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Investigating the decline of the African lion population in Lake Nakuru National Park using diet analysis

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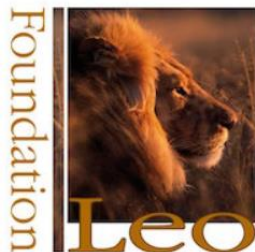
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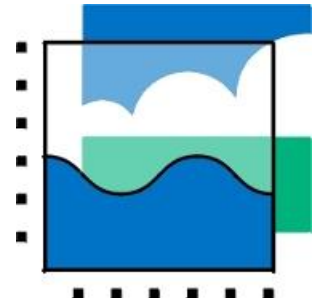
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Abstract

During the last decades the global African lion (*Panthera leo*, L. 1758) population has decreased rapidly. In Lake Nakuru National Park (LNNP) in Kenya this decline has also been observed. One of the possible reasons of this decline is the increasing African buffalo (*Syncerus caffer*) population in LNNP. Large herds can cause danger for lions in hunting attempts and result in deaths. The pressure of the African buffaloes can result in prey switching towards smaller prey species and species such as reptiles, birds and very small mammals (<5kg). Hence, by investigating the foraging behaviour and predator-prey interactions of the lions in LNNP it can be possible to get insight into factors that are causing the decrease of these lions. This study assessed the foraging behaviour in LNNP, with the use of three different methods of diet analysis: carcass counts, hair morphology analysis and DNA analysis. This study especially focusses on the effect of the large population of the African buffalo in LNNP. Does the diet of the African lion in LNNP indeed reflect an avoidance of the African buffalo compared to the lions in the nearby wildlife conservancy (Soysambu Conservancy, SC)? Furthermore, this study investigates whether this possible pressure results in prey switching. This study showed that the current lion population in LNNP is still in decline (with a decrease from approximately 20 to 9 lions in two years) and that the African buffalo is one of the drivers for this decline. This study confirmed that there is a high avoidance for African buffalo by lions in LNNP, while this is not observed with the lions from SC. The general diet in LNNP and SC were very different. Lions in LNNP consumed a larger amount of species under 100kg, as the preferred weight is around 350kg, this can indicate a possible prey switch towards smaller prey. DNA analysis showed that slightly more non-mammalian prey species and very small mammals were eaten in LNNP than in SC, of which most were positive for reptiles. These results imply that park management should reduce the number of African buffaloes in LNNP to counter the decline of lions in LNNP.

Keywords: African lion, Lake Nakuru National Park, Soysambu Conservancy, Environmental DNA, prey avoidance, prey switching, African buffalo

Introduction

The global population of the African lion (*Panthera leo*, L. 1758) has declined with approximately 43% over the last 21 years (Bauer *et al.*, 2016). In parts of its range the lion has disappeared completely. The African lion has therefore been listed as Vulnerable on the global IUCN Red List (category A2abcd ver.3.1). The decline is most prominently observed in Western and Central Africa (Riggio *et al.*, 2013). The most important causes for the decline in lion populations are habitat destruction, prey depletion and human wildlife conflicts (Bauer *et al.*, 2016). The increasing human population has resulted in a decline of the surface of Savanna habitat and an increase of habitat fragmentation (Riggio *et al.*, 2013). Subsequently lions are forced to live in smaller areas where migration is limited due to fragmentation, which leads to less gene flow and more conflicts with humans (Craigie *et al.*, 2010).

The population of the African lion (*Panthera leo melanochaita*, S. 1842) in Kenya has also shown a drastic decline over the last 30 years (Hazzah *et al.*, 2009). According to a study of Chardonnet (2002), the lion population consisted of 2,780 individuals in 2002. This dropped to 2,439 in 2004 (Bauer & van der Merwe, 2004) and to 1,970 in 2009 (M. Chege in prep). Due to the increasing

conflicts with humans and the decrease in habitat size it is expected that national parks and conservancies might provide a sustainable habitat for the African lion in the future. Previous research showed that stable management with a high capital investment and fencing can contribute to an increase in lion populations (Packer *et al.*, 2013).

Lake Nakuru National Park (LNNP) (187.9 km²) in Kenya is a small enclosed and protected wildlife conservation area. In 2002 the lion population in the park was estimated to be 65 (approximately 35 lions per 100 km²) (Muller, 2018). However, in the year 2011-2012 this number dropped to 56 individuals, which is a 14% decrease in population size (Muller, 2018, Ogutu *et al.*, 2012). A more recent study of the KWS found approximately 18 to 22 individuals in the park (KWS, 2017). In 2014 there was an increase of the lion population reported in Soysambu Conservancy (SC) which is only separated from LNNP by a fence (M. Chege, in prep.). This conservancy previously held no lion population and currently has a population of about 14 lions. This results in the belief that lions escaped from LNNP and dispersed to the nearby conservancy via burrow paths under the fences (Kassily *et al.*, 2008). According to KWS researchers a plausible reason for this dispersion is pressure from the large African buffalo population in LNNP on lions. By dispersing towards SC the lions experienced less hunting risks and easier foraging.

The estimated size of the African buffalo population in LNNP is about 6,000 individuals, while the carrying capacity is approximately 400 (A. Bett, 2019, personal communication). Mwasi (2002) demonstrated that the African buffalo population in LNNP increased rapidly from 1976 until 1999. In the Annual report of KWS from 2015 the African buffalo was the dominant species in the entire park. The density of the African buffalo in LNNP is further increasing due to the decrease of land area. The water level in LNNP has increased from 30.46 km² in 2009 to 57.55 km² in 2014 (Gichuru & Waithaka, 2015). At this moment the African buffalo density in LNNP is high enough that KWS is considering to translocate part of the population to other parks (H. H de Jongh, 2019, personal communication). Even though the African buffalo is one of the main prey of lions, the lions are decreasing rapidly in LNNP. (Davidson *et al.*, 2013; Hayward & Kerley, 2005).

The large herd sizes of the African buffalo result in more safety from predation by increased detection of the predator, and from better protection by the group, as they are stronger together (Hamilton, 1971). Moreover, the African buffalo is a prey species which defends itself and is capable of killing a lion (Tambling *et al.*, 2012). This can lead to avoidance of African buffalo as prey by its predators due to the possible risks involved during hunting (Hayward and Kerley, 2005). This would increase the density of the African buffalo in LNNP further. It is suggested that the lions in LNNP are in decline because of the high density of the African buffalo in the park (J.S.K Shonko, 2019, personal communication).

