

Review and critical evaluation of the different survey methods to estimate population size of leopard (*Panthera pardus pardus*) in Africa

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1. Introduction

Leopards (*Panthera pardus*) are the most widely distributed and adaptable large cat in the family *Felidae* (Mondol *et al.* 2009; Jackson *et al.* 2006; Du Preez *et al.* 2014; Havmøller *et al.* 2018). Of the nine genetically identified sub species, this report will focus on leopards in Africa (*Panthera pardus pardus*). Leopards are subject to many of the anthropomorphic threats driving carnivore declines globally and have lost 37% of their range in Africa alone (Balme, Slotow and Hunter, 2010). These animals play an important role in ecosystem dynamics through top down predatory effects (Rodgers and Janečka, 2013). However, due to their solitary, cryptic, nocturnal and wide-ranging behaviour; they remain some of the least understood mammalian species (Balme, Hunter and Slotow, 2009). This makes obtaining population estimates of this species a challenging task (Havmøller *et al.* 2018). To ensure populations of leopards persist, conservationists need to have an in-depth knowledge of the species population trends over manageable periods of time. The census methodologies must be accurate, reliable, cost-effective and easy to apply (Jackson *et al.* 2006). Effectiveness of management decision-making is often dependent upon the timeliness and accuracy of the relevant ecological data upon which the decisions are based (Hodgson *et al.* 2018). When assessing the different methods to obtain this ecological data, careful consideration needs to be given as to whether it deals adequately with the geographic variation and detectability, thereby ensuring the methods can be used, and results compared with different leopard populations across Africa. In this study I will examine and evaluate the role that different methodologies play in the monitoring of leopard populations.

2. Track Counts

Track counts have been frequently used throughout Africa to measure the relative abundance of carnivore populations (Silveira, Jacomo and Diniz-Filho, 2003; Gusset and Burgener, 2005; Funston *et al.* 2010). However, indices of abundance may be useful for specific management purposes, it is generally more desirable to establish absolute abundance indicators as abundance indicators are generally not comparable in different habitats or consistent over a large geographic area (Silvy, 2012). This view is confirmed by Karanth *et al.* (2004) when reviewing the efficiency of using tiger pugmarks to calculate population estimate. They found that the methodology was fundamentally flawed and lacked the necessary statistical rigour. Fortunately, a new suite of non-invasive techniques e.g. camera traps and metabarcoding offer hopeful predictions for estimating population abundance with greater accuracy, precision and scientific rigour (Jackson *et al.* 2006).

3. Camera Traps

Camera trapping is the use of remotely triggered cameras that automatically take videos or pictures of the study subjects passing in front of them (Rovero and Zimmermann, 2016). Camera traps can be used to address large scale questions in ecology by providing a systematic review of an array of wildlife ranging species (Swanson *et al.* 2015; Schneider Taylor and Kremer, 2018). Motion sensitive camera traps are a non-invasive method of monitoring wildlife (He *et al.* 2016; Rovero and Zimmermann, 2016). Due to their relative low cost, rapid deployment, and easy maintenance camera traps are now being used across the world, at various spatial and temporal scales (He *et al.* 2016; Dong *et al.* 2016; Rovero and Zimmermann, 2016; Silvy, 2012). The proliferation of digital still and video camera traps

as a primary source of ecological data has led to a 50% annual growth of publications using camera traps to assess population sizes between 1998 and 2008 (Schneider *et al.* 2019; Schneider, Taylor and Kremer, 2018). When applying the camera trap methodology to monitoring leopard populations, the most effective studies are those that use PIR sensors and a xenon white flash. This setup will allow the researcher to collect high-quality photos in terms of clarity, sharpness and resolution, which is essential for the data analysis process (Rovero and Zimmermann, 2016). Capture mark recapture (CMR) studies of individuals provide a powerful approach to construct a database in order to make inferences on the traits and parameters of populations (Drechsler, Helling and Steinfartz, 2015). CMR relies upon the recognition of individuals within a population over a certain period of time (Rovero and Zimmermann, 2016). A method for individual recognition is to apply a mark on the body of the animal. However, attaching marks upon an individual can alter the behaviour leading to bias in the parameter estimates and creating ethical issues (Buehler *et al.* 2019). In the case of large bodied, endangered mammals that are difficult to capture, non-invasive methods of individual recognition, such as photography of unique natural markers that are stable and robust over time are preferred (Jackson *et al.* 2006; Hiby *et al.* 2009; Du Preez *et al.* 2014; Goswami *et al.* 2012). For species with distinct fur patterns (e.g. leopard), data from camera trapping can be analysed with a CMR model framework to estimate the abundance and density (Rovero and Zimmermann, 2016).

