population performance of Black Rhino (Dicerosbicornismichaeli) in six Kenyan rhino sanctuaries.*University of Kent.

xOkita-Ouma B., Amin B, Kock R, (2007) Conservation and management strategy for the black rhino (Diceros bicornis michaeli) and management guidelines for the white rhino (Ceratotherium simum simum) in Kenya (2007-2011).Nairobi: KWS. p. 157

Park, Kenya. *African Journal of Ecology*.38, pp. 62-71.

xPaul Udoto (2012) Wildlife as a Lifeline to Kenya's Economy: Making Memorable Visitor Experiences.

Rayaa G. (2002) The Use of Genetic Markers to Measure Genomic Response to Selection in Livestock.Genetics. 162: 1381-1388.

Rozas J, Sanches-DelBarrio JC, Messenguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19 (2496-2497).

Rubinoff D, Holland BS (2005) Between two extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Systematic Biol 54 (6):952-961.

Schwartz MK, Luikart G, Waples RS (2006) Genetic monitoring as a promising tool for conservation and management. Trends EcolEvol 22 (1):25-33.

xSoka GE, Rija AA, Owino A (2014) Modeling Black Rhino (*Dicerosbicornis*) Population Performance in East Africa: The Case of Lake Nakuru National Park, Kenya . J Biodivers Endanger Species 2: 126. doi:10.4172/2332-2543.1000126.

Sommer S (2003) Effects of habitat fragmentation and changes of dispersal behaviour after a recent population decline on the genetic variability of noncoding and coding DNA of a monogamous Malagasy rodent. Molecular Ecology 12 (10):2845-2851.

Soulé ME (1980) Thresholds for survival: maintaining fitness and evolutionary potential. Conservation biology: an evolutionary-ecological perspective. Sinauer, Sunderland, Massachusetts.

Soulé ME. In: Soulé ME, Wilcox BA (1980) Eds. Conservation Biology: An evolutionary ecological perspective. Sunderland: SinauerAsso-ciates; pp. 151-69.

Swart MKJ, Ferguson JWH, du Toit R. Flamand JRB. (1994) Substantial genetic variation in southern African black rhino. *Journal of Heredity* 85, p. 261–266.

Swofford DL (2002) PAUP* 4.0b: Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.

Taberlet P, Bouvet J (1994) Mitochondrial-DNA Polymorphism, Phylogeography, and Conservation Genetics of the Brown Bear *Ursusarctos*in Europe. P Roy SocLond B Bio 255 (1344):195-200.

Talukdar BK, Emslie R, Bist SS, Choudhury A, Ellis S, Bonal BS, Malakar MC, Talukdar BN, Barua M (2008)*Rhino unicornis*.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. MolBiolEvol 28 (10):2731-2739. doi:Doi 10.1093/Molbev/Msr121.

Tamura K, Dudley J, Nei M, Kumar S. (2007) MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0.*MolecularBiology and Evolution* 24, pp. 1596-1599.

The IUCN Red List of Threatened Species.Version 2014.3.<www.iucnredlist.org>. Downloaded on **04 February 2015**.

Toews DPL, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. MolEcol 21:3907-3930.

Tougard C, Delefosse T, Hanni C, Montgelard C (2001) Phylogenetic relationships of the five extant rhino species (Rhinocerotidae, Perissodactyla) based on mitochondrial cytochrome

b and 12S rRNA genes. MolPhylogenetEvol 19 (1):34-44. doi:Doi 10.1006/Mpev.2000.0903.

Tracy LN, Wallis GP, Efford MG, Jamieson IG (2011) Preserving genetic diversity in threatened species reintroductions: how many individuals should be released? AnimConserv 14 (4):439-446.

Traill LW, Brook BW, Frankham RR, Bradshaw CJA (2010) Pragmatic population viability targets in a rapidly changing world. BiolConserv 143 (1):28-34.

Van Hooft WF, Groen AF, Prins HHT (2002) Phylogeography of the African buffalo based on mitochondrial and Y-chromosomal loci: Pleistocene origin and population expansion of the Cape buffalo subspecies. MolEcol 11:267-279.

Waits LP, Talbot SL, Ward RH, Shields GF (1998) Mitochondrial DNA phylogeography of the North American brown bear and implications for conservation.ConservBiol 12 (2):408-417.

xWalpole MJ, Morgan-Davies M, Milledge M, Bett P, Leader-Williams N (2001) Population dynamics and future conservation of a free-ranging black Rhino (Dicerosbicornis) population in Kenya. Biological Conservation 99: 237-243.

Watson LH, Chadwick P (2007) Management of Cape mountain zebra in the Kammanassie Nature Reserve, South Africa. S Afr J Wildl Res 37 (1):31-39.

Western D, Vigne L (1985) The deteriorating status of African rhinos. Oryx 19:215-220,

Williams JG (*1990)* DNA polymorphisms amplified by arbitrary primers are useful as genetic marker *Nucleic Acids Res.;18(22):6531-5.* PMID: 1979162

Zenger KR, Richardson BJ, Vachot-Griffin AM (2003)A rapid population expansion retains genetic diversity within European rabbits in Australia. MolEcol 12 (3):789-794.

Zhang Y, Wang X, Ryder OA, (2002) Genetic diversity and conservation of endangered animal species. Pure ApplChem 74:575–584.

Zheng Zhang, Scott Schwartz, Lukas Wagner, Webb Miller (2000), "A greedy algorithm for aligning DNA sequences", *J Comput.Biol.***7**(1-2):203.

Zukowsky L (1965) Die systematic der gattung*Diceros*Gray, 1821. ZoologischeGarten 30:1178.

APPENDICES

Appendix A

Complete details of the samples

No	label No	Name	Age	Sample type	Location
1	555	BRNak1	A	Tissue	Lake Nakuru National park
2	537	BRNak2	A	Tissue	Lake Nakuru National park
3	571	BRNak3	A	Blood	Lake Nakuru National park
4	560	BRNak4	A	Tissue	Lake Nakuru National park
5	505	BRNak5	A	Blood	Lake Nakuru National park
6	567	BRNak6	A	Tissue	Lake Nakuru National park
7	564	BRNak7	A	Blood	Lake Nakuru National park
8	572	BRNak14	A	Blood	Lake Nakuru National park
9	577	BRNak8	A	Blood	Lake Nakuru National park
10	556	BRNak15	A	Tissue	Lake Nakuru National park
11	559	BRNak9	A	Tissue	Lake Nakuru National park
12	538	BRNak10	A	Tissue	Lake Nakuru National park
13	570	BRNak11	A	Tissue	Lake Nakuru National park
14	559	BRNak12	A	Blood	Lake Nakuru National park
15	544	BRNak13	A	Tissue	Lake Nakuru National park