Lions are opportunistic predators and need a daily uptake of 10.4 kg for males and 7.5 kg for females (Schaller, 1972). They mostly prey upon species ranging from 190 to 550 kg, but 350 kg is the preferred weight (Hayward & Kerley, 2005). The most common prey is zebra (*Equus sp.*), wildebeest (*Connochaetes sp.*), African buffalo (*Syncerus caffer*), giraffe (*Giraffa sp.*), pigs (*Sus sp.*) and different species of antelopes (Schaller, 1972). Because of their opportunistic foraging behaviour, they also prey upon small species like hares and warthogs or even on birds, reptiles or very small mammals that weigh less than 5kg (also known as “snacking”) (Davidson *et al.*, 2013; Schaller, 1972). This is mostly observed during periods of common prey scarcity and is called prey

switching. It is hypothesized that prey switching might also be observed when lions are experiencing threats of large herds of prey such as the African buffalo.

The foraging behaviour of the lion and the prey species included in its diet can give insights into processes that are contributing to the decrease in population size in LNNP and the dispersion towards SC. It can give insight in prey preference which allows measuring avoidance of certain prey like the African buffalo and switching towards other groups of prey. This can result in less energy uptake when smaller prey is captured instead (Carbone et al., 2017). Change in diet towards different weight classes or taxonomic groups can provide information on changes in prey populations. It is therefore important to assess the diet of the lion to get a better understanding of the previously mentioned processes that can contribute to the current decrease in population size.

The aim of this research was to study the decline of the lions in LNNP with the use of diet analysis, with a focus on the pressure from the African buffalo population and the potential switch in prey species of the lion. This was done with the use of three complementary methods for diet analysis. The results of the dietary analysis in LNNP were compared to the diet of the lions from SC. With diet analysis can be assessed whether the current population of the African buffalo in LNNP results in an avoidance of this prey by the lion population, and whether this possible pressure results in prey switching towards prey such as birds, reptiles and very small mammals (<5kg). Lion diet analyses gives important insight into predator-prey dynamics, prey (African buffalo) avoidance and prey switching. These results inform management how to counter the decrease of the lion population size in LNNP. Studying these processes is there for important for conservation purposes of the African lion.

Methods

Study site

This study was performed in 2019 from the 6th of February until the 19th of April in Lake Nakuru National Park and Soysambu Conservancy. LNNP is situated in the Great Rift Valley region of Kenya. (Kenya Wildlife Service, 2018; Kassily, 2002). The fenced park was established in 1961 and occupies an area of 189.7km² (up to 57km² maximum can be occupied by the lake) (Mwasi, 2002). The landscape consists of grasslands, swamps and marsh, with rocky cliffs and outcrops. There are areas of woodland and rocky hillsides covered with bush land and forest (Kassily, 2008). The saline lake is a hotspot for birds, in total Lake Nakuru holds more than 500 bird species. (Ham, 2018).

To the East of LNNP is Soysambu Conservancy surrounding Lake Elementeita (Figure 1). SC is a partly enclosed protected wildlife area and has about the same habitat as LNNP dominated by Acacia and woodland (Muller, 2018). In total SC occupies an area

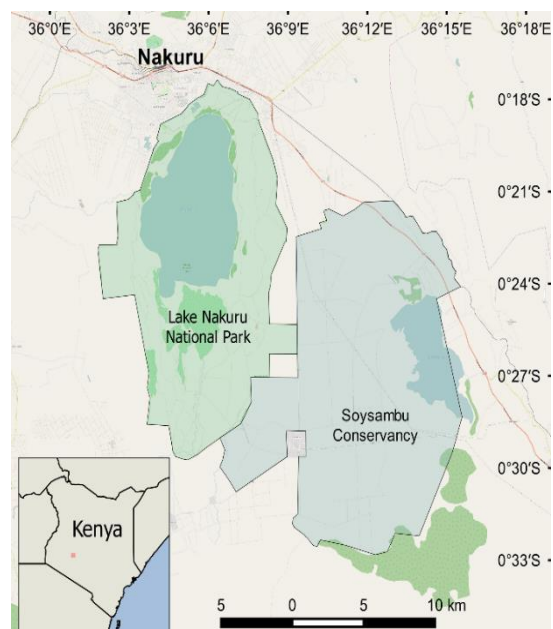


Figure 1: map of Lake Nakuru National Park and the adjacent Soysambu Conservancy. (Muller, 2018)

of 194km² and is a refuge for about 50 mammalian species and 450 bird species (Muller, 2012; Soysambu Conservancy, 2015). The conservancy is dominated by the zebra and the African buffalo. SC is not only a wildlife conservancy but also holds a livestock ranch. The livestock ranch consisted of 8,700 boran cows (*Bos indicus*), 2,000 sheep and 1,200 goats (Soysambu Conservancy, 2015).

Lion sightings

To assess whether the lion population in LNNP is still in decline, individual lions were identified to compare the population size with previous years. This was also done in SC to see how the lions perform in that area and what the current population size is. Lions can be distinguished from each other by their unique whisker spot pattern or scars (when present) (Pennycuik & Rudnai, 1970). Number of cubs were identified via the mother they were observed with (Pennycuik & Rudnai, 1970).

Transects

In order to calculate the proportion of prey species in the lion's diet relative to the prey abundance, transect counts were done in LNNP and SC. Transects were performed to assess the distribution, diversity, abundance and density of potential lion prey species. With the use of the Mileseey Golf laser Rangefinder the distance of the prey to the transects were measured. All mammalian prey species within 500 meters on each side of the transect were counted and identified, some birds (ostrich and guinea fowl) were also included. Species were identified using the pocket guide of mammals in East Africa (Stuart & Stuart, 2009). A route consisting of seven transects of 2 km in length by 1 km in width along LNNP were performed ten times (Figure 2). Together these transects cover 7% of the total park and are situated in a number of habitats, tall and short grassland, acacia woodlands and swamps. Due to time constraints because of driving distance, only three transects were selected in SC which covers approximately 3% of the total area (Figure 2). The transects in SC were performed seven times. All transects were performed clockwise and anti-clockwise alternating in the morning (starting at 06:30) and afternoon (starting at 15:00).



Figure 2: Transects in Lake Nakuru National Park and Soysambu Conservancy.

Diet analysis

To get a good insight in the general diet and preferred prey species of the lion from LNNP and SC, three methods for diet analysis were performed. i) Prey carcass counts, ii) microscopic hair analysis of prey hair morphology and iii) environmental DNA (eDNA) analysis. For the last two methods scat samples of the lions were needed. Scat and carcasses were obtained by opportunistic surveys and were identified using the pocket guide of mammals in East Africa (Stuart & Stuart, 2009). Three methods were conducted because they all have their own limitations but together complement each other (Lesilau, 2019). In this study eDNA was only used to detect pre-selected species or taxonomic groups. Hair analysis limits the number of taxonomic groups which can be investigated, as some prey don't have hairs or feathers (Perrin & Campbell, 1980), carcass counts are most likely to underestimate the number of kills. More specifically the kills of mammals ranging from 5-50kg and especially very small mammals under 5kg (Rapson & Bernard, 2007).