One of the most important aspects of survey design is to cite the camera traps on well used travel routes, in order to maximise the possibility of encountering the focal species (Silvy, 2012). These can be based on specific species signs (e.g. tracks, scent marks and scat). The camera traps should be set perpendicular to the trail and to avoid overexposure due to the other camera trap flash, the cameras should not be facing each other (Rovero and Zimmermann, 2016). There is contention in the scientific community in regard to what role baits play in camera trap surveys. It is possible that baits could attract individuals from outside the survey area and thereby inflate population densities (Silvy, 2012). Contrary to this, Du Preez *et al.* (2014) found that baits significantly increase leopard capture rates and detected dependant cubs, which un-baited camera traps failed to do. They also found that there was no significant difference in size and location of the leopard's home range during the baited surveys. Although this is only one example it provides evidence that baits can be used to enhance capture probability which will increase the precision of abundance and density estimates significantly, whilst having little bias on the survey.

Careful consideration needs to be given to the period of the study, as female leopards with young have reduced mobility during the period of birth and lactation (Rovero and Zimmermann, 2016). This would impose serious bias of the study if it encompasses this period as the population of leopards in a given area would be underrepresented.

In camera trapping studies the sampling effort is usually expressed as the number of camera trap nights or days accumulated (Rovero and Zimmermann, 2016). Therefore, the greater the effort, the more pictures of different individuals are likely to be accumulated. Typically, CMR employs a catalogue of images that are matched against new images in order to assess if they are "re-captures" that have been previously photographed or unknown individuals ("captures") which are then included in the catalogue (Sacchi *et al.* 2016). The new individuals get an individual ID which accompanies other information (age, sex, mother if available, location of sightings etc). In order to identify each of the individuals photographed, at least two different investigators should be involved in the identification process (Rovero and Zimmermann, 2016) in order to reduce misidentification error as much as possible.

However, even with these considerations, misidentification is still an issue which can lead to serious miscalculations in species parameter estimates (Drechsler, Helling and Steinfartz, 2015).

The encounter history of leopards can be used as an input format for modelling abundance using the CAPTURE model in the programme MARK. The number of different leopards and the cumulative number of encounters is plotted against the number of sampling occasions. This allows you to ascertain the number of individuals within a given area. The software R offers a flexible framework to manage observations from different time zones and seasonal notations making more accurate estimations of leopard numbers.

There are many studies that attempt to calculate animal density from camera traps. Cusack *et al.* (2015) suggests a random encounter model as a novel method for estimating animal density without the need for individual recognition. However, this study highlights that accuracy is dependant upon reliable estimates of the model parameters (average lion speed and camera detection zone dimensions). Lion behaviour varies amongst individuals and differing metapopulations. This makes this model ineffective at obtaining accurate data for the model parameters which can be comparable to other such studies.

Camera trapping generates large volumes of data, often at a large temporal and spatial scales (Gomez *et al.* 2016; He *et al.* 2016; Willi *et al.* 2019; Sacchi *et al.* 2016). Our ability to process this data remains a bottleneck in turning these data into ecological information (Buehler *et al.* 2019). Small catalogues (e.g. 50 individuals), may be feasible by eye. However, when a catalogue exceeds 75 individuals or more, the identification process becomes hugely time consuming and misclassification errors occur frequently (Sacchi *et al.* 2016). To support CMR at large scales, cyber infrastructure needs to be developed to cope with large quantities of collection, analysis and management of multimodal wildlife sensor data (He *et al.* 2016). One such method to cope with large quantities of data is through citizen science. This is scientific research conducted, in whole, or in part by volunteers from the general population and has used the collective labour of thousands of citizen scientists, which can result in accurate classifications whilst reducing the time to process large data sets (He *et al.* 2016; Willi *et al.* 2019). Swanson *et al.* (2015) outlines a successful citizen science project called Snapshot Serengeti. This project has accumulated 99,241 camera trap days and produced 1.2 million pictures between 2010 and 2013. Multiple users view each species on the online database and record each species, number of individuals, associated behaviour and presence of young. The 28,000 registered users contributed 10.8 million classifications and an algorithm was used to aggregate the individuals into a final census dataset. This online platform provided scientists with means of managing large volumes of data. This same principle could be used to ascertain individual leopards in a CMR survey. However, there is a higher chance of misidentification of individual leopards than misidentification of species. These inaccuracies would lead to bias in the overall census of the leopard population.

Advances in machine learning, especially deep learning, allow for automatic image classification (Hiby *et al.* 2009; Yousif *et al.* 2019; Schneider *et al.* 2019; Willie *et al.* 2019). Machine learning has already been applied to predict the extinction risk of thousands of species (Wearn, Freeman and Jacoby, 2019). Deep learning systems no longer require “hard coded” feature extraction. Instead the algorithms can learn, through exposure to large amounts of data, and the particular features that allow for the discrimination of individuals (Schneider *et al.* 2019). There are many different algorithms that can be used; PCA, LDA, SVM, however, Deep Convolutional Neural Networks (DCNN's) model is one of the most

popular deep learning models (Villa, Salazar and Vargas, 2017) with accuracies of 98% being recorded (Trnovszky *et al.* 2017). It consists of convolution, pooling and classification layers. There have been many examples that have been used to automatically identify particular species from camera trap images (Gomez, Salazar and Diaz, 2016; Nguyen *et al.* 2017; Yousif *et al.* 2019). However, there have been fewer examples that show successful automated individual animal recognition. These programmes utilise algorithms to find unique individual characteristics and compare images to a catalogue of known individuals (Buehler *et al.* 2019). This makes leopards particularly suitable for deep learning due to their unique patterning. There is various software that utilise these (DCNN); APHIS (Moya *et al.* 2015), SLOOP (Duyck *et al.* 2015), Siamese networks (Schneider *et al.* 2019) AMPHIDENT (Goedbloed *et al.* 2017), SIFT algorithm and SPACECAP (Gopalaswamy *et al.* 2012). These software's have three processing steps: i) regional interest is selected, the image is rotated, scaled and spatially corrected, ii) automated comparison between the sample and the library of images, which arrange candidates by matching probability or likelihood values, iii) visual comparison between sample-candidate pairs for a limited number of plausible matches. DCNN's are often very complex and contain millions of parameters which have to be learned from the training data (Willi *et al.* 2019). Large volumes of training data are required to learn suitable parameter values, sometimes over 1000 images (Moya *et al.* 2015; Sacchi *et al.* 2016; Bolger *et al.* 2012; Yousif *et al.* 2019; Willi *et al.* 2019). To acquire this volume of images the scientist is generally dependent upon publicly available databases, or large volumes of leopard images with an individual ID (Schneider *et al.* 2019). This is not always possible, and at present with the technological level of DCNN's requiring large amounts of training data, it can be a serious drawback.

4. Genetics

The use of environment DNA (eDNA) to detect animals of interest from DNA that they leave in the environment has gained promise as genetics techniques have advanced (Quasim, MacDonald and Sarre, 2018). This non-invasive technique normally involves the sampling of hair or faeces, and can generate genetic data without having to catch, handle or in some cases, even observe the study species (Carroll *et al.* 2018; Rodgers and Janečka, 2013). The sensitivity of eDNA methods makes them ideal for detecting the presence of endangered, low density, transient, and cryptic species (Adams *et al.* 2019; Mondol *et al.* 2009), such as leopards (Rodgers and Janečka, 2013; Perez, Geffen and Mokady, 2006; Jackson *et al.* 2006; Janečka *et al.* 2011; Janjua *et al.* 2019; Busby *et al.* 2009; Mondol *et al.* 2009; Spong Johansson and Björklund, 2000). Genetic data can be used to estimate many biological parameters, including abundance, occupancy, hybridisation, disease, effective population size, diet, sex ratios (Carroll *et al.* 2018; Rodgers and Janečka, 2013). Abundance is of interest to scientists and conservationists because of its ecological and behavioural significance (4). Several different approaches have been used to estimate animal abundance through genetic data; rarefaction curve from unique genotypes, genetic capture-recapture method, Jackknife estimators and Lincoln-Peterson estimator (Mondol *et al.* 2009). Among these, capture-mark-recapture methods (CMR) have been the primary method for many studies, due to them being well founded theoretically and empirically. With the rise of molecular approach in population biology, highly variable molecular markers can also be used to distinguish individuals from each other across a population (Jackson *et al.* 2006; Karmacharya *et al.* 2011). The most common method for identifying the species from DNA samples is direct sequencing. DNA is extracted using a widely used Qiagen DNA extraction kit (Janečka *et al.* 2011; Mondol *et al.* 2009; Mondol *et al.* 2009). The Mitochondrial DNA (mtDNA) are amplified by polymerase chain reaction (PCR) using leopard specific primers;

which are then sequenced, aligned with reference sequence and placed in specific clades using a maximum likelihood or neighbour joining tree construction (Rodgers and Janečka, 2013).

To identify individual leopards, microsatellite markers that are polymorphic within a population are used (Mondol *et al.* 2009). The number of microsatellite loci needed to confidently distinguish between individuals, depends on the layout of the genetic diversity within the population and the expected heterozygosity of the loci used (Rodgers and Janečka, 2013). Perez, Geffen and Mokady (2006) found that a low genetically diverse population of Arabian leopards in which 7 of the 11 microsatellite loci that were amplified were monomorphic, and the remaining 4 had few alleles. This highlights that individual identification within a small inbred population that has undergone recent bottlenecks may be challenging. On the other hand, in larger more diverse populations of leopards, Mondol *et al.* (2009) revealed that analysis of 8 loci provided adequate probability and identifiability rate for individual recognition, which led to population estimates on a larger landscape. Programmes such as; CERVUS, GeneA1Ex, GENECAAP, API-CALC, GIMLET, (Jackson *et al.* 2006; Janečka *et al.* 2008; Mondol *et al.* 2009; Busby *et al.* 2009), are used to match genotypes as individuals across a large dataset. However, which type of model is most appropriate will depend on what the temporal and spatial distribution of the samples collected were (Rodgers and Janečka, 2013).

Each genetic “capture” of an individual is then assigned to the appropriate sampling occasion to generate the individual “capture” histories in the standard X Matric format (Mondol *et al.* 2009). These can then be analysed with the software CAPTURE, the leopard’s abundance estimates derived from genetic CMR sampling closely match the abundance estimates generated by camera trap CMR sampling that has been previously discussed. However, through camera trapping data, a study period can be defined as the pictures are annotated with a date or time. Generally, with genetic analysis samples are grouped into one temporal period, however, as the temporal period remains undefined and the scats can persist in the environment for an unknown period of time (Janečka *et al.* 2011).

New approaches such as single nucleotide polymorphism (SNP) analysis may be more effective for use in leopard studies than the PCR approach because they are ubiquitous and codominant (Korani *et al.* 2019). Unlike more conventional DNA markers SNPs have relatively few alleles per locus and often have more varied applications across species than microsatellite markers which are often species specific (Carroll *et al.* 2018). SNPs are numerous, broadly distributed across various genomic regions, and amenable to high-throughput technologies (Fitak *et al.* 2015). SNP based applications have great potential for non-invasive genotyping as; (i) large number of loci can be serving simultaneously, and (ii) the relative ease of scoring, analysis and modelling of SNP genotype data due to it being digital/binary, this contrasts with the near continuous distribution of microsatellite alleles that can be difficult to categorise, and therefore cause scoring errors. Whilst using one of the SNP platforms (Fluidigm SNPTYPE assay) (Dewoody *et al.* 2017) had error rates of 0.2%. Low error rates are essential in order to accurately calculate individual identification. Without this accuracy population estimates would be inaccurate leading to ineffective conservation and management of leopards. To date, the use of SNPs in wildlife conservation and management programmes remains relatively uncommon (Fitak *et al.* 2015). But the large panels of markers from next generation sequencing will allow the more efficient identification of individual leopards, especially in the case of close kin CMR models.

Indirect DNA sampling is reliant upon the subject species leaving eDNA in the environment (e.g. hair or faeces). There is the potential for a particular age/sex/class being undetectable due to behavioural reasons. For example, transient leopards rarely defecate on regular travel routes to avoid detection from territorial residents (Mizutani and Jewell, 1998), therefore, scat sampling may underestimate that segment of the population. Scat detection dogs have been employed in order to increase detection rates of carnivore scat, thereby mitigating such bias. Surveys using dogs have grown increasingly popular as an efficient means to gather data, particularly for low density species such as carnivores (Reed *et al.* 2011; Dematteo *et al.* 2009; Long *et al.* 2007a; Kerley and Salkina, 2007; Long *et al.* 2007b). Dogs have been trained to locate scats of target species, and to ignore others, both on land and water (Silvy, 2012). In the case of Wasser *et al.* (2009), three dogs correctly matched 25 out of 28 samples from 6 captive leopards of known identity. However, the literature still highlights relatively large margins of error with individual identification (Silvy, 2012; Wasser *et al.* 2009), therefore relying on it entirely may produce inaccurate results. This method may be useful in conjunction with scat sampling. To date it has not been used to survey leopards, however, the use of canines will make data collection more efficient.

In recent years new DNA collection methods have been developed, which involve analysing the genetic material that is ingested by invertebrates in order to categorise the biodiversity of the animals that served as host (iDNA). This method has most commonly used flies and leeches (Kampmann *et al.* 2017; Weiskopf *et al.* 2018; Drinkwater *et al.* 2019; Schubert *et al.* 2015; Rodgers *et al.* 2017). Schnell *et al.* (2018) found that terrestrial blood feeding leeches contain informative iDNA from various invertebrate classes both taxonomically (mammals, birds, amphibians and reptiles) and functionally (arboreal, and terrestrial, small and large, predator and prey). Abrams *et al.* (2018) found consistent estimate occupancy probabilities of camera traps and leech dataset. This indicates that metabarcoding of leech iDNA provides a reasonable estimate of occupancy and could be a suitable method of studying leopards. iDNA from flies has been similarly effective. A study by Rodgers *et al.* (2017) using iDNA surveys documented 67% of mammal species that were identified during eight years of transect counts and camera traps combined. This highlights the potential for iDNA to be a powerful, cost-effective and efficient tool for collecting genetic material of species and individuals (Schubert *et al.* 2015). However, in order to carry out a study with a similar robustness to a camera trapping survey, vast quantities of flies and leeches need to be collected using a similar standardised sampling scheme as camera trapping. Even so the use of iDNA shows great potential for complimenting camera trapping studies, as some species that were identified from the iDNA approach were not identified using camera traps (Abrams *et al.* 2018). At present, individual recognition from iDNA has been unsuccessful with high error rates (Schubert *et al.* 2015). However, with the recent technological advances in genetic methods (Adams *et al.* 2019), this could soon be a possibility, thereby making leopard studies incorporating iDNA much more effective and applicable for monitoring target species.

5. Conclusion

In conclusion, both non-invasive genetic and camera trapping techniques are promising for generating quantitative data on the abundance and distribution of leopards. When comparing the survey methods Mondol *et al.* (2009) found that the abundance estimates from both approaches were very similar for Bengal tigers. However, Jackson *et al.* (2006) found that with the aid of an automatic recognition algorithm called AMPHIDENT allowed camera traps to outperform genetic surveys. This is contradicted by Janečka *et al.* (2011) who found that collection and genetic analysis of scat offers a more effective means of data collection

than camera trapping; in terms of field effort, total effort, and financial expenditure. These contrasting results highlight the pros and cons of these two methods. However, while molecular technologies have been used to distinguish individual animals, real-time individual identification in the field can be more effective. Camera trapping covers all the requirements for data collection; accurate, reliable, cost-effective and easy to apply. However, new technological advances have seen DNA analysis become cheaper. If this trend continues, this method could soon be a more applicable method to study leopard populations.

However, even with the improved data collection methods, leopard populations are still declining across Africa (IUCN Red List, 2019), more effective conservation action, needs to be taken to halt this trajectory. Balme, Slotow and Hunter (2009) and Balme, Slotow and Hunter (2010) have found that with management intervention leopard populations in Africa can increase, however, this needs to be replicated at a larger scale.

6. References

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