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USING PHOTO IDENTIFICATION TO ESTIMATE THE POPULATION SIZE OF NYALA (*TRAGELAPHUS ANGASII*) IN UMKHUZI GAME RESERVE.

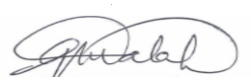
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A research report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Environmental Science.

13 June 2015

Declaration

I, Gisbertus Shanyengange Nakale, declare that this research report is my own, unaided work. It is being submitted for the Degree of Masters by coursework and research at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

13th day of June 2015

Abstract

Wildlife conservation and management requires an in-depth understanding of the demographics and dynamics of the population concerned to enable sound management decisions. Yet it is often very challenging to obtain reliable information of cryptic and highly migratory species. I used photo identification and capture-recapture methods to study the population of nyala, a highly secretive species, in the Umkhuzi game reserve. The nyala species is individually identifiable by the mark pattern on their body. Photographs used for this project were taken at a waterhole in Umkhuzi game reserve between June 23rd and 01 July 2014, representing nine sampling occasions. Identification of individuals was achieved with a computer-assisted technique using open source pattern identification software called Wild-ID version 1.0.1. A total of 652 photographs were taken at the sampling site and from these pictures wild-ID identified 372 distinct animals. An encounter history for each individual during the nine sampling occasions was also produced using Wild-ID. The encounter histories of all individuals were entered into Program MARK. I used the closed population models in Program MARK to obtain population estimates. Program MARK indicated that M_h was the most appropriate model to fit this data as indicated by the AIC_c ranking. M_h showed population estimates as follows: adult males: 111.90 ± 16.07, adult females: 298.01 ± 36.66; young adult males: 21.33 ±19.34; juvenile males: 37.15 ±16.84; yearling males: 37.73 ±8.51; yearling females: 96.48 ±22.75 and juvenile unidentified: 69.03. ±28.96 Closure test performed to ascertain demographic and geographic closure during the sampling period showed a χ^2= 21.74, p= 0.08, df = 14, for the Stanley & Burnham test and a p and z-values of 0.06 and -1.51 respectively for the Otis *et al test.* These results shows marginal violation of population closure, nevertheless closed population models were used to estimate population abundance due to the fact the violations are marginal and the sampling period

was very short, nine days. The study revealed that there is as much as twice the number of females compared to males.

Dedication

I dedicate this research report to my parents. Thank you for moulding me into the person I have become.

Acknowledgements

I would like to use this opportunity to express my gratitude to my supervisor, Dr Jason Marshal for supporting and guiding me throughout this research project. I would also thank all the members of our weekly laboratory meeting team for their support and information sharing. Lastly I will thank everyone who directly and indirectly to made a contribution to this research project. I am thankful to all of you for your inspiring guidance, invaluably constructive criticism and friendly advice during the project work.

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CHAPTER 1

1.1 Introduction

Wildlife conservation and management requires an in-depth understanding of the demographics and dynamics of the population concerned to enable sound management decisions. Yet it is often very challenging to obtain reliable information of cryptic and highly migratory species (Graham and Roberts 2007). Obtaining such information entails *inter alia* studying the interactions between species as well as among individuals within populations. The process of studying intra-population interaction often entails identifying individual animals between sightings taken at different places and times (Lahiri *et al.* 2011). According to Anthony and Blumstein (1999), interactions between individuals within a population can alter the population dynamics through at least three different mechanisms: 1. by changing the population size through immigration and emigration as well as births and deaths; 2. changing the population growth rate by changing the population fecundity and 3. by increasing reproductive skew (i.e. the degree to which different members in a population contribute to the total reproductive output, Cant 2008). Interactions between members of a population can yield important information that can help researchers draw empirical conclusions about the population vitalities such as the survival rates, emigration rates, state transition rates, abundance and the rate of population change (Lukacs and Burnham 2005) and studying group compositions as well as individuals' fidelity to the groups (Wursig and Jefferson 1996).

To be able to study interactions between individuals within a population, one needs to identify individual animals so that their fates can be followed through time (Speed *et al*. 2006).

The method traditionally used to achieve this study is referred to as capture mark recapture (CMR) where animals are captured, marked, released and recaptured as many times as possible by subsequent sampling accessions (Pradel 1996). A record of different recaptures of an individual makes up that individual's capture history which can inform population estimations (Pradel 1996). Capture-mark recapture method can provide accurate long-term analysis of abundance and other population parameters such as survival and longevity, and can be very useful for geographically well-defined populations (Lettink 2012) using the principle of detection probability. Detection probability may however vary spatially and temporally as a result of the nature of organisms and environmental factors prevailing in the study area making estimation of population parameters difficult (MacKenzie *et al.* 2005). The problem of varying encounter probability can be diminished through repeated capture-mark- recapture by estimating encounter probability of observed animals and accounting for individuals not observed during a survey (Williams *et al*. 2002). Estimating population parameters using this method is done on the basis of a proportional relationship that exists between the number of marked animals and the total population (Nichols 1992).

Earlier in wildlife population studies, tagging of and applying artificial markings as a form of CMR was widely used to identify individuals within a population (Wursig and Jefferson1996). Tagging was used to study migration patterns (Homel and Budy 2008), survival rates (Kremers 1988), site fidelity (Jud and Layman 2012) and population dynamics (Koch and Schoonbee 1980) of wild animals. A lot of success has been recorded with this method.

However, Beausoleil *et al.* (2004) noted that artificial tags imposed on animals have been proven to affect some animals by altering: their behaviours or interactions with their own or other species; their health and welfare; their capacity to survive or reproduce; population dynamics; ecological balance and other factors. In addition, tags can be

damaged or shed and reliably accessing and identifying heavily fouled tags for cryptic species may prove to be challenging (Marshall and Pierce 2012).

According to Schofield *et al.* (2008), the use of tags on animals stresses the animals through capturing, handling and attaching the tag. They also indicated that some tags (e.g. flipper bands in sea turtle) have been shown to increase mortality of animals. Arnemo *et al.* (2006) indicated that capturing and handling animals for tagging and other scientific studies causes death either directly through overdose with drugs used to anaesthetise the animals or indirectly through stress. High stress hormone levels as a result of handling can also result in negative consequences such as depression of the animals' immune system (Marketon and Glaser 2008). Burley *et al*. (1982) observed in zebra finches (a bird species) that certain colour banded males and females were more or less attractive to the opposite sex than ones that were unbanded. Some conservationists therefore believe that tagging animals for the purpose of population studies affects their recapture probability and hence leads to inaccurate estimation of population parameters (Hammond *et al.* 1990). Coupled with the need to catch and physically handle the animal, this problem is exacerbated by the fact that some species have elusive tendencies coupled with marked habitat preference differences between the sexes (Kerby *et al.* 2008) therefore making it difficult to use traditional methods of tagging to study them.

Other methods, with varying degrees of intrusiveness, have been used in conservation to identify individuals in population studies. Beausoleil (2004) summarised some of the methods used on reptiles and marine fauna in New Zealand as follows: hot branding used to mark Gulf Coast toats (*Bufo vallicepts*); freeze branding used in tailed frogs (*Ascaphus truit*); chemical branding and tattooing used in hylid frogs and bullfrogs respectively. The success and appropriateness of each method depends on the type animals under study (Beausoleil *et al.* 2004).

