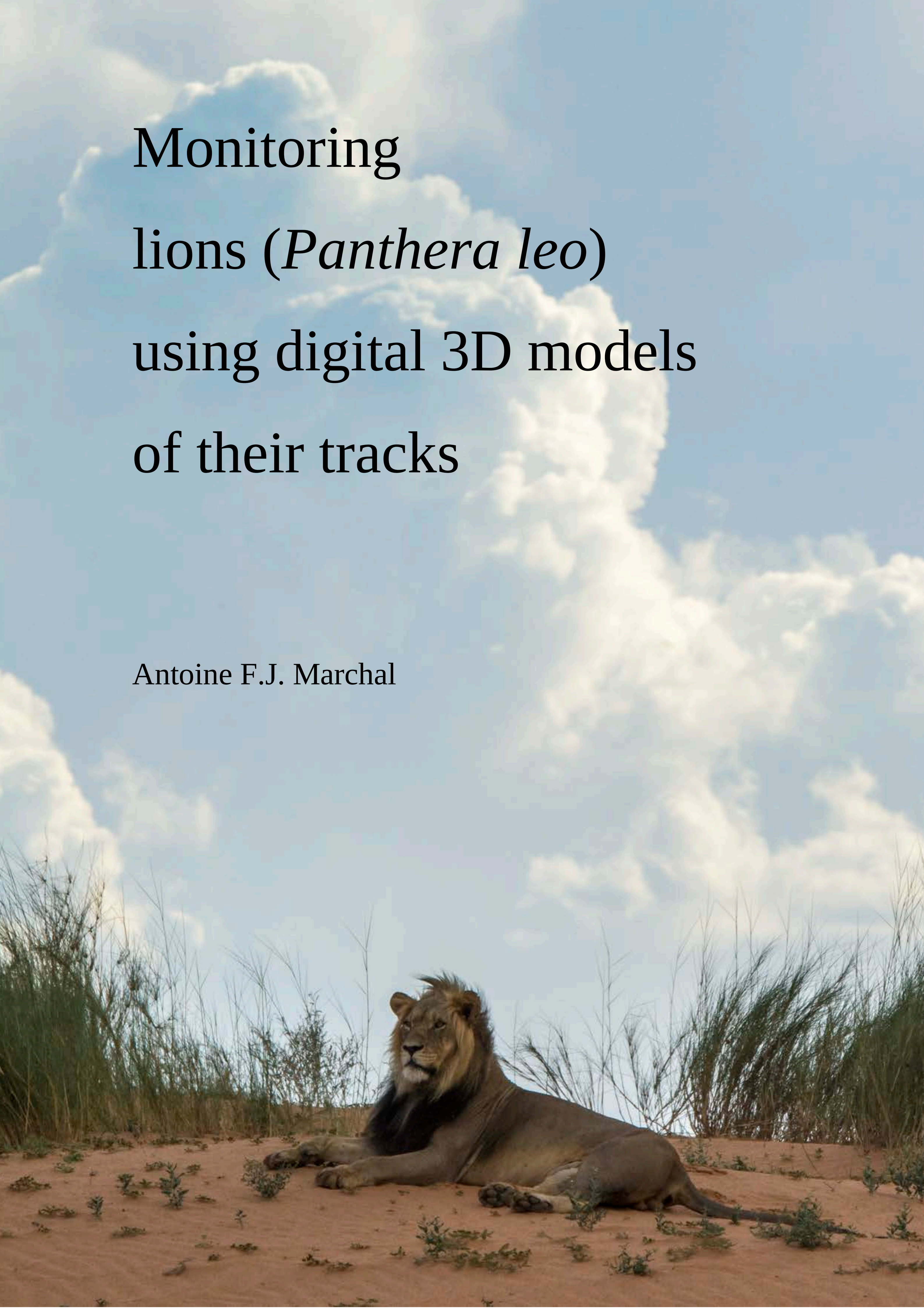

Monitoring lions (*Panthera leo*) using digital 3D models of their tracks

Antoine F.J. Marchal

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by

Antoine F.J. Marchal

Submitted in partial fulfilment of the requirements for the joint-degree

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Faculty of Natural and Agricultural Sciences	Gembloux Agro-Bio Tech
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‘The Art of Tracking may well be the origin of science. After hundreds of thousands of years, traditional tracking skills may soon be lost. Yet tracking can be developed into a new science with far-reaching implications for nature conservation’

- Louis Liebenberg

Declaration

I, Antoine F.J. Marchal, declare that the thesis/dissertation, which I hereby submit for the joint-degree PhD Zoology, and PhD Agricultural Sciences and Bio-engineering at the University of Pretoria (South Africa) and the University of Liège (Belgium), is my own work and has not previously been submitted by me for a degree at these or any other tertiary institution.

SIGNATURE:

Marchal

DATE: 21 March 2017

University of Pretoria / University of Liège

South Africa / Belgium

Summary

Title: Monitoring lions (*Panthera leo*) using digital 3D models of their tracks

Student : Antoine F.J. Marchal

Supervisors : Prof. P.J. Nico de Bruyn

Prof. Philippe Lejeune

Joint-degree : PhD (Zoology)

PhD (Agricultural Sciences and Bio-engineering)

Department : Zoology and Entomology, University of Pretoria, South Africa

Biosystems Engineering, University of Liège, Belgium

Summary: As the Earth may be entering a new human-influenced geological time, the Anthropocene, the pressure on biodiversity due to increasing human populations and activities is unprecedented. Monitoring animal populations is essential to gain demographic data and assess their viability. Because of the difficulties linked to invasive methods that involve direct observation or handling, many researchers have explored alternative approaches such as using tracks.

Tracks are the material by-product of the interaction between terrestrial animals and their physical environment due to gravity. The interpretation of tracks exists since the dawn of mankind and is a crucial factor for human evolution. As an integral part of hunting, the art of tracking involves cognitive thinking that may well be part of the origin of science. However, monitoring species through their tracks is controversial due to several reasons: unreliable recording techniques limited to two-dimensions, manipulator bias, substrate variation, misidentification of the foot from which each track originates, and subjective identification of the age, sex and/or individual. The aim of this thesis was to evaluate the possibility of monitoring lions *Panthera leo* through digital three-dimensional (3D) models of their tracks.

The first step was to assess close-range digital photogrammetry as a low-cost, rapid, practical and reliable field technique for the digital 3D modelling of lion paws, tracks and trails. A trail, that is a continuous sequence of tracks made by the same individual, reflects the morphology and kinematics of that individual. The use of traditional morphometrics enabled the extraction of variables such as distances, angles and areas from the digital 3D trails originating from individuals of known age, sex and/or identity. When comparing adult lions walking at the same gait (i.e. same kinematics), the trail variables provided sufficient information to identify the sex and individual. The digital solution permitted the extraction of more variables than the usual pace, stride and straddle. These additional variables enabled a better discrimination between the sexes and individuals. As for the paw and track morphology, traditional morphometrics conveys limited information about the geometric structure, and fails to quantify the shape variations along curves and surfaces. To overcome these drawbacks, geometric morphometrics allowed the extraction of the form (i.e. size and shape) by means of superimposed fixed landmarks, and curve- and surface-slider semi-landmarks. Using fixed landmarks, the paws and tracks presented enough shape variation to identify their position along the anteroposterior (front or hind) and mediolateral (right or left) axes. We used fixed landmarks, with and without curve- and surface-sliders, on paws and tracks from lions of known age, sex and/or identity. The identification of the age and sex from the paws, and the age, sex and individual from the tracks achieved higher accuracies when using size and shape variables together rather than independently. The information from curves and surfaces offered an advantage for the identification from the tracks but not from the paws.

Due to the variation in anatomy, paw morphology and individuality in walking, tracks and trails contain information about the individual lion that created them. Recent advances in digital close-range photogrammetry and geometric morphometrics allow the recording and extraction of that information, which can then provide data to help monitor lion populations using a non-invasive approach.

Keywords: Lion; *Panthera leo*; paw; track; trail; digital 3D model; digital close-range photogrammetry; traditional morphometrics; geometric morphometrics; foot, age, sex and individual identification; non-invasive; ecological monitoring.

Résumé

A une époque où la Planète Terre est potentiellement sur le point d'entrer dans une nouvelle ère géologique marquée par l'influence de l'homme, l'Anthropocène, la pression sur la biodiversité émanant d'une augmentation de la population humaine et de ses activités, n'a jamais été aussi importante. Le suivi écologique des populations animales est essentiel pour obtenir des informations sur leur démographie et leur viabilité. Les méthodes invasives impliquant une observation directe ou une immobilisation des individus étudiés présentent certains désavantages qui ont poussé les chercheurs à développer des méthodes alternatives telles que l'utilisation des traces.

Les traces sont le produit de l'interaction entre animaux terrestres et leur environnement physique sous l'effet de la gravité. L'interprétation des traces existe depuis l'avènement de l'humanité et est un facteur crucial de l'évolution de l'homme. Etant une part intégrale de la chasse, l'art du pistage implique un raisonnement cognitif qui serait même à l'origine des sciences. Cependant, l'utilisation des traces comme outil de suivi écologique de la faune est un sujet controversé pour plusieurs raisons: techniques d'échantillonnage non fiables limitées à deux dimensions, biais du manipulateur, variation liée au substrat, erreur d'identification de la patte qui est à l'origine de chaque trace et identification subjective de l'âge, du sexe et/ou de l'individu. L'objectif de cette thèse est d'évaluer les possibilités de suivi écologique des lions *Panthera leo* grâce à la modélisation digitale en trois dimensions (3D) de leurs traces.

La première étape a été de tester l'utilisation de la photogrammétrie digitale rapprochée pour l'échantillonnage en 3D de pattes, traces et séquences de traces de lions de façon fiable, rapide, pratique et à moindre coût. Une séquence continue de traces provenant d'un même individu reflète la morphologie et cinématique de cet individu. La morphométrie traditionnelle a permis l'extraction de variables telle que des distances, angles et aires à partir de modèles 3D de séquences de traces provenant d'individus d'âge, de sexe et/ou d'identité connus. La comparaison des variables provenant de lions adultes marchant à la même allure (i.e. même cinématique) a permis l'identification du sexe et de l'individu. La photogrammétrie représente une solution digitale permettant l'extraction de variables autres que celles utilisées de façon classique, à savoir, le pas, l'enjambée et l'écartement. L'utilisation des variables additionnelles a produit une meilleure discrimination entre les sexes et les individus. Dans le cas des pattes et des traces, la morphométrie traditionnelle présente plusieurs désavantages en matière de restitution de l'information sur la structure géométrique. De plus, elle est incapable de mesurer les différences de forme le long des courbes et surfaces. Pour pallier à ces limitations, nous avons utilisé la morphométrie géométrique afin d'extraire la conformation géométrique (i.e. taille et forme) par l'intermédiaire de points de repère fixes et mobiles (sur courbes et surfaces). Nous avons montré que la forme des pattes et traces extraites à partir de points de repère fixes permet d'identifier leurs positions selon l'axe antéro-postérieur (avant ou arrière) et médio-latéral (droite ou gauche). Nous avons utilisé des points de repère fixes, complétés ou non de points de repère mobiles sur courbes et sur surfaces, sur des pattes et traces de lions d'âge, de sexe et/ou d'identité connus. L'identification de l'âge et du sexe à partir des pattes et de l'âge, du sexe et de l'identité à partir des traces a atteint de meilleurs résultats lorsque les variables de taille et de forme étaient utilisées de façon combinée plutôt que séparément. L'information issue des courbes et des surfaces a offert un avantage dans l'identification à partir des traces, mais pas dans celle à partir des pattes.

A cause des différences d'anatomie, de morphologie des pattes et de démarche, les traces et séquences de traces possèdent un certain niveau d'information sur l'individu qui les a créées. Les avancées récentes en matière de photogrammétrie digitale rapprochée et de morphométrie géométrique permettent l'échantillonnage et l'extraction de cette information qui peut ensuite être utilisée dans le suivi écologique de façon non invasive des populations de lions.

Mots-clés: lion; *Panthera leo*; patte; trace; séquence de traces; modèle 3D digital; photogrammétrie digitale rapprochée; morphométrie traditionnelle; morphométrie géométrique; identification de l'âge, du sexe et de l'individu; non invasive; suivi écologique.

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A doctoral adventure is a challenging journey, but along this path I have met the most amazing people and lived the greatest experiences. I also had the chance to meet and work with those who selflessly sacrifice their life to protect our wildlife. These people will recognise themselves and naming them is not necessary because glory and recognition are not their goal.

To all, including all the ones that I have unintentionally forgotten, I thank you!

Contents

List of abbreviations and acronyms

2D: Two-dimensions

3D: Three-dimensions or three-dimensional

a.s.l.: above the sea level

AF: Adult Female

AM: Adult Male

ANOVA: analysis of variance

bTB: bovine tuberculosis

CBD: Convention on Biological Diversity

CI: Confidence Interval

CR: Critically Endangered

Dg: Digital measurement

DMVR: Dense Multi-View 3D Reconstruction

DNA: Deoxyribonucleic Acid

Dr: Direct measurement

e.g.: *exempli gratia* (for example)

EKZNW: Ezemvelo KZN Wildlife

EN: Endangered

FIV: Feline Immunodeficiency Virus

FL: Front Left

FR: Front Right

GPA: Generalised Procrustes Analysis

GPS: Global Positioning System

HiP: Hluhluwe-iMfolozi Park

HL: Hind Left

HR: Hind Right

ID: identification

IUCN: International Union for Conservation of Nature

JF: Juvenile Female

JM: Juvenile Male

KZN: KwaZulu-Natal

LDA: Linear Discriminant Analysis

MANOVA: multivariate analysis of variance

MP: Main Pad

PC: Principal Component

PCA: Principal Component Analysis

PS: PhotoScan

SAF: SubAdult Female

SAM: SubAdult Male

SFM: Structure-From-Motion

T1: Toe 1

T2: Toe 2

T3: Toe 3

T4: Toe 4

TEP: Tembe Elephant Park

VU: Vulnerable

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Scientific outputs

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Marchal A, Lejeune P, Bruyn P. Digital 3D foot identification of lion paws and tracks using geometric morphometrics. Submitted to African Journal of Wildlife Research. Accepted with major modifications.

Chapter 1 - General introduction

Figure 1. 1 - Adult male lion in Kgalagadi Transfrontier Park, Botswana/South Africa.

ECOLOGICAL MONITORING

Biodiversity refers to all interdependent levels of nature variation including genetic, species and landscape levels (Wilson and Peter 1988). Another definition used in the Convention on Biological Diversity (CBD) is the variability within species, between species and between ecosystems (United Nations 1992). Conservative estimates indicate that there are approximately seven million species of eukaryotes (i.e. all living organisms excluding bacteria and cyanobacteria) and it is estimated that only 20% of these have been recorded (May 2000). The impact of human activities, mainly through habitat destruction, has accelerated the loss of biodiversity to a level that may well be considered a sixth mass extinction (Pimm and Raven 2000; Ceballos et al. 2015). Today's species extinction rates are 1,000 times the background rate and future rates are likely to reach 10,000 times the background rate (De Vos et al. 2014; Pimm et al. 2014; Joppa et al. 2016). The background rate is the rate of extinction recorded between the five preceding mass extinctions (Ceballos et al. 2015). By July 2015, the International Union for Conservation of Nature (IUCN) assessed 82,845 species in its Red List of Threatened Species (IUCN 2016). Out of 55,317 extant wild terrestrial species, 8,684 species are considered vulnerable (VU), 6,331 species are endangered (EN) and 4,109 species are critically endangered (CR). Amongst the terrestrial vertebrates (subphylum Vertebrata), 32% of amphibians, 22% of mammals, 19% of reptiles and 13% of birds are threatened with extinction (i.e. VU, EN or CR).

Monitoring species enables us to assess the distribution and status of their populations (Gese 2001; Wilson and Delahay 2001). The word 'monitor' originates from the Latin '*moneo*' which means 'to warn'. Therefore, ecological monitoring can be used as a warning mechanism to highlight ecological changes over space and time (Grimsdell 1978). According to Grimsdell (1978), wildlife monitoring can be divided into three main components: (i) numbers, (ii) distributions and (iii) body condition plus population dynamics. In other words, monitoring enables us to answer the most commonly asked questions about wildlife populations: how many animals are there, where are they and what is the trend of their populations? The answers to these basic questions are fundamental for the achievement of any scientific or management objectives regarding wildlife populations (Yoccoz et al. 2001).

Animal abundance can be classified into two different categories: absolute and relative abundance (Gese 2001). Estimating the absolute abundance (i.e. true abundance) necessitates the use of methods to count the actual number or density of animals, while relative abundance (i.e. index of abundance) uses indices of animal abundance (e.g. animal droppings, bird calls, den counts, scent-post visitation rates or track counts) (Schwarz and Seber 1999; Gese 2001; Wilson and Delahay 2001). The former can be considered as a direct measure of abundance in comparison to the latter that is an indirect measure of abundance. The relative abundance is assumed to correlate in some way to the true abundance (Schwarz and Seber 1999). However, this relation is rarely tested and it can be influenced by many factors such as environmental conditions (habitat, substrate, season and weather), animal behaviour and movements, population status and manipulator bias (Wilson and Delahay 2001; Hayward et al. 2005). It is therefore crucial to repeat the validation between index and true parameter across local, temporal and spatial scales (Hayward et al. 2005). Both absolute and relative abundance estimates made at different time intervals can be used to assess population trends. Information on animal distribution can originate from any abundance survey (absolute or relative) or by means of methods such as habitat mapping, questionnaires, interviews, sighting reports, camera trapping and radio-telemetry (Gese 2001). Body condition and population dynamics provide information on potential population trends (Grimsdell 1978). Body condition is related to animal's health, quality or vigour (Peig and Green 2010), and it serves as an indication of the nutritional status and prevalence of diseases amongst individuals or populations. It is traditionally assessed through morphometric measurements and visual scores (Grimsdell 1978; Hilderbrand et al. 1998). More advanced techniques, which are sometimes destructive, include biochemical and physiological metrics (e.g. bioelectrical impedance analysis, body electrical conductivity, densitometry, isotopic water dilution and kidney fat index) (Grimsdell 1978; Hilderbrand et al. 1998; Peig and Green 2010). Population dynamics are linked to demographic processes such as age structure, sex ratio, survival, fecundity, immigration and emigration (Grimsdell 1978; Gese 2001). These criteria enable the assessment of the overall condition of the population.

A monitoring method that does not require the studied animals to be directly observed or handled by the researcher is considered as being non-invasive (i.e. non-intrusive) (MacKay et al. 2008). Under this definition, all methods measuring indices of animal abundance can be classified as non-invasive. Conversely, methods measuring true abundance can be either invasive (e.g. traditional capture- or observation-based methods) or non-invasive (e.g. camera traps or DNA analysis on faeces and hair). However, the distinction between invasive and non-invasive monitoring is not that straightforward. For example, camera trap flashes can result in trap shyness (Wegge et al. 2004; Schipper 2007), and the mere presence of scat detection dogs, humans or survey equipment can notably disturb wildlife (MacKay et al. 2008). Furthermore, misemployed equipment for non-invasive survey such as barbed-wire hair collection device or nails to tighten baits can induce injuries, while GPS (Global Positioning System) collars may be virtually non-invasive once the collars are fitted (Garshelis 2006; MacKay et al. 2008).

CARNIVORE CONSERVATION

With a total of 286 described species, the Carnivora rank as the fifth largest mammalian order (of 29 extant orders) in terms of species diversity after the Rodentia (2,277 species), Chiroptera (1,116 species), Soricomorpha (428 species) and Primates (376 species) (Wilson and Reeder 2005). The total number of mammal species is 5,416 distributed amongst 1,229 genera (Wilson and Reeder 2005), thus, carnivore species represent about 5% of all mammal species. Of all mammalian orders, carnivores present the greatest interspecific variation for important biological traits such as body size, reproductive rate, habitat selection, home range and social structure (see Eisenberg 1981; Gittleman et al. 2001). For example, the body sizes range from the 100 g least weasel *Mustela nivalis* to the 800 kg polar bear *Ursus maritimus*. The reproductive rates can be as low as one progeny every seven years as noted with the black bear *Ursus americanus*, to as much as three litters of up to eight young per year for certain species of mongooses. Carnivores are found in every major habitat on Earth, from short grasslands (meerkat *Suricata suricatta*) to sparse woodlands (dwarf mongoose *Helogale parvula*), deserts (fennec fox *Vulpes zerda*), dense tropical forests (kinkajou *Potos flavus*), oceans (sea otter *Enhydra lutris*) and Arctic ice sheets (polar bear). Home ranges vary from fairly small (0.20 km^2 for red fox *Vulpes vulpes*) to extremely large areas (2,000 km^2 for African wild dog *Lycaon pictus*). The social structure varies from solitary individuals with brief mating encounters (ermine *Mustela erminea*) to monogamous pairs (golden jackal *Canis aureus*) and social groups with up to 80 individuals (spotted hyena *Crocuta crocuta*). Beyond this interspecific variation, the variation and flexibility within species are also significant (Gittleman et al. 2001).

Within the context of conservation, we often classify species according to the following categories (Caro, 2010; Gittleman et al. 2001):

- Flagship species which attract much attention from the general public;
- Indicator species which may suggest critical environmental damages;
- Keystone species which exert an essential role in the ecosystems;
- Umbrella species which require large areas and their protection would benefit other species;
- Threatened species which are more prone to extinction.

These subsets of species are called surrogate species and they can be defined as ‘species that are used to represent other species or aspects of the environment to attain a conservation objective’ (Caro, 2010). The fact that many carnivore species and even entire carnivore clades match all these labels makes carnivores particularly important in the context of conservation (Gittleman et al. 2001).

There are 245 species of terrestrial carnivores in the world (Hunter and Barrett 2011). All members of the order Carnivora descend from a small civet-like carnivorous ancestor (from the extinct family Miacidae) that lived over 60 million years ago (Wang et al. 2010). Carnivores possess a shared heritage of subsisting primarily on meat and are naturally rare due to their position at the top of the food chain (Hunter and Barrett 2011; Ripple et al. 2014). Among them, 31 species have an average adult body mass of more than 15 kg and they belong to five families: Canidae, Felidae, Mustelidae, Ursidae and Hyaenidae (Ripple et al. 2014). These species tend to have large energetic requirements, slow life histories and low population densities. They roam widely in search of larger prey, which brings them into conflict with humans and their livestock (Carbone et al. 1999; Cardillo et al. 2004; Cardillo et al. 2005). Large carnivores are some of the world's most iconic, charismatic and revered species, but also some of the most imperilled (Hunter and Barrett 2011; Ripple et al. 2014). Many of these species (61%) are listed as threatened (i.e. VU, EN or CR) and most (77%) are experiencing population decline. Many carnivores have experienced substantial decline in their populations and geographic ranges, and fragmentation of their habitats over the past two centuries (Ceballos and Ehrlich 2002; Morrison et al. 2007). Their main threats are habitat loss and degradation, persecution, utilisation and prey depletion (Ripple et al. 2014).

As apex predators, large carnivores play an important role in limiting herbivore populations through predation (Ripple and Beschta 2012; Mech and Peterson 2003) and meso-carnivore populations through intra-guild competition (Prugh et al. 2009; Ritchie and Johnson 2009; Ripple et al. 2013). Large carnivores are therefore responsible for structuring the ecosystems along multiple food-web pathways, both directly and indirectly (Ripple et al. 2014). Effects of trophic cascades include changes in abundance or richness in various taxa (e.g. mammalian, avian, invertebrate and herpetofauna), subsidies to scavengers, altered disease dynamics, carbon sequestration, modified stream morphology and crop production (Ripple et al. 2014). Due to their iconic and charismatic nature, large carnivores also provide direct economic benefits as a result of tourism (Chambers and Whitehead 2003; Richardson and Loomis 2009; Naidoo et al. 2011). For example, a single lion *Panthera leo* from the 1980s in Amboseli National Park, Kenya, generated an estimated income of $128,750 per year (Western 1984), while the re-introduction of this species in the Pilanesberg National Park, South Africa, generated an estimated income of $9 million per year (McNeely 2000).

LION (*Panthera leo*)

Taxonomy and description

Lion *Panthera leo* (Linnaeus, 1758) belongs to the Order Carnivora, Suborder Feliformia, Family Felidae and Genus Panthera. Linnaeus originally described the species from a northern African specimen from Constantine, Algeria (Allen 1942). Nowell and Jackson (1996) listed eight subspecies, namely, *P. l. azandica* (north-eastern Democratic Republic of Congo), *P. l. bleyenberghi* (southern Democratic Republic of Congo, and neighbouring parts of Zambia and Angola), *P. l. krugeri* (northern and eastern South Africa), *P. l. leo* (Algeria, Morocco and Tunisia, extinct), *P. l. melanochaita* (Cape region of South Africa, extinct), *P. l. nubica* (north-eastern and eastern Africa), *P. l. persica* (Asia), and *P. l. senegalensis* (western Africa). Based on genetic studies, O'Brien et al. (1987) recognised two subspecies: African lion *P. l. leo* and Asiatic lion *P. l. persica*. However, more recent studies argue that the species should be split differently with *P. l. leo* in Asia and central, northern and western Africa, and *P. l. melanochaita* in eastern and southern Africa (Barnett et al. 2014).

Lion fur is typically tawny or sandy with pale under parts, with black tail-tips and backs of the ears (Hunter and Barrett 2011). The rare white lions from the Kruger National Park region, South Africa, are leucistic with normal yellow pigment in their eyes (as opposed to the pink-red colour observed in albinos) (Cruikshank and Robinson 1997). Only males bare a mane that usually starts growing from the age of six months (Mills and Bester 2005; Hunter and Barrett 2011). Lion shoulder heights are typically recorded at 100-128 cm and the adult females weigh approximately 110-168 kg, while adult males weigh 150-272 kg (Hunter and Barrett 2011). Lions from the Indian population are usually between 10-20% smaller than their African relatives, with generally smaller manes (Joslin 1973; Hunter and Barrett 2011). This species is Africa's largest felid and the world's second largest after the tiger (Haas et al. 2005). In Africa, lions from hot areas (e.g. Tsavo National Park in Kenya and Sahelian countries) may have a 'maneless' appearance (Hunter and Barrett 2011; Kays and Patterson 2002). Mane length and colour influence the sexual selection and temperature regulation of males (West and Packer 2002). An important anatomical feature is that lions, along with other members of the genus Panthera (i.e. jaguar *P. onca*, leopard *P. pardus*, tiger *P. tigris* and snow leopard *P. uncia*) have the ability to roar due to the presence of a ligament in the hyoid apparatus (i.e. suspensorium) (Weissengruber et al. 2002; Mills and Bester 2005).

Distribution and habitat

Lion populations are distributed in patches, mainly in and around protected areas, across most sub-Saharan countries (Bauer et al. 2015) (Figure 1.2). The species is absent or possibly extinct in Djibouti, Equatorial Guinea, Eritrea, Gambia, Ghana, Guinea-Bissau, Guinea, Ivory Coast, Lesotho, Liberia, Mali, Mauritania, Republic of Congo, Sierra Leone and Togo (Chardonnet 2002; Henschel et al. 2010; Bauer et al. 2015). Riggio et al. (2013) identified 67 lion areas in Africa, which represent a total of 3.4 million km² (17% of their historical range). However, based on recent records, the IUCN estimates the lion range to be approximately 1.8 million km², or 8% of its historical range (Bauer et al. 2016) (Figure 1.2). The historical range spread across northern Africa to southwest Asia, west into Europe and east into India (Nowell and Jackson 1996). Lions became extinct in most of southwest Asia over the last 150 years, while they disappeared from Europe almost 2,000 years ago (Nowell and Jackson 1996). The last Asian population is present in India's Gir Forest (Singh and Gibson 2011).

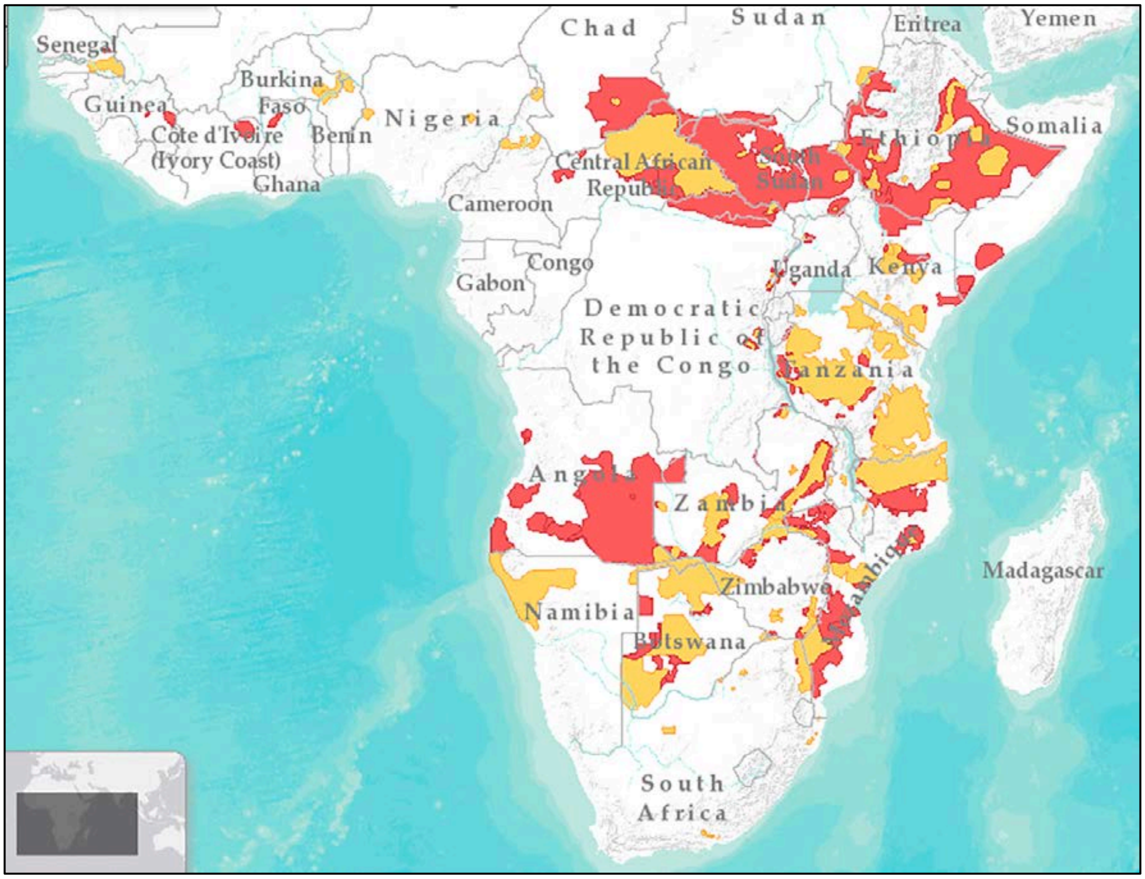


Figure 1. 2 - Confirmed lion populations (in orange) and areas where the species is possibly extinct (in red) (Bauer et al. 2016). This map does not show the single Asian population present in India.

The species is absent from true deserts and rainforests (Mills and Bester 2005). However, lions persisted at the edge of the Aïr Mountains in Niger until the 1930s (Rosevear 1974; Nowell and Jackson 1996). Lions are present in semi-deserts such as the Kalahari Desert in Botswana, Namibia and South Africa, and in parts of western Africa (Eloff 1973; Mills and Bester 2005). Individuals were recorded at altitudes of 3,600 m above sea level (a.s.l.) on Kenya's Mount Elgon (Guggisberg 1963) and 4,240 m a.s.l. on Ethiopia's Bale Mountains (Yalden et al. 1980). Important habitat requirements are availability of medium and large prey, shade to rest during the heat of the day and vegetation cover for successful stalking (Mills and Bester 2005). The optimum habitats are open woodland and grassland savannah (Nowell and Jackson 1996; Hunter and Barrett 2011).

Feeding ecology

Maximum charging speed for a lion is approximately 48-59 km/h (13-16 m/s), which is rarely maintained for more than 100 m (Estes 1991). Therefore, lions require skill, patience and judgement to capture prey either by stalking, ambushing or communal hunting (Estes 1991). Lions are predominantly nocturnal and most of the hunting crepuscular or at night (Schaller 1972; Hunter and Barrett 2011). However, hunting during daylight hours is frequently observed, especially in places with suitable vegetation cover (Van Orsdol 1984; Estes 1991). Over different spatial and temporal scales throughout their range, lions preferably prey on species with a body weight of 190-550 kg (Hayward and Kerley 2005). The preferred prey species are gemsbok *Oryx gazella*, buffalo *Syncerus caffer*, blue wildebeest *Connochaetes taurinus*, giraffe *Giraffa camelopardalis* and plains zebra Equus quagga (Hayward and Kerley 2005). Unusual prey, such as Cape fur seals *Arctocephalus pusillus* (Bridgeford 1985), black rhinoceros *Diceros bicornis* (Elliot 1987; Brain et al. 1999; Matipano 2004), African elephant *Loxodonta africana* (Ruggiero 1991) and hippopotamus *Hippopotamus amphibius* (Pienaar 1969), have also been recorded. The largest mean daily intake rate recorded for the species was 11.4 kg/day from Etosha National Park, Namibia (Stander 1991; Hayward et al. 2009). Lion hunting success rates range from 15% in Etosha National Park (Namibia) (Stander 1992a) to 23% in Serengeti National Park (Tanzania) (Schaller 1972) to 29% in Queen Elizabeth National Park (Uganda) (Van Ordsol 1982) and 38% in Kalahari Gemsbok National Park (Eloff 1984). Lions often appropriate kills from other predators, and are common scavengers. Scavenging contributes 5.5% and almost 40% of their food intake in Etosha National Park (Namibia) and Serengeti National Park (Tanzania) respectively (Hunter and Barrett 2011). In the absence of their normal prey, lions may prey on livestock and also become potential man-eaters (Mills and Bester 2005; Packer et al. 2005). Though they drink regularly when water is available, lions can obtain their moisture requirements from their prey and even plants (e.g. desert melons) (Nowell and Jackson 1996; Mills and Bester 2005).

Social and spatial behaviour

Lions, aside from occasional coalitions of male cheetahs *Acinonyx jubatus*, are the only distinctly social felids (Mills and Bester 2005; Hunter and Barrett 2011). The species live in prides that are fission-fusion social units comprising a few individuals to 30 or more (Mills and Bester 2005). The average pride size varies from 15 individuals (range = 4-37) in Serengeti National Park (Tanzania) (Schaller 1972) to 12.5 (range = 9-20) in Etosha National Park (Namibia) (Stander 1991), 11.8 (range = 7-12) in Kruger National Park (South Africa) (Smuts 1976), 10.8 (range = 7-12) in Kaudom Game Park (Namibia) (Stander 1997) and 4.7 (range = 2-16) in Kgalagadi Transfrontier Park (Botswana/South Africa) (Eloff 2002). Mosser and Packer (2009) demonstrated that larger prides have a significant advantage in territorial competition. A pride usually contains four to eleven related females, their offspring and two to four immigrant males that are unrelated to the breeding females (Hunter and Barrett 2011). Females represent the most stable nucleus of a pride (Bertram 1973). The pride members defend a territory against other prides and unknown males, but they are rarely seen altogether (Estes 1991; Hunter and Barrett 2011). Territory size, which is correlated to habitat condition and prey abundance, varies greatly: 30-400 km^2 in Serengeti National Park (Tanzania) (Schaller 1972), 50-248 km^2 in Kruger National Park (South Africa) (Mills and Bester 2005), 150-2,075 km^2 in Etosha National Park (Namibia) (Stander 1991) and 266-4,532 km^2 in Kgalagadi Transfrontier Park (Botswana/South Africa) (Funston 2001). For comparison, the country Luxembourg is 2,586 km^2. Females generally spend their entire life within the same territory, unless they disperse following pride take-over by a new male(s) or to avoid inbreeding (Mills and Bester 2005; Hunter and Barrett 2011). Following their eviction or dispersion from the natal pride around 25-48 months, young males become nomadic for two to three years before trying to acquire their own pride (Hunter and Barrett 2011). They either remain solitary or form a coalition with other males, related or not, containing up to nine individuals (Pusey and Packer 1987; Estes 1991). A single male or a coalition of males can hold tenure over one or more prides (Packer et al. 1991). The average pride tenure is no more than two (Packer et al. 1988) to three years (Stander 1991) due to intense competition between males. Population densities varies from 0.05-0.62 lions/100 km^2 in northern Namibia to 1.5-2 lions/100 km^2 in the Kalahari Desert (Botswana, Namibia and South Africa), 6-12 lions/100 km^2 in Kruger National Park (South Africa) and 38 lions/100 km^2 in Lake Manyara National Park (Tanzania) (Hunter and Barrett 2011).

