

Research Article

Estimating Leopard population sizes in western Mozambique using SNP-based capture–mark–recapture models

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Abstract

Reliable population size estimates are imperative for effective conservation and management, but are notoriously difficult to obtain for rare, cryptic species such as large carnivores. Genetic capture–mark–recapture (CMR) models can provide robust population size estimates and may be conducted noninvasively during a single sampling period. African leopards (*Panthera pardus*) are listed as vulnerable, with declining populations. However, population size estimates are rare for much of their range. Here, we provide population size estimates for leopards in the Limpopo (LNP) and Banhine (BNP) national parks and the Lebombo Conservancy (LC), in western Mozambique. We estimate population sizes using SNP-based CMR models derived from genetic information acquired noninvasively from scats collected across the sites. Additionally, we compare our density estimates with those derived from trophic scaling to provide inferences on the drivers of leopard density in the region. We estimate populations of 87, 15, and 13 leopards in LNP, BNP, and LC, respectively. Population size estimates derived from trophic scaling suggest that leopards are limited by bottom-up prey resources in LNP and LC, but there is evidence for top-down regulation in BNP. Given the precariousness of leopard populations in the region, we urge use of population monitoring using genetic CMR models to inform conservation and management.

Key words: genotyping, Greater Limpopo Transfrontier Conservation Area, noninvasive, *Panthera pardus*, scat, trophic scaling.

Effective management and conservation are dependent on reliable population size estimates (Katzner et al. 2011). These are inherently challenging to obtain for rare, cryptic species such as large carnivores (Creel and Rosenblatt 2013). Various noninvasive methods including spoor and scat counts, call-up surveys, camera trapping, and aerial surveys are available for estimating carnivore population sizes (Wilson and Delahay 2001). However, these methods carry biases related to detection probabilities (Redfern et al. 2002; Drøge et al. 2020) and to obtaining absolute population sizes from frequency data, and are frequently imprecise (Ringvall et al. 2000; Anderson 2001). Capture–mark–recapture (CMR) models satisfy many of the abovementioned biases but are often constrained by logistical issues such as the need to often handle the animal (e.g., Muñoz-Igualada et al. 2008) and biased catchability (e.g., Tuytens et al. 1999). Genetic CMR models, which use the genotype of an individual as a unique identifier (Palsbøll 1999), are advantageous over traditional CMR methods since they can be conducted noninvasively, during a single sampling period, and hold great potential in providing robust population size estimates (Kohn et al. 1999).

African leopards (*Panthera pardus*) are listed as Vulnerable by the IUCN and populations have declined by 42% across Africa in the last 24 years (Stein et al. 2020). Leopard population strongholds now center on protected areas (PAs; Jacobson et al. 2016), but many PAs across their range are deteriorating in terms of conserving biodiversity (Lindsey et al. 2017). Given their elusive and cryptic nature, estimating leopard population sizes is inherently difficult, with such estimates typically being derived from density estimates from spatially explicit capture–recapture (SECR) models using camera traps (e.g., Strampelli et al. 2020; Briers-Louw et al. 2024). Although previous studies have used noninvasive genetic sampling (microsatellites) to assess leopard gene flow (Dutta et al. 2013a), population structure (Dutta et al. 2013b), and presence (Busby et al. 2009), only 1 study has previously used genetic CMR models to assess leopard population sizes (Sugimoto et al. 2014)—this despite the utility of genetic CMR models in providing robust population size estimates. This lack of CMR models highlights the need for genetic CMR techniques to be applied more broadly, particularly in African PAs that are deteriorating (Lindsey et al. 2017).

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and experiencing high levels of human encroachment (Witemyer et al. 2008).

Leopards are limited by prey availability (Stander et al. 1997), but human activity is becoming an increasingly limiting and pervasive factor (e.g., Strampelli et al. 2018; Rogan et al. 2022). Comparing potential Leopard density derived from trophic scaling based on prey availability with observed densities allows for inferences to be drawn on the drivers of Leopard density in a region (Hayward et al. 2007)—such information may aid in effective conservation of the species.

Southern Africa's flagship transfrontier conservation area—the Greater Limpopo Transfrontier Conservation Area (GLTCA)—is of global conservation importance (Mabunda et al. 2012) and consists of national parks, private game reserves, and communal lands in South Africa, Mozambique, and Zimbabwe. However, the Mozambique portion of the GLTCA—that constitutes its greatest area—is heavily impacted by human activity, with Mozambique's civil war and decades of poaching decimating wildlife in the region (Grossmann et al. 2014). Furthermore, population size estimates for various mammals, including leopards, in the Mozambique portion of the GLTCA are rare.

Here we provide Leopard population estimates for Mozambique's Limpopo (LNP) and Banhine (BNP) national parks and the Lebombo Conservancy (LC), within the GLTCA, using genetic information obtained noninvasively from scats. Additionally, we compare these estimates with predicted Leopard densities in LNP, BNP, and LC,

testing the hypothesis that Leopard populations are limited by top-down anthropogenic activities. These population estimates can help guide conservation and management efforts in the region, and highlight the utility of genetic CMR models in determining population sizes.

Materials and methods.

Study area.

The study was conducted in 3 PAs in the Mozambique portion of the GLTCA: LNP (11,233 km²); BNP (7,250 km²); and LC (1,320 km²; Fig. 1). The climate is warm, dry tropical, with low and variable rainfall (Stalmans et al. 2004; Stalmans and Wishart 2005). Vegetation is savanna woodland and scrubland (Stalmans et al. 2004; Stalmans and Wishart 2005). Human activities in the PAs include subsistence cropping and pastoralism, bushmeat hunting, logging, mining, charcoal production, and commercial-scale Lion (*P. leo*), African Savanna Elephant (*Loxodonta africana*), White Rhinoceros (*Ceratotherium simum*), and Black Rhinoceros (*Diceros bicornis*) poaching (Grossmann et al. 2014; Everatt et al. 2019a). Wildlife populations are well below estimated carrying capacity (Lindsey et al. 2017) due to the decimation of wildlife during Mozambique's civil war (Hatton et al. 2001), and poor management capacity due to inadequate conservation funding (Baghai et al. 2018), allowing for decades of poaching (Grossmann et al. 2014).

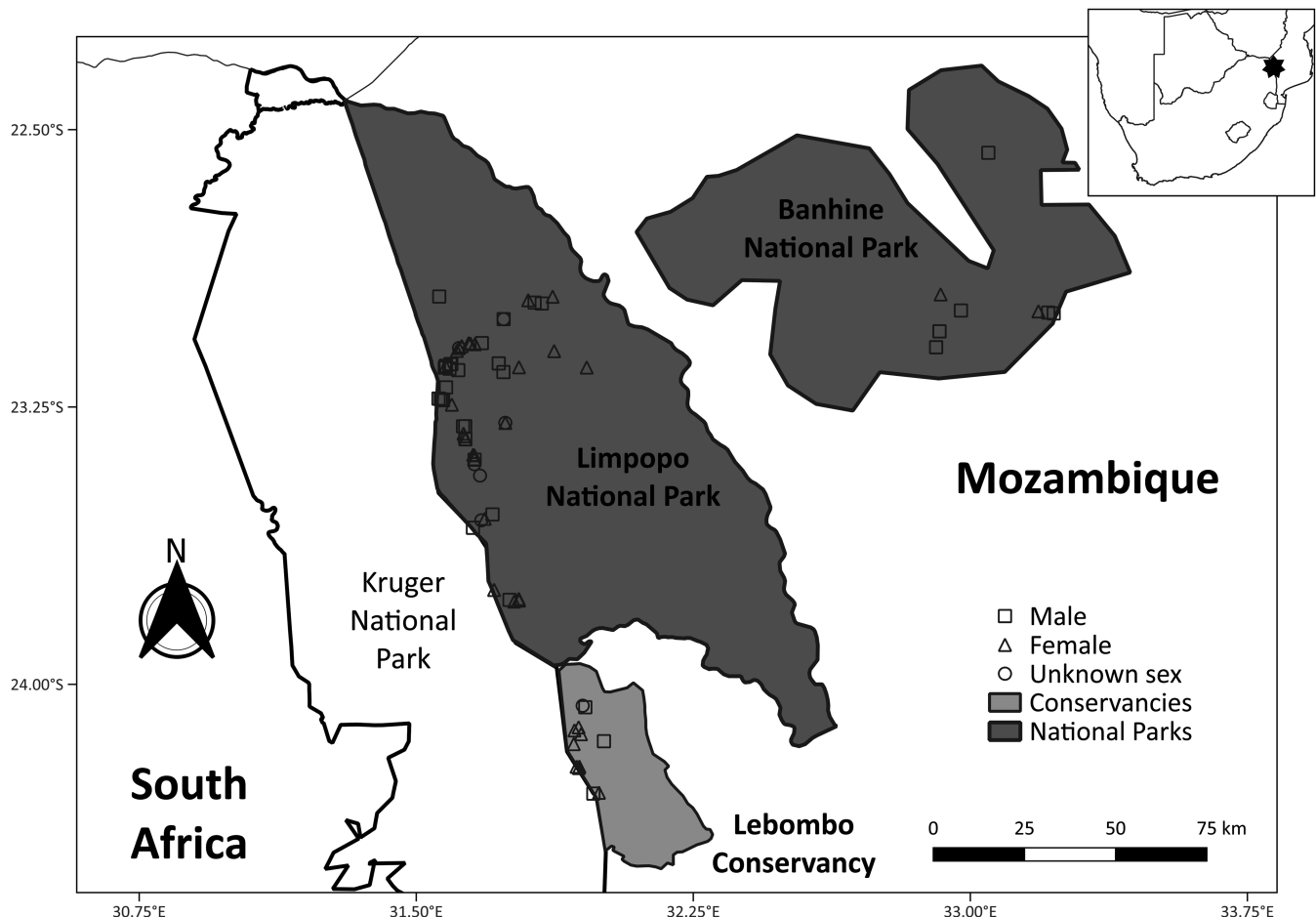


Fig. 1. Study area (shaded) consisting of Mozambique's Limpopo and Banhine national parks and the Lebombo Conservancy. Points refer to locations where genotyped male (squares), female (triangles), and unknown sex (circles) Leopard scats were collected.

Scat sampling.

Scats were collected in 2014 in BNP, 2014 and 2015 in LNP, and 2013 and 2015 in LC using a trained detection dog, within a random sampling design following MacKay et al. (2008). Because detector dogs search for scents on the air, they are equally likely to find scats on or off of trails and to find cryptically placed scats as prominently placed male marking scats, thus reducing sex bias within the sampling (MacKay et al. 2008). Locations of collected scats were recorded, and scats stored in airtight double plastic bags with silica gel in the inner bag.

Dried scats were moistened with distilled water, after which Q-tips were used to sample the outer surface. Both ends of each Q-tip were used to sample each scat, such that 2 subsamples per scat were obtained. Q-tips were then placed in small envelopes, air-dried for 24 h, and stored in an airtight container with silica gel. These samples were then sent to the Molecular Ecology Group, Swedish University of Agricultural Sciences (Umeå, Sweden) for DNA extraction and genotyping.

DNA extraction and genotyping.

DNA was extracted using Zymo's Quick-DNA fecal/soil extraction kit. A total of 252 scats identified as leopards in the field were genotyped on a Fluidigm Biomark using panels of 96 SNPs identified for leopards (PID 2.45×10^{-35} and PIDsib 6.53×10^{-17}). Markers were selected based on their ability to generate consistent and accurate genotypes from low- and high-quality DNA extracts, which was validated using replicates and empirically determined thresholds following methods previously described (Norman et al. 2013; Blåhed et al. 2018; Spitzer et al. 2020). The panel is comprised of 3 monomorphic sex markers and 5 mitochondrial markers—see Mamugy et al. (2024) for a full description of the SNP panel used in this study as well as the design, validation, and QC protocols. Negative controls were included in all runs. Samples where heterozygosity was <0.05 and amplification success was $<90\%$ were removed from our analyses. Genotyping errors were calculated from autosomal SNPs by counting the number of allele mismatches at each locus, divided by the total number of amplified alleles.

Data analysis.

Population size estimation.

Data analysis was performed using R software (R Core Team 2023). We empirically determined the threshold amplification rate required to generate genotypes of sufficient quality by comparing allelic differences between all sample pairs. When comparing genotypes of all sample pairs in a CMR data set, 2 distributions of allelic differences manifest. One distribution will hold the allelic differences between unique individuals. For 96 markers, this distribution typically centers around 50 to 60 differences depending on variation in the population. The other distribution shows mismatches between samples from the same individual caused by genotyping error. If genotyping error is high and genetic variation in the population is low, these distributions may partially overlap, rendering separation of unique genotypes (individuals) from spurious genotypes caused by error impossible. Once the amplification success needed to ensure genotypes of sufficient quality had been established, we assessed the number of unique Leopard individuals sampled across PAs using the package “allelematch” (Galpern et al. 2012). Based on the above, we allowed for a maximum of 13 mismatches for samples to be categorized as the same individual. We manually screened the output to ensure accurate matching of duplicate samples, and that the same samples were not identified as different individuals. Our data held 9 technical replicates, all of which were correctly binned.

Outputs from the individual identifications were then used to estimate Leopard population sizes using CMR analyses.

We estimated population sizes using a maximum likelihood estimate (multinomial probability distribution) in the package “capwire” (Pennell et al. 2013). In “capwire,” we ran 2 CMR models that: (1) assume all individuals were sampled equally (equal capture model); and (2) account for differences in individual detectability (2-innate rate model). We selected the best-fitting model using a likelihood ratio test in “capwire” (Pennell et al. 2013). We bootstrapped population size estimates 1,000 times to acquire 95% confidence interval estimates for each PA. We used population sizes from the best-fitting models to calculate Leopard density per PA, expressed as the number of leopards per 100 km².

Additionally, we calculated the effective population size (N_e) for each PA as:

$$N_e = \frac{4 \times N_m \times N_f}{N_m + N_f}$$

where N_m is the number of males, and N_f is the number of females in a population (Wright 1933).

Genetic diversity.

We calculated population-level genetic diversity for leopards as observed (H_o) and expected (H_e) heterozygosity, and inbreeding coefficients (F_{IS}) in GENALEX 6.5 (Peakall and Smouse 2006).

Trophic scaling.

We estimated Leopard carrying capacity for LNP and BNP using a Leopard/prey regression model (Hayward et al. 2007), which produces expected Leopard densities based on the biomass of their significantly preferred prey. We calculated prey biomass using $\frac{3}{4}$ mean adult female body mass (Hayward et al. 2007) for each preferred Leopard prey species (Hayward et al. 2006), multiplied by the availability of the preferred prey species. Significantly preferred prey included in our trophic scaling estimates were Impala (*Aepyceros melampus*), Northern Bushbuck (*Tragelaphus scriptus*), and Bush Duiker (*Sylvicapra grimmia*; Hayward et al. 2006). Data on prey availability were acquired from raw animal counts derived from driven transects conducted in LNP, BNP, and LC (Everatt et al. 2023).

Results

Individual identification.

Genotyping error for the filtered data set was 6%. Following filtering, manual screening, and removal of duplicate samples, we obtained 118 genotype matches from 103 scats of which 63 were unique individuals and 12 genotypes had multiple matches, across LNP, BNP, and LC (Table 1). LC's Leopard population was strongly female-biased (1 male:3.5 female), whereas the inverse was recorded for BNP (1 male:0.35 females), and LNP's sex ratio approximated parity (1 male:1.1 females; Table 1), thus indicating that there was no sex bias associated with the sampling.

Population size.

Model selection based on the hypothesis that there are individual differences in detectability was accepted for leopards in LNP (P -value for all likelihood ratio test <0.01). We therefore applied the 2-innate rate model to assess population size for LNP, and the equal capture model for Leopard populations in BNP and LC. Leopard population estimates were 84 in LNP, 12 in BNP, and 13 in LC, with the 2-innate rate model providing larger population size estimates for all PAs (Table 2). N_e for LNP, BNP, and LC were 40, 6, and 6, respectively (Table 2).

Table 1. Number of genotype matches, unique individuals, multiple matches, and unclassified samples from “allelematch” outputs and the number of unique male, female, and unknown sex samples for the Limpopo (LNP) and Banhine (BNP) national parks and the Lebombo Conservancy (LC).

Protected area	Genotype matches	Unique individuals	Multiple matches	Unclassified	Males	Females	Unknown
LNP	95	45	12	1	19	21	5
BNP	10	9	0	0	7	2	0
LC	13	9	0	0	2	7	0

Table 2. Realized population size estimates and 95% confidence intervals, derived from each CMR model, and potential population sizes based on trophic scaling for leopards in the Limpopo (LNP) and Banhine (BNP) national parks and the Lebombo Conservancy (LC). Realized and potential Leopard densities from selected CMR model and trophic scaling, respectively, and effective population sizes N_e .

Protected area	Realized population size		Potential population size	Realized density (leopards/100 km ²)	Potential density (leopards/100 km ²)	N_e
	Two-innate rate model	Equal capture model				
LNP	87 (78 to 118) ^a	56 (46 to 66)	62.91	0.75	0.56	40
BNP	19 (10 to 39)	15 (8 to 22) ^a	34.08	0.17	0.47	6
LC	19 (10 to 45)	13 (8 to 18) ^a	8.1	0.98	0.36	6

^aBest-fitting model.**Table 3.** Genetic diversity, expressed as observed (H_o) and expected (H_e) heterozygosity, and inbreeding coefficients (F_{is}) for leopards in the Limpopo (LNP) and Banhine (BNP) national parks and the Lebombo Conservancy (LC).

Protected area	n	H_o (± SE)	H_e (± SE)	F_{is}
LNP	89	0.35 (0.02)	0.40 (0.02)	0.05
BNP	13	0.36 (0.02)	0.36 (0.02)	−0.01
LC	13	0.41 (0.02)	0.37 (0.02)	−0.08

n: number of genotypes.

Genetic diversity.

Genetic diversity metrics were broadly similar across sites, with H_o being greatest in LC (0.41), followed by BNP (0.36), and LNP (0.35; Table 3). F_{is} values were also similar across sites, being lowest in LC (−0.08) followed by BNP (−0.01) and LNP (0.05; Table 3).

Trophic scaling.

Everatt et al. (2023) estimated 1,579, 543, and 75 preferred Leopard prey individuals for LNP, BNP, and LC, respectively. This estimate produced preferred prey biomass estimates of 4.09, 2.05, and 1.7 kg/km² in LNP, BNP, and LC, respectively. Trophic scaling estimates of potential Leopard density were 0.56, 0.47, and 0.36 individuals per 100 km², yielding potential Leopard population size estimates of 63, 34, and 8 individuals in LNP, BNP, and LC, respectively. These estimates closely approximate estimates produced by our genetic CMR models for LNP, but are higher and lower than the estimates in BNP and LC (Table 2).

Discussion

Our findings represent the first Leopard population size estimates for LNP and BNP and provide baseline population estimates for the region. The density estimates are among the lowest recorded for leopards in southern Africa and are similar to those in landscapes of comparable habitat type and land use in Botswana (Boast and Houser 2012) and northwestern Zimbabwe (Loveridge et al. 2022), and to those of Xonghile Game Reserve within LC (Strampelli et al. 2020). Furthermore, our density estimates approximate those in unprotected Fynbos and Succulent Karoo landscapes in the Western Cape of South Africa (Martins 2010; Devens et al. 2018),

where primary productivity is low (Read and Mitchell 1983) and carnivores have faced intense persecution (Skead 2011). The Western Cape Leopard densities are recognized as being particularly low, and these are relic populations that have persisted for over 250 years in the face of persecution (Skead 2011). This result suggests that despite the low densities recorded here, these Mozambique populations may persist. However, under conditions of favorable conservation management and prey availability, such as in the Greater Kruger National Park, Leopard densities may exceed 12 individuals per 100 km² (Maputla et al. 2013; Balme et al. 2019)—an order of magnitude higher than our density estimates of <1/100 km².

Contrary to our hypothesis that leopards in our study area are limited by top-down anthropogenic activity, our population size estimates closely approximate those derived from trophic scaling based on preferred prey biomass. This result suggests that Leopard populations in our study area are predominantly limited by prey availability rather than top-down anthropogenic activity, as seen elsewhere (Rosenblatt et al. 2016). Conversely, lions in our study area are strongly limited by human activities (Everatt et al. 2014, 2019b). Lions are usually more persecuted relative to other African carnivores, since they: (1) consume cattle (*Bos* spp.) more than other livestock, with cattle having greater cultural and spiritual value in African communities relative to other livestock (Schneider 1984); (2) defend their kills from humans, unlike other carnivores; and (3) are social and vocal carnivores, with these behavioral traits enabling humans to find and kill them more easily than other carnivores (Kissui 2008). The secretive and solitary nature of leopards, and their strong livestock avoidance (Forbes et al. 2024), possibly explain why their populations in our study area approximate potential

prey-based population size estimates, unlike lions. However, we note the evidence for top-down anthropogenic activity limiting BNP's Leopard population as seen by the lower realized population size relative to the potential population size. Poaching for Leopard body parts and skins, and bycatch from bushmeat snaring limits populations both in Mozambique (Briers-Louw et al. 2024) and across their range (Stein et al. 2020). Further investigation into this matter, particularly in BNP, is required.

We note the variability in sex ratios estimated here, with LNP approximating parity and contrasting with the male-biased BNP and the female-biased LC estimates. Leopard sex ratios are typically female-biased, with 1 male:1.8 females (Nowell and Jackson 1996), whereas the sex ratios recorded in our study vary across sites. These ratios for BNP and LC may reflect chance demographic effects that are particularly pronounced in very small populations (Caughley 1994), but may have long-term, currently unknown, demographic implications. This is, in part, reflected in the low effective population sizes estimated here.

Conservation and management implications.

Given that various noninvasive techniques used for assessing Leopard population sizes face numerous biases and are often inaccurate (Redfern et al. 2002; Dröge et al. 2020), genetic CMR models can serve as a robust, alternative method for assessing population sizes (Kohn et al. 1999). SECR models derived from camera trap data can produce precise population size estimates (Efford 2004) and are the most commonly used method for assessing Leopard density. Our Leopard density estimate for LC is in line with those derived using SECR models (Strampelli et al. 2020), with data collected during the same period in both studies. The precision of our density estimates potentially underscores that both methods (genetic CMR and SECR) can produce robust population size estimates for leopards. However, large-scale camera trap studies are expensive and logistically challenging in terms of camera trap setup and maintenance (Foster and Harmsen 2012). Additionally, camera traps need to be operated for an extensive survey period to yield accurate population size estimates (Wang and Macdonald 2009). Conversely, genetic CMR methods can be conducted during a single, short sampling period. Many large mammal species (e.g., lions) are also difficult to recognize individually based on external features using cameras, which can lead to inflated population size estimates (Treves et al. 2010). This issue can be mitigated by identifying individuals from their unique genotype (Palsbøll 1999). Furthermore, genetic data derived from noninvasively collected samples can also be used to meet other objectives including drawing inferences on survival, reproductive success, dispersal, and describing diets. For example, we have previously described Leopard diets in LNP using the same scats that were used in this study (Forbes et al. 2024). Additionally, genetic CMR models can be used for providing robust assessments of sustainable hunting quotas, since a lack of robust population size estimates and assessments of sustainable off-takes (Spong et al. 2000; Balme et al. 2009; Creel et al. 2015) has been shown to have devastating outcomes for Leopard populations (Loveridge et al. 2022). Based on the above information, we urge future carnivore population assessments to be conducted using genetic CMR models as described here.

In conclusion, the baseline Leopard population size estimates provided here for LNP, BNP, and LC clearly highlight the need to improve management and conservation of the species in the region. We reemphasize the need for urgent prey population rehabilitation, which would promote the recovery of Leopard populations. Potential top-down Leopard population regulation in BNP needs further investigation. Given the precariousness of the Leopard

population in the region, continued monitoring of population sizes using noninvasive CMR models would reveal population responses to improved conservation practices.

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Conflict of interest

None declared.

Data availability

Data are available at <https://doi.org/10.25408/mandela.28172882.v1>.

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