However, all these methods entail physically handling the animals which make them intrusive and in some species may alter the behaviour and physiology of captured animals (Wilson and McMahon 2006).

It is not always possible to artificially mark certain types of animals, either they are difficult to catch and handle or because the law prohibits any type of disturbances of such animals (Hammond et al. 1990). This fact coupled with the element of disturbance associated with physically handling animals in order to mark them gave rise to the introduction of less invasive methods to identify animals in their natural habitats for population studies. Two examples include photo identification and genetic capture-recapture. Genetic recapture uses DNA obtained from scat, hair or other body part to identify the individuals without necessarily physically handling the animal (Miller *et al*. 2005). It has however been shown that this type of sampling method is prone to technical error due to allelic dropout as well as the possibility of two closely related individuals having identical genotypes (Bacon *et al.* 1999).

Photo identification (photo-ID), a relatively new method has recently become popular with environmental conservationists to identify animals in their natural habitats in the late sixties and early seventies. Photo-ID became more popular over traditional marking methods because photo-ID avoids problems associated with physically handling of animals (Cutler and Swann 1999).

Moreover, in a reasonably short period of study, natural marks remains stable, cannot be, removed or fouled, and, depending on field practices, photo-ID can also minimize the risk of confounding stress or behavioural issues (Marshall and Pierce 2012). The photographs can be stored and become permanent record of the individual for rest of its life span (Hammond 1990).

This method has gained popularity over the others because it less disruptive to the animals (Speed *et al.* 2007). It is designed particularly for species that have unique natural markings on their bodies. Scars bite marks, fic morphology and deformities may be useful in the absence of intrinsic patterns (Marshall *et al*. 2012).
The underlying principle is that every individual in the population or species have unique patterns of natural marking, which do not change throughout the duration of the study (Auger-Methe and Whitehead 2007).

Despite the growing popularity of photo-ID due to its non-invasiveness, it can be prone to misidentifications especially where there is little variations in the natural marking patterns between individuals and if the natural marks can change during the sampling period leading to an individual being identified as two distinct individuals which can cause population size being overestimated (Morrison *et al* 2011). Incidences of misidentification can be eliminated with the natural markings are adequately different between individuals, are constant throughout the study period and the researcher has sufficient training (Vincent *et al*.2001). Photo-ID allows the storage of photos in a library for subsequent cross-matching and generation of capture-history matrices which can be matched (Speed *et al* 2007).

The process of matching photographs using natural markings can be done manually but it is a laborious and strenuous process and is prone to human error hence matching photographs manually mainly suitable for small populations (Beausoleil 2004). To minimise the element of mismatching of photographs with manual matching, matches or new sightings must be confirmed by more than one experienced persons (Hammond et al 2000). New computer software such as the (3-D) computer-matching system (Kelly 2001) have been developed to aid with the identification of individuals especially where large populations and databases are involved.

To date, photo identification has been used extensively to study wild animals in their natural habitats especially in the cetacean family (Graham and Roberts 2007, Baird *et al*. 2009, Neumann *et al.* (2002). Photo identification was also used to study the seasonal migration of giraffes (*Girrafa camelopardalis*) in Niger (Le Pendu and Ciofolo 1999), identification of zebra (*Aguus burchellii*) in Nairobi National Park, Kenya (Petersen 1972) and for the identification of cheetahs (*Acinonyx jabatu*) in Seregetti (Kelly 2001), African elephants, (*Loxodonta africana)* (Ardovini *et al*. 2007), African penguins, *Spheniscusdemersus linnaeus* (Sherley *et al.* 2010) and salamanders (Church *et al.* 2007). The use of photo identification has led to the establishment of numerous widely used databases mostly of cetaceans in the last few decades. These include the Whale shark (*Rhincodon typus*) in Ningaloo Marine Park in Western Australia (Holmberg *et al*. 2008), Gray seals, *Halichoerus grypus* (Beaumont (2007), tree frogs (Del Lama *et al*. 2011). Grellie (2003) used this technique to quantify mother–calf association patterns in bottlenose dolphins (*Tursiops truncates*) by using unique natural markings such as nicks and notches in the dorsal fin and tooth- rake marks, scratches, scars, and skin lesions on the dorsal fin and back.

Langtim *et al.* (2004) successfully used photo identification to estimate the survival rates of Florida manatees (*Trichechus manatuslatirostris*) using the scars that animals sustained during collisions with watercraft as primary identification features. Karanth *et al.* 1997 used photo ID to estimate the density of tiger (*Panthera tigrid*) in India using their stripe patterns as the identification mark. Maddock and Mills (1993) estimated the population of African wild dogs (*Lycaon pictus*)using unique combinations of black, white and tan markings on the animals, Maputla *et al.* (2012) studied the population of leopards and Marnewick *et al.* (2014) evaluated the status of wild dogs (*Lycaon pictus)* and cheetahs (*Acinonyx jubatus*) in the Kruger National Park.

Kumbhar *et al*. (2013) used photo-ID to estimate the population size and density of the Indian mouse deer (*Moschiola indica).* The Indian mouse deer moves mainly at night and it is thus very difficult to study using any other methods.

Study species

Nyala (*Tragelaphus agasii*) is a medium sized species of antelopes occurring in Southern Africa. Their natural range includes Malawi, Mozambique, South Africa, Swaziland and Zimbabwe (Grobler *et al*. 2005). The species was introduced to Botswana and Namibia, and reintroduced to Swaziland, where it had become extinct in the 1950s (Grobler *et al.* 2005). Its population is stable and it has been listed as Least Concern by the International Union for Conservation of Nature (IUCN 2014). The principal threats to the species are poaching and habitat loss resulting from human settlement (IUCN redlist of threatened species, 2014).

Nyala is an economically important species. Records from the Hunting and Extension Division, Ezemvelo KwaZulu-Natal Wildlife (EKZNW), South Africa, show that approximately ZAR 8.1 and 2.8 million were generated from nyala trophy hunting and live sales respectively in KwaZulu-natal province in 2002 alone (Coates and Downs 2005).

The species is characterised by white vertical stripes along the body with a few white spots on the hips and a white chevron mark between the eyes (Haagner 1920). The tail is bushy, black above and white below and reaches the hocks. The males are dark blue-grey in colour with up to 14 white vertical stripes (Smithers and Abbott 1986) and stockinged legs (Cillie 1987). Mature males have spiral horns reaching lengths of 60 cm (Skinner and Meakin 1988). The mane in the neck and back is white tipped. Old males have long

fringed hair in the belly and against the backside of the buttocks (Cillie 1987). Females and immature males are reddish-brown to chestnut with similar stripes and marks but lack horns (Cillie 1987). They have a distinct black dorsal line which runs along the back form the crown of the head to the root of the tail (Haagner 1920).

This species exhibits marked sexual dimorphism with mature males being substantially larger in body size than mature females and mature females having more stripes than mature males (Tello and van Gelder 1975).

The animals live in thickets and dense bush along rivers in dry woodlands (Rautenberg 1982). They are gregarious, living in herds ranging from 3 up to 30 individual, usually comprised of a bull, females and the young. Members move freely between groups. Old males are usually solitary or form small herds (Cillie 1987).

Nyala normally feed in the late afternoons and during the night and rest during the hot part of the day. They feed mainly on leaves, freshly resprouting grass, fruit, flowers and pods (Cillie 1987). Breeding occurs throughout the year but peaks in May and between August and December. They have a life expectancy of 13 years (Cillie 1987).

Due to the elusive nature and marked differences in habitat preference as well as social behaviour between male and female nyala Kirby *et al.* 2008), it is difficult to use traditional large-mammals sampling methods such as line transects and distance sampling to accurately study the population size of this species. This is because this species prefers the thicket and it has highly secretive tendencies.

On the basis that nyala are individually recognisable by the marks on their bodies and that population size of an individually recognisable species can be estimated with photo identification using the CMR theory (Karanth 1995), in this study, I estimated abundance of nyala in Umkhuzi game reserve in KwaZulu Natal, South Africa. A survey about the status and management of bushbuck and nyala in KwaZulu-Natal showed growing concern from conservationists that the population of Nyala in several protected areas is increasing rapidly (Coates and Downs, 2005). This rapid increase in abundance can have adverse effects on the environment as well as other ungulates living within the same or overlapping geographical areas. Nyala are mixed feeders preferring brows during winter but can also supplement their diets with graze when browse is in short supply (Anderson 1979). They can therefore outcompete other sympatric ungulates by having access to forage at a higher feeding level and potentially creating brows lines and consequently excluding the smaller species such as bushbucks and forest duikers that rely exclusively on browse (Bowland 1990).

The objectives of this study are two folds;

1. To develop a database with photographic records of individual nyala in the region around Masinga hide in Umkhuzi Game Reserve.

2. Estimate abundance of nyala based on encounter histories from that database and compares that with data published from previous studies.

CHAPTER 2

2.1 Methods and materials

2.1.1 Study Area

Umkhuzi Game Reserve is a 360 square kilometre fenced-off game reserve located between 27°32′30″S and 27°48′30″S and between 32°06′00″E and 32°26′00″E (Taylor et al. 2007) on the coastal plain east of the Lebombo Mountains in northern KwaZulu-Natal, South Africa (White and Goodman 2009). It was proclaimed a protected area on 15 February 1912. The reserve has a diversity of natural vegetation communities which include forest and closed woodland, mixed bush land, mixed woodland, mixed thicket and scrub, wooded grassland, grassland and wetland (Goodman, 1990).The game reserve is home to a variety of wild mammals including the black rhino (*Diceros bicornis*), whiterhino (*Ceratotherium simum*), Cape buffalo (*Syncerus caffer*), leopard (*Panthera pardus*), elephant (*Loxodonta africana*), cheetah (*Acinonyx jubatus*), potted hyaena (*Crocuta crocuta*), giraffe (*Giraffa camelopardalis*) and many antelope species (Weladji and Laflamme-Meyer 2011). It is also home to the largest nyala population in Southern Africa, with over 5000 individuals (White and Goodman 2009).

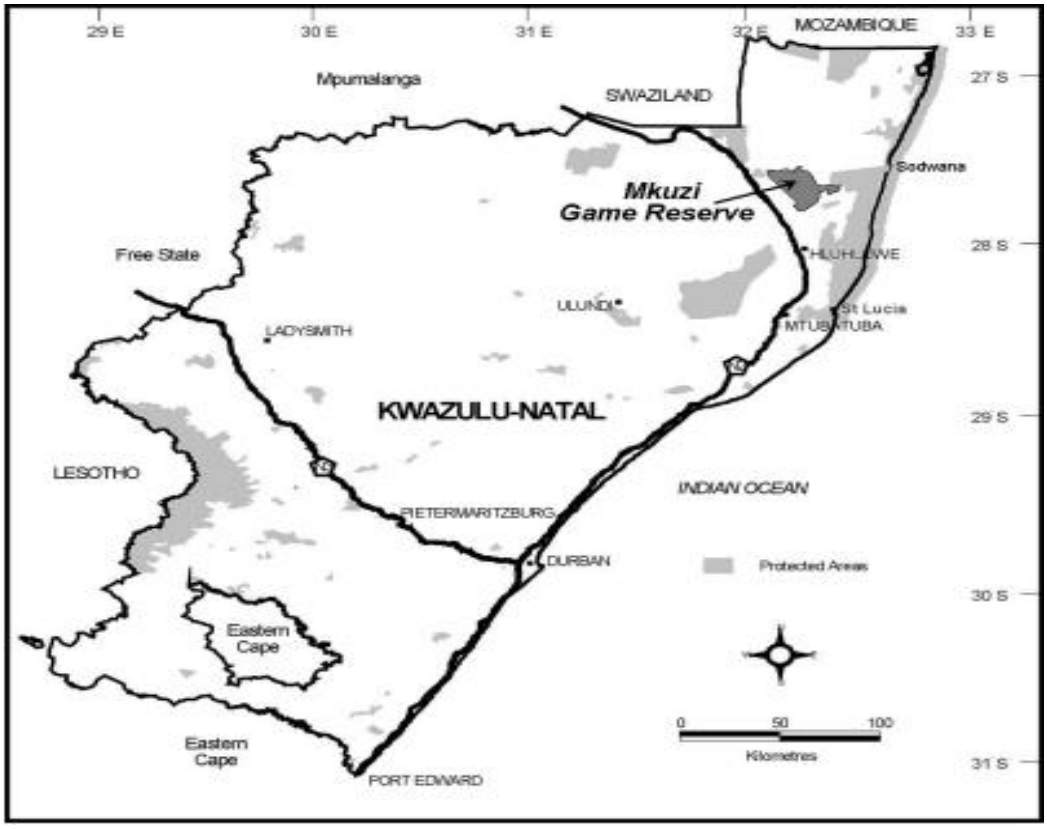


Figure 2.1 The location of Umkhuzi Game Reserve in KwaZulu Natal (Mulqueeny, 2005)

Balme *et al.* (2010) describes the climate as warm to hot with two distinct seasons; a warm, dry winter from April to September and a hot, humid summer from October to March with daily temperatures averaging 33^{o}C and 19^{o}C in January and July respectively. The area receives an annual average rainfall of 664 ml (Weladji and Laflamme-Mayer 2011).

2.1.2 Methods

2.1.2.1 Data collection

Data in the form of photographs was collected between June 23rd and 01 July 2014. The timing for this data collection was planned to coincide with the winter season when water sources in the reserve are limited so as to increase the probability of sightings at the limited available water sources. During this time of year, most temporary water resources available to the animals during the rainy season become dry. A total of 9 photographing occasions were undertaken. Sampling was done at Masinga hide, an open and reliable water source in Umkhuzi game reserve. . Photographs of nyala were taken as they approached the water point to drink, whilst they drank and as they left the water point to return to the bushes. A deliberate effort was made to photograph all animals in the shooting range of the camera at random. Photographing was not discriminative of the distinctiveness of the animals, the size and sex of the animals and whether the animal appeared to have been photographed before. An animal was considered to have been encountered when it was photographed both sides. In the case where more than one nyala approached the water at the same time, attention was given to one animal until it was photographed both sides before moving to the next animal. At the end of every sampling occasion, the pictures were compared to one another to avoid multiple captures of the same individual on the same day. Where an animal was photographed more than one time the same day, it would only count as one encounter.