Carcass counts

Carcasses of prey were searched for opportunistically in LNNP and SC. Carcasses of prey were inspected and identified, GPS data, date, the age and whether the carcass is old or new were recorded for each carcass. To confirm that these prey species were indeed killed by a lion, teeth marks were identified and lion tracks and scat were searched for around the carcass.

Microscopic hair analysis

Microscopic analysis of prey hair morphology was used to assess the diet of the lion in LNNP and SC. This method is able to capture almost every animal with hairs that was eaten by the lion. Entire scats were collected in plastic bags and were sundried. In the KWS vet lab in Nairobi the dry scats were put in stockings of 15 denier and were soaked in cold water for 15-20 minutes. Thereafter the stocking with scat sample was washed two times for 15 minutes to separate the organic components (Huqa, 2015; K. Groen, personal communication, 2019). After washing the scats, the stockings were put in the drier two times for 5 minutes. Hairs, bones and feathers remain in the stocking for further analysis. Pictures were made of the bones and feathers in the scat. Twenty hairs were randomly selected from the scat and cleaned with 96% ethanol (Tommissen, 2017). From these randomly selected hairs, five complete hairs were selected for identification. Hairs were partly identified based on the colour, size and shape. Thereafter the cuticle pattern of the hairs was used to determine the species of prey. A solution of 1.7 grams of gelatine (one sheet Dr. Oetker white) and 40 mL demi-water was used to make the imprints on. The solution was placed on a hot plate (65°C) until the gelatine was completely dissolved (Tommissen, 2017). A layer of gelatine was spread over a microscope slide, the hairs were placed on the gelatine before it completely dried (Huqa, 2015). When the gelatine layer was dry, the hairs were removed. The scale patterns were observed with a microscope at Leiden University. The hairs were identified with the use of a reference library of prey hair morphology from Beveridge & van den Hoogen. (2013).

DNA analysis

For DNA analysis the scat samples were also collected. From each scat sample five picks from the inside of the scat were taken with a tweezer distributed over the centre of the scat. DNA was collected from the centre of the sample minimizing the parts with DNA degradation and minimizing the number of epithelial cells from the lion in the sample. The samples were put in 2ml tubes preserved on 99% ethanol and stocked in the fridge to prevent DNA degradation. To minimize contamination with human DNA gloves were put on. For each sample the date of collection, GPS data and freshness of the scat (1: fresh- 4: only hairs left) were recorded.

At Leiden University the QIAamp DNA stool mini kit (Qiagen) was used to extract the DNA from the lion scat (Groen, in prep.; Mumma et al., 2016). A droplet digital PCR (ddPCR) was performed using specific and general primers for the species of interest. Because in ddPCR the reaction mix is partitioned in a lot of tiny reactions of one nano litre, the DNA can be measured very sensitively (Bio-Rad Laboratories, 2020). All ddPCR experiments were conducted on the Bio-Rad QX200 Droplet Digital PCR system.

Two species and three taxonomic groups of interest were selected to perform DNA analysis on. The African buffalo (*Syncerus caffer*) was selected to study the presence or absence of African buffalo avoidance by lions in LNNP and SC. The Boran cattle (*Bos indicus*), only present in SC, was selected to get an insight in the proportion of livestock in the diet of the lion. For these species two specific primers were designed with the use of Geneious (version 2019.1), GenBank and Primer-BLAST (Ye et al., 2012; NCBI, 2019). To increase the specificity of these primers a TaqMan probe was designed with the Primer3 software.

To detect whether lions also eat very small mammals, birds and reptiles, five primers were made. A general primer for mongoose (Herpestidae) as a representation of very small mammals, a general primer for birds (Aves) together with a specific primer for ostrich (*Struthio*) and a general primer for reptiles (Squamata) in combination with a specific primer for monitor lizard (*Varanus niloticus*). General primers were also made with the use of Geneious, GenBank and Primer-BLAST. With these primers the samples were tested with the use of the Biorad Evagreen protocol. The list of all the used primers can be found in Appendix A. The ddPCR mix and schedule for the specific and the general primers are listed in Appendix B. With the use of general primers multiple species can be detected. The lists of species that each general primer works on are stated in Appendix C.

All primers were tested for selectivity and specificity on positive DNA samples of the target species and closely related species. Positive samples that were used to test the primers and that were used during sample analysis are shown in Appendix D. The results from the ddPCR were analysed in QuantaSoft (version. 1.7.4.0917). A threshold value was determined with the use of a positive sample and a negative control (Milli-Q) and was also used to determine whether the scat samples were truly positive.

Data analysis

The population densities of the prey species were calculated with the Hayne estimator (1949) using the prey transect data: Hayne's estimator (D_H) = $\frac{n}{2L} \left(\frac{1}{n} \sum \frac{1}{r_i} \right)$.

In this equation n is the number of the animals spotted, L is the length of the transect and r_i is the distance to each animal i (Krebs, 1989). This equation was used to estimate the incorporation of a certain species in the lion's diet relative to its abundance.

To calculate the proportion of each prey in the diet of the African lion the following formula is used: $Proportion\ in\ diet\ (r) = \frac{Frequency\ of\ occurrence\ in\ diet\ of\ one\ prey\ species}{Total\ frequency\ of\ occurrence\ in\ diet\ of\ all\ prey\ species}$

(Upadhyaya et al., 2018). This formula was used for both hair morphology analysis and DNA analysis results.

In order to calculate whether there is avoidance or preference for prey species by the lion, the Jacobs' Index was used: Jacobs' Index (D) = $\frac{(r-p)}{(r+p-2rp)}$ (Jacobs, 1974).

In this equation r is the proportion of how much a prey species is part of the diet of the lion and p is the proportional availability of the prey species in abundance. The outcome of the index is between -1 (avoidance) and +1 (preference). For this equation the results of the hair morphology analysis were used with the exception of the African buffalo and boran cattle for which DNA analysis results were used.

To test for significant difference in proportion of prey consumed by lions between LNNP and SC a Fisher's exact test was performed.

Results

During opportunistic sightings for lions in LNNP only six adult lions were found with an equal male to female ratio. There were three cubs found belonging to the oldest female of the park. Lions in LNNP were often spotted two at a time or alone. In SC only nine lions were found, but the Soysambu lions are known to disperse towards Naivasha (Personal communication, K. Combes, 2019). The nine lions (two lionesses and seven cubs) belong to the same pride and were always spotted together.

The total prey density in LNNP was 63 prey animals per km², the total prey density in SC was estimated to be slightly higher with 67 prey animals per km². Densities of prey species differed in both parks. As expected, the African buffalo population in LNNP had the highest density with 19.6 individuals per km² (Figure 3). In SC the zebra population was the largest with approximately 20 individuals per km² (Figure 4). Relative comparisons between the densities per park are represented in Figure 5 and 6.

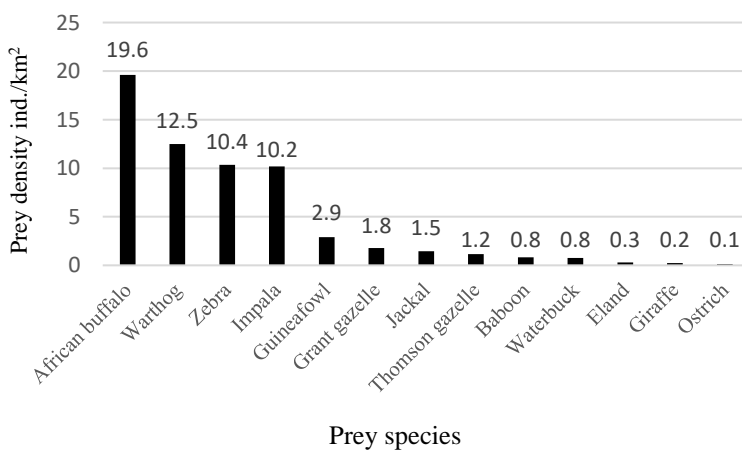


Figure 3: Prey density estimation based on transect counts in LNNP from highest to lowest. In total 10 transect days performed. N=145

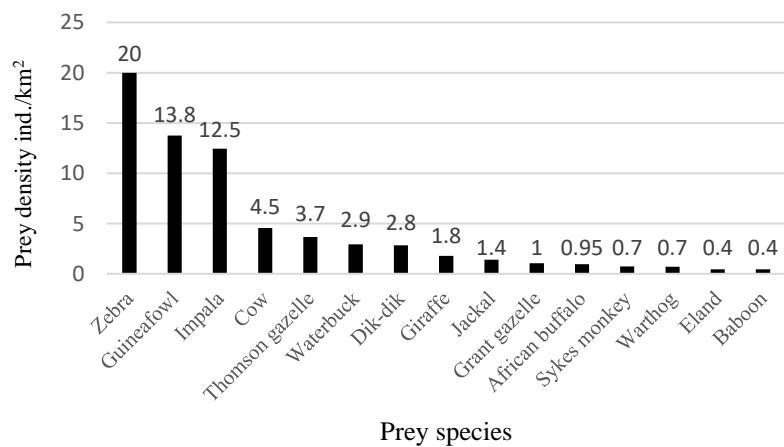


Figure 4: Prey density estimation based on transect counts in SC from highest to lowest. In total 7 transect days performed. N=39

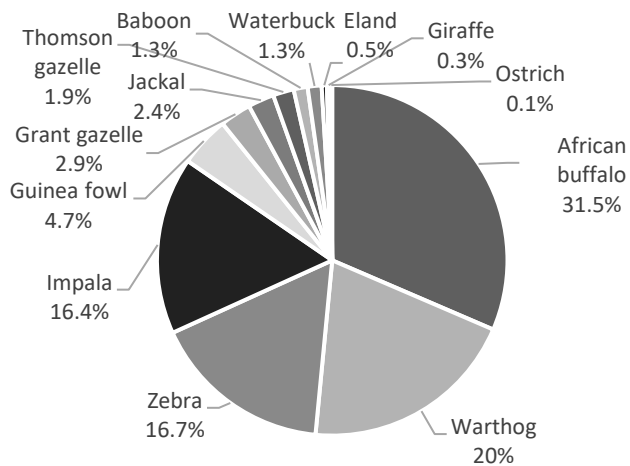


Figure 5: Relative densities of prey species counted in LNNP based on 10 transect days. N=145

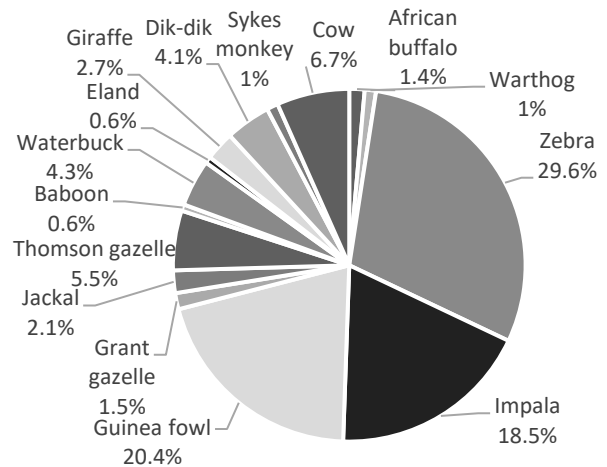


Figure 6: Relative densities of prey species counted in SC, based on 7 transect days. N=39

During this study five carcasses were found in LNNP and six in SC, the results of the carcass counts can be found in Appendix E. The locations of the scat collections can be found in Appendix F. The proportion of prey species in the diet of the lion was analysed using hair morphology for prey species and DNA analysis for the African buffalo, boran cattle, birds, reptiles and very small mammals. Figure 7 and 8 presents the proportion of the prey species in the diet of the lion with the use of hair morphology. Most prey ranged from 23kg to 590kg in weight. In total 13 different species were found in the scats from LNNP and 12 species were found in SC. The species that were eaten the most in LNNP were the African buffalo (22% of prey eaten was African buffalo), Thomson's gazelle (20%) and the zebra (15%). According to hair morphology 5% of the eaten prey were birds or very small mammals (rodent and rock hyrax). In SC 56% of the prey eaten were zebra and therefore contributed the most to the diet of the lion, 10% of the diet consisted of bird, rodent, rock hyrax and springhare. The Fisher's exact test showed that the proportion of the prey species that were eaten by lions (the diet) differs significantly between the two parks ($p=1.874e-07$).

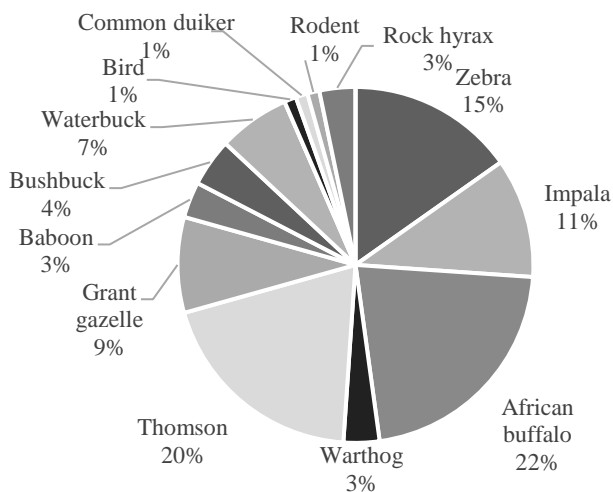


Figure 7: Proportion of prey species in the diet of the African lion, (hair morphology analysis) in LNNP. Based on 58 samples

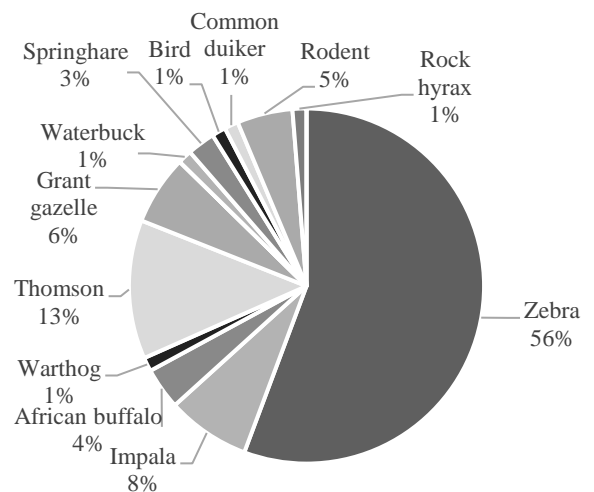


Figure 8: Proportion of prey species in the diet of the African lion, (hair morphology analysis) in SC. Based on 55 scat samples.

With the use of DNA analysis was confirmed that lions in SC ate the domesticated boran cattle. In six scat samples DNA from this species was found. None of the scats from LNNP contained boran cattle DNA. Despite the huge difference in density of the African buffalo between the parks, the amount of scat samples that were positive for African buffalo DNA in both parks did only differ by three (Figure 9).

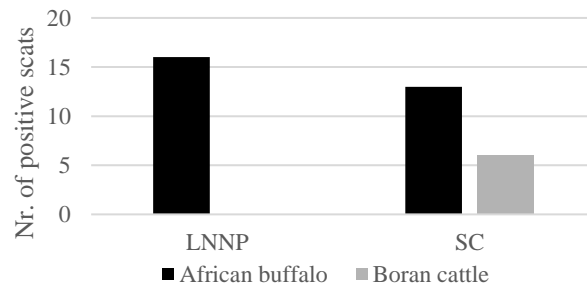


Figure 9: Number of positive samples per park for Buffalo and Cow (boran cattle) based on DNA analysis. With a total amount of 60 scats in LNNP and 57 in SC.

Presence of a species in the diet does not mean that this species is preferred. Density of the prey species and the proportion of this prey in the diet of the lion should be combined in the Jacobs' index, in order to look for preference or avoidance for this prey (Figure 10). Only species that were counted during the transects and were present in the scats during hair analysis and DNA analysis (African buffalo and boran cattle) were taken into account for the Jacobs' index. Although African buffaloes were eaten quite a lot in LNNP (27% of the scats were positive with DNA analysis), based on the proportion in the diet relative to their abundance in the park the Jacobs' index came out negative. Meaning lions showed the highest avoidance for African buffaloes, warthogs and birds. Lions in LNNP mostly preferred Thomson's gazelle, waterbuck and Grant's gazelle. In SC lions preferred African buffaloes, Grant's gazelle and zebras the most and mainly avoided baboons, birds and waterbucks. Boran cattle is not present in LNNP, the Jacobs' index for boran cattle in SC indicated a slight preference with a value of 0.06.

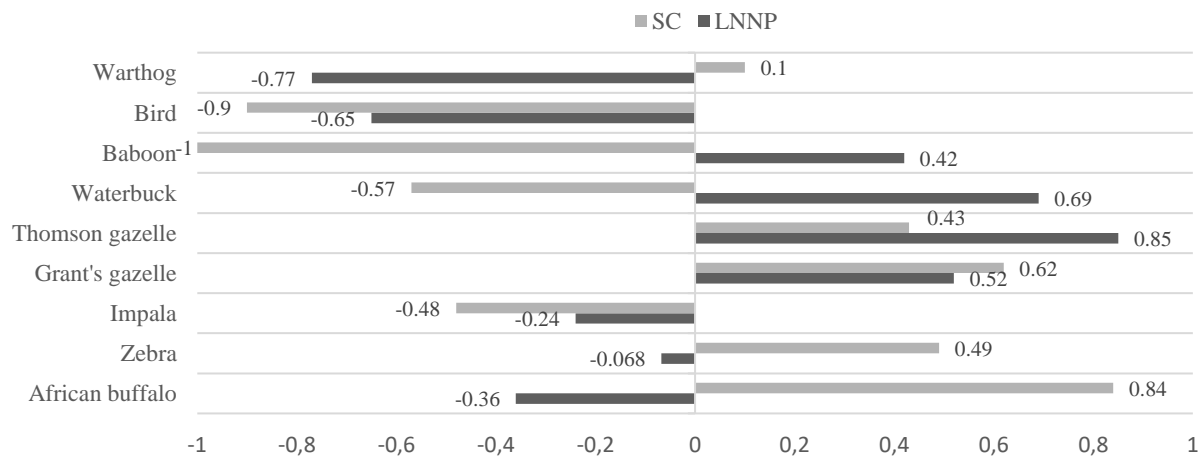


Figure 10: Prey preference of the African Lion in LNNP and SC. -1 is avoidance of prey and +1 is preference for that prey species. Only species taken into account which were spotted during transect counts and found in the hair analysis. The values of the African buffalo are based on DNA analysis.)

It is hypothesized that the pressure from the African buffalo can result in a shift in diet towards prey such as birds, reptiles and very small mammals. Via DNA analysis the proportion of these prey species in the diet was analysed and compared between LNNP and SC. In LNNP 32% of the 60 scats contained these prey species, and from the 57 scats in SC 23% were positive. In LNNP and SC reptiles were eaten the most (Figure 11 and 12). In SC no mongoose DNA was found in the scats.

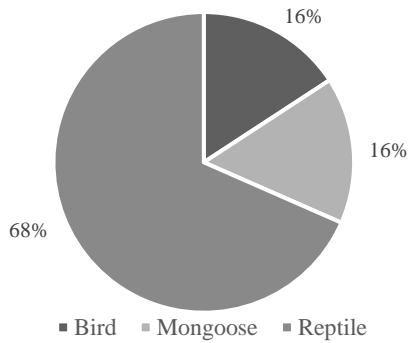


Figure 11: Proportion of Reptiles, birds and mongoose in LNNP. (19 positive samples).

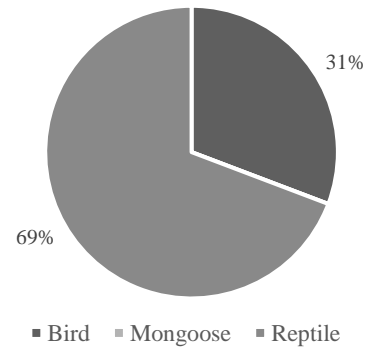


Figure 12: Proportion of Reptiles, birds and mongoose in SC (13 positive samples).

Different kinds of diet analyses were performed during this study. The different methods yielded different results (Figure 13). DNA analysis and hair analysis had ten positive samples in common for African buffalo and one sample for birds but not one for the boran cattle, reptiles and mongoose.

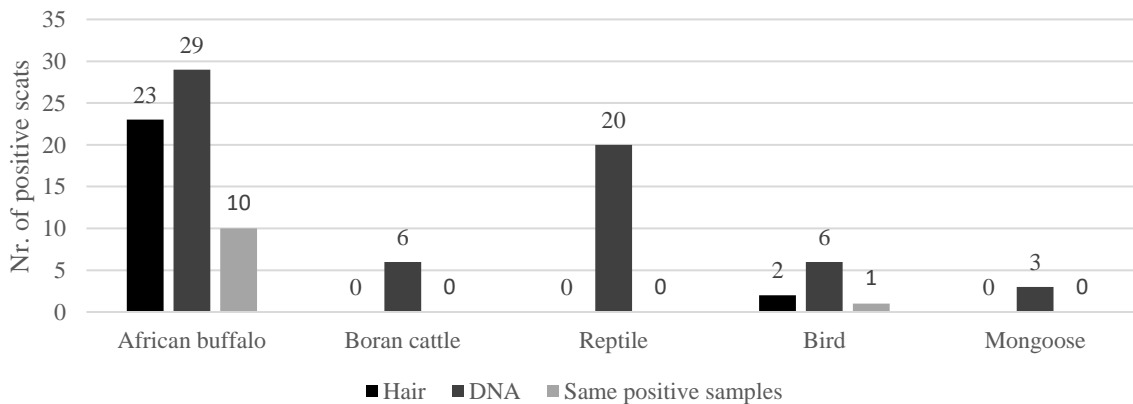


Figure 13: Differences in results of DNA and hair analysis during this study. Based on both samples from LNNP and SC. For example, 23 scats were positive for African buffalo with hair analysis and 29 in DNA analysis. From these samples only 10 were positive in both methods.

Discussion

The purpose of this study was to assess drivers for the decline of the African lion in Lake Nakuru National Park with the use of diet analysis, and a focus on the African buffalo population as a potential threat and prey switching towards smaller prey, reptiles, birds and very small mammals <5kg. This was done with the use of three methods for diet analysis. In this study an ongoing decline of the lion population in LNNP has been observed. As expected, lions showed an avoidance for the African buffalo in LNNP (-0.36), in SC the African buffalo was preferred by the lions (0.84). This can be an indication that the avoidance of the African buffalo by lions in LNNP is a result of the large population size which can cause trouble for the lion during foraging. According to hair analysis, the diet between the parks is very different ($P= 1.874e-07$). In comparison to SC, the lions in LNNP seem to shift towards smaller prey as they eat prey of less than 100 kg more frequently. Reptiles, birds and mongoose were also consumed more often in LNNP according to DNA analysis.

African lion density

This study supports the long observed declining trend of the lion population in LNNP. In this study three lionesses, three lions and three newly born cubs were observed. The previous lion population survey in 2018 by local researchers estimated the lion population in LNNP to be between 18 and 22 lions (M. Chege, personal communication, 2019). The lower population size observed in this study can be a result of dispersion towards SC in the past (K. Combes, personal communication, 2019). Dispersion of male lions is common, it mostly happens when males leave their natal pride to become nomadic and search for new prides (Packer & Pusey, 1987). Females however, rarely leave their natal area. In a previous study was stated that this reduces their fitness because of less knowledge of the area and no advantage of nearby close relatives which result in less successful rearing of cubs (Packer & Pusey, 1987). Dispersion towards SC can be explained for males as there are very few prides in LNNP, but for females this dispersion is less likely to occur. A possible explanation can be the large African buffalo population that motivates dispersion towards SC. A few months prior to this study one lioness in LNNP was killed due to an African buffalo attack, indicating the importance of this study and the threat African buffaloes can pose (J.S.K Shonko, personal communication, 2019).

In SC two lionesses with seven cubs were observed frequently. SC is a non-fenced conservancy, and according to local researchers it is home to more lions than LNNP (R. White, personal communication, 2019). According to news messages and conversations with the local researchers, male lions from SC were spotted around Naivasha, an area close to SC. Therefore, the population in SC could be higher than observed.

Prey abundancy

This study showed that the African buffalo population in LNNP is the highest of all prey species (19.6 individuals/km²). This is in large contrast with the African buffalo population in SC (0.95 individuals/km²). The density from SC is more in accordance with previous studies in Kenya on prey densities (Table 1). According to local researchers from LNNP the density of the African buffalo is too high and exceeds the carrying capacity of 400 individuals (A. Bett, Personal communication, 2019).

Table 1: African buffalo population densities in different areas in Kenya.

Area	African buffalo density (individuals/km ²)	Source
Amboseli NP	0.14	Huqa et al., 2015
Masai Mara	0.99	Kiambi et al., 2010
Meru Conservation Area	0.19	Mwangi et al., 2007
Laikipia/Samburu ecosystem	0.20	Omondi et al., 2002

Diet of the African lion in LNNP and SC

Lions are opportunistic feeders, as is seen in the diverse diet of the lions in LNNP and SC. Overall, most species eaten in LNNP had a much lower weight than the preferred 350kg, indicating a possible switch to smaller prey species. In total, 56% of the species in the diet of lions from LNNP were <100kg, in comparison to 38% in SC. This switch towards smaller and less abundant prey is in contrast with previous studies where lions switched their diet to species that were increasing in population size and not by avoiding the species which is most abundant (Bissett et al., 2012; Rapson & Bernard, 2007). This switch is probably due to the threat of large African buffalo population in combination with the small lion prides in LNNP (Funston et al., 2001; Packer et al., 1990).

African buffalo avoidance

This study supports the hypothesis that lions avoid African buffaloes in LNNP. Although hair and DNA analysis concluded that African buffaloes were eaten relatively often (22% of scat positive during hair analysis, 27% of the scat positive for DNA), due to the high density of African buffalo in the park the Jacobs' index was -0.36, indicating an avoidance. Previous studies showed that the Jacobs' index is often positive for African buffaloes, such as is seen in Amboseli NP (0.68), in Hwange NP, Zimbabwe (± 0.8) and in an average Jacobs' index from 30 surveys (0.32) (Huqa, 2015; Davidson et al., 2013; Hayward & Kerley, 2005). A positive index was also observed in SC, with a value of 0.84. Previous studies showed that lions are more likely to kill the most abundant prey species in an area (Rapson & Bernard, 2007; Schaller, 1972; Pienaar, 1969). This was observed in SC where zebra was the most abundant prey and also the most consumed prey (56%) with a positive Jacobs' index of 0.49. Contrastingly, in LNNP the most consumed prey species was the African buffalo, but the Jacobs' index was negative.

This avoidance can be explained by the pressure of the large population size of African buffaloes in LNNP, resulting in hunting risks. According to previous studies, this avoidance can also be a consequence of small prides (Funston et al., 2001). In LNNP the prides consist of only one or two lions. In previous studies it was concluded that a large group of lions is needed to kill an African buffalo (Packer et al., 1990). The success rate for hunting on African buffalo in females is best with four lionesses. Because the prides in LNNP are so small, lions are responding to the threat of African buffaloes by reduced killings. However, diet analysis also showed that a large part of the diet still consists of the African buffalo. Despite the threat, lions still hunt on the African buffalo, possibly resulting in lower lion population size in LNNP throughout the years.

Hair analysis and DNA analysis gave very different results, from the 29 positive DNA samples and the 23 positive hair samples only 10 were positive for both methods. This discrepancy could be the

result of mis-identification of the hairs during hair analysis. A previous study found that hairs are hard to identify because scale patterns can differ in individuals of the same species (Perrin & Campbell, 1980). Moreover, a complete reference database is needed for identification, which is still lacking, some species were not included and only one type of scale pattern was shown. Due to time constraints only five hairs were randomly selected, which is not in accordance with the method used by Huqa (2015), in that study 20 hairs were identified. During analysis it was observed that one scat can contain multiple prey species, a possibility is that some prey species were missed because of the adaptation to the method that was used. The Jacobs' Index takes proportion of prey in the diet into account, due to the limitations of the hair morphology analysis this index should be considered as a rough estimation for avoidance and preference.

Specificity tests of designed primers resulted in successful amplification of the target species only. Closely related species were used for these tests, it is therefore unlikely that further related species would give a positive result.

Birds, reptiles and mongoose in the diet based on DNA analysis

More scats were positive for DNA of reptiles, birds and mongoose in LNNP (32%) than SC (23%). Reptiles contributed the most to the diet of lions in both parks. In a previous study, lions have been spotted eating snakes, monitor lizards, crocodiles and tortoises (Schaller, 1972). The higher contribution of these species in the diet can be due to the prey switching discussed earlier, or because of a higher availability in LNNP. Birds were rarely spotted during the transects and reptiles and mongooses were not spotted at all, so nothing can be concluded about their abundance in the parks. Although thorough specificity testing has been done, universal reptile primers are known to amplify non-intended targets, this is because of their old evolutionary lineages (Vences et al., 2012). Reaching a consensus sequence for all reptiles is therefore difficult and non-intended targets could be an issue for this universal primer. This study confirms that lions eat reptiles, birds and very small mammals, which according to other studies are not their preferred kind of prey and is a sign of prey switching (Hayward & Kerley, 2005; Schaller, 1972).

In conclusion, during this study six adult lions and three new born cubs were found in LNNP. This is a large decline from the survey in 2018 which estimated 18-22 individuals. In SC two adult lionesses and seven cubs were observed while the other lions probably dispersed towards Naivasha. Prey abundance assessment showed that the density of the African buffalo in LNNP is the highest in the park with 19.6 individuals/km². As hypothesized, scat analysis showed an avoidance for the African buffalo in LNNP (-0.36) and a high preference in SC (0.84). Furthermore, warthogs and impala were strongly avoided by lions in LNNP. While waterbucks, baboons and Thomson's gazelle were preferred. Waterbuck and impala were avoided. The general diet between the two parks was very different ($P= 1.874e-07$). More scat samples were positive for prey species under 100kg in LNNP (56%) than in SC (38%), indicating a possible prey switch towards smaller prey of the lions in LNNP. Moreover, frequency of occurrence of birds, reptiles or mongoose was higher in LNNP compared to scats of lions in SC, which supports the prey switching hypothesis. Prey switching might be a result of the high African buffalo population size in LNNP in combination with the small lion prides. Overall, it can be recommended that hair analysis should be improved with a more complete reference material. DNA analysis is a reliable alternative in case of species-specific targets are needed. Results are less reliable when a consensus sequence is considered.

Multiple observed lion deaths in the past due to African buffalo attacks, a higher incorporation of prey under 100kg, dispersion of lions from LNNP to SC and an observed African buffalo avoidance (-0.36) indicate that the high African buffalo population in LNNP has an impact on the current lion population and is a plausible driver for the reduced lion population. For future management it should be considered to relocate a part of the African buffalo population and/or introduce lions that are known to often kill African buffaloes to counter this decline of the lion population in LNNP.

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Appendix A

Table A-1: Primers and probes created with the use of Geneious, Primer blast, primer3 and GenBank.

Name	Forward primer	Reverse Primer	Probe	TM used
African buffalo	GCATGGACTTCCCTCACCAC	CCGGAGCGAGAAGAGAAAT	TTAGCATGCCGCGTGAAACC	52
Cow (Boran cattle)	GGTGAACCGCTATCAAAGG	TGAGATGCTGTCATTGGTTGTC	AAGGCCAACTTTAAATTAAGC GCC	52
Bird	CATCTACCTCCACATCGGACG	CGGTTGCTATGAGGGTGAG	-	60
Ostrich	ACCCAGTATGCGCTAGTTCA	AACCAGTGGTCTTCAAGCGA	-	60
Snake	CTAACCCGATTCTTCGCCCT	TGGGTTGTTGGAGCCTC	-	55.7
Monitor lizard	TGAATGTATCTGGACTGTCCCT	AGGAGGCTTATGGCGGTT	-	55.7
Mongoose	CTCTCGCAGCAGTACACCT	TTCGATGGTCCGGAGATGG	-	60

Appendix B

Table B-1: African buffalo and Boran cattle (cow) ddPCR schedule and mix

African buffalo and cow ddPCR schedule			ddPCR mix
Temperature	Time	Cycle	1 µl probe African buffalo
95	15 min	1	1 µl forward primer African buffalo
95	30 sec	40	1 µl reverse primer African buffalo
52	30 sec		1 µl probe boran cattle
72	1 min		1 µl Forward primer boran cattle
72	10 min		1 µl reverse primer boran cattle
4	inf.	1	11 µl probe supermix 5 µl sample DNA

Table B-2: Bird and ostrich ddPCR schedule and mix

Bird and ostrich ddPCR schedule			ddPCR mix
Temperature	Time	Cycle	1 µl forward primer bird
95	5 min	1	1 µl reverse primer bird
95	30 sec	40	1 µl forward primer ostrich
60	1 min		1 µl reverse primer ostrich
4	5 min	1	11 µl Evagreen mix
90	5 min	1	5 µl Milli-Q
4	inf.	1	2 µl sample DNA

Table B-3: Reptile and monitor lizard ddPCR schedule and mix

Reptile and monitor lizard ddPCR schedule			ddPCR mix
Temperature	Time	Cycle	1 µl forward primer reptile
95	5 min	1	1 µl reverse primer reptile
95	30 sec	40	1 µl forward primer monitor lizard
55.7	1 min		1 µl reverse primer monitor lizard
4	5 min	1	11 µl Evagreen mix
90	5 min	1	5 µl Milli-Q
4	inf.	1	2 µl sample DNA

Table B-4: Mongoose ddPCR schedule and mix

Mongoose ddPCR schedule			ddPCR mix
Temperature	Time	Cycle	1 µl forward primer mongoose
95	5 min	1	1 µl reverse primer mongoose
95	30 sec	40	11 µl Evagreen mix
60	1 min		7 µl Milli-Q
4	5 min	1	2 µl sample DNA
90	5 min	1	
4	inf.	1	

Appendix C

Table C-1: Species that the general primers work on based on testing in Primer Blast and Geneious

Bird and Ostrich primer			
Southern ground stork	Saddle billed stork	Egyptian goose	Secretary bird
Greater flamingo	Lesser flamingo	Guinea fowl	Great cormorant
Great white pelican	Grey crowned crane	Grey heron	Little egret
Great egret	African sacred ibis	Yellow billed stork	Glossy ibis
Ostrich			
Reptile and monitor lizard			
Puff adder	White lipped snake	Brown house snake	Common egg eater
Black necked spitting cobra	Central African python	Black mamba	Boom slang
Brown house snake	Rhombic night adder	Oliva grass snake	Stiletto snake
Monitor lizard			
Mongoose			
Slender mongoose	Water mongoose	Banded mongoose	White tailed mongoose

Appendix D

Table D-1: Positive samples used for selectivity and specificity

Positive sample	Type	Extraction method	Species
African buffalo	Stool	QIAamp DNA stool mini kit (Qiagen)	<i>Syncerus caffer nanus</i>
Mongoose	Blood	DNeasy blood & tissue kit protocol nucleated blood	<i>Suricata suricata</i>
Ostrich	Blood	DNeasy blood & tissue kit protocol non nucleated blood	<i>Struthio</i> sp.
Boran cattle	Tissue	DNeasy blood & tissue kit protocol for tissue samples	<i>Bos indicus</i>
Reptiles	Already extracted	-	<i>Psammophis mossambicus</i>
Birds	Already extracted	-	<i>Phoenicopterus chilensis</i>
Waterbuck	Tissue	DNeasy blood & tissue kit protocol for tissue samples	<i>Kobus ellipsiprymnus</i>
Spotted hyena	Already extracted	-	<i>Crocuta Crocuta</i>
Lion	Already extracted	-	<i>Panthera leo</i>

Appendix E

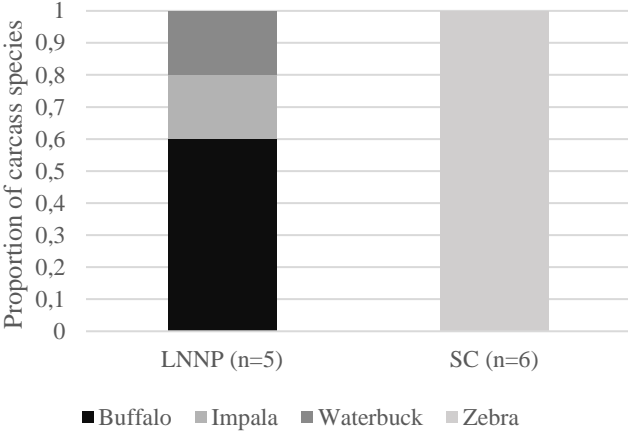


Figure E-1: Carcass counts in LNNP and SC

Appendix F

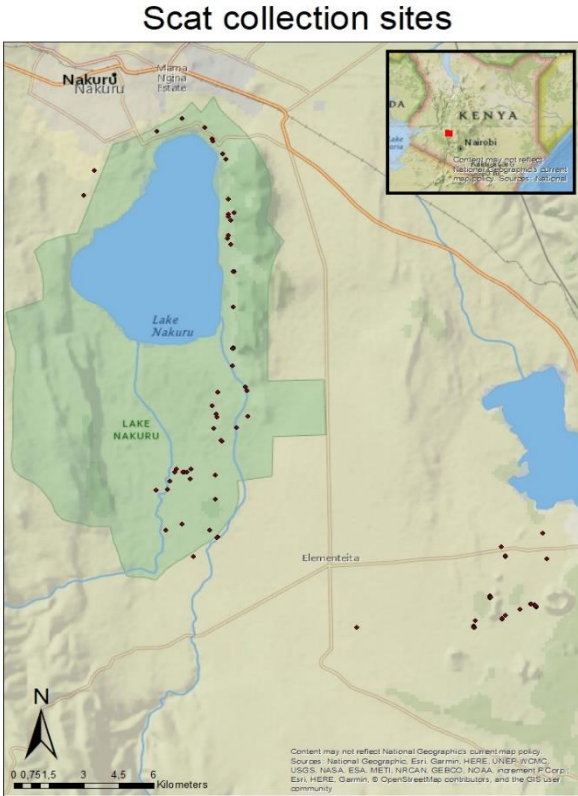


Figure F-1: Scat collection sites in LNNP and SC.