

ASSESSING THE IMPACTS OF PROTECTION GRADIENTS ON LARGE AFRICAN
CARNIVORE DENSITY AND SURVIVAL: AN EXAMPLE WITH AFRICAN LION
AND LEOPARD IN THE LUANGWA VALLEY, ZAMBIA

by

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ABSTRACT

Large carnivores are in rapid global decline, primarily due to anthropogenic pressures. Human activities on the periphery of protected areas can limit carnivore populations, but measurements of the strength of such effects are limited. Both African lion (*Panthera leo*) and leopard (*Panthera pardus*) are declining throughout their ranges, and thus accurate monitoring of key populations is critical. Both of these species face pressure from encroaching human populations, particularly from trophy hunting, illegal bushmeat harvest, and human-carnivore conflict. In Zambia, South Luangwa National Park and its buffer areas are thought to contain the country's largest lion and leopard populations. However, this protection gradient is experiencing rapid human population growth and activities that are known to threaten large carnivore populations elsewhere. Here we examined the status and major anthropogenic drivers of the South Luangwa lion and leopard populations. First, we estimated population size, trends, survival rates and demography for the South Luangwa lion population from 2008 to 2012. These data indicated that trophy hunting was impacting the South Luangwa lion population, and potential management actions exist and should be implemented to mitigate impacts from trophy hunting. Second, we measured how the density and survival rates of South Luangwa's leopard population varied across this gradient of protection using remote camera trap surveys from 2012-2014 during a ban on trophy hunting. We estimated that leopard density was higher inside South Luangwa National Park as compared to an adjacent buffer area with lower levels of protection, but could not detect differences in leopard survival across these two areas. This difference in density was most likely driven by prey depletion in the buffer areas, and this limitation is likely an issue for other sympatric large carnivore species. Finally, we developed a rapid survey method based on pedigree reconstruction to estimate population size, with validation based on a simulated population. This method shows promise for surveying unstudied large carnivore populations. Overall, large carnivore populations face growing anthropogenic pressures worldwide, and management action to mitigate population declines must be informed by intensive monitoring of key large carnivore populations to identify the drivers and dynamics of such declines.

CHAPTER ONE

INTRODUCTION TO THESIS

Overview of Thesis

Large carnivores are under threat worldwide, primarily due to anthropogenic activities (Vitousek et al. 1997; Ripple et al. 2014). Not only are large carnivores iconic, but also provide important ecosystem, economic, and cultural services (Estes et al. 2011; Ripple et al. 2014). Anthropogenic activities directly and indirectly limit large carnivore populations through the conversion of habitat for agriculture and livestock, the legal and illegal harvest of wildlife, and increased risk of human-carnivore conflict (Ripple et al. 2014). With the rapid growth of human populations across the globe, the majority of large carnivore populations now reside within or adjacent to protected areas (Ripple et al. 2014). Though these populations may appear to be fully protected, they face increasing pressure from rapid human population growth on their periphery (Woodroffe and Ginsberg 1998; Wittemyer et al. 2008).

Large carnivores are difficult to protect from anthropogenic pressures as they are usually elusive, low-density species that are vulnerable to conflict with people (Ripple et al. 2014). Large carnivores consequently require large areas of intact habitat and are sensitive to nearby human activities (Woodroffe and Ginsberg 1998; Brashares et al. 2001; Woodroffe 2000; Cardillo et al. 2004; Creel et al. 2013). In developing countries, many of which contain key large carnivore populations, human encroachment around protected areas is problematic. Rural human population growth in many developing

countries is highest around protected areas (Wittemyer et al. 2008). These high rates of human encroachment and associated activities can limit carnivore populations within protected areas (Pulliam 1988; Woodroffe & Ginsberg 1998) and can draw individuals areas of lower protection (Loveridge et al. 2010).

Large carnivores are difficult to study for the same reasons they are difficult to protect. Demographic data from intensive monitoring of known individuals are of prime importance to inform and guide conservation efforts for large carnivore species. However, their wide-ranging and elusive nature hinders the collection of these data critical in identifying the drivers and dynamics of population declines (Durant et al. 2007). As a result, several rapid and economical survey methods have been developed to monitor large carnivore populations. While these rapid survey methods can produce estimates of large carnivore abundance, the precision of these estimates is usually insufficient to detect population declines beyond large reductions. Also, these rapid survey methods provide little demographic information, which are critical for identifying drivers of and potential management actions for declining large carnivore populations. While these alternative survey methods have some use for large carnivore studies and warrant further development, intensive monitoring of known individuals is our primary means to precisely estimate population abundance, trends, and vital rates, and to evaluate the drivers behind the declines of large carnivore populations.

The African lion (*Panthera leo*) and leopard (*Panthera pardus*) typify the issues facing large carnivore research and conservation. Both species have suffered reductions in their distributions due to human encroachment (Riggio et al. 2012; Ripple et al. 2014),

primarily due to the depletion of herbivore populations, habitat loss, conflict with humans, poaching, and trophy hunting (Yamazaki 1996; Ogada et al. 2003; Loveridge et al. 2007, 2010; Henschel et al. 2008; Bauer, Nowell, & Packer 2013; Packer et al. 2009, 2011; Becker et al. 2013a,b, Groom et al. 2014). Intensive monitoring for both of these species is difficult, and thus rapid survey methods have been developed to estimate population sizes. Zambia contains regionally-important lion and leopard populations (Purchase, Mateke, & Purchase 2007; Riggio et al. 2012). However, the majority of Zambia's lion and leopard populations have not been intensively studied (Yamazaki 1996; Ray 2011; Becker et al. 2013b; Midlane 2014) and face growing threats from peripheral, rapidly growing human populations (Watson et al. 2014). The Luangwa Valley in eastern Zambia is particularly important as it contains what are thought to be Zambia's largest lion and leopard populations (ZAWA 2010; Ray 2011). Of the protected areas in the Luangwa Valley, South Luangwa National Park (SLNP) is thought to contain the largest populations of lion and leopard. However, SLNP is bordered by buffer areas, or Game Management Areas (GMAs), that are intended to provide some protection for wildlife, but contain rapidly growing human populations, habitat conversion, illegal bushmeat harvest and legal trophy hunting of both species (Yamazaki 1996; Lewis & Phiri 1998; Becker et al. 2013a,b; Lindsey et al. 2013; Watson et al. 2013, 2014). Therefore, SLNP and its protection gradient provide an opportunity to intensively study the dynamics of these important populations facing threats widespread across Zambia and Southern Africa, and develop alternative methods of surveying other unstudied large carnivore populations.

The South Luangwa (SL) lion population faces pressure from legal trophy hunting and illegal bushmeat harvest in the GMAs, as both of these activities can increase lion mortality and deplete herbivore populations (Yamazaki 1996; Lewis & Phiri 1998; Becker et al. 2013a,b; Lindsey et al. 2013; Watson et al. 2013, 2014). Therefore, in chapter 2, I used capture-mark-recapture models to assess the trend, demography, and survival rates of the SL lion population facing both of these harvest pressures, and discuss the future for this important lion population. I conclude this chapter with recommendations for managing lion trophy hunting, and highlight the importance of maintaining such intensive monitoring efforts.

The SL leopard population faces similar pressures from legal and illegal harvests both within SLNP and in surrounding GMAs. Leopard survival and density has been documented to decrease outside of protected areas due to prey depletion and increased anthropogenic mortality for leopards (Henschel et al. 2008, 2011; Balme, Slotow & Hunter 2009, 2010; Swanepoel et al. 2015). Therefore, the SL leopard population may experience higher levels of mortality and lower densities in buffer areas around SLNP. In chapter 3, I used remote camera trap surveys and capture-mark-recapture models to estimate densities and survival rates for the SL leopard population within this protection gradient during a temporary ban on all trophy hunting. This study is the first of its kind for the SL leopard population, and provides methodological improvements for future studies of how leopard densities and vital rates change across space and time.

As discussed previously, intensive monitoring of individuals in most carnivore populations is not possible due to the high costs and duration of such studies (Durant et

al. 2007). Therefore, we must continue to develop rapid surveys to learn baseline information about the abundance of unstudied populations. In Chapter 4, I developed a population size estimator using pedigree reconstruction methods as a tool for initial assessments of large carnivore populations, and validated this approach with a simulated lion population. This method appears to be a promising approach to rapidly surveying multiple carnivore populations for regional assessment of the status of carnivore populations.

Finally, in chapter 5, I provided general conclusions and discuss how this work can contribute to our efforts to conserve large African carnivore populations in the face of growing anthropogenic pressures. I also explored how these approaches can be improved for monitoring Zambia's important lion and leopard populations.

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CHAPTER TWO

DETECTING DECLINES OF APEX CARNIVORES AND
EVALUATING THEIR CAUSES: AN EXAMPLE
WITH ZAMBIAN LIONS

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Abstract

Large carnivores are in rapid global decline, with a broad array of consequences for the ecosystems they inhabit. To efficiently detect and address these declines requires unbiased and precise demographic data. Unfortunately, the characteristics that make large carnivores extinction-prone also pose serious challenges to obtaining these data. Rapid survey methods exist, but provide only relative measures of abundance, cannot detect declines before they become large, and provide little or no information about the causes of decline. African lions (*Panthera leo*) are declining throughout their range, making accurate monitoring of remaining populations urgent. We provide statistically rigorous estimates of population size, trends and survival rate from Zambia's South Luangwa lion population from 2008-2012, just prior to cessation of hunting in 2013. Mark-recapture models fit to data from intensive monitoring of 210 individual lions in 18 prides and 14 male coalitions indicated a declining population, low recruitment, low sub-adult and adult male survival, depletion of adult males, and a senescing adult female population. Trophy hunting was the leading cause of death, with 46 males harvested. Based on these data we recommend continuing the hunting ban at least to 2016 to allow recovery, with substantially reduced quotas, age-limits, and effective trophy monitoring mandated thereafter should hunting resume. Similar data from intensive monitoring of key Zambian lion populations is required to evaluate effects of the hunting ban and provide management guidance. Effectively integrating intensive long-term monitoring and rapid survey methods should be a primary priority for future large-scale management and monitoring of carnivore species.

Keywords: African lion, survival, population estimate, intensive monitoring, precision, mark-recapture.

Introduction

The decline and extinction of large carnivores is one of the most pervasive human impacts on earth's ecosystems (Vitousek et al., 1997). While our understanding of carnivores' far-reaching ecological impacts continues to broaden (Estes et al., 2011), losses continue to accelerate, and the majority of the world's large carnivores are currently threatened (Ripple et al., 2014). Large carnivores are typically low-density, wide-ranging, and elusive, with a propensity to conflict with humans; consequently, these species are very sensitive to human impacts, even in strictly protected areas, and usually require large areas of relatively intact, contiguous tracts of habitat (Woodroffe and Ginsberg, 1998; Brashares et al., 2001; Woodroffe, 2000; Cardillo et al., 2004; Creel et al., 2013). Demographic data are of prime importance to inform and guide conservation efforts, but the characteristics that make large carnivores extinction-prone also hinder monitoring, particularly when populations are small and declining.

Population monitoring to describe dynamics typically yields data constrained by a trade-off between scale and precision. Intensive long-term studies of known individuals provide good precision (e.g. Packer et al., 1998; Peterson, 1999; Kelly and Durant, 2000), but are rare and relatively small scale due to their logistical difficulty, expense, and time-consuming nature (Durant et al., 2007). Consequently, a variety of rapid and economical survey methods have been developed to monitor large carnivore populations, including

spoor counts (Van Dyke et al., 1986; Stander, 1998; Houser et al., 2009; Funston et al., 2010; Ferreira et al., 2013; Bauer et al., 2014; Midlane et al., 2014), audio lures (Ogutu and Dublin, 1998; Mills et al. 2001; Kiffner et al. 2008; Ferreira and Funston, 2010; Cozzi et al., 2013; Groom et al., 2014), camera trapping (Karanth and Nichols, 1998; Jackson et al., 2006; Balme et al., 2009; Karanth et al., 2011; Schuette et al., 2013), distance sampling (Durant et al., 2011), detection dogs (Smith et al. 2001), extrapolation from prey density (Karanth et al., 2004), and noninvasive genetic surveys (Kohn et al., 1999; Creel et al., 2003; Mondol et al., 2009; Creel and Rosenblatt, 2013). While these survey methods avoid some of the constraints inherent to intensive monitoring of known individuals, they often provide population estimates with confidence intervals too broad to provide clear guidance for management and conservation. Thus large (or unmeasured) variance in estimates of population size remains a substantial impediment to detecting carnivore declines, prioritizing areas for conservation, and assessing the effectiveness of management actions.

This problem is exemplified with Africa's largest carnivore, the lion (*Panthera leo*), which has declined throughout its range (Riggio et al., 2012) due to a combination of prey depletion and habitat loss, direct conflict and retaliatory killing, wire-snare poaching, and trophy hunting (Yamazaki, 1996; Ogada et al., 2003; Loveridge et al., 2007, 2010; Bauer, 2013; Packer et al., 2009, 2011; Becker et al., 2013a, Groom et al., 2014). The broad range of threats to lion population viability creates an urgent need for accurate data to describe population trends, identify underlying demographic changes and understand their causes. To address many of the difficulties inherent to studying lions,

indices of abundance (IOA; Conroy, 1996) such as spoor counts (Stander, 1998) have been widely adopted. While spoor counts can provide unbiased estimates of lion population size, the precision of these estimates must be carefully considered in practical assessments of lion and other large carnivore populations. As noted by Midlane (2014), the coefficient of variation has been calculated incorrectly in a sequence of studies that have estimated lion density from spoor counts in a manner that substantially overestimates precision (Stander, 1998; Funston et al., 2010; Ferreira et al. 2013, Bauer et al., 2014). Consequently, the ability of spoor counts to describe and evaluate trends in lion populations has been overstated. Another common lion monitoring strategy employs audio playback experiments (i.e. call-in surveys). However, these population estimates also have low precision and can be biased by variation in detection probability and methodology (Mills et al., 2001; Whitman, 2006; Kiffner et al., 2009, Cozzi et al. 2013). While IOA for lions and other carnivores are important conservation tools, precise estimates of population density, trends and vital rates and information about the probable causes of demographic patterns still depend primarily on intensive monitoring of known individuals.

Zambia is one of eight remaining African countries containing a lion stronghold (Riggio et al 2012); however its lion populations are geographically and numerically limited by human encroachment, direct mortality due to wire-snare poaching by-catch, prey depletion due to poaching, trophy hunting, disease, and human-lion conflict (Yamazaki, 1996; ZAWA, 2009; Becker et al., 2013a, 2013b; Berentsen et al., 2013; Watson et al., 2013, 2014; Midlane et al., 2014; Lindsey et al., 2014). The relative

importance of these factors, their trends through time, and the associated demographic impacts on lions are poorly understood. In response to growing concern over the status of Zambian lions and a lack of data on population size, distribution, and trends, the Zambia Wildlife Authority (ZAWA) developed a National Conservation Strategy and Action Plan for the Lion, with the overall intent being "... to establish a science-based Conservation Strategy and Action Plan for the African Lion" (ZAWA, 2009) In January 2013, the government of Zambia enacted a ban on lion trophy hunting due to concern over potentially excessive quotas, alleged mismanagement, possible lion declines, and a lack of scientific data to assess the status of lions and other species (Mfula, 2013). To address these issues we use data from intensive monitoring of known individuals in a five-year (2008-2012) study of lions in South Luangwa National Park and the adjacent Lumimba and Lupande Game Management Areas to estimate age- and sex-specific survival rates and population size, density and growth rate using mark-recapture models. We use these results to evaluate lion management policies in Zambia, and more broadly as an example of the importance of intensive monitoring for detecting, understanding and addressing large carnivore declines.

Material and Methods

Study Area and Data Collection

Our 2 775 km² intensive study area was located along the eastern boundary of South Luangwa National Park (SLNP) and the adjoining Lupande and Lumimba Game Management Areas (GMAs), which collectively support a substantial portion of the

largest lion population in Zambia and Zambia's prime photo tourism and trophy hunting area (Figure 2.1; ZAWA 2010). While national parks are strictly protected, GMAs are classified as IUCN Category VI areas that serve as buffer zones to national parks and allow a variety of natural resource-based uses (Dudley, 2008; Chomba et al., 2011), including trophy hunting of male lions (Yamazaki, 1996; Becker et al., 2013b), and human settlement and development (see Section 4.3). Our study area thus encompassed two wildlife management regimes, with associated variations in human influence, available habitats, and potential prey.

The study area included a mosaic of edaphic grassland, deciduous riparian forest, miombo (*Brachystegia spp*) woodland, mopane (*Colophospermum mopane*) woodland and scrubland, dry deciduous forest, and undifferentiated woodland (Astle, 1988; Astle et al., 1969; White, 1983). The perennial Luangwa River flows through the study area from North to South and forms most of the eastern border of the park, though lions and other wildlife move freely between SLNP and adjacent GMAs. The Luangwa valley experiences two distinct seasons: a rainy season (December-April) with extensive flooding and a dry season (May-November) during which availability of water is severely restricted, even in major rivers. Within the dry season, there is a cold dry season (May-August) and a hot dry season (September-November). Both wildlife and human activity is centered along the Luangwa River at the boundary of SLNP and adjacent GMAs, particularly during the height of the dry season. This pattern results in high carnivore and herbivore densities close to the Luangwa River, with diminishing densities further from

the river. Thus, our data come from what is thought to be the prime lion habitat within and around the park.

We recorded all lion sightings from intensive monitoring of known individuals in 18 prides and 14 male coalitions (hereafter referred to as ‘coalitions’) from 2008-2012, during which all lions were individually-identified using whisker-spot patterns, scarring, and tooth breakage (Pennycuick and Rudnai, 1970; Becker et al., 2013a). Since mid-2009, with permission from the Zambia Department of Veterinary and Livestock Development and ZAWA, one adult female lion in each of eight resident prides and one adult male lion in each of five resident male coalitions was instrumented with a combination of VHF and GPS collars. Because lions live in stable social units, VHF radio collars allowed regular resighting of uncollared individuals in our focal study groups, allowing better monitoring of population size and survival than would otherwise have been possible. Data from peripheral, uncollared prides and coalitions sighted opportunistically were used in conjunction with sighting data from the collared resident prides and coalitions, with the presence or absence of a collar considered in statistical analysis.

Field teams worked daily from March-December (> 1 000 person-days) to cover the intensive study area and locate individuals using a combination of telemetry and opportunistic sightings. At each lion sighting, photographs were taken of each animal and compared to identification photos of known individuals. Group affiliation, reproductive status, and fate (alive, dead or not detected) were recorded for each individual at each sighting. Birth dates for individuals that were first identified as adults

were estimated using established standards for nose-pigmentation pattern, tooth wear and coloration, and facial scarring (Whitman et al., 2004; Whitman and Packer, 2007), and by comparison with known-age individuals in this population. We obtained additional data from sightings and photographs by commercial safari guides, clients, and film crews operating in the intensive study area. When possible, causes of mortality were determined via observations, necropsies, and harvest data from hunting concessions. As the identity of a harvested adult male lion could not always be confirmed (see Section 3.1), the disappearance of a known lion was attributed to a harvest when coinciding with the reported harvest of a lion within that known lion's home range. Data used in this study were restricted to the 8-month period (April-November) that was dry enough for intensive monitoring throughout the study area.

Estimation of Population Size, Growth Rate and Density

We used closed mark-recapture models to estimate population size as the number of lions counted divided by the estimated probability of detection (Otis et al., 1978). For each individual lion in each of five years (2008 – 2012), we constructed an eight month detection history from April through November that recorded whether each known lion was detected (1) or not (0) in each month. We used Program MARK to fit capture-mark-recapture models to these detection histories, estimating the monthly probability of initial detection (\hat{p}), subsequent detection (\hat{c}), and population size (\hat{N}) (White and Burnham, 1999). Analyzing each year's data separately, we used Akaike's Information Criteria corrected for small sample size (AICc) to evaluate a set of models that allowed \hat{p} and \hat{c} to vary by age, sex, season and location. We used the same model for population estimation

in each year, as is desirable for unbiased estimation of population trends (Williams et al., 2002).

Two preliminary results confirm that the population was geographically closed as assumed by the models we fit. First, detection rates were high (see Section 3.3) for the age-sex class that most commonly disperses in lions, sub-adult and young adult male lions (Schaller, 1972; Hanby et al., 1995). Second, the proportion of male coalitions that were detected for the first time remained small and constant as the study progressed (see Section 4.3). The models' assumption of demographic closure over eight months is also reasonable for these data, which were characterized by long inter-birth intervals, reproduction spread across all seasons and relatively low annual natural mortality in most age classes (Packer et al., 1988; Packer et al., 2001).