Photographs were taken using a hand-held digital single-lens reflex (SLR) camera (Nikon D90, Tokyo, Japan) and a zoom lens Nikkor 70—300 mm, f4.5, Ø67 mm, Tokyo, Japan) with image resolution of 12.3 megapixels.

The furthest distance at which photographs were taken that allowed recognition of individuals was approximately 70 m (i.e. the distance across the pioshere). At the time of photographing the animals, descriptions of each image are made using visible features. Features used to describe the images include sex of the individual, date and time of photographing the age group of the individual. The animals were classified into seven age groups mainly; adult male, adult female, young adult male, juvenile male, yearling male, yearling female and juvenile unidentified. These age groups were mainly based on the body size and in males colour on body colouration and the length of their horns (Tello and Gelder 1975). Adult males are dark grey in colour with horns, young adult males are dark brown to slate grey, yearling male are darker in colour with visible horns while adult females are rust red without horns and their lower part of the legs are lighter than the upper part (Skinner and Chimimba 2005). Juvenile males and females look very similar and cannot be distinguished unless males have horn buds, therefore juveniles without horn buds were classified as juvenile unidentified.

Once the photographing process is completed for each day, the pictures were screened and poor quality photographs discarded so that only those of exceptional quality were used for in the matching process. All photographs which were deemed of workable quality were stored in the camera SIM card from where they were downloaded onto a desktop computer where the matching would take place.

2.1.2.2 Individual identification

The identification of individuals was achieved with a computer-assisted technique using an open source pattern identification software called Wild-ID version 1.0.1;http://www.dartmouth.edu/~envs/faculty/bolger.html (Bolger *et al.* 2012) which uses pigmentation patterns on the digital photographs to match individuals. This software has been extensively used in wild animals' studies and has been used to accurately identify wildebeest (*Connochaetes taurinus*; Morrison and Bolger 2012), giraffe (*Giraffa camelopardis;* Bolger *et al*. 2012) and a species of toads (*Melanophryniscus montevidensis*; Elgue *et al*. 2014). Wild-ID uses the Scale Invariant Feature Transform (SIFT) algorithm to characterize variable patterns within photographs and compare all combinations of photographs in the database (Bendik *et al*. 2013). SIFT is designed to compare unique image characteristics invariant to image scale, rotation, viewpoint, local distortion and illumination (Lowe 2004). The photographs are cropped (see Figure 2) and entered into the database. Cropping of images is done to accentuate the area of interest for matching, ensure faster processing as well as to increase matching accuracy (Bolger *et al*. 2012).



Figure 2.2 Cropped photographs to accentuate the area of interest for matching.

Wild-ID allocates each photograph entered a unique number in a sequential fashion starting with the first entry. Wild-ID pairs the images and the paired images are scored and ranked based on the similarity of their keypoint maps (Bendik *et al*. 2013). Photograph similarities are scored from 0.0 to 1.0, with the score of zero signifying lack of similarity and 1.0 denoting perfect similarity. Similarity scores provide a standardized measure of how similar the patterns in paired pictures are, the higher the score, the more accurate the match. Similarity scores are determined by iteratively comparing geometrically self-consistent subsets of keypoints within pairs of images (Bendik *et al*. 2013). Matching of photographs to those in the database is done in one direction in the order that the photographs are entered that is when starting a new database, the first photograph has nothing to be compared to, the second picture is compared to the first, the third picture is compared to the first and second and so forth. Once the matching is completed, the Wild-ID interface shows the focal pictures in turns, one at a time, together with the top twenty ranked potential matches from the database (Bolger 2012).

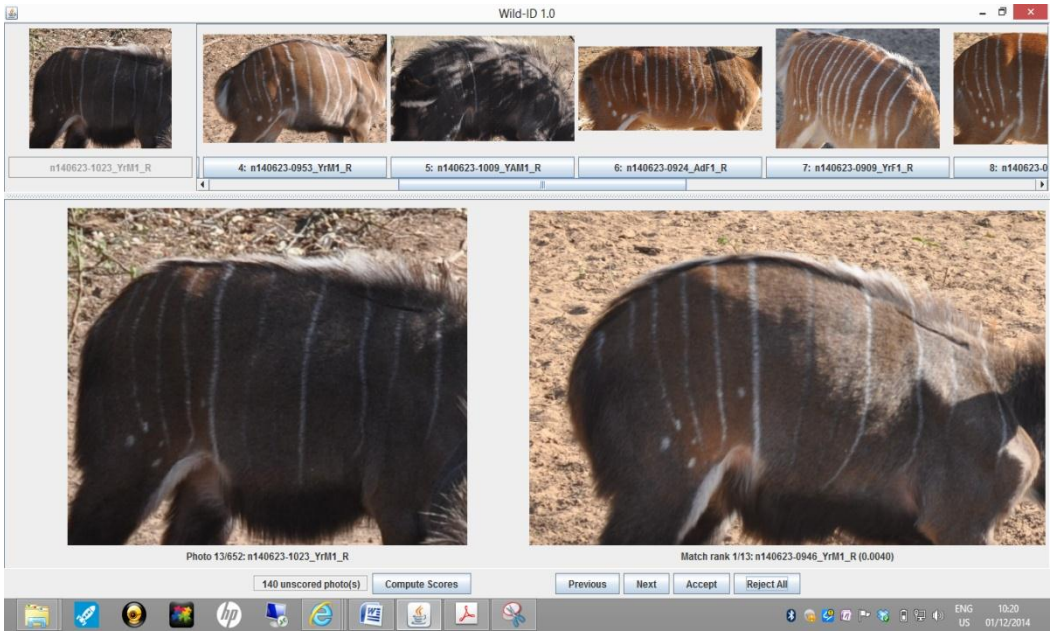


Figure 2.3. The user interface of wild-ID. The top and bottom left images are the focal images and the rest of the pictures in the top row are the ranked potential matches. Bottom right is the comparison image.

The researcher verifies the images, picks the correct match and if no match is found, the focal picture is entered into the database as a new entry. Once the matching process by the software was completed, photographs were again verified manually by comparing matched and unmatched photographs to one another between the various sampling occasions. This was merely a quality control measure to ensure that the software did not mismatch potential matches. At the end of the matching process, an encounter history of all individuals was compiled by the researcher. These encounter histories were summarised and used to estimate population abundance.

2.1.2.3 Data analysis

Encounter histories of the nyala were described using the binary matrix with 0 and 1, respectively denoting a no-sighting and sighting during a particular sampling occasion. Each sampling day was considered a distinct sampling occasion. Where during a sampling occasion an animal was encountered more than one time, only one sighting is considered.

100000000 0 0 0 0 0 0 1 0;
100000000 0 0 0 0 0 1 0 0;
100000000 1 0 0 0 0 0 0 0;
100000000 1 0 0 0 0 0 0 0;
100000000 0 0 0 0 0 0 0 1;
100000000 0 1 0 0 0 0 0 0;
100000000 0 0 0 0 0 1 0 0;
100000000 0 0 0 0 0 1 0 0;
100000000 0 0 1 0 0 0 0 0;
100000000 0 1 0 0 0 0 0 0;
100000000 0 0 0 1 0 0 0 0;
100000000 0 0 0 0 0 0 0 1;

Figure 2.4. The binary matrix representing the individual animal's encounter history. This matrix shows nine encounter occasions with eight groups.

I conducted a mark-recapture analysis using program MARK (White and Burnham 1999) assuming a closed population. Program MARK produces population abundance estimates using eight closed population models which differ from one another in their assumption for capture probabilities which are based on individual heterogeneity, behavioural response to initial capture and time-dependent probabilities (Garrote *et al.* 2010).

Estimating abundance for a closed population can be generally demonstrated using a basic two sampling occasions in which a sample of n_1 animals is captured, marked and released. After a short period of time, a second random sample of n_2 animals is captured, m_2 of which are marked.

Assuming that the population is closed between the two sampling occasions, the ration of the proportion of marked animals in the second sample is approximately equal the proportion of marked animals in the population **(**Kendall 1999), known as the classic Lincoln-Petersen estimator and denoted by formula:

$$\frac{\boldsymbol{n_1}}{\boldsymbol{N}} = \frac{\boldsymbol{m_2}}{\boldsymbol{n_2}}$$

Where n_1 is the number of marked animals in the population, n_2 is the total number of marked and unmarked animals seen, and m_2 is the number of marked animals seen. This formula aims to get the probability distribution which for the two occasions can be written in terms of probability as:

$$P(n_1,n_2,m_2/N,p_1,p_2)=\frac{N!}{m_2!(n_1-m_2)!(n_2-m_2)!(N-r)!}(p_1p_2)^{m}{}_2(p_1q_2)^{n}{}_1{}^{-m}{}_2(q_1p_2)^{n}{}_2{}^{-m}{}_2(q_1q_2)^{N-r}$$

Where N is the population size, $qi=1-pi$ and $r = n_1+n_2-m_2$ = number of unique animals captured in study.For equation above to be true, two assumptions have to be met; 1) all animals in the population should have the same chance of being caught and 2) that no marks or tags are lost or overlooked which could lead to misidentification of individuals (William *et al.*2002).

I used the closed captures full likelihood data type to estimate abundance using the various close models (White 1998) in Table 2.1. The various closed models are distinguishable from one another by their encounter probabilities described by Otis 1978 as follow; 1) detection probability varying from one occasion to the next (M_t), 2)

detection probability varying as a result of the animals responding behaviourally to the sampling technique after an individual has been caught for the first time (M_b).
This model assumes different capture probabilities for animals encountered for the first time and those that were encountered before without a variation in temporal or individual capture probabilities, and 3) the encounter probability varying due to the inherent differences amongst the individuals being sampled (M_h). Model M_h assumes that each individual in the population has a distinct capture probability which is the same over the entire sampling period irrespective of the previous encounters. The null model (M_o) assumes that there is no difference in capture probabilities between sampling occasion and amongst individuals (Karanth and Nichols 1998).M_b, M_{tb} the models with behavioural responses and time varying behavioural responses respectively were not considered for estimating abundance because it is biologically unlikely for the photo-identification, a non-invasive method to affect the animal's behaviour during sampling. π in that equation represents the mixture parameter, the probability that the individual occurs in mixture A or B.

Table 2.1 Closed population models. M_o, the null model representing homogenous capture probabilities, M_t representing time varying capture probabilities, M_b representing behavioural response and M_h representing heterogeneous capture probabilities

Otis notation	Expanded notation	Description
M_o	{N,p(.)=c(.)}	Constant p
M_t	{N,p(t)=c(t)}	Time varying p
M_b	{N,p(.),c(.)	Behavioural response
M_h or M_{h2}	$\{N,p_a(.)=c_a(.),p_b(.)=c_b(.),\pi$	Heterogeneous p

The best model was selected using the Akaike Information Criterion which is based on a penalised likelihood of a general form: AIC = -2 log L(θ)+2k, where L(θ) is the maximized likelihood function, and k is the number of free parameters in the model (Bozdogan 2000). This criterion aims to find the best balance between the fit of the model to the data and its complexity (Cubaynes *et al.* 2012). The best model was chosen by selecting the model with the smallest AIC value (Bozdogan 2000) presented by program MARK.

I used the encounter histories of sighted individuals to perform a closure test on the data using the software CloseTest (Stanley and Richards 2005) to ascertain that there were no individuals added or removed from the population during the sampling period.

A population assumed to be geographically closed when there is no immigration or emigration and demographically closed when there are no births and deaths occurring in the population during the sampling period (White *et al.* 1982).

I assumed that the population was closed during the sampling period because nyala are long lived relative to the sampling period which spanned only nine days. Program CloseTest computes two closure tests; 1) the Stanley and Burnham (1999, Environmental and Ecological Statistics, 6:197-209) test, developed under a null model allowing for time-specific variation in capture probabilities under closure, and 2) the Otis *et al.* (1978, Wildlife Monographs) test, which was developed under a null model allowing for heterogeneity in capture probabilities under closure (Stanley and Richards 2005).

CHAPTER 3

3.1 Results

3.1.1 Database

A total of 652 processed photographs were entered into Wild-ID software for matching. The photographs were each given unique numbers in a chronological fashion in the order of their entry into the software. The age-sex structure of photographs entered into the Wild-ID is as follows: adult females: 269, adult males: 93, juvenile males: 60, juvenile unidentified: 46, young adult males: 25, yearling females: 116 and yearling males: 43.

After all photographs were matched, the software produced a database (Figure 4) containing all the matching information.

#Serial	Relpath	Match-serial	Match-relpath	Choice-rank	Score
1	140623_Mkhuze\n140623-0909_YrF1_R.JPG	-1	NONE	0	NaN
2	140623_Mkhuze\n140623-0909_YrM1_R.JPG	-1	NONE	0	NaN
3	140623_Mkhuze\n140623-0924_AdF1_R.JPG	-1	NONE	0	NaN
4	140623_Mkhuze\n140623-0924_AdF2_R.JPG	-1	NONE	0	NaN
5	140623_Mkhuze\n140623-0924_JvU1_R.JPG	-1	NONE	0	NaN
8	140623_Mkhuze\n140623-0947_AdM1_R.JPG	-1	NONE	0	NaN
9	140623_Mkhuze\n140623-0953_YrM1_R.JPG	-1	NONE	0	NaN
12	140623_Mkhuze\n140623-1009_YrM1_R.JPG	7	140623_Mkhuze\n14062	8	0.000002
13	140623_Mkhuze\n140623-1023_YrM1_R.JPG	7	140623_Mkhuze\n14062	1	0.004001
14	140623_Mkhuze\n140623-1024_YAM1_R.JPG	11	140623_Mkhuze\n14062	5	0.000004
15	140623_Mkhuze\n140623-1040_AdM1_R.JPG	-1	NONE	0	NaN
16	140623_Mkhuze\n140623-1041_JvM1_R.JPG	-1	NONE	0	NaN
17	140623_Mkhuze\n140623-1041_JvU1_R.JPG	-1	NONE	0	NaN
19	140623_Mkhuze\n140623-1045_AdF2_R.JPG	-1	NONE	0	NaN
21	140623_Mkhuze\n140623-1047_AdM2_R.JPG	6	140623_Mkhuze\n14062	3	0.000505
22	140623_Mkhuze\n140623-1056_AdM1_R.JPG	10	140623_Mkhuze\n14062	4	0.000503
23	140623_Mkhuze\n140623-1057_YAM1_R.JPG	14	140623_Mkhuze\n14062	1	0.004002
24	140623_Mkhuze\n140623-1106_AdF1_R.JPG	-1	NONE	0	NaN
25	140623_Mkhuze\n140623-1107_AdF1_R.JPG	-1	NONE	0	NaN
26	140623_Mkhuze\n140623-1107_AdF2_R.JPG	-1	NONE	0	NaN
35	140623_Mkhuze\n140623-1140_AdF1_R.JPG	-1	NONE	0	NaN
39	140623_Mkhuze\n140623-1147_AdF1_R.JPG	-1	NONE	0	NaN
40	140623_Mkhuze\n140623-1148_AdM1_R.JPG	-1	NONE	0	NaN
42	140623_Mkhuze\n140623-1155_AdF1_R.JPG	-1	NONE	0	NaN

Figure 3.1 The database output screen.

Figure 3.1 shows an illustration of the database base matching outputs. Program Wild-ID assigns a unique number to every photograph entered; this number is denoted in the first column labelled '#serial'.

The relpath column shows the photographs identification number as well as the folder wherein the photographs are nested. The Match-serial column indicates the unique number that the photograph in relpath has been successfully matched with. A -1 indicates that there was no match for the corresponding relpath photograph. Match-relpath column indicates the identification number for the matched picture and folder where it is nested. Choice rank column indicates the user designated matching image from the potential matches offered and the score column indicates the similarity score between the matched photographs.

The database identified 372 as the total number of distinct individuals observed during the study period. The frequency of encounter of these individuals ranges from 1 to 7 encounters.

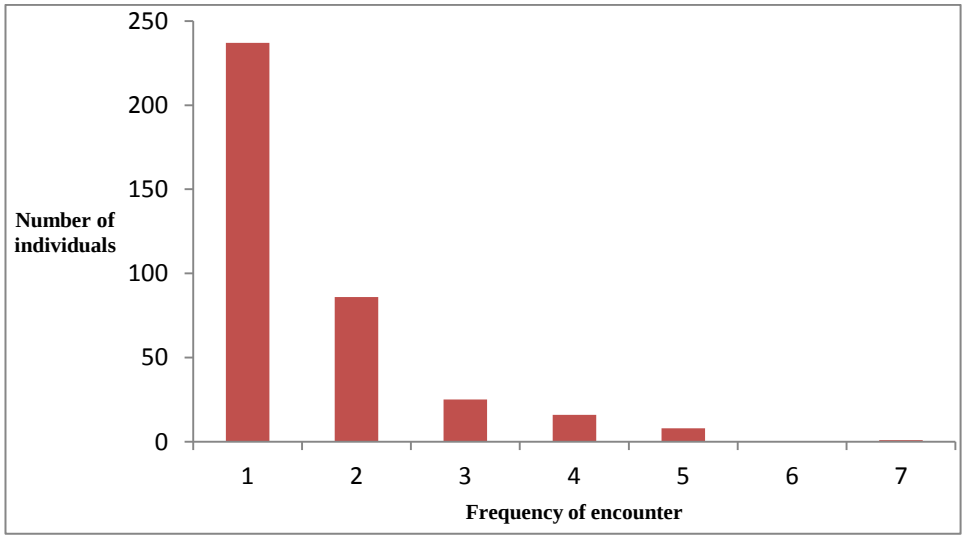


Figure 3.2 The number of nyala observed at respective frequencies.

The matched data show that out of the 372 distinct individuals' encountered, 156 were adult females, 64 were adult males, 28 were juvenile males, 26 were juvenile unidentified, 11 were young adult males, 64 were yearling females and 23 were yearling males.

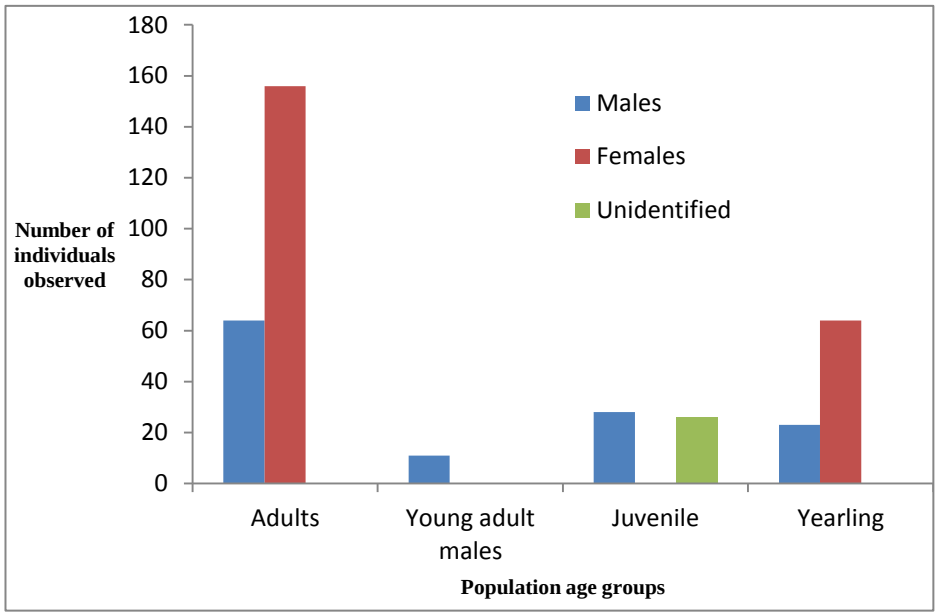


Figure 3.3 The total number of individuals per sex and age group.

3.1.2 Closure test

Population closure test yielded χ^2= 21.74, *p*= 0.08, df = 14, for the Stanley & Burnham Closure test and a *p* and z-values of 0.06 and -1.51 respectively for the Otis *et al*. (1978) Closure Test. These low *p*-values suggest a weak violation of population closure. Both these tests have intrinsic shortfalls.

The Otis *et al*. (1978) closure test has type I error rates exceeding nominal rates in the presence of time or behavioural variation in capture probabilities (White *et al.*1982) and the is insensitive to temporary violation of closure which occurs in the middle of the study (Otis *et al. 1978)* while the Stanley & Burnham closure test rejects at rates greater

than the nominal error rates in the presence of behavioural variation or heterogeneity capture probabilities and no migration (Stanley and Burnham 1999). The two tests should therefore be used in conjunction with each other to better detect closure violations in a capture-recapture data set (Stanley and Burnham, 1999).

I used closed population models to run the analysis because the sampling period was reasonably short (i.e. nine days) which is acceptable time to assume closure (Wang &Macdonald2009) and the deviations from population closure were minimal (Karanth *et al*. 2006). The criterion used to determine the acceptability of the sampling period in order to assume closure was the duration of the sampling process in relation to life span of the species.

3.1.3 Population size

According to the AIC model selection criterion in program MARK, model M_h was chosen as the most appropriate model for this set of data as shown by table 3.1. This model is based on the assumptions that capture probabilities vary by individual animal (White et al 1982).

Table 3.1 Model selection based on AIC_c.

Model	**AIC_c**	**Delta AIC_c**	**AIC weight**
M_h	497.60	0.00	0.93
M_t	502.92	5.33	0.06
M_o	510.15	12.54	0.00
M_b	511.03	13.43	0.00

M_h population size estimates show high abundance of adult females and young adult males being the least abundant group in the population see table 3.2 below for summary. Capture probabilities are summarised in Figure 3.3. As shown in the figure, each group has two capture probabilities, each representing a mixture group.

Table 3.2 Nyala population estimates along with their 95% confidence interval.

Group	Population estimates	Standard Error (SE)	LCU	UCL
Adult males (ADM)	111.90	16.07	89.25	154.87
Adult females (ADF)	298.01	36.66	242.32	389.64
Young adult males (YAM)	21.33	19.34	11.93	125.42
Juvenile males (JVM)	37.15	16.84	28.84	127.23
Yearling males (YRM)	37.73	8.51	28.14	65.20
Yearling females (YRF)	96.48	22.75	73.83	177.19
Juveniles unidentified (JVU)	69.03	28.96	38.98	168.61

Table 3.3 Capture-recapture probability estimates along with their 95% confidence interval for model M_h with two mixture groups with initial capture and recapture probabilities being the same (p=c).

Parameter p=c	Probability estimates	SE	LCI	UCI
1	0.214	0.000	0.214	0.214
1	0.089	0.016	0.063	0.125
2	0.074	0.013	0.053	0.103
2	0.444	0.079	0.298	0.600
3	0.270	0.180	0.058	0.689
3	0.058	0.092	0.002	0.630
4	0.279	0.069	0.165	0.431
4	0.075	0.124	0.002	0.729
5	0.097	0.030	0.052	0.174
5	0.097	0.372	0.000	0.998
6	0.090	0.061	0.022	0.300
6	0.298	0.098	0.144	0.515
7	0.338	0.261	0.049	0.834
7	0.048	0.025	0.018	0.126

The numbers in the parameters column (1-7) represents the various groups. Each group has two mixtures as denoted by the repeated numbers, each number representing one mixture. Average capture probability was 0.17 with the highest encounter probability observed in adult females with probability of 0.44, SE =0.07 and the lowest encounter probability was observed in the group juveniles unidentified with 0.04, SE = 0.025.

Heterogeneity between the two mixture groups in the different age/sex categories is summarised in Table 3.4 below. The heterogeneity between the groups ranged from zero for adult males to 0.96 for yearling males.

Table 3.4 Heterogeneity estimates of the various groups of nyala.

Group	**Estimates**	**Standard Error**	**LCI**	**UCI**
Adult males	0.00	0.00	0.00	1.00
Adult females	0.95	0.01	0.90	0.98
Young adult males	0.17	0.22	0.01	0.81
Juvenile males	0.54	0.29	0.10	0.92
Yearling males	0.96	13.80	0.00	1.00
Yearling females	0.74	0.20	0.26	0.96
Juveniles unidentified	0.02	0.03	0.00	0.33

All the groups were individually run in program CAPTURE. This was done to assess the reliability of the population estimates from program MARK given the too large and too small standard errors shown by program MARK. Results from population estimates in program CAPTURE along with their 95% confidence interval are indicated in Table 3.5 below.

Table 3.5 Population estimates in program CAPTURE along with their 95% confidence interval.

Group	Estimates	Standard Error (SE)	LCL	UCL
ADF	371	39.46	307	463
ADM	112	14.47	91	149
YAM	34	9.55	23	63
JVM	41	7.02	34	64
YRM	42	10.27	30	58
YRF	132	19.53	104	183
JVU	58	12.41	42	92

The probability of detecting an individual on at least one sampling occasion given in program CAPTURE for each of the groups is as follow: ADF: 0.07, ADM: 0.09, YAM: 0.07, JVM: 0.17, YRM: 0.06 and YRF: 0.10.

CHAPTER 4

4.1 Discussion

4.1.1 Photo identification and database

Photo identification technique was successful to estimate the population of nyala as well as create a database of photographs of nyala in the vicinity of Masinga hide. This method has demonstrated to be a viable method for estimating population parameters (i.e. abundance) of secretive individually recognizable species that traditionally are difficult to study because of species elusive tendencies, large home ranges and low population densities (Silver et al. 2004). Photo-ID in the form of capture-mark-recapture can provide a pragmatic solution to population studies of cryptic species by computing the capture probabilities of individuals never observed during sampling (Williams *et al.* 2002). It can help understand the ecology of wildlife populations, and therefore help in the process of recommending conservation and management actions. Moreover, the technique provides a relatively inexpensive way to collect data in a way that is not invasive to the study population (McClintock and White 2009). The nyala species is individually identifiable by the colour patterns on their coats and this was used as the main identifying feature. However visible scars and abrasion on the animal's skin were also used to aid the identification process. This study also showed that markings on nyala are bilaterally asymmetrical. Therefore matching of photographs for the purpose of identifying animals from photographs had to be done consistently on the same flank. For this study I used the right side of the animals. The choice to use the right side was merely arbitrary.

The duration of the study is crucial when using photo identification to study nyala. Nyala are known to undergo physiological changes on the pelage with age. New borns of both sexes bear a resemblance to the adult females -reddish-brown to chestnut in colour (Cellie 1987) and the adult males are dark blue grey (Smithers and Abbott 1986).

Young males undergo a colour transformation as they grow and mature until they are fully grown bulls which can take up to 24 months after birth (Anderson 1984). It is therefore important to note that a photo identification study spanning over a year may be prone to misidentifications as some males would have changed some of their primary identification features. Photographic databases normally of species forms permanent records of individuals within their geographic confinements (Hammond 1990), this is however not necessarily the case with nyala especially the young males. Due to the pelage transformation that males undergo with ageing, photographs of young males may not be usable for identifying these individuals later in their lives.

Comparing the input and output data, there seems to be a disproportional amount of juveniles unidentified in the two data. Juvenile nyala are difficult to ascertain their sexes. This is because juveniles or both sexes are so alike (Cellie 1987).Therefore through the process of matching, some of the juveniles which their sex could not be ascertained during sampling were correctly matched in other age groups hence the drastic reduction in number of juvenile unidentified in the output data. .

Wild-ID, the software that was used to facilitate the matching process has also proven to be reliable. Wild-ID performs exceptionally well especially with small data. However as the amount of data increase, its matching accuracy drops. The accuracy is denoted by the score that the software allocates the matches, with high score indicative of high accuracy and vice versa (Bendik 2013). The quality of photographs also played a major role in the matching process by the software. Some photographs appeared to be clear but were shiny due to the light reflecting from the animals backs; these photographs also got a low score indicating low accuracy especially during the initial iterations with many photographs being processed.

We also observed that photographs that were taken at the same angle were easier to match by Wild-ID than those taken at different angles. The best matches were produced from photographs captured at a perpendicular angle to the animal's body. For this reason I had to run the photographs through the software several times before I could come to the final output. With every run where new matching occurred, the number of unmatched photographs reduced, thus increasing the accuracy of the subsequent run. Moreover, some photographs had to be verified manually.

Wild-ID has been extensively used previously for estimating individually identifiable elusive and vulnerable animals including giraffes (Bolger *et al*. 2013, Halloran *et al.* 2014), Wildebeest (Morrison and Bolger 2014) and Melanophryniscus monitividensis (Elgue *et al.* 2014). It patently reduces the time need to identify compared to manual analysis of photos. However good quality photographs are paramount to achieving good accuracy.

Photo-ID has been proven to be very efficient in animal ecology studies. Silveira *et al*. (2003) compared this method to direct animal counting along transects lines and track counts to assess the faunal richness and abundance in the greenlands of Emas National Park in central Brazil. They discovered that track count was the most efficient detecting species richness and relative abundance but had a lot of shortcomings. This method requires a minimum of two people and a vehicle to be able to cover extensive area. It is also largely constrained by environmental conditions which determine the detectability of tracks. Line transects was found to be the least effective because it was also limited by environmental conditions and its success is to a large extend dependent on how experienced the researcher is. Photo-ID on the other hand can work under almost all environmental conditions and it has the advantage over the other methods of being able to determine the sex and age of the organisms.

The ability to determine the animal's sex is however dependent on a number of factors such study site, season, animal species, behaviour and counting methods.

4.1.2 Population estimates

Our results provide objective estimates of the nyala population in the Masinga hide area. Population estimates from program MARK and program CAPTURE were fairly close and this gives better confidence about the estimates despite the unusual standard errors. Results show a marked skewed ratio towards females. Without counting the unidentified juveniles which sex is unknown, the ration of males to females in our study is 1:1.5. This finding is consistent with Vincent *et al.* (1968), where they observed that male to female ratio of new born nyala was 1:1.3 and with Tello and van Gelder (1975) where they observed that only 39% of their encounters during the two years survey were males, 61% were females. Barnes (1972) found the male to female ratio of nyala in False bay Park in Zululand to be 1:2 in 1969 and 1:1.5 in 1970.

Beside the high proportion of female Vincent *et al.* (1968), the disproportion in the adult population can be attributable to selective hunting where males are particularly targeted for trophy hunting. Hunting in Umkhuzi game reserve is strictly controlled and is allowed for both sexes but trophy hunters prefer to hunt bulls because of their spectacular spiralling horns.

Fifty-nine percent of our study population was made up of adults of both sexes. From the adult population, 66 % were female adult and only 34 were males. Female nyala have been shown to reach sexual maturing as early as at 11 months (Vincent *et al.* 1968).

Males on the other hand take longer to reach the size where they are considered adults even though they might be capable of reproduction before they attain that adult size (Vincent *et al.* 1968).

Most or all of the young adult males might in fact be matured males who had not just reached size that they can be called adults. This may explain the disproportional high number of adult females compared to their male counterparts.

Sampling was done at the drinking hole. The positioning of the sampling area was particularly important for producing unbiased estimates of the population size where all ages and sexes well represented. Nyala is a cryptic species, spending most of their time in the thickets. Moreover, new born nyala are kept hidden in the thickets by their mothers for the first two weeks or so (Tello and van Gelder 1978). Therefore sampling at the water hole enabled all sexes and ages to be seen as they came to drink, giving an unbiased representation of the entire population in the area.

Population estimates from this study does not represent the entire Umkhuzi Game Reserve but only restricted to the area around Masinga hide. For this reason we observed a marked difference in abundance from the estimates of this study compared to the findings of White and Goodman 2009 which was representative of the entire Umkhuzi game reserve. Tello and van Gelder (1975) indicated that the general average home range of nyala is 5.5 km^2. Therefore estimates from this study are only indicative of abundance of nyala found within the limits of their home range around Masinga hide.

4.1.3 Closure test

The closure test indicated a lack of closure for this population. This could be due to the large number of animals which were only sighted once. The Closure test is done to ascertain that the population was constant throughout the sampling period. In our case the large number of animals with one encounter history may seem to indicate animals entering the population. It are however difficult in practice to make certain closure of a biological population as even during the shortest sampling time death and birth can occur as well as animals can move on and off the sampling area (Soisalo and Cavalcanti 2006). Moreover, the closure assumption has been shown in a controlled environment to have been violated when it is known that it was in reality not violated (White *et al* 1982). A closure test for a closed population may indicate that the population is not closed which is a reaction to variations in capture probabilities which may seem like there was an ingress or egress of animals (White *et al*, 1982). This shows that the closure test is not always 100% accurate and it is therefore highly recommended that the researchers do what they can to ensure that the assumption of closure is met; one way of achieving this is by keeping the sampling period as short as possible relative to the lifespan of the study species (Soisalo and Cavalcanti 2006).

4.1.4 Model selection

Model M_h was chosen as the most appropriate model for this set of data. This model assumes that each individual animal has a distinct encounter probability which is independent of all the other members of the population and is not affected by the animal's response to traps or time (Otis et al. 1978).

This was a logical assumption for this study where sampling was done by photographing animals from a distance, a practice that does not in any way interfere with the animals and can therefore not affect the animal's behaviour. This model signifies that the variation in the capture probabilities is attributable to the inherent unobservable heterogeneity amongst the members of the population. Normally closed capture-recapture models assumes that all animals have the same detection probability but the reality is that individual attributes of the animals such as sex, age, body mass and even genetic make-up create variations in the detection probabilities between individuals (Cubaynes *et al.* 2009). This heterogeneity created by varying individual attributes can lead to underestimation of abundance in a closed population if they are not considered during process (Hwang and Huggins 2005). This heterogeneity can be accounted for by considering classes for each individual or group with distinct constant detection probabilities mixture models (Agresti 1994). For our study we considered two mixtures for each group. Choosing two group mixture was arbitrary and does not necessarily imply that there are two mixture groups of animals, it is really aimed at reducing the bias which is caused by the simplistic assumption of homogeneity (Pledger and Phillpot 2008).

The population size estimator of this model, M_h, is known to be robust to violation of underlying assumptions (Otis *et al.* 1978).

CHAPTER 5

5.1 Recommendations

Photo identification has proven to be the future in population studies of animals with individually identifiable physical features and more so for cryptic, rare and vulnerable through the framework of capture-mark-recapture. The method is non-invasion and for this reason it is preferred as it avoids problems associated handling animals during the traditional capture mark recapture practices.

A number of photo matching software have been developed and are available to aid with the matching especially where many photographs are involved. These software are very useful in helping the researcher deal with large numbers of photographs to be matched. Most software declare that they are scale invariable, meaning that they can recognise and correctly match photographs irrespective of their rotation or change in size. This study has revealed that this statement is not always 100% accurate and that the software, no matter how good can be prone to errors. These errors can lead to false positive or negative identifications. It is therefore good practice to verify the matching done by the software manually as far as possible. More importantly, it is crucial to have only the best quality photographs used for matching. This can be achieved through properly planning the study to ensure that the best equipment are available, sampling is done at the most suitable site and time of day to get the best quality photographs. Failing to take care of this problem during the project planning phase will result in a lot of photographs having to be discarded due to their poor quality. Discarding photographs after the sampling is completed will reduce the input data available for analysis and may defeat the whole purpose of the study, which may necessitate further sampling due to insufficient raw data. That will inevitably add unduly to the costs for the project.

Population parameter studies are important to ensure correct stocking of animals so as to avoid overstocking or understocking which could inevitably and invariably lead to the degradation of the environment. In addition, animal population studies for economically important species such the nyala is important to ensure that the species continues to provide the maximum economic returns in a sustainable manner. It is therefore recommendable that regular population studies be carried out at appropriate intervals to assess the status of the species in the wild to ensure sustainable development. To inform management and conservation decisions, population estimates studies in an enclosed facility such as Umkhuzi game reserve need to be carried out at various strategic places so that it can give estimates of the entire population in the game reserve.

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