Reproduction and demography

The adult sex ratio is heavily skewed in favour of females, with one male to seven females in the Kruger National Park, South Africa (Van Orsdol et al. 1985). Females reach sexual maturity around 30-36 months of age, but typically have their first litter around 42-48 months (Hunter 1998; Hunter and Barrett 2011). Males reach sexually maturity around 26-28 months, but usually do not get the opportunity to mate before they reach an age of four to five years (Smuts 1982; Hunter and Barrett

2011). Individuals are generally grouped into the following age classes: small cubs (0-1 years), large cubs (1-2 years), subadults (2-4 years) and adults (>4 years) (Schaller 1972; Smuts et al. 1978a; Stander 1991). Breeding is seasonal but females generally synchronise their births within a pride and care communally for the cubs (Estes 1991). Oestrus lasts four to five days, the gestation averages 110 days, the litter size ranges from one to four cubs and the inter-litter interval is approximately two years if the cubs survive (Estes 1991; Packer et al. 2001). During oestrus, a couple mates at a rate of 2.2 times per hour, where copulation lasts 21 seconds (Estes 1991). At birth, the sex ratio is at parity and cubs of either sex weigh approximately 1.5 kg (Mills and Bester 2005). The cub mortality within the first year can be as high as 50-70% due to infanticide, predation and starvation (Hunter and Barrett 2011). Infanticide occurs during take-overs of a pride by new males causing the mothers to conceive again within four months (Packer and Pusey 1983). Inbreeding only occurs under unusual conditions such as small population or limited dispersion (Pusey and Packer 1987; Packer et al. 1991). Females typically stop breeding at 15 years of age and can live up to 20 years in the wild (Packer et al. 1988; Eloff 2002). Although males can still produce viable sperm up to 16 years old, they usually stop breeding at the end of pride tenure at an age of 8-10 years (Smuts et al. 1978b; Packer et al. 1988). Male longevity in the wild may be up to 16 years, but rarely exceeds 12 years (Smuts et al. 1978b; Hunter and Barrett 2011). In captivity, a lion’s lifespan may reach 25-30 years (Nowell and Jackson 1996).

Status, threats and diseases

The species *Panthera leo* is listed as vulnerable in the IUCN Red List of Threatened Species (Bauer et al. 2016). Chardonnet (2002) estimated the African population to be 37,945 [28,854-47,132] lions. Independently, Bauer and Van Der Merwe (2004) provided a more conservative estimate of 22,143 [16,500-30,000] lions. The numbers in square brackets provide the minimum and maximum estimates. The main difference between these two surveys is that Chardonnet (2002) aimed for a realistic estimate by filling the gaps with extrapolations and guess-estimations, while Bauer and Van Der Merwe (2004) presented an inventory using only known research data. The most recent survey from Riggio et al. (2013) found an estimated population of 32,000-35,000 lions across 67 lion areas. Out of the larger total (i.e. 35,000 lions), eastern Africa holds 57% of the total population, southern Africa 35%, central Africa 7% and western Africa 1% (Riggio et al. 2013). The western African population is considered critically endangered (Henschel et al. 2010; Henschel et al. 2015). Globally, the populations in Africa are declining except in intensively managed areas (Bauer et al. 2015). Alarming models indicate a 67% and 37% chance that the lions in western Africa and central Africa, respectively, will decline by one-half over the next two decades (Bauer et al. 2015). The Asiatic lion is currently considered as a separate subspecies, *P. leo persica* (O'Brien et al. 1987) which is concentrated into a single population in India’s Gir forest and is listed as endangered with only 411 individuals in 2010 (Breitenmoser et al. 2008; Singh and Gibson 2011).

The main threats to lions, similar to the threats faced by large carnivores in general, are indiscriminate killing due to human-lion conflict, prey depletion, habitat loss and transformation, use of bones and body parts in traditional medicine, and unsustainable trophy hunting (Bauer et al. 2016). Lions are susceptible to certain diseases such as bovine tuberculosis (bTB) (Ferreira and Funston 2010; Viljoen et al. 2015), canine distemper virus (Roelke-Parker et al. 1996), stomoxys plague (Fosbrooke 1963) and feline immunodeficiency virus (FIV) (Bull et al. 2003).

MONITORING LIONS

Monitoring lions, such as population census, may be accomplished using various methods depending on the objectives and desired accuracy. The most accurate census method within an area is to individually identify each lion. This can be done through natural features such as whisker spots, scars and nose colour (Pennycuick and Rudnai 1970; Creel and Creel 1997; Ogutu and Dublin 1998), or by means of man-made features such as brands and radio-collars (Mills et al. 1978; Orford et al. 1988; Stander 1992b; Castley et al. 2002; Funston 2011). The nose varies from pink to fully black according to the following scheme: black speckling (two to four years), 25% black (four to five years), 50% black (five to eight years) and 75% black (eight to ten years) (Creel and Creel 1997). Miller et al. (2016) recognised a total of ten phenotypic traits that can be used to age male lions: mane development, teeth colour, teeth wear, facial scarring, slack jowl, nose darkness, and colour variations of the chest mane, neck mane, shoulder mane and forehead mane.

Lion capture for branding and collaring usually involves the technique described by Smuts et al. (1977). During night capture, lions are attracted to chained bait using recorded calls of an animal in distress or spotted hyena vocalisations. Once the lions are feeding, a wildlife veterinarian immobilises the targeted individuals by using hypodermic darts. The chemical restraint used in darts is typically Zoletil (1-3 mg/kg) in combination with Medetomidine (0.05-0.08 mg/kg) (Kock and Burroughs 2012; Fahlman et al. 2005). In addition, the capture enables blood sampling and the implantation of microchips behind the ears. Fitting radio-collars, with or without integrated GPS, to individual lions enables the estimation of abundance by combining territory size and number of individuals per pride (Gese 2001).

Lion call-ups (i.e. call-ins) can also be used without capture, and with or without bait, in order to estimate lion abundance (Ogutu and Dublin 1998; Bauer 2007). This method implies the use of a calibration factor to estimate the true abundance from the response rate (i.e. number of individuals that were attracted by the calling stations). Another method that requires a calibration factor involves track counts along roads; the track density or number of tracks per 100 km of road enables the estimation of the true density (Stander 1998; Funston et al. 2010). Lion track surveys may also be used as the detection method in occupancy modelling (Midlane et al. 2014).

Other studies used the theory of capture-recapture to estimate lion abundance (Schwarz and Seber 1999; Castley et al. 2002). In a closed population, the Petersen estimator of a population size N works as follows: during a first sampling, n_1 individuals are captured and marked; during a second sampling, n_2 individuals are captured including m_2 recaptures; the proportion of captured individuals in the total population (n_1/N) is equal to the proportion of recaptured individuals in the second sampling (m_2/n_2), therefore, $N = n_1 n_2/m_2$ (Schwarz and Seber 1999).

Another approach, which is more opportunistic, is to use reports and pictures from staff members or tourists (Bauer and Van Der Merwe 2004). Camera traps can be used for individual identification if artificial or natural features are visible on the pictures (Tumenta et al. 2010). The least accurate method for estimating lion population size is ‘guess-estimation’ from resident researchers or based on secondary data such as prey or spotted hyena numbers, size of the area, rainfall and habitats (East 1984; Van Orsdol et al. 1985; Bauer and Van Der Merwe 2004).

The majority of lion surveys use a combination of the above-mentioned methods. Beyond the estimation of population size, these methods also provide information on the species presence and distribution, age structure, sex ratio and body condition. For example, radio telemetry permits the estimation of animal movement and habitat selection (Schwarz and Seber 1999; Gese 2001). Furthermore, it allows the location of collared individuals and their pride, thus, providing the opportunity to assess the pride number, age structure, sex ratio and body condition. The body condition may also be assessed from photographs or during captures. In the absence of sightings, track assessment provides another mean to gain information on populations.

THE ART OF TRACKING

Tracking is not only about looking at, recognising or following tracks; it also involves other aspects such as a total awareness of the environment, the use of the senses of smell and hearing, and the ability to recognise signs other than tracks (Gutteridge and Liebenberg 2013). Beyond ‘reading everything that is written in the sand', a tracker must be able to 'read between the lines' (Liebenberg 1990a). The art of tracking is used in persistence hunting, which consists of running down a prey to exhaustion during the hottest temperatures of the day (Liebenberg 1990a; Liebenberg 2006). The San people from southern Africa, such as the !Xo and /Gwi hunters, continue the practice of persistence hunting in the Kalahari Desert of Botswana (Silberbauer 1981; Liebenberg 2006). There is evidence that other hunter-gatherers, such as the Native Americans and Australian aborigines, also used this type of hunting (Sollas 1915; Nabokov 1981). Carrier (1984) hypothesised that persistence hunting, including tracking, may have been a crucial factor in human evolution, either anatomically or intellectually. The modern brain evolved in size and neurological complexity over millions of years, and first appeared at a time where humans were hunter-gatherers (Washburn 1978).

Liebenberg (1990a) described three levels of tracking: simple, systematic and speculative. Simple tracking is the easiest form of tracking as it involves following tracks under ideal conditions (e.g. on a soft substrate without vegetation and other animal tracks). Systematic tracking is a refined form of simple tracking where the tracker systematically gathers all the available information from the tracks and other signs. Finally, speculative tracking is the most advanced level of tracking where the tracker creates a working hypothesis based on the tracks and signs, and includes knowledge of both the animal behaviour and the local environment. The first two levels are based on inductive-deductive reasoning, while the latter is based on hypothetico-deductive reasoning. During speculative tracking, the tracker constantly searches for evidence to accept or reject the hypothesis (Liebenberg 1990a; Stander et al. 1997).

The art of tracking practiced by some of the earliest members of anatomically modern *Homo sapiens* may have been the first creative science (Liebenberg 1990a). Beyond the hypothetico-deductive approach, this science is characterised by the role of critical discussion and empathy in art (e.g. Palaeolithic paintings and engravings). This ingenious and sophisticated intellect was a necessity for the survival of hunter-gatherer societies (Liebenberg 1990a). The earliest evidence of tracking is seen by the depiction of tracks in prehistoric art. Animal tracks are represented in the early Magdalenian painting in the cave of El Castillo (Spain) (more than 40,000 years old) (Rink et al. 1997) and in the Late Stone Age rock engravings or petroglyphs in Twyfelfontein World Heritage Site in (Namibia) (aged between 6,000 to 2,000 years old) (Viereck and Rudner 1957; Rudner and Rudner 1968). Amongst other species, Twyfelfontein petroglyphs contain lion tracks.

Bicchieri (1972), and Blurton-Jones and Konner (1976) emphasised the knowledge of animal behaviour and exceptional hunting skills of the contemporary San trackers. Western scientists used the abilities of the San trackers to recognise tracks and follow trails (i.e. continuous sequence of tracks made by the same individual) in behavioural ecology studies of large carnivores (e.g. leopard, brown hyena *Hyaena brunnea* and spotted hyena - Eloff 1984; Bothma and Le Riche 1984; Bothma and Le Riche 1990; Mills 1990). Stander et al. (1997) quantified the accuracy of the tracking skills of four San trackers. For carnivore and herbivore species identification, the trackers were 100% correct in 147 cases. The age, sex and individual identification of three carnivore species, namely, cheetah, lion and leopard, were 92.75% (69 cases), 97.1% (69 cases) and 93.75% (32 cases) correct, respectively.

THE CURRENT USE OF TRACKS IN WILDLIFE STUDIES

A track (i.e. footprint, pawprint, pugmark or spoor) can be defined as the impression of a foot on a surface. Frey (1973) defined tracks as being biogenic sedimentary structures. Due to gravity, the interaction between living organisms and their abiotic environment is often marked by tracks and other signs (e.g. leaf rolling in the wind). Signs such as claw scratches on a tree (interaction biotic-biotic) or rolling marks in the dust (interaction biotic-abiotic) also indicate specific behavioural activities. Tracks represent the signature and evidence of the passage of animals or humans that are no longer visible or that may even be extinct, such as dinosaurs (Petti et al. 2008; Remondino et al. 2010; Falkingham 2012; Falkingham et al. 2014; Razzolini et al. 2014) and earliest hominids (Bennett et al. 2009; Bennett et al. 2013; Bennett et al. 2014; Bennett et al. 2016). The science of organism-substrate interaction is named ichnology; from the Greek word '*ichnos*' that means 'trace' or 'track' (Remondino et al. 2010). Ichnology can be divided into palaeoichnology when dealing with fossil traces (invertebrates) and tracks (vertebrates), and neoichnology when dealing with present day living organisms (Remondino et al. 2010). In other words, modern traditional and expert trackers are neoichnologists.

Tracks offer a low-cost non-invasive alternative to study elusive species that are otherwise difficult to observe (Heinemeyer et al. 2008). The use of tracks in wildlife studies may be classified into three categories that present an increasing level of complexity: track identification, track counts and track measurements. Track identification merely comprises subjective recognition of tracks with the help of a tracking book such as Liebenberg (1990b), Stuart and Stuart (2000), Gutteridge and Liebenberg (2013), and Stuart and Stuart (2013) for Africa, and Smith (1982), Halfpenny (1986), Murie and Elbroch (2005) and Halfpenny (2008) for North America. This type of track identification method, which involves the comparison between a drawn or photographed track in a reference guide and an actual track, is classified as a qualitative approach.

In track counts, the occurrence of tracks along transects or on prepared plots (track stations) is used as an index of relative abundance (i.e. number of tracks detected per unit of sampling effort) (Wilson and Delahay 2001). Track counts along transects usually implies driving at slow speed (~20 km/h) along dirt roads, with observers sitting on the front of the vehicle (Stander 1998). In some instances, the surveys are executed on foot or from a motorbike (Fitzhugh and Gorenzel 1985; Smallwood and Fitzhugh 1995). The observers may either be traditional or expert trackers, or simply use tracking books for species identification. Tracks from the same individual are recorded only once in a day. Track counts along transects were implemented for population size estimation for canids such as African wild dog (Stander 1998) and dingo *Canis lupus dingo* (Edwards et al. 2000), and felids such as cheetah (Houser et al. 2009), leopard (Stander 1998; Gusset and Burgener 2005; Balme et al. 2009), lion (Stander 1998) and mountain lion *Puma concolor* (Van Dyke et al. 1986; Smallwood and Fitzhugh 1995; Beier and Cunningham 1996).

Track stations with artificial or smoothed substrates improve the quality of the tracks, and scented attractants may be used to lure individuals to the site (Wilson and Delahay 2001). Track stations have been used for canids (e.g. coyote *Canis latrans* - Linhart and Knowlton 1975; dingo - Allen et al. 1996), and ursids (e.g. black bear - Lindzey et al. 1977). The index of relative abundance may be compared between sampling occasions that are separated in space or time (Wilson and Delahay 2001). However, various factors may influence this index (e.g. differences in habitats, seasons, substrate quality and topography). In some studies, the index of relative abundance was validated against true abundance, thus providing a calibration factor (Stander 1998; Funston et al. 2010).

With the aim of attaining more practicable, objective and quantifiable results, the art of tracking and the use of tracking books were replaced by track measurements and multivariate analyses. The measurements are either recorded directly on the tracks or on their replicas. The *in situ* approach, which is usually done with a calliper, may be time consuming, poses a higher risk of destroying the tracks and limits the number of measurements that can be recorded. There are various techniques that may be used to replicate the tracks: plaster casts, drawings and photographs. Once replicated, the tracks may be stored and the measurements extracted *ex situ*. Morphometrics (i.e. morphometry), which is the study of shape variation and its covariation with other variables (Bookstein 1991; Zelditch et al. 2012), enables the extraction of measurements from the tracks. The majority of studies using track measurements used variables such as distances, angles and areas (Figure 1.3; 1.4; 1.5; 1.6; 1.7). Along with track measurements, Sharma et al. (2005) also included trail measurements such as stride and straddle (Figure 1.6).

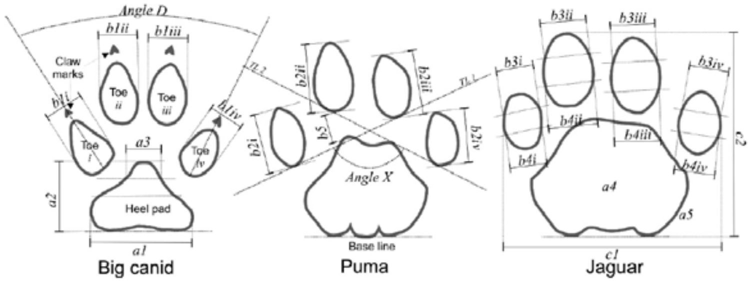


Figure 1. 3 - Examples of track measurements for big canid (domestic dog and maned wolf), mountain lion (i.e. puma) and jaguar (De Angelo et al. 2010).

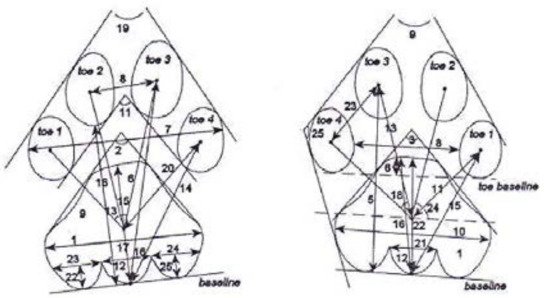


Figure 1. 4 - Examples of track measurements for lion (Van Bommel et al. unpublished data).

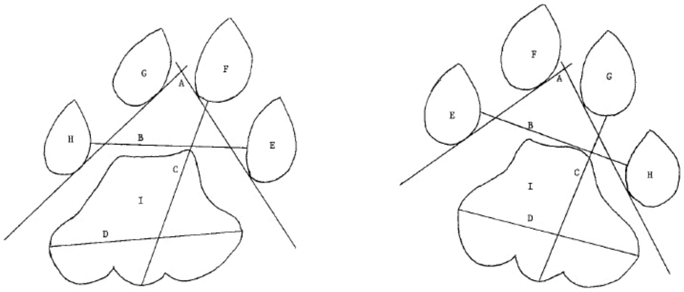


Figure 1. 5 - Examples of track measurements for mountain lion (Grigione et al. 1999).

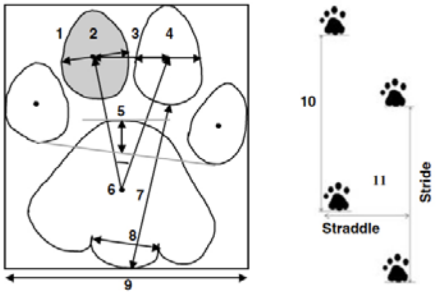


Figure 1. 6 - Examples of track and trail measurements for tiger (Sharma et al. 2005).

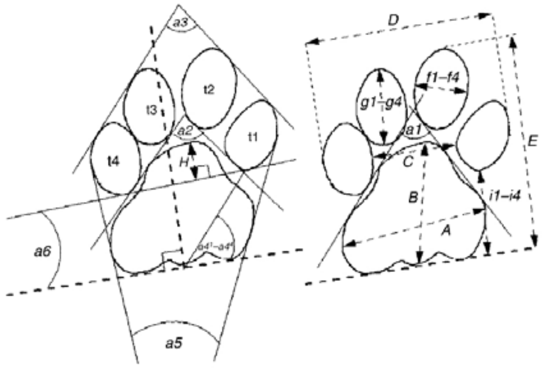


Figure 1. 7 - Examples of track measurements for snow leopard and tiger (Riordan 1998).

Track measurements used to identify the species, age, sex and/or individuals have been applied to:

- Canidae species such as African wild dog (Scharis et al. 2015), black-backed jackal *Canis mesomelas* (Gusset and Burgener 2005) and maned wolf *Chrysocyon brachyurus* (De Angelo et al. 2010);
- Felidae species such as African wildcat *Felis silvestris* (Gusset and Burgener 2005), caracal *Caracal caracal* (Gusset and Burgener 2005), cheetah (Jewell et al. 2016), jaguar (De Angelo et al. 2010), leopard (Stander et al. 1997; Gusset and Burgener 2005), lion (Stander et al. 1997; Van Bommel et al. unpublished data), mountain lion (Smallwood and Fitzhugh 1993; Grigione et al. 1999; Lewison et al. 2001; De Angelo et al. 2010; Jewell et al. 2014), serval *Leptailurus serval* (Gusset and Burgener 2005), snow leopard (Riordan 1998) and tiger (Gore et al. 1993; Riordan 1998; Sharma et al. 2003, Sharma et al. 2005);
- Rhinocerotidae species such as black rhinoceros (Jewell et al. 2001) and white rhinoceros *Ceratotherium simum* (Alibhai et al. 2008).

The most significant example of track use in wildlife monitoring is the ‘pugmark census method’ that was mainly described in the grey literature (Karanth et al. 2003). Invented in 1966, this single approach was implemented for more than 30 years to survey the tiger populations in India (Choudhury 1970; 1972). For one or two weeks per annum, thousands of rangers search for tracks across the entire country. The aim is to record, using plaster casts or tracings, the hind left paw’s tracks of supposedly each individual tiger. The track replicates were then compared to subjectively discriminate the different individuals and estimate the national population size. Beyond the use of a poor analytical framework and the violation of several statistical assumptions, this method was widely criticised for various reasons: misidentification of the foot from which the track originates, use of recording technique extremely affected by manipulator bias, no consideration of the variation due to the substrate and highly subjective identification of the individuals. Furthermore, the pugmark census method and all the above-mentioned studies using track measurements were limited to two-dimensions (2D), thus, lacking crucial depth information.

THESIS

Study sites

Location, size and history

Hluhluwe-iMfolozi Park (HiP) and Tembe Elephant Park (TEP) are located in the eastern province of KwaZulu-Natal (KZN), South Africa (Figure 1.8). HiP (28°17’S, 31°44’E) comprises approximately 900 km^2, while TEP (27°01’S, 32°24’E) is approximately 300 km^2 in size, Both parks are completely fenced and managed by the provincial conservation agency, Ezemvelo KZN Wildlife (EKZNW). Situated in Zululand, Hluhluwe and Umfolozi Game Reserves were established in 1895, making them along with St Lucia Game Reserve, the oldest game reserves in Africa (Charlton-Perkins and De la Harpe 1995). Along with the corridor area that separated them, the two reserves were amalgamated in 1989 to form the current HiP (Brooks 2000). Located in Maputaland at the international border with Mozambique, TEP was established in 1983 and was opened to the public in 1991.

Topography, climate and habitats

HiP is characterised by a rugged topography with altitudes ranging from 40 to 560 m a.s.l. Three major rivers, namely, Black iMfolozi, White iMfolozi and Hluhluwe, traverse the park. Sandy plains with ancient littoral dunes characterise TEP. The highest dunes are approximately 129 m a.s.l., while the lowest lying areas are approximately 50 m a.s.l. (Muzi swamp).

Characterised by a sub-tropical climate, the mean annual rainfall ranges from 650 to 985 mm in HiP and 700 mm in TEP. In HiP, the rainfall gradient follows the altitude gradient that is stretched from north to south. The major habitat of HiP is classified as woodland savannah interposed with shrub thicket. Semi-deciduous forests are present in the north of the park and open savannah woodland in the south. TEP is mainly comprised of sand forests and mixed woodlands. According to Mucina and Rutherford (2006), the two study sites both fall within the Savanna Biome. The predominant vegetation types are Northern Zululand Sourveld, Zululand Lowveld and Scarp Forest in HiP and Tembe Sandy Bushveld and Sand Forest in TEP (Mucina and Rutherford 2006).

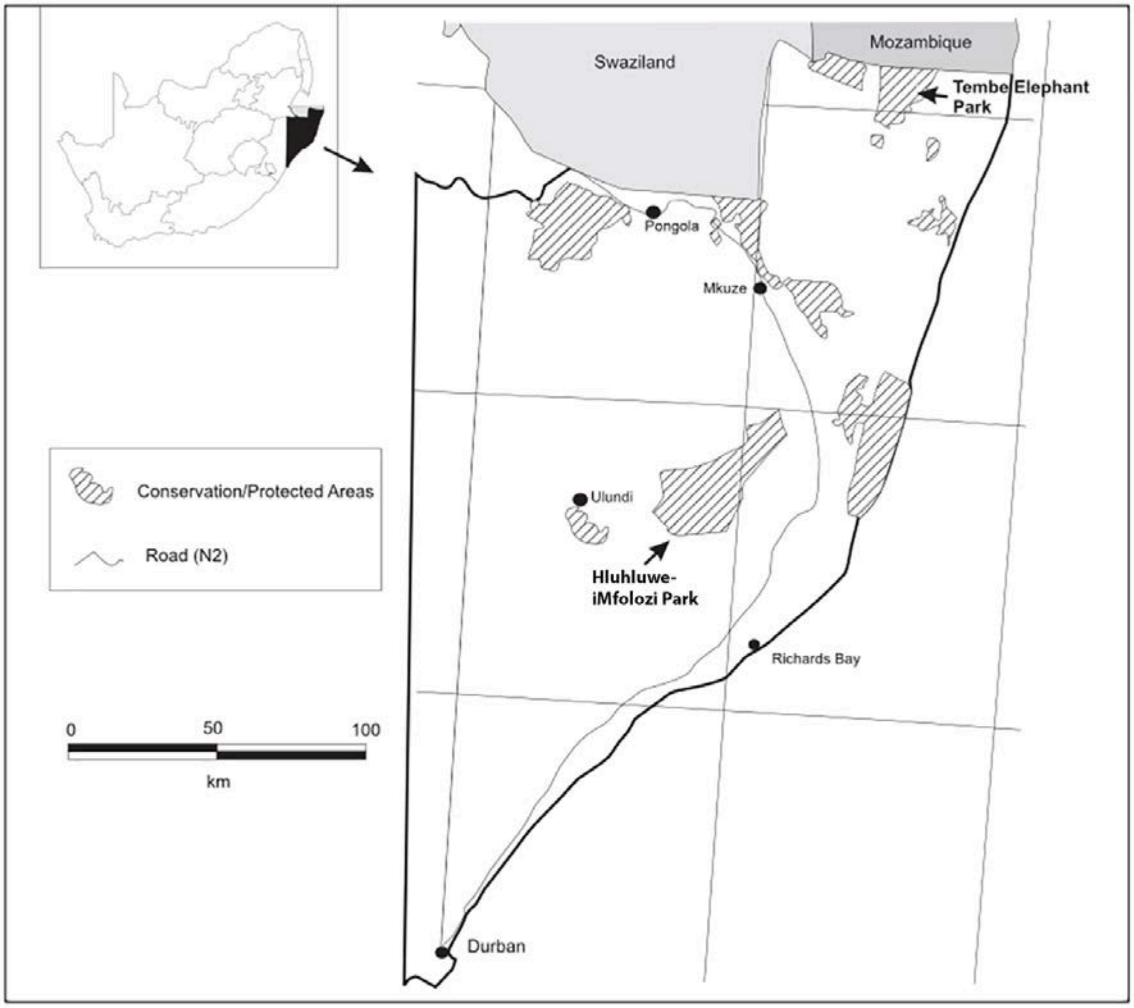


Figure 1. 8 - Location of Hluhluwe-iMfolozi Park and Tembe Elephant Park in northern KwaZulu-Natal, South Africa (adapted from Gaugris et al. 2004).

Lion populations

Resident lions were extirpated from both Maputaland and Zululand in 1938 (Steele 1970; Rautenbach et al. 1980; Rowe-Rowe 1992). In 1958, a lone male wandered down from either Mozambique or Swaziland, and settled in Umfolozi Game Reserve (Steele 1970). In 1965, three adult females and three cubs were released into Umfolozi Game Reserve (Steele 1970; Maddock et al. 1996). From these seven individuals (i.e. one male, three females and three cubs), the population increased to approximately 140 individuals in 1987 (Maddock et al. 1996). Due to an herbivore reintroduction programme, most of the lions in the Hluhluwe part of the park were destroyed between 1988 and 1992 (Maddock et al. 1996). The population was estimated at 80 lions in 1999 (Trinkel et al. 2008). At that time, the individuals showed evidence of inbreeding, with signs of abscesses, high cub mortality, low immunity, poor body condition and poor genetic variation (Maddock et al. 1996; Trinkel et al. 2008). To introduce new genes, 16 lions were released between 1999 and 2001 in HiP (Trinkel et al. 2008). The individuals were sourced from Pilanesberg National Park and Madikwe Game Reserve, South Africa. By 2006, the translocated lions and their descendants formed the entire population (Trinkel et al. 2008). The population was estimated at ~120 lions in 2015 (Somers et al. unpublished data). In 2002, four lions (two males and two females) were reintroduced in TEP from Pilanesberg National Park and Madikwe Game Reserve, South Africa (Millspaugh et al. 2015). From these four founders, the population increased to ~40 individuals in 2015 (Hanekom, TEP's ecologist, unpublished data).

Fieldwork and data

We collected all the data reported in this thesis, with the exception of the paw sampling where we had the assistance of several field biologists (see 'Acknowledgements'). The data, which was collected in HiP and TEP between January 2013 and April 2016, comprises digital photographs of:

- 20 tracks and 20 paws (the number of individuals was irrelevant for this part of the research) (Chapter 2);
- 26 trails (from at least 13 different individuals) (Chapter 3);
- 24 paws (four paws from both male and female lions of three different age categories) and 170 tracks from ten different trails (from unknown number of individuals) (Chapter 4);
- 81 lion paws (40 front left and 41 hind left paws sampled from 45 different individuals) and 116 tracks (from 25 different individuals) (Chapter 5).

Some of the sampled paws, tracks and trails were used more than once across the different chapters. The paws were opportunistically sampled during captures organised by EKZNW staff for management purposes. Both EKZNW and the University of Pretoria's Animal Ethics Committee (EC021-16) approved the paw sampling part of this study. Lions were chosen as the study species for various reasons: (i) their populations are highly managed in small, fenced-off protected areas of South Africa, (ii) individuals are regularly captured for management purposes, (iii) many individuals are known due to branding, collaring and scar pattern identification, (iv) the age and sex of unknown individuals are relatively easy to identify, (v) they live in prides which facilitate the sampling of a greater number of individuals, (vi) most of the prides contain collared individuals therefore facilitating their observation (vii) they tend to walk in riverbeds and on sandy roads, (viii) their foot structure is similar to other carnivore species (i.e. digitigrade) and thus can be used as a model species.

When tracks are recorded for analysis, they are subjected to variations due to the paw morphology, recording technique (including manipulator bias), substrate, slope, gait and age of the track. To focus on the form of the paws (i.e. size and shape) and their variation between different age and sex classes, and individuals, we sampled all the tracks using the same recording technique (i.e. digital close-range photogrammetry) (described in ‘Chapter 2’). Tracks were recorded on flat sandy riverbeds and roads. They were less than 24 hours old and were characteristic of walking individuals. The paws were sampled with the same recording technique by different manipulators with different high-resolution digital cameras. The trail sampling used the same recording technique with a slightly different protocol (described in ‘Chapter 3’) under the same conditions as track sampling. Whenever possible, known individuals were identified by means of an identification kit, which combines information regarding collars, brands and scar patterns. In the case that individual identification was unsuccessful, we considered the trails of individuals walking side-by-side in the same direction as belonging to different individuals. Working with separate prides across two different study sites enabled us to sample tracks from different individuals without systematically confirming their identification.

Aims and structure

The main objective of this thesis was to evaluate the possibility of monitoring lions through digital 3D models of their tracks. To achieve this goal, we aimed to overcome some of the controversies linked to the use of tracks in ecological monitoring by: (i) developing an innovative technique using digital photogrammetry to 3D record lion paws and tracks (Chapter 2), (ii) applying digital photogrammetry to 3D sample lion trails in order to extract the gait information used to discriminate between sexes and individuals (Chapter 3), (iii) identifying the position of the paws and tracks along the anteroposterior (front or hind) and mediolateral (right or left) axes using shape variables extracted by means of 3D landmarked-based geometric morphometrics, and (iv) discriminating the paws according to the age and sex, and the tracks according to the age, sex and individual, using size and shape variables extracted by means of 3D landmarked-based geometric morphometrics (Chapter 5) (Figure 1.9). For consistency, all of the chapters follow the referencing style from *Polar Biology*.

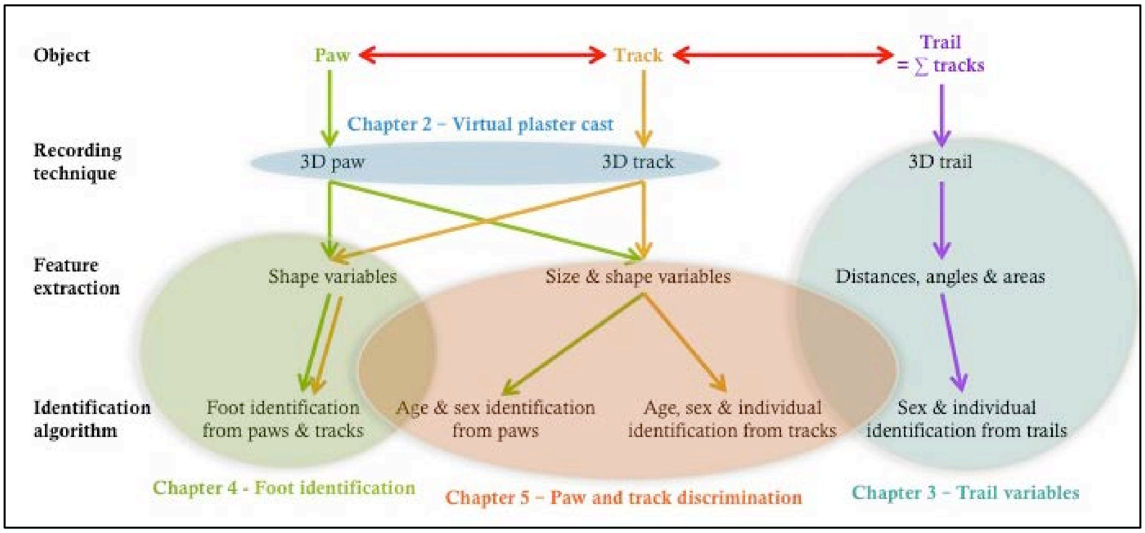


Figure 1. 9 - Thesis aims and structure.

Chapter 2 - Virtual plaster cast: digital 3D modelling of lion paws and tracks using close-range photogrammetry

In this chapter, we demonstrated that digital close-range photogrammetry provides a low-cost, rapid, practical and reliable field technique for the digital 3D modelling of lion paws and tracks. We therefore:

- Tested the impact of three reconstruction parameters (i.e. masking, calibration and optimisation) on the 3D model quality;
- Compared direct linear measurements on paws and tracks with the same measurements on their 3D models;
- Assessed the minimum number of photographs required for the 3D reconstruction of paws and tracks.

Chapter 3 - 3D trails: investigating lion gaits from tracks using digital photogrammetry

The relative positioning of tracks within a trail reflects the gait of an individual. The gait is linked to the morphology and kinematics of that individual. Previous studies employed a measuring tape to record a limited number of trail variables. In this chapter, we assessed whether close-range digital photogrammetry may be used to sample portions of lion trails and whether the variables extracted from these 3D trails provide better information on the sex and identity of different adult lions. We specifically:

- Compared direct linear measurements from the trails versus the same measurements on their 3D models;
- Analysed the differences in pace, stride and straddle between sex classes from overstepping adults;
- Compared the accuracy of predictions for sex and individual identification from discriminant analysis when using information from pace, stride and straddle, or from 40 variables, including distances, angles and areas, extracted from the 3D trails.

Chapter 4 - Digital 3D foot identification of lion paws and tracks using geometric morphometrics

The comparison of tracks originating from different feet leads to significant errors in analysis. For example, when assigning the tracks made by two different feet from the same individual to two different individuals. The objective of this chapter was to show that lion paws and tracks present shape variation along the anteroposterior (front or hind) and mediolateral (right or left) axes. To achieve this objective, we:

- Extracted the shape variables, using 3D landmark-based geometric morphometrics, from all four paws of various individuals of different age and sex, and from the tracks for which the position right or left and front or hind was identified;
- Measured and visualised the shape variation between paws and tracks, and between paws and between tracks, either they are front or hind and right or left;
- Compared the accuracy of prediction for foot identification using discriminant analysis with the shape variables extracted from paws and tracks.

Chapter 5 - Age, sex and individual identification from lion paws and tracks

Track measurements usually involve traditional morphometrics that require the use of linear, angular and area metrics. These basic measurements convey limited information on the geometric structure of tracks. This chapter aimed at applying 3D landmark-based geometric morphometrics to analyse the size and shape of lion paws and tracks, and their variations related to age, sex and/or individual. The sub-objectives were as following:

- Extract the size and shape variables using three different landmark scenarios, namely, fixed landmarks, fixed landmarks with curve-slider semi-landmarks or fixed landmarks with curve- and surface-sliders semi-landmarks;
- Measure and visualise the size and shape variation of the paws in terms of age and sex;
- Compare the accuracy of prediction for the age and sex identification from the paws, and for the age, sex and individual identification from the tracks using discriminant analyses with size and/or shape variables extracted through the different landmark scenarios.

Chapter 6 - Synthesis and conclusions

In this last chapter, we briefly summarised the results presented in Chapters 2-5 and their potential outcomes. Then we conclude with future research points that will enable improvement of the method introduced in this thesis.

Chapter 2 - Virtual plaster cast: digital 3D modelling of lion paws and tracks using close-range photogrammetry

Figure 2. 1 - Subadult male lion feeding on chained bait during a call-up operation in Hluhluwe-iMfolozi Park, South Africa. The spotlight was fitted with a red filter to decrease the disturbance on the targeted individuals.

ABSTRACT

The ecological monitoring of threatened species is vital for their survival as it provides the baselines for conservation, research and management strategies. Wildlife studies using tracks to identify individuals are controversial mainly due to unreliable recording techniques limited to two-dimensions. We assess close-range photogrammetry as a low-cost, rapid, practical and reliable field technique for the digital three-dimensional (3D) modelling of lion *Panthera leo* paws and tracks. Firstly, we tested three reconstruction parameters affecting the 3D model quality. We then compared direct measurements on the paws and tracks versus the same measurements on their digital 3D models. Finally, we assessed the minimum number of photographs required for the 3D reconstruction. Masking, auto-calibration and optimisation provided higher reconstruction quality. Paws masked semi-automatically and tracks masked manually were characterised by a geometric deviation of 0.23 ± 0.18 cm and 0.50 ± 0.33 cm respectively. Unmasked tracks delineated by means of the contour lines had a geometric deviation of -0.06 ± 0.39 cm. The use of a correction factor reduced the geometric deviation to -0.03 ± 0.20 cm (pad-masked paws), -0.04 ± 0.35 cm (pad-masked tracks) and -0.01 ± 0.39 cm (unmasked tracks). Based on the predicted error, the minimum number of photographs required for an accurate reconstruction is seven (paws) or eight (tracks) photographs. This field technique, using only a digital camera and a ruler, takes less than one minute to sample a paw or track. The introduction of the 3D facet provides more realistic replications of paws and tracks that will enable a better understanding of their intrinsic properties and variation due to external factors. This advanced recording technique will permit a refinement of the current methods aiming at identifying species, age, sex and individual from tracks.

Keywords: Tracking, digital 3D model, digital photogrammetry, computer vision, Agisoft PhotoScan, foot, footprint, *Panthera leo*.

INTRODUCTION

Ecological monitoring provides basic information on status and distribution of animal populations that is crucial for conservation, research and management strategies (Gese 2001). Using tracks is often considered as a non-invasive, cost- and time-effective way of gaining information on species that are difficult to observe (Gese 2001; Long et al. 2008). As an integral part of hunting, the earliest human beings have developed the art of tracking that is still used by modern hunter-gatherers such as the San people of Southern Africa (Liebenberg 1990a). A study in Namibia showed that modern-day San trackers were 96% accurate in interpreting the species, age, sex and individual from tracks for 317 cases (Stander et al. 1997). Track measurements were used to achieve similar levels of identification as that of the San trackers for larger felids such as leopard *Panthera pardus* (Stander et al. 1997; Gusset and Burgener, 2005), tiger *P. tigris* (Gore et al. 1993; Sharma et al. 2003; Sharma et al. 2005), lion *P. leo* (Stander et al. 1997) and mountain lion *Puma concolor* (Smallwood and Fitzhugh 1993; Grigione et al. 1999; Jewell et al. 2014), and for black *Diceros bicornis* and white *Ceratotherium simum* rhinoceroses (Jewell et al. 2001; Alibhai et al. 2008). The most significant example of track use in wildlife studies is the ‘pugmark census method’ that has been implemented for more than three decades to monitor the tiger populations in India (Karanth et al. 2003; Sharma et al. 2005). Designed in 1966, this census involves thousands of rangers that are searching for tracks across India for set periods of time (Choudhury 1970; Choudhury 1972). Tracings of the left hind paw’s tracks of purportedly nearly all the tigers are then compared for individual identification. This type of census using tracks is highly controversial since the protocol does not take into consideration the variation due to different manipulators and substrates, and the individual identification is highly subjective (Karanth et al. 2003). The pugmark census method and all the above-mentioned track measurement methods are using recording techniques limited to two-dimensions: direct measurement, drawing on acetate sheets or taking photographs. More recently, a Microsoft Kinect depth sensor was used to capture depth images of tracks from captive tigers (Lokare et al. 2014).

The rigorous use of tracks in ecological monitoring requires the variables extracted from them to be sensitive to variation between animals (species, age, sex, individual and body condition) and insensitive to external factors (such as substrate and manipulator bias). Three-dimensional (3D) reproduction of an object that is inherently 3D inevitably provides a better representation of reality that will improve the understanding of its intrinsic properties and their variation. Photogrammetry, the ‘science of measuring in photos’ (Linder 2009), provides a potentially useful tool for such 3D reconstruction. However, any innovative application first requires validation. Here we determine whether close-range photogrammetry can be used as a rapid, practical and reliable field technique for the digital 3D modelling of lion paws and tracks. We first tested the influence of reconstruction parameters on the alignment step, before comparing direct versus digital measurements and finally we assessed the number of photographs required for the 3D reconstruction. This technique was developed with the practical considerations of remote study sites and proximity to potentially dangerous animals in mind. Additionally, digital 3D reconstruction can be computed with a commercially available low-cost non-customised software package that implements both digital photogrammetry and computer vision techniques.

MATERIALS AND METHODS

Study areas

The two study sites, Hluhluwe-iMfolozi Park (HiP, ~900 km^2) and Tembe Elephant Park (TEP, ~300 km^2), are located in the sub-tropical province of KwaZulu-Natal (KZN), eastern South Africa. These two fenced areas are managed by a provincial conservation agency, Ezemvelo KZN Wildlife (EKZNW). Situated in Zululand, HiP is characterised by hilly topography ranging from 40 m to 560 m above sea level with a mean annual rainfall of 650-985 mm. Three major rivers (Hluhluwe, Black iMfolozi and White iMfolozi River) traverse the park. TEP is located in Maputaland along the international border of South Africa with Mozambique and is characterised by sandy plains with ancient littoral dunes and a mean annual rainfall of 700 mm. Dry riverbeds in HiP and sandy roads in TEP provide optimal substrate for tracks. Current lion populations (July 2015) are estimated at ~120 individuals in HiP (Somers et al. unpublished data) and ~40 individuals in TEP (Hanekom unpublished data).

Paw and track sampling

Twenty lion paws were opportunistically sampled during nocturnal captures in TEP (Figure 2.2a). The captures were part of management activities unrelated to this project (Animal Population Control plan, proposed by Tembe Management Team and accepted by EKZNW Board). Twenty clear lion tracks were sampled in HiP after a direct observation, in front of a camera trap [Cuddeback Attack, Green Bay, Wisconsin, USA] or after identification by means of a tracking book such as Liebenberg (1990b) and Gutteridge and Liebenberg (2013) (Figure 2.2b).

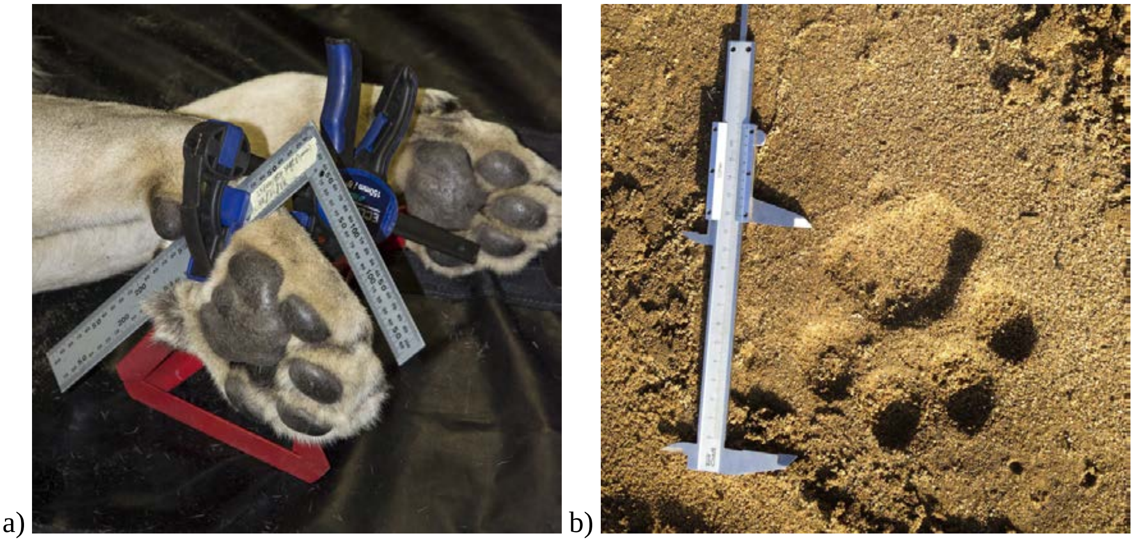


Figure 2. 2 - Paw and track sampling. (a) During the paw sampling, the motionless paw is positioned on a stand with a clamp holding the ruler and orientating the paw upward. (b) A vernier calliper was used for the direct measurements of paws and tracks.

Both paw and track sampling consisted of (i) directly measuring the length and width of the main pad and toes with the help of a 0-150 mm vernier calliper [Tork Craft, Midrand, South Africa] (Figure 2.2b), and (ii) taking photographs to create digital 3D models using close-range photogrammetry. The same manipulator, A.F.J.M., did all the sampling and two different digital single-lens reflex cameras were used: Nikon D7100 (24.1 megapixels) with Nikkor 18-70 mm f/3.5-4.5 and Nikon D80 (10 megapixels) with Nikkor 50 mm f/1.8 for photographing the paws and tracks [Nikon Corporation, Tokyo, Japan]. The sampling was carried out following the guidelines provided in the photogrammetric package's user manual (Agisoft LLC 2014a), as well as those described in De Bruyn et al. (2009). The manipulator took 10-15 photographs of the object (i.e. paw or track) with the same focal length from different angles and distances (Figure 2.3). During image acquisition, the paw was positioned off the ground on a stand with a clamp making it strictly motionless (Figure 2.2a). The photographs have to cover each side of the object to avoid blind spots and they have to overlap with each other (Figure 2.3). The object must fill the frame but a feature can be absent in one photograph provided that it appears in others. A ruler, that needs to be visible on at least three photographs, was positioned near the object and remained motionless between photographs to provide a scaling measure (Figure 2.2a).

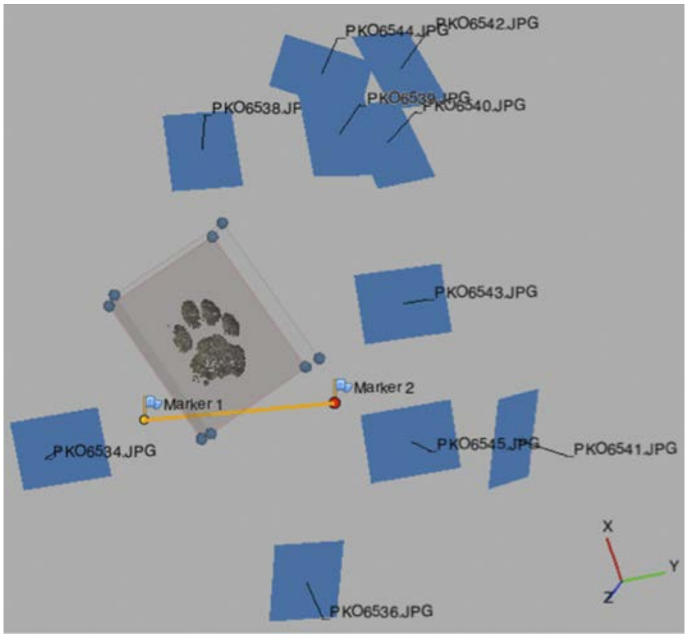


Figure 2. 3 - Placement of camera stations (blue frames) around the object of interest. The sparse point cloud as well as the camera positions and orientations are the outcomes of the camera alignment step. Note the two markers and the scale bar.

3D modelling and re-projection error

The 3D modelling was performed with Agisoft PhotoScan Professional Edition version 1.1.4 build 2021 [Agisoft LLC, Saint Petersburg, Russia] (hereafter PS). PS is an image-based 3D modelling solution that can process arbitrary photographs taken in either controlled or uncontrolled conditions and that can reconstruct any visible object from at least two photographs (Verhoeven 2011; Agisoft LLC 2014a). PS implements both 'Structure-From-Motion' (SFM) and 'Dense Multi-View 3D Reconstruction' (DMVR) algorithms (Verhoeven 2011). The reconstruction of a 3D model comprises three main steps: camera alignment (building sparse point cloud) (Figure 2.3; 2.4a), building dense point cloud (Figure 2.4b) and building polygonal mesh (Figure 2.4c). The mesh can then be exported to external programs for further analyses (Figure 2.4d). The camera alignment step applies the 'bundle adjustment' method to search the feature points (i.e. key points) and match them between photographs (i.e. providing the tie points), find the external orientations (i.e. camera positions and orientations) and estimate the internal orientations (i.e. camera calibration parameters) (Figure 2.3; 2.4a) (Triggs et al. 2000; Szeliski 2010; Agisoft LLC 2014a). The second step applies DMVR algorithms on the aligned image set by operating on the pixel values (Scharstein and Szeliski 2002; Verhoeven 2011). The outcome is a dense point cloud (Figure 2.4b) that can then be transformed into a polygonal mesh (Figure 2.4c). Following the alignment step, PS estimates the 'camera error' or 're-projection error' in pixels that can be defined as the ‘root mean square re-projection error calculated over all feature points detected on the photograph’ (Agisoft LLC 2014a). The re-projection error is basically the distance between a projected point and the measured one (Gargallo et al. 2007). This error provides crucial information about the quality and accuracy of the alignment step (Verhoeven et al. 2012).

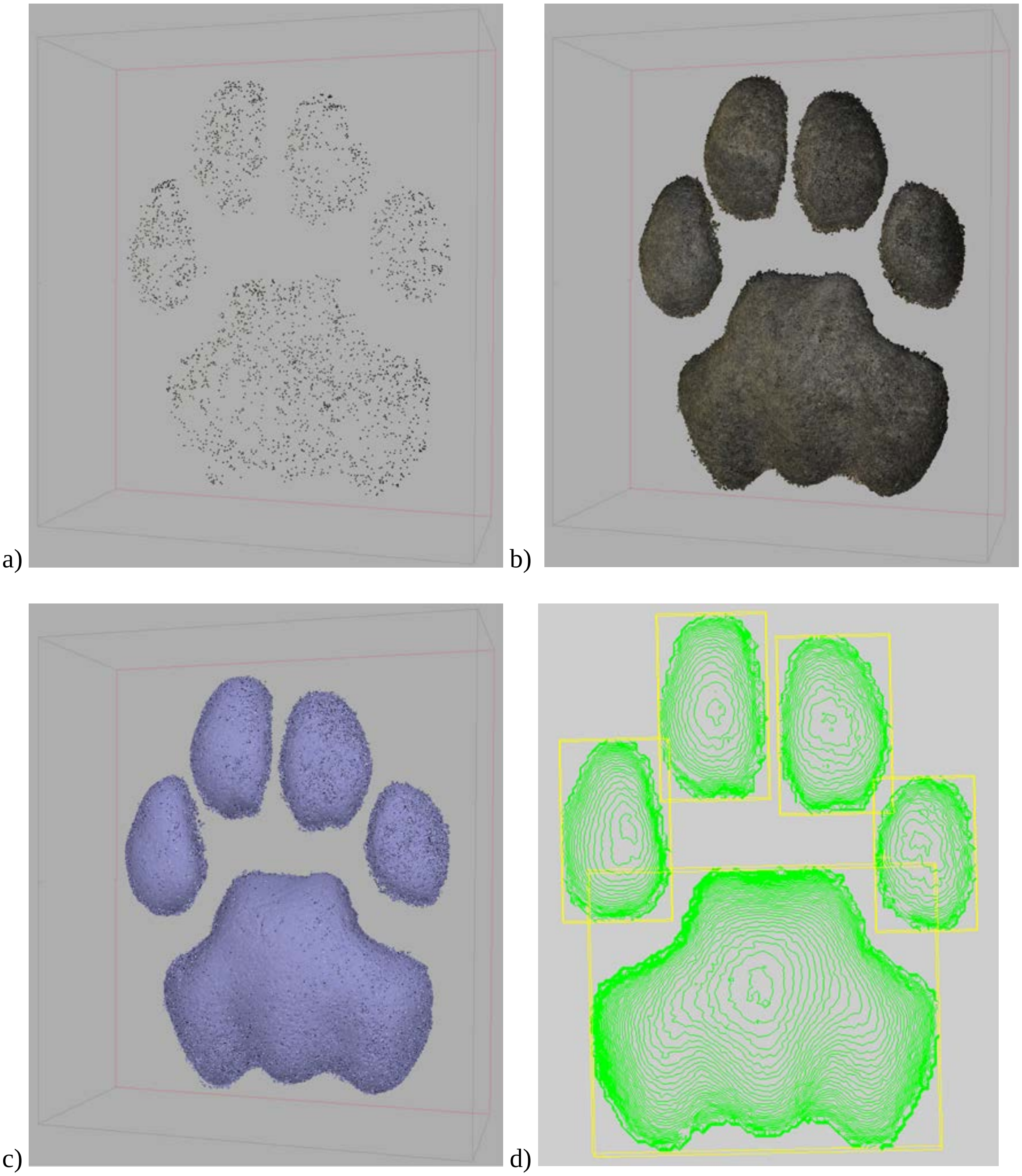


Figure 2. 4 - General workflow in PhotoScan and contour lines. (a) Sparse point cloud (2,812 points). (b) Dense point cloud (4,572,854 points). (c) Polygonal mesh (916,402 faces). (d) 0.5 mm contour lines with non-axis-orientated bounding boxes computed in CloudCompare.

Preferred reconstruction parameters

There are three reconstruction parameters that can influence the camera alignment and that are tested here: (i) masking, (ii) calibration and (iii) optimisation. For scenario (i), the pictures were either not masked (unmasked) or masked around everything except the main pad and toes (pad-masked). In scenario (ii), the cameras were either automatically calibrated by PS (auto-calibrated) or manually pre-calibrated (pre-calibrated) in external software. For the third scenario, either we did not apply an optimisation step (non-optimised) or we did (optimised). We selected three paw and three track datasets that contain between 11 to 12 photographs and 10 to 15 photographs respectively. These datasets were representative of our database and complete for the following testing procedures. We manually discarded any blurred photographs and those of lower quality (less than 0.5 units) by using the tool ‘estimate image quality’ in PS. We then aligned the photographs using the highest accuracy (i.e. using original size photographs) and the default settings (Table 2.1). We positioned two markers (with two projections per marker) by using the ‘guided marker placement approach’ - placing the marker projections on a single aligned photograph and the program automatically projects predictor rays onto the remaining photographs to reduce the chance of misplacing a marker. For each scaled 3D model originating from a specific dataset, we re-launched the alignment step three times for each possible combination of reconstruction parameters (i.e. eight combinations; e.g. combination 1: unmasked/auto-calibrated/non-optimised) and recorded the re-projection error.

Masking is a tool to exclude parts of the photographs, particularly the background, from the processing. The paws were semi-automatically masked in Photoshop Creative Cloud [Adobe, San Jose, California, USA]. After applying the options ‘sharpen edge’ and ‘auto-contrast’ to enhance the edges, we used the ‘quick selection tool’ with an automatic edge refinement of 10-pixel-radius and 50% contrast (see Adobe Photoshop Cloud Creative help file). The ‘quick selection tool’ was not successful for the tracks as the colours and texture were too uniform. Therefore, the tracks were manually masked in PS using the tool ‘intelligent scissors’. For the pre-calibration, we manually estimated the camera calibration parameters using the software Agisoft Lens version 0.4.1 beta build 2021 [Agisoft LLC, Saint Petersburg, Russia] that uses the computer screen as calibration target. The calibration parameters were then imported into PS and used for aligning the photographs through the unfixed calibration mode. Aligning the photographs using image data only (i.e. through the tie points) leads to non-linear deformations originating from calibration errors (Agisoft LLC 2014a). The optimisation step offers a refined bundle adjustment by adding ground control points to the calculations. We used the ‘scale bar based optimisation’ with the default settings (Table 2.1) by using the two markers as ground control points.

Table 2. 1 - Settings used in the camera alignment, optimisation, build dense cloud and build mesh step.

Align photos	
Accuracy	High
Pair pre-selection	Disabled
Key point limit	40,000
Tie point limit	1,000

Optimisation	
Camera accuracy (m)	10
Marker accuracy (m)	0.005
Scale bar accuracy (m)	0.001
Projection accuracy (pix)	0.1
Tie point accuracy (pix)	4

Build dense cloud	
Quality	Ultra high
Depth filtering	Moderate

Build mesh	
Surface type	Arbitrary
Source data	Dense cloud
Face count	High
Interpolation	Disabled

Direct versus digital measurements

To test for differences between direct measurements on the actual paws and tracks (i.e. length and width of the main pad and the four toes), and the same measurements on their digital 3D models, we reconstructed the 3D polygonal mesh of 20 paws and 20 tracks. The paw and track datasets contain between 12 and 14 photographs and between 10 and 15 photographs respectively. After discarding blurred and lower quality photographs, we launched the camera alignment step using the same settings as above, with two markers and two projections per marker, and using masking, auto-calibration and optimisation. Once the photographs aligned, we built the dense point cloud with the highest accuracy (i.e. using full photograph resolution) and moderate depth filtering (Table 2.1). Using the dense point cloud as a data source, we then built the mesh with the highest possible details (i.e. highest face count) for arbitrary surface type (i.e. non-topographic photogrammetry) and without automatic interpolation (i.e. only areas corresponding to dense point cloud are reconstructed) (Table 2.1). We cleaned the meshes by gradually selecting and removing all the patches that did not define the main pad or any of the toes. After automatically closing all the gaps in the meshes, we exported the five shapes. In CloudCompare, we used the tool ‘Principal Component Analysis (PCA) fit’ to create a bounding box that was not axis-orientated (Figure 2.4d) (CloudCompare 2015). This allowed us to automatically extract the lengths and widths of each shape.

To avoid the subjective manual masking of the tracks, we reconstructed the 3D meshes from the same track photographs with the same settings as above but unmasked. After using the tool ‘PCA fit’, we created the contour lines starting at the minimum height (i.e. bottom of the track) with a step of 0.5 mm (Figure 2.4d). For each shape, we selected the highest isolated (i.e. non-connected to another shape) contour line as the shape delineation. As with the pad-masked 3D models, the length and width were then automatically extracted.

For the pad-masked paws, pad-masked tracks and unmasked tracks, we calculated the mean geometric deviation as the difference between digital and direct measurements. We estimated a correction factor to adjust the digital measurements using the following equation:

$$\mathit{Direct} = \mathit{Digital} - \mathit{Digital} * \mathit{Correction\ Factor} \leftrightarrow \mathit{Correction\ Factor} = 1 - \mathit{Direct}/\mathit{Digital}.$$

Number of photographs required

To assess the minimum number of required photographs, we selected three paw and three track datasets that all contained more than 10 photographs. Same as for the reconstruction parameters, these datasets were representative of our database and complete for the following testing procedures. We reclassified each dataset into subsets with an increasing number of pad-masked photographs randomly selected with replacement. In each subset, two photographs were always the same as they were used to position the two markers and the scale bar. Thus cancelling the influence of subjective marker positioning on the final 3D models. The random selection of photographs was repeated three times per dataset and per category of number of photographs. We reconstructed, cleaned and measured the mesh of each subset using the same procedure described above. For each category of number of photographs ranging from five to ten, we calculated the predicted error as the percentage of the absolute difference between the corrected digital and direct measurement. The total volume was also recorded.

Data analysis

We processed all our statistical analyses with the program R (R Development Core Team 2014). We used a Mann-Whitney U test to analyse the difference between the mean re-projection errors for the paw and track datasets taken separately, while a Wilcoxon signed ranks test was used to study the effects of the reconstruction parameters on the same error value. We plotted both the direct versus digital and the direct versus corrected digital measurements for each case (pad-masked paws, pad-masked tracks and unmasked tracks) and we calculated the coefficient of correlation using a Spearman's rank-order correlation test. We plotted the mean predicted error with 95% confidence intervals against the number of photographs for the pad-masked paws and pad-masked tracks. Using a Mann-Whitney U test, we estimated the category of number of photographs in which the asymptote is reached (i.e. when the mean predicted error for that category is not significantly different from that of the category with 10 photographs). The probability values are considered statistically significant at $p \leq 0.05$.

RESULTS

Reconstruction parameters

There is a significant difference (Mann-Whitney U test, $p < 0.001$) between the mean re-projection error for the paws (1.03 ± 0.39 pix) and for the tracks (0.47 ± 0.09 pix). Paws and tracks were therefore considered independently for testing the effects of masking, calibration and optimisation on the 3D model quality. Masking has a significant influence (Wilcoxon signed ranks test, $p < 0.001$) on the alignment of paw photographs. Mean re-projection error is lower for pad-masked (0.68 ± 0.13 pix) than for unmasked (1.39 ± 0.18 pix) paw photographs. The same influence (Wilcoxon signed ranks test, $p < 0.001$) is observed for the alignment of track photographs, with a mean re-projection error that is again lower for pad-masked (0.42 ± 0.09 pix) than for unmasked (0.53 ± 0.02 pix) photographs. Calibration does not have a significant influence on the alignment of paw photographs (Wilcoxon signed ranks test, $p = 0.822$). However, it has a significant influence (Wilcoxon signed ranks test, $p < 0.01$) on the alignment of track photographs, with a mean re-projection error that is lower for auto-calibrated (0.46 ± 0.07 pix) than for pre-calibrated (0.49 ± 0.10 pix) photographs. Optimisation has a significant influence (Wilcoxon signed ranks test, $p < 0.001$) on the alignment of both paw and track photographs. The mean re-projection error of the optimised alignment for paw photographs is lower (1.03 ± 0.39 pix) than in the non-optimised case (1.04 ± 0.39 pix). Similarly, lower re-projection error was observed when optimisation was applied (0.47 ± 0.07 pix) than when it was not (0.48 ± 0.10 pix) for the track photographs.

Direct versus digital measurements

The 3D models of both pad-masked paws and tracks present a positive geometric deviation of 0.23 ± 0.18 cm and 0.50 ± 0.33 cm respectively (Table 2.2; Figure 2.5a; 2.5b), while a negative geometric deviation of -0.06 ± 0.39 cm (Table 2.2; Figure 2.5c) is observed for the unmasked tracks. The calculated correction factor is 0.06 ± 0.05 for pad-masked paws, 0.11 ± 0.07 for pad-masked tracks and -0.01 ± 0.12 for unmasked tracks (Table 2.2). These factors may be used in predictive equations to adjust the overestimation in the case of the pad-masked paws and tracks, and the underestimation in the case of the unmasked tracks (Table 2.2). The use of the appropriate correction factor reduces the geometric deviation to -0.03 ± 0.20 cm for pad-masked paws, -0.04 ± 0.35 cm for pad-masked tracks and -0.01 ± 0.39 cm for unmasked tracks (Table 2.2; Figure 2.5). The coefficient of correlation, Spearman's r, is 0.98 for pad-masked paws, 0.96 for pad-masked tracks and 0.93 for unmasked tracks (Table 2.2; Figure 2.5).

Table 2. 2 - Geometric deviation (non-corrected and corrected) and predictive equations to approximate the length and width of the main pad and toes for the pad-masked paw, pad-masked track and unmasked track models. r values are the resultant linear regression fit of direct to digital measurements calculated with Spearman's rank-order correlation test. 'Dr' stands for 'direct measurement' and 'Dg' for 'digital measurements'.

Model	**Geometric deviation (cm)**		**Equation**	***N***	***r***
	Non corrected	Corrected			
Pad-masked paws	0.23 ± 0.18	-0.03 ± 0.20	Dr = Dg - Dg * (0.06 ± 0.05)	200	0.98
Pad-masked tracks	0.50 ± 0.33	-0.04 ± 0.35	Dr = Dg - Dg * (0.11 ± 0.07)	200	0.96
Unmasked tracks	-0.06 ± 0.39	-0.01 ± 0.39	Dr = Dg - Dg * (-0.01 ± 0.12)	194	0.93

Number of photographs

For both the pad-masked paws and tracks, the mean predicted error decreases with an increasing number of photographs used to reconstruct the 3D models (Figure 2.6). For the paws, an asymptote is reached between six and seven photographs, as the predicted error for seven photographs (5.15 ± 4.05%) is not significantly different from that of 10 photographs (4.22 ± 3.75%) (Mann-Whitney U test, p = 0.09) (Figure 2.6a). The asymptote is reached for the tracks between seven and eight photographs (Mann-Whitney U test, p = 0.06), with a predicted error of 6.00 ± 3.37% for eight photographs and 5.07 ± 3.20% for 10 photographs (Figure 2.6b). Other than observing an increasing predicted error when decreasing the amount of photographs, the 3D model volume also shrinks with a decreasing number of photographs. The mean volume for five photographs represents 67.78 ± 5.91% and 84.89 ± 9.31% of the mean volume for 10 photographs for the paws and tracks.

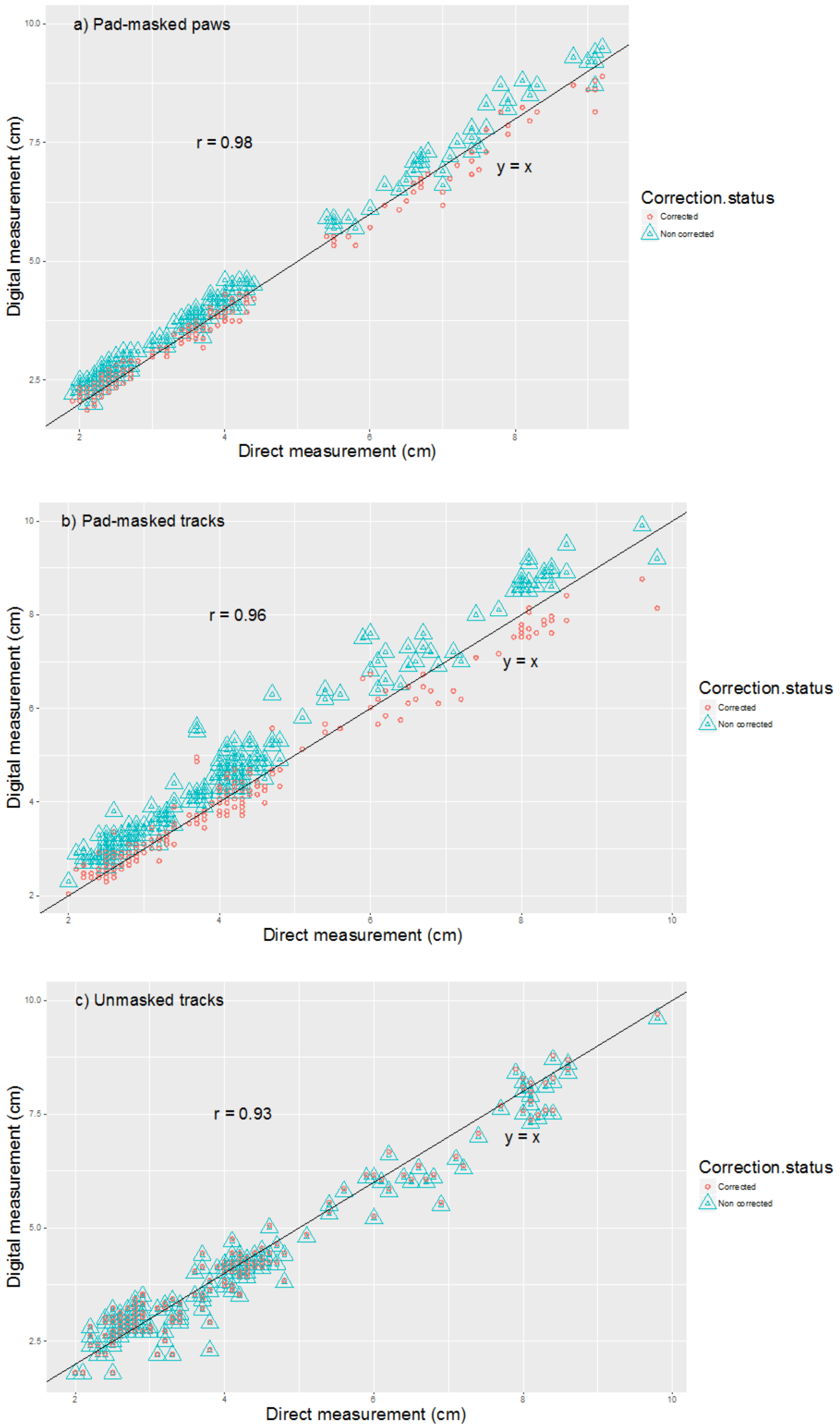


Figure 2. 5 - Regression of direct versus digital measurements (corrected and non corrected) for (a) pad-masked paws, (b) pad-masked tracks and (c) unmasked tracks. The line represents the true regression line (intercept = 0, slope = 1) and r is the coefficient of correlation using the Spearman's rank-order correlation test.

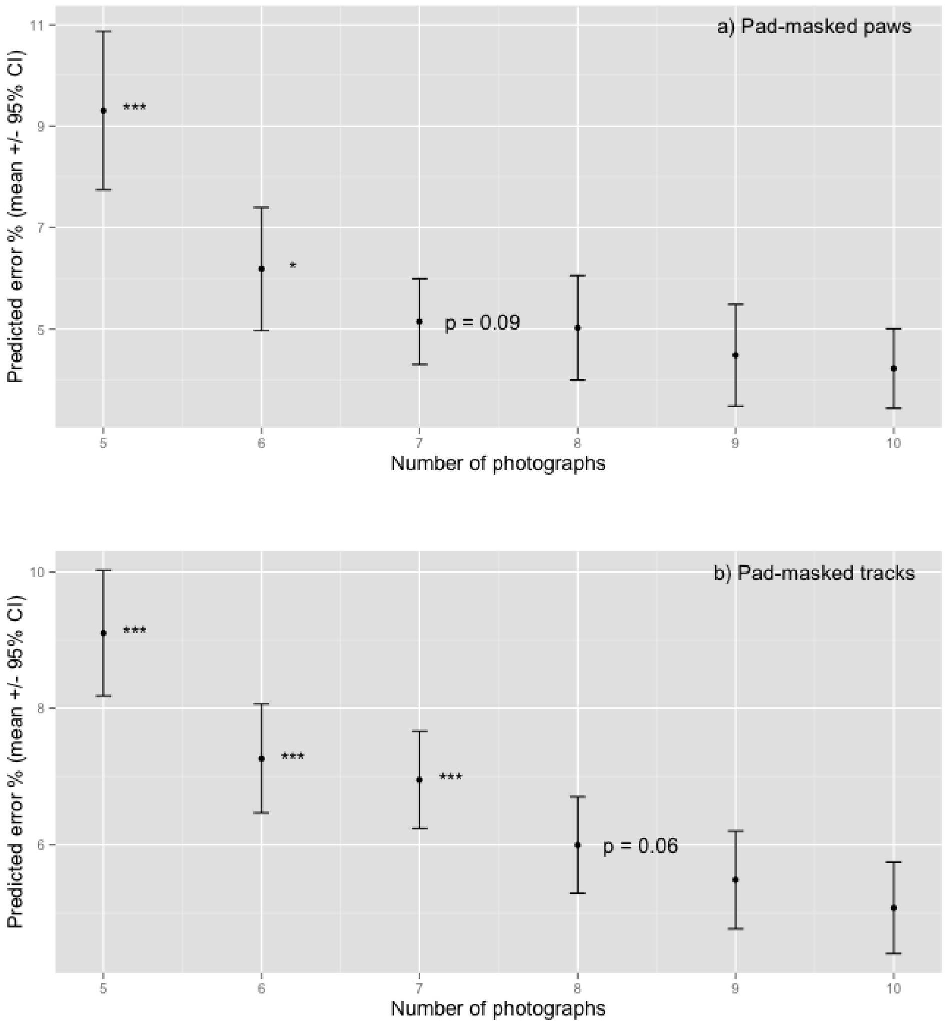


Figure 2. 6 - Mean predicted error (%) and 95% confidence interval (CI) for each category of number of (a) paw and (b) track photographs. The predicted error is the percentage of the absolute difference between the corrected digital and the direct measurement. An asymptote is reached between 6 and 7 paw photographs, and between 7 and 8 track photographs as the mean predicted error (%) for 7 paw photographs and 8 track photographs is not significantly different from that for 10 photographs.

Sampling and processing time considerations

The image acquisition (i.e. photography) of either the paws or tracks took less than 1 minute per object. The manual masking in PS took on average 1.50 minutes per photograph (range: 1.15 to 2.14 minutes) and the semi-automatic masking in Photoshop took on average 3.4 minutes (range: 2.42 to 4.20 minutes). For the processing of datasets containing 10 pad-masked photographs, two paw and one track datasets were computed with a laptop Mac Book Pro OSX Yosemite 2.8 GHz Intel Core i7 8GB memory (hereafter MAC), and one paw and two track datasets were computed with a desktop computer Windows 7 Enterprise 3.60 GHz Intel Core i7 16GB memory (hereafter PC). The MAC mean total processing time for paws (tracks) was 53.03 ± 22.86 minutes (5.82 ± 0.32 minutes) with the following breakdown in percentage for the three steps: 2% (5%) photograph alignment, 55% (79%) dense cloud building and 43% (16%) mesh building. Processing with the PC reduced the mean total processing time for paws (tracks) to 11.59 ± 0.94 minutes (1.70 ± 0.30 minutes) with the following breakdown in percentage for the three steps: 3% (8%) photograph alignment, 58% (77%) dense cloud building and 39% (16%) mesh building. Using the same datasets but with only five photographs, the total processing time for paws (tracks) becomes 22.12 ± 1.27 minutes (2.68 ± 0.38 minutes) using the MAC and 9.33 ± 3.98 minutes (0.58 ± 0.11 minutes) using the PC. Five track datasets containing 12 photographs each were processed with the PC in both unmasked and pad-masked condition. The mean total processing time was 46.32 ± 2.86 minutes for unmasked photographs and 2.25 ± 0.64 minutes for pad-masked photographs.

DISCUSSION

In ichnology (i.e. science studying the interaction between organism and substrate), dinosaur tracks have previously been sampled using photogrammetry (Petti et al. 2008; Remondino et al. 2010). To our knowledge, this study represents the first application of close-range photogrammetry to record paws and tracks of extant animals in 3D. This innovative field technique provides an objective and reliable solution to obtain digital 3D models of both paws and tracks. The image acquisition time, less than a minute per paw or track, is ideal for minimising the interaction with immobilized individuals and for working with potentially dangerous species. Furthermore, the necessary equipment for the field data collection is essentially limited to a digital camera and a ruler.

The reconstruction parameters have a significant impact on the alignment step and therefore on the quality of the final 3D models. The comparison of the mean re-projection errors between the different possible combinations showed that masking, auto-calibration and optimisation yielded more accurate 3D reconstruction of both paws and tracks. Other than decreasing the processing time, another advantage of masking is the delineation of the object of interest (paw or track). However, it is important to use a delineation process that is not affected by the manipulator’s subjectivity. This was successfully achieved for the paws by means of a semi-automatic masking tool in Photoshop. The tool could easily pick up the interface between the pads and the hair due to a high contrast in colour and texture. This clear contrast is not present in the track photographs and the masking tool showed limited success with the delineation between an imprint and the surface that enables its existence. While subjective manual masking of tracks led to a significant overestimation of the digital measurement (Table 2.2; Figure 2.5b), the semi-automatic segmentation using unmasked tracks and contour lines led to the lowest geometric deviation (Table 2.2; Figure 2.5c). Unmasked tracks present a higher mean re-projection error compared to pad-masked tracks, however this error remains less than that of pad-masked paws (see section Reconstruction parameters). In the case of the pad-masked paws, we believe that the overestimation of the digital measurement might in fact be due to an underestimation of the direct measurement. Since the pads are made of a thick elastic mass of connective tissue (Gutteridge and Liebenberg 2013), the manipulator tends to compress the calliper on the pads leading to an underestimated measurement. The use of specified correction factors for the measurement estimation from the digital 3D models reduces the geometric deviation by two decimals of a centimetre (Table 2.2). The accuracy advertised by Agisoft for close-range photogrammetry with PS is 0.1 cm (Agisoft LLC 2014b). For both paw and track 3D models, we showed that the predicted error increases and the total volume decreases, as the number of photographs used in the reconstruction decreases (Figure 2.6). The suggested minimum of seven and eight photographs for paws and tracks respectively, represents a theoretical minimum number of photographs to process the 3D models. From our experience, approximately 7% of the photographs were discarded due to poor quality. Furthermore, it is not only the quantity of photographs that matters but also their position in the 3D space, as they must overlap without any blind spots (Figure 2.3). Since more photographs make better models and to avoid a lack of two-dimensions (2D) information, we advise capturing twice as many photographs than the theoretical minimum (i.e. between 14 and 16 photographs). We further recommend masking the paws but not the tracks, and using both the auto-calibration and optimisation functions.

Previous studies using 2D have shown high accuracy (>90%) for objective individual identification from tracks made by black and white rhinoceroses (Jewell et al. 2001; Alibhai et al. 2008), mountain lions (Smallwood and Fitzhugh 1993; Grigione et al. 1999; Jewell et al. 2014) and tigers (Sharma et al. 2005). Felid tracks were mainly sampled on dusty roads (i.e. producing shallow tracks) as other substrates, such as sand, generated greater variability of the track contour. Unfortunately, optimal dusty roads are not present everywhere. This is particularly the case in our study sites as TEP largely comprises sandy roads while HiP’s unpaved roads are often too hard. The above-mentioned studies of wild felids sampled a limited number of individuals (from 3 to 17 individuals). Additionally, the identification accuracy was dependent on the number of tracks sampled per individual. Sharma et al. (2005) suggested a minimum of ten tracks per individual. Recording techniques in 2D are affected by the manipulator posture (Smallwood and Fitzhugh 1993) and experience (Karanth et al. 2003) during tracing, while photographs are affected by the time of the day and cloud cover (Grigione et al. 1999). Furthermore, photographs that are not aligned directly over the object can introduce a parallax error. In the same way that 3D has improved facial recognition methods (Chang et al. 2003), we are confident that it will enable a more rigorous, objective and repeatable use of tracks in future studies. By providing more information, 3D replicas of tracks should enable the correct identification of more individuals on a greater variety of substrates with fewer tracks required per individual. Analysing the intrinsic properties of the paws will lead to a better understanding of the tracks they produce. The nature of the sampling technique, which requires several photographs taken from different distances and angles, is expected to be less affected by manipulator bias. This paper shows that working with digital 3D models ought to improve the track segmentation and feature extraction by decreasing the human input. Given the results of our innovative technological approach, we are currently working on improving the technique (e.g. understanding manipulator bias and using different types of cameras) and applying it to identify individual lions from their paws and tracks. Identifying individuals from their tracks would have major implications in behavioural ecology, conservation biology and wildlife management. Tracking is less invasive than camera trapping (e.g. flash avoidance and presence of a foreign object with human scent), requires less investment and logistics while not being prone to hardware failure and theft.

Chapter 3 - 3D trails: investigating lion gaits from tracks using digital photogrammetry

Figure 3. 1 - Lion trail on a sandy road in Tembe Elephant Park, South Africa.

ABSTRACT

In the absence of sightings, elusive species can be studied through their tracks. Previous studies have mainly focused on separate tracks without looking at their position within a trail (i.e. continuous sequence of tracks made by the same individual). Trails are morphologic and kinematic signatures as they reflect animal gaits. We aimed to determine whether digital photogrammetry can be used as a rapid, practical and reliable field technique for sampling lion (*Panthera leo*) trails, and whether this digital solution improves the feature extraction and discrimination between sexes and individuals from trail variables. Lion trails were sampled in Hluhluwe-iMfolozi Park and Tembe Elephant Park (South Africa) from June to December 2015. First, we compared direct measurements on trails versus the same measurements on their digital 3D models. Then, we analysed the differences in pace, stride and straddle between sexes from overstepping adults. Finally, we compared the sex and individual identification accuracy from Linear Discriminant Analysis (LDA), without and with jack-knifed prediction, when using either the information from pace, stride and straddle, or from 40 variables, including distances, angles and areas, extracted from the 3D trails. The mean geometric deviation between direct and digital measurements was 0.06 ± 2.38 cm. A significantly larger stride and straddle was observed for adult males compared to adult females. Using the LDA without jack-knife, the sex identification reached 100% in both cases, and improved from 87.1% to 100% when using more information than only pace, stride and straddle for individual identification. Using more information also improved the accuracy of prediction with jack-knife for sex identification from 96.9% to 100% and for the individual identification from 77.4% to 90.32%. Photogrammetry provides a digital solution that enables better feature extraction and discrimination between sexes and individuals from trails. Trail variables could be integrated to track measurements to refine current methods aimed at identifying species, age, sex and individual from tracks. Furthermore, trails may provide information such as behaviour, speed of locomotion and body condition, not available from single tracks.

Keywords: Digital 3D model, footprint, gait, lion, *Panthera leo*, photogrammetry, track, trail.

INTRODUCTION

Human activities may well be the cause of a sixth biodiversity mass extinction (Barnosky et al. 2011). Habitat destruction, a main factor in species extinction, leads to the confinement of many species to fragmented areas (Pimm and Raven 2000). Assessing the distribution and population status of threatened species, over space and time, is vital for reaching conservation, research and management objectives (Yoccoz et al. 2001; Witmer 2005). Large carnivores are increasingly identified as major role-players in ecosystem functions and biodiversity conservation (Chapron et al. 2014; Ripple et al. 2014). However, monitoring carnivores represents a challenge due to their elusive nature (Gese 2001; Wilson and Delahay 2001). In the absence of sightings, the targeted species can be studied through their characteristic signs, such as tracks and scats, that they leave behind (Gese 2001; Wilson and Delahay 2001; Heinemeyer et al. 2008).

Track variables have been used for identifying the species, age, sex and/or individual for larger felids such as cheetah (*Acinonyx jubatus*) (Jewell et al. 2016), jaguar (*Panthera onca*) (De Angelo et al. 2010), leopard (*P. pardus*) (Stander et al. 1997; Gusset and Burgener 2005), lion (*P. leo*) (Stander et al. 1997), tiger (*P. tigris*) (Choudhury 1970; Gore et al. 1993; Riordan 1998; Sharma et al. 2003; Sharma et al. 2005), snow leopard (*P. uncia*) (Riordan 1998) and mountain lion (*Puma concolor*) (Smallwood and Fitzhugh 1993; Grigione et al. 1999; De Angelo et al. 2010; Jewell et al. 2014). In few cases, direct linear measurements were used to record the relative positioning between the different tracks from continuous sequences made by the same individuals (i.e. trails). Sagar and Singh (1990) developed a technique to discriminate between adult leopards and tiger cubs through stride measurements. Stander et al. (1997) aimed to quantify the differences between leopards and lions when stalking, walking, trotting and galloping. Sharma et al. (2005) used trail variables along with track variables to identify individual tigers. In these studies, the measurements were recorded with a tape measure and were limited to 1 variable (stride) (Sagar and Singh 1990), 2 variables (stride and straddle) (Sharma et al. 2005) or 3 variables (pace, stride and straddle) (Stander et al. 1997). A pace is the “distance of the step between the leading front foot and trailing back foot on either left or right sides” (i.e. distance between the tracks from the same track set) (Stander et al. 1997:331) and a stride is the “distance between 2 impressions made by the same foot” (Stander et al. 1997:331) (Figure 3.2). The straddle is the perpendicular distance between the tracks on opposing sides (Sharma et al. 2005).

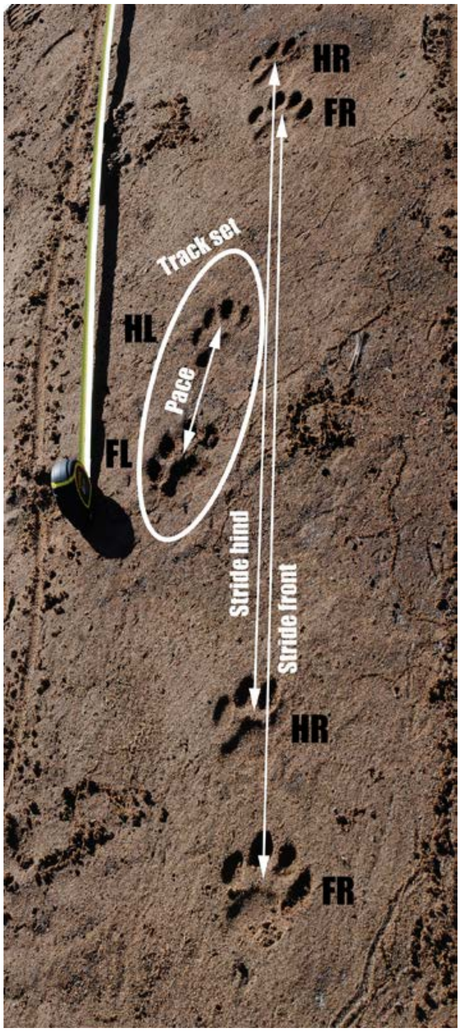


Figure 3. 2 - Picture of a partial trail made by an overstepping (i.e. hind foot touches down beyond front track) lion sampled in Hluhluwe-iMfolozi Park (South Africa) between June and December 2015. This trail portion contains 3 track sets (i.e. 6 tracks). The pace is the distance between 2 tracks from the same track set and the stride is the distance between 2 consecutive tracks made by the same foot. "H" = hind, "F" = front, "R" = right and "L" = left. Note the measuring tape that provides a scale.

A trail reflects the gait of an animal that is the repetitive and regular manner of foot movement describing the pattern of locomotion (Hildebrand 1989; Liebenberg et al. 2010). Different gaits are related to speed, behaviour, anatomy and morphology (Liebenberg et al. 2010). For humans, the gait is used as a novel biometric trait to identify individuals (Cunado et al. 2003; Juefei-Xu et al. 2012). As trails reflect gaits, they can therefore be considered as morphologic and kinematic signatures (Meldrum et al. 2011). Tracks enable scientists to study the gait of individuals that are no longer visible or even extinct (e.g. dinosaurs and earliest hominids) (Day et al. 2002; Meldrum et al. 2011).

Beyond the outdated, unpractical and time-consuming tape measure, three-dimensional (3D) technologies such as laser scanning and photogrammetry are used to record tracks and trails made by dinosaurs (Petti et al. 2008; Remondino et al. 2010; Falkingham 2012; Falkingham et al. 2014; Razzolini et al. 2014) and earliest hominids (Meldrum et al. 2011). Photogrammetry or the "science of measuring in photographs" enables the digital 3D modelling of a surface using photographs taken by a consumer digital camera (Linder 2009; Marchal et al. 2016; Chapter 2). This provides a practical alternative to cumbersome and expensive laser scanners (Falkingham 2012; Bennett et al. 2013). In this article, we aimed to determine whether photogrammetry can be used as a rapid, practical and reliable field technique for sampling lion trails. We assessed whether this digital solution improves the feature extraction and discrimination between sexes and individuals from trail variables, as compared to classical measuring techniques.

STUDY AREA

The study sites were Hluhluwe-iMfolozi Park (HiP) (~900 km^2) and Tembe Elephant Park (TEP) (~300 km^2) that are located in the sub-tropical province of KwaZulu-Natal (KZN), eastern South Africa. The two parks were fenced and managed by a provincial conservation agency, Ezemvelo KZN Wildlife. HiP had a mean annual rainfall of 650 mm to 985 mm. Three main rivers (Hluhluwe, Black iMfolozi and White iMfolozi) traverse the hilly topography that ranges from 40 m to 560 m above mean sea level (amsl). TEP was characterised by a mean annual rainfall of 700 mm. Sandy plains and ancient littoral dunes, with an altitude ranging from 50 m to 129 m amsl, cover the park. The predominant vegetation types are Northern Zululand Sourveld, Zululand Lowveld and Scarp Forest in HiP, and Tembe Sandy Bushveld and Sand Forest in TEP (Mucina and Rutherford 2006). The dry riverbeds in HiP and sandy roads in TEP provided optimal substrate for sampling trails. In 2015, lion populations were estimated at ~120 individuals in HiP (D. Druce, HiP's ecologist, unpublished data) and ~40 individuals in TEP (C. Hanekom, TEP's ecologist, unpublished data).

METHODS

Trail sampling

A total of 26 lion trails containing between 4 and 33 track sets (Table 3.1) were sampled between June and December 2015. A track set includes the 2 tracks made by front and hind feet from the same side and within the same walking cycle (Figure 3.2). The tracks were less than 24 hours old and were sampled on flat terrain following a direct observation, in front of a camera trap (Cuddeback, Green

Bay, Wisconsin, USA) or after species identification using a book on animal tracks such as Liebenberg (1990b) or Gutteridge and Liebenberg (2013). The age of the tracks was estimated from the time of observation during sightings or from the time of capture by the camera traps. The age, sex and/or individual identity were recorded whenever possible during direct observations or on the pictures taken by the camera traps.

Table 3. 1 - Details of the 26 lion trails sampled in Hluhluwe-iMfolozi Park and Tembe Elephant Park (South Africa) from June to December 2015. A track set includes the 2 tracks made by front and hind feet from the same side and within the same walking cycle. The flight pattern describes the sampling protocol: either the manipulator walked 4 times along the trail while taking overlapping photographs (i.e. full), 3 times (i.e. 3 flights) or 2 times (i.e. 2 flights). Overstep means that the hind foot touches down beyond front track, register means that the hind foot touches down directly on top of front track and mix is a combination of both.

Trail number	Gait	Sex	Age	Identification	Number track sets	Number pictures	Flight pattern
1	Overstep	Male	Adult	Qaqeni Pride	4	27	Full
2[a]	Overstep	Male	Adult	Sontuli Pride	7	30	2 flights
3	Overstep	Male	Adult	HiP310	17	113	2 flights
4	Overstep	Male	Adult	HiP310	5	37	2 flights
5[a]	Overstep	Male	Adult	HiP626	6	47	Full
6	Overstep	Male	Adult	HiP626	7	46	Full
7	Overstep	Female	Adult	HiP618	14	95	Full
8	Overstep	Female	Adult	HiP618	9	70	Full
9[a]	Overstep	Unknown	Juvenile	Bridge Pride 1	5	32	Full
10[a]	Overstep	Unknown	Juvenile	Bridge Pride 2	7	44	Full
11[a]	Overstep	Unknown	Juvenile	Bridge Pride 3	5	24	3 flights
12[a]	Overstep	Unknown	Unknown	Unknown	29	186	2 flights
13[a]	Overstep	Unknown	Unknown	Unknown	33	183	Full
14[a]	Overstep	Unknown	Unknown	Unknown	12	89	Full
15[a]	Overstep	Unknown	Unknown	Unknown	12	83	Full
16[a]	Overstep	Unknown	Unknown	Unknown	10	83	Full
17[a]	Overstep	Unknown	Unknown	Unknown	5	34	Full
18[a]	Overstep	Unknown	Unknown	Unknown	4	27	Full
19[a]	Overstep	Unknown	Unknown	Unknown	4	22	Full
20[a]	Overstep	Unknown	Unknown	Unknown	9	76	Full
21[a]	Mix	Female	Adult	HiP404	16	90	Full
22[a]	Mix	Unknown	Unknown	Unknown	8	30	2 flights
23[a]	Register	Female	Adult	HiP509	10	57	3 flights
24[a]	Register	Male	Sub-adult	Hluhluwe Pride 1	10	56	Full
25[a]	Register	Male	Sub-adult	Hluhluwe Pride 2	11	62	Full
26	Overstep	Female	Adult	Tembe Pride	13	61	3 flights

[a] Trail for which we applied direct measurements using a measuring tape.

The trails were grouped into 3 categories (Table 3.1): 1) 21 trails originating from an overstep walk (i.e. hind foot touches down beyond front track) (hereafter overstep) (Figure 3.2), 2) 3 trails from a direct register (i.e. hind foot touches down directly on top of front track) (hereafter register), and 3) 2 trails from a combination of both (hereafter mix). Direct registration indicates a normal walk, while overstepping reveals a fast walk (Liebenberg et al. 2010). These categories represent a symmetrical gait in which the footfalls of a pair of feet (either front or hind feet) are regularly spaced in time (Peters 1983; Hildebrand 1989).

Five trails (3 oversteps, 1 register and 1 mix) belonged to 4 different adult females (N=4), 6 trails (all overstep) to 4 different adult males (N=4), 2 trails (both register) to 2 different sub-adult males (N=2) and 3 trails (all overstep) to 3 juveniles of unknown sex (N=3) (Table 3.1). The remaining 10 trails (all overstep except 1 mix) were from unknown individuals of unknown age and sex (Table 3.1). The known trails originating from a total or partial overstep walk were separated for analysis as follows (Table 3.1): 10 trails for the sex (4 females and 6 males) and 9 trails for the individual identification. The 9 trails belong to 6 different individuals (N=6) (3 adult females and 3 adult males).

We photographed all the trails to create digital 3D models using photogrammetry, 20 of which were also measured in situ with a tape measure (Table 3.1). The same manipulator (AFJM) took the photographs and measurements. Photographs were taken with a digital single-lens reflex camera Nikon D80 (10 megapixels) with a Nikkor 50 mm f/1.8 lens (Nikon Corporation, Tokyo, Japan). The sampling protocol closely followed the guidelines from aerial photogrammetry as the manipulator took overlapping photographs while walking the length of the trail (i.e. flight) in opposite directions along each side of the trail (Figure 3.3) (Agisoft LLC 2014a). Two clusters of 3 photographs were also captured on each extremity of the trail (Figure 3.3). Eighteen trails were sampled according to this protocol (i.e. 4 flights) while 8 trails were sampled using a partial sampling pattern (e.g. 2 or 3 flights) due to time constraints (Table 3.1). The reference point on a track from which measurements were taken, either directly or on digital 3D model, was located between the main pad and the 4 toes (Figure 3.2). Direct measurements included: 1) the pace, 2) stride made by the front feet (hereafter stride front), and 3) stride made by the hind feet (hereafter stride hind) (Figure 3.2).

Digital 3D modelling

The 3D reconstruction was processed with the photogrammetric package Agisoft PhotoScan Professional Edition version 1.1.4 build 2021 (Agisoft LLC, Saint Petersburg, Russia). PhotoScan is an image-based 3D modelling solution that implements both “Structure-From-Motion” (SFM) and “Dense Multi-View 3D Reconstruction” (DMVR) algorithms (Verhoeven 2011; Agisoft LLC 2014a). The reconstruction of a textured 3D model consists of 4 main steps: 1) building sparse point cloud (i.e. camera alignment), 2) building dense point cloud, 3) building polygonal mesh, and 4) building texture (Figure 3.3).

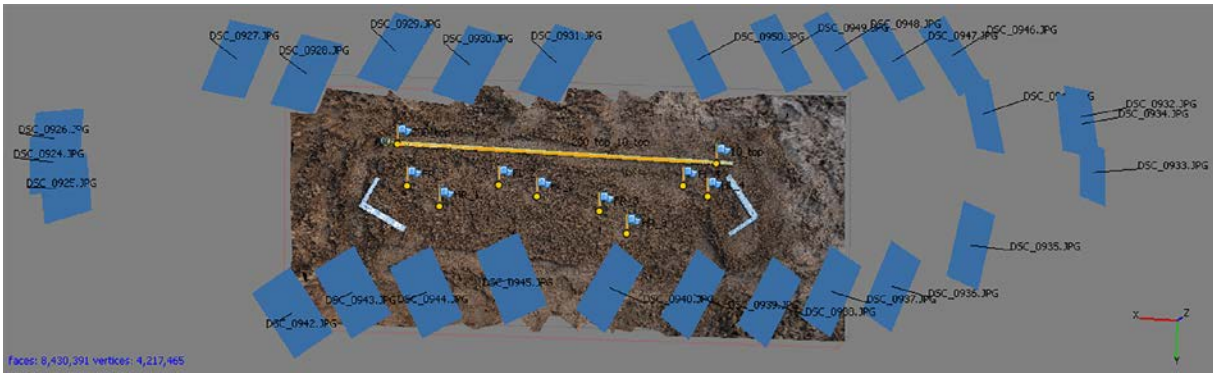


Figure 3. 3 - Textured 3D model of a lion trail reconstructed from 27 photographs. The frames represent the position and orientation of each camera station (i.e. where the photograph was taken). Note the specific flight pattern: 2 opposite flights on each side and 2 clusters of 3 photographs at each extremity of the trail. The flags represent markers positioned on the scale bar (i.e. measuring tape) and on the reference point of each track. The square rulers are used to visualise the start and the end of the sampled trail. We sampled lion trails in Hluhluwe-iMfolozi Park and Tembe Elephant Park (South Africa) from June to December 2015.

Our trail data sets contain between 22 and 186 photographs (Table 3.1). We aligned the photographs, optimised the sparse point clouds, and built the dense point cloud, mesh and texture using the settings described in Appendix A. We referenced the digital 3D models by positioning 2 projections of 3 markers located at the extremities of the scale bar and on an arbitrarily selected track.

Feature extraction

Each trail was divided into trail units. A trail unit consisted of 8 tracks belonging to 2 consecutive right track sets (Front Right_i-Hind Right_i and Front Right_{i+1}-Hind Right_{i+1}) and 2 consecutive left track sets (Front Left_i-Hind Left_i and Front Left_{i+1}-Hind Left_{i+1}) (Figure 3.4a). A marker was positioned on the reference point of each track (Figure 3.2; 3.3). From these markers we extracted a total of 40 variables per trail unit, which comprised 28 lengths (Figure 3.4b; 3.4c), 6 angles (Figure 3.4d) and 6 areas (Figure 3.4e). Figure 3.4c illustrates the straddle front and straddle hind that can be defined as the perpendicular distance between the front left and front right tracks, and the hind left and hind right tracks, respectively. A full description of the 40 variables is available in Appendix B.

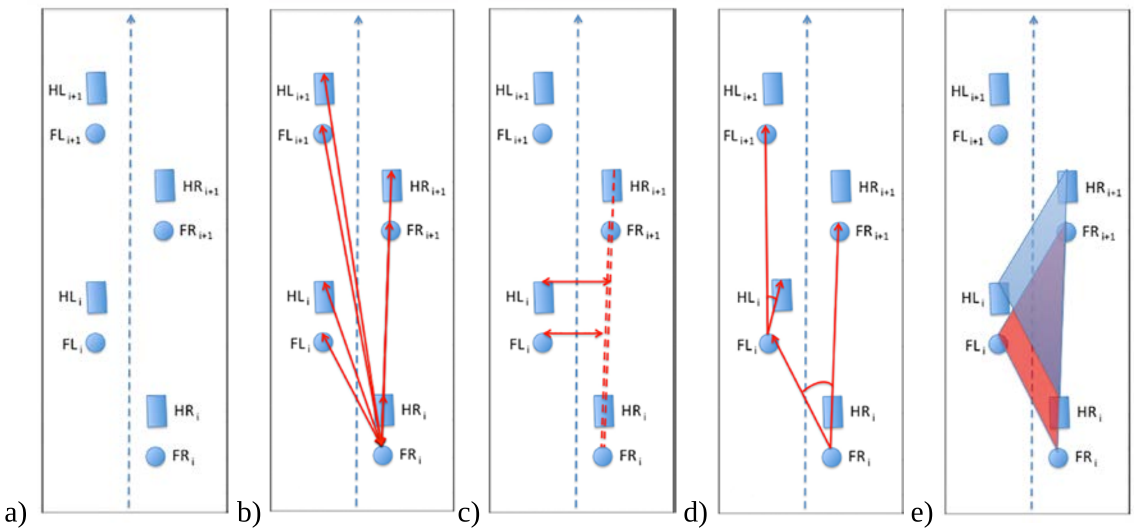


Figure 3. 4 - (a) Trail unit and examples of (b and c) lengths, (d) angles and (e) areas extracted from it. The pace (FR_i-HR_i) and the stride (FR_i-FR_{i+1}) are illustrated in (b), while the straddle front and straddle hind are illustrated in (c). 'H' = hind, 'F' = front, 'R' = right and 'L' = left. A full description of the 40 variables extracted from a trail unit is available in Appendix B.

Data analysis

We processed our statistical analyses in the program R (R Development Core Team 2014) and the probability values were considered statistically significant at $P \leq 0.05$. We plotted direct versus digital measurements (i.e. measurements extracted from the digital 3D models of trails) and used a Spearman's rank-order correlation test to analyse the correlation between the 2 variables. A Kruskal–Wallis test assessed the influence of the type of measurements and flight pattern on the mean geometric deviation. To describe the variation of the pace, stride and straddle as the speed increases, we selected the trail of an adult female switching from register to overstep (Table 3.1, trail number 21). We plotted the gait measurements for each of the 14 steps. We used a Mann–Whitney test to analyse the difference between mean pace, stride and straddle for overstepping adult females and adult males. We processed the 10 variables linked to pace, stride and straddle (i.e. pace left and right, stride front left and right, stride hind left and right, straddle front left and right, and straddle hind left and right; Appendix B) and the 40 variables (including 10 variables linked to pace, stride and straddle, with additional distances, angles and areas; Appendix B) extracted from the 3D trails made by overstepping individuals through independent Principal Component Analysis (PCA). For each group, we subjected an increasing number of principal components, up to a cumulative variance of >90%, to Linear Discriminant Analysis (LDA) without and with jack-knifed prediction. We compared the accuracy of prediction for the sex and individual identification for the trails for which these classifiers were known (Table 3.1).

The LDA is a supervised classification method based on the fact that the identity of each individual is known (i.e. we know the group to which each individual belongs). This discriminant analysis investigates how the explanatory variables contribute to the correct classification of the individuals to their group. Once the model is computed using all the observations, the predicted groups may then be matched to known groups in order to calculate the accuracy of the prediction. In this case, the LDA uses the same observations for the adjustment of the allocation model and the estimation of the error rate know as the resubstitution error. The consequence is that the resubstitution error is biased downward. To overcome this limitation, the LDA with jack-knife predictions creates an allocation model using all the observations with the exception of one. This observation is then used to estimate the predicted group. A loop analysis applies this procedure to each observation and the sum of all the individual errors on the jack-knife adjusted models provides the resubstitution error of the LDA. The LDA with jack-knife predictions therefore provides a more realistic estimation for the accuracy of the prediction using new observations.

RESULTS

Direct versus digital measurements

Except for 3 outliers that are most likely linked to direct measurement errors, the regression between direct and digital measurements was true (Fig. 4). The Spearman's rank-order correlation indicated significant correlation ($S = 63271$, $P < 0.01$) with a coefficient of correlation (r) equal to 0.99 (Fig. 4). The mean geometric deviation was 0.06 ± 2.38 cm, with the following breakdown per component: -0.02 ± 1.16 cm (pace), 0.01 ± 2.55 cm (stride front) and 0.19 ± 3.04 cm (stride hind). There was no difference in terms of mean geometric deviation between the type of measurements (Kruskal–Wallis test: $\chi^2 = 0.39$, df = 2, $P = 0.82$) and flight pattern (Kruskal–Wallis test: $\chi^2 = 2.12$, df = 2, $P = 0.35$).

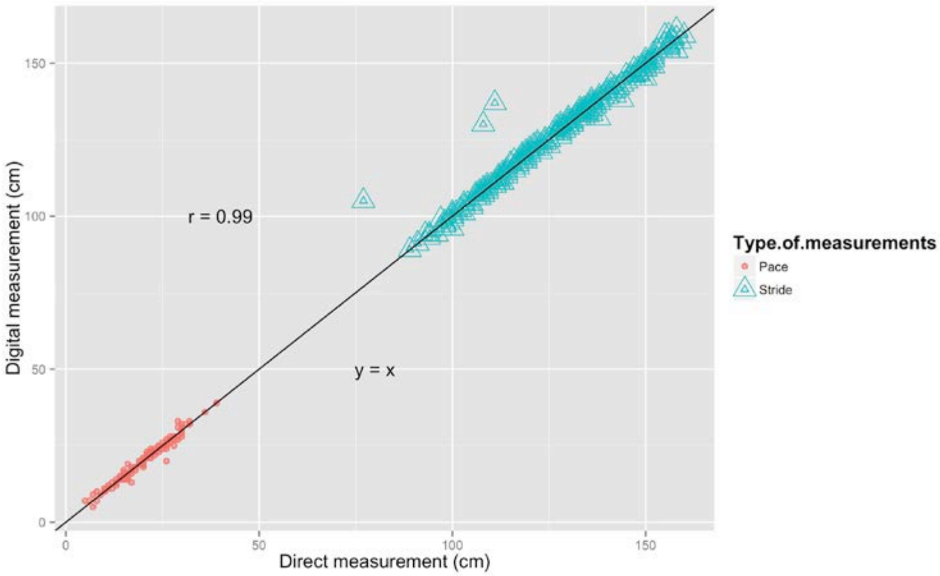


Figure 3. 5 - Regression of direct versus digital measurements. Colour code indicates the type of measurements. The line represents the true regression line (intercept = 0, slope = 1) and *r* is the coefficient of correlation using the Spearman's rank-order correlation test. The measurements originate from lion trails sampled in Hluhluwe-iMfolozi Park (South Africa) from June to December 2015.

Pace, stride and straddle

As the lioness (trail number 21) switched from register (step 2 to 5) to overstep (step 6 to 14), the pace increased while the stride and straddle for both front and hind feet decreased (Figure 3.6). The straddle of the front feet was generally smaller than the straddle of the hind feet along the trail (Figure 3.6). However, this difference decreased as the pace increased (Figure 3.6).

The mean pace was 17.05 ± 4.38 cm for overstepping adult females and 26.75 ± 6.71 cm for overstepping adult males. The mean stride was 122.44 ± 7.96 cm and 153.2 ± 7.09 cm for overstepping adult females and males, respectively. The mean straddle was 11.33 ± 3.52 cm for overstepping adult females and 15.12 ± 4.86 cm for overstepping adult males. The difference in mean pace (Mann–Whitney test: $W = 20$, $P = 0.11$) was not statistically significant, while the differences in mean stride (Mann–Whitney test: $W = 24$, $P = 0.01$) and mean straddle (Mann–Whitney test: $W = 23$, $P = 0.02$) were significant, between overstepping adult females and males.

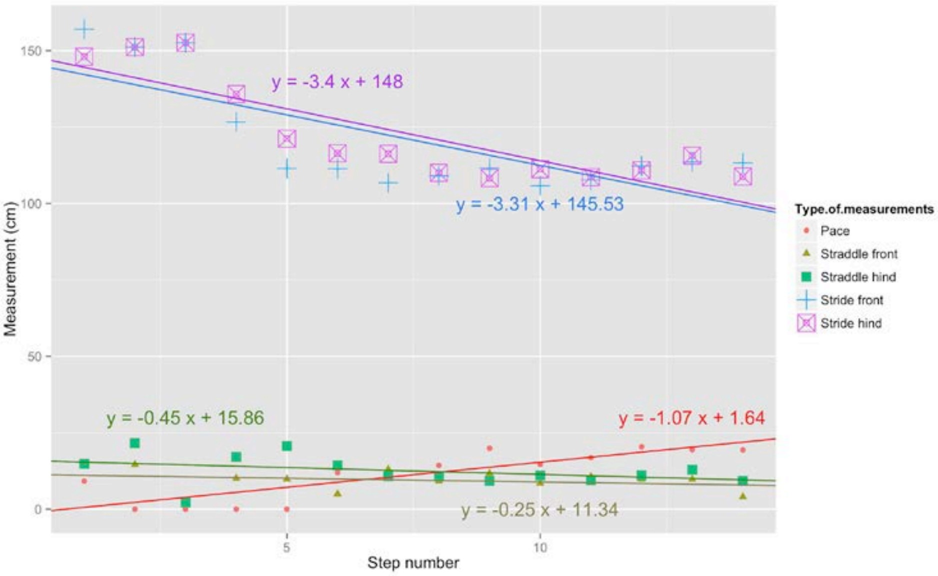


Figure 3. 6 - Pace, stride and straddle variation for the same adult female (trail number 21), sampled in Hluhluwe-iMfolozi Park (South Africa) between June and December 2015, switching from register (step 2 to 5) to overstep (step 6 to 14) on flat riverbed. Colour code indicates the type of measurements. Regression lines and equations are shown for each type of measurements.

Sex and individual discrimination

Using the LDA without jack-knife, the sex identification reached 100% either we used the information from pace, stride and straddle or from the 40 variables (using 2 principal components), and improved from 87.1% to 100% when using more information than only pace, stride and straddle (using 4 and 7 principal components respectively) for individual identification. The LDA with jack-knifed prediction produced a maximum accuracy of 96.88% for the sex identification and 77.42% for the individual identification when using the principal components computed from the 10 variables linked to pace, stride and straddle. The maximum accuracy was reached with 1 principal component for the former and 4 principal components for the latter. Using the principal components computed from the 40 variables provided a maximum accuracy of 100% for the sex identification and 90.32% for the individual identification. The maximum accuracy was reached with 2 principal components for both sex and individual identification.

Number of photographs, sampling and processing time considerations

The mean number of photographs per meter per flight was 2.92 ± 0.72, which represents an average of 1.67 ± 0.58 pictures per track set per flight. In other words, a photograph was taken every 34 cm while walking along the trail. Capturing the photographs took on average 0.39 ± 0.19 min per track set. Our smallest trail containing 4 track sets took 1 min to take 22 photographs and our longest trail containing 33 track sets took 7 minutes to take 183 photographs. We processed the digital 3D models with a desktop computer Windows 7 Enterprise 3.60 GHz Intel Core i7 16GB memory. Total 3D reconstruction time was 15 ± 9.41 min per track set with the following breakdown in percentage for the 4 steps: 7.14% photograph alignment, 78.19% dense cloud building, 7.02% mesh building and 7.66% texture building. The smallest trail took 29.57 min while the longest trail took 423.23 min to reconstruct the textured digital 3D model from 22 and 183 photographs respectively.

DISCUSSION

Trail sampling using photogrammetry involves 2 main steps: 1) capturing the photographs in the field, and 2) processing the digital 3D models in the laboratory. The fieldwork is often critically time constrained, especially when working in remote areas with potentially dangerous animals. In our study, we took 183 photographs for the longest trail (which stretched over 20 meters and contained 66 tracks) in 7 minutes. Recording basic linear distances such as pace, stride and straddle from a similar trail with a measuring tape is unpractical, effort demanding and time consuming. Furthermore, physically measuring trails increases the chance to damage the tracks and introduce a manipulator bias. The field equipment required for photogrammetric measurement included only a consumer digital camera and a scale bar. This affordable and portable apparatus represents an advantage for remote study sites in resource-limited areas. In the laboratory, the digital 3D models are computed with a commercially available low-cost non-customised photogrammetric package. The processing time may present a drawback (e.g. it took more than 7 h to 3D reconstruct the longest trail). However, this step is *ex situ*, and can be implemented automatically and continuously on computers with incessantly improving processing capacities. The mean geometric deviation between pace and stride measured directly in the field and on the digital 3D models of the same trails was significantly low, less than 1 mm. When considering the recording time, required equipment and geometric deviation, we can conclude that this photogrammetric application represents a rapid, practical and reliable field technique to sample lion trails. Although the geometric deviation was independent of the flight pattern, we recommend the use of the full sampling protocol (i.e. 4 flights and 2 clusters) in order to avoid blind spots and to facilitate the 3D reconstruction by increasing the overlap between photographs. While walking along the trail, one photograph should be taken for every step of approximately 30 cm.

Pace, stride and straddle are common variables used to describe the gait. As shown in Figure 3.6, these variables change according to the speed of locomotion (i.e. kinematics). Therefore, we only compared these trail variables between sexes and individuals for the same speed of locomotion characterised by an overstep walk. Overstepping adult males presented significantly larger mean stride and straddle than adult females. Morphologic traits, particularly body shape and weight, determine the gait of an animal (Dagg 1973) and larger animals usually have longer strides than smaller ones moving at a similar speed (Alexander 1984). An adult male (female compared in brackets) lion weighs on average 190 kg (126 kg), has a shoulder height of 120 cm (110 cm), a thorax girth of 125 cm (105 cm) and vertebral column length of 145 cm (125 cm) (Smuts et al. 1980; Mills and Bester 2005; see ‘Taxonomy and description’ in Chapter 1). These morphological dissimilarities can therefore explain the stride and straddle differences between sexes. Our dataset contained a small number of juveniles (n = 3) but preliminary results showed that these overstepping juveniles have a significantly smaller stride than overstepping adults (Appendix C). This difference can be explained by morphological changes during ontogenesis (see ‘Reproduction and demography’ in Chapter 1). However, additional individuals, particularly juveniles and sub-adults, should be included in future studies to address the possible overlap between certain age-sex categories (e.g. between adult females and sub-adult males or between juvenile males and sub-adult females).

The discrimination using the information originating from the 40 variables (including distances, angles and areas) produced higher accuracies for the prediction of both the sex and individual than when using the information from pace, stride and straddle. This highlights the necessity of using a digital solution, such as photogrammetry, to record and extract the maximum information from the trails. Photogrammetry was previously used in ichnology (i.e. science studying the interaction between organism and substrate) to record and/or digitally rescue dinosaur and early hominid tracks (Petti et al. 2008; Remondino et al. 2010) and trails (Meldrum et al. 2011; Falkingham 2012; Falkingham et al. 2014; Razzolini et al. 2014). More recently, close-range photogrammetry was used to sample lion paws and tracks (Marchal et al. 2016; Chapter 2). However, to our knowledge, this study represents the first use of photogrammetry to record trails of extant animals in the wild.

The use of tracks in wildlife studies, particularly the “pugmark census method” that was implemented for 3 decades to monitor tigers in India (Choudhury 1970), is a controversial matter. In fact, the protocols often ignore the variation due to different manipulators and substrate, and the identification between individuals is highly subjective (Karanth et al. 2003). In this study, we showed that trails contain enough information to discriminate between adult males and females with an accuracy of 100%, and between 6 different individual adults (3 males and 3 females) with an accuracy of 90.32%. We realise that our dataset is limited, but it enabled us to describe an innovative technique to 3D record lion trails in the field and to demonstrate that this digital solution brings significant advantages over the use of basic measurements such as pace, stride and straddle. We are currently working on the integration of trail and track variables, both extracted from digital 3D models (see Marchal et al. 2016), to objectively identify a larger number of individual lions along with their age and sex. Studies on gait analysis are usually classified into 2 categories: image- or accelerometer-based (Juefei-Xu et al. 2012). The former requires a direct observation, while the latter necessitates the attachment of a device to the subject. Therefore, analysing gaits from tracks offers a non-invasive alternative to the 2 above-mentioned categories. Gait patterns (e.g. stalking, walking, trotting and galloping) can be linked to behavioural activities such as hunting, charging, patrolling the territory or travelling from one hunting ground to another. Thus, studying the gait from trails could provide information on individual behaviour in specific conditions (e.g. habitat and group composition) and without observer interference. Furthermore, an atypical gait could reveal information on the body condition (e.g. sick, limping or snared individuals, pregnancy and satiety) (see ‘Feeding ecology’ and ‘Status, threats and diseases’ in Chapter 1).

Monitoring wildlife populations from tracks would have various implications in conservation biology, behavioural ecology and wildlife management. It would provide a non-invasive alternative to direct observations, and to other invasive techniques such as branding and radio-collaring. Invasive techniques are often costly, practically and technically difficult to implement, and may have negative impacts on the targeted species due to intrusiveness, immobilisation, handling and tagging effects (Alibhai et al. 2001; Wilson and McMahon 2006; McIntyre 2015). This method could be extended to other species for which the speed of locomotion can be identified from their trails. Sex and age could be identified for any species presenting sexual dimorphism, and various body size and shape as they mature. Furthermore, trail variables should enable us to discriminate between different species presenting similar foot anatomy but characterised by different gaits. In the conflict between humans and wildlife that frequently occurs in the vicinity of protected areas, tracks are often the only evidence left by problem animals following predation on livestock or raiding of crops. Using tracks to determine the species, age, sex, body condition and/or individual problem animal would be extremely valuable for a better understanding of this complex issue and to avoid indiscriminate killing of the wrong individual or even species. Due to the affordability of equipment, easy field deployment and minimal need for logistical support (Bennett et al. 2013), photogrammetry offers an ideal tool for community based monitoring, provided that a third party handles the 3D processing step, feature extraction and statistical analysis (for example through the use of an online platform).

APPENDIXES

Appendix A: Settings used in PhotoScan

Step	Description	Setting
Align cameras	Accuracy	High
	Pair pre-selection	Disabled
	Key point limit	40,000
	Tie point limit	1,000
Scale bar optimisation	Camera accuracy (m)	10
	Marker accuracy (m)	0.005
	Scale bar accuracy (m)	0.001
	Projection accuracy (pix)	0.1
	Tie point accuracy (pix)	4
Build dense cloud	Quality	Ultra high
	Depth filtering	Moderate
Build mesh	Surface type	Height field
	Source data	Dense cloud
	Face count	High
	Interpolation	Disabled
Build texture	Mapping mode	Generic
	Blending mode	Mosaic
	Texture size/count	4096x1
	Color correction	Disable

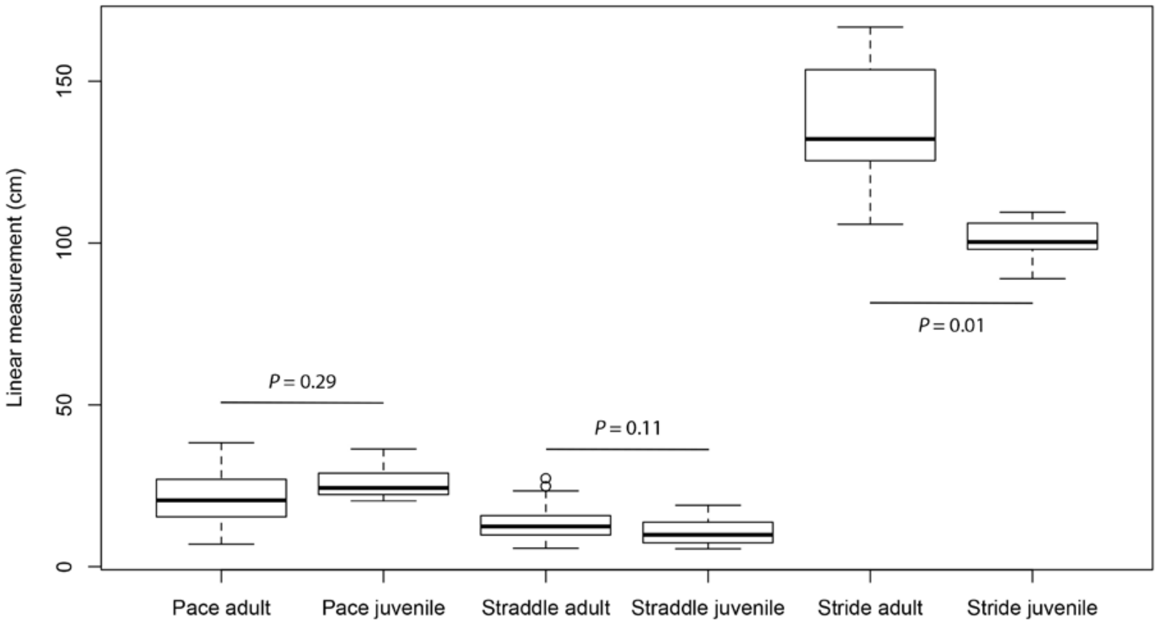
Appendix B: Details of the 40 trail variables extracted from a trail unit

Type of variables	Variable	Description	Remark
Length	FR_i-HR_i	Distance between front right track and hind right track	Pace right
Length	FL_i-FL_{i+1}	Distance between front left track and front left of the next cycle	Stride front left
Length	FR_i-FR_{i+1}	Distance between front right track and front right track of the next cycle	Stride front right
Length	HL_i-HL_{i+1}	Distance between hind left and hind left of the next cycle	Stride hind left
Length	HR_i-HR_{i+1}	Distance between hind right track and hind right track of the next cycle	Stride hind right
Length	Height triangle (FL_i, FL_{i+1}, FR_{i+1})	Height of the triangle between front left, front right of the next cycle and front left of the next cycle	Straddle front left
Length	Height triangle (FR_i, FR_{i+1}, FL_i)	Height of the triangle between front right, front left and front right of the next cycle	Straddle front right
Length	Height triangle (HL_i, HL_{i+1}, HR_{i+1})	Height of the triangle between hind left, hind right of the next cycle and hind left of the next cycle	Straddle hind left
Length	Height triangle (HR_i, HR_{i+1}, HL_i)	Height of the triangle between hind right, hind left and hind right of the next cycle	Straddle hind right
Length	FL_i-FR_{i+1}	Distance between front left track and front right track of the next cycle	
Length	FL_i-HL_i	Distance between front left track and hind left track	
Length	FL_i-HL_{i+1}	Distance between front left track and hind left of the next cycle	
Length	FL_i-HR_{i+1}	Distance between front left track and hind right of the next cycle	
Length	FR_i-FL_i	Distance between front right track and front left track	
Length	FR_i-FL_{i+1}	Distance between front right track and front left track of the next cycle	
Length	FR_i-HL_i	Distance between front right track and hind left track	
Length	FR_i-HL_{i+1}	Distance between front right track and hind left track of the next cycle	
Length	FR_i-HR_{i+1}	Distance between front right track and hind right track of the next cycle	
Length	Height triangle (FL_i, FL_{i+1}, HL_i)	Height of the triangle between front left, hind left and front left of the next cycle	
Length	Height triangle (FR_i, FR_{i+1}, HR_i)	Height of the triangle between front right, hind right and front right of the next cycle	
Length	HL_i-FL_{i+1}	Distance between hind left and front left of the next cycle	
Length	HL_i-FR_{i+1}	Distance between hind left and front right of the next cycle	
Length	HL_i-HR_{i+1}	Distance between hind left and hind right of the next cycle	
Length	HR_i-FL_i	Distance between hind right track and front left track	
Length	HR_i-FL_{i+1}	Distance between hind right track and front left track of the next cycle	
Length	HR_i-FR_{i+1}	Distance between hind right track and front right track of the next cycle	
Length	HR_i-HL_i	Distance between hind right track and hind left track	

(Continued)

Length	HR_i-HL_{i+1}	Distance between hind right track and hind left track of the next cycle
Area	Area (FL_i, FL_{i+1}, FR_{i+1})	Area of the triangle between front left, front right of the next cycle and front left of the next cycle
Area	Area (FL_i, FL_{i+1}, HL_i)	Area of the triangle between front left, hind left and front left of the next cycle
Area	Area (FR_i, FR_{i+1}, FL_i)	Area of the triangle between front right, front left and front right of the next cycle
Area	Area (FR_i, FR_{i+1}, HR_i)	Area of the triangle between front right, hind right and front right of the next cycle
Area	Area (HL_i, HL_{i+1}, HR_{i+1})	Area of the triangle between hind left, hind right of the next cycle and hind left of the next cycle
Area	Area (HR_i, HR_{i+1}, HL_i)	Area of the triangle between hind right, hind left and hind right of the next cycle
Angle	Angle (FL_i-FL_{i+1},FL_i-HL_i,)	Angle between front left-front left of the next cycle and front left-hind left
Angle	Angle (FL_i-FR_{i+1},FL_i-FL_{i+1})	Angle between front left-front right of the next cycle and front left-front left of the next cycle
Angle	Angle (FR_i-FL_i,FR_i-FR_{i+1})	Angle between front right-front left and front right-front right of the next cycle
Angle	Angle (FR_i-FR_{i+1},FR_i-HR_i,)	Angle between front right-front right of the next cycle and front right-hind right
Angle	Angle (HL_i-HR_{i+1},HL_i-HL_{i+1})	Angle between hind left-hind right of the next cycle and hind left-hind left of the next cycle
Angle	Angle (HR_i-HL_i,HR_i-HR_{i+1})	Angle between hind right-hind left and hind right-hind right of the next cycle

Appendix C: Differences in pace, stride and straddle between overstepping adult and juvenile lions; P-values from a Mann–Whitney test



Chapter 4 - Digital 3D foot identification of lion paws and tracks using geometric morphometrics

Figure 4. 1 - A lioness walks on a sandy road in Tembe Elephant Park, South Africa.

ABSTRACT

Estimating the distribution and status of animal populations is crucial in various fields of biology. Monitoring species through their tracks is controversial due to unreliable recording techniques, manipulator bias and substrate variation. Furthermore, subjective identification of the foot that produces each track can lead to significant initial errors, for example, when assigning tracks made by different feet from the same individual to different individuals. The aim of this research is to develop an accurate, consistent and objective foot identification algorithm for lion *Panthera leo* paws and tracks using geometric morphometrics. We manually positioned 12 fixed landmarks on 24 paws and 170 tracks recorded in three-dimensions (3D) using digital photogrammetry. We used geometric morphometrics, including procrustes analysis, to evaluate and visualise the shape variation between paws and tracks, between paws, and between tracks along the anteroposterior (front or hind) and mediolateral (right or left) axis. The foot prediction accuracy (with jack-knifed predictions in brackets) reached a maximum of 100% (95.83%) and 95.29% (92.94%) for paws and tracks respectively. We recommend the use of this objective foot identification algorithm in future studies where tracks are compared between individual lions. Identifying individuals from their tracks would represent a robust, practical, low-cost and non-invasive monitoring tool.

Keywords: Digital 3D model, footprint, geometric morphometrics, *Panthera leo*, photogrammetry, tracking.

INTRODUCTION

Reliable population estimates are essential for planning conservation and management actions, allocating and prioritising resources, and evaluating the impact and success of conservation programmes (Nowell and Jackson 1996). Large carnivores are particularly challenging to monitor due to their elusive nature (Balme et al. 2009). When sightings are scarce, information can be drawn from tracks (Heinemeyer et al. 2008). For more than three decades, the monitoring of tigers in India was based on the ‘pugmark census method’ (Karanth et al. 2003; Sharma et al. 2005). Tracings or plaster casts of tracks made by the hind left paws were sampled countrywide for individual tiger identification (Karanth et al. 2003; Sharma et al. 2005). Besides a poor analytical framework and subjective identification, this method received criticism for ignoring the variation related to different manipulators and substrates (Karanth et al. 2003). Furthermore, the misidentification of the foot from which each track originates led to significant errors. For example, when census personnel are not able to find clear tracks made by the four feet in order to identify the hind left one, they tend to sample tracks made by the different feet of the same individual from different locations (Karanth et al. 2003). This error can lead to an over-estimation by assigning tracks made by different feet of the same individual to different individuals. To overcome this challenge, we aim to illustrate a consistent and accurate approach to objectively identify the anteroposterior (front or hind, i.e. manus or pes) and mediolateral (right or left) position from digital three-dimensional (3D) models of lion *Panthera leo* paws and tracks using landmark-based geometric morphometrics.

MATERIALS AND METHODS

Paw and track sampling using photogrammetry

Lion paws and tracks were sampled in Hluhluwe-iMfolozi Park (HiP, ~900 km^2) and Tembe Elephant Park (TEP, ~300 km^2) that are located in the province of KwaZulu-Natal (KZN), South Africa, and managed by Ezemvelo KZN Wildlife (EKZNW). We opportunistically sampled the four paws (i.e. front right (FR), front left (FL), hind right (HR) and hind left (HL)) from both male and female lions of three different age categories (juvenile, sub-adult and adult) (N=6) during nocturnal captures (i.e. total of 24 paws). All lions were captured as part of management operations that were unrelated to this project and conducted by EKZNW’s staff, which included a wildlife veterinarian. We recorded a total of 170 tracks from 10 different trails (Table 4.1). A trail is a continuous sequence of tracks belonging to one individual (Liebenberg et al. 2010). Tracks, that belonged to both male/female and adult/juvenile individuals, were sampled in riverbeds and on dirt roads after a direct observation, in front of a camera trap [Cuddeback Attack, Green Bay, Wisconsin, USA] or after identification of the species with the help of a tracking book (e.g. Liebenberg 1990b) (Table 4.1). The foot from which each track originated was identified by the track's relative position within the sequence. All trails were less than 24 hours old and were characteristic of an overstep walk where the hind foot registered beyond the front track (Liebenberg 1990b; Liebenberg et al. 2010). Thirty tracks (11 FR, 12 FL, 5 HR and 2 HL) from the trails were missing or discarded due to poor quality.

Table 4. 1 - Details of the sampled tracks.

Trail number	Source of information	Age	Sex	FR	FL	HR	HL	Total
1	Camera trap	Adult	Male	1	1	3	4	9
2	Camera trap	Adult	Male	3	3	2	3	11
3	Camera trap	Adult	Female	1	6	4	6	17
6	Direction observation	Adult	Female	4	2	3	3	12
4	Direction observation	Juvenile	NA	2	2	2	2	8
5	Direction observation	Juvenile	NA	3	2	2	2	9
7	Direction observation	Juvenile	NA	3	3	3	4	13
8	Tracking book	NA	NA	4	2	6	5	17
9	Tracking book	NA	NA	3	4	5	5	17
10	Tracking book	NA	NA	15	13	15	14	57
Total				39	38	45	48	170

'H' = hind, 'F' = front, 'R' = right and 'L' = left.

As described in Marchal et al. (2016), we took between 9 and 15 overlapping photographs at different distances and angles from the object of interest (i.e. paw or track) and a scale bar. Two digital single-lens reflex cameras were used for the sampling: Nikon D7100 (24.1 megapixels) with Nikkor 18-70 mm f/3.5-4.5 and Nikon D80 (10 megapixels) with Nikkor 50 mm f/1.8 [Nikon Corporation, Tokyo, Japan]. We used the photogrammetric package Agisoft PhotoScan Professional Edition version 1.1.4 build 2021 [Agisoft LLC, Saint Petersburg, Russia] to process digital 3D meshes. Masking the photographs (i.e. to remove the background) was applied for the paws but not for the tracks, and we used both auto-calibration and optimization in the camera alignment step (Marchal et al. 2016). The scale bar enabled scaling of the 3D models.

Landmark-based geometric morphometrics

Twelve fixed landmarks were digitised on the 3D meshes of paws (Figure 4.2a) and tracks (Figure 4.2b) using the Geomorph package (Adams and Otárola-Castillo 2013; Adams et al. 2015) in the R program (R Development Core Team 2014). Landmarks are discrete endpoints that are biologically homologous anatomical loci (Zelditch et al. 2012). We only used landmarks type II (i.e. curvature extrema of a local structure) (Bookstein 1991) that were manually positioned at the extremes of the two outer bottom lobes and the two top pseudo-lobes of the main pad, and the two extremes located on the longest axis of each toe (Figure 4.2a; 4.2b). We used the same landmark digitisation sequence for all paws and tracks by taking into consideration rotation symmetry between them (Figure 4.2a; 4.2b). To assess repeatability of the manual landmark digitisation, we selected three paws and three tracks on which the landmarks were positioned three times, on different days, by the same manipulator. This subsample was representative of our database and complete for testing the digitisation repeatability.

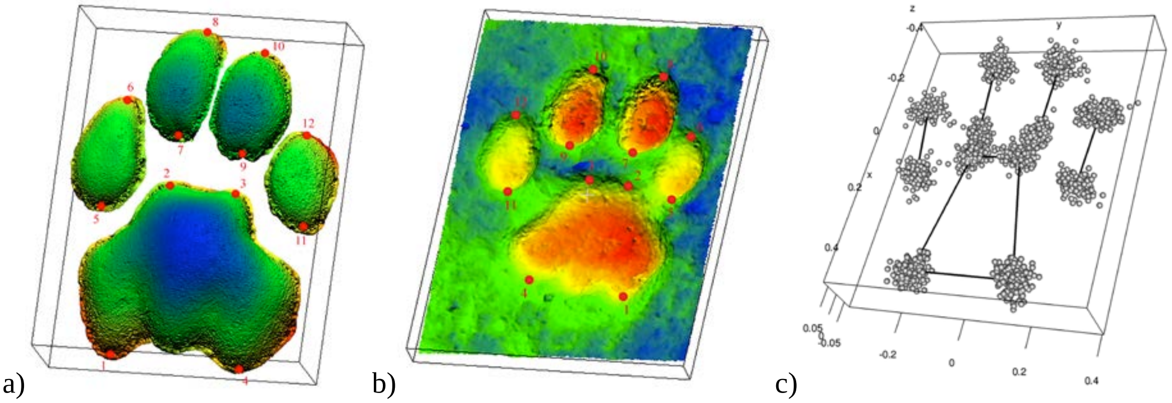


Figure 4. 2 - Digital 3D model of (a) paw and (b) track with 12 fixed landmarks, and c) procrustes coordinates of paws (24 specimens) and tracks (170 specimens). Note the two different landmark positioning sequences due to rotation symmetry between paw and track. In the procrustes coordinates, the specimens (grey points) surround the mean shape points. Links between the mean shape points were added for better visualisation of the main pad and four toes.

We used geometric morphometrics to extract features from paws and tracks. Geometric morphometrics involved a Generalised Procrustes Analysis (GPA) that superimposes all the specimens to a common coordinate system by removing variation due to differences in position, scale and orientation (Rohlf and Slice 1990; Zelditch et al. 2012) (Figure 4.2c). Due to superimposition, the morphometric analysis can be applied directly to the procrustes coordinates.

Data analysis

We used the subsample containing three paws and three tracks to test the intra-observer landmark digitisation error by calculating the repeatability using repeated-measures nested ANOVA (Zelditch et al. 2012). Following a GPA and procrustes ANOVA to test the influence of the individual objects and three repetitions on shapes, we calculated the repeatability by using the formula (Zelditch et al. 2012):

$$\textit{Repeatability} = \textit{Individual variance} / (\textit{Mean Squares}_{\textit{Repetitions}} + \textit{Individual variance})$$

with

$$\textit{Individual variance} = (\textit{Mean Squares}_{\textit{Individuals}} - \textit{Mean Squares}_{\textit{Repetitions}}) / \textit{Number of repetitions}$$

Secondly, we independently applied a GPA to (i) combination paws and tracks, (ii) paws and (iii) tracks from the entire database. For each group, we ran a Principal Component Analysis (PCA) on the procrustes coordinates. We used a procrustes ANOVA to test the influence of the factor object (i.e. paw or track) on the procrustes data combining paws and tracks, and the influence of the factor foot (i.e. FR, HR, FL or HL) on the procustes data of the paws and tracks independently. We applied a Linear Discriminant Analysis (LDA) (with and without jack-knifed predictions) and compared accuracy of the foot prediction for paws and tracks by using an increasing number of principal components originating from the PCA on the procustes coordinates. The LDA without jack-knife prediction uses the same observations to adjust the allocation model and estimate the error rate. The jack-knife prediction creates the allocation model with all the observations except one. This observation is then used to estimate the predicted group. The procedure is applied to each observation and the sum of the individual errors provides the error rate. The jack-knife prediction enables a better estimation of what the prediction error would be if all the observations tested were entirely new. Finally, we plotted the procrustes-aligned paws and tracks, together and independently, according to the first two principal components (i.e. PC1 versus PC2) and drew the thin-plate spline deformation grids representing the shape at the extremes of the range of variability along the two axes. Probability values were considered statistically significant at $p \leq 0.05$.

RESULTS

Landmark error and foot identification

The landmark digitisation repeatability was 97.61% for the paws and 92.49% for the tracks. The interaction between the factor object and shape was significant (procrustes ANOVA, $F_{1,192} = 26.05$, $p = 0.001$). The factor foot had a significant influence on both the shape of paws (procrustes ANOVA, $F_{1,22} = 5.22$, $p = 0.001$) and tracks (procrustes ANOVA, $F_{1,168} = 20.68$, $p = 0.001$). The first 8 and 16 principal components explained more than 90% of the variability between the paws and tracks respectively. The foot prediction accuracy (with jack-knifed predictions in brackets) reached a maximum of 100% (95.83%) and 95.29% (92.94%) for paws and tracks respectively (Table 4.2).

Shape variation between paws and tracks, between paws and between tracks

PC1, explaining 22.09% of the variation, displaid a significant discriminating power between paws and tracks (Figure 4.3a). Deformation grids showed the main difference between paws and tracks to be the split of the toes (Figure 4.3a). When paws and tracks are analysed separately, PC1 discriminated right from left, while PC2 discriminated front from hind (Figure 4.3b, 4.3c). PC1 and PC2 explained

40.88% and 18.41% of the variability between paws, and 23.13% and 15.89% of the variability between tracks. Considering that the dewclaw (located higher up on the inner side) is not represented on paws and tracks, the lateral position was identified by the small digit (i.e. little finger or toe that is located on the outside) and the middle digit (i.e. between small digit and dewclaw, located two digits away from the small digit) (Figure 4.3b, 4.3c). The small digit was shorter and positioned closer to the main pad. The middle digit was placed further away from the main pad; therefore, the upper tip marked the highest point of the paw or track. Particularly in tracks, the inner bottom lobe of the main pad (i.e. opposite side from the small digit) tent to be positioned higher than the outer bottom lobe. The front paws and tracks were larger and rounder than the hind paws and tracks (Figure 4.3b, 4.3c).

Table 4. 2 - Accuracy of foot prediction (%) for the paws and tracks using an increasing number of principal components in the Linear Discriminant Analysis (with and without jack-knifed predictions). The maximum number of principal components used represents >90% of the cumulative proportion of the variance, viz., 8 and 16 principal components for paws and tracks respectively.

Number of principal components	Foot prediction (%)		Foot prediction with jack-knife (%)	
	Paws	Tracks	Paws	Tracks
2	91.67	84.71	75.00	84.71
3	95.83	88.82	95.83	87.06
4	100.00	90.00	95.83	90.00
5	100.00	90.59	91.67	90.00
6	100.00	91.18	79.17	90.59
7	100.00	91.18	83.33	90.00
8	100.00	92.35	91.67	90.59
9	NA	91.76	NA	90.59
10	NA	92.94	NA	89.41
11	NA	94.71	NA	90.00
12	NA	94.71	NA	92.94
13	NA	95.29	NA	92.35
14	NA	95.29	NA	91.76
15	NA	95.29	NA	91.76
16	NA	95.29	NA	91.76

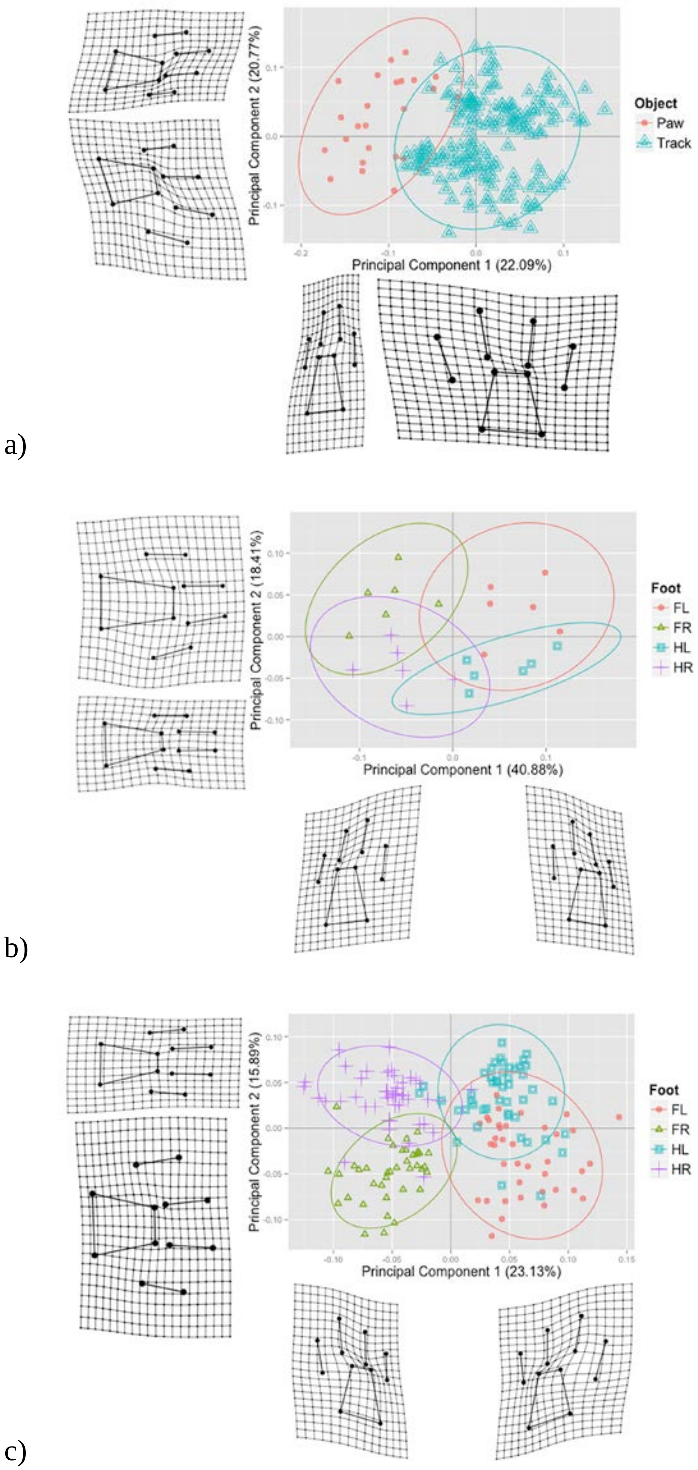


Figure 4. 3 - Principal components of shape variation between (a) paws and tracks, (b) paws and (c) tracks. The thin-plate spline deformation grids (with 1.5 magnification) show the shape difference between extremes of each principal component axis and mean shape. Shape and colour code indicates the object (i.e. paw or track) (a) or the foot (b and c). 'H' = hind, 'F' = front, 'R' = right and 'L' = left. The ellipses represent the 95% confidence intervals.

DISCUSSION

Lion paws and tracks present shapes that are complex enough to accurately and consistently identify their position along both anteroposterior and mediolateral axis. A simple photogrammetric application enabled the shape recording in 3D, while geometric morphometrics permitted the study of shape variation and its covariation with other variables. The foot identification from the paws reached higher levels of prediction (100% without jack-knifed and 95.83% with jack-knifed) than for the tracks (95.29% without jack-knifed and 92.94% with jack-knifed) probably due to the distortion created by the complex interaction between paw and substrate. This distortion seems to be more significant in the front tracks as a higher number of front tracks (>75%) were discarded from the analyses due to poor quality. This can be explained by the fact that lions (as with most quadruped species) carry the majority of their weight on the front legs. We recommend the use of this foot identification algorithm in future studies aiming to compare tracks between different lions. Identifying individuals from their tracks represents a practical low-cost non-invasive monitoring tool that would have major implications in the development of conservation, management and research strategies.

Chapter 5 - Age, sex and individual identification from lion paws and tracks

Figure 5. 1 - A lioness and her two cubs in Hluhluwe-iMfolozi Park, South Africa.

ABSTRACT

Gaining information on abundance and distribution of animal populations is key element in the field of ecology and wildlife conservation. In the absence of direct observations, tracks offer a non-invasive low-cost approach to study elusive species such as certain carnivores. However, monitoring species through their tracks is controversial due to unreliable recording techniques, manipulator bias, substrate variation, misidentification of the foot from which each track originates and subjective identification of the age, sex, and/or individual. Furthermore, previous studies were limited to traditional morphometrics using basic measures such as distances, angles, and areas that convey no information about the geometric structure. The objective of this research is to apply geometric morphometrics on digital three-dimensional (3D) models of lion *Panthera leo* paws and tracks to (i) discriminate the age and sex from the paws, (ii) analyse the size and shape variation of the paws in terms of age and sex, and (iii) discriminate the age, sex and individual from the tracks. Using digital close-range 3D photogrammetry, we sampled 40 front left and 41 hind left paws belonging to 45 different individuals, and 123 hind left tracks extracted from 25 trails belonging to 25 individuals. The samples were representative of both sexes and three different age classes (juvenile, subadult, and adult). The geometric form (i.e. size and shape variables) was extracted from the paws and tracks by means of landmarked-based geometric morphometrics using three types of landmarks: fixed landmarks, fixed landmarks with curve-sliders and fixed landmarks with curve- and surface-sliders. The landmark coordinates were superimposed through a Generalized Procrustes Analysis. Centroid sizes (i.e. size variables) and principal components originating from the Procrustes coordinates (i.e. shape variables) were then used in Linear Discriminant Analysis (LDA), with or without jack-knifed predictions, to assess the accuracy of age and sex identification from the paws, and age, sex and individual identification from the tracks. Improved classifications occur when the size and shape variables are combined than when they are analysed independently. The information from curves and surfaces showed an advantage for the tracks, but not for the paws. Accuracy of prediction from the LDA without jack-knife predictions (results from the LDA with jack-knife predictions in brackets) attained a maximum accuracy of 100% (73.17%) for the age-sex identification from paws, 100% (85.11%) for the age-sex identification from tracks and 97.41% (45.69%) for the individual identification from tracks. Identifying the age, sex and individual from tracks would have major implications for the non-invasive monitoring of lions.

Keywords: digital 3D model, footprint, geometric form, geometric morphometrics, paw, photogrammetry, track.

INTRODUCTION

Gaining basic information on animal populations, such as abundance and distribution, is key element in the field of ecology and wildlife conservation (Caughley 1977; Grimsdell 1978; Yoccoz et al. 2001). Amongst other species, large carnivores represent a priority as they exert strong regulatory effects on ecosystems (Schmitz et al. 2010; Estes et al. 2011). However, monitoring them presents a challenge as many species are secretive, nocturnal, wide-ranging, potentially dangerous to humans and/or live at low-densities in remote areas (Gese 2001; Wilson and Delahay 2001). Tracks, the by-product of the interaction between terrestrial animals and their physical environment under the effect of gravity, offer an indirect alternative to gain information on certain species in the absence of sightings (Heinemeyer et al. 2008). The only limitations are that species must have a characteristic foot anatomy and that the substrate must allow the impression of tracks with sufficient quality. For millennia, the earliest humans have used tracks as an essential part of hunting (Liebenberg 1990a). The art of tracking, which is still used by modern-day hunter-gatherer communities such as the San people of southern Africa (Liebenberg 2006), implies a hypothetico-deductive approach that may well be the origin of science (Liebenberg 1990a). Traditional San trackers were involved in behavioural studies of large carnivores, as they possess the skills to interpret tracks and follow trails (i.e. continuous sequence of tracks made by the same individual) (Bothma and Le Riche 1984; Eloff 1984; Mills 1990; Bothma and Le Riche 1993; Bothma and Le Riche 1995). Following the trail of various predators while interpreting their activities reveal information on hunting, cover and water use, territorial behaviour, range, movements, and interactions with conspecifics and other predators (see ‘Social and spatial behaviour’ and ‘Reproduction and demography’ in Chapter 1). A group of San trackers from Namibia were 98% accurate when identifying the species, age, sex, individual and/or behaviour from tracks in 569 cases (Stander et al. 1997).

To achieve greater practicability, objectivity and quantification, the art of tracking was replaced by measurements, either directly on the tracks or on their replicas (plaster casts, drawings and photographs). Multivariate analyses were used to identify the species, age, sex and/or individual from these measurements. Track measurements were applied to canid species such as African wild dog *Lycaon pictus* (Scharis et al. 2015), black-backed jackal *Canis mesomelas* (Gusset and Burgener 2005) and maned wolf *Chrysocyon brachyurus* (De Angelo et al. 2010), to felid species such as African wildcat *Felis silvestris* (Gusset and Burgener 2005), caracal *Caracal caracal* (Gusset and Burgener 2005), cheetah *Acinonyx jubatus* (Jewell et al. 2016), jaguar *Panthera onca* (De Angelo et al. 2010), leopard *P. pardus* (Stander et al. 1997; Gusset and Burgener 2005), lion *P. leo* (Stander et al. 1997; Van Bommel et al. unpublished data), mountain lion *Puma concolor* (Smallwood and Fitzhugh 1993;

Grigione et al. 1999; Lewison et al. 2001; De Angelo et al. 2010; Jewell et al. 2014), serval *Leptailurus serval* (Gusset and Burgener 2005) and tiger *P. tigris* (Gore et al. 1993; Sharma et al. 2003, Sharma et al. 2005), and to rhinoceros species such as black rhinoceros *Diceros bicornis* (Jewell et al. 2001) and white rhinoceros *Ceratotherium simum* (Alibhai et al. 2008).

All these above-mentioned examples involved the study of tracks by means of traditional morphometrics. Morphometrics may be defined as the quantitative study of shape variation and its covariation with other variables (Bookstein 1991; Zelditch et al. 2012; Adams and Otárola-Castillo 2013). Traditional morphometrics, which implies the use of basic measurements such as linear, angular and area metrics, possesses many limitations: the measurements overlap, the values are not completely independent, they are cumbersome and redundant, and they convey no information about geometric structure (Marcus 1990; Zelditch et al. 2012). Additionally, traditional morphometrics fail to quantify subtle variations between the landmarks used to extract the measurements (i.e. along curves or surfaces) and the results are a list of numbers that does not allow visualisation of the shape variation. To overcome these drawbacks, geometric morphometrics defines the shape as being 'all the geometric information that remains when location, scale and rotational effects are filtered out from an object' (Kendall 1977) and it utilises 'the coordinates of landmarks to record the relative positions of morphological points, boundary curves and surfaces as the basis of shape quantification' (Adams and Otárola-Castillo 2013). The objective of this research is to apply three-dimensional (3D) landmark-based geometric morphometrics, with and without curve- and surface-sliders semi-landmarks, on lion paws and tracks to (i) discriminate the age and sex from the paws, (ii) analyse the size and shape variation of the paws in terms of age and sex, and (iii) discriminate the age, sex and individual from the tracks.

MATERIALS AND METHODS

Study sites

Hluhluwe-iMfolozi Park (HiP, ~900 km^2) and Tembe Elephant Park (TEP, ~300 km^2) are both entirely fenced and located in the province of KwaZulu-Natal (KZN), eastern South Africa. HIP experiences a sub-tropical climate with a mean annual rainfall between 650 mm and 985 mm across a north to south rainfall gradient. The hilly topography, ranging from 40 m to 560 m above the sea level, is traversed by three major rivers, namely, the Hluhluwe, Black iMfolozi and White iMfolozi rivers. The vegetation is mainly savannah woodland with patches of shrub thickets. Semi-deciduous forests are present in the north of the park, while open savannah woodland is found in the south. Located along the international border with Mozambique, TEP is characterised by sandy plains with ancient littoral dunes. The sub-tropical climate and mean annual rainfall of 700 mm sustains the combination of vegetation of open and closed woodlands, sand forests and reed beds.

The dry riverbeds of HiP and the sandy roads of TEP offer an optimal substrate for tracks. In July 2015, the number of individual lions were estimated at ~120 (Somers et al. unpublished data) and ~40 (Hanekom unpublished data) in HiP and TEP respectively.

Paw and track sampling using photogrammetry

Following the guidelines described in Marchal et al. (2016), 81 paws (Table 5.1) and 123 tracks (Table 5.2) were replicated in 3D using digital close-range photogrammetry. The samples included 40 front left (hereafter FL) and 41 hind left (hereafter HL) paws belonging to 45 different individual lions from both sexes and three age classes (juvenile, subadult and adult) (Table 5.1). The age classification used by research staff from the study sites followed Smuts (1976) and Smuts et al. (1978) with the following breakdown: juvenile (less than two years old), subadult (between two and four years old) and adult (more than four years old). The opportunistic sampling was carried out on immobilised lions that were captured for management reasons (e.g. branding, collaring, blood sampling and/or implanting micro-chips). EKZNW staff members, including a wildlife veterinarian, captured the lions following a strict ethics protocol.

All tracks in this analysis originated from the HL paws. Tracks made by the front paws may be missing due to direct registration (i.e. hind paws stepping at the same location as the front paws). Furthermore, front tracks are often distorted due to various possible reasons: most quadrupeds carry the majority of their weight on the anterior part of their body (Jewell et al. 2001), the lateral movement of the head and neck, and the slight kick-back movement of the front paws. Tracks were sampled from 25 trails belonging to 25 different individuals (Table 5.2). Out of the 123 tracks sampled, seven tracks were discarded due to poor quality. Therefore, each trail contained between 2 and 9 hind left tracks of good quality. Tracks were less than 24 hours old and were sampled following direct observations or in front of a camera trap [Cuddeback Attack, Green Bay, WI, USA]. We sampled quality tracks in dry riverbeds or on sandy roads representing flat terrain (Table 5.2). In a few instances, the substrate in front of the camera traps was prepared with a rake (Table 5.2). All trails were characteristic of walking individuals with the exception of two trails where the individuals were courting and mating.

Table 5. 1 - Details of the sampled paws. Lions sampled in Hluhluwe-iMfolozi Park (HiP) and Tembe Elephant Park have a unique identification (i.e. ID) that starts with 'HiP' and 'Tembe' respectively. 'FL' stands for front left and 'HL' for hind left.

Age	Sex	ID	FL	HL
Adult	Female	HiP309	X	X
Adult	Female	HiP311	X	X
Adult	Female	HiP404	X	X
Adult	Female	HiP406	X	
Adult	Female	HiP507		X
Adult	Female	HiP618	X	X
Adult	Female	HiP618	X	X
Adult	Female	HiP625		X
Adult	Female	HiP630	X	X
Adult	Female	HiP636	X	X
Adult	Female	HiP639	X	X
Adult	Male	HiP312	X	X
Adult	Male	HiP405	X	
Adult	Male	HiP523		X
Adult	Male	HiP707	X	X
Adult	Male	Tembe5	X	
Subadult	Female	HiP631	X	X
Subadult	Female	HiP633		X
Subadult	Female	HiP634	X	X
Subadult	Female	HiP641	X	X
Subadult	Male	HiP 614	X	
Subadult	Male	HiP527	X	X
Subadult	Male	HIP614		X
Subadult	Male	HiP628	X	X
Subadult	Male	HiP628	X	
Subadult	Male	HiP629	X	X
Subadult	Male	HiP635	X	X
Subadult	Male	HiP640	X	X
Subadult	Male	HiP718	X	X
Subadult	Male	HiP730	X	X
Subadult	Male	HiP731	X	X
Subadult	Male	HiP732	X	X
Subadult	Male	HiP733	X	X
Subadult	Male	HiP734	X	X
Subadult	Male	HiP735	X	X
Subadult	Male	Tembe4	X	X
Juvenile	Female	HiP623	X	X
Juvenile	Female	HiP719	X	
Juvenile	Female	HiP722		X
Juvenile	Female	Tembe2	X	X
Juvenile	Female	Tembe3	X	X
Juvenile	Male	HiP407	X	X
Juvenile	Male	HiP408	X	X
Juvenile	Male	HiP621		X
Juvenile	Male	HiP721	X	X
Juvenile	Male	Tembe1	X	X
Juvenile	Male	Tembe6	X	X
	Total		40	41

Table 5. 2 - Details of the sampled hind left (HL) tracks belonging to 25 individuals of different age and sex.

ID	Age	Sex	Type of substrate	Substrate status	Sampled HL	Damaged HL	Quality HL
Bridge Pride 1	NA	NA	Riverbed	Non prepared	8	0	8
Bridge Pride 2	NA	NA	Riverbed	Non prepared	10	1	9
Bridge Pride 3	NA	NA	Riverbed	Non prepared	4	0	4
Bridge Pride 4	NA	NA	Riverbed	Non prepared	3	0	3
Bridge Pride 5	Juvenile	NA	Riverbed	Non prepared	4	0	4
Bridge Pride 6	Juvenile	NA	Riverbed	Non prepared	2	0	2
Cengeni Pride 1	Adult	Female	Riverbed	Non prepared	2	0	2
HiP310	Adult	Male	Riverbed	Prepared	9	0	9
HiP312	Adult	Male	Riverbed	Non prepared	4	0	4
HiP404	Adult	Female	Riverbed	Non prepared	8	2	6
HiP626	Adult	Male	Sandy road	Prepared	3	0	3
HiP630	Adult	Female	Riverbed	Non prepared	4	0	4
Hluhluwe Pride 1	Subadult	Male	Riverbed	Non prepared	5	2	3
Hluhluwe Pride 2	Subadult	Male	Riverbed	Non prepared	6	1	5
Qaqeni Pride 1	NA	NA	Riverbed	Non prepared	5	0	5
Qaqeni Pride 2	NA	NA	Riverbed	Non prepared	4	0	4
Qaqeni Pride 3	NA	NA	Riverbed	Non prepared	9	0	9
Siyembeni Pride 1	NA	NA	Riverbed	Prepared	7	0	7
Siyembeni Pride 2	NA	NA	Riverbed	Prepared	4	0	4
Sontuli Pride 1	NA	NA	Riverbed	Non prepared	4	0	4
Sontuli Pride 2	NA	NA	Riverbed	Prepared	2	0	2
Sontuli Pride 3	NA	NA	Riverbed	Prepared	2	0	2
Sontuli Pride 4	NA	NA	Riverbed	Prepared	2	0	2
Tembe Pride 1	Adult	Female	Sandy road	Non prepared	6	1	5
Tembe Pride 2	Adult	Female	Sandy road	Non prepared	6	0	6
			Total		123	7	116

The digital 3D modelling was performed with the photogrammetric package Agisoft PhotoScan Professional Edition version 1.1.4 build 2021 [Agisoft LLC, Saint Petersburg, Russia]. The reconstruction of a 3D mesh from photographs comprises three main steps: (i) build the sparse point cloud (i.e. camera alignment), (ii) build the dense point cloud and (iii) build the polygonal mesh. The background was masked for the paws but not for the tracks, and both auto-calibration and optimisation were used for the camera alignment step (Marchal et al. 2016). A ruler positioned near the paws and tracks enabled the scaling of the digital 3D models.

Feature extraction using geometric morphometrics

Paws and tracks were segmented into five components: main pad (hereafter MP), first toe (inner toe; hereafter T1), second toe (hereafter T2), third toe (hereafter T3) and fourth toe (smallest and outer toe; hereafter T4) (Figure 5.2). Segmentation of the paws occurred in the photographs (i.e. before 3D reconstruction) through semi-automatic masking in Photoshop Creative Cloud [Adobe, San Jose, CA, USA] (Marchal et al. 2016) (Figure 5.2a). Minimal manual editing was applied to the 3D paws to eliminate imperfections from the 3D reconstruction, particularly, to manually remove the fur that was not properly eliminated through masking. Using CloudCompare, we applied the 'Principal Component Analysis (PCA) fit' tool to align the 3D tracks according to their principal components and create a non axis-orientated bounding box (CloudCompare 2015). This re-orientation enabled the addition of gradient colours according to the depth (z-axis) in order to facilitate the manual segmentation of the track components (Figure 5.2b).

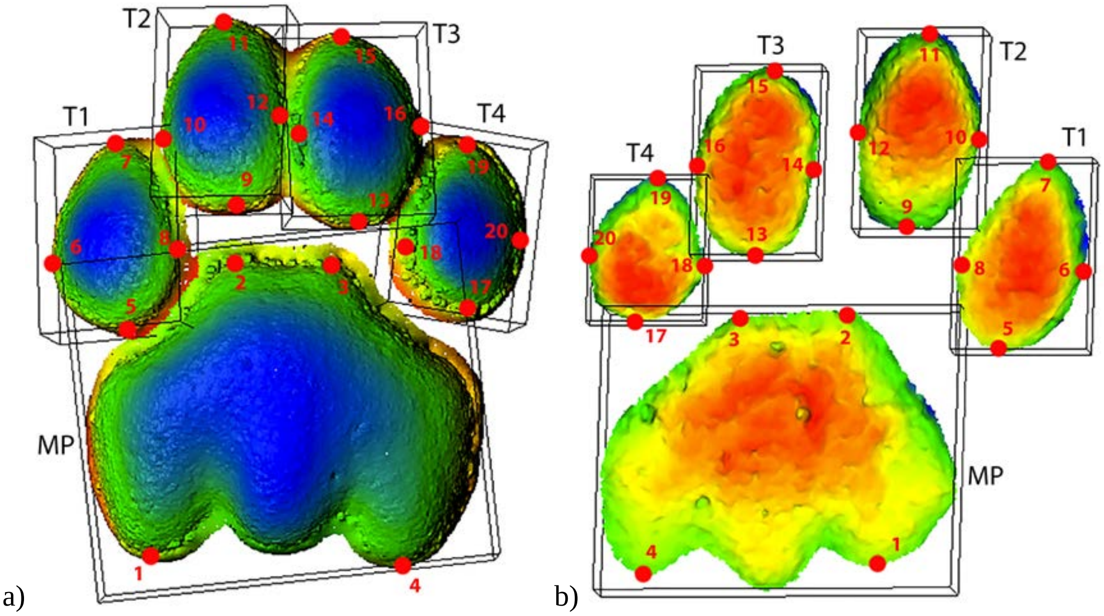


Figure 5. 2 - Segmented digital 3D models of (a) paw and (b) track with 20 fixed landmarks. Gradient colours were added according to the depth (z-axis) for better visualisation and to facilitate the manual segmentation of the tracks. Note the difference in landmark digitisation sequences due to rotation symmetry between paw and track. 'MP' = main pad, 'T1' = first toe, 'T2' = second toe, 'T3' = third toe and 'T4' = fourth toe.

We used the Geomorph package (Adams and Otárola-Castillo 2013; Adams et al. 2015) in the R program (R Development Core Team 2014) to digitise fixed landmarks, compute curve- and surface-sliders semi-landmarks and superimpose their coordinates. A landmark is defined as a 'biologically homologous anatomical loci' (Zelditch et al. 2012). We manually placed 20 fixed landmarks type II positioned on the curvature extrema of local structures (Bookstein 1991) (Figure 5.2). Four landmarks were positioned on MP: two on the outer bottom lobes and two on the top pseudo-lobes (Figure 5.2; 5.3a). For each toe, we placed two landmarks representing the maximum length and two representing the maximum width (Figure 5.2). We automatically computed the curve-sliders by positioning 50 equidistant landmarks along the border of MP (Figure 5.3b) and 20 around each toe. For each component of FL paws, HL paws or HL tracks, we built a template of equally spaced points on the surface (i.e. surface-sliders) (Gunz et al. 2005) using 50 landmarks for MP (Figure 5.3c) and 20 for each toe. Using one-to-one matching with the template, the surface-sliders are then positioned on the other specimens (Adams and Otárola-Castillo 2013). We investigated three possible types of landmarks depending on whether the features were extracted from: (i) fixed landmarks (hereafter fixed) (Figure 5.3a), (ii) fixed landmarks with curve-sliders (hereafter fixed-curves) (Figure 5.3b) or (iii) fixed landmarks with curve- and surface-sliders (hereafter fixed-curves-surfaces) (Figure 5.3c). For each foot (i.e. FL or HL), each component (i.e. MP, T1, T2, T3 or T4) and each type of landmarks (i.e. fixed, fixed-curves or fixed-curves-surfaces), we applied a Generalised Procrustes Analysis (GPA) that superimposes the specimens to a common coordinate system by holding constant variation in their position, size and orientation (Gower 1975; Rohlf and Slice 1990) (Figure 5.4). During GPA, curve and surface semi-landmarks slide along tangent lines of the respective curve or tangent planes of the respective surface using Procrustes distance as a criterion to optimise their position (Gunz et al. 2005; Gunz and Mitteroecker 2013). The GPA generates the Procrustes coordinates as well as the centroid sizes before the size difference during superimposition is cancelled.

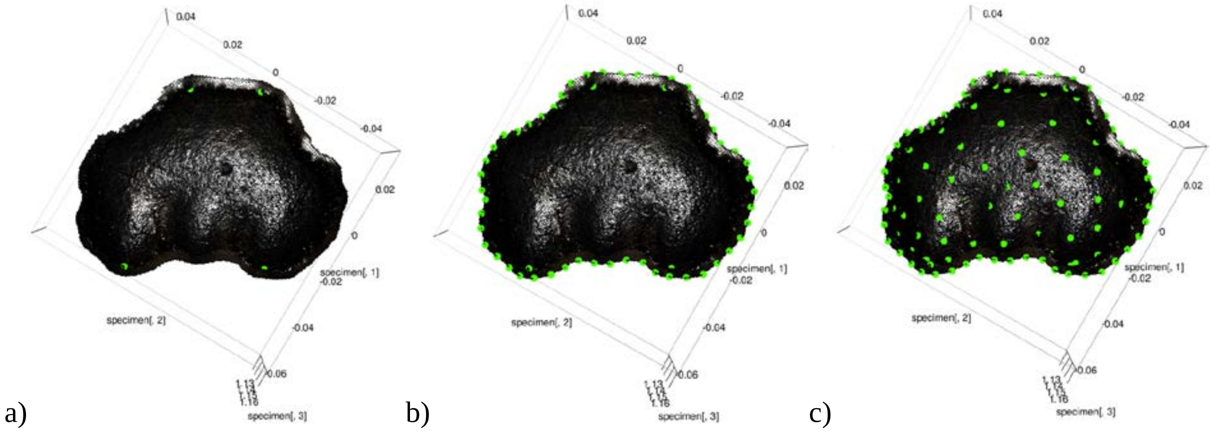


Figure 5. 3 - The three types of landmarks: (a) fixed landmarks (fixed), (b) fixed landmarks with curve-sliders (fixed-curves) and (c) fixed landmarks with curve- and surface-sliders (fixed-curves-surfaces) positioned on the segmented main pad of a paw.

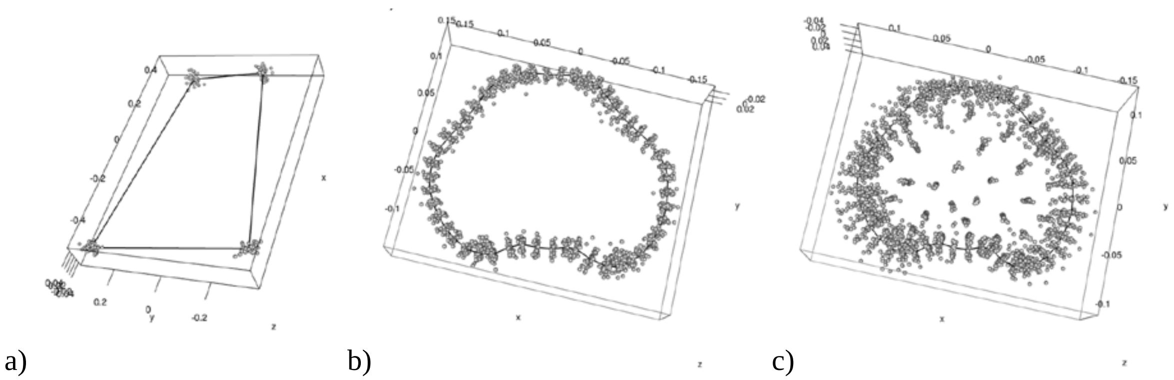


Figure 5. 4 - Procrustes coordinates using (a) fixed landmarks (fixed), (b) fixed landmarks with curve-sliders (fixed-curves) and (c) fixed landmarks with curve- and surface-sliders (fixed-curves-surfaces) on the segmented main pad from front left paws (40 specimens). The specimens (grey points) surround the mean shape points. Links between mean shape points of the fixed landmarks (a) and curve-sliders (b and c) were added for a better visualisation.

Data analysis

We applied a PCA on the Procrustes coordinates from each component from FL paws, HL paws and HL tracks to decrease the dimensionality and extract the principal components (PC) that explain most of the shape variations. These principal components and the centroid sizes represent the shape and size variables respectively. Size and shape variables provide information on the geometric form.

We used a Linear Discriminant Analysis (LDA) to assess the accuracy of prediction for the identification of the combination age-sex (i.e. juvenile female (JF), juvenile male (JM), subadult female (SAF), subadult male (SAM), adult female (AF) and adult male (AM)) from FL and HL paws. Additionally to the three types of landmarks, we used three types of variables in the LDA: (i) size variables, (ii) shape variables, and (iii) size and shape variables combined. We plotted the accuracy of prediction against the number of PC per component for two types of variables (shape or combination size-shape) and three types of landmarks (fixed, fixed-curves or fixed-curves-surfaces). Furthermore, we applied a LDA with jack-knife predictions (i.e. leave one out) to assess the accuracy of prediction for the identification from FL and HL paws of the combination age-sex using size and shape variables combined, and the three types of landmarks.

Using the features extracted through fixed landmarks with curve-sliders (i.e. fixed-curves), we plotted the combined centroid size (i.e. sum of the centroid sizes from the five components) for each category age-sex for FL and HL paws separately. We used a t-test to analyse the difference in combined centroid size of FL and HL paws between each possible pair of age-sex categories. We drew the mean shape for each age-sex category to visualise the shape variation of FL and HL paws between age group and sex classes. We applied a MANOVA to test the influence of the combination age-sex on the size and shape of the paws.

Among the 25 individuals, the number of individuals for which the age and sex was known was 10 (3 AM, 5 AF and 2 SAM). We applied the same discriminant analysis to identify the age, sex and individual lions using the size and shape variables extracted from the HL tracks by means of the three types of landmarks. For an increasing number of PC (i.e. increasing number of shape variables) per component, we plotted the accuracy of prediction for the identification age-sex and for the individual identification. We used a MANOVA to analyse the influence of the combination age-sex and individual identification on the size and shape of the tracks. Similarly to the paws, we used a LDA with jack-knife predictions to measure the accuracy of prediction for the identification from HL tracks of the combination age-sex and the individual, using size and shape variables combined, and the three types of landmarks.

RESULTS

Age and sex identification from paws

Using only the combined centroid size of each paw component, the LDA provided accuracy of prediction for the age-sex identification of 72.5% (fixed), 67.5% (fixed-curves) and 67.5% (fixed-curves-surfaces) for the FL paws. The accuracy of prediction for the age-sex identification for the HL paws reached higher levels of identification of 85.37% (fixed), 80.49% (fixed-curves) and 73.17% (fixed-curves-surfaces).

Independent of the number of PC per paw component, the use of size and shape variables combined in the LDA yielded higher accuracy of prediction for both FL (Figure 5.5a) and HL paws (Figure 5.5b). Age-sex accuracy of prediction, using size and shape variables combined extracted by means of the three types landmarks, increased with the number of shape variables (i.e. number of PC) that are added to the LDA. The accuracy reached 100% for both FL and HL paws (Figure 5.5).

The LDA with jack-knife predictions provided lower levels of accuracy (Table 5.3). The highest accuracy of prediction for the combination age-sex using size and shape variables combined reached 42.50% for the FL paws and 73.17% for the HL paws (Table 5.3). The former accuracy of prediction was reached through the use of fixed landmarks with curve-sliders and one PC per component, while the latter was reached through the use of fixed landmarks with one PC per component.

Size and shape variation between paws

For FL and HL paws, and for both sexes, the paw size (i.e. combined centroid size) increases with age (Figure 5.6). At any given age (i.e. juvenile, subadult or adult), the males have bigger paws than the females (Figure 5.6). The paw size tends to increase quicker with age for the males than for the females, for both FL and HL paws.

Across the different sexes, there is no difference in FL paw size between juvenile male and subadult female (*t*-test, $t = 1.57$, $df = 6$, $P = 0.17$), and between juvenile male and adult female (*t*-test, $t = 0.54$, $df = 12$, $P = 0.60$) (Figure 5.6a). As for the HL paw size, there is no difference between juvenile male and subadult female (*t*-test, $t = 1.73$, $df = 8$, $P = 0.12$) and between juvenile male and adult female (*t*-test, $t = -0.81$, $df = 14$, $P = 0.43$) (Figure 5.6b). An assessment of the mean shape indicates that the male paws appear broader than those of the females with a smaller gap between MP and toes (Figure 5.6). The front of the MP appears flatter for the males and rounder for the females (Figure 5.6).

Using the size and shape variables combined (with five PC per paw component) extracted through fixed landmarks with curve-sliders, we found that the combination age-sex significantly influences the size and shape of the FL (MANOVA, $F = 1.55$, $df = 5$, $P = 0.04$) and HL (MANOVA, $F = 1.66$, $df = 5$, $P = 0.02$) paws.

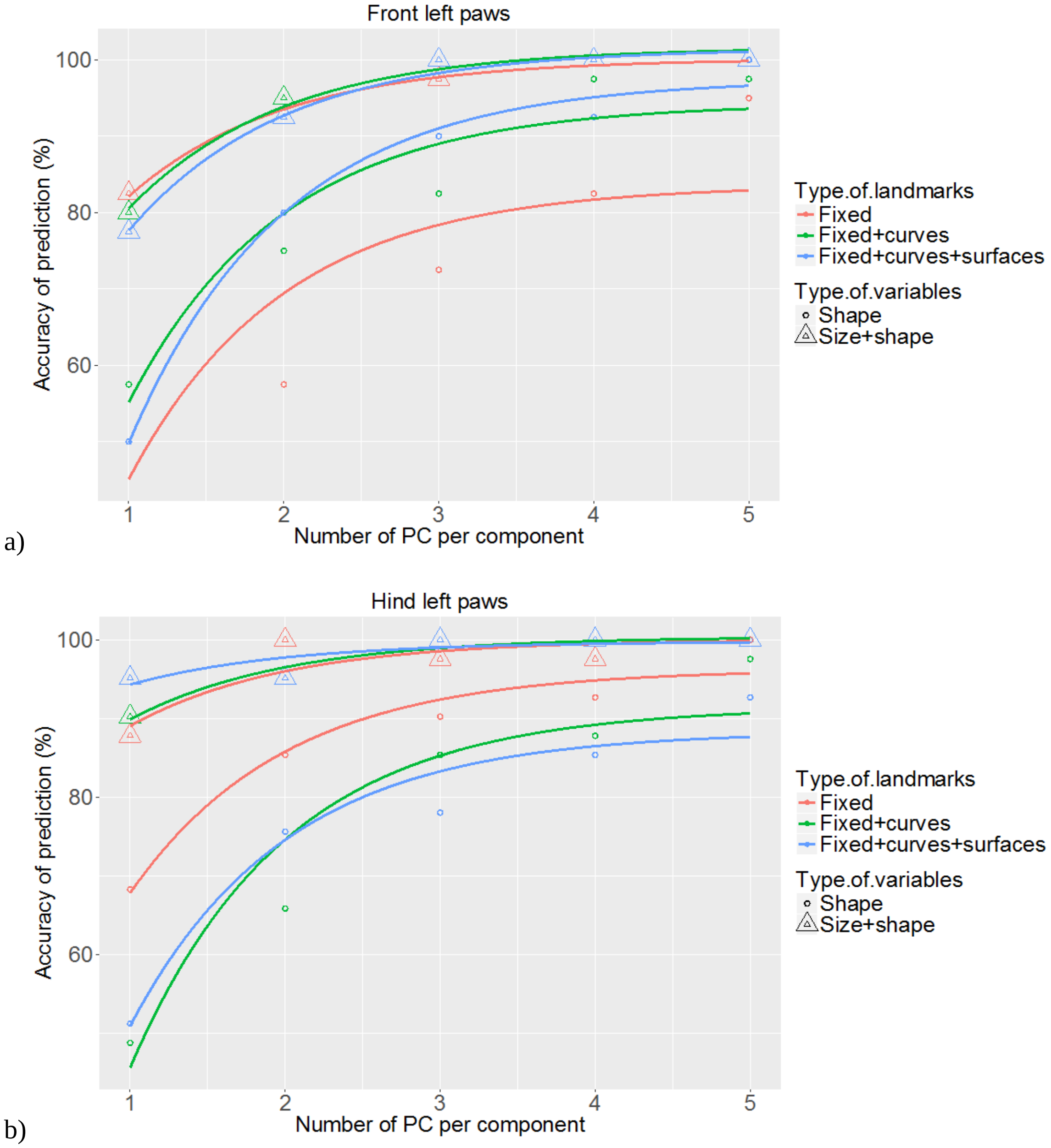


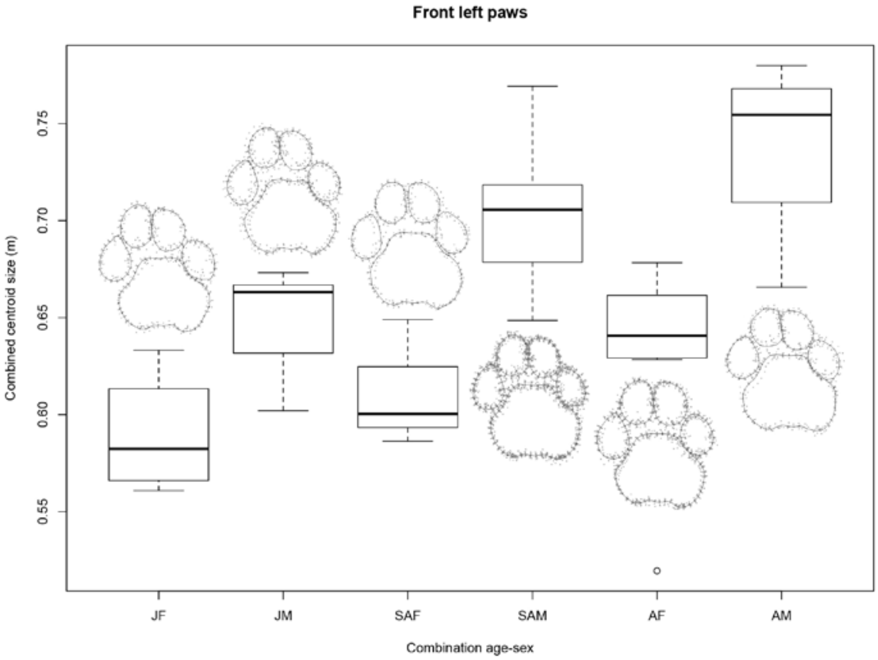
Figure 5. 5 - Accuracy of prediction (%) for the identification of the combination age-sex against the number of principal components (PC) (i.e. shape variables) per paw component for the (a) front left and (b) hind left paws. The types of landmarks are fixed, fixed with curve-sliders (fixed+curves) or fixed with curve- and surface-sliders (fixed+curves+surfaces). The types of variables are shape variables only (shape) or combination size and shape variables (size+shape). Negative exponential smooth curves were added for better visualisation of the trends.

Table 5. 3 - Maximum accuracy of prediction (%) for identification of the combination age-sex using Linear Discriminant Analysis (LDA) with jack-knife predictions for front left (FL) and hind left (HL) paws using three types of landmarks (fixed, fixed with curve-sliders, and fixed with curve- and surface-sliders). Both size and shape variables were used in the analysis and the number in brackets indicates the number of principal components (PC) per paw component for which the maximum accuracy of prediction was reached.

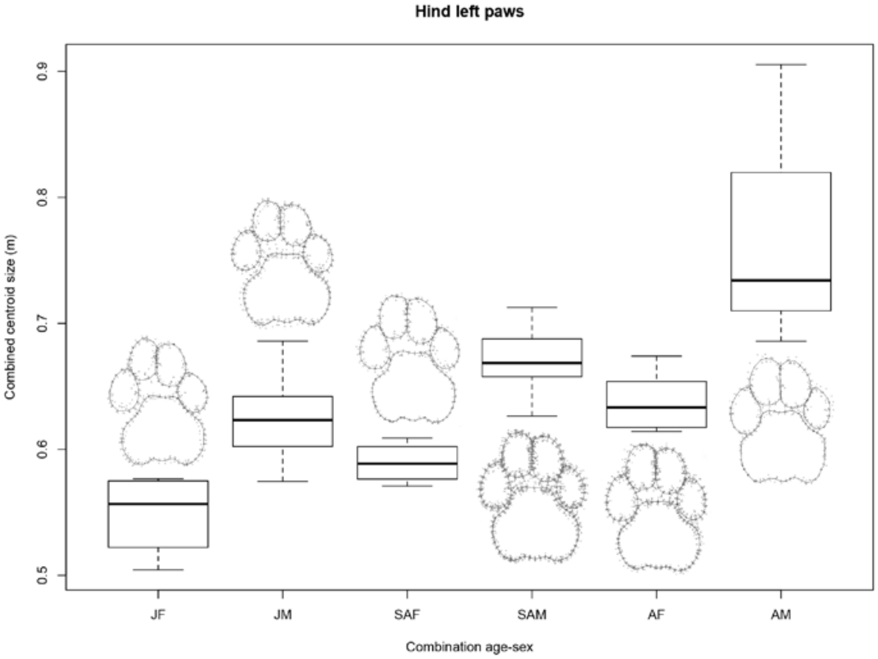
	FL paws	**HL paws**
Fixed	40.00% (4 PC)	73.17% (1 PC)
Fixed with curve-sliders	42.50% (1 PC)	58.54% (2 PC)
Fixed with curve- and surface-sliders	37.50 % (1 PC)	63.41% (2 PC)

Age, sex and individual identification from tracks

Similarly to the paws, the accuracy of prediction for the identification of the combination age-sex (Figure 5.7a) and individual (Figure 5.7b) increases with the number of PC that provides information on the track shape. The identification of the age and sex reached an accuracy of 100% when using fixed landmarks with curve- and surface-sliders for the feature extraction from the tracks (Figure 5.7a). The use of fixed landmarks with curve- and surface-sliders also provided higher accuracy of prediction at the individual level (Figure 5.7b). The highest accuracy of prediction for individual identification reached 97.41% (Figure 5.7b). Size and shape (with five PC per paw component) extracted through fixed landmarks with curve- and surface-sliders are significantly influenced by the combination age-sex (MANOVA, $F = 3.58$, $df = 2$, $P < 0.001$) and the individual identification (MANOVA, $F = 1.71$, $df = 24$, $P < 0.001$). The LDA with jack-knife predictions provided a maximum of 85.11% for the identification of the age-sex and 45.69% for the identification of the individual using fixed landmarks with curve- and surface-sliders in both cases (Table 5.4).

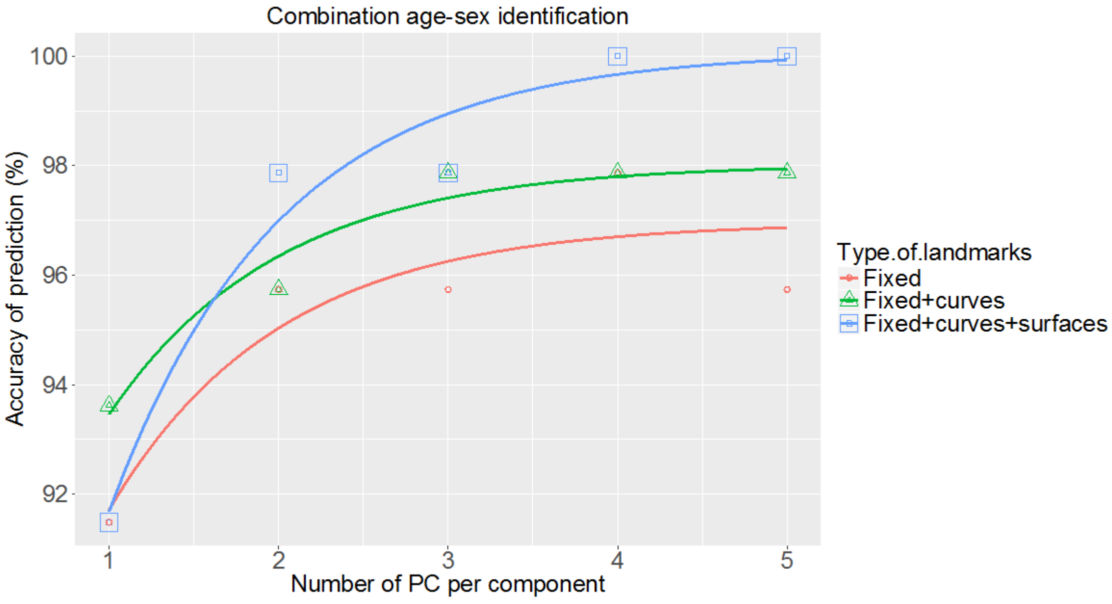


a)

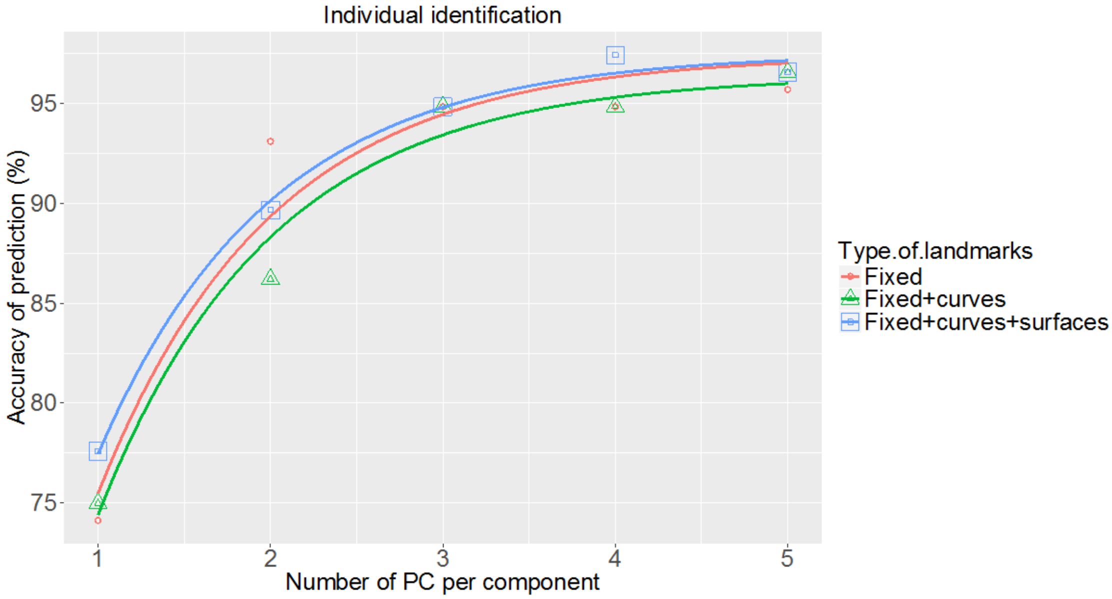


b)

Figure 5. 6 - Combined centroid size (m) and mean shape of each category age-sex for the (a) front left and (b) hind left paws. The categories are juvenile female (JF), juvenile male (JM), subadult female (SAF), subadult male (SAM), adult female (AF) and adult male (AM).



a)



b)

Figure 5. 7 - Accuracy of prediction (%) against the number of principal components (PC) (i.e. shape variables) for the (a) identification of the combination age-sex and (b) individual identification using hind left tracks. The type of variables is a combination size and shape and the types of landmarks are fixed, fixed with curve-sliders (fixed+curves) or fixed with curve- and surface-sliders (fixed+curves+surfaces). We added negative exponential smooth curves for better visualisation of the trends.

Table 5. 4 - Maximum accuracy of prediction (%) for identification of the combination age-sex and individual using Linear Discriminant Analysis with jack-knife prediction for hind left tracks using three types of landmarks (fixed, fixed with curve-sliders, and fixed with curve- and surface-sliders). Both size and shape variables were used in the analysis and the number in brackets indicates the number of principal components (PC) per paw component for which the maximum accuracy of prediction is reached.

	Combination age-sex	**Individual**
Fixed	78.72% (1 PC)	44.83% (3 PC)
Fixed with curve-sliders	78.72% (2 PC)	41.38% (3 PC)
Fixed with curve- and surface-sliders	85.11% (2 PC)	45.69% (4 PC)

DISCUSSION

Lion paws - and therefore tracks - contain characteristics that can be interpreted by indigenous trackers. To understand these characteristics and their variation across the different individuals, scientists need a reliable technique to record paws and tracks, and to extract the information they contain. Geometric morphometrics provides a useful tool for the extraction of both size and shape variables from lion paws and tracks. Regardless of the position of the paw (i.e. FL or HL) and the type of landmarks used for feature extractions (i.e. fixed, fixed-curves or fixed-curves-surfaces), the accuracy of prediction reached 100% when using both size and shape variables in the LDA (Figure 5.5). The exclusive use of size or shape variables in the same discriminant analysis yielded less accurate predictions. This can be explained by the overlap between the paw sizes of certain age-sex categories, particularly between juvenile males, subadult females and adult females. In other words, the size and shape alone do not entirely explain the differences between age classes and sex groups, but they do explain these differences when examined together as each age-sex category presents a unique combination of size and shape variables (i.e. a unique form).

Using an LDA with jack-knife predictions, the accuracy to identify the age-sex category from paws was greater for the HL paws (73.17%) than for the FL paws (42.50%) (Table 5.3). We can thus conclude that the hind paws contain more information about the age and sex of the individuals than the front paws. This represents an advantage as the tracks made by the front paws are often highly distorted due to the centre of mass located in the front part of the body of most quadrupeds. Furthermore, front tracks may be missing due to direct registration.

The addition of information from curves and surfaces do not hold any improvements for the identification of the age and the sex of the individual from their paws. The information provided by the morphological points (i.e. fixed landmarks) appears sufficient to explain the variation of the paw forms (i.e. size and shape) between age and sex classes. Additional information on curves and surfaces may introduce noise into the discriminant analysis, thus decreasing the accuracy.

In the case of the tracks, the situation is different. For both the LDA and LDA with jack-knife predictions, the accuracy of the prediction of the age-sex category from HL tracks increase with additional information from curves and surfaces. This may be explained by the difference of weight in the age classes and between the two sexes. For example, an adult male weighs on average ~190 kg, while an adult female weighs ~126 kg. In soft substrates, such as sand, the weight inevitably affects the penetration depth of the tracks and the shape of the curves around the main pad and toes, which are made of thick elastic masses of connective tissue. Similarly to age-sex identification from tracks, the two types of LDA offered higher levels of accuracy for individual identification when the information originated from points, curves, and surfaces. Weight, but also the individual manner of walking may influence the way tracks are cast into the sand, and the information obtained from fixed landmarks is not sufficient to explain these individual variations.

Despite 97.41% of predicted individuals being correctly identified by an allocation model built from all the observations (Figure 5.7a), the jack-knife prediction for the individual identification of 25 individuals does not exceed 45% (Table 5.4). This may be due to a sample size that is too small (i.e. insufficient repetitions of tracks made by the same paw from one individual) to capture the complexity of the variations. These variations are particularly high as tracks were sampled from wild lions in sub-optimal conditions. Previous studies using photographs and traditional morphometrics revealed high accuracy (>90%) for individual identification from tracks made by mountain lions (Smallwood and Fitzhugh 1993; Grigione et al. 1999; Jewell et al. 2014) and tigers (Sharma et al. 2005). However, these studies were either limited to captive animals where tracks were sampled off optimal artificial substrates, from a small number of wild individuals and/or dusty roads producing shallow tracks. The introduction of 3D has improved facial recognition methods (Chang et al. 2003) by removing parallax errors and the influence of light on the recording techniques. We believe that digital photogrammetry and geometric morphometrics can provide higher accuracy if we increase the sample size and introduce the use of more sophisticated statistical methods such as neural networks. The potential identification of the age, sex, and individual from their tracks will enable the non-invasive monitoring of lions. Furthermore, the low-cost and practical aspect of this track recording technique will enable the longitudinal sampling of data in remote and resourceless areas. This innovative method can involve traditional trackers and local community members in the conservation efforts.

Chapter 6 - Synthesis and conclusions

Figure 6. 1 - A lioness stares through the bushes in Hluhluwe-iMfolozi Park, South Africa.

The first chapter explained the importance of ecological monitoring and carnivore conservation. We introduced the emblematic lion and the various methods currently used to monitor their populations. We then presented the art of tracking and the use of tracks in wildlife studies. Our study sites, fieldwork and data collection details were also described in this chapter along with the aims and structure of the thesis.

In Chapters 2 and 3, we showed that digital close-range photogrammetry provides a useful tool to enable the 3D recording of lion paws, tracks and trails in the field. A trail reflects the manner in which an individual walks. When comparing different lions moving with the same gait under the same conditions in terms of the substrate and terrain, the trail variables provide indications of the sex and identity of the individuals. These variations are due to anatomical and morphological variations between the sexes, and to the specific manner in which each individual walks. Investigating trails, rather than single tracks, enables us to gain information from the tracks that may not have been of sufficient quality on their own due to substrate conditions. In the case where tracks are individually readable, the information gathered from trails could be used to cross-validate or supplement the information from the tracks themselves. Furthermore, trail variables could provide information on body condition that would not always be visible in single tracks, such as the tendency to limp (due to sickness, wounds or snares), pregnancy or satiety (see ‘Feeding ecology’ and ‘Status, threats and diseases’ in Chapter 1). Contrary to the dated time consuming method of sampling with a measuring tape which is prone to manipulator bias and may be destructive to the tracks, trail sampling using photogrammetry enables digital replications of entire trail sections. This digital solution facilitates feature extraction and the storage of information. A significant number of variables such as distances, angles and areas, that are practically impossible to measure in the field, can be extracted from the digital replica. In the case of paws and tracks, the 3D replicas provide crucial information on the thickness of the paws and the depth of the tracks. The use of contour lines or gradient colours indicating depth enables a better visualisation of the track delineation. Under natural light, the boundary between a track and the substrate that allows its existence is often unclear due to poor contrast in both colour and texture. This contrast is more visible for the paws where the main pad and toes are bounded by fur, therefore, allowing the segmentation of the paws directly on the photographs before 3D reconstruction. At the present level of study, the depth information assists the manual segmentation of the tracks. However, future studies would be able to use this information to automate the segmentation process.

Once the paws and tracks are delineated, their features may be extracted by means of basic metrics such as lengths, angles and areas. This is the approach used in traditional morphometrics that was widely used in previous studies involving track measurements. However, these basic metrics convey little information on the geometric structure and fail to measure the variation along curves and surfaces. To overcome these limitations, we have used geometric morphometrics that permits the comprehensive study of the form (i.e. size and shape) variations among paws and tracks. Using only the shape variables extracted from fixed landmarks, Chapter 4 showed that paws and tracks present sufficient shape variation to identify their position along the anteroposterior (front or hind) and mediolateral (right or left) axis. The foot identification by means of discriminant analysis attained better prediction accuracies for the paws than for the tracks. This was due to the noise resulting from the complex interaction between paw and substrate. The correct identification of the foot that created each track will enable reliable extraction of the trail variables and more objective comparisons between individuals i.e. by comparing tracks made by the same foot (e.g. hind left tracks). In Chapter 5, we compared the size and shape variation of front left and hind left paws, and hind left tracks from different individuals of various ages and sexes. Using the size and/or shape variables extracted by means of fixed landmarks, fixed landmarks with curve-sliders, or fixed landmarks with curve- and surface-sliders, we investigated whether curves and surfaces provided valuable information to discriminate the individuals according to their age and sex from their paws, or according to their age, sex and identity from their tracks. The independent use of size and shape variables did not provide as clear discriminations compared to the use of a combination of both types of variables. The hind paws offered more characteristics related to the age and sex of the individuals than the front paws, while the curves and surfaces did not provide additional information to describe the differences between females and males, and juveniles, subadults and adults. The situation was different for the tracks where the information from the curves and surfaces enabled better discrimination between age and sex categories, and between individuals. This can be explained by the differences in anatomy, morphology and individual motion pattern that influence both the depth and the contour of the tracks. The accuracy of prediction for the identification of the individual (among 25 individuals) using a Linear Discriminant Analysis with jack-knife predictions is no higher than 50%. However, we strongly believe that individual discrimination may be improved through the implementation of more advanced statistical methods, such as neural networks, that necessitate bigger sample size. The accuracy of prediction (using jack-knifed predictions) for the identification of the age-sex category reached 73.17% and 85.11% from paws and tracks respectively.

As continuous improvement is an important part of science, we recommend that future studies should focus on the following points:

- Measure the manipulator bias that can influence the 3D recording technique of paws, tracks and trails, as well as the manual segmentation and landmarking;
- Analyse the impact of environmental factors, such as sun inclination and cloud cover, on the photographs, and thus, on the reconstructed 3D models;
- Test various digital cameras across the quality/price range, compare different photogrammetric packages (including freeware), and investigate the possibility of using videogrammetry (i.e. the source data are videos) and lasergrammetry;
- Decrease or eliminate the human input by automating the segmentation and landmarking processes;
- Take a step backward to compare the identification algorithms based on 2D versus those based on 3D;
- Refine the age identification using known aging traits (see ‘Monitoring lions’ in Chapter 1; Miller et al. 2016) or by working with captive animals;
- Integrate track and trail variables into identification algorithms;
- Expand on the age identification from trails;
- Study the influence of the body condition, substrate and terrain on tracks;
- Introduce the use of unsupervised classification methods for which initial reference groups are not necessary;
- Apply the method to population ecology in different contexts and compare the results with well-established methods.

Monitoring lions through their tracks is non-invasive, as it does not imply any kind of contact between observer and studied individuals. For invasive methods that involve direct observations, the mere presence of the observer can affect natural animal behaviour. Other invasive methods, for which capture, immobilisation and handling are necessary, can have negative effects such as pain, suffering and distress for the individuals (Alibhai et al. 2001; Hawkins 2004). A common technique to identify individual lions is to mark them using a hot brand (see ‘Monitoring lions’ in Chapter 1). However, the effects of such mutilation technique have not been evaluated (Murray and Fuller 2000). The use of attached or implanted devices introduces tagging effects that may cause physical and physiological impacts (Hawkins 2004; Wilson and McMahon 2006; McIntyre 2015). Furthermore, the colour and odour of the device can expose the tagged individuals to increased levels of predation (Hawkins 2004). The use of tracks in wildlife monitoring will decrease the implementation of invasive methods but will never entirely replace them. For example, when a GPS-collared lion breaks out of a protected area, the park management can almost instantaneously be aware of the escape and quickly locate the individual. Additionally, telemetry enables the analysis of large-scale animal movement, the location of animals during key life stages (e.g. dening), the access of carcasses for post-mortem examination, the study of kill sites and the habitat use (MacKay et al. 2008). Invasive methods are expensive, labour intensive and can be logistically difficult to implement. For example, in many countries capturing wild animals necessitates an ethics clearance and a wildlife veterinarian to be present.

Identifying the species, age, sex, body condition and individual from tracks, either from single tracks and/or trails, may be integrated into survey methods for monitoring populations. Here, we can differentiate two main categories depending on the context in which the tracks are sampled: (i) natural tracks originating from the normal daily activities of an animal (Heinemeyer et al. 2008) or (ii) tracks sampled on prepared track stations where the animal may have been lured by an attractant (Ray and Zielinski 2008). Track stations overcome one of the weaknesses of natural tracks that are limited by the availability of adequate substrate and conditions for tracking.

The most basic form of track surveys, using either natural tracks or track stations, aims to confirm the presence of a species in a certain area (i.e. occurrence). Under certain conditions and using standardisation, they can also provide information on species distribution, relative and absolute abundance, habitat use and trends in population status (Heinemeyer et al. 2008; Ray and Zielinski 2008).

Assessing the occurrence, distribution and relative abundance only requires a correct identification of the species from tracks. Single location surveys aim at detecting at least a single individual to confirm the presence of a species of interest in a certain area. To save time, scientists should first look at the habitat preference of the target species in order to focus their efforts in areas where they will most likely find evidence of species presence (Gese 2001). In the case of lions, efforts at finding tracks should be localised in their favoured habitats and near waterholes (see ‘Distribution and habitat’ and ‘Feeding ecology’ in Chapter 1). Gathering information from several single location surveys or from a survey at multiple locations enables the assessment of a species distribution.

Occurrence surveys can be concluded once the species is detected. However, it is more difficult to judge the duration of the survey if the species has not yet been detected. Failing to detect a species that is actually present leads to false-negative error (i.e. false absence). The probability to commit that type of error is $1-p$, with p being the detectability (i.e. probability to detect a species that is present). Occupancy modelling permits the estimation of the detectability by using repeat sampling occasions at multiple sites (i.e. providing detection or encounter history) (Long and Zielinski 2008). The detectability is then used to adjust the presence estimate into an occupancy probability by incorporating the effects of false absences (MacKenzie et al. 2006). Detection-nondetection survey data can be linked to site-specific covariates (e.g. prey biomass, habitat class, law-enforcement effort, as well as the proximity to water, park boundary, external human activities, tourist camps, hunting camps and authority outposts) in order to predict species occurrence at unsurveyed locations (MacKenzie et al. 2006; Long and Zielinski 2008; Midlane et al. 2014). Assessing the occurrence and distribution of lions in Africa seems to be a priority as there are many gaps where the species presence is still unknown (see 'Distribution and habitat' in Chapter 1; Figure 1.2).

Counting natural tracks along transects (e.g. riverbeds and roads) or considering the proportion of track stations that receive a visit can provide indices of relative abundance. Under certain conditions, there is a positive relationship between the relative and absolute abundance (Schwarz and Seber 1999; Gese 2001). Unfortunately, this relationship and the sensitivity of a particular index to true changes in absolute abundance have rarely been tested (Heinemeyer et al. 2008). Furthermore, many factors can affect the quantity of tracks and associated detections: conditions linked to the animals (behaviour, status and movement), to the environment (habitat, substrate, season and weather) and to the survey (manipulator bias) (Wilson and Delahay 2001; Hayward et al. 2005; Heinemeyer et al. 2008; Ray and Zielinski 2008; see 'Ecological monitoring' in Chapter 1).

Identifying individuals from their tracks can be used for total counts in small populations. In the case of lions that tend to live in prides (see 'Social and spatial behaviour' in Chapter 1), the total count using tracks can be carried out pride per pride, and can also be added to information gathered during direct observations. For example, when a pride of lions is located opportunistically or by using telemetry, it is often difficult to count the exact number of individuals, to identify the age and sex of each individual and to assess their body condition. In that case the manipulator could wait for the lions to move off and then collect their tracks to extract the missing information. Furthermore, identifying the age, sex and body condition of each identified individual would provide information on the age structure, sex ratio and general condition of each pride and sub-population. If a total count is not possible, the individual identification from tracks may be used in a capture-recapture framework, either along transects or at track stations, to estimate the absolute abundance (see 'Monitoring lions' in Chapter 1). Capture-recapture models are based on the assumption of population closure (i.e. there are no variations in population size during the survey). The sampling approach involves either two (known as Lincoln-Peterson model) or multiple sampling occasions (known as K-sample) (Long and Zielinski 2008). The latter enables the modelling of heterogeneity in capture probability (i.e. detectability). The ultimate goal of any capture-recapture approach is to maximise detectability while minimising detection heterogeneity. The variation in detection probabilities may be linked to time of the day, animal behaviour (e.g. detection response), individual characteristics (e.g. age, sex and body condition) and site-specific properties. The correct identification of the age, sex and body condition of the captured individuals from their tracks would enable the detection variability to be accounted for during statistical analysis (Long and Zielinski 2008; Amstrup et al. 2010). A new approach that has not yet been tested on carnivore datasets is the abundance-induced heterogeneity model (Long and Zielinski 2008). This approach is based on the assumption that detectability and abundance are positively related, therefore allowing direct estimation of absolute abundance to be derived from detection-nondetection data (i.e. occupancy surveys) (Royle and Nichols 2003; Long and Zielinski 2008). Monitoring can be defined as the “repeated assessment of the status of some quantity, attribute or task within a defined area over a specified amount of time” (Thompson et al. 1998). Monitoring programmes, often referred to as trend studies, baseline monitoring, inventory monitoring or long-term ecological studies, enable the detection of changes in distribution, occupancy, relative abundance or abundance (Long and Zielinski 2008; see ‘Ecological monitoring’ in Chapter 1). Monitoring is sometime linked to adaptive management programmes to decide if specific management activities should be modified or not (Long and Zielinski 2008).

Tracks could potentially be used in the various above-mentioned surveys, including monitoring surveys. The choice of the survey type and its design will depend on the survey objectives, the ecology and population status of the target species, the location of the study site and logistical constraints (e.g. time, financial and human resources). Thus, any surveys which may include animal tracks should first take all these considerations into account.

Tracks offer a practical low-cost alternative that will enable longitudinal data sampling. The recording technique presented in this thesis uses digital close-range photogrammetry. It follows a simple sampling protocol, requiring only a digital camera and a scale in the field. Due to its affordability, practicability and low logistical requirements, this recording technique can be implemented in remote resourceless areas. It will be possible for park staff to opportunistically sample tracks during their daily routine. Local community members and traditional trackers who live in the vicinity of protected areas may be encouraged to participate in the conservation effort by locating and sampling tracks. Furthermore, citizen scientists from around the world, whether they already have a background in tracking or not, can participate in the global effort by recording tracks from different species. The tracks that are digitally replicated in 3D may be stored and shared using online virtual catalogues, therefore, facilitating data sharing and international collaboration.

The method that has been applied in this study on lions may be extended to any species with a sufficiently complex foot anatomy, as in most of the digitigrades and plantigrades. Humans can be one of these studied species, particularly when we realise that anti-poaching units across the world still use out-dated plaster casts to record poachers’ footprints. The extension of this method to other carnivore species would represent a significant advantage in their conservation, which is a critical aspect of biodiversity preservation (see ‘Carnivore conservation’ in Chapter 1).

Tracks are often the only evidence left by problem animals that have predated on livestock or raided crops in farmlands or community lands surrounding protected areas. The identification of the species, age, sex, body condition and/or individual identity of these problem animals will enable a better understanding of the human-wildlife conflict. This would also decrease the indiscriminate killing of the wrong individual or species. Furthermore, this correct species identification from tracks will assist park managers who are sometimes required to financially compensate local community members and farmers for the loss of livestock caused by specific species. An example of this occurs in the periphery of Hluhluwe-iMfolozi Park where the local community members receive compensation when African wild dogs that have escaped from the park predate on their livestock. However, in many instances the African wild dogs are wrongly blamed when the livestock are actually predated upon by stray domestic dogs or spotted hyenas.

By promoting the conservation of animals and land, trophy hunting can play an important role in nature conservation (Miller et al. 2016). On the other hand, unsustainable hunting can lead to species decline (Packer et al. 2009). Sustainable trophy hunting of lions relies on the ability of aging *pre-mortem* quarry in the field with high precision (Miller et al. 2016). The accurate age identification from tracks would represent a useful tool for professional hunters.

The innovative method investigated in this thesis includes the use of (i) digital close-range photogrammetry to record lion paws, tracks and trails, (ii) traditional and geometric morphometrics to extract their features, and (iii) multivariate analysis to identify the foot, age, sex and/or individual from size and/or shape variables.

It is important to note that this research is a first attempt to explore the possibilities of monitoring lions through digital 3D models of their tracks. As mentioned above, further studies will need to focus on the variation of tracks and trails across different substrates and terrains, the impact of manipulator bias and camera types on the recording technique, the automation of track segmentation and landmark digitisation, and the use of advanced statistical analyses in the identification algorithms. Furthermore, continuous and rapid developments in digital photogrammetry, computer vision, laser scanning, morphometrics and multivariate statistics will permit further improvements of this method.

The science of conservation biology is often considered as a crisis discipline (Soulé 1985). As the problems increase, and they will do so exponentially, innovative solutions must be implemented to preserve our biodiversity. Among this biodiversity, carnivores may be considered as a ‘renaissance taxa' involving conservation problems, causal factors and solutions (Gittleman et al. 2001). The loss of apex carnivores may be humankind's most prevalent influence on nature (Estes et al. 2011) and we, as humans, are part of the environment that we need to conserve for our collective good (Ehrlich and Ehrlich 1992).

Tracks are ubiquitous in our life, from forensic to anthropology and palaeontology, they provide the only evidence left by criminals, they help us to understand the origin of mankind and they remind us that dinosaurs inhabited the planet before us. They are there to commemorate the stars of Hollywood and to mark the first step of mankind on the moon. In the field, tracks remind us that we share our world with other living creatures. They also remind us that these were the very same tracks that our ancestors, and perhaps humans' predecessor *Homo erectus*, learnt to follow in order to survive and to evolve into the modern humans that we are today (Smillie 2016). As a signature of their passage, tracks contain crucial information about the animals that made them. Only experienced trackers can interpret their meaning as it has been done since the rise of mankind, over the last two-million years (Smillie 2016). It is our privilege to conclude with a quote by Louis Liebenberg: 'the Art of Tracking may well be the origin of science. After hundreds of thousands of years, traditional tracking skills may soon be lost. Yet tracking can be developed into a new science with far-reaching implications for nature conservation' (Liebenberg 1990a).

References

Adams DC, Collyer ML, Sherratt E (2015) geomorph: Software for geometric morphometric analyses. R package version 3.0.1. http://cran.r-project.org/web/packages/geomorph /index.html. Accessed 26 June 2016

Adams DC, Otárola-Castillo E (2013) geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods in Ecology and Evolution 4:393–399.

Agisoft LLC (2014a) Agisoft PhotoScan User Manual, Professional Edition, Version 1.1. Agisoft LLC. http://www.agisoft.com/pdf/photoscan-pro_1_1_en.pdf. Accessed 1 August 2015

Agisoft LLC (2014b) PhotoScan: fully automated professional photogrammetric kit. Agisoft LLC. http://airgon.com/Agisoft_PhotoScan.pdf. Accessed 1 August 2015

Alexander RM (1984) The gaits of bipedal and quadrupedal animals. The International Journal of Robotics Research 3:49–59.

Alibhai SK, Jewell ZC, Law PR (2008) A footprint technique to identify white rhino *Ceratotherium simum* at individual and species levels. Endangered Species Research 4:205–218.

Alibhai SK, Jewell ZC, Towindo SS (2001) Effects of immobilization on fertility in female black rhino (*Diceros bicornis*). Journal of Zoology 253:333–345.

Allen GM (1942) Extinct and vanishing mammals of the western hemisphere: with the warine species of all the oceans. American Committee for International Wild Life Protection, New York

Allen L, Engeman R, Krupa H (1996) Evaluation of three relative abundance indices for assessing dingo populations. Wildlife Research 23:197–205.

Amstrup SC, McDonald TL, Manly BF (2010) Handbook of capture-recapture analysis. Princeton University Press, Princeton

Balme GA, Hunter LT, Slotow R (2009) Evaluating methods for counting cryptic carnivores. Journal of Wildlife Management 73:433–441.

Barnett R, Yamaguchi N, Shapiro B, et al (2014) Revealing the maternal demographic history of *Panthera leo* using ancient DNA and a spatially explicit genealogical analysis. BioMed Central Evolutionary Biology 14:1–11.

Barnosky AD, Matzke N, Tomiya S, et al (2011) Has the Earth's sixth mass extinction already arrived? Nature 471:51–57.

Bauer H (2007) Status of large carnivores in Bouba Ndjida National Park, Cameroon. African Journal of Ecology 45:448-450.

Bauer H, Chapron G, Nowell K, et al (2015) Lion (*Panthera leo*) populations are declining rapidly across Africa, except in intensively managed areas. Proceedings of the National Academy of Sciences 112:14894–14899.

Bauer H, Packer C, Funston PF, et al (2016) *Panthera leo*. The IUCN Red List of Threatened Species. http://www.iucnredlist.org. Accessed 1 August 2016.

Bauer H, Van Der Merwe S (2004) Inventory of free-ranging lions *Panthera leo* in Africa. Oryx 38:26–31.

Beier P, Cunningham SC (1996) Power of track surveys to detect changes in cougar populations. Wildlife Society Bulletin 24:540–546.

Bennett M, Reynolds S, Morse S, Budka M (2016) Laetoli's lost tracks: 3D generated mean shape and missing footprints. Scientific Reports 6:1–8.

Bennett MR, Falkingham P, Morse SA, et al (2013) Preserving the impossible: conservation of soft-sediment hominin footprint sites and strategies for three-dimensional digital data capture. PloS one 8:e60755.

Bennett MR, Harris JW, Richmond BG, et al (2009) Early hominin foot morphology based on 1.5-million-year-old footprints from Ileret, Kenya. Science 323:1197–1201.

Bennett MR, Morse SA, Liutkus-Pierce C, et al (2014) Exceptional preservation of children's footprints from a Holocene footprint site in Namibia. Journal of African Earth Sciences 97:331–341.

Bertram BC (1973) Lion population regulation. African Journal of Ecology 11:215–225.

Bicchieri MG (1972) Hunters and gatherers today. Holt, Rinehart and Winston, New York

Blurton-Jones N, Konner MJ (1976) !Kung knowledge of animal behavior. In: Lee RB, De Vore I (eds) Kalahari hunter-garherers. Harvard University Press, Cambridge, pp 325–348

Bookstein FL (1991) Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge

Bothma J du P, Le Riche E (1993) Disturbance bias when tracking Kalahari leopards *Panthera pardus* by spoor. Koedoe 36:109–112.

Bothma J du P, Le Riche E (1995) Evidence of the use of rubbing, scent-marking and scratching-posts by Kalahari leopards. Journal of Arid Environments 29:511–517.

Bothma J du P, Le Riche EA (1984) Aspects of the ecology and the behaviour of the leopard *Panthera pardus* in the Kalahari desert. Koedoe 27:259–279.

Bothma J, Le Riche E (1990) The influence of increasing hunger on the hunting behaviour of southern Kalahari leopards. Journal of arid environments 18:79–84.

Brain C, Forge O, Erb P (1999) Lion predation on black rhinoceros (*Diceros bicornis*) in Etosha National Park. African Journal of Ecology 37:107–109.

Breitenmoser U, Mallon DP, Ahmad Khan J, Driscoll C (2008) *Panthera leo ssp. persica*. The IUCN Red List of Threatened Species. http://www.iucnredlist.org. Accessed 1 August 2016.

Bridgeford P (1985) Unusual diet of the lion *Panthera leo* in the Skeleton Coast Park. Madoqua 14:187–188.

Brooks S (2000) Re-reading the Hluhluwe-Umfolozi game reserve: constructions of a ‘natural’ space. Transformation 44:63–79.

Bull ME, Kennedy-Stoskopf S, Levine JF, et al (2003) Evaluation of T lymphocytes in captive african lions (*Panthera leo*) infected with feline immunodeficiency virus. American journal of veterinary research 64:1293–1300.

Carbone C, Mace GM, Roberts SC, Macdonald DW (1999) Energetic constraints on the diet of terrestrial carnivores. Nature 402:286–288.

Cardillo M, Mace GM, Jones KE, et al (2005) Multiple causes of high extinction risk in large mammal species. Science 309:1239–1241.

Cardillo M, Purvis A, Sechrest W, et al (2004) Human population density and extinction risk in the world’s carnivores. PLoS Biology 2: 0909–0914.

Caro T (2010) Conservation by proxy: indicator, umbrella, keystone, flagship, and other surrogate species. Island Press, Washington

Carrier DR (1984) The energetic paradox of human running and hominid evolution. Current Anthropology 483–495.

Castley J, Knight M, Mills M, Thouless C (2002) Estimation of the lion (*Panthera leo*) population in the southwestern Kgalagadi Transfrontier Park using a capture–recapture survey. African Zoology 37:27–34.

Caughley G (1977) Analysis of vertebrate populations. Wiley, New York

Ceballos G, Ehrlich PR (2002) Mammal population losses and the extinction crisis. Science 296:904–907.

Ceballos G, Ehrlich PR, Barnosky AD, et al (2015) Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances 1:e1400253.

Chambers CM, Whitehead JC (2003) A contingent valuation estimate of the benefits of wolves in Minnesota. Environmental and Resource Economics 26:249–267.

Chang K, Bowyer K, Flynn P (2003) Face recognition using 2D and 3D facial data. In: Workshop on Multimodal User Authentication. Association for Computing Machinery, p 25–32

Chapron G, Kaczensky P, Linnell JD, et al (2014) Recovery of large carnivores in Europe's modern human-dominated landscapes. Science 346:1517–1519.

Chardonnet P (2002) Conservation of the African lion: contribution to a status survey. International Foundation for the Conservation of Wildlife, Paris and Conservation Force, Metairie

Charlton-Perkins W, De la Harpe R (1995) Hluhluwe-Umfolozi Park. Struik, Cape Town

Choudhury S (1970) Let us count our tigers. Cheetal 14:41–51.

Choudhury S (1972) Tiger census in India. Cheetal 15:67–84.

CloudCompare (2015) CloudCompare, Version 2.6.1. EDF, R&D, Telecom, ParisTech. http://www.cloudcompare.org/. Accessed 1 August 2015

Creel S, Creel N (1997) Lion density and population structure in the Selous Game Reserve: evaluation of hunting quotas and offtake. African Journal of Ecology 35:83–93.

Cruikshank K, Robinson T (1997) Inheritance of the white coat colour phenotype in African lions (*Panthera leo*). In: Symposium on lions and leopards as game ranch animals. Wildlife group of the South African Veterinary Association, p 92–95

Cunado D, Nixon MS, Carter JN (2003) Automatic extraction and description of human gait models for recognition purposes. Computer Vision and Image Understanding 90:1–41.

Dagg AI (1973) Gaits in mammals. Mammal Review 3:135–154.

Day JJ, Norman DB, Upchurch P, Powell HP (2002) Biomechanics: dinosaur locomotion from a new trackway. Nature 415:494–495.

De Angelo C, Paviolo A, Di Bitetti MS (2010) Traditional versus multivariate methods for identifying jaguar, puma, and large canid tracks. Journal of Wildlife Management 74:1141–1153.

De Bruyn PJN, Bester MN, Carlini AR, Oosthuizen WC (2009) How to weigh an elephant seal with one finger: a simple three-dimensional photogrammetric application. Aquatic Biology 5:31–39.

De Vos JM, Joppa LN, Gittleman JL, et al (2014) Estimating the normal background rate of species extinction. Conservation Biology 29:452–462.

East R (1984) Rainfall, soil nutrient status and biomass of large African savanna mammals. African journal of Ecology 22:245–270.

Edwards G, De Preu N, Shakeshaft B, Crealy I (2000) An evaluation of two methods of assessing feral cat and dingo abundance in central Australia. Wildlife Research 27:143–149.

Ehrlich PR, Ehrlich AH (1992) The value of biodiversity. Ambio 21:219–226.

Eisenberg JF (1981) The mammalian radiation: An analysis of trends in evolution, adaptation, and behavior. University of Chicago Press, Chicago

Elliot W (1987) Possible predation of a black rhinoceros calf by a lion. Lammergeyer 38:68.

Eloff F (1973) Ecology and behavior of the Kalahari lion. The world’s cats 1:90–126.

Eloff F (1984) Food ecology of the Kalahari lion *Panthera leo vernayi*. Koedoe 27:249–258.

Eloff F (2002) Hunters of the dunes: the story of the Kalahari lion. Sunbird Publishers, Cape Town

Estes JA, Terborgh J, Brashares JS, et al (2011) Trophic downgrading of planet Earth. Science 333:301–306.

Estes R (1991) The behavior guide to African mammals. University of California Press, Berkeley

Fahlman A, Loveridge A, Wenham C, et al (2005) Reversible anaesthesia of free-ranging lions (*Panthera leo*) in Zimbabwe. Journal of the South African Veterinary Association 76:187–192.

Falkingham PL (2012) Acquisition of high resolution three-dimensional models using free, open-source, photogrammetric software. Palaeontologia Electronica 15:1–15.

Falkingham PL, Bates KT, Farlow JO (2014) Historical photogrammetry: Bird’s paluxy river dinosaur chase sequence digitally reconstructed as it was prior to excavation 70 years ago. PloS one 9:e93247.

Ferreira SM, Funston PJ (2010) Estimating lion population variables: prey and disease effects in Kruger National Park, South Africa. Wildlife Research 37:194–206.

Fitzhugh EL, Gorenzel WP (1985) Design and analysis of mountain lion track surveys. Transactions of the Western Section of the Wildlife Society 21:78–87.

Fosbrooke H (1963) The stomoxys plague in Ngorongoro, 1962. African Journal of Ecology 1:124–126.

Frey RW (1973) Concepts in the study of biogenic sedimentary structures. Journal of Sedimentary Research 43: 6–19.

Funston P (2001) Kalahari Transfrontier Lion Project: Population-ecology and long term monitoring of a free-ranging population in an arid environment. Endangered Wildlife Trust, Johannesburg

Funston P, Frank L, Stephens T, et al (2010) Substrate and species constraints on the use of track incidences to estimate African large carnivore abundance. Journal of Zoology 281:56–65.

Funston PJ (2011) Population characteristics of lions (*Panthera leo*) in the Kgalagadi Transfrontier Park. South African Journal of Wildlife Research 41:1–10.

Gargallo P, Prados E, Sturm P (2007) Minimizing the reprojection error in surface reconstruction from images. In: 11th IEEE International Conference on Computer Vision. Institute of Electrical and Electronics Engineers, p 1–8

Garshelis DL (2006) On the allure of noninvasive genetic sampling-putting a face to the name. Ursus 17:109–123.

Gaugris J, Matthews W, Van Rooyen M, Bothma J du P (2004) The vegetation of Tshanini Game Reserve and a comparison with equivalent units in the Tembe Elephant Park in Maputaland, South Africa. Koedoe 47:9–29.

Gese EM (2001) Monitoring of terrestrial carnivore populations. In: Gittleman JL, Funk S, Macdonald D, Wayne RK (eds) Carnivore Conservation. Cambridge University Press, Cambridge, pp 372–396

Gittleman JL, Funk SM, Macdonald DW, Wayne RK (2001) Why “carnivore conservation”? In: Gittleman JL, Funk SM, Macdonald DW, Wayne RK (eds) Carnivore conservation. Cambridge University Press, Cambridge, pp 1–7

Gore A, Paranjape S, Rajgopalan G, et al (1993) Tiger census: role of quantification. Current Science 64:711–714.

Gower JC (1975) Generalized procrustes analysis. Psychometrika 40:33–51.

Grigione MM, Burman P, Bleich VC, Pierce BM (1999) Identifying individual mountain lions *Felis concolor* by their tracks: refinement of an innovative technique. Biological Conservation 88:25–32.

Grimsdell JJR (1978) Ecological monitoring. African Wildlife Foundation, Nairobi

Guggisberg CAW (1963) Simba: the life of the lion. Chilton Books, Philadelphia

Gunz P, Mitteroecker P (2013) Semilandmarks: a method for quantifying curves and surfaces. Hystrix, the Italian Journal of Mammalogy 24:103–109.

Gunz P, Mitteroecker P, Bookstein FL (2005) Semilandmarks in three dimensions. In: Slice DE (ed) Modern morphometrics in physical anthropology. Kluwer Academic/Plenum Publishers, New York, pp 73–98

Gusset M, Burgener N (2005) Estimating larger carnivore numbers from track counts and measurements. African Journal of Ecology 43:320–324.

Gutteridge L, Liebenberg L (2013) Mammals of Southern Africa and their Tracks and Signs. Jacana Media, Sunnyside

Haas SK, Hayssen V, Krausman PR (2005) *Panthera leo*. Mammalian Species 1:1–11.

Halfpenny J (1986) Tracks and tracking: a“ how to” guide. A Naturalist’s World, Gardiner

Halfpenny J (2008) Scats and tracks of North America: a field guide to the signs of nearly 150 wildlife species. Rowman and Littlefield, Lanham

Hawkins P (2004) Bio-logging and animal welfare: practical refinements. Memoirs of National Institute of Polar Research Special issue 58:58–68.

Hayward MW, Boitani L, Burrows ND, et al (2015) Ecologists need robust survey designs, sampling and analytical methods. Journal of Applied Ecology 52:286–290.

Hayward MW, Hayward GJ, Druce DJ, Kerley GI (2009) Do fences constrain predator movements on an evolutionary scale? Home range, food intake and movement patterns of large predators reintroduced to Addo Elephant National Park, South Africa. Biodiversity and Conservation 18:887–904.

Hayward MW, Kerley GI (2005) Prey preferences of the lion (*Panthera leo*). Journal of zoology 267:309–322.

Heinemeyer KS, Ulizio TJ, Harrison RL (2008) Natural sign: tracks and scats. In: Long RA, MacKay P, Zielinski WJ, Ray JC (eds) Noninvasive survey methods for carnivores. Island Press, Washington, pp 45–74

Henschel P, Azani D, Burton C, et al (2010) Lion status updates from five range countries in West and Central Africa. CatNews 52:34–39.

Henschel P, Bauer H, Sogbohoussou E, Nowell K (2015) *Panthera leo* (West Africa subpopulation). The IUCN Red List of Threatened Species. http://www.iucnredlist.org. Accessed 1 August 2016.

Hildebrand M (1989) The quadrupedal gaits of vertebrates. BioScience 39:766–775.

Hilderbrand GV, Farley SD, Robbins CT (1998) Predicting body condition of bears via two field methods. Journal of Wildlife Management 62:406–409.

Houser A, Somers M, Boast L (2009) Spoor density as a measure of true density of a known population of free-ranging wild cheetah in Botswana. Journal of Zoology 278:108–115.

Hunter L, Barrett P (2011) Carnivores of the world. Princeton University Press, Princeton

Hunter LT (1998) The behavioural ecology of reintroduced lions and cheetahs in the Phinda Resource Reserve, KwaZulu-Natal, South Africa. Dissertation, University of Pretoria

IUCN (2016) The IUCN Red List of Threatened Species. http://www.iucnredlist.org. Accessed 1 August 2016.

Jewell ZC, Alibhai SK, Evans JW (2014) Monitoring mountain lion using footprints: A robust new technique. Wild Felid Monitor 7:26–27.

Jewell ZC, Alibhai SK, Law PR (2001) Censusing and monitoring black rhino (*Diceros bicornis*) using an objective spoor (footprint) identification technique. Journal of Zoology 254:1–16.

Jewell ZC, Alibhai SK, Weise F, et al (2016) Spotting Cheetahs: Identifying Individuals by Their Footprints. Journal of Visualized Experiments 111:e54034–e54034.

Joppa L, O'Connor B, Visconti P, et al (2016) Filling in biodiversity threat gaps. Science 352:416–418.

Joslin P (1973) The Asiatic lion: a study of ecology and behaviour. Dissertation, University of Edinburgh

Juefei-Xu F, Bhagavatula C, Jaech A, et al (2012) Gait-id on the move: Pace independent human identification using cell phone accelerometer dynamics. In: Fifth International Conference on Biometrics: Theory, Applications and Systems (BTAS). Institute of Electrical and Electronics Engineers, p 8–15

Karanth KU, Nichols JD, Seidenstricker J, et al (2003) Science deficiency in conservation practice: the monitoring of tiger populations in India. Animal Conservation 6:141–146.

Kays RW, Patterson BD (2002) Mane variation in African lions and its social correlates. Canadian Journal of Zoology 80:471–478.

Kendall DG (1977) The diffusion of shape. Advances in applied probability 9:428–430.

Kock MD, Burroughs R (2012) Chemical and Physical Restraint of Wild Animals. Second Edition. IWVS, Greyton

Lewison R, Fitzhugh E, Galentine SP (2001) Validation of a rigorous track classification technique: identifying individual mountain lions. Biological Conservation 99:313–321.

Liebenberg L (1990a) The art of tracking, the origin of science. David Philip, Cape Town

Liebenberg L (1990b) A Field Guide to the Animal Tracks of Southern Africa. David Philip, Claremont

Liebenberg L (2006) Persistence hunting by modern hunter-gatherers. Current Anthropology 47:1017–1026.

Liebenberg L, Louw A, Elbroch M (2010) Practical tracking: a guide to following footprints and finding animals. Stackpole Books, Mechanicsburg

Linder W (2009) Digital Photogrammetry, Third Edition. Springer, Berlin

Lindzey FG, Hodges JI, Thompson SK (1977) Scent station index of black bear abundance. Journal of Wildlife Management 41:151–153.

Linhart SB, Knowlton FF (1975) Determining the relative abundance of coyotes by scent station lines. Wildlife Society Bulletin 119–124.

Lokare N, Ge Q, Snyder W, et al (2014) Manifold learning approach to curve identification with applications to footprint segmentation. In: Computational Intelligence for Multimedia, Signal and Vision Processing. Institute of Electrical and Electronics Engineers, p 1–8

Long RA, MacKay P, Ray J, Zielinski W (2008) Noninvasive survey methods for carnivores. Island Press, Washington

Long RA, Zielinski WJ (2008) Designing effective noninvasive carnivore surveys. In: Long RA, MacKay P, Zielinski WJ, Ray JC (eds) Noninvasive survey methods for carnivores. Washington, pp 8–44

MacKay P, Zielinski WJ, Long RA, Ray JC (2008) Noninvasive research and carnivore conservation. In: Long R, MacKay P, Zielinski W, Ray J (eds) Noninvasive survey methods for carnivores. Island Press, Washington, pp 1–7

MacKenzie DI, Nichols JD, Royle JA, et al (2006) Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, Oxford

Maddock A, Anderson A, Carlisle F, et al (1996) Changes in lion numbers in Hluhluwe-Umfolozi Park. Lammergeyer 6–18.

Marchal A, Lejeune P, Bruyn P (2016) Virtual plaster cast: digital 3D modelling of lion paws and tracks using close- range photogrammetry. Journal of Zoology 300:111–119.

Marcus LF (1990) Traditional morphometrics. In: Michigan morphometrics workshop, p 77-122

Matipano G (2004) Black rhinoceros mortality in Matusadona National Park, Zimbabwe: 1992–2003. Pachyderm 109–112.

May RM (2000) The dimensions of life on earth. In: Raven PH (ed) Nature and human society. The quest for a sustainable world. National Academy Press, Washington, pp 30–45

McIntyre T (2015) Animal telemetry: Tagging effects. Science 349:596–597.

McNeely JA (2000) Practical approaches for including mammals in biodiversity conservation. In: Entwistle A, Dunstone N (eds) Priorities for the Conservation of Mammalian Diversity: Has the Panda Had its Day? Cambridge University Press, Cambridge, pp 355–368

Mech LD, Peterson RO (2003) Wolf-prey relations. In: Mech LD, Boitani L (eds) Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, pp 131–160

Meldrum D, Lockley MG, Lucas SG, Musiba C (2011) Ichnotaxonomy of the Laetoli trackways: the earliest hominin footprints. Journal of African Earth Sciences 60:1–12.

Midlane N, O'Riain M, Balme G, et al (2014) On tracks: A spoor-based occupancy survey of lion *Panthera leo* distribution in Kafue National Park, Zambia. Biological Conservation 172:101–108.

Miller JR, Balme G, Lindsey PA, et al (2016) Aging traits and sustainable trophy hunting of African lions. Biological Conservation 201:160–168.

Mills M, Wolff P, Le Riche E, Meyer I (1978) Some population characteristics of the lion *Panthera leo* in the Kalahari Gemsbok National Park. Koedoe 21:163–171.

Mills MGL (1990) Kalahari Hyaenas: Comparative Behavioural Ecology of Two Species. Unwin Hyman, London

Mills MGL, Bester MN (2005) Order Carnivora. In: Skinner JD, Chimimba CT (eds) The mammals of the southern African sub-region. Cambridge University Press, Cambridge, pp 358–526

Millspaugh J, Rittenhouse C, Montgomery R, et al (2015) Resource selection modeling reveals potential conflicts involving reintroduced lions in Tembe Elephant Park, South Africa. Journal of Zoology 296:124–132.

Morrison JC, Sechrest W, Dinerstein E, et al (2007) Persistence of large mammal faunas as indicators of global human impacts. Journal of Mammalogy 88:1363–1380.

Mosser A, Packer C (2009) Group territoriality and the benefits of sociality in the African lion, *Panthera leo*. Animal Behaviour 78:359–370.

Mucina L, Rutherford MC (2006) The vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria

Murie OJ, Elbroch M (2005) A field guide to animal tracks. Houghton Mifflin Harcourt, Boston

Murray DL, Fuller MR (2000) A critical review of the effects of marking on the biology of vertebrates. In: Boitani L, Fuller TK (eds) Research techniques in animal ecology: controversies and consequences. Columbia University Press, New York, pp 15–64

Nabokov P (1981) Indian running: Native American History and Tradition. Aneburt City Press, Santa Fe

Naidoo R, Stuart-Hill G, Weaver LC, et al (2011) Effect of diversity of large wildlife species on financial benefits to local communities in northwest Namibia. Environmental and Resource Economics 48:321–335.

Nowel K, Jackson P (1996) Wild cats: status survey and conservation action plan. International Union for Conservation of Nature, Gland

O’Brien SJ, Martenson JS, Packer C, et al (1987) Biochemical genetic-variation in geographic isolates of African and Asiatic lions. National Geographic Research 3:114–124.

Ogutu J, Dublin H (1998) The response of lions and spotted hyaenas to sound playbacks as a technique for estimating population size. African Journal of Ecology 36:83–95.

Orford H, Perrin M, Berry H (1988) Contraception, reproduction and demography of free-ranging Etosha lions (*Panthera leo*). Journal of Zoology 216:717–733.

Packer C, Gilbert DA, Pusey A, O’Brien SJ (1991) A molecular genetic analysis of kinship and cooperation in African lions. Nature 351:562–565.

Packer C, Herbst L, Pusey AE, et al (1988) Reproductive success of lions. In: Clutton-Brock TH (ed) Reproductive success. University of Chicago Press, Chicago, pp 363–383

Packer C, Ikanda D, Kissui B, Kushnir H (2005) Conservation biology: lion attacks on humans in Tanzania. Nature 436:927–928.

Packer C, Kosmala M, Cooley HS, et al (2009) Sport hunting, predator control and conservation of large carnivores. Plos One 4:e5941.

Packer C, Pusey AE (1983) Male takeovers and female reproductive parameters: a simulation of oestrous synchrony in lions (*Panthera leo*). Animal Behaviour 31:334–340.

Packer C, Pusey AE, Eberly LE (2001) Egalitarianism in female African lions. Science 293:690–693.

Peig J, Green AJ (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Functional Ecology 24:1323–1332.

Pennycuick C, Rudnai J (1970) A method of identifying individual lions *Panthera leo* with an analysis of the reliability of identification. Journal of Zoology 160:497–508.

Peters SE (1983) Postnatal development of gait behaviour and functional allometry in the domestic cat (*Felis catus*). Journal of Zoology 199:461–486.

Petti FM, Avanzini M, Belvedere M, et al (2008) Digital 3D modelling of dinosaur footprints by photogrammetry and laser scanning techniques: integrated approach at the Coste dell'Anglone tracksite (Lower Jurassic, Southern Alps, Northern Italy). Studi Trentini di Scienze Naturali, Acta Geologica 83:303–315.

Pienaar U de V (1969) Predator-prey relationship amongst the larger mammals of the Kruger National Park. Koedoe 108–187.

Pimm SL, Jenkins CN, Abell R, et al (2014) The biodiversity of species and their rates of extinction, distribution, and protection. Science 344:1246752.

Pimm SL, Raven P (2000) Biodiversity: extinction by numbers. Nature 403:843–845.

Prugh LR, Stoner CJ, Epps CW, et al (2009) The rise of the mesopredator. Bioscience 59:779–791.

Pusey AE, Packer C (1987) The evolution of sex-biased dispersal in lions. Behaviour 101:275–310.

R Development Core Team (2014) R: A Language and Environment for Statistical Computing, version 3.2.2. R Foundation for Statistical Computing. http://www.R-project.org/. Accessed 14 August 2015

Rautenbach I, Skinner J, Nel J (1980) The past and present status of mammals of Maputaland. In: Bruton MN, Cooper KH (eds) The Ecology of Maputaland. Rhodes University, Grahamstown, pp 322–345

Ray JC, Zielinski WJ (2008) Track stations. In: Long RA, MacKay P, Zielinski WJ, Ray JC (eds) Noninvasive survey methods for carnivores. Island Press, Washington, pp 75–109

Razzolini NL, Vila B, Castanera D, et al (2014) Intra-trackway morphological variations due to substrate consistency: The El Frontal dinosaur tracksite (Lower Cretaceous, Spain). PloS one 9:e93708.

Remondino F, Rizzi A, Girardi S, et al (2010) 3D Ichnology—recovering digital 3D models of dinosaur footprints. The Photogrammetric Record 25:266–282.

Richardson L, Loomis J (2009) The total economic value of threatened, endangered and rare species: an updated meta-analysis. Ecological Economics 68:1535–1548.

Riggio J, Jacobson A, Dollar L, et al (2013) The size of savannah Africa: a lion's (*Panthera leo*) view. Biodiversity and Conservation 22:17–35.

Rink W, Schwarcz H, Lee H, et al (1997) ESR dating of Mousterian levels at El Castillo cave, Cantabria, Spain. Journal of Archaeological Science 24:593–600.

Riordan P (1998) Unsupervised recognition of individual tigers and snow leopards from their footprints. Animal Conservation 1:253–262.

Ripple WJ, Beschta RL (2012) Large predators limit herbivore densities in northern forest ecosystems. European Journal of Wildlife Research 58:733–742.

Ripple WJ, Estes JA, Beschta RL, et al (2014) Status and ecological effects of the world's largest carnivores. Science 343:1241484.

Ripple WJ, Wirsing AJ, Wilmers CC, Letnic M (2013) Widespread mesopredator effects after wolf extirpation. Biological Conservation 160:70–79.

Ritchie EG, Johnson CN (2009) Predator interactions, mesopredator release and biodiversity conservation. Ecology Letters 12:982–998.

Roelke-Parker ME, Munson L, Packer C, et al (1996) A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). Nature 379:441–445.

Rohlf FJ, Slice D (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Biology 39:40–59.

Rosevaer D (1974) The carnivores of west Africa. British Museum (Natural History), London

Rowe-Rowe D (1992) The Carnivores of Natal. Natal Parks Board, Pietermaritzburg

Royle JA, Nichols JD (2003) Estimating abundance from repeated presence–absence data or point counts. Ecology 84:777–790.

Rudner J, Rudner I (1968) Rock-art in the thirstland areas. The South African Archaeological Bulletin 23:75–89.

Ruggiero RG (1991) Opportunistic predation on elephant calves. African Journal of Ecology 29:86–89.

Sagar S, Singh L (1990) Technique to distinguish tracks of leopard and tiger cub. Indian Forester 116:214–219.

Schaller G (1972) The Serengeti lion. University of Chicago Press, Chicago

Scharis I, Rasmussen GS, Laska M (2015) Using morphometrics to quantitatively differentiate African wild dog footprints from domestic dog footprints–a pilot study. African Journal of Ecology 1:3–8.

Scharstein D, Szeliski R (2002) A taxonomy and evaluation of dense two-frame stereo correspondence algorithms. International Journal of Computer Vision 47:7–42.

Schipper J (2007) Camera-trap avoidance by Kinkajous *Potos flavus*: rethinking the “non-invasive” paradigm. Small Carnivore Conservation 36:38–41.

Schmitz OJ, Hawlena D, Trussell GC (2010) Predator control of ecosystem nutrient dynamics. Ecology Letters 13:1199–1209.

Schwarz CJ, Seber GA (1999) Estimating animal abundance: review III. Statistical Science 14:427–456.

Sharma S, Jhala Y, Sawarkar VB (2003) Gender discrimination of tigers by using their pugmarks. Wildlife Society Bulletin 31:258–264.

Sharma S, Jhala Y, Sawarkar VB (2005) Identification of individual tigers (*Panthera tigris*) from their pugmarks. Journal of Zoology 267:9–18.

Silberbauer GB (1981) Hunter and habitat in the central Kalahari Desert. Cambridge University Press, Cambridge

Singh H, Gibson L (2011) A conservation success story in the otherwise dire megafauna extinction crisis: the Asiatic lion (*Panthera leo persica*) of Gir forest. Biological Conservation 144:1753–1757.

Smallwood KS, Fitzhugh EL (1993) A rigorous technique for identifying individual mountain lions *Felis concolor* by their tracks. Biological Conservation 65:51–59.

Smallwood KS, Fitzhugh EL (1995) A track count for estimating mountain lion *Felis concolor californica* population trend. Biological Conservation 71:251–259.

Smillie S (2016) In the past we knew how to run for our livelihood. Business Day. http://www.businesslive.co.za/bd/life/2016-11-08-in-the-past-we-knew-how-to-run-for-our-livelihood/ Accessed 08 November 2016

Smith RP (1982) Animal tracks and signs of North America. Stackpole books, Mechanicsburg

Smuts G (1976) Population characteristics and recent history of lions in two parts of the Kruger National Park. Koedoe 19:153–164.

Smuts G (1982) Lion. MacMillan, Johannesburg

Smuts G, Anderson J, Austin J (1978) Age determination of the African lion (*Panthera leo*). Journal of Zoology 185:115–146.

Smuts G, Hanks J, Whyte I (1978b) Reproduction and social organization of lions from the Kruger National Park. Carnivore 1:17–28.

Smuts G, Robinson G, Whyte I (1980) Comparative growth of wild male and female lions (*Panthera leo*). Journal of Zoology 190:365–373.

Smuts G, Whyte I, Dearlove T (1977) A mass capture technique for lions. African Journal of Ecology 15:81–87.

Sollas WJ (1915) Ancient hunters and their modern representatives. MacMillan and Company, London

Soulé ME (1985) What is conservation biology? BioScience 35:727–734.

Stander P (1991) Demography of lions in the Etosha national park, Namibia. Madoqua 18:1–9.

Stander P (1992a) Foraging dynamics of lions in a semi-arid environment. Canadian Journal of Zoology 70:8–21.

Stander P (1992b) Cooperative hunting in lions: the role of the individual. Behavioral ecology and sociobiology 29:445–454.

Stander P (1997) The ecology of lions and conflict with people in north-eastern Namibia. In: Symposium on lions and leopards as game ranch animals. Wildlife group of the South African Veterinary Association, p 10–17

Stander P (1998) Spoor counts as indices of large carnivore populations: the relationship between spoor frequency, sampling effort and true density. Journal of Applied Ecology 35:378–385.

Stander P, Ghau I, Tsisaba D, et al (1997) Tracking and the interpretation of spoor: a scientifically sound method in ecology. Journal of Zoology 242:329–341.

Steele N (1970) A preliminary report on the lions in the Umfolozi and Hluhluwe Game Reserves. Lammergeyer 11:68–79.

Stuart C, Stuart M (2013) Field guide to tracks and signs of southern, central & east African wildlife. Random House Struik, Cape Town

Stuart C, Stuart T (2000) A field guide to the tracks and signs of southern and east African wildlife. Struik, Cape Town

Szeliski R (2010) Computer vision: algorithms and applications. Springer, Berlin

Thompson WL, White GC, Gowan C (1998) Monitoring vertebrate populations. Academic Press, San Diego

Triggs B, McLauchlan PF, Hartley RI, Fitzgibbon AW (2000) Bundle adjustment—a modern synthesis. In: Triggs B, Zisserman A, Szeliski R (eds) Vision algorithms: theory and practice. Springer-Verlag, Berlin, pp 298–372

Trinkel M, Ferguson N, Reid A, et al (2008) Translocating lions into an inbred lion population in the Hluhluwe-iMfolozi Park, South Africa. Animal Conservation 11:138–143.

Tumenta P, Kok J, Van Rijssel J, et al (2010) Threat of rapid extermination of the lion (*Panthera leo leo*) in Waza National Park, Northern Cameroon. African Journal of Ecology 48:888–894.

United Nations (1992) Convention on biological diversity. United Nations, Rio de Janeiro

Van Dyke FG, Brocke RH, Shaw HG (1986) Use of road track counts as indices of mountain lion presence. Journal of Wildlife Management 50:102–109.

Van Ordsol K (1982) Lion predation in Rwenzori National Park, Uganda. Dissertation, University of Cambridge

Van Ordsol K, Hanby JP, Bygott J (1985) Ecological correlates of lion social organization (Panthers, leo). Journal of zoology 206:97–112.

Van Orsdol KG (1984) Foraging behaviour and hunting success of lions in Queen Elizabeth National Park, Uganda. African Journal of Ecology 22:79–99.

Verhoeven G (2011) Taking computer vision aloft–archaeological three-dimensional reconstructions from aerial photographs with photoscan. Archaeological Prospection 18:67–73.

Verhoeven G, Doneus M, Briese C, Vermeulen F (2012) Mapping by matching: a computer vision-based approach to fast and accurate georeferencing of archaeological aerial photographs. Journal of Archaeological Science 39:2060–2070.

Viereck A, Rudner J (1957) Twyfelfontein: a centre of prehistoric art in south west Africa. The South African Archaeological Bulletin 12:15–26.

Viljoen IM, van Helden PD, Millar RP (2015) Mycobacterium bovis infection in the lion (*Panthera leo*): current knowledge, conundrums and research challenges. Veterinary microbiology 177:252–260.

Wang X, Tedford RH, Antón M (2010) Dogs: their fossil relatives and evolutionary history. Columbia University Press, New York

Washburn SL (1978) The evolution of man. Scientific American 239:194–208.

Wegge P, Pokheral CP, Jnawali SR (2004) Effects of trapping effort and trap shyness on estimates of tiger abundance from camera trap studies. Animal Conservation 7:251–256.

Weissengruber G, Forstenpointner G, Peters G, et al (2002) Hyoid apparatus and pharynx in the lion (*Panthera leo*), jaguar (*Panthera onca*), tiger (*Panthera tigris*), cheetah (*Acinonyx jubatus*) and domestic cat (*Felis silvestris f. catus*). Journal of anatomy 201:195–209.

West PM, Packer C (2002) Sexual selection, temperature, and the lion’s mane. Science 297:1339–1343.

Western D (1984) Amboseli National Park: human values and the conservation of a savanna ecosystem. In: McNeely JA, Miller KR (eds) National Parks, Conservation and Development: the Role of Protected Areas in Sustaining Society. Smithsonian Institution Press, Washington, pp 93-100

Wilson DE, Reeder DM (2005) Mammal species of the world: a taxonomic and geographic reference. The Johns Hopkins University Press, Baltimore

Wilson EO, Peter FM (1988) Biodiversity. National Academy Press, Washington

Wilson GJ, Delahay RJ (2001) A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation. Wildlife Research 28:151–164.

Wilson RP, McMahon CR (2006) Measuring devices on wild animals: what constitutes acceptable practice? Frontiers in Ecology and the Environment 4:147–154.

Witmer GW (2005) Wildlife population monitoring: some practical considerations. Wildlife Research 32:259–263.

Yalden DW, Largen M, Kock D (1980) Catalogue of the mammals of Ethiopia. 4. Carnivora. Italian Journal of Zoology 8:169–272.

Yoccoz NG, Nichols JD, Boulinier T (2001) Monitoring of biological diversity in space and time. Trends in Ecology and Evolution 16:446–453.

Zelditch ML, Swiderski DL, Sheets HD (2012) Geometric morphometrics for biologists: a primer, Second Edition. Academic Press, Amsterdam

Supplementary material

SUPPLEMENTARY MATERIAL 1 - R SCRIPT FOR FEATURE EXTRACTION USING GEOMORPH PACKAGE (FIXED LANDMARKS)

```
library(geomorph)
## Data collection (digitizing landmarks on main pad)
# Importing 3D surface files (read.ply)
        filename_1 <- /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Considerations/R/Track variation/HL/Track_ID_MP_fixed/Specimen001_MP
        Specimen001_MP <- read.ply(filename_1, ShowSpecimen = FALSE, addNormals = TRUE)
        filename_2 <- /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Considerations/R/Track variation/HL/Track_ID_MP_fixed/Specimen002_MP
        Specimen002_MP <- read.ply(filename_2, ShowSpecimen = FALSE, addNormals = TRUE)
        filename_3 <- /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Considerations/R/Track variation/HL/Track_ID_MP_fixed/Specimen003_MP
        Specimen003_MP <- read.ply(filename_3, ShowSpecimen = FALSE, addNormals = TRUE)
# 3D data collection: fixed landmarks (digit.fixed)
        digit.fixed(Specimen001_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
        digit.fixed(Specimen002_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
        digit.fixed(Specimen003_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
## Data input: importing landmark data
# Multiple NTS files of single specimens (readmulti.nts)
        filelist <- list.files(pattern = ".nts")
        filelist
        mydata <- readmulti.nts(filelist)
        mydata
        dim(mydata)
## Data preparation: manipulating landmark data and classifiers
# Making a factor: group variables
        classifier <- read.csv("classifier.csv", header=T, row.names=1)
## Generalized Procrustes Analysis (gpagen)
```

```
        Y <- gpagen(mydata[,,])
        Y
        Y$coords
        Y$Csize
# Plot all specimens
        plotAllSpecimens(Y$coords,mean=T)
#Check for outliers
        plotOutliers(Y$coords, groups = NULL)
        outliers <- plotOutliers(Y$coords) # function returns dimname and address of outliers
        outliers
        plotRefToTarget(mshape(Y$coords),Y$coords[,,outliers[1]],method="vector", label = T)
## Data analysis
# Principal Components Analysis
PCA <- plotTangentSpace(Y$coords, verbose=TRUE, warpgrids = FALSE)
PCA$pc.summary$importance
PCA$pc.scores
```

=> Y$Csize represent the size variables and PCA$pc.scores the shape variables

SUPPLEMENTARY MATERIAL 2 - R SCRIPT FOR FEATURE EXTRACTION USING GEOMORPH PACKAGE (FIXED LANDMARKS WITH CURVE-SLIDERS)

```
library(geomorph)
## Data collection (digitizing landmarks on main pad)
# Importing 3D surface files (read.ply)
    filename_1 <-
    /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Con
    siderations/R/Track variation/HL/Track_ID_MP_fixed/Specimen001_MP
    Specimen001_MP <- read.ply(filename_1, ShowSpecimen = FALSE, addNormals = TRUE)
    filename_2 <-
    /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Con
    siderations/R/Track variation/HL/Track_ID_MP_fixed/Specimen002_MP
    Specimen002_MP <- read.ply(filename_2, ShowSpecimen = FALSE, addNormals = TRUE)
    filename_3 <-
    /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Con
    siderations/R/Track variation/HL/Track_ID_MP_fixed/Specimen003_MP
    Specimen003_MP <- read.ply(filename_3, ShowSpecimen = FALSE, addNormals = TRUE)
# 3D data collection: fixed landmarks (digit.fixed)
    digit.fixed(Specimen001_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
    digit.fixed(Specimen002_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
    digit.fixed(Specimen003_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
# Define sliding semilandmarks (50 sliders for main pad)
    filelist <- list.files(pattern = ".nts")
    filelist
    mydata <- readmulti.nts(filelist)
    mydata
    dim(mydata)
    filelist_curve <- list.files(pattern = ".txt")
    filelist_curve
    for (j in 1:3) {
      curve <- as.matrix(read.table(filelist_curve[j]))
      start <- c(mydata[1,1,j], mydata[1,2,j], mydata[1,3,j])
      lmks <- digit.curves(start, curve, nPoints=49, closed = TRUE)
      distance_18_2 <- sqrt((lmks[18,1]-mydata[2,1,j])^2+(lmks[18,2]-
    mydata[2,2,j])^2+(lmks[18,3]-mydata[2,3,j])^2)
```

```
      distance_18_4 <- sqrt((lmks[18,1]-mydata[4,1,j])^2+(lmks[18,2]-
    mydata[4,2,j])^2+(lmks[18,3]-mydata[4,3,j])^2)
      if(distance_18_2 > distance_18_4) {lmks=lmks[order(nrow(lmks):1),]}
      for (i in 1:50) {
        write(lmks[i,],file=filelist[j],append=TRUE)}
      filenames <- filelist[j]
      for( f in filenames){
        x <- readLines(f)
        y <- gsub("1 4 3 0 dim=3","1 54 3 0 dim=3",x)
        cat(y,file=f,sep="\n")
      }}
## Data input: importing landmark data
# Multiple NTS files of single specimens (readmulti.nts)
    filelist <- list.files(pattern = ".nts")
    filelist
    mydata <- readmulti.nts(filelist)
    mydata
    dim(mydata)
# Define sliders
    define.sliders(mydata[,,1], nsliders=50)
## Data preparation: manipulating landmark data and classifiers
# Making a factor: group variables
    classifier <- read.csv("classifier.csv", header=T, row.names=1)
## Generalized Procrustes Analysis (gpagen)
    curves <- as.matrix(read.csv("curveslide.csv", header=T))
    Y <- gpagen(mydata[,,], curves=curves, ProcD=TRUE)
    Y
    Y$coords
    Y$Csize
# Plot all specimens
    plotAllSpecimens(Y$coords,mean=T)
#Check for outliers
    plotOutliers(Y$coords, groups = NULL)
    outliers <- plotOutliers(Y$coords) # function returns dimname and address of outliers
    outliers
```

```
    plotRefToTarget(mshape(Y$coords),Y$coords[,,outliers[1]],method="vector", label = T)
## Data analysis
# Principal Components Analysis
PCA <- plotTangentSpace(Y$coords, verbose=TRUE, warpgrids = FALSE)
PCA$pc.summary$importance
PCA$pc.scores
```

=> Y$Csize represent the size variables and PCA$pc.scores the shape variables

SUPPLEMENTARY MATERIAL 3 - R SCRIPT FOR FEATURE EXTRACTION USING GEOMORPH PACKAGE (FIXED LANDMARKS WITH CURVE- AND SURFACE-SLIDERS)

```
library(geomorph)
## Data collection (digitizing landmarks on main pad)
# Importing 3D surface files (read.ply)
    filename_1 <- /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Considerations/R/Track variation/HL/Track_ID_MP_fixed/Specimen001_MP
    Specimen001_MP <- read.ply(filename_1, ShowSpecimen = FALSE, addNormals = TRUE)
    filename_2 <- /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Considerations/R/Track variation/HL/Track_ID_MP_fixed/Specimen002_MP
    Specimen002_MP <- read.ply(filename_2, ShowSpecimen = FALSE, addNormals = TRUE)
    filename_3 <- /Users/antoinemarchal/Documents/My_Studies/PhD_Zoology_Bioengineering/Statistical_Considerations/R/Track variation/HL/Track_ID_MP_fixed/Specimen003_MP
    Specimen003_MP <- read.ply(filename_3, ShowSpecimen = FALSE, addNormals = TRUE)
# 3D data collection: fixed landmarks (digit.fixed)
    digit.fixed(Specimen001_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
    digit.fixed(Specimen002_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
    digit.fixed(Specimen003_MP, 4, index = FALSE, ptsize = 1, center = FALSE)
# Define sliding semilandmarks (50 sliders for main pad)
    filelist <- list.files(pattern = ".nts")
    filelist
    mydata <- readmulti.nts(filelist)
    mydata
    dim(mydata)
    filelist_curve <- list.files(pattern = ".txt")
    filelist_curve
    for (j in 1:3) {
      curve <- as.matrix(read.table(filelist_curve[j]))
      start <- c(mydata[1,1,j], mydata[1,2,j], mydata[1,3,j])
      lmks <- digit.curves(start, curve, nPoints=49, closed = TRUE)
      distance_18_2 <- sqrt((lmks[18,1]-mydata[2,1,j])^2+(lmks[18,2]-mydata[2,2,j])^2+(lmks[18,3]-mydata[2,3,j])^2)
```

```
        distance_18_4 <- sqrt((lmks[18,1]-mydata[4,1,j])^2+(lmks[18,2]-mydata[4,2,j])^2+(lmks[18,3]-mydata[4,3,j])^2)
        if(distance_18_2 > distance_18_4) {lmks=lmks[order(nrow(lmks):1),]}
        for (i in 1:50) {
          write(lmks[i,],file=filelist[j],append=TRUE)}
        filenames <- filelist[j]
        for( f in filenames){
          x <- readLines(f)
          y <- gsub("1 4 3 0 dim=3","1 54 3 0 dim=3",x)
          cat(y,file=f,sep="\n")
        }}
## Data input: importing landmark data
# Multiple NTS files of single specimens (readmulti.nts)
      filelist <- list.files(pattern = ".nts")
      filelist
      mydata <- readmulti.nts(filelist)
      mydata
      dim(mydata)
# Define sliders
      define.sliders(mydata[,,1], nsliders=50)
# Add surface sliders
      buildtemplate(Specimen001_MP, mydata[,,1], 50, ptsize = 1, center = FALSE)
      digitsurface(Specimen002_MP, mydata[,,2], ptsize = 1, center = FALSE)
      digitsurface(Specimen003_MP, mydata[,,3], ptsize = 1, center = FALSE)
## Data input: importing landmark data
# Multiple NTS files of single specimens (readmulti.nts)
      filelist <- list.files(pattern = ".nts")
      filelist
      mydata <- readmulti.nts(filelist)
      mydata
      dim(mydata)
## Data preparation: manipulating landmark data and classifiers
# Making a factor: group variables
      classifier <- read.csv("classifier.csv", header=T, row.names=1)
## Generalized Procrustes Analysis (gpagen)
```

```
        curves <- as.matrix(read.csv("curveslide.csv", header=T))
        sliders <- as.matrix(read.csv("surfslide.csv", header=T))
        Y <- gpagen(mydata[,,], curves=curves, surfaces=sliders, ProcD=TRUE)
        Y
        Y$coords
        Y$Csize
# Plot all specimens
        plotAllSpecimens(Y$coords,mean=T)
#Check for outliers
        plotOutliers(Y$coords, groups = NULL)
        outliers <- plotOutliers(Y$coords) # function returns dimname and address of outliers
        outliers
        plotRefToTarget(mshape(Y$coords),Y$coords[,,outliers[1]],method="vector", label = T)
## Data analysis
# Principal Components Analysis
PCA <- plotTangentSpace(Y$coords, verbose=TRUE, warpgrids = FALSE)
PCA$pc.summary$importance
PCA$pc.scores
```

=> Y$Csize represent the size variables and PCA$pc.scores the shape variables

SUPPLEMENTARY MATERIAL 4 - R SCRIPT FOR LINEAR DISCRIMINANT ANALYSIS USING MASS PACKAGE

```
library(MASS)
## Import data
        Complete_tracks <- read.csv("Complete_tracks.csv", header=T, row.names=1)
## Using fixed landmarks with curve and surface sliders
        Data <-
Complete_tracks[which(Complete_tracks$Type.of.landmarks=='Fixed+curves+surfaces'),]
## Linear Discriminant analysis (without jack-knife) using size variables and five principal
components of the shape variables for individual identification
        model<-lda(Data$ID~
                Data$MP.Csize+
                Data$MP.PC1+
                Data$MP.PC2+
                Data$MP.PC3+
                Data$MP.PC4+
                Data$MP.PC5+
                Data$T1.Csize+
                Data$T1.PC1+
                Data$T1.PC2+
                Data$T1.PC3+
                Data$T1.PC4+
                Data$T1.PC5+
                Data$T2.Csize+
                Data$T2.PC1+
                Data$T2.PC2+
                Data$T2.PC3+
                Data$T2.PC4+
                Data$T2.PC5+
                Data$T3.Csize+
                Data$T3.PC1+
                Data$T3.PC2+
                Data$T3.PC3+
                Data$T3.PC4+
```

```
        Data$T3.PC5+
        Data$T4.Csize+
        Data$T4.PC1+
        Data$T4.PC2+
        Data$T4.PC3+
        Data$T4.PC4+
        Data$T4.PC5)
    predict(model)
    predict(model)$class

    # Assess the accuracy of the prediction
    # percent correct for each category of G
    ct <- table(Data$ID, predict(model)$class)
    diag(prop.table(ct, 1))
    # total percent correct
    sum(diag(prop.table(ct)))
## Linear Discriminant analysis (with jack-knife) using size variables and five principal components of the shape variables for individual identification
    model<-lda(Data$ID~
        Data$MP.Csize+
        Data$MP.PC1+
        Data$MP.PC2+
        Data$MP.PC3+
        Data$MP.PC4+
        Data$MP.PC5+
        Data$T1.Csize+
        Data$T1.PC1+
        Data$T1.PC2+
        Data$T1.PC3+
        Data$T1.PC4+
        Data$T1.PC5+
        Data$T2.Csize+
        Data$T2.PC1+
        Data$T2.PC2+
        Data$T2.PC3+
```

```
        Data$T2.PC4+
        Data$T2.PC5+
        Data$T3.Csize+
        Data$T3.PC1+
        Data$T3.PC2+
        Data$T3.PC3+
        Data$T3.PC4+
        Data$T3.PC5+
        Data$T4.Csize+
        Data$T4.PC1+
        Data$T4.PC2+
        Data$T4.PC3+
        Data$T4.PC4+
        Data$T4.PC5, CV=TRUE)
model

# Assess the accuracy of the prediction
# percent correct for each category of G
ct <- table(Data$ID, model$class)
diag(prop.table(ct, 1))
# total percent correct
sum(diag(prop.table(ct)))
```

View of the sand forest in Tembe Elephant Park, South Africa.

View of the White iMfolozi River in Hluhluwe-iMfolozi Park, South Africa.

End