AICc scores consistently provided the strongest support for Huggins closed-capture models with individual heterogeneity in the likelihood of detection (Huggins, 1989; Huggins, 1991). Within the Huggins heterogeneity model, the data consistently supported models with variation in detection probability among two seasons (April-September, and October-November), roughly corresponding to the cold-dry and hot-dry seasons respectively, and in which the probability of initial detection differed from the probability of subsequent detection ($\hat{p} \neq \hat{c}$), probably because decreasing water availability caused prey to concentrate, and thus increased lion detection with the progression of the dry season. In each year, we excluded the detection histories of individuals that were known to have died during that year, and added these individuals to \hat{N} from the mark-recapture model to produce a final estimate of population size at the

beginning of each year's study period. Because we detected ecologically meaningful differences among annual estimates of population size (with $p < 0.05$) power analysis was not necessary.

Annual population growth rates (λ_t) were estimated as $(\hat{N}_{t+1}/\hat{N}_t)$, with 80%, 90%, and 95% confidence intervals estimated by parametric bootstrapping in R (R Core Team 2014) with 10 000 replications, assuming that population sizes were distributed normally with the mean and variance estimated by the mark-recapture model. The population growth rate (λ) for the entire period was estimated as the geometric mean of annual growth rates with a confidence intervals estimated using the *deltamethod()* function in the **msm** package (Jackson, 2011) of R (Seber, 1982). We estimated 95%, 90% and 80% confidence limits to provide retrospective power analysis.

We converted estimates of population size to two estimates of population density. We estimated 'maximum density' with a minimum bound on the area used by these lions by dividing the estimated population size by the area of the 95th percentile isopleth of a kernel utilization distribution (Worton, 1989) fit to all lion locations using Hawth's Tools (Beyer et al., 2004) in ArcGIS 9.2 (ESRI, 2006; Figure 2.4). This isopleth (hereafter referred to as utilized area) represents the area that was unquestionably utilized by the Luangwa Valley lion population. Because this method is likely to overestimate the true density (Sollman et al. 2012), we also estimated 'minimum density' with a maximum bound on the area sampled, equal to the area of our study site (2 775 km²). We did not apply a buffer to this maximum area because search effort was lower at the periphery of the study site, it is likely that unidentified lions entered the periphery of the study area

(particularly from the west) more often than identified lions left it, and several patterns in the data suggest that such movements were not common (see Results and Discussion).

Estimation of Age- and Sex-Specific Survival

We used a multiple-age Cormac-Jolly-Seber (CJS) model to estimate age- and sex-specific annual apparent survival rates ($\hat{\phi}$) and annual detection probabilities (\hat{p}) for five biologically meaningful age classes: cub (0-1 years old), sub-adult (2-3 years old), and sexually-mature young adult (4-5 years old), prime adult (6-7 years old) and old adult (8+ years old). We collapsed the eight-month annual encounter histories described above into two four-month occasions per year, where the detection or non-detection of each individual was recorded for each four month period (July 31st and November 31st) resulting in a 10-occasion encounter history for each individual for the entire 5-year study period. This collapsed encounter history yielded adequate probability of detection for all prides and coalitions in the study area. Given the small number of individuals known to have died (directly observed or identified from a carcass) during the study period (see Section 3.1), these individuals were excluded from this analysis, because including them would require a combination of recovery and recapture models that also estimate a site fidelity parameter for each age and sex class (Burnham, 1993), and separation was observed during model selection without these additional parameters (see below). To control for possible heterogeneity in capture probability and meet the assumptions of CJS models, covariates of survival and detection were included for each individual indicating their sex and whether they were ever part of a radio-collared group (a covariate hereafter referred to as group, with collared groups having a higher detection probability but no

impact on survival). Age class was assigned to each individual based on its age on July 31st of the year it was first observed (July 31st was the first capture occasion of each year). This allowed individuals to be grouped into age-class cohorts and age with subsequent July 31st occasions. We allowed detection probability to vary among age classes, sexes, and group, but assumed that the detection probability for each of these classes was constant across the study period.

We constructed an *a priori* set of models in which survival and detection rates varied by age and sex (the effects of primary interest) and by the presence/absence of a radio collar within a pride and the sex of the collared animal (because males and females differ in patterns of association). All models were corrected for extrabinomial variation in survival rates by using a \hat{c} (estimated as deviance/df) as a variance inflation factor. We compared models using Akaike's Information Criterion with correction for sample size and extrabinomial variation (QAICc), and used model averaging over all models within 2 QAIC scores from the best-supported model, implemented with the *model.avg()* function of the R package MuMIn (Barton, 2013), to account for uncertainty in model selection to produce estimates of unconditional apparent survival (Burnham and Anderson, 1998). When implementing the *model.avg()* function to estimate standard errors, we used the revised variance estimator of Burnham and Anderson (2004, their equation 4) to improve coverage. Separation occurred in models that estimated $\hat{\phi}$ and \hat{p} separately for each sex in each age class (indicated by MLE of parameter standard errors), so we limited the final model list to those that pooled the effect of sex across two or more age classes.

The average age at which we first detected cubs was 4.29 months (SE=0.32), so we corrected sex-specific $\hat{\phi}$ and \hat{p} for cubs by exponentiation (exponent = $12/(12-4.29)$ = 1.56) to yield annualized survival rates. This adjustment assumes that the survival rate in the first 4.29 months of a cub's life is similar to the following 7.71 months of the lion's first year. This assumption will yield over-estimates if cub survival is lower in the first months of life, particularly during turnovers of territorial male coalition and low prey availability (Pusey and Packer, 1987; Packer et al., 1988). We calculated 95% binomial confidence intervals for cub $\hat{\phi}$ and \hat{p} using the Wilson method as implemented by the *binconf()* function of the Hmisc package (Harrell, 2014) in R.

Results

Observed Group Structure, Known Mortality, and Age and Sex Distribution

A total of 210 lions were individually identified in the South Luangwa study area, in 18 prides ranging in size from 1 to 9 adult females with a mean size of 3.27 (95% CI = 2.80 - 3.74) and 14 male coalitions ranging in size from 1 to 4 subadult and adult males with a mean size of 1.89 (95% CI = 1.42 - 2.36). Fifteen of these individuals were known to have died from natural and human causes including trophy hunting (5), natural injuries (4), infanticide (3), disease (1), wire snare injuries (1), and unknown causes (1). An additional 8 males likely died through trophy hunting because their last sighting coincided with a lion harvest in the same area. Despite CITES reporting requirements, it was difficult to obtain photographs or tissue samples for genetic analysis from hunted lions to confirm their identity; of the 46 males harvested during the study period based on

annual harvests reported to ZAWA and to ZCP we received these data for 11 % (n=5) of the hunted lions during the study period. Estimated age of individuals known/suspected to be harvested ranged from 3.75 to 7.65 years old, with a median age of 4.86 years old (mean age was 5.21 years old (SE= 0.31)).

The proportion of males reaching the prime and old age classes was consistently small, even following years with relatively large cohorts of subadults and young adults that potentially could have been recruited to older age classes (Figure 2.2). Old adult males never comprised more than 3% of the population, and the total of prime and old adult males never exceeded 6% of the population. The age composition of the female population shifted over the study period, with a large cohort born around 2006 moving through successive age classes and relatively low recruitment thereafter, yielding an increasingly senescent female population (Figure 2.2).

Population Size, Density, and Trend Estimates

Estimates of the local lion population size (excluding cubs <1 year old) declined from a maximum of 125 in 2009 (95% CI: 108 -167) to a minimum of 94 lions in 2012 (95% CI: 92 - 106) (Table 2.1, Figure 2.3a). The coefficient of variation for annual population estimates ranged from 0.30 – 1.14 (Table 2.1). Locations from GPS collared lions and direct observations in the study area covered an area of 1 065 km² (95% isopleth from kernel utilization distribution), centered along the Luangwa River, including areas in SLNP and Lumimba and Lupande GMAs (Figure 2.4).

Assuming that this area of lion usage was constant between years and given that field crews covered the study area with similar effort throughout the study period, mean

maximum population density across the study period was estimated to be 10.4 lions (excluding cubs <1 year old) per 100 km² (95% CI: 9.4-13.3 lions per 100 km², Table 2.1). Mean minimum population density was estimated to be 4.0 lions per 100 km² (95% CI: 3.6-5.3 per 100 km², Table 2.1). Annual estimates of population growth (λ) declined through the study period (Figure 2.3b) and by the final year was 0.81 (95% CI: 0.66 - 1.05; Figure 2.3b). The geometric mean of the annual growth rates suggests a decline of 2% per year, though the uncertainty of this estimate is large despite intensive monitoring ($\bar{\lambda} = 0.98$, 95% CI: 0.79 - 1.22).

Survival Rates and Detection Probabilities

Age- and sex-specific apparent survival rates ($\hat{\phi}$) from the best model and from model-averaging of *a priori* models within 2 QAICc scores of the best model (Table 2.2) are shown in Figure 2.5. These models differed in their parameterization of effects of sex on ϕ and p (top model parameterization presented in Table 2.3), the presence/absence of at least one radio collar within the pride (radio-collared prides were better monitored), and an interaction between sex and the presence/absence of a radio collar on p (radio-collaring improved the detection of females more than males; Table 2.2). We found no effect of sex on survival among cubs, but sub-adult males had lower survival than sub-adult females. As individuals sexually matured and entered the adult age classes, males continued to show survival rates 30% - 35% below the survival of females in the same age class. The low precision of the adult male survival estimates was largely due to the small numbers of males surviving to these classes (given their high probability of

detection). There was no difference in survival between collared and uncollared individuals.

The annual probability of detection (\hat{p}) differed between sexes, and was affected by the presence of a radio-collared individual within an individual's group (Figure 2.6). Detection did not differ between collared and uncollared groups for males, and increased with age, with male cubs having the lowest \hat{p} . The detection of females in the cub and sub-adult age classes was similar for collared and uncollared groups, but was substantially different for collared and uncollared groups in older age classes; adult lionesses in collared groups maintained high \hat{p} (approximately 0.8), but \hat{p} was low (approximately 0.2) for adult lionesses in uncollared groups.

Discussion

The Status of the South Luangwa Lion Population

Using five years of intensive monitoring data for a substantial portion of the South Luangwa lion population studied with well-established mark-recapture methods, our estimates of maximum density indicate that this portion of Zambia's largest lion population attains local densities comparable to other stronghold lion populations (8-13 adult lions per 100 km² in Selous Game Reserve: Creel and Creel 1997; 10 lions per 100 km² in Serengeti National Park: Hanby et al. 1995; 10.5-15.5 lions per 100 km² in Kruger National Park: Smuts 1976). Relative to previous estimates of lion density for South Luangwa, our maximum density estimate of 8.8-11.7 lions (>1 year old) is focused on the area known to be used by the core lion population of the national park. Prior estimates

from this population were for a larger area that includes and surrounds this core. Becker et al. (2013b) reported 3.5 lions (>2 years old) per 100 km for 2 775 km² (the same intensive study area covered in this study), including low-lion density areas outside of the utilization distribution used in this study and away from the Luangwa River. When our estimates of known population size were applied to this broader area, we estimated densities of 3.7-4.5 lions (>1 year old) per 100 km². Thus it is likely that lion density in much of the Luangwa ecosystem is substantially lower than the area we studied, and even in this stronghold population, estimated lion density at broad spatial scales is considerably lower than has been reported for several other areas.

Our analyses also detected a significant decrease in population size (or density) between 2009 and 2012 and an overall decline of 2% per year, although no single annual estimate of population growth differed from $\lambda=1$ with certainty greater than 80%. Thus, intensive monitoring provided data sufficiently precise to detect a decline over a period of 5 years with >95% confidence. Even for a period of one year, the data were sufficiently precise to provide substantial confidence in the inference that a decline is in progress. In addition to this population decline, we detected low cub recruitment, low apparent survival among subadult and adult males, and a senescing female population. Because this study was centered on the core of a stronghold lion population that is considered to be the densest and most secure in Zambia, the observed decline, though modest, could threaten the viability of the population in the long-term. For this population and others, early detection of declines, before the magnitude of becomes large, is critical. Given that similar dynamics are likely elsewhere in the country's less

robust populations, the reasons for this decline should be addressed to help secure a viable lion populations for the long-term ecological benefits and for Zambia's tourism industry.

Drivers in the Dynamics of the South Luangwa Lion Population

Modeling Zambian lion populations under different harvest management scenarios, Becker et al. (2013b) found that age-based harvest coupled with reduced quotas were likely to be effective strategies, provided that net growth was stable, but that more conservative management strategies were necessary where net growth was negative. Our results, collected during the five years leading to the 2013 ban on lion trophy hunting, provide the first statistically rigorous estimates of trends in population size and demography for lions in Zambia and indicate a declining population. Together, the two analyses suggest that measures beyond adjustments to harvest strategies and quotas are needed to allow for population recovery in the short term.

CJS models cannot distinguish between permanent emigration and death, so it might be argued that some male lions were simply not detected or that their disappearance was often due to permanent emigration, and that trophy hunted males were also largely immigrants from outside the study population. We cannot rule out the possibility that some individuals permanently emigrated, but in the 5 year study period we did not document any collared or uncollared lions dispersing out of the intensive study area (Figure 2.4). It seems likely that permanent emigration and potential violations of the closure assumption had little effect on the patterns we observed for

several reasons. Firstly, detection rates of sub-adult and young adult males, the age classes most likely to disperse, were closely comparable to those of collared sub-adult and adult females, who very rarely disperse (Figure 2.6). Secondly, it was uncommon for unknown lions to immigrate into the intensive study area, while we regularly detected multiple dispersals of subadult and adult males between known prides within the study area. Thirdly, the intensive study area (2 775 km²) is much larger and includes areas peripheral to the utilized area (1 065 km²), indicating that the study population rarely used the periphery of the intensive study area. Fourthly, we documented 46 males >3 years old disappearing from the study population from 2009-2012 (excluding 2008 given it was the first study year and could not be calculated), which closely matches the 46 males harvested in the study area from 2008-2012. Despite this study being a collaborative ZAWA project, it was difficult to obtain reports and photographs of harvested lions from hunting operators and thus we were unable to confirm the identity of most harvested individuals. However, all of the harvested lions for which we were able to obtain photographs were known males from within the study area. Finally, spatial movements of coalitions and known fates of male lions indicated that the GMAs acted as an attractive sink for males in the study area, with coalition turnover due to trophy hunting continually creating open territories and weakening established coalitions by removing their members. These vacancies attracted male coalitions from within the population's core via the 'vacuum effect' described by Loveridge et al. (2007, 2010), and observed coalition turnover over the five year period seldom was the result of direct confrontation between coalitions.

Natural mortality and anthropogenic sources of mortality other than trophy hunting (such as snaring by-catch) contributed to the patterns we observed, but the annual loss of 8-11 males to trophy hunting made it the primary source of mortality for lions in the area. Snaring by-catch would likely have significant impacts on lion dynamics in our study area if not controlled, but the majority (n=13, or 87%) of known snared lions on our site were immobilized for snare-removal and successfully treated for injuries during the study period (Becker et al., 2013a). Snaring of target herbivore species is likely to have more serious long-term impacts on the underlying dynamics of this lion population, but the loss of potential prey to wire snares would be detrimental to all age and sex classes and has yet to be quantified. For these reasons, it is unlikely that factors other than trophy hunting significantly contributed to the severe male depletion of the South Luangwa lion population.

Additional data on fecundity and cub recruitment are needed to better understand the dynamics of this population and other Zambian lion populations, but we found consistently low cub recruitment throughout the study period. Infanticide following turnover in male coalitions is well-documented in lions (Bertram, 1975; Packer and Pusey, 1984; Pusey and Packer, 1994; Packer et al., 2001). Consequently, increased turnover of male coalitions from trophy hunting is expected to produce the low cub recruitment that we observed (Whitman et al., 2004). While we did not observe most suspected cases of infanticide, at a minimum, high rates of male loss and scarcity of adult males did not positively influence cub survival, and the observed lack of recruitment caused the majority of adult females in the study population to shift into the older age

classes during the study (Figure 2.2). Similar data from years following the hunting ban will clarify the influence of male loss from trophy hunting on population dynamics, cub survival and the observed sex and age structure of the South Luangwa lion population.

Future Management and Conservation of Zambian Lions

While overharvesting of lions has been well-documented throughout Africa (Loveridge et al., 2007, 2010; Packer et al., 2009, 2011; Groom et al. 2014), a number of practices have been proposed to help ensure that hunting is sustainable, including conservative quotas (0.5 lions/1 000km²; Packer et al., 2011), harvest restricted to older age-classes, changes in the quota allocation structure and accurate, and transparent trophy monitoring and enforcement (Lindsey et al., 2013b). Annual lion harvests in the 4 308 km² of hunting concession comprising portions of our study area ranged from 1.86 – 2.56 lions/1 000 km², substantially higher than the 0.5 lions/1 000km² recommended by Packer et al. (2011) and heavily concentrated along the park boundaries. Not surprisingly, sustained high offtake resulted in a reduction in the age of harvested males, with an increasing number of sub-adult and young adult males shot because few males older than the recommended 6 years (Whitman et al., 2004) remained to fill the allotted quotas. Given the severe male depletion, poor cub recruitment and declining population in South Luangwa prior to the 2013 ban, we recommend that the lion hunting ban should continue until at least 2016 to allow for recovery of the population's sex and age structure. This recovery period would help stabilize the female age distribution, improve cub recruitment for both sexes, and restore the adult male age classes. However, hunting of lions should only resume with substantially lower quotas, age-restricted harvesting and improved

reporting, trophy monitoring and revised fee and quota allocation structures that discourage unsustainable harvesting. Because effective implementation of these best practices is complicated and difficult, such a time frame is equally necessary to ensure their effective planning and development well before any potential resumption of hunting. In addition, given lion hunting largely occurred in the Kafue and Luangwa ecosystems, rotation of hunting between these two lion populations on a three year cycle could be considered to further ensure hunting is sustainable. To evaluate the impacts of the hunting ban and other factors on lion demography and dynamics, the intensive monitoring undertaken for this study should be duplicated during the hunting ban to test whether survival and population dynamics respond as predicted.

While our results suggest a recovery period for lions from trophy hunting is warranted, a serious side-effect of the complete ban on all trophy hunting in Zambia was the loss of wildlife-based tourism throughout 167 000 km² of lion habitat in the GMAs where these activities occurred and no wildlife-based revenue is now generated. Many of these areas are typically not highly suitable for photo-tourism, either inherently or at present, and they are undergoing rapid human change in the form of encroachment and bushmeat poaching (Becker et al., 2013a; Lindsey et al., 2013b, 2014; Watson et al., 2013, 2014). Bushmeat poaching with wire-snares is widespread and increasing in Zambia (Becker et al., 2013b; Lindsey et al., 2013b; Midlane, 2014), and is strongly correlated with human development (primarily in the form of roads, agriculture, charcoal, and settlements), which is estimated to be increasing at a rate of 18 ha per daylight hour in Zambia's two largest lion populations in the Luangwa and Kafue regions (Watson et

al., 2013, 2014). Removal of wildlife-based economies in these areas can have severe negative consequences for wildlife populations and habitat and should not be undertaken without careful consideration and implementation of alternatives. Given the difficulties in establishing alternatives, improved management of hunting is often the most effective immediate solution, though options for promoting and expanding photo-tourism should definitely be considered given their economic benefits (Lindsey et al. 2014). Lindsey et al. (2012) estimated that reforming lion hunting in Africa, rather than banning it altogether, potentially conserved 52 533 km² of habitat for lion and other wildlife species. Thus while hunting can be well-managed if best practices are adhered to, the increasing impacts of bushmeat poaching and human encroachment are likely to present much greater threats to the long-term viability of Zambian lions and their habitat than hunting, and conservation of large tracts of critical lion habitat is not currently likely without an array of wildlife-based uses that include hunting (Lindsey et al. 2014).

The Case for Intensive Monitoring Studies

Assessing the status of and threats to large carnivore populations will require accurate and precise estimates of population size and growth, with sufficient demographic information to identify and address the limiting factors that are most influential on a population's future. The necessary information cannot be obtained through indices of abundance and at present can only be acquired through intensive monitoring. Even with this intensive monitoring effort spanning five years and requiring high levels of effort, there was appreciable uncertainty around the estimates reported in this study, owing to the difficulties in finding and monitoring low density, wide-ranging,

and declining species. This uncertainty, however, was much less than is typical of results from other methods commonly used for large carnivores. For example, Midlane (2014) reported corrected CV estimates of 2.22 and 0.95-1.00 for spoor-count and call-in estimates (respectively) of lion density and abundance in northern Kafue National Park, part of the second-largest lion population in Zambia. In the year with the most precise abundance estimate (2012), we estimated a coefficient of variation of 0.3 for our estimates of population density. To our knowledge, other lion spoor counts did not use the correct methods to estimate the coefficient of variation, and thus were not comparable, but are likely to have similarly broad uncertainties when corrected.

These differences in uncertainty and the need for evaluation of the underlying causes of carnivore declines both highlight the importance of intensive, long-term monitoring efforts and the need for closely linking them with larger-scale IOA methods to obtain a more thorough understanding of large carnivore dynamics within remaining stronghold populations. Indices of abundance are valuable over larger scales and when budgets, resources, and time are limited, the biology of the species and/or the ecosystems they are found in prohibit direct monitoring of individuals, and when little is known about a large carnivore population in a given area. These conditions are reflective of many remaining large carnivore populations; however, the most common research and monitoring objective for any carnivore conservation project is to determine population size, structure, and trends, and to do this precise estimates are needed. Without intensive monitoring in stronghold populations of large carnivores, data from currently available methods are not likely to detect population declines until they are large, and are not likely

to produce clear guidance for management priorities and policies. Thus, given the respective advantages and information yielded from both intensive studies and IOA methods, the priority for effective conservation and management of large carnivores is to effectively mesh both techniques to provide precise data over large spatial scales for conservation of the planet's rapidly diminishing large carnivore populations.

Conclusions

As global large carnivore declines continue to accelerate, precise estimates of population sizes and trends are of paramount importance to facilitate early detection and evaluate causes of population declines, and to implement corrective management and conservation measures. While rapid and economical survey methods based on indices of abundance are an important conservation tool, they are often inadequate to provide the necessary precision and demographic evaluations needed to inform and guide management policies. Intensive long-term monitoring of key large carnivore populations is still a critical component for detecting and addressing declines and should be considered wherever possible for stronghold populations of large carnivores. A primary priority for large carnivore conservation and management should be to effectively combine both indices of abundance and intensive monitoring approaches to provide precise data over large scales.

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Table 2.1: Estimates of annual population and density and coefficient of variation for the SL lion population from 2008-2012. Estimates of density were calculated as the number of individuals older than 1 year of age per 100 km² using two methods to determine the area occupied. Maximum density estimates came from the area (1 065 km²) enclosed by the 95% isopleth of a kernel utilization distribution fit to known lion locations. Minimum density estimates came from the entire intensive study area (2 775 km²).

Year	Count	SL Lion Pop. Estimate	SE	Coefficient of Variation	95% CI	Max. Density (95% CI)	Min. Density (95% CI)
2008	77	102	8.08	0.70	90- 123	9.58 (8.45- 11.55)	3.67 (3.24- 4.43)
2009	95	125	14.1	1.10	108- 167	11.74 (10.14- 15.68)	4.50 (3.89- 6.82)
2010	99	118	8.81	0.74	106- 142	11.08 (9.95- 13.33)	4.25 (3.82- 5.12)
2011	106	116	12.8	1.14	106- 171	10.89 (9.95- 16.06)	4.18 (3.82- 6.16)
2012	91	94	2.93	0.30	92- 106	8.83 (8.64- 9.95)	3.38 (3.31- 3.82)

Table 2.2: The five best-supported Cormack-Jolly-Seber models of survival, as determined by QAICc scores. All models were required to estimate $\hat{\phi}$ and \hat{p} for each of the five age classes. The effects of sex on ϕ and p , as well as the presence of a collar and an interaction between sex and collar on p were modeled as constant for all adults (adult(.)), two classes of adults (YA=PA, PA=OA) or constant for individual age classes (YA = young adult age class; PA = prime adult age class; OA = old adult age class).

Model	Delta QAICc	QAICc Weights	Number of Parameters
$\phi(\text{age \& sex-Adult}(.)), p(\text{age, sex-Adult}(.), \text{collar-Adult}(.), \text{sex*collar-Adult}(.))$	0.00	0.33	22
$\phi(\text{age \& sex-Adult}(.)), p(\text{age, sex-Adult}(.), \text{collar-Adult}(.))$	1.14	0.19	19
$\phi(\text{age \& sex-Adult}(.)), p(\text{age, sex-PA=OA}, \text{collar-Adult}(.), \text{sex*collar-Adult}(.))$	1.43	0.16	23
$\phi(\text{age \& sex-YA=PA}), p(\text{age, sex-PA=OA}, \text{collar-Adult}(.), \text{sex*collar-Adult}(.))$	2.17	0.11	23
$\phi(\text{age \& sex-YA=PA}), p(\text{age, sex-Adult}(.), \text{collar-Adult}(.))$	3.28	0.06	20

Table 2.3: Coefficient estimates from the best-supported Cormack-Jolly-Seber model, which included effects of age and sex on apparent survival (ϕ), and effects of age, sex, the presence of a radio-collared animal, and the sex of the collared animal on detection (p) $\{\phi(\text{age \& sex-Adult}(.)), p(\text{age, sex-Adult}(.), \text{collar-Adult}(.), \text{sex*collar-Adult}(.))\}$.

β	Effect of:	Parameter	Estimate	SE	95% LCL	95% UCL
		Affected				
1	F Cub	ϕ	0.962815	0.535858	-0.08747	2.013096
2	F Sub-Adult	ϕ	1.61072	0.615069	0.405185	2.816256
3	F Young Adult	ϕ	2.884205	0.776886	1.361509	4.406901
4	F Prime Adult	ϕ	3.012064	0.864877	1.316905	4.707223
5	F Old Adult	ϕ	2.880182	0.90587	1.104676	4.655688
	M Cub					
6	Adjustment	ϕ	0.159858	0.695888	-1.20408	1.523798
	M SA					
7	Adjustment	ϕ	-1.46767	0.706414	-2.85224	-0.08309
	M Adult					
8	Adjustment	ϕ	-2.33482	0.781898	-3.86734	-0.8023
9	F Cub	p	0.429533	0.656112	-0.85645	1.715512
10	F Sub-Adult	p	-0.42469	0.41533	-1.23873	0.389358
11	F Young Adult	p	-1.3242	0.349468	-2.00916	-0.63924
12	F Prime Adult	p	-1.38331	0.345124	-2.05975	-0.70687
13	F Old Adult	p	-1.79636	0.34891	-2.48023	-1.1125
	M Cub					
14	Adjustment	p	0.085186	0.88182	-1.64318	1.813553
	M SA					
15	Adjustment	p	1.534648	0.581216	0.395464	2.673831
	M Adult					
16	Adjustment	p	2.671828	0.598422	1.498922	3.844734
	Collar Cub					
17	Adjustment	p	-1.26707	0.852558	-2.93808	0.403945
	Collar SA					
18	Adjustment	p	1.589845	0.614089	0.386231	2.793459
	Collar Adult					
19	Adjustment	p	2.818953	0.343646	2.145408	3.492498
	M x Collar Cub					
20	Adjustment	p	1.721782	1.183512	-0.5979	4.041465
	M x Collar SA					
21	Adjustment	p	-0.46639	1.377435	-3.16617	2.233378
	M x Collar Adult					
22	Adjustment	p	-2.59707	0.996547	-4.5503	-0.64384

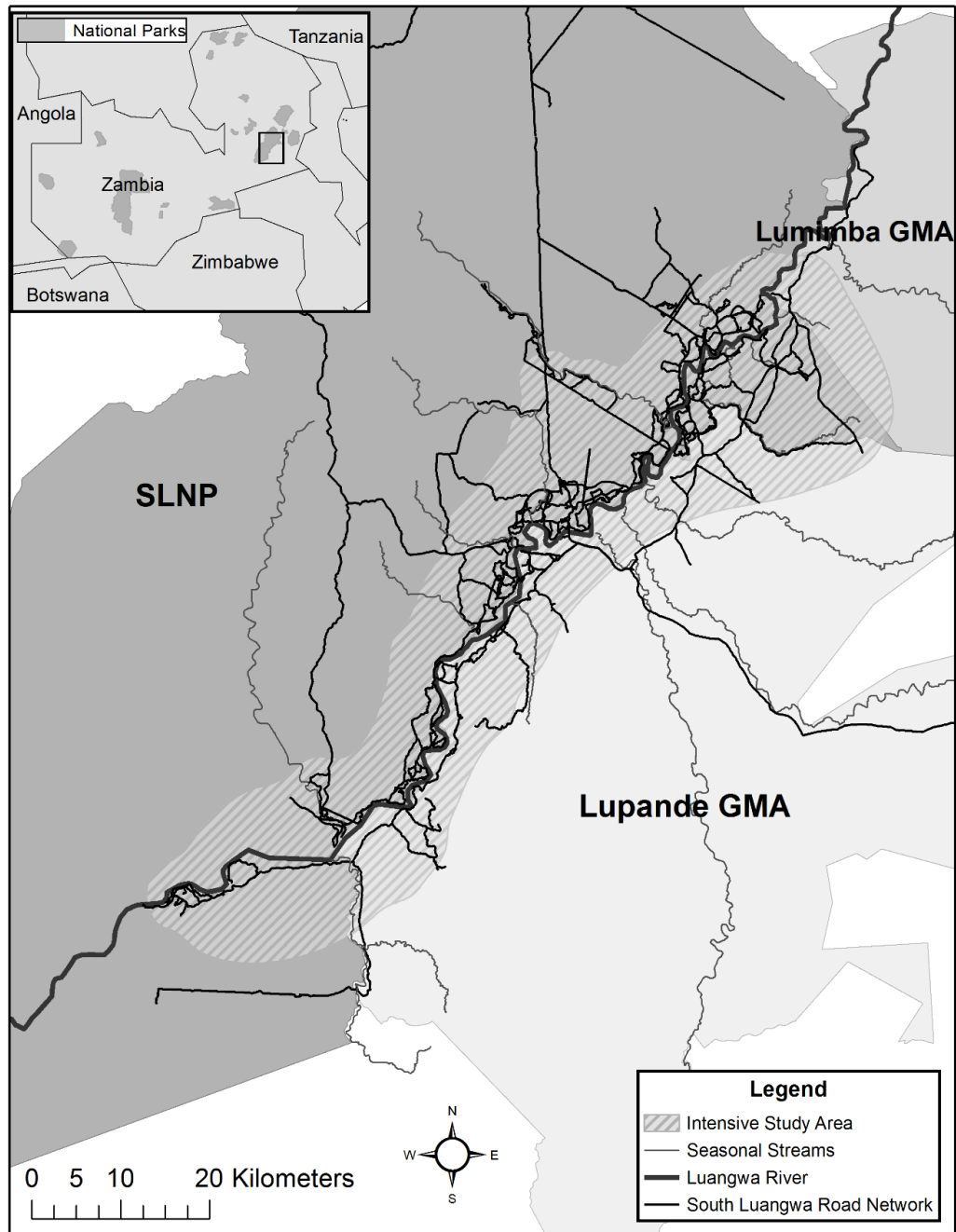


Figure 2.1: Intensive study area of the South Luangwa lion population in Eastern Province, Zambia (S13.07958 E31.77407). Comprising both strictly protected areas and community Game Management Areas the study encompassed the full array of biological and human factors likely to impact Zambian lions.

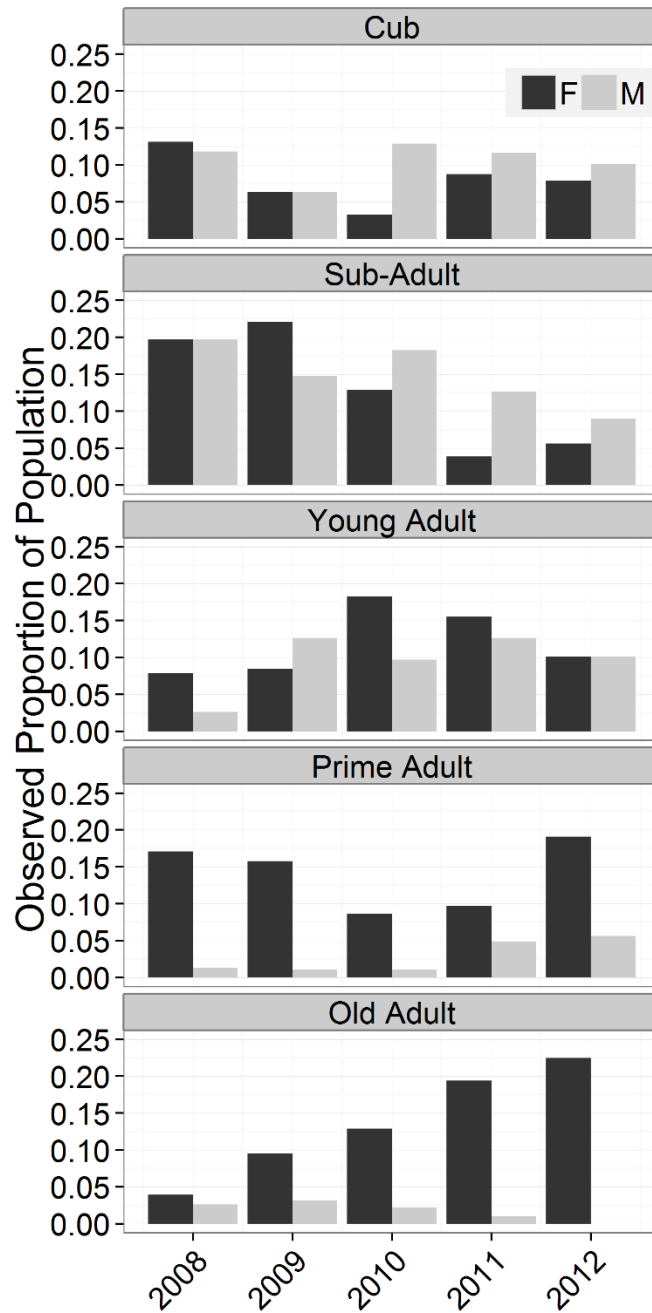


Figure 2.2: Observed age and sex distribution for the South Luangwa lion population during the study period (2008-2012), indicating a male-depleted (M) and senescing adult female (F) population.

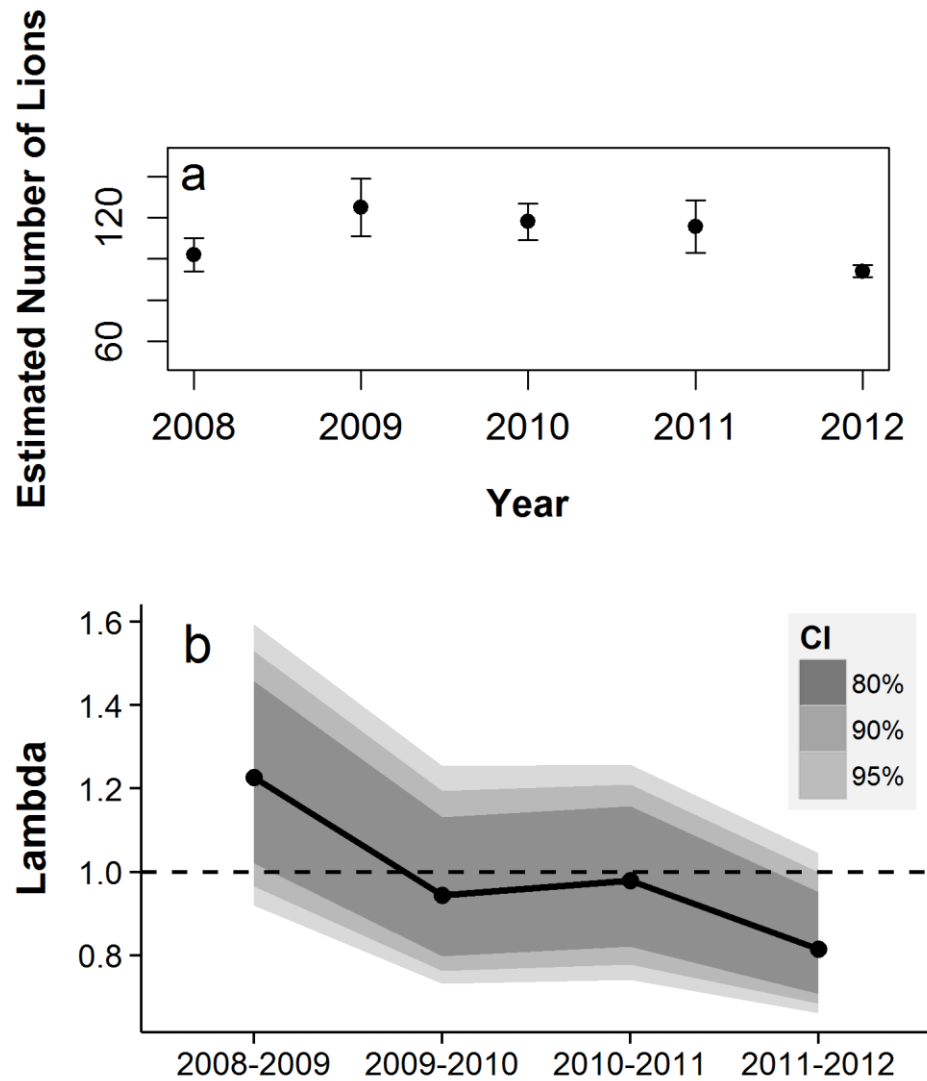


Figure 2.3: (a) South Luangwa lion population estimates from a Huggins closed capture model with individual heterogeneity in detection probability from 2008-2012. (b) Trends in annual population growth rate (λ) through time. The dashed line indicates a stable population ($\lambda = 1.0$). Shaded areas indicate 80%, 90%, and 95% confidence intervals (CI) from parametric bootstrapping.

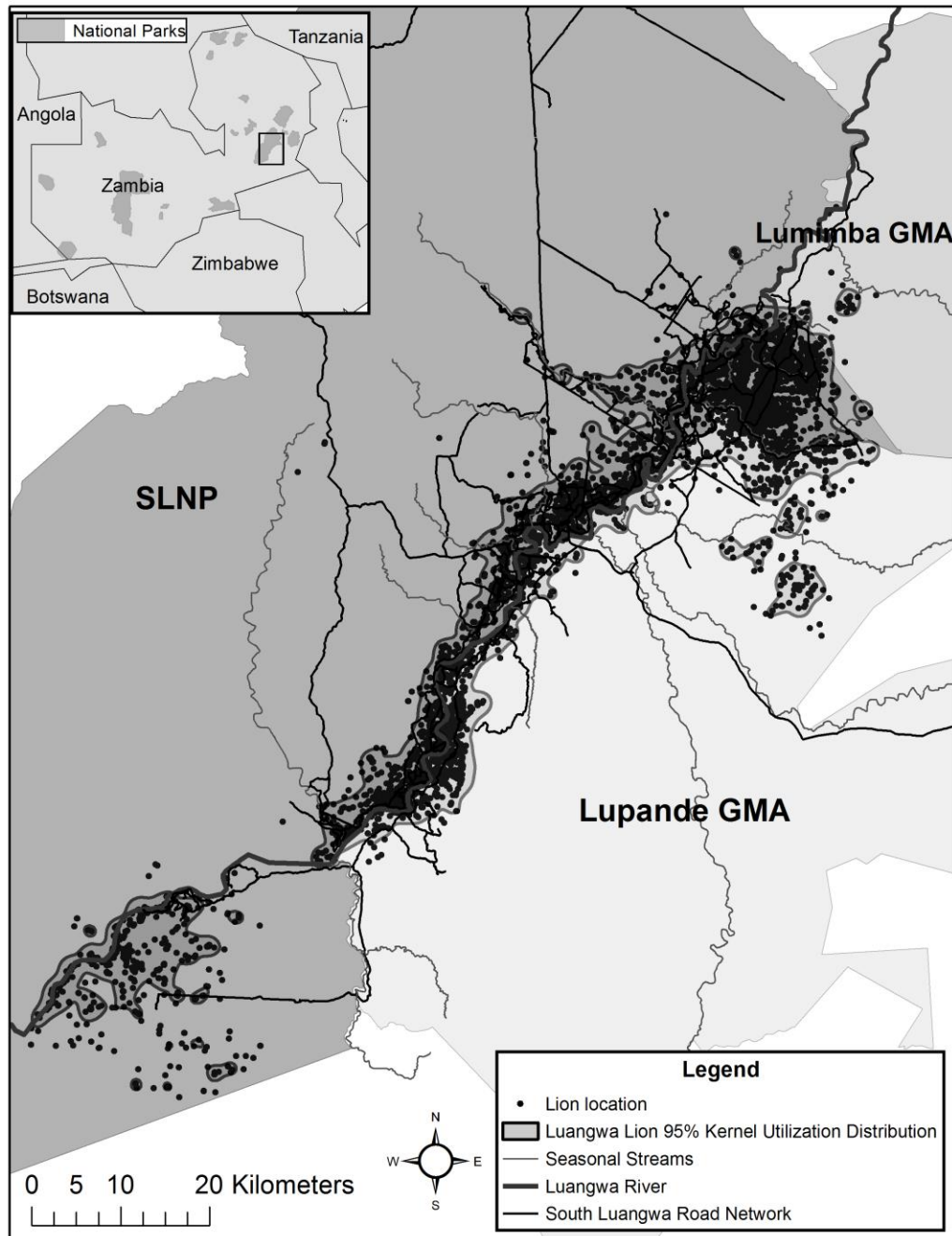


Figure 2.4: 95% Kernel utilization distribution mapped to determine the utilized area (1 065 km²). This area was used to estimate the maximum density for the South Luangwa lion population. In this study population, lion locations were tightly linked to prey distributions and permanent water, so that the area typically used by study animals was appreciably smaller than the intensive study area (2 775 km², see Figure 2.1).

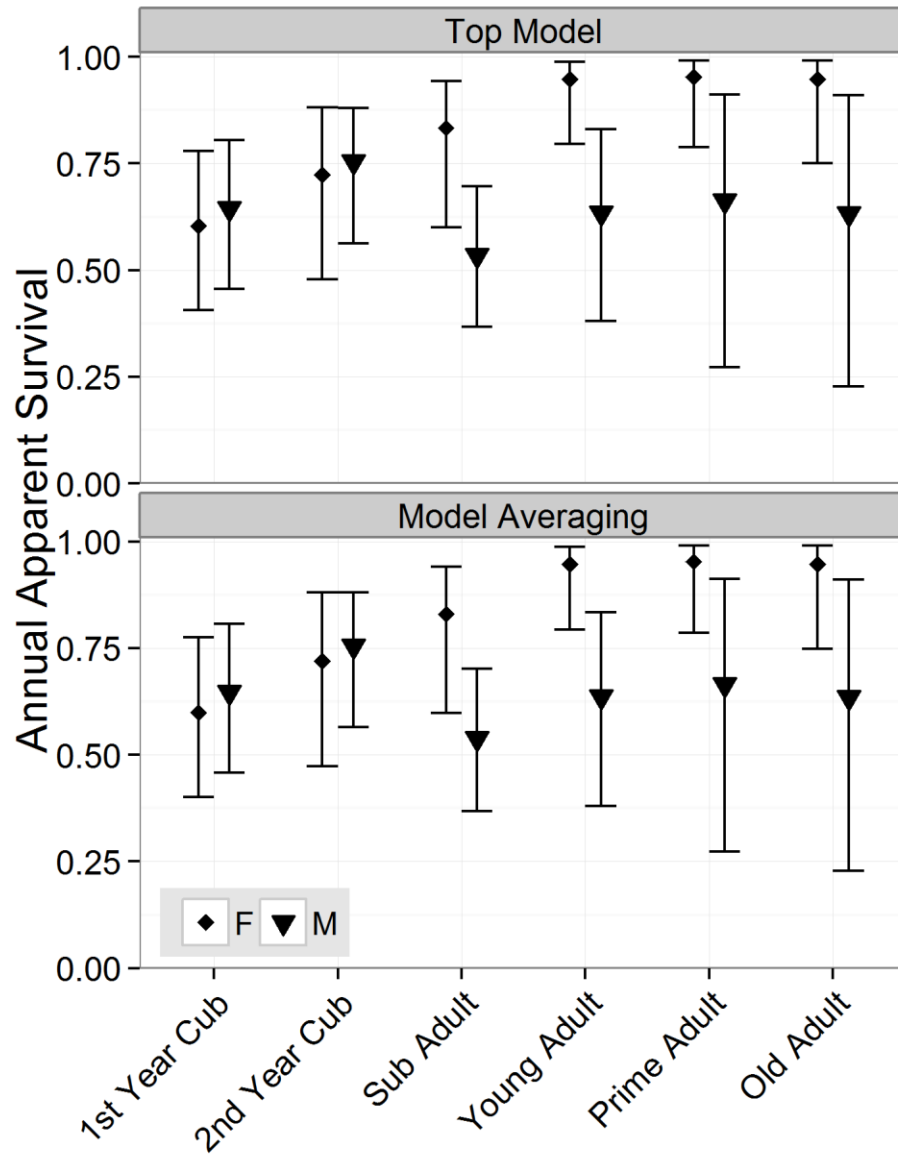


Figure 2.5: Annual apparent survival rates ($\hat{\phi}$) for the South Luangwa lion population, estimated by the single best supported Cormack-Jolly-Seber model (upper panel) and by model averaging (lower panel; see Table 2.2). Error bars indicate 95% binomial confidence intervals. While male and female annual apparent survival are similar in the early age classes, male annual apparent survival beginning in the sub-adult age classes consistently tended to be lower than apparent survival for females (though not statistically significant given overlapping 95% confidence intervals).

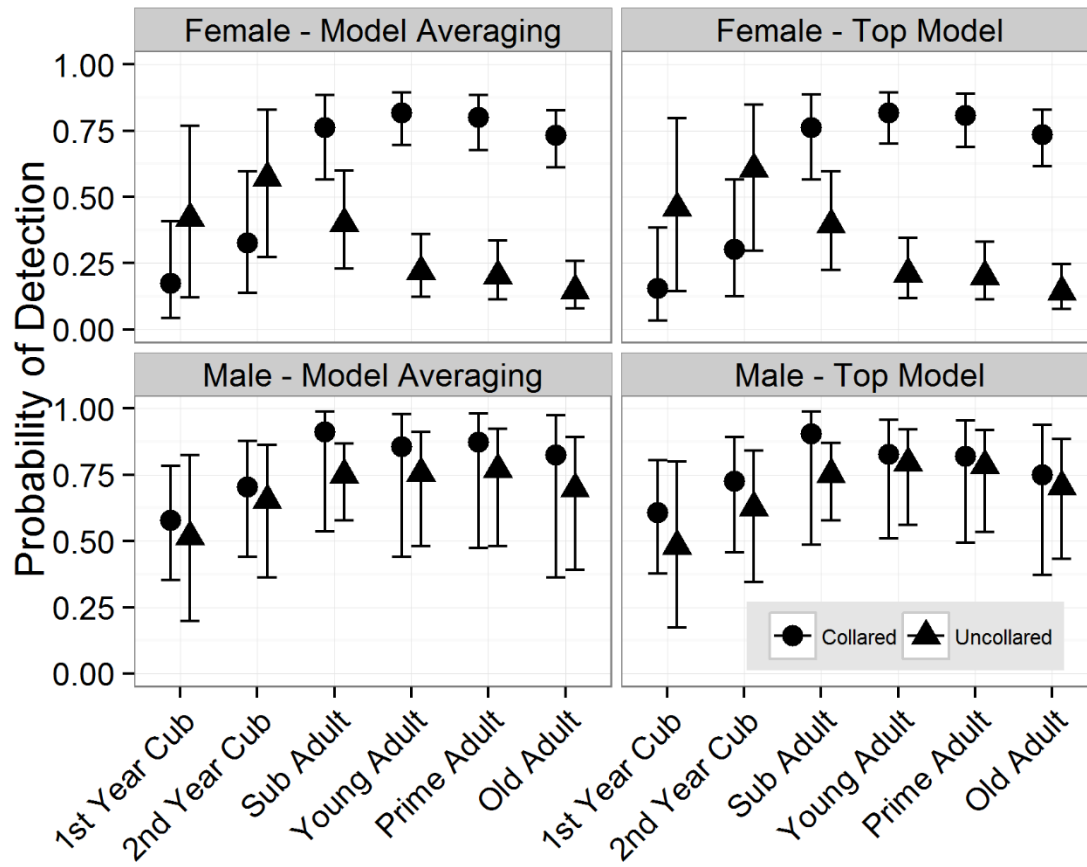


Figure 2.6: Detection probabilities (\hat{p}) for the South Luangwa lion population, estimated by the single best supported model (see Table 2.1) and by model averaging. Error bars indicate 95% binomial confidence intervals. Uncollared females had significantly lower detection probabilities relative to collared females, while collared and uncollared males had similarly high detection probabilities.

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CHAPTER THREE

EFFECTS OF A PROTECTION GRADIENT ON CARNIVORE DENSITY
AND SURVIVAL: AN EXAMPLE WITH LEOPARDS IN
THE LUANGWA VALLEY, ZAMBIA

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Abstract

Human activities on the periphery of protected areas can limit carnivore populations, but measurements of the strength of such effects are limited, largely due to difficulties of obtaining precise data on population density and survival. We measured how density and survival rates of a previously unstudied leopard population varied across a gradient of protection and evaluated which anthropogenic activities accounted for observed patterns. Insights into this generalist's response to human encroachment are likely to identify limiting factors for other sympatric carnivore species. Motion-sensitive cameras were deployed systematically in adjacent, similarly-sized and ecologically similar study areas inside and outside Zambia's South Luangwa National Park (SLNP) from 2012-2014. The sites differed primarily in the degree of human impacts: SLNP is strictly protected, but the adjacent area was subject to human encroachment and bushmeat poaching throughout the study, and trophy hunting of leopards prior to 2012. We used photographic capture histories with robust design capture-recapture models to estimate population size and sex-specific survival rates for the two areas. Leopard density within SLNP was 67% greater than in the adjacent area, but annual survival rates and sex ratios did not detectably differ between the sites. Prior research indicated that wire-snare occurrence was 5.2 times greater in the areas adjacent to the park. These results suggest that the low density of leopards on the periphery of SLNP is better explained by prey depletion, rather than by direct anthropogenic mortality. Long-term spatial data from concurrent lion studies suggested that interspecific competition did not produce the observed patterns. Large carnivore populations are often limited by human activities, but

science-based management policies depend on methods to rigorously and quantitatively assess threats to populations of concern. Using non-invasive robust design capture-recapture methods, we systematically assessed leopard density and survival across a protection gradient and identified bushmeat poaching as the likely limiting factor. This approach is of broad value to evaluate the impacts of anthropogenic activities on carnivore populations that are distributed across gradients of protection.

Keywords: Anthropogenic effects, harvest, bushmeat, leopard, *Panthera pardus*, prey depletion, robust design.

Introduction

Large carnivore populations are declining globally, often due to anthropogenic effects (Vitousek et al. 1997; Ripple et al. 2014), despite their ecological, economic and social importance (Estes et al. 2011; Ripple et al. 2014). These species are difficult to study due to their cryptic and often solitary nature, requiring substantial effort to accurately describe their status and viability (Durant et al. 2007). Most carnivore populations reside within and adjacent to protected areas (PAs), which face increasing pressure from rapid human population growth on their periphery (Wittemyer et al. 2008). Peripheral human encroachment and activities can limit carnivore populations within PAs (edge effects; Pulliam 1988; Woodroffe & Ginsberg 1998) and can draw individuals from PAs (attractive sinks; Loveridge et al. 2010). To lessen edge effects and illegal activity within PAs, buffer zones are often established, or fences constructed, but the efficacy of

these measures has recently been questioned (Geldmann et al. 2013; Lindsey et al. 2014; Durant et al. 2015). With growing human populations there is a clear need to identify how gradients of protection from anthropogenic activities affect carnivore populations, to inform wildlife management and land-use planning.

Leopards (*Panthera pardus*; Figure 3.1) are broadly distributed throughout Africa and Asia in ecosystems with a wide array of prey and habitats (Hayward et al. 2006; Henschel et al. 2008). Despite the species' generalist ecology, recent studies indicate range contraction due to human encroachment, poorly-regulated harvest, poaching and conflict (Henschel et al. 2008). Zambia contains regionally significant leopard populations (Purchase, Mateke & Purchase 2007) in a complex of national parks and adjacent Game Management Area buffer zones (GMAs; Lewis and Alpert 1997). Most of these populations have received little study (Ray 2011) and face rapid human population growth, encroachment and wire snaring for bushmeat. These anthropogenic pressures have reduced the ecological functionality of GMA buffer zones, and are thought to directly and indirectly affect carnivore populations through snaring by-catch, prey depletion and habitat loss (Watson et al. 2013; Watson et al. 2014; Lindsey et al. 2014). The effects of anthropogenic activities on protected leopard populations have been investigated in several ecosystems across their distribution (Balme, Slotow & Hunter 2009,2010; Henschel et al. 2011; Swanepoel et al. 2015), but the status of most leopard populations remains unknown, while anthropogenic pressures around them are intensifying. Therefore improving our understanding of leopards' responses to anthropogenic pressures is critical for their conservation, and will likely indicate limiting

factors for other sympatric, unstudied carnivore populations facing the same anthropogenic pressures. Developing methods that address these questions efficiently is a high priority.

South Luangwa National Park (SLNP) is Zambia's premiere photo-tourism destination and the GMAs adjacent to SLNP are categorized by the Zambian Department of National Parks and Wildlife as 'prime' for trophy hunting. The Luangwa Valley, which encompasses SLNP, contains what is thought to be Zambia's largest leopard population, but there are limited data to support this assertion (Ray 2011). Though most of SLNP is surrounded by GMAs, human encroachment is increasing with demonstrated effects on other carnivores. The drivers of these effects include illegal bushmeat harvest (Lewis & Phiri 1998; Becker et al. 2013a; Lindsey et al. 2013; Watson et al. 2013), high levels of legal trophy and resident hunting (Yamazaki 1996; Becker et al. 2013b; Rosenblatt et al. 2014), and habitat conversion (Watson et al. 2014). Therefore, SLNP and adjacent GMAs provide an opportunity to compare leopard densities and survival rates between a fully protected area with relatively minimal impact by humans, and an immediately adjacent buffer zone with growing direct and indirect anthropogenic effects. Estimated differences in density or survival areas across this protection gradient can help identify which anthropogenic pressure(s) limit leopards and other sympatric carnivores.

In this study we use motion sensitive cameras and mark-recapture methods to estimate leopard density and survival rates in a portion of the South Luangwa leopard population from 2012-2014. We use these estimates to determine how density and survival change across a gradient of protection, and identify the likely drivers. This study

contributes to national and regional efforts to reduce the negative effects of human population growth around PAs, and establishes effective tools to identify anthropogenic threats and guide large carnivore management.

Materials and Methods

Study Area

Our study was conducted in two adjoining areas on the boundary of SLNP (S13.07958 E31.77407; 9 050 km²) and Lupande GMA (5 660 km²), allowing comparison of leopard densities across management regimes differing in the degree and type of human activity (Figure 3.2). SLNP is strictly protected as an IUCN Category II Protected Area, with photographic safari tourism and law enforcement patrols, though some illegal wire-snare and rifle poaching does occur. GMAs are IUCN Category VI areas intended as buffer zones allowing a variety of natural resource-based uses (Chomba, Mwenya & Nyirenda 2011). Human settlements are permitted and increasing in the Lupande GMA (and most GMAs in Zambia), causing an array of conservation concerns (Lewis & Phiri 1998; Becker et al. 2013a; Watson et al. 2013,2014). Legal trophy hunting of adult male leopards, other large carnivores and herbivores occurred in Lupande and other GMAs, except during a January 2013-April 2015 moratorium. Livestock densities were locally low, making human-carnivore conflict uncommon relative to other studies (e.g., Marker & Dickman 2005).

The Luangwa River forms most of the eastern border of SLNP and the western border of Lupande GMA. As the largest perennial water source in the region, wildlife and

human activity is centered along the river boundaries of SLNP and adjacent GMAs, particularly during the dry season (May-November). Large mammals move across the Luangwa River freely, particularly in the late dry season (Rosenblatt et al. 2014); thus we considered our two study areas as segments of the same leopard population, which we termed the western study area (WSA) and eastern study area (ESA) (Figure 3.2). The WSA (172 km²) is located in SLNP on the western side of the Luangwa River ranging between the seasonal Katete and Luwi Rivers, and includes areas within 6 km of the Luangwa River. The ESA (141 km²) is located on the eastern bank of the Luangwa River, bounded by the seasonal Mwangazi and spring-fed Chichele streams and also includes areas within 6 km of the Luangwa River. The ESA includes portions of the Lupande GMA and the Nsefu sector of SLNP, a small portion of the park situated on the eastern side of the Luangwa River. Other than differences in human use, the ESA and WSA were selected to be ecologically similar, with comparable compositions of edaphic grassland, deciduous riparian forest, miombo (*Brachystegia spp*) woodland, mopane (*Colophospermum mopane*) woodland and scrubland, dry deciduous forest and undifferentiated woodland (Astle, Webster & Lawrance 1969; White 1983; Astle 1988). Our sampling on the ESA and WSA was designed to provide strong inferences in the following ways: (1) The two sites were selected to be similar for variables (other than those directly related to the level of protection) that would be expected to influence leopard density or demography (e.g. vegetation type and proximity to permanent water). (2) The two sites were of the same size, were spatially close, and were sampled over

highly overlapping time periods. (3) Within primary sampling periods, the sampling design for both sites was identical.

Study Design

We used a systematic camera grid to photograph leopards within each study area and used closed robust design capture-recapture models to estimate population size and annual survival rates (Pollock 1982; Kendall, Pollock & Brownie 1995). In both study areas we placed cameras using a square grid that was random in its origin and orientation. Spacing for this grid followed established procedures for large felids to meet the assumptions of closed mark-recapture models (Otis et al. 1978; Karanth & Nichols 1998; Balme, Hunter & Slotow 2009). We based grid cell size on the smallest home range estimate available (14 km²) for an adult female leopard in Zambia's Luambe National Park (approximately 60km from our study; Ray, 2011), and spaced trap sites 2.5 km from each other (Figure 3.2). This spacing was intended to place multiple trap sites within the home range of each individual (Karanth & Nichols 1998).

We established 25 and 26 unbaited camera trap sites (hereafter sites) in the ESA and WSA respectively (Figure 3.2). We selected sites by searching for leopard tracks within 100m of each grid point (Silver et al. 2004). If we encountered no tracks, we selected the most active game trail within 100m of the point. Cameras were attached to trees at a height and angle intended to maximize the likelihood of being triggered by leopards. One site was located more than 100m from the grid point, because no trees were available within the 100m radius. Vegetation varied between sites but all were in vegetation types used by leopards (Balme, Hunter & Slotow 2007). At each site we set

two Reconyx Hyperfire PC800 cameras ([Reconyx](#), Inc., Wisconsin, USA) facing each other to photograph both sides of passing leopards, set to take five photos in succession upon detection of movement. We visited sites on foot in small groups to minimize our potential impact on subsequent detections. We downloaded photographs when cameras were moved between locations (see below). We identified individuals using spot patterns and sexed them using genitalia and sexually dimorphic traits such as body and head size and the prominence of their neck dewlap (Balme, Hunter & Braczkowski 2012). We did not assign ages due to the limitations of image quality and the difficulty of aging leopards accurately (Balme, Hunter & Braczkowski 2012). We created capture histories for each individual denoting detections (1) and non-detections (0) on each day of camera trapping.

Robust Design Model Selection

We used an extended robust design model to estimate population size (N) for each study area, annual survival (S), detection and redetection probabilities (p and c respectively), and rates of temporary emigration (γ' , the probability of an individual temporarily moving off of the study area and becoming unavailable for capture and γ , the probability of an individual remaining outside of the study area and thus remaining unavailable for capture). We hypothesized that density would vary by study area and annual survival rates would vary by gender, study area and time, with a potential interaction between gender and study area. We compared models with random, Markovian or no temporary emigration (Kendall, Nichols & Hines 1997). Finally, we tested for effects on detection probability of study area and season (see below) and

whether detection probability differed from redetection probability to evaluate whether our activity at trap sites were impacted leopard behavior.

For each primary sampling occasion on each study area, we estimated population size during an 87-day period, a period we selected *a priori* to satisfy the assumption of population closure based on other large felid camera trap studies (Karanth & Nichols 1998). Each of these primary sampling occasions fell entirely within the cold dry season (CD, May - August) or the hot dry season (HD, September - November), so we refer to primary sampling occasions as seasons hereafter. Wet season data collection was not possible because portions of both study areas were inaccessible. Because we had too few cameras to survey all locations simultaneously, we deployed camera traps in a random rotation across four 'sections' within each study area (Karanth 1995). Each section consisted of 6-7 sites and was sampled for 21 days (21 days/section X 4 sections + 3 days to redeploy cameras = 87 days). We created encounter histories for each individual pooled across sections for a 21-day period for each study area in each season. We broke each primary occasion into three seven-day secondary occasions *a priori* to maximize detection probability.

In total, five seasons were surveyed over three years in the WSA (CD 2012, CD 2013, HD 2013, CD 2014, and HD 2014) and four seasons were surveyed over two years in the ESA (CD 2013, HD 2013, CD 2014, and HD 2014). With the rotation design to maximize spatial replication, there were staggered periods of 69-70 days between CD and HD sampling. We considered these periods as open to contribute to survival estimates, in addition to the open periods during wet seasons between HD and CD sampling (CD 2012

– CD 2013 = 349 days, HD2013 - CD2014 = 262 days). One ESA section was not sampled in the HD season of 2013 due to early rains, so our estimates for this season is based on 76% of the camera-days used for estimate in the other seasons. Survival rates are expressed as annual rates, exponentiating as needed to account for the time over which survival was estimated.

The robust design model includes several parameters (p , c , γ' and γ'') that must be estimated to provide unbiased estimates of population size and survival rates, but were not of direct interest in this study. To focus on the parameters of interest, we evaluated models in two steps, using Akaike's Information Criteria corrected for small sample size and overdispersion (QAICc) in the RMark package of R (Laake 2013). To correct for overdispersion, we estimated a median \hat{c} value by collapsing the secondary sessions within each season and fitting a time-varying Cormack-Jolly-Seber model to the data, as suggested by Laake (personal communication). All confidence intervals were then corrected for overdispersion in survival rates by using this median \hat{c} value as a variance inflation factor. In the first step of model selection we identified the best model of annual survival out of 10 candidate models (Table 1) with a single estimate of detection probability $\hat{p}(\cdot)$ and no temporary emigration ($\gamma'' = 0$ and $\gamma' = 1$) The model(s) receiving the majority of the QAICc weight was selected as the most likely parameterization for annual survival. In the second step, we used the best model(s) for annual survival to test our hypotheses for each of the remaining parameters, resulting in a set of 72 candidate models. In fitting these 72 candidate models we eliminated any that showed signs of overparametrization. We identified the top models of remaining candidate models (n=32;

Table A1) using QAICc, and used model averaging (with the *collect.modelsl()* and *model.average()* functions of the RMark package) to estimate parameter values across sex, study area and time. We calculated overdispersion-corrected 95% confidence intervals for parameters in each study area using model-averaged seasonal estimates with pooled variances.

We estimated density for each study area by dividing population estimates by the area surveyed. We estimated this area by calculating the mean maximum distance moved (MMDM; Stickel, 1954; Wilson and Anderson, 1985) across all individuals from both study areas and buffering each trap site by half of the MMDM distance (HMMDM). Balme, Hunter & Slotow (2009) found that without telemetry data, using HMMDM and buffering each camera trap site (Silver et al. 2004) was the least biased estimator for leopard density when compared to independent estimates of density from intensive telemetry data. Some recent research questions whether MMDM or HMMDM compares more closely to density estimates derived from telemetry data and spatially-explicit capture-recapture models (SECR; Efford 2004) in large felids, and that HMMDM may overestimate density estimates by underestimating space use of individuals (e.g. Tobler & Powell 2013). We chose to follow Balme, Hunter & Slotow (2009)'s leopard-specific recommendation for density estimates, but we provide density estimates based on average MMDM measures in the Appendix (Table A2). A strength of our study design is that the choice of HMMDM versus MMDM has no effect on differences in density between the ESA and WSA (the primary interest of this study). In this study, the choice is relevant only for comparisons of our density estimates to those from other studies. We did not

implement spatially-explicit capture-recapture models (SECR; Efford 2004) because current implementations require assumptions about space-use distributions that are not likely to be met by leopards, and our sampling was carefully designed to limit differences in the area sampled for the two sites. By sampling in immediately adjacent areas with identical sampling grids and methods, we minimized problems related to estimation of sampling area that can arise during conversion of population size to population density, thus avoiding the primary problem that SECR attempts to address.

Results

We photographed 51 leopards over the three year study, from 8730 successful camera trap days. Of these 51 leopards, twenty leopards were photographed only from one side due to photo angles and camera failure or theft. To avoid double counting individual leopards we created encounter histories using only right-sided photographs, resulting in 43 individually-recognized leopards used in this analysis. We documented 28 individuals in WSA and 15 individuals in ESA on 99 occasions, with 26 individuals photographed on more than one occasion. No individuals were detected in both study areas. We observed male:female ratios of 1:1.5 and 1:1.8 in the ESA and WSA, respectively (1:1.7 overall). Five leopards were not sexed on the basis of genitalia, and were designated as females for the analysis based on their size and neck characteristics (Balme, Hunter & Braczkowski 2012). In the WSA, leopards were photographed at 77% of the trap sites, with most leopards detected along seasonal streams and the Luangwa

River (Figure 3.3). In the ESA, leopards were photographed at 48% of trap sites, most frequently along the Luangwa River (Figure 3.3).

Using QAICc scores (median $\hat{c} = 1.92$) to compare models with constant detection and no temporary emigration (Table 1), the best supported model of survival (S) had a single mean rate for all individuals, but appreciable QAICc weight also went to models with a difference between the sexes (slightly lower survival in males) and with a difference between sites (slightly lower survival within the ESA). When we varied the parameterizations of temporary emigration and detection probability, QAICc scores identified 10 models within 4 units of the best-supported model (Table 2). Model-averaged survival estimates were higher for females and did not differ by area (Table 3), but confidence intervals on these estimates were broad compared to observed differences. Model-averaged estimates indicated very low levels of temporary emigration, with precision insufficient to determine whether movements were Markovian or random (Table 3). The model-averaged detection probability estimate was higher in the WSA than the ESA, but this difference was small relative to the confidence intervals (Table 3). Model-averaged estimates of detection (p) and redetection (c) probabilities were nearly equal, confirming that unbaited cameras did not influence recapture probability.

The overall mean WSA population estimate (18.66 leopards; 95% CI: 16.50-20.81) was 78% larger than the overall mean ESA population estimate (10.50 leopards; 95% CI: 7.90-13.10), with non-overlapping 95% confidence limits for the two areas (Table 4). The HMMDM for 14 leopards recaptured at multiple sites was 2.04 km (range: 1.22 km – 6.25 km). We used this HMMDM buffer to estimate the effectively sampled

areas as 219.51 km² and 206.48 km² for WSA and ESA, respectively. Thus, overall mean leopard density was 67% higher in the WSA (8.50 leopards/100 km²; 95% CI = 7.52-9.48) relative to the ESA (5.08 leopards/100 km²; 95% CI = 3.83-6.34; Table 4). Population size estimates varied seasonally in both study areas, but did not show systematic increase/decline or seasonal changes in local density (Table 4).

Discussion

Large carnivores throughout Africa are rarely studied in a manner that yields data on population density or demography that is sufficiently precise to facilitate science-based management. Improving our understanding of how leopard populations respond to growing peripheral human activities is important for leopard conservation, but may also help identify factors that limit sympatric carnivore populations. In this study we detected 67% higher density within the National Park (WSA) relative to the bordering areas (ESA), demonstrating that leopard density responds very strongly to management regimes. Our results align with other studies that have found a negative relationship between human encroachment around PAs and leopard population density (Marker & Dickman 2005; Balme, Slotow & Hunter 2010; Henschel et al. 2011; but see Stein et al. 2011). Despite large differences in density, we found little evidence that survival differed between study areas and that temporary emigration was occurring at low levels (though estimates for these last two parameters had low precision, likely due to the difficulty of sampling a large number of individual leopards, and inability to estimate age from camera-trap photos, largely taken at night). While these limitations must be recognized,

these are the first methodologically and statistically rigorous estimates of leopard density and survival in a population considered critical for regional carnivore conservation.

These data identify differences in leopard density between areas differing in management and anthropogenic effects, and help to identify factors limiting carnivore populations (see below).

Determining Abundance in Comparable Study Areas

Estimating population density is a common objective for camera trap studies, but the best methods are debated (Foster & Harmsen 2012). A central problem lies in estimating the area that has been surveyed, because some detected animals are likely to move beyond the perimeter of a camera trap array. The width of the average home range of the target species is often used to estimate this area (Dice 1938). A common alternative method relies on the mean maximum distance moved (MMDM) across trap sites for all captured animals. Studies have compared density estimates derived from telemetry and MMDM data, but their conclusions are inconsistent (Foster & Harmsen 2012; Parmenter et al. 2003). Spatially-explicit capture-recapture models can simultaneously estimate population size and the area surveyed, yielding a direct estimate of density (Efford 2004). Rather than relying on these solutions, our study design reduced the problem of estimating the surveyed area, relative to prior studies that placed camera traps at sites frequently used by the target species (Balme, Hunter & Slotow 2009) or used lures to increase detection probability (Du Preez, Loveridge & Macdonald 2014). Instead, we used two highly similar randomized sampling grids. By using a randomized grid, our sampling was not biased to areas of heavy use, and thus is more likely to produce a

representative estimate of density for the sampled area. By comparing samples from two study areas of the same size, using the same sampling grid and design, our primary analysis is not complicated by the conversion of population size to population density (though this issue does affect comparison of our estimates to other studies). The application of our design hinges on retaining adequate detection probability for the desired analysis, which is a limitation for wide-ranging felids, but has the advantage of providing estimates of population density that can be directly compared between surveyed areas, and that are representative of those areas.

Drivers Behind Observed Differences in Leopard Densities

Density differences between the WSA and the ESA could stem from three limiting factors. First, prey populations in the ESA could be depleted due to ongoing illegal poaching and past legal harvests. Second, the ESA leopard population density could be lower due to direct leopard mortality from poaching or residual effects from trophy harvests prior to the 2012 moratorium. Finally, interspecific competition with the local lion population could limit this leopard population. Below we consider how these three factors may have contributed to the observed results.

Prey depletion provides a coherent explanation for low leopard density in the ESA. A reduction in prey density would be expected to reduce leopard density (Hayward, O'Brien & Kerley 2007; Balme et al. 2013), and this hypothesis is compatible with the observation that survival rates did not differ between the two areas. Our two study areas differed dramatically in their exposure to wire-snare poaching. The ESA's median probability of snare occurrence was 5.2-fold greater than that of the WSA (ESA

median= 0.74, range:0.01-0.99; WSA median=0.14, range:0.01-0.70; Watson et al. 2013; Figure 3.4a) due to differences in law enforcement and land use. Herbivore biomass in Lupande GMA was recently estimated as 1/10th of capacity (Lindsey et al. 2014), and in a rough calculation based on interviews in one Lupande GMA community (south of the ESA), Lewis & Phiri (1998) roughly estimated that the community harvested 2 428 animals with wire snares during a 6-month dry season, the majority of which were impala (*Aepyceros melampus*) and other small- and medium-sized ungulates commonly preyed upon by leopards (Hayward et al. 2006; Ray 2011). Finally, in the Congo Basin, Henschel et al. (2011) documented similar responses of leopard density to exploitative competition with local hunting community. Given the rapid rates of human encroachment for Lupande and the majority of Zambia's GMAs (Watson et al. 2014), illegal bushmeat harvest and consequent prey depletion is probably a strong limiting factor for leopards (and other large carnivores) in GMAs.

Past legal harvests of herbivores may also contribute to prey depletion in GMAs. For decades prior to the 2013-2015 moratorium on trophy and resident hunting, there was active trophy and resident hunting in the ESA for leopard and their prey. Legal hunting did not occur during this study, but prior decades of poorly regulated harvest of herbivores could contribute to prey depletion in the ESA, although legal hunting offtakes were lower than estimated poaching offtake by an order of magnitude. Nevertheless future management of legal hunting should consider the potentially additive effects of poaching, legal harvest and rapid rates of habitat loss on prey populations for leopard and other carnivores.

Direct mortality of leopards due to legal or illegal hunting could result in lowered leopard numbers outside of SLNP, as has been demonstrated for the South Luangwa lion population (Rosenblatt et al. 2014), but evidence for this hypothesis is lacking. In the Luangwa valley wire snare poaching is known to be a source of mortality for other large carnivores (Becker et al. 2013a,b; Rosenblatt et al. 2014), but snared leopards are uncommon relative to wild dogs or lions (Rosenblatt unpublished data). Point estimates of survival were lower for males than for females, but this difference was small relative to the width of the confidence intervals, did not differ between the ESA and WSA, and could be due to natural causes. Though legal trophy hunting was not a source of leopard mortality during this study, the effects of trophy hunting on large carnivore density and population structure are likely to persist for some time after the harvest period (Balme, Slotow & Hunter 2009; Davidson et al. 2011). Leopard quotas in hunting concessions in the Luangwa Valley are high relative to those allocated in the rest of Zambia (Ray 2011), and the ESA included portions of a hunting concession that on average removed 4 male leopards per year. Based on the estimated average population size and the observed sex ratio for the ESA, this rate of offtake would remove most resident males in this study area. Furthermore, male leopards were removed all along the periphery of SLNP, creating the potential for ‘attractive sink’ dynamics (Loveridge et al. 2010) and lowered cub survival due to increased rates of infanticide (Whitman et al. 2004; Balme, Slotow & Hunter 2009; Packer et al. 2011). Finally given the difficulty in aging leopards (Balme, Hunter & Braczkowski 2012) documented by Ray (2011) in nearby Luambe National Park and the high potential for harvesting female leopards in trophy hunts (Spong,

Hellborg & Creel 2000), the impacts of past leopard harvests, particularly those that did not adhere to sex- or age-based limits (Balme, Hunter & Braczkowski 2012), are likely to persist for several years. Despite these potential problems, our data provide no evidence that differences in management during the hunting moratorium directly affected leopard mortality rates.

Finally, leopard density can be limited through interspecific competition, mainly with lions (Stander, Haden & Kagece 1997; Balme et al. 2013). If the South Luangwa lion population was the limiting factor for the leopard population, we would expect decreasing leopard densities in areas with recovering lion populations during the hunting ban of 2013-2015. This hypothesis was not supported for two reasons. First, prior to the hunting ban, the WSA was more heavily utilized by lions than the ESA (Figure 3.4b), yet after the ban was implemented the WSA had higher leopard population estimates than the ESA. Second, the frequency of leopard captures across space paralleled the distribution of the local lion population, suggesting that leopards did not avoid areas frequently used by lions in this ecosystem (Figure 3.4b). Therefore, there is little evidence that differences in leopard densities were due to interspecific competition with lions.

Conclusions

The conservation of African large carnivores is increasingly critical given their ecological, economic and social value, large declines in range and numbers, and the growing threats they face. By quantifying the status and trend of carnivore populations and identifying the factors that control them we can inform management priorities to mitigate threats. In our study we detected lower leopard density on the periphery of an

important Zambian National Park, likely due to prey depletion driven by bushmeat poaching. If prey depletion limits leopards, it is likely to affect other carnivores, and therefore further research objectives should include rigorous monitoring of both carnivore and herbivore populations across protection gradients. Additionally, with leopard trophy hunting resuming in Zambia in 2015, monitoring should continue to quantify how the density and distribution of leopard respond to altered legal harvest. Our data, combined with this change in policy, provide an ideal opportunity to apply the principles of adaptive management in a manner that is rare for large carnivores. As human encroachment increases adjacent to PAs around the world with strong limiting effects on large carnivores and their prey, research providing reliable and precise estimates of critical population parameters must be in place to evaluate the effectiveness of management decisions.

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Table 3.1: Model selection results using QAICc to determine the best supported robust design model of survival (S): in the text, this is step one of model selection. Models varied only by their parameterization of S . In all models there was no temporary emigration ($\gamma''=0$, $\gamma'=1$) and a single detection probability ($p(\cdot)$), and population size was estimated by season and study area (N). From these results we used the top three parameterizations of S for the second stage of model selection.

Model	Parameters	Delta QAICc	QAICc weight
$S(\cdot), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	8	0	0.5
$S(\text{sex}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	9	1.61	0.22
$S(\text{area}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	9	2.37	0.15
$S(\text{sex+area}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	10	3.99	0.07
$S(\text{area*sex}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	11	6.44	0.02
$S(\text{time}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	11	6.94	0.02
$S(\text{time+sex}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	12	8.56	0.01
$S(\text{area+time}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	12	9.34	0
$S(\text{time+sex+area}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	13	11	0
$S(\text{time+area*sex}), \gamma''(0), \gamma'(1), p(\cdot), N(\text{season+area})$	14	13.62	0

Table 3.2: The best supported robust design models from 72 candidate models, as determined by QAICc scores: in the text, this is step two of model selection. In addition to the three best parameterizations of S , these top models supported non-existent ($\gamma''(0)$, $\gamma'(1)$) or random ($\gamma''(\cdot)=\gamma'$) temporary emigration and p and c to be equal and constant ($p(\cdot)$), unequal and constant ($p(\cdot)$, $c(\cdot)$), or equal and differing by area ($p(\text{area})$). These models were used for model-averaged estimates of S , γ'' , γ' , p , c , and N .

Model	Parameters	Delta QAICc	QAICc weight
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $N(\text{season+area})$	8	0	0.19
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $N(\text{season+area})$	9	0.94	0.12
$S(\text{sex})$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $N(\text{season+area})$	9	1.61	0.08
$S(\cdot)$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $N(\text{season+area})$	9	1.93	0.07
$S(\text{area})$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $N(\text{season+area})$	9	2.37	0.06
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	9	2.42	0.06
$S(\text{sex})$, $\gamma''(\cdot)=\gamma'$, $p(\text{area})$, $N(\text{season+area})$	10	2.71	0.05
$S(\cdot)$, $\gamma''(\cdot)=\gamma'$, $p(\text{area})$, $N(\text{season+area})$	10	3.02	0.04
$S(\text{area})$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $N(\text{season+area})$	10	3.19	0.04
$S(\cdot)$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	10	3.33	0.03
$S(\text{sex})$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $N(\text{season+area})$	10	3.6	0.03

Table 3.3: Model-averaged parameter estimates of survival (S), temporary emigration (γ' and γ''), detection (p), and redetection (c) probabilities for the South Luangwa leopard population.

Parameter	Estimate	SE	95% LCL-UCL
\hat{S} - male, ESA	0.68	0.24	0.20-0.95
\hat{S} - female, ESA	0.73	0.21	0.25-0.96
\hat{S} - male, WSA	0.68	0.18	0.30-0.91
\hat{S} - female, WSA	0.73	0.14	0.40-0.91
$\hat{\gamma}'$	0.81	0.35	0.04-1.00
$\hat{\gamma}''$	0.05	0.14	0.00-0.96
\hat{p} - ESA	0.22	0.08	0.10-0.41
\hat{p} - WSA	0.25	0.07	0.15-0.40
\hat{c} - ESA	0.21	0.07	0.10-0.38
\hat{c} - WSA	0.25	0.06	0.15-0.37

Table 3.4: Model-averaged estimates of seasonal and overall average population size (\hat{N}) and density (leopards per 100 km²) calculated using HMMDM. There was no apparent trend across cold dry (CD) and hot dry (HD) seasons on population estimates within each study area. \hat{N} for HD 2013 and CD 2014 in both study areas were identical due to the same number of individuals captured. On the ESA in HD 2013, 24% of trap sites could not be sampled due to early onset of the rainy season.

Season	Number Captured	\hat{N}	SE	95% LCL-UCL	Density	95% LCL-UCL Density
CD 2013, ESA	8	12.53	5.35	8.45-53.85	6.07	4.09-26.08
HD 2013, ESA	5	8.67	4.37	5.36-42.72	4.20	2.59-20.69
CD 2014, ESA	5	8.67	4.37	5.36-42.72	4.20	2.59-20.69
HD 2014, ESA	7	12.11	5.78	7.56-53.91	5.87	3.66-26.11
CD 2012, WSA	16	22.29	5.91	17.00-55.65	10.15	7.74-25.35
CD 2013, WSA	10	18.21	5.88	12.02-43.39	8.30	5.48-19.77
HD 2013, WSA	9	15.71	5.20	10.47-39.66	7.16	4.77-18.07
CD 2014, WSA	9	15.71	5.20	10.47-39.66	7.16	4.77-18.07
HD 2014, WSA	12	21.35	6.44	14.42-48.04	9.72	6.57-21.88
Average, ESA	-	10.50	1.33	7.90-13.10	5.08	3.83-6.34
Average, WSA	-	18.66	1.10	16.50-20.81	8.50	7.52-9.48

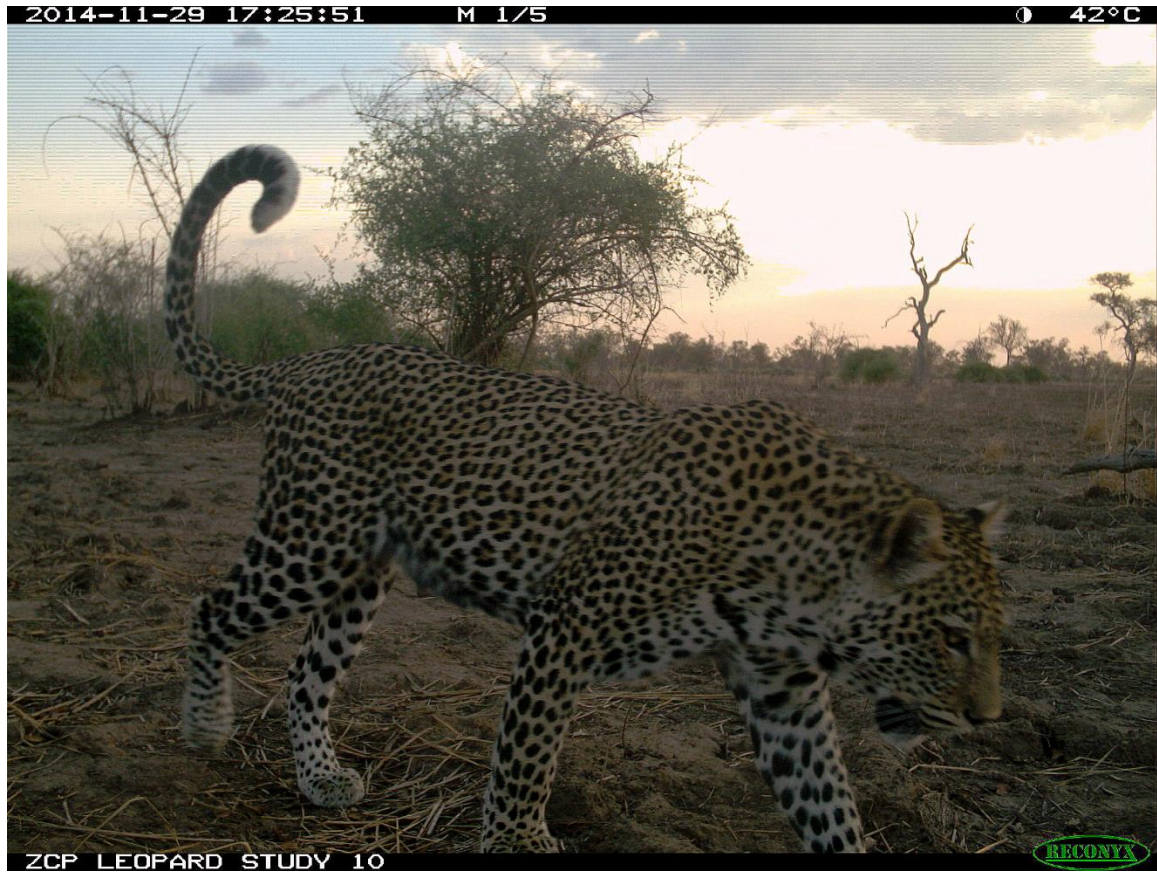


Figure 3.1: A leopard photographed by a remote camera trap travelling in the late afternoon in South Luangwa National Park, Zambia.

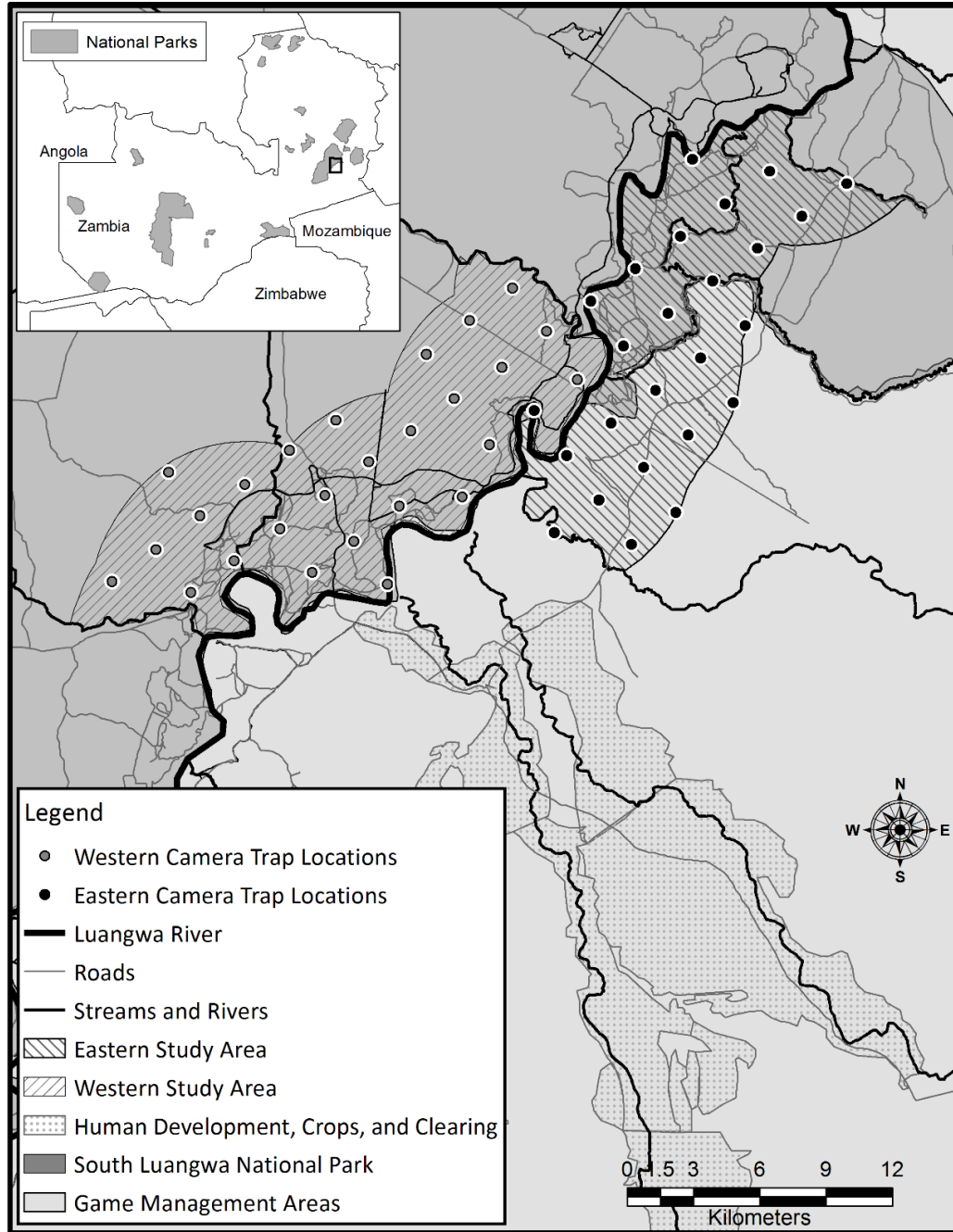


Figure 3.2: Our two study areas spanned the border of South Luangwa National Park, Eastern Province, Zambia. Camera traps surveyed strictly protected areas (Western Study Area – WSA) and community Game Management Areas (Eastern Study Area – ESA) encompassing a gradient of management regimes and accompanying human impacts likely to influence density and survival.

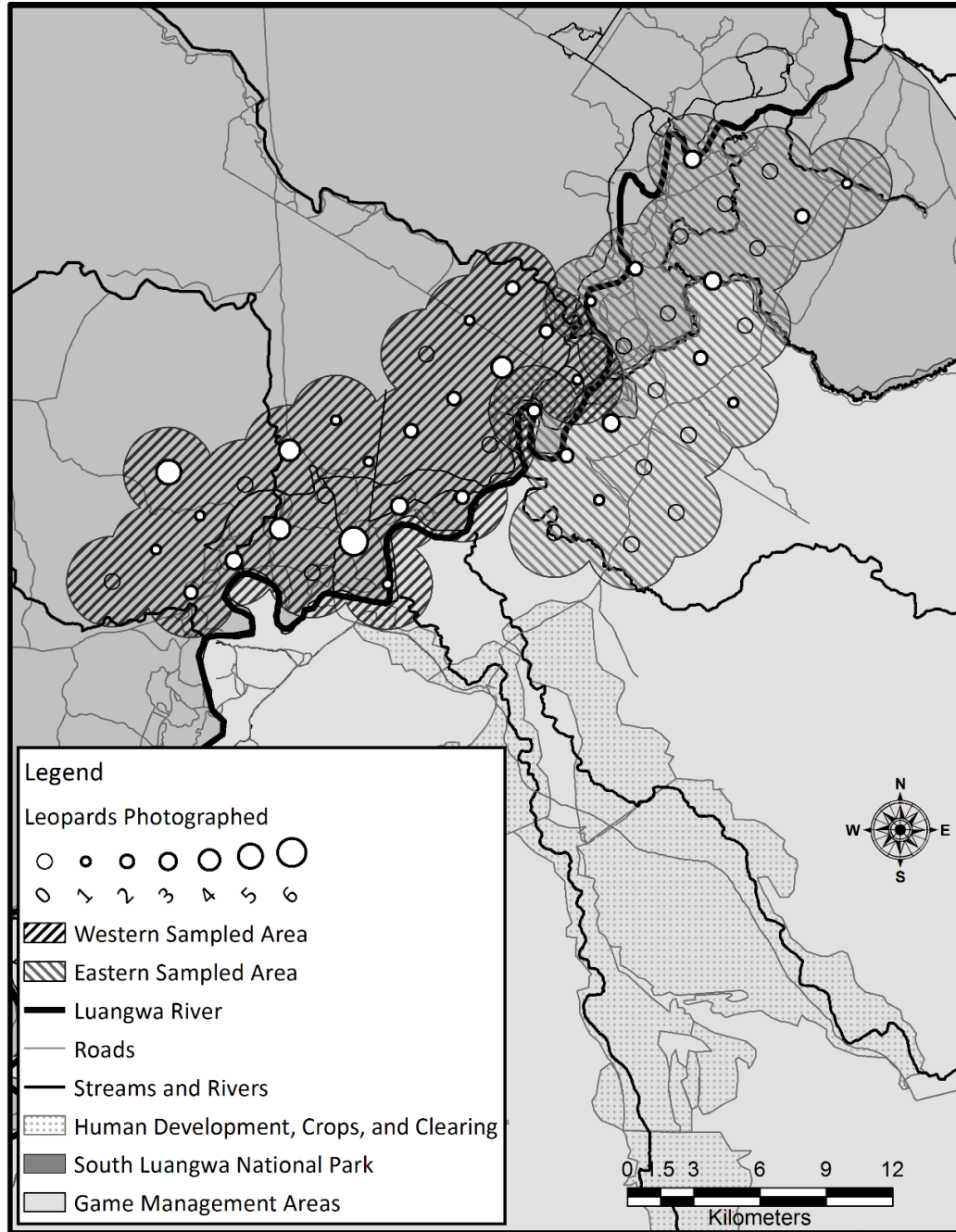


Figure 3.3: The distribution of leopard detections across the ESA and WSA. The size of the circles indicates the number of individual leopards that were detected at each camera trap site. The shaded polygons indicate each study area's trap-buffer, i.e. the area effectively sampled for the calculation of density.

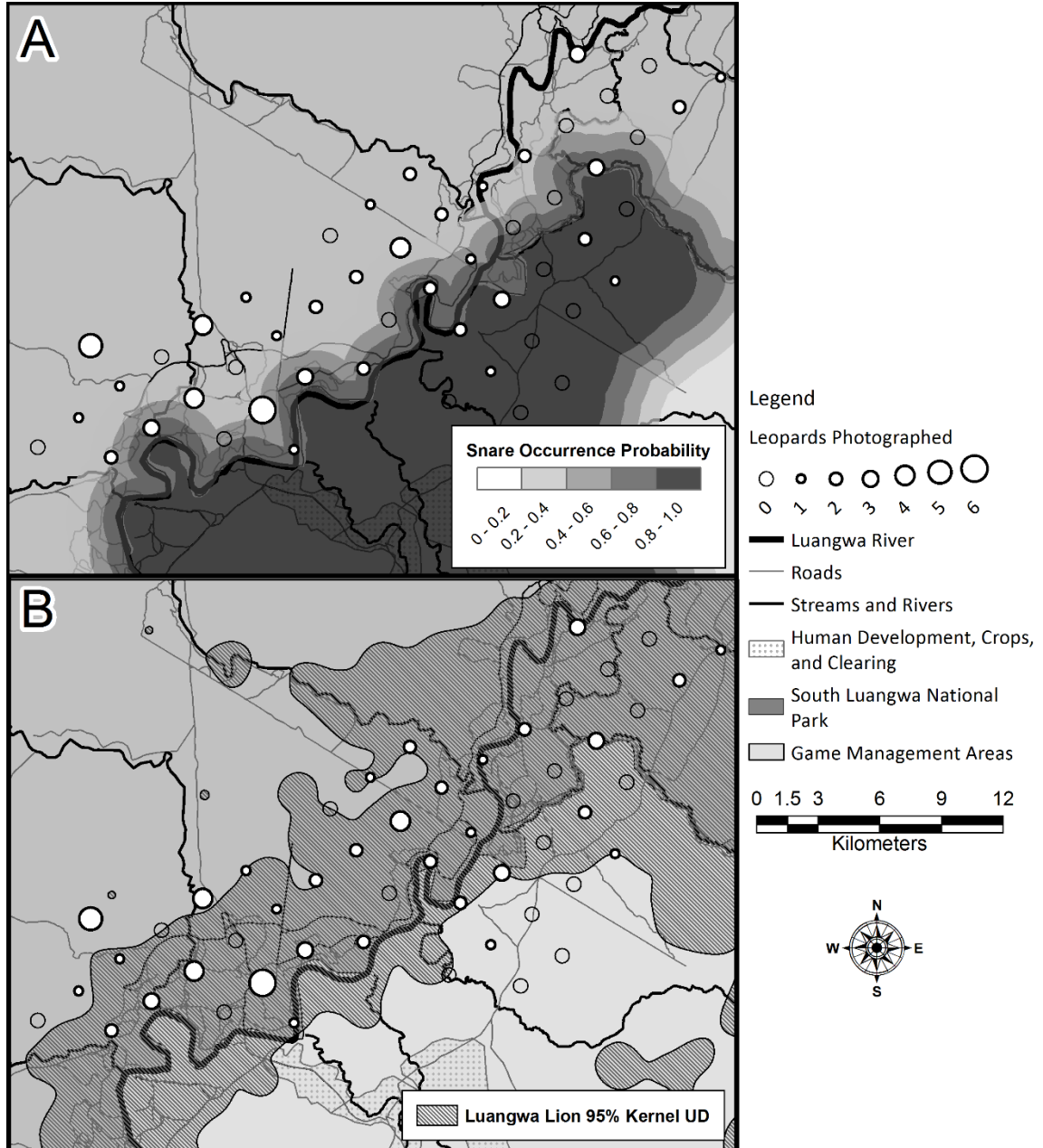


Figure 3.4: The distribution of leopard encounters compared to (A) gradients of probability of wire snare occurrence (from Watson et al. 2013) and (B) patterns of African lion use (95% kernel utilization distribution – from Rosenblatt et al. 2014). Overall, wire snare occurrence was higher in the ESA relative to the WSA, and fewer leopards were photographed in areas of high wire snare occurrence. Leopards commonly used areas of high lion density and thus do not appear strongly limited by interspecific competition.

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CHAPTER FOUR

USING PEDIGREE RECONSTRUCTION TO ESTIMATE POPULATION SIZE:
GENOTYPES ARE MORE THAN INDIVIDUALLY UNIQUE MARKS

Contribution of Authors and Co-Authors

Manuscript in Chapter Four

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Abstract

Estimates of population size are critical for conservation and management, but accurate estimates are difficult to obtain for many species. Noninvasive genetic methods are increasingly used to estimate population size, particularly in elusive species such as large carnivores, which are difficult to count by most other methods. In most such studies, genotypes are treated simply as unique individual identifiers. Here, we develop a new estimator of population size based on pedigree reconstruction. The estimator accounts for individuals that were directly sampled, individuals that were not sampled but whose genotype could be inferred by pedigree reconstruction, and individuals that were not detected by either of these methods. Monte Carlo simulations show that the population estimate is unbiased and precise if sampling is of sufficient intensity and duration. Simulations also identified sampling conditions that can cause the method to overestimate or underestimate true population size; we present and discuss methods to correct these potential biases. The method detected 2–21% more individuals than were directly sampled across a broad range of simulated sampling schemes. Genotypes are more than unique identifiers, and the information about relationships in a set of genotypes can improve estimates of population size.

Keywords: Census, lion, mark-recapture, pedigree reconstruction, population estimate, population size.

Introduction

Conservation and management of wildlife populations require information on population size, but this is usually difficult to obtain for species that are rare or elusive. Many endangered species (exemplified by large carnivores) are both rare and elusive, and accurate estimates of total population size for such species are not common. Methods have been developed to extract DNA and determine microsatellite genotypes from hair (Goossens et al. 1998; Flagstad et al. 1999; Woods et al. 1999; Sloane et al. 2000), feces (Taberlet et al. 1996, 1999; Gagneux et al. 1997; Kohn and Wayne 1997; Kohn et al. 1999) and less direct sources of cells (Parsons et al. 1999; Valiere and Taberlet 2000). Because hair and fecal samples can be collected without capturing or handling the animal, these methods have great promise for population estimation. Genotypes can be used to estimate population size in several ways. Most directly, the number of genotypes is an estimate of the minimum population size, which can be identified by the asymptote of a curve relating the number of distinct genotypes to the number of samples (Kohn et al. 1999) or by rarefaction (Kalinowski 2005). Capture-mark-recapture (CMR) methods of estimating population size can be applied to genetic data if individuals are sampled sufficiently often to estimate capture probabilities (Otis et al. 1978; Seber 1982; Seber 1986; Boulanger et al. 2004; Kendall et al. 2009). Genetic CMR methods all rely on the logical argument that population size (\hat{N}) can be estimated by the number of genotypes (or 'captures', C) divided by the probability of capture (\hat{p}). With repeated sampling, \hat{p} can be estimated directly from individual capture histories (Otis et. al 1978). These direct

genetic census methods have been applied to several large carnivore species, including brown bears (*Ursus arctos*: Taberlet et al. 1999; Boulanger et al. 2004; Kendall et al. 2009), wolves (*Canis lupus*: Creel et al. 2003), mountain lions (*Panthera concolor*: Ernest et al. 2000; Sawaya et al. 2011), and coyotes (*Canis latrans*: Kohn et al. 1999). Direct genetic census methods are virtually identical to other CMR methods in their underlying logic; genotypes simply substitute for any other type of mark that allows individuals to be identified.

The use of genotypes as individual identifiers does not necessarily eliminate bias in estimates of population size (and survival rates) due to ‘misread’ marks. If the number or variability of genetic markers is low, two individuals may have the same genotype. In this case, the number of unique captures (C) is underestimated and the probability of capture (\hat{p}) is overestimated, leading to underestimation of population size (\hat{N}) (Mills et al. 2001). This ‘shadow effect’ can be corrected by the use of additional genetic markers or markers with greater variation among individuals. If individuals are sampled repeatedly and genotyping errors occur (allelic dropout and misprinting are relatively common with noninvasive sample types that have low DNA yield and poor preservation), then (C) is overestimated and (\hat{p}) is underestimated, leading to overestimation of (\hat{N}). This ‘ghost effect’ (Creel et al. 2003) can be corrected by procedures to check for and eliminate genotyping errors (Taberlet et al. 1996, 1999) or by using methods that allow for imperfect genotyping (Creel et al. 2003, Kalinowski et al. 2006). Ironically, the ghost effect becomes stronger if the number of loci is increased to eliminate the shadow effect, or if the number of samples is increased to better estimate \hat{p} (Creel et al. 2003). Finally,

it can be difficult to identify distinct sampling occasions for population estimates using genetic CMR if noninvasive samples (such as hair) can persist in the environment for long periods. As with CMR studies using other types of marks, population estimates using genetic CMR often have wide confidence limits because recapture probabilities are low (Mills et al. 2001; Lukacs and Burnham 2005).

Indirect genetic census methods based on pedigree reconstruction have also been developed (Jones and Avise 1997a, 1997b; Pearse et al. 2001; Israel and May 2010). For example, in a study of painted turtles (*Chrysemys picta*), the set of genotypes ‘captured’ was extended to include individuals who were never directly sampled, but whose genotype could be inferred by pedigree reconstruction (Pearse et al. 2001). This study took advantage of the opportunity to sample many offspring in a single nest. With multi-locus microsatellite genotypes from many offspring and one parent (the female attending the nest), the likely genotype of an un-sampled parent (in this case the father) could be inferred with confidence. The resulting set of genotypes was then analyzed with the normal CMR logic to estimate population size. The inclusion of individuals inferred to be present from pedigree reconstruction improved the population estimate substantially, compared to estimates based on direct CMR analysis (Pearse et al. 2001).

Pedigree reconstruction can detect the genetic fingerprint (and thus the presence) of un-sampled individuals. This distinguishes pedigree reconstruction from typical direct genetic census methods, and presents a potential alternative method to estimate population size. This alternative may be more powerful and precise, because it does not reduce the information in a multi-locus genotype into a ‘mark’ that simply identifies an

individual in the same manner that a colored leg band or ear tag would identify it. In addition to serving as individually-unique identifiers, genotypes contain information about population structure (genetic relationships among individuals), and that information can be used to improve estimates of population sizes and trends (Luikart et al. 2010; Tallmon et al. 2010).

Several excellent recent reviews discuss the range of genetic markers suitable for pedigree reconstruction, and appropriate methods of analysis (Blouin 2003; Morin et al. 2004; Anderson and Garza 2006; Kalinowski et al. 2006; Wagner et al. 2006; Koch et al. 2008; Pemberton 2008; Wang and Santure 2009; Jones et al. 2010; Riester et al. 2010). For the purposes of this paper, we restrict our discussion to comparing of the genotypes of a set of offspring to the genotype of one parent, and thus inferring the genotype of the other parent even though it was not directly sampled. Some methods of pedigree reconstruction can provide insight into secondary relationships between individuals (and thus can potentially be used to infer the existence of un-sampled individuals across generational gaps or past first-order relationships), but we leave this for later.

Until now, pedigree reconstruction methods have been used to estimate the number of breeding individuals in a population, rather than total population size (Jones and Avise 1997; Nielsen et al. 2001; Pearse et al. 2001; Koch et al. 2008; Israel and May 2010). Because an individual must breed in order to appear in a pedigree, this constraint initially seems unavoidable. Here, we use a simulation model to show that pedigree reconstruction can be used to estimate total population size. From the perspectives of conservation and management, total population size is often of greater interest than the

number of breeders (for example, to evaluate the effect of human harvest on population dynamics [Creel and Rotella 2010; Packer et al. 2011; Cooley et al. 2009]). We present formulas to estimate the size of a population by estimating the numbers of both breeding and non-breeding adults, and use Monte Carlo simulation to evaluate the bias and precision of the estimates. The simulated population has demographic properties derived from African lions in Zambia (*Panthera leo*; Figure 4.1; Becker et al. 2013b), so that the method is tested for a scenario that exemplifies a species of conservation and management concern. Finally, we use the simulation to explore the effects of variation of sampling methods and sampling intensity on the bias and precision population estimates.

Using Pedigree Reconstruction to Estimate Population Size,
Including Individuals that did not Breed

We can estimate total population size (\hat{N}) as the sum of individuals directly sampled (N_s), individuals that bred and whose presence was inferred by pedigree reconstruction (N_{in}), and individuals who did not breed and therefore remained ‘invisible’ to pedigree reconstruction (N_{iv}).

$$\hat{N} = N_s + N_{in} + N_{iv} \quad (\text{Equation 4.1})$$

where

$$N_{iv} = P_{iv}(N_s + N_{in}) \quad (\text{Equation 4.2})$$

$$P_{iv} = P_{nb} \cdot P_{in} \quad (\text{Equation 4.3})$$

$$P_{nb} = 1 - \frac{B_s}{N_s} \quad (\text{Equation 4.4})$$

and

$$P_{in} = \frac{N_{in}}{N_s + N_{in}} \quad (\text{Equation 4.5})$$

The variables are:

N_s = number of individuals sampled

N_{in} = number individuals inferred by pedigree reconstruction

N_{iv} = number of individuals invisible to pedigree reconstruction (unsampled nonbreeders)

P_{iv} = probability of an individual being ‘invisible’

P_{nb} = probability of an individual not breeding

P_{in} = probability of inferring an individual’s presence by pedigree reconstruction

B_s = number of breeders sampled

The number of individuals directly sampled and genotyped (N_s) requires no special explanation. N_{in} is the number of individuals that were not directly sampled, but whose presence could be inferred by pedigree reconstruction because they bred with sampled mates and left offspring that were also sampled. Logically, the number of ‘invisible’ individuals (N_{iv}) that were not detected by sampling or by pedigree reconstruction can be estimated as the proportion of individuals that were invisible to the pedigree (P_{iv} , animals that neither bred nor were directly sampled) multiplied by the total size of the pedigree ($N_s + N_{in}$) (Equation 4.2). Under any given sampling scheme, the proportion of individuals invisible to both direct sampling and pedigree reconstruction (P_{iv}) is simply the product of two known quantities: the proportion of detected individuals that were detected only by pedigree reconstruction and not by direct sampling (P_{in}), and the proportion of individuals that did not breed, and thus could not be inferred by

pedigree reconstruction (P_{nb}) (Equation 4.3). Equation 4.3 assumes that the probability of obtaining a sample from an individual is independent of its breeding status, which is likely to be correct for many methods of sampling. Both of these probabilities can be estimated by the data. The probability of breeding (and thus the probability of not breeding, P_{nb}) can be estimated from the number of directly sampled individuals (N_s) and the subset of these that were known to breed (B_s) because they had descendants in the pedigree (Equation 4.4). The probability of being detected only by reconstruction (and not by a direct sample) is also easily estimated from the pedigree itself (Equation 4.5). Equations 4.1 – 4.5 simplify by substitution to:

$$\hat{N} = N_s + 2N_{in} - \frac{N_{in}B_s}{N_s} \quad (\text{Equation 4.6})$$

This corrected equation differs slightly from the published equation due to a minor substitution error. The simulation study in this paper uses equations 4.1-4.5, so this error does not affect the inferences of this manuscript. This estimator uses the information in a set of genotypes to estimate population size as the sum of three segments of a population: (1) the number of individuals directly sampled, (2) the number of individuals who were not sampled but whose existence could be directly inferred by pedigree reconstruction, and (3) the number of individuals that were not sampled and whose existence could not have been inferred by pedigree reconstruction, because they left no offspring. The logic of this estimator is similar in one way to the logic of CMR estimators, because the number of individuals ‘captured’ (N_s here, C in the context of CMR) is adjusted upward to account for individuals that were not captured. The logic differs because this process of adjustment does not simply treat genotypes as individual

identifiers, but instead takes advantage of the information that genotypes provide about population structure. Below, we use simulation to confirm that the estimator is unbiased and precise with reasonable sampling effort.

A Caveat about Demographic Closure

This method estimates the size of the adult population within the sampling period. The method (as presented above) does not account for the possibility that a pedigree can contain a set of individuals that were not all alive at the same time. For population surveys based only on direct genotypes, non-invasive genetic samples can usually be collected intensively within a period that has been tested for population closure by species-specific mark-recapture surveys (ie. 60 days for snow leopards (*Uncia uncia*; Jackson et al. 2005), 59 days for jaguars (*Panthera onca*; Silver et al. 2004), 250 days for leopards and tigers (*Panthera pardus fusca* and *Panthera tigris tigris*; Wang and MacDonald 2009). However, it is advisable for users of this method to test and confirm population closure using one of multiple software packages available, or using direct data on the rates (and timing) of mortality, reproduction, immigration and emigration. We return to this issue in the results and discussion, because the assumption of demographic closure is more likely to be violated when the set of genotypes includes inferred parents (which can be dead by the time that their existence is inferred).

Simulations to Evaluate Bias and Precision
of the New Population Size Estimator

We evaluated the population size estimator (Equation 4.6) by simulation, sampling from a modeled lion population that we created using demographic data from a lion population in Eastern Zambia's Luangwa Valley (Becker et al. 2013b). By creating a simulated population and then sampling from it stochastically, we could (1) compare the population estimate to the true population size across a range of realistic sampling schemes (e.g. including or excluding samples from juveniles) and sampling intensities (ranging from 10% to 90% of the population that was not sampled in previous years), and (2) test the inferential gains from including inferred and 'invisible' individuals in the estimate. We based the simulated population on demographic data from lions because they typify large, wide ranging endangered carnivores, for which there are few logistically-tractable methods that provide precise estimates of population size. We used data from Luangwa Valley lions to parameterize the model, because this population is now being sampled for an empirical test of the method, and because it is affected by two conservation issues of importance for the species (and for large carnivores in general): harvest by trophy hunters (Creel and Creel 1997; Whitman et al. 2007; Packer et al. 2009; Creel & Rotella 2010) and mortality and prey depletion due to illegal harvest, in this case wire snaring (Becker et al. 2013a,b).

We emphasize that the model was designed to test the population size estimator with a simulated population of known size, with known parent-offspring relationships. The intention of the simulation was not to make detailed inferences about the dynamics

of a specific lion population. Using MS Excel, we created an individual-based model with a binomially-distributed initial adult sex ratio with a mean of 0.92 (proportion females; the Luangwa Valley lion population used to parameterize the model is male-depleted as a consequence of trophy hunting), a binomially-distributed cub sex ratio with a mean of 0.50, and an initial age-distribution matching that observed in the Luangwa population. The initial distribution of individuals across age classes 0 to 8 years was 0.10, 0.17, 0.17, 0.18, 0.12, 0.07, 0.08, 0.07, 0.05 (Becker et al. 2013b). Age-specific mean survival rates from age classes 0 to 12 were 0.63, 0.91, 0.93, 0.95, 0.95, 0.94, 0.91, 0.82, 0.46, 0.26, 0.18, 0.10, 0.05 (Becker et al. 2013b) and survival for each age class was assumed to be a binomial process (that is, we did not model extra-binomial variation in survival rates within age classes). Becker et al. (2013b) reported fecundity as number of cubs per female, but our simulation tracked newborns by drawing from a normal distribution of litter sizes, and then assigning the value drawn to each female that reproduced (i.e., reproduction was modeled as a binomial process qualitatively matching that observed in Luangwa lionesses: see below). The assignment of stochastic litter sizes used Schaller's (1972) mean litter size of 2.4 (± 0.5 S.D.) cubs per litter, because we lacked sufficient data from Luangwa.

We assumed that females began reproducing at 4 years of age and that once a female reproduced, she did not reproduce for two years regardless of the fate of her cubs. The composition of mating pairs was assigned randomly among adults alive in that year. A useful refinement of this model for a more species-specific application would be to examine the effect of population subdivision and mating within and among prides

(Gilbert et al. 1991; Packer et al. 1991; Spong et al. 2002). The initial population size was 100 individuals, and the individual-based model was run for a period of 15 years. Individuals were tracked throughout their lifespan to identify parent-offspring relationships. The first 10 years were used as burn-in, and years 11-15 were used to simulate the collection of genotypes that we then used to estimate population size with Equation 4.6.

Varying the intensity of sampling from 10% to 90% (in increments of 10%) of the previously unsampled portion of the population each year, we ran 100 replicates of the simulation for each sampling intensity level, for each of two sampling schemes. As sampled individuals accumulate in a population, the annual sampling effort required to maintain constant sampling coverage declines. Figure 4.2 shows the sampling intensity that was required in each year to maintain sampling coverage of 10% to 90% of the lion population in 9000 iterations of our Monte Carlo model. The details of this pattern are expected to vary in a manner that depends on the rate of individual turnover in a population. In general, long generation times and low population growth rates (both typical of large carnivores) will allow sampling effort to asymptote at a lower value for a given level of coverage.

We modeled two sampling schemes. One sampling scheme mimicked biopsy-darting (Karesh et al 1987), which provides high quality tissue samples for extraction of DNA, but cannot be used with juveniles of many species. For the purposes of this model, individuals less than 2 years old were excluded from sampling. The other sampling scheme mimicked the collection of fecal samples, a technique with theoretically no age

limitation (though the yield and quality of DNA is lower and very young lions [$\leq 3-4$ weeks] may be difficult to sample due to lower visibility). We tracked the cumulative number of sampled, inferred and ‘invisible’ individuals, which of these individuals were still alive in each year, the population size in each year, the number of sampled breeding individuals, and \hat{N} (following Equation 4.6). We inferred the existence of an un-sampled individual if at least one of its offspring and the other parent were sampled; that is, we assumed that genotypes were sufficiently powerful to infer parents without error. This assumption is likely to hold for pedigree analysis based on a large number of SNPs (e.g., genotyping by sequencing can now yield 100,000s of SNPs per genotyped individual, and species-specific SNP chips can efficiently provide 96 or 384 SNPs). Although it is conceptually possible to extend the method using inferences about second-order genetic relationships with such data, we did not address this possibility.

Results & Discussion

Comparing estimated population size (\hat{N}) to true population size across in 9000 simulations with a broad range of sampling scenarios confirms that the estimator is fundamentally unbiased. The population estimate converges on the true population size with adequate duration and intensity of sampling (Figure 4.3B, lower right panels). The method can also provide precise estimates, particularly in comparison to many of the methods used to estimate population size in difficult-to-count species like large carnivores (Figure 4.3B, lower right panels). However, two issues related to sampling can cause the population estimate to be biased.

First, the method can potentially overestimate population size if the pedigree includes individuals that were sampled, or whose presence was inferred by reconstruction, over a long period (Figure 4.3A, particularly lower right panels). This problem is not unique to this method (Pollock et al. 1990). For any method that relies on data aggregated over an appreciable interval, the population estimate might include individuals that died before the end of the period. Pedigree reconstruction tends to amplify this basic problem, because an individual can be inferred by pedigree reconstruction after it is already dead. If one has an estimate of the annual rate of mortality, then it is conceptually simple to remove this overestimation bias by accounting for mortality during the sampling period. Following Equation 4.7, the population estimate at the end of the period is a weighted sum of the number of individuals first detected in each year, with weights equal to the probability of survival (s) from the year of detection to the end of the entire sampling period. The annual N_i values in this summation should include only the first (or last) detection of each individual, to avoid double counting.

$$\hat{N} = \hat{N}_t + \hat{N}_{t-1}s^1 + \hat{N}_{t-2}s^2 + \dots + \hat{N}_{t-\omega}s^\omega \quad (\text{Equation 4.7})$$

Equation 4.7 reduces to yield equation 4.8,

$$\hat{N} = \sum_{i=0} \hat{N}_t s^i \quad (\text{Equation 4.8})$$

where i is an index of years (or other intervals with equal lengths) prior to the final sample. One could also reduce or eliminate this overestimation problem by sampling intensively over an interval short enough for little mortality to occur, though obtaining adequate samples in a short period may not be tractable for many species (Figs. 1 & 2).

In this situation, one might treat \hat{N} as a count rather than a final estimate of population size, and use well-established CMR methods to produce the final estimate (Pollock et al. 1990, Kendall 1999, Lukacs & Burnham 2005).

Second, the type, duration and intensity of sampling must be adequate, or population size will be underestimated. Not surprisingly, the method works better if juveniles can be sampled, because this increases the likelihood of inferring the existence of parents that were not directly sampled. Sampling for a period of two years (with juveniles sampled), the population estimate is typically within 10% of true population size if $\geq 50\%$ of the population is sampled. As the duration of sampling increases, the sampling intensity required to maintain this level of accuracy decreases, but not by much. Thus, the method is best suited to species and contexts in which it is reasonable to expect that approximately 40% of previously unsampled individuals can be sampled, if \hat{N} is to be considered a direct estimate of population size. For species and contexts in which this intensity of sampling is not likely to be possible, one could also avoid underestimation by treating \hat{N} from Equation 4.6 as a count, rather than a population estimate (Pollock et al. 1990, Kendall 1999). If we consider $\hat{N}_{pedigree}$ to be a count (rather than an estimate of population size) and apply standard CMR logic, then

$$\hat{N}_{final} = \frac{\hat{N}_{pedigree}}{\hat{p}},$$

where \hat{p} is an estimate of the proportion of the population included in the pedigree. This might be estimated simply using the logic of the Lincoln-Peterson method or its extensions (Seber 1973; Williams et al. 2002). Finally, one might adjust the count to produce a final estimate of population size by estimating the asymptote of an

accumulation curve or rarefaction analysis, as is sometimes done with simple counts of genotypes (Kohn et al. 1999; Gotelli & Colwell 2001).

Underestimation and overestimation biases can fortuitously negate one another (e.g. Fig 2A, upper middle panel for 3 years of sampling with cubs not sampled), but this outcome should not be mistaken for validation of the method. The method reliably provided unbiased estimates of population size only when juveniles were sampled, sampling intensity exceeded 40% of the population and extended over several years, and individuals that had died were excluded from the estimate. These limitations are important, but the simulations also confirm the basic premise that pedigree reconstruction can provide an unbiased and precise estimate of population size, by taking advantage of the information about population structure that is contained within genotypes. Figure 4.4 illustrates the way in which this information increased the estimate of population size, relative to a simple count of the individuals sampled. Under the sampling scenarios we considered, pedigree reconstruction increased the number of individuals detected by 2% to 20%. In general, the biggest gains occurred when 30% to 40% of the population was sampled, including juveniles. Sampling juveniles is important to take advantage of the method, because this increases the likelihood of inferring the presence of an un-sampled parent. At very low sampling intensities, it is unlikely that an offspring and one of its parents will be sampled, so little power is gained over simply counting unique genotypes. At high sampling intensities, it is likely that any individuals that could be inferred would also be directly sampled.

Given the strong effect of age-restricted sampling methods in our simulations, it is important that field studies carefully consider the method of obtaining DNA. The ideal use of this method would be in a study that intensively collected non-invasive (fecal or hair) and/or non-destructive (tissue or blood) samples intensively across a large area with the intent of collecting samples from as many individuals as possible. Using multiple sample types would increase sampling efficiency and reduce the possibility of sampling that was biased by age or sex.

The ideal genetic marker for any pedigree reconstruction would provide high resolution to provide reliable relatedness estimates beyond first-order relationships, thereby facilitating pedigree reconstruction. There is an emerging preference for single-nucleotide polymorphisms (SNPs) as a low-cost, stable marker that can provide suitable genetic resolution (Smouse 2010). Though microsatellites require one-half to one-quarter as many markers to provide equivalent information content, SNPs provide stable, plentiful markers, the effect of mutation on these markers is predictable by simple mutation models, genotyping errors are minimal, and markers are present even in severely damaged genetic samples (Morin et al. 2004; Anderson and Garza 2006; Jones et al. 2010; Mesnick et al. 2011). We anticipate that the method outlined in this paper will be applied with SNPs, perhaps in the context of genotyping by sequencing, which can provide more than 100,000 SNPs per genotyped individual.

In summary, genotypes uniquely identify individuals, but they provide information beyond identity that can be used to estimate population size. In simulations that assumed that genotypes would provide enough information to identify an un-sampled

parent when the other parent and at least one offspring was sampled, pedigree reconstruction increased the count of sampled individuals by 2% to 20%, depending on sampling methods. This constitutes a valuable increase in the power to detect individuals with no extra sampling, relative to methods that simply treat genotypes as unique identifiers. Particularly if combined with capture-mark-recapture methods of population estimation, pedigree reconstruction offers a promising method of increasing the power of noninvasive genetic methods to estimate population size.

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Figure 4.1: Lion cubs with their resting mother in South Luangwa National Park, Zambia.

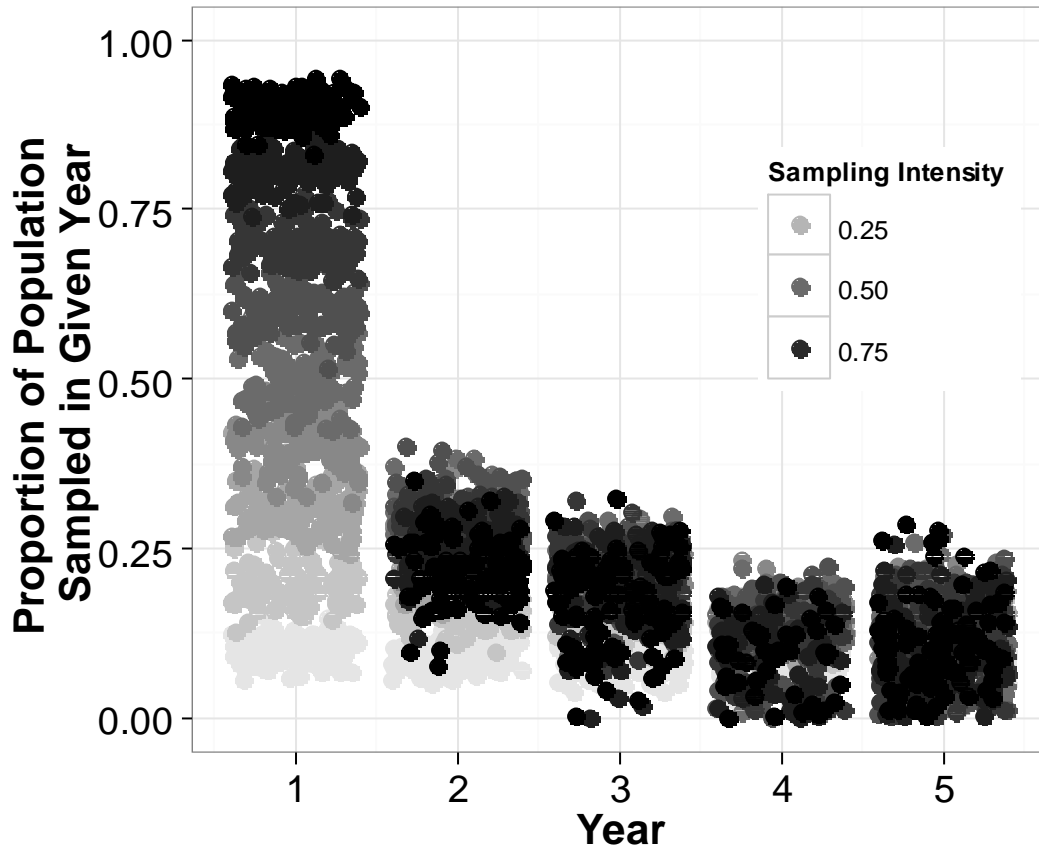


Figure 4.2: The intensity of sampling required to obtain samples from a fixed proportion of the population declines through time, in a scenario with realistic turnover of individuals within a population. The results from 4500 Monte Carlo simulations of sampling 10% to 90% of the individuals in a lion population showed, as expected, that this decrease was most pronounced for the most intensive sampling. The proportion of the population that must be sampled in a given year to maintain the desired sampling coverage typically dropped to $\leq 20\%$ within 3 years. These results are from simulated sampling that included juveniles, which increased the rate of turnover and thus increased the required sampling.

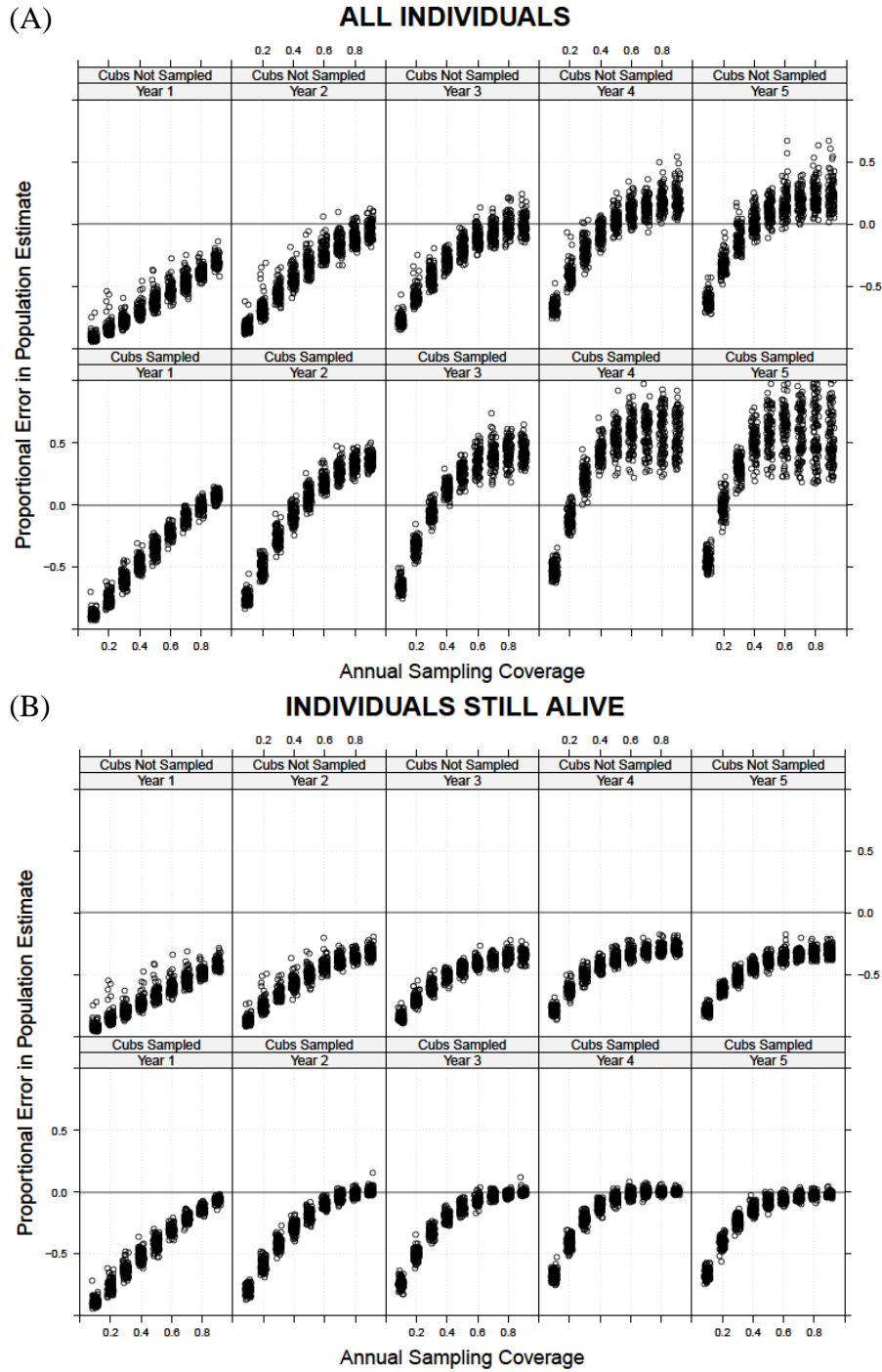


Figure 4.3: Results of 9000 Monte Carlo simulations comparing population estimates from pedigree reconstruction (N^{\wedge} from Equation 4.6) to the true population size of the simulated lion population described in the text. In each panel, the ordinate shows the difference between estimated population size and true population size, as a proportion of

Figure 4.3 Continued

true population size, so that zero represents an unbiased estimate, negative values indicate underestimation, and positive values indicate overestimation. In each panel, the abscissa shows the proportion of the population sampled annually. The five panels in each row show changes across five consecutive years of sampling, with estimates from one year of sampling on the left, and five years of sampling on the right. The bottom row shows results from simulations in which juveniles were sampled, and the top row shows results from simulations in which juveniles were not sampled. (A) In this implementation, the population estimator includes individuals that died after being sampled. With sampling over a long period of sampling, population size is often overestimated (particularly with high sampling coverage) because the population estimate includes individuals that were not alive at the end of the sampling interval. (B) In this implementation, the population estimator excludes individuals that were sampled or inferred to exist, but died prior to the year for which the population estimate is produced. With adequate sampling, the population estimator is unbiased and precise.

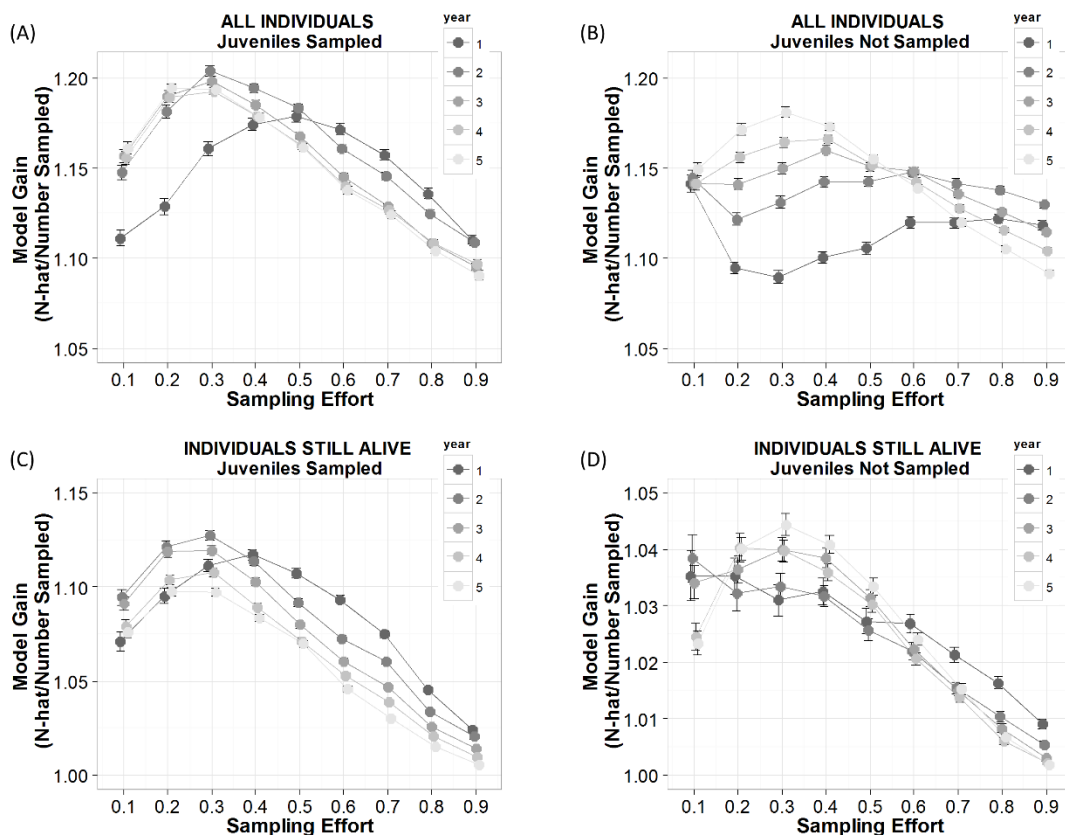


Figure 4.4: Gains in the detection of unsampled individuals through the use of Equation 4.6, relative to a count of directly sampled individuals. The ordinate plots the increase in estimated population size, relative the number of individuals detected by direct sampling, as functions of sampling effort (from 10% to 90% of the population sampled), years of sampling (from 1 to 5), and sampling type (juveniles included or excluded). (A, B) Estimates of population size include individuals that died prior to the year of the estimate. (C, D) Estimates of population size exclude individuals that died prior to the year of the estimate.

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CHAPTER FIVE

CONCLUSION TO THESIS

Overall Conclusions

My research overall focused on African lion and leopard populations that are under pressure from rapidly growing human populations and activities, circumstances that threaten the majority of protected large carnivore populations in Southern Africa and across the globe (Vitousek et al. 1997; Ripple et al. 2014). My research indicated that trophy hunting and illegal bushmeat harvests are impacting the South Luangwa lion and leopard populations. I suggested that trophy hunting regulations in Zambia need further reform and enforcement if trophy hunting is to continue, as well as intensive monitoring in place for key populations subject to trophy offtake. I also suggested that we need more information on the dynamics of prey depletion to assess the status of herbivore populations both in South Luangwa National Park and the surrounding Game Management Areas (GMAs) and more effectively combat illegal bushmeat poaching. This research constitutes the first rigorous scientific study of the South Luangwa lion and leopard populations, and will contribute to the conservation of these critical populations.

In Chapter Two, I presented data from an intensive five-year study of the South Luangwa lion population prior to a temporary moratorium on all trophy hunting. These data indicated that the South Luangwa lion population was in decline and was experiencing low levels of recruitment, low male sub-adult and adult survival rates, a depletion of adult males, and a senescing adult female population. These findings are

corroborated by conclusions in other study areas where the increased removal of male lions can impact the greater lion population through increased rates of coalition turnover and infanticide, both within and outside of protected areas (Loveridge et al. 2007,2010; Packer et al. 2009, 2011). In order to mitigate the population level impacts of lion trophy hunting, I suggested that a combination of reduced quotas, enforced age limits, and future recovery periods for the South Luangwa lion population be incorporated into long-term lion management plans for what is thought to be Zambia's largest lion population.

With the ongoing debate regarding the role and presence of trophy hunting, intensive studies like the one I presented in Chapter Two must be continued to validate the efficacy of management actions. Recovery periods (including the current moratorium ending in 2016) and major changes in lion harvest management (changes in quotas and age-limits) provide discrete study periods for assessing impacts on the South Luangwa lion population, and thus feedback into future adaptive management actions. Although we did not detect any impacts of illegal bushmeat harvest on the South Luangwa lion population, the likely depletion of herbivore populations in the GMAs will be a long-term issue for dependent large carnivore populations (Lewis & Phiri 1998; Becker et al. 2013; Lindsey et al. 2013; Watson et al. 2013). This issue requires more attention in order to understand the dynamics of herbivore depletion in the face of rapid human encroachment in this and other Zambian protection gradients (Watson et al. 2013; Watson et al. 2014; Lindsey et al. 2014).

In Chapter Three, I presented data from a three-year camera trap study of the South Luangwa leopard population primarily during the temporary trophy hunting

moratorium. These data indicated lowered leopard density in areas of lower protection, but did not detect differences in survival across the two study areas. The most logical explanation for detectable differences in density but not survival is the depletion of herbivore populations in this protection gradient, most likely driven by bushmeat poaching. There could be differences in survival in these two study areas due to lingering effects of previous trophy harvests or leopard mortality due to wire snaring, but this study provided no evidence of any difference in survival across this protection gradient. The depletion of herbivore populations remains a primary concern for leopard and other large carnivore populations in the area and warrants further attention.

A novel aspect of the study design presented in Chapter Three was the systematic placement of camera traps in two study areas of similar sizes. This design differs from previous leopard camera trap studies that aim to maximize detection probability either by placing camera traps where leopards are seen frequently (Balme, Hunter & Slotow 2009), or by using lures (Du Preez, Loveridge & Macdonald 2014). Our design allows for unbiased comparison of densities and survival rates between study areas, and thus can be used for long-term monitoring of leopard populations. We were not able to assign age classes for leopards photographed by the camera traps (Balme, Hunter & Braczkowski 2012), and this likely resulted in the low precision around our survival estimates for the South Luangwa leopard population. Therefore, this study design could be improved with advances in leopard aging methods. Additionally, an increase in the number of leopards sampled would help overall with the precision of survival, and thus increasing the size of the study area would help increase the number of leopards monitored.

Finally, in Chapter Four, I presented a novel pedigree reconstruction approach for rapid abundance estimates for unstudied large carnivore populations, and validated this approach with a simulated population based on the South Luangwa lion population. This new population estimate is unbiased and precise if enough individuals are sampled over a short enough period (at least 50% of the population within 2 years), and sampling methods allow juveniles to be included. If sampling takes place over multiple years and includes juveniles, individuals will be inferred that no longer exist in the population and thus population abundance will be overestimated. Approaches to mitigate this overestimation are discussed in Chapter Four. With the support of this simulation-based study, this approach should be applied to a well-studied population of known individuals to validate its accuracy. If this approach is validated with a real population, this method could be an option for rapidly estimating unbiased population size for unstudied carnivore populations facing anthropogenic pressures and regulated harvests.

In conclusion, as large carnivore populations are under threat worldwide, precise estimates of population size, trend, demography, and vital rates are critical in detecting population declines, determining their causes, and informing management action. Given the dynamic nature of anthropogenic pressures that are increasing adjacent to protected areas, intensive monitoring of key large carnivore populations remains currently the only approach for gathering the necessary data to evaluate and inform critical management decisions. However, the high cost and duration of intensive monitoring prevent it from being used to study all large carnivore populations. Therefore, combining rapid surveys with intensive monitoring for regional carnivore monitoring and management remain a

high priority for large carnivore conservation facing population declines and threats that are only intensifying.

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APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER THREE

Table A1: The 32 candidate robust design models from the 72 possible candidate models that were not overparameterized. In addition to the three best parameterizations of S , these models included non-existent ($\gamma''(0)$, $\gamma'(1)$), Markovian ($\gamma''(\cdot)$, $\gamma'(\cdot)$) or random ($\gamma''(\cdot)=\gamma'$) temporary emigration and p and c to be equal and constant ($p(\cdot)$), unequal and constant ($p(\cdot)$, $c(\cdot)$), equal and differing by area ($p(\text{area})$), unequal and differing by area ($p(\text{area})$, $c(\text{area})$), or equal and differing by season ($p(\text{season})$).

Model	Parameters	Delta QAICc	QAICc weight
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $N(\text{season+area})$	8	0	0.19
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $N(\text{season+area})$	9	0.93	0.12
$S(\text{sex})$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $N(\text{season+area})$	9	1.61	0.08
$S(\cdot)$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $N(\text{season+area})$	9	1.93	0.07
$S(\text{area})$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $N(\text{season+area})$	9	2.37	0.06
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	9	2.42	0.06
$S(\text{sex})$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $N(\text{season+area})$	10	2.71	0.05
$S(\cdot)$, $\gamma''(\cdot)=\gamma'$, $p(\text{area})$, $N(\text{season+area})$	10	3.02	0.04
$S(\text{area})$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $N(\text{season+area})$	10	3.19	0.04
$S(\cdot)$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	10	3.33	0.03
$S(\text{sex})$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $N(\text{season+area})$	10	3.6	0.03
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{season})$, $N(\text{season+area})$	12	4.05	0.02
$S(\text{sex})$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	10	4.09	0.02
$S(\cdot)$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\cdot)$, $N(\text{season+area})$	10	4.23	0.02
$S(\text{area})$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $N(\text{season+area})$	10	4.39	0.02
$S(\text{area})$, $\gamma''(0)$, $\gamma'(1)$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	10	4.84	0.02
$S(\text{sex})$, $\gamma''(\cdot)=\gamma'$, $p(\text{area})$, $N(\text{season+area})$	11	4.85	0.02
$S(\text{sex})$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	11	5.06	0.01
$S(\cdot)$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $c(\text{area})$, $N(\text{season+area})$	11	5.28	0.01
$S(\cdot)$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\text{area})$, $N(\text{season+area})$	11	5.5	0.01
$S(\cdot)$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	11	5.55	0.01
$S(\text{area})$, $\gamma''(\cdot)=\gamma'$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	11	5.85	0.01
$S(\text{sex})$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $N(\text{season+area})$	13	5.88	0.01
$S(\text{sex})$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\cdot)$, $N(\text{season+area})$	11	5.93	0.01
$S(\text{area})$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{season})$, $N(\text{season+area})$	13	6.69	0.01
$S(\text{area})$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\cdot)$, $N(\text{season+area})$	11	6.77	0.01
$S(\text{sex})$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $c(\text{area})$, $N(\text{season+area})$	12	7.11	0.01
$S(\text{sex})$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\cdot)$, $c(\cdot)$, $N(\text{season+area})$	12	7.29	0
$S(\text{sex})$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\text{area})$, $N(\text{season+area})$	12	7.37	0
$S(\text{area})$, $\gamma''(0)$, $\gamma'(1)$, $p(\text{area})$, $c(\text{area})$, $N(\text{season+area})$	12	7.87	0
$S(\text{area})$, $\gamma''(\cdot)$, $\gamma'(\cdot)$, $p(\cdot)$, $N(\text{season+area})$	12	8.14	0
$S(\text{sex})$, $\gamma''(\cdot)=\gamma'$, $p(\text{area})$, $c(\text{area})$, $N(\text{season+area})$	13	8.38	0

Table A2: Model-averaged estimates of seasonal and overall average population size (\hat{N}) and density (leopards per 100 km²) calculated using mean maximum distance moved (MMDM). Using a MMDM buffer, the effectively sampled areas were estimated as 381.64 km² and 358.58 km² for the WSA and ESA, respectively. As discussed in the text, the proportional difference between average WSA and ESA density estimates remains the same regardless of whether HMMDM or MMDM is used to calculate effectively sampled areas (Table 3.4).

Season	Number Captured	\hat{N}	SE	95% LCL-UCL	Density	95% LCL-UCL Density
CD 2013, ESA	8	12.53	5.35	8.45-53.85	3.49	2.36-15.02
HD 2013, ESA	5	8.67	4.37	5.36-42.72	2.42	1.49-11.91
CD 2014, ESA	5	8.67	4.37	5.36-42.72	2.42	1.49-11.91
HD 2014, ESA	7	12.11	5.78	7.56-53.91	3.38	2.11-15.03
CD 2012, WSA	16	22.29	5.91	17.00-55.65	5.84	4.45-14.58
CD 2013, WSA	10	18.21	5.88	12.02-43.39	4.77	3.15-11.37
HD 2013, WSA	9	15.71	5.2	10.47-39.66	4.12	2.74-10.39
CD 2014, WSA	9	15.71	5.2	10.47-39.66	4.12	2.74-10.39
HD 2014, WSA	12	21.35	6.44	14.42-48.04	5.59	3.78-12.59
Average, ESA	-	10.5	1.33	7.90-13.10	2.93	2.20-3.65
Average, WSA	-	18.66	1.1	16.50-20.81	4.89	4.32-5.45