

Intraspecific and interspecific olfactory interactions and
density of leopards *Panthera pardus* (Linnaeus, 1758) in Sabi
Sands Game Reserve, South Africa

by

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A thesis submitted in fulfilment of the requirements for the degree

Master of Science (Wildlife Management)

In the Faculty of Natural & Agricultural Sciences,

University of Pretoria

Pretoria

(December 2021)

Supervisor: Prof. M. J. Somers

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Declaration

I, Agathe Gervais declare that the thesis, which I hereby submit for the degree MSc. Wildlife Management at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:

Date: November 19th 2021

A handwritten signature in blue ink, appearing to be 'Agathe Gervais', enclosed within a faint rectangular border.

Agathe Gervais

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Summary

The olfactory behavioural ecology of large solitary carnivores is still poorly understood. Meanwhile, these species represent challenging cases in conservation and management. They are frequently involved in conflicts with farmers, depredating livestock and being killed in retaliation. Low densities and large territories also make it hard to assess their population status accurately. This thesis aimed to improve our understanding of the African leopard (*Panthera pardus*) olfactory ecology and investigate whether this knowledge could help mitigate farmer-leopard conflicts or facilitate the management of the species.

Chapter 1 described the critical role of olfactory communication in animals, large carnivores, and leopards. I reviewed the evidence for the use of intraspecific and interspecific carnivore scents as deterrent strategies and movement-manipulating tools in wildlife

management. Peer-reviewed evidence is limited, and conclusions vary between studies. Manipulative experiments observed target carnivores being either repelled or attracted to the scent-marked area. I concluded that, if any, the deterrence potential of scent marks against leopards likely relied on its two strongest sympatric competitors' scents, lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*).

Chapter 2 experimentally characterized leopard intra and interspecific olfactory interactions at marking sites and assessed the effects of lions' and spotted hyenas' scents on leopards. Camera traps monitored leopard scent-marking sites in the western sector of the Sabi Sands Game Reserve. Lion and spotted hyena activity at marking sites was simulated with the addition of their scats. If successful in deterring leopards from an area, these scents could further be synthesized and used around farms to prevent conflicts. Results supported territory advertising and reproductive functions of leopard scent marking and revealed a higher marking frequency in females than previously reported. Frequent olfactory behaviours included spraying, rubbing, and investigating scents. Generalized linear mixed effect models revealed no deterrent effects of lion and spotted hyena scents on leopards. Additionally, leopards spent significantly more time marking and investigating sites where lion scats were present. Thus, olfactory cues of guild members did not spatially displace or affect leopards over time. Instead, leopards used scent-marking sites to gather information about conspecifics and heterospecifics and to advertise their presence reciprocally. This behavioural strategy does not enable the use of intraguild scent interactions to manipulate the movements of leopards.

Chapter 3 estimated leopard density and assessed the impact of intraguild interactions on the estimate precision. I used data from the previous scent survey and implemented a control survey with 15 paired camera traps at road junctions along a 5-km² grid. Spatially-Explicit-Capture-Recapture analyses under both Bayesian and maximum likelihood frameworks estimated leopard densities. As expected, the accuracy and precision of estimates were

relatively close between surveys. Neither the translocated scats or habitat types covariates impacted density results. However, the scent survey better described the sexually mature leopard population, whereas the control survey sampled individuals representative of the entire population structure. Cost-effectively and parameter-wise results provided more support for the control survey and Bayesian approach. Thereby, the estimated leopard density in the study area was 12.81 individuals (± 0.07)/100 km².

This type of small scale *in situ* experimental study helps field biologists to make optimal decisions and better defines the range of management actions available to conservation.

Key words: Camera trapping, conflict mitigation, landscape of fear, manipulative experiments, olfactory behaviour, SECR modelling, species interaction.

Acknowledgements

I would like to thank all the people who supported and helped me with my thesis. I have gained invaluable experience and have been truly amazed by the kindness of those I met and sought help from.

A special thanks to my supervisors Prof. Michael Somers and Dr. Alexander Braczkowski, for your precious insights and advice in shaping and achieving this project. Michael, I am extremely grateful for the support, guidance, trust and academic freedom you gave me throughout this year. Alex, I am particularly grateful for your meticulous and constructive feedback and for being so patient and resourceful with my analyses.

Thank you to the overwhelming generosity of the Dulini team, who welcomed me so warmly and enabled me to carry out my fieldwork in such amazing conditions. I enjoyed every minute of my time amongst you. In particular, I thank Iain Garratt for so kindly opening the doors of the Dulini property to me, supporting my project and encouraging and coordinating with the other lodges to give me access to the entire western sector for this research. Heartfelt thanks to the Dulini rangers and trackers too. You have patiently shared your knowledge of the bush with me and provided invaluable help during my entire fieldwork. Thank you for your everyday enthusiasm, for getting organised to count me in in your schedule, taking me on game drives or on long days to check camera traps, letting me know where the best lions' and hyenas' scats were, and for accompanying me to pick them up.

I am also grateful to all the other lodges of the western sector, Savana, Leopard Hills, Idube, Inyati, and Ulusaba, for allowing my research on your properties. Your cooperation with the Dulini teams to show me the best camera trapping sites and leopard spots on your land, let me know when camera traps were knocked over, or insights into where lions' and hyenas' scats were, was very precious to me.

I also thank the University of Pretoria for all the camera trapping equipment without which this research would not have been possible, and for awarding me the UP Postgraduate Masters Research Bursary 2021 for this research.

Finally, my deepest thanks to my parents and siblings for their support and, above all, for always standing behind me and having faith in me and my projects.

Ethical considerations

The research has been conducted in agreement with ecologists from the Sabi Sands Wildtuin (SSW) and managers of the western sector of the Sabi Sands Game Reserve. It has also received approval from the Animal Ethics Committee of the University of Pretoria (NAS007/2021) and a provincial permit (MPB. 5685) from the Mpumalanga Tourism and Park Agency (MTPA).

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CHAPTER 1

Scent marking as an olfactory communication strategy in large carnivores: a focus on the African leopard

Abstract

Scent marking is a common communication strategy across the animal kingdom and particularly in the order Carnivora. It facilitates mating interactions, advertises territories, and facilitates encounters or avoidance of conspecifics depending on the situation. Multiple studies also suggest an important role of olfactory communication in interspecific interactions, particularly for solitary carnivores. A review of carnivore studies emphasized that species exposed to scents of conspecific or sympatric competitors either exhibit avoidance or attraction for scent-marked areas. This observation is a key argument for the potential use of scent marking in wildlife management and human-carnivore conflict mitigation. I showed that few human-wildlife conflict studies have investigated an olfactory mitigation approach. I further detailed mechanisms and principles of olfactory interactions in carnivores, focusing on potential applications for wildlife management and conflict mitigation. Finally, as a model species, I used the African leopard (*Panthera pardus*), a cryptic solitary carnivore frequently involved in conflicts with farmers. I demonstrated the importance of olfactory interactions in the ecology of the species and described hypotheses relative to their potential applications in wildlife management and conflict mitigation.

Key words: Carnivore distribution, conflict mitigation, dear enemy effect, landscape of fear, wildlife management tools.

I – The importance of scent marking as a form of animal communication and as a tool for wildlife management

Olfactory communication is a core component of communication across most animal taxa on earth (Hoover 2010, Apps 2013). It facilitates the transmission of information between animals through chemical signals (Eisenberg and Kleiman 1972), and is thought to be the most widespread communication means in the *Animalia*, often surpassing visual or physical cues in terms of its importance (Mykytowycz and Goodrich 1974, Kitchener et al. 2010, Ausband et al. 2013). Examples of animal communication include pheromones of insects and fish (Silva and Antunes 2017), piles of herbivore dung (Marneweck et al. 2018), reptile urine (Silva and Antunes 2017), and oily deposits like those found in preorbital glands of antelopes (Burger et al. 1997) or sebaceous glands of carnivore body parts (e.g., interdigital, salivary, cheek, and anal glands) (Eisenberg and Kleiman 1972). Oily deposits are secreted during tree scraping, licking, rubbing, rolling or brushing against various substrates (Eisenberg and Kleiman 1972, Silva and Antunes 2017). This variety of scent marking serves many different functions such as territory demarcation, mate selection, raising of young, group bonding, herding, spatial orientation, and defensive behaviour (Cheal and Sprott 1971, Eisenberg and Kleiman 1972). For instance, concentrations of (*Z*)-7-dodecanyl acetate increase in the urine of female Asian elephants (*Elephas maximus*) when approaching ovulation (Rasmussen 2001). Male elephants investigate urine through flehmen behaviour, and analyse urine chemical composition with their vomeronasal organ (Jacobson's organ) to determine females' readiness to mate (Rasmussen 2001). Male elephants also exude frontalin pheromone during musth (Rasmussen and Greenwood 2003). This repulses younger immature males but increases female receptivity to breeding (Rasmussen and Greenwood 2003). Another compound, 4-methylphenol, serves in the mating interactions of three herbivores (horses—*Equus caballus*, water buffalo—*Bubalus bubalis*, moose—*Alces alces*) and several insects (*Stomoxys calcitrans*, *Glossina* spp) (Soso

and Koziel 2017). Contrastingly, 2-methylpropyl acetate and seven other compounds are found in the preorbital glands of klipspringers (*Oretragus oretragus*), which are deposited when they rub their face against a substrate to demarcate territory (Burger et al. 1997). Pronghorns (*Antilocapra americana*) release 2-pyrrolidinone from scent glands on their rumps when alarmed (Wood 2001); house mice (*Mus musculus*) stand still in response to detecting a compound of wolf (*Canis lupus*) urine (2,5-dimethylpyrazine) (Osada et al. 2013); and *Locusta migratoria manilensis* aggregate (defensive behaviour) in response to the same 2,5-dimethylpyrazine compound (Shi et al. 2011). In summary, olfactory communication takes many forms, serves diverse purposes, either in a species-specific way or commonly to different phylogenetic groups, and elicits behavioural and physiological responses. Additionally, it may be a one-way only form of communication, or allows for two-way interactions if receivers scent mark in response to detected scents (Reiger 1979, Apps et al. 2019, Rafiq et al. 2020).

The effectiveness of scent marking as an olfactory communication strategy relies on both the persistence and detectability of olfactory cues (Johnson 1973, Allen et al. 2017a). Persistence refers to the time period during which scent information is available for a receiver to find (Wemmer and Scow 1977, Rafiq et al. 2020). Atmospheric phenomena (e.g., rainfall, snow, and intense heat) and the passage of other animals may enhance the degradation of a scent mark and reduce its persistence (Wemmer and Scow 1977). Detectability determines the receiver's ease of finding the information (Macdonald 1980), and may be enhanced by the selection of specific sites frequently visited or susceptible to being visited by the receiver (Allen et al. 2017a). Ideally, senders should maximize detectability and persistence, leave multiple messages to increase the receiver's encounter probability, and check sites often to renew cues (Allen et al. 2017a, Rafiq et al. 2020). However, achieving such maximal communication efficiency is energetically costly and has evolutionary consequences (Allen et al. 2017a, Rafiq et al. 2020). In the process, scent-marking individuals may advertise their presence to

eavesdroppers like prey and competitors. This advertising potentially increases prey vigilance, reduces hunting success, or intensifies direct competition (kleptoparasitism), as well as risks of hostile encounters with both conspecifics or heterospecifics (Rafiq et al. 2020). These factors collectively impact the survivorship of both scent-marking individuals and individuals interacting within the same community and shape their respective behavioural strategies over time.

The role of olfactory interactions as a tool for conflict mitigation between animals and humans has been largely unexplored compared to other methods, which provides motivation for this thesis. Rates of animal habitat loss have increased over the past three decades, and this has placed human communities at increased risk of conflict with many animal species (Woodroffe et al. 2005, Potapov et al. 2017, Jones et al. 2018). Most conflicts resolve around food resources or involve carnivores. Science on solving these conflicts has also increased and adopted multiple approaches (Smith et al. 2000, Miller et al. 2016), but few investigated olfactory solutions. Frequently successful mitigation examples include fortified bomas (Lichtenfeld et al. 2015), livestock guarding Anatolian dogs to deter carnivores in Africa (Leijenaar et al. 2015), fences of beehives and playback of carnivore growls (leopard–*Panthera pardus*, and tiger–*Panthera tigris*) to deter elephants (*Loxodonta africana*, *Elephas maximus*) from raiding crops in Asia and Africa (King et al. 2009, Thuppil and Coss 2013), livestock husbandry practices adapted in space and time to the surrounding carnivore community in Africa (Ogada et al. 2003, Woodroffe et al. 2006, Melzheimer et al. 2020), visual deterrents against carnivores like fladries in North America (Musiani et al. 2003), and compensation schemes for farmers losing livestock to carnivores (Bauer et al. 2017). In comparison, only a handful of studies investigated the potential of olfactory communication as a tool of wildlife management (Table 1.1). First, a study at the Nordens Ark Zoo, Sweden, investigated solutions to wolverine (*Gulo gulo*)/farmer conflicts over livestock and tested the feeding reaction of four

captive wolverines to various olfactory compounds (Landa and Tømmerås 1997). Anal secretions of a male brown bear (*Ursus arctos*) and urine of a male wolf (*Canis lupus*) did not affect captive wolverines (Landa and Tømmerås 1997). Anal secretions of wolves increased alertness in captive wolverines but did not deter them from eating the given meat (Landa and Tømmerås 1997). On the contrary, anal secretions of foreign wolverines had a significant aversive effect and successfully deterred wolverines from eating the meat (Landa and Tømmerås 1997). Secondly, two experiments respectively tested the aversive effect of foreign coyote (*Canis latrans*) urine on coyotes of a sanctuary in the USA (Shivik et al. 2011) and synthesized red fox (*Vulpes vulpes*) urine on urban red foxes in the UK (Arnold et al. 2011). Experiments were unsuccessful in that coyotes and male red foxes spent significantly more time in artificially scent-marked areas (Shivik et al. 2011, Arnold et al. 2011). Next, in Botswana, the urine of a foreign pack of wild dogs (*Lycaon pictus*) has been used to manipulate the movements of a local pack and keep it within the Northern Tuli Game Reserve (Jackson et al. 2012). Wild dogs were monitored with GPS collars. They moved away from added scents each time, and the procedure successfully redirected them into the reserve when they established a den outside (Jackson et al. 2012). A similar experiment on gray wolves in the USA used urine of a foreign pack to form a biofence over two consecutive summers and prevent a local pack from preying upon sheep in a close-by field (Ausband et al. 2013). The pack has been known to kill sheep over the four years preceding the experiment (Ausband et al. 2013). During the deployments of the biofence, wolves did not kill any sheep from the targeted field. However, inconsistent refreshing of the biofence's scent may have resulted in trespassing events (Ausband et al. 2013). Two further studies also respectively investigated the *in situ* behavioural response of foxes to scents of cats (*Felis catus*) and dogs (*Canis lupus domesticus*), sympatric competitors in mallee wheatlands of Australia (Banks et al. 2016), and the behavioural response of captured wild stoats (*Mustela erminea*) to scents of ferrets (*Mustela furo*) and cats, sympatric

apex predators in New Zealand, and wild dogs (*Lycaon pictus*), a novel predator (Garvey et al. 2016). Foxes and stoats both spent significantly more time investigating the scents of sympatric heterospecifics than control, conspecifics or novel scents. Both studies concluded that olfactory communication serves as an antipredator adaptation that significantly reduces the cost of unnecessary flights, and decreases both time of species identification and time of adequate response when encountering heterospecifics (Banks et al. 2016, Garvey et al. 2016). However, these experiments failed at providing solutions to human-wildlife conflicts. Next, an experimental field study investigated scent-marking interactions of cheetahs (*Acinonyx jubatus*) with other members of the large carnivore guild in the Hluhluwe-iMfolozi Park and Phinda Private Game Reserve, South Africa (Cornhill and Kerley 2020). Cheetah marking sites serve as communication hubs to the species and inform conspecifics of territory ownership or oestrus status in females (Cornhill and Kerley 2020). Other large carnivores visit these sites and sometimes scent mark (Cornhill and Kerley 2020). Visits of leopards and lions (*Panthera leo*) and experimentally placed fresh faeces of lions at cheetah marking sites induced a temporary change in the spatial occupancy of cheetahs and delayed oestrus signalling in females (Cornhill and Kerley 2020). Additionally, identifying cheetah communication hubs across ~ 1 000 farms in central Namibia and advising farmers to avoid leaving herds with young calves (most likely prey for cheetahs) in these areas reduced livestock loss to cheetahs by 86% (Melzheimer et al. 2020). Lastly, experiments were conducted on five semi-tamed African elephants in the Adventures with Elephants facility, Limpopo province, South Africa (Valenta et al. 2020). The study synthesized the scent of lion scats, implemented a biofence, and compared the time response and treats (oranges) required for elephants to cross the biofence to a control situation. Despite a small sample size, the study is considered successful with only one individual that crossed the biofence (after multiple treats and significantly more time than during control), three elephants did not cross the biofence, and one individual stopped participating in the trials

after the first exposure to lion scent (Valenta et al. 2020). The limited number of studies investigating scent interactions for conflict mitigation, the lack of consistency across methods, and the lack of replicate studies stand out from this short literature review. However, the few successful experiments suggest that scent marking has the potential in wildlife management to manipulate the movement of species and mitigate conflicts with humans (Table 1.1). Garnering knowledge on wildlife interactions also helps to understand how conflicts with humans arise (e.g., related to behaviours or space use), identify a few target species with significant potential to impact their community, and allows to more confidently evaluate areas of focus for conservation efforts (Campbell-Palmer and Rosell 2011, Krebs et al. 2012, Jones et al. 2016). In summary, studying animals that primarily rely on olfactory communication could reveal significant relationships between community members. These olfactory interactions could be used as a strategy in reducing conflict with humans and assets they value (e.g., crops, livestock).

II – Scent marking in large carnivores

Scent marking and olfactory communication are critical for large carnivores, particularly solitary species (Rafiq et al. 2020), preceding auditory cues that can often only be heard for a few kilometres (Wemmer and Scow 1977). Scent marking is the primary form of mate selection and territory demarcation in large carnivores (Apps 2013, Allen et al. 2016a). It aids in detecting close conspecifics before making visual contact. Thereby, olfactory communication facilitates desirable encounters with conspecifics and reduces unwanted ones that can be highly antagonistic and result in lethal injuries given carnivores' defensive physical attributes (e.g., claws, teeth, and musculature) (Wemmer and Scow 1977, Macdonald 1980, Palomares and Caro 1999, Allen et al. 2016a, Cornhill and Kerley 2020).

Table 1.1 Experimental studies manipulating mammalian olfactory interactions for wildlife management purposes.

Study species	Stakes	Compounds	Interaction level	Conclusion	Site/Country	Authors & Year
Red foxes (<i>Vulpes vulpes</i>)	Mitigate conflicts with livestock farmers (poultry) and in urban areas (garbage) by deterring red foxes from specific human infrastructures	Synthesised urine of red fox	Intraspecific	Failed: males significantly increased their time spent in the artificially scent-marked areas.	Urban area in northwest Bristol, UK (GPS collared red foxes)	Arnold et al. 2011
Coyotes (<i>Canis latrans</i>)	Mitigate conflicts with livestock farmers by keeping coyotes away from livestock	Urine of foreign coyotes	Intraspecific	Failed: coyotes spent more time in areas artificially scent marked.	Welder Wildlife Refuge in southern Texas, USA (wild coyotes equipped with GPS collars) and Predator Research Facility, Utah, USA (captive coyotes)	Shivik et al. 2011
Wild dogs (<i>Lycaon pictus</i>)	Mitigate conflicts with livestock farmers and conserve wild dogs, a critically endangered species, by keeping them inside protected areas where they cannot be shot	Urine and faeces of a foreign wild dog pack to the resident one	Intraspecific	Successful: after being exposed to translocated foreign pack scent marks, the resident pack moved away in opposite direction. It abandoned the den site outside of the reserve two days before the females gave birth, and moved back inside the reserve.	Northern Tuli Game Reserve, Botswana (GPS collared wild dogs)	Jackson et al. 2012
Gray wolves (<i>Canis lupus</i>)	Mitigate conflicts with livestock farmers by keeping gray wolves away from livestock and conserve gray wolves, a keystone species in its ecosystem	Urine and faeces of foreign wolf packs to the three resident ones	Intraspecific	Successful: during the two consecutive summers with active biofence, attacks on sheep in the targeted field stopped. Inconsistencies in scent refreshing procedures (origin of the compounds, frequency of renewal, etc) did not fully deter wolves from intruding in the biofenced area but over short distances and short time periods only.	Mountainous forest near Garden Valley, Idaho, USA	Ausband et al. 2013

Study species	Stakes	Compounds	Interaction level	Conclusion	Site/Country	Authors & Year
Wolverines (<i>Gulo gulo</i>)	Mitigate conflicts with livestock farmers by deterring wolverines from depredating livestock, and conserve wolverines, an apex predator in its ecosystem	Urine and anal secretions of wolf, anal secretions of brown bear, anal secretions of foreign wolverine	Intraspecific & Interspecific	Successful: scents of foreign wolverine had significant effects on captive wolverines and even deterred some individuals from eating the meat. Failed: scents of wolf and brown bear did not deter wolverine from eating the meat.	Nordens Ark Zoo, Sweden (captive wolverines)	Landa et al. 1997
Wild stoats (<i>Mustela erminea</i>)	Gain understanding on interspecific interactions between sympatric carnivore species	Scents of domestic cats, ferrets, African wild dogs	Interspecific	Failed: stoats were significantly attracted to scents of sympatric cats and ferrets, they did not significantly react to scents of wild dogs.	Landcare Research, Lincoln, New Zealand	Banks et al. 2016
Red foxes (<i>Vulpes vulpes</i>)	Mitigate conflicts with livestock farmers (poultry) and in urban areas (garbage) by deterring red foxes from specific human infrastructures	Urine of domestic cats, domestic dogs, foreign red foxes	Intraspecific & Interspecific	Failed: foxes investigated all scents and particularly those of cats and dogs, and did not significantly react to foreign fox urine. Foxes were significantly attracted to heterospecific scents rather than repelled by any of the scents.	Mallee wheatland, Victoria, Australia	Garvey et al. 2016
Cheetahs (<i>Acinonyx jubatus</i>)	Mitigate conflicts with livestock farmers by adapting their practices to the ecology of cheetahs, and conserve cheetahs, a species classified vulnerable	Faeces of lions and leopards	Interspecific	Successful: scents of lion and leopard temporarily physically displaced cheetahs from marking sites (longer visit interval).	Hluhluwe-iMfolozi Park and Phinda Game Reserve, KwaZulu-Natal, South Africa	Cornhill and Kerley 2020
Elephants (<i>Loxodonta africana</i>)	Mitigate conflicts with crop farmers by deterring elephants from foraging in crop fields, and conserve elephants, an endangered species	Faeces of lion, faeces of cheetah, and synthesised scent of lion faeces (phenol and indole)	Interspecific	Successful: elephants took significantly longer and more treats to cross the biofence of cheetah faeces than control biofence. Elephants did not cross biofences of lion faeces or synthesized lion faeces scent.	Adventure with elephant facility Limpopo, South Africa (semi-tamed elephants)	Valenta et al. 2020

For example, during a field experiment in the greater Moremi area, Botswana, 52 wild lion individuals from three male coalitions and four female prides were presented urine scent marks from resident or non-resident male or female lions on 68 occasions (Gilfillan et al. 2017). On 69.2% of the trials, lions reacted to the translocated scents (counter-marked, investigated, flehmened). Females responded significantly more to non-resident female and resident male urines than to resident female urine, whereas males responded similarly to resident and non-resident female urine (Gilfillan et al. 2017). Since males and non-resident females represent greater risks to resident lionesses (kleptoparasitism, infanticides) and that resident male lions mate opportunistically with both resident and non-resident females (Gilfillan et al. 2017), these experimental results support the use of olfactory cues by lions to anticipate direct interactions. Similarly, free-ranging cheetahs dispersed on about 1 000 private farms in central Namibia use communication hubs (areas of intense scent-marking activity for neighbouring individuals) to navigate territories and limit conflicts (Melzheimer et al. 2020). Dominant males (n=67) spent 50% of their time patrolling and marking these areas to keep foreigners and nomadic individuals (n=25) away (Melzheimer et al. 2020). Females (n=10) raising cubs avoided these hubs to minimise risks of infanticide (Melzheimer et al. 2020). Thus, in both case studies, carnivore scent marks informed foreigners on territory ownership and provided an early warning of an individual's presence in an area, increasing time for reaction and minimising costly conflicts.

Carnivore olfactory communication may also facilitate friendly intraspecific interactions such as finding mates or group members (Apps 2013, Allen et al. 2016a). For example, 12 cheetah communication hubs were monitored over two years in Phinda Private Game Reserve (n=4) and Hluhluwe-iMfolozi Park (n=8), KwaZulu-Natal province, South Africa (Cornhill and Kerley 2020). Female cheetahs in oestrus visited communication hubs significantly more times (n=44) than non-oestrus females (n=2), and scent marked to indicate their readiness to mate to males (Cornhill and Kerley 2020). Upon detecting females in oestrus,

male tigers (n=3) of the Royal Chitwan National Park, Nepal, also increased their scent-marking frequency by four to advertise their presence and facilitate encounters with females (Smith and al. 1989). Finally, in group or pair-living carnivores, for example, grey wolves, lions, wild dogs, spotted hyenas (*Crocuta crocuta*), or red foxes, olfactory communication assists in social organisation and the identification of individuals, group membership, and hierarchy (Pageat and Gaultier 2003). For example, grey wolves in a pack perform squat urination accompanied by friendly behaviours and urinate at the same spot as alpha individuals (Macdonald 1980); lions in a pride rub against each other and lick each other (Matoba et al. 2013); wild dogs of a pack often roll in each other's urine, overmark and investigate one another (Parker 2010); spotted hyenas of a clan deposit faeces in the same latrines (Macdonald 1980). The creation of a group odour is essential to the sociality of group-living carnivores (Macdonald 1980, Marneweck et al. 2020). This knowledge has been key to reintroduction programs releasing together individuals from different groups (e.g., wild dogs): scents of the different individuals must be mixed together by rubbing individuals against each other to create a new group odour (Marneweck et al. 2020).

In summary, all species must retain a minimum level of sociality to successfully reproduce, disperse, and sustain genetically viable populations (Uphyrkina et al. 2001, Graw et al. 2019). In group-living carnivores where frequent direct interactions occur, olfactory communication is an important component of sociality, but only part of a multimodal communication that involves arguably equally importantly auditory, physical, and visual communication (e.g., body posture, tail flicking, playing, licking, fur, growling) on a daily basis (Higham and Hebets 2013). In solitary carnivores, scent marking constitutes the core component of communication. It overcomes three challenges: advertise territories to prevent conflicts, keep the reproductive status of surrounding potential mates in check, and find them when females are in oestrus (Wemmer and Scow 1977, Allen et al. 2016b). Therefore, scent

marking is essential for large solitary carnivores that typically occur at low densities and feature home ranges and territories of hundreds or thousands of square kilometres (Wemmer and Scow 1977, Bothma and Coertze 2004, Allen et al. 2016a, Rafiq et al. 2020).

Few studies have documented carnivore interspecific olfactory interactions as the primary focus of their work (Table 1.1), which provides additional motivation to this thesis. The main evidence for the existence of these interactions is that olfactory compounds of a species can elicit specific responses in other species (Soso and Koziel 2017). For instance, a field study in the greater Moremi ecosystem of Botswana recorded strong behavioural responses and frequent countermarking activity by small-spotted genets (*Genetta genetta*), slender mongooses (*Galerella sanguinea*), and African civets (*Civettictis civetta*) to artificial tomcat thiol (a natural compound of leopard urine) dispensers (Apps et al. 2017). Moreover, carnivores involved in these interactions tend to be solitary species which correlates the “dear enemy” principle. It suggests that solitary carnivores are more likely to encounter heterospecifics than conspecifics (Banks et al. 2016, Garvey et al. 2016). Keeping track of the heterospecifics and reciprocally signalling its own presence may be critical to minimise interspecific competition and mediate interspecific killing (Banks et al. 2016, Garvey et al. 2016). Therefore, interspecific olfactory interactions are important for solitary carnivores (Apps et al. 2017, Rafiq et al. 2020). Additionally, in an environment where risks with heterospecifics are permanent, carnivores cannot habituate to the surrounding scent-marking compounds (Valenta et al. 2020). Adequately manipulating these compounds could be a critical avenue of research for conservation and conflict management (Campbell-Palmer and Rosell 2011).

Understanding the underlying mechanisms of carnivore olfactory interactions is essential prior to exploiting scent marking in wildlife management. The chemical transmission of precise information is facilitated by the diversity of odorous compounds (Volatile Organic Compounds—VOC) produced by a species (Mykytowycz and Goodrich 1974, Reiger 1979,

Soso and Koziel 2017), and the great number of scent glands on different carnivore body parts (Reiger 1979, Kitchener et al. 2010). Faeces and urine are common scent marks across carnivores and are often accompanied of anal-sac secretions (Macdonald 1980, Asa 1993). Many species also possess scent glands on their back (caudal), under their feet (pedal), between digits (interdigital), and in anal sacs or around the genital area (Reiger 1979), and can exude multiple VOC. About 55 VOC have been reported in lion marking fluids (Andersen and Vulpius 1999), 27 in the urine of cheetahs (Burger et al. 2006), and 26 in brown bears' (*Urus arctos*) pedal scent (Sergiel et al. 2017). The composition of scent marks further seems to correlate the transmission of specific information. Six of the compounds found in brown bear pedal scent were only present in males, suggesting a sex specific function (Sergiel et al. 2017); amounts of 3-methylcyclopentanone fluctuate with mating season in European badgers (*Meles meles*), potentially helping mate selection (Soso and Koziel 2017). Some compounds are also species-specific, while others are common to multiple species, suggesting the importance of olfactory communication at both intraspecific and interspecific levels (Soso and Koziel 2017). For example, the tomcat thiol (3-mercapto-3-methylbutanol) is only found in the urine of domestic cats (*Felis silvestris catus*) and African leopards and is thought to function as a pheromone aiding mate selection (Apps et al. 2014). Lions share 3-methylcyclopentanone with cheetahs' urine (Soso and Koziel 2017), phenol and 4-methylphenol with Iberian wolves' (*Canis lupus signatus*) faeces (Barja and Lopez 2010), 2,5-dimethylpyrazine with both wolf urine and wild dog faeces, at least 11 other compounds with wild dog urine (Soso and Koziel 2017), and lipid-based anal-sac secretions with tigers (Asa 1993). Lipid-based secretions accompany urine sprays, delay the vanishing of volatile odorous compounds, and inform conspecifics of an individual's overall health, aiding mate selection (Asa 1993).

Additionally, carnivores combine chemical content and scent-marking strategies to improve message delivery. For instance, spotted hyenas use clan-specific latrines and anal

pastings (substrates fermented by symbiotic bacteria) to create strong odorous borders (Theis et al. 2013). Wolves also urinate and defecate at the same sites as alpha pack members while patrolling boundaries and depositing anal-sac secretions along with faeces (Macdonald 1980, Asa 1993). They preferentially select road junctions that foreign wolves are likely to follow when trespassing (Paquet 1990, Ausband et al. 2013). Polar bears (*Ursus maritimus*) mainly rely on interdigital and pedal glands to leave a scent trail and overcome the rarity of vertical substrate in their primary sea ice habitat (Owen et al. 2014). Finally, scent-marking behaviours are relatively consistent across the Felidae, and cheek rubbing, urinating, scraping, defecating, and tree scratching are common (Wemmer and Scow 1977, Allen et al. 2016b). Felids, however, adapt strategies to maximise communication efficiency in their habitat. In Canada, Eurasian lynx (*Lynx lynx*) urinate on young conifers and vertical mossy rocks that reach approximately the height of a lynx's head, and on main routes used by lynx while travelling, aiding the detection of urine in the snow by other lynx and maximising odorous persistence (Allen et al. 2017a). In high elevation environments where vegetation might be scarce, snow leopards (*Panthera uncia*) spray urine and rub sebaceous glands within their cheeks on rock hangovers that protect the marks from rain and wind (Wemmer and Scow 1977, Allen et al. 2017b). In the dense Neotropical Forest, both pumas (*Felis concolor*) and jaguars (*Panthera onca*) leave ubiquitous scrape marks on the ground, sometimes accompanied by faeces or urine in the centre (Harmsen et al. 2010). The purpose of tree scratching in felids is uncertain, specifically when felids engage in claw grooming and sharpening. However, pheromones exuding from felid interdigital glands have been given as one possibility (Wemmer and Scow 1977, Bothma and Coertze 2004). Thus, given the complexity of olfactory interactions, wildlife management applications of scent marking must consider the chemical content, detectability, and persistence of scents and the behavioural ecology of the targeted species.

III - The African leopard (*Panthera pardus pardus*) as a model for a scent-marking investigation and consequences for conflict mitigation

In this thesis, I use leopards and their interactions with lions and spotted hyenas as a model of scent communication. Leopards represent an ideal candidate species to study the importance of scent marking as a communication strategy and as a potential tool for human-carnivore conflict resolution for five key reasons: 1) they are solitary, cryptic, and territorial carnivores, and regularly occupy extensive home ranges in a diversity of habitats ranging from extremely arid areas like the Kalahari Desert (Bothma and Bothma 2012), to areas of high anthropogenic activities like in central Kenya (Van Cleave et al. 2018). Leopards can patrol territories spanning as small as 16.4 km² for males and 5.6 km² for females in the Kruger National Park (Friedmann and Traylor-Holzer 2008), a mesic area of rich prey abundance, up to 2 182 km² for males and 1 258.5 km² for females in the arid Kalahari Desert, where resource abundance is extremely low (Bothma and Bothma 2012). On average, individuals travel 10 to 30 km a day depending on weather and body condition (Bothma 1998). Thus, in low-density areas, leopards are unlikely to encounter conspecifics at random and require indirect communication (scents) to interact. In high leopard density areas, scent communication likely facilitates conspecific avoidance. 2) Leopards rely strongly on scent marking during breeding even though they are non-seasonal breeders and females are not synchronised, entering oestrus every 20 to 50 days once sexually mature, between 2.5 and 3 years old (Friedmann and Traylor-Holzer 2008, Balme and Hunter 2013). 3) Leopards naturally occur in sympatry with strong competitors (lions, spotted hyenas) and are significantly smaller than lions, potentially leaving them exposed to antagonistic and potentially fatal interactions with the species (Bothma 1998). 4) Leopards are a conflict species and regularly kill livestock and domestic animals, even occasionally attacking humans (Jacobson et al. 2016). Conflicts also spread to subspecies in other countries (*P. pardus pardus* in Africa, *P. pardus kotiya* in Sri Lanka, *P. pardus fusca* in

India, *P. pardus orientalis* in Russia and north-eastern China) (Uphyrkina et al. 2001, Balme et al. 2014, Jacobson et al. 2016, Kumbhojkar et al. 2021, Vitekere et al. 2021). Yet no satisfying long-term solution exists; the adaptability of leopards overcomes any protective infrastructure and system (e.g., boma, electric fence, livestock guarding dogs) (Balme et al. 2014). 5) Leopards are of conservation concern: they are often shot in retaliation for livestock depredation; their populations are declining; the species is already classified as Vulnerable; significant populations occur outside of protected areas (Jacobson et al. 2016). It is estimated that the species overall has lost roughly 65% of its global historical range and 48-67% of its historic range across Africa (Jacobson et al. 2016). For these reasons, leopards represent an interesting and apt model study species for this thesis which aims to investigate how scent-marking interactions between individuals and sympatric competitors could have ramifications for leopard-farmer conflicts and camera trap density estimates of the species.

To date, only a few studies have investigated scent-marking behaviours of leopards but suggest the importance of scent marking in the species' interactions (Table 1.2). Allen et al. (2016b) gathered reports from the literature and classified scent-marking associated behaviours of leopards into three categories: investigation through olfaction and flehmen (flehmen response had been observed only once in Bailey (1993) and was later confirmed by Rafiq et al. (2020)), body rubbing such as cheek rubbing, rolling, tail wrapping, and claw scratching, and scent marking via faeces deposition, urine spraying, and hindfeet scraping. Leopards perform both squat urination on the ground (vegetation or bare soil) and spraying from a standing posture on vertical objects such as trees and shrubs (Rafiq et al. 2020). They also scrape dust with hindfeet, forming small monticules, and urinate or defecate in the slight depression formed (Rafiq et al. 2020). Grimbeek (1992) further recorded African leopards preferentially rolling in aromatic plants like the lavender bush (*Lippia rehmannii*), which has coarse hair retaining the leopard's hair and scent, and the fever tea bush (*Lippie javanica*). Finally, leopards scratch bark

off camel thorn trees (*Vachellia erioloba*) preferentially, but also off Kalahari-sand acacias (*Vachellia luederitzii*), shepherd's trees (*Boscia albitrunca*) and worm-cure albizzia trees (*Albizzia anthelmintica*) (Bothma and le Riche 1995). Grimbeek (1992) further observed leopard scratches on water pearl (*Syzygium guineense*), water berry, (*Syzygium cordatum*) and common white pear (*Dombeya rotundifolia*) trees. Drawing parallels to other carnivores with similar ecology, scent-marking behaviours of leopards fulfil mating, asserting dominance status, and territory advertising (Rafiq et al. 2020). Additionally, scent marking of leopards may serve other purposes. Of the 39 extant African carnivore species, 24, including leopards, frequently visit, investigate and sometimes countermark one another (roll, rub body parts against scrapes, urinate on top of a previous mark) at scent-marking sites of other carnivores like at spotted hyena latrines (Apps et al. 2017, Apps et al. 2019). Similar dynamic interspecific activities around scent-marked objects of pumas (*Felis concolor*) earned puma scrapes the name of communal scraping in North America (Allen et al. 2017b, Apps et al. 2017). Apps et al. (2019) suggest that scent marking nearby or on top of another scent may be an olfactory way to increase detectability. In turn, Allen et al. (2017b) hypothesized that subdominant carnivores like grey foxes (*Urocyon cinereoargenteus*) soak up the scent of dominant carnivores like pumas in a “self-burdening process” (Reiger 1979) to repel competitors and larger predators as coyotes (*Canis latrans*) and bobcats (*Lynx rufus*) through deception (Hauser and Nelson 1991). Likewise, leopards rolling in gemsbok (*Oryx gazella*) and red hartebeest (*Alcelaphus buselaphus*) urine or aromatic plants may serve to conceal their scent from prey. Leopards being specialized stalk and ambush predators (Stander et al. 1997), improves their stalking distance and hence hunting success (Allen et al. 2017b, Apps et al. 2017). So, although the literature is limited on leopard’s scent-marking behaviours, there is evidence for several olfactory interactions with heterospecifics (Table 1.2).

Table 1.2. Original literature reports on scent-marking behaviours of *Panthera pardus*.

Behaviour	Objects	Subspecies	Country	Author & year
Scratching bark, spraying, investigating, rubbing	Tree	<i>P. pardus pardus</i>	Wilpattu National Park, Sri Lanka	Eisenberg 1970
Scraping	Ground			
Rubbing, spraying, scratching	Bush, tree	<i>P. pardus pardus</i>	Serengeti National Park, Tanzania	Schaller 1972
Spraying	Bush	<i>P. pardus pardus</i>	Tsavo National Park, Kenya	Hamilton 1976
Scraping	Ground			
Scratching bark	<i>Syzygium guineense</i> , <i>Syzygium cordatum</i> , <i>Dombeya rotundifolia</i>	<i>P. pardus pardus</i>	Waterberg mountain range, South Africa	Grimbeek 1992
Rolling	Aromatic plants like <i>Lippia rehmannii</i> and <i>Lippie javanica</i>			
Spraying, rubbing	Bush, tree, grass tufts, <i>Grewia hexamite</i>	<i>P. pardus pardus</i>	Kruger National Park, South Africa	Bailey 1993
Scraping	Ground, grass, road junction, game path, dry streambed, culvert, bridge			
Investigating, flehmening	Scents of vegetation along trails			
Rolling	Elephant dung			
Scratching bark, spraying urine, rubbing	<i>Acacia erioloba</i> , <i>Acacia luederitzii</i> , <i>Boscia albitrunca</i> and <i>Albizzia anthelmintica</i> , <i>Acacia haematoxylon</i>	<i>P. pardus pardus</i>	Kalahari Gemsbok National Park, South Africa	Bothma and le Riche 1995
Scraping, defecating	Along trails and paths	<i>P. pardus pardus</i>	Tai National Park, Ivory Coast	Jenny 1996
Scratching bark	<i>Tieghemella heckelii</i>			
Scratching bark	<i>Kirkia acuminata</i> , <i>Cassia abbreviate</i> , <i>B. albitrunca</i>	<i>P. pardus pardus</i> (captivity)	Farm Masequa, Limpopo province, South Africa	Van Wyk 2003
Gland secretions, defecating, urinating, squat urinating, rolling, scraping	Grass tufts, ground			
Spraying	Shrubs, especially <i>Combretum apiculatum</i> during the wet season, large tree trunks, especially <i>Boscia albitrunca</i> and <i>Kirkia acuminata</i>			
Rubbing	Long grasses			

Behaviour	Objects	Subspecies	Country	Author & year
Spraying urine Scratching bark Rolling	Grass tufts and open sand areas, lower tree branches, tree trunk and stumps, and small shrubs <i>Acacia erioloba</i> , <i>Acacia luederitzii</i> , <i>Boscia albitrunca</i> , <i>Albizia anthelmintica</i> Own urine, gemsbok urine, old carcass, red hartebeest urine, other animal faeces	<i>P. pardus pardus</i>	Kgalagadi Transfrontier Park, South Africa	Bothma and Coertze 2004
Spraying Rolling	Wood log On the ground, close to its own urine	<i>P. pardus pardus</i>	Nambiti Private Game Reserve, KwaZulu-Natal, South Africa	Castenada 2018
Scraping, licking, flehmening, rolling, spraying, rubbing, squat urinating, defecating, scratching bark	Grass, shrubs, trees	<i>P. pardus pardus</i>	Okavango Delta, Botswana	Rafiq et al. 2020
Urinating, scratching bark, rubbing, defecating	Leopard and cheetah marking sites	<i>P. pardus pardus</i>	Farmlands, Namibia	Verschueren et al. 2021
Cheek rubbing, squat defecating, spraying	Camera trap unit and trail	<i>P. pardus nimr</i>	Wadi Rabkhut, Wadi Arah and Arkak, Saudi Arabia	Spalton et al. 2006
Scraping, defecating, spraying urine, cheek rubbing	Alongside travel routes	<i>P. pardus nimr</i>	South-western Highlands, Saudi Arabia	Islam et al. 2018
Roll, investigating, spraying urine or other secretions, cheek rubbing, scratching, urinating, defecating	Ground, objects and enrichment in the enclosure	<i>P. pardus orientalis</i> (captivity)	Beijing zoological garden, China	Yu et al. 2009
Investigating, scratching, urinating, defecating, spraying, cheek rubbing	Objects and enrichment in the enclosure	<i>P. pardus orientalis</i> (captivity)	Jackson Zoological Park, Mississippi, USA	McWhorter 2014
Scraping, defecating, urinating Scratching bark	On the ridge top trails Five trees	<i>P. pardus saxicolor</i>	Bamu National Park, Fars province, Iran	Ghoddousi et al. 2008
Spraying urine, investigating	<i>Berberis</i> tree	<i>P. pardus saxicolor</i>	Sarigol National Park, Iran	Farhadinia et al. 2009

Importantly, scent-marking interactions of the African leopard could provide information on leopards' movements and distribution patterns outside of protected areas and into human settlements/farms. For example, leopards may be subordinates to lions and sometimes spotted hyenas (Palomares and Caro 1999, Ramesh et al. 2017). Interactions with these species could exert a pressure on leopards and negatively impact their spatial distribution (Rafiq 2016, Comley 2020). An increase in lion or spotted hyena densities could expand the landscape of fear of leopards (spatial and/or temporal unit avoided due to its association with greater risks), and turn a suitable territory into a non-suitable territory. Eventually, leopards could move out of the area and establish more suitable territories, potentially outside protected areas (e.g., farms) where lion and spotted hyena densities are lower (Mills and Hofer 1998, Mésochina et al. 2010). However, the reputation of roaming and stock raiding behaviours of leopards often makes them unpopular with farmers and results in retaliatory killings (Pitman 2012, McManus et al. 2015). The empty territories may create a vacuum effect, where the farm forms an attractive sink for new individuals (Balme et al. 2009, Balme et al. 2010a, 2010b). Furthermore, male leopards are particularly prone to infanticidal behaviours: new permanent resident males will kill cubs of other males (Palomares and Caro 1999, Balme and Hunter 2013). Individual removal by farmers, the accompanying vacuum effect, and the constant instability in ownership of neighbouring territories place female leopards in a parenting dead end, causing an overall decline in the density of leopards (Balme et al. 2009, Pitman 2012). To conclude, evidence across carnivores suggest that olfactory interactions are likely to influence leopards on multiple levels (e.g., behaviours, distribution, reproduction). Understanding these interactions could benefit wildlife management and facilitate the resolution of human-leopard conflicts on non-protected areas.

IV – Research questions, relevance and hypotheses

In Africa, habitat loss, unsustainable trophy hunting, poaching, retaliatory killing, and cultural practices using leopard's body parts have reduced African leopards to 43% of their historical range (Jacobson et al. 2016, Naude et al. 2020). The species still occurs in 78% of its remaining global range, but notably, all nine extant subspecies of *Panthera pardus* cause conflicts across most of their range (Jacobson et al. 2016). Leopards being highly adaptable and generalist carnivores (relatively flexible prey preference size), are not limited by the range of conditions that limits larger felids (Hayward et al. 2006). However, leopards spatial distribution can be negatively affected by lions (Rafiq 2016, Ramesh et al. 2017, Comley 2020). Understanding their interactions with other large carnivore species could improve our knowledge of their activity pattern, temporal overlap and antagonism. Through the use of artificially synthesised scent molecules, olfactory communication could potentially manipulate leopard distribution and repel them from livestock areas (Jones et al. 2016). Therefore, this study centres on interactions occurring at leopards' marking posts and aims to answer the following questions: 1) What type(s) of communication associated behaviours occur at marking sites of African leopards? 2) Do interspecific olfactory interactions of sympatric species affect leopard behaviour? 3) Does the monitoring of scent-marking sites enable the estimation of the leopard density, and do scents of competitors influence the associated estimate precision? Recent improvements in camera trapping technology and the associated density estimation techniques have increased their usefulness for behavioural ecology studies (e.g., Moqanaki et al. 2020, Dupont et al. 2021). Amongst others, videos on camera traps help capture indirect interspecific and intraspecific interactions occurring over a period of time with minimal human disturbances (Grant 2012). Therefore, video-triggered camera traps are the main data recording technique of my thesis project.

My project featured several important hypotheses. In chapter 2, I aimed at characterising leopard scent-marking activities. I hypothesized that intraspecific communication occurred at leopard marking sites for mating and territory advertising purposes. I expected 20 to 50-day cyclic peaks of non-pregnant females scent marking and visiting marking sites more intensively over short time periods (~14 days) corresponding to oestrus periods, and no females attending or raising cubs (Bothma and Coertze 2004). I also predicted cyclic detections of territorial adults at marking sites, with a greater activity of dominant males compared to females and nomadic subadult males. Regarding marking sites characteristics, I expected a greater incidence of leopard marking activities on some plant species compared to others across the sampled marking sites. Finally, I hypothesized that interspecific interactions with lions and spotted hyenas occurred at leopard marking sites and that fresh scents of these apex intraguild competitors would repel leopards from marking sites (shorter visit durations at sites, increased interval between visits).

In chapter 3, I assessed both the potential of camera trapping leopard scent-marking sites to estimate the species density, and the impact of lions' and spotted hyenas' scents on these estimates. I made two alternative hypotheses depending on the outcomes of chapter 2. If scents of lions and spotted hyenas showed to deter leopards in chapter 2, then I hypothesized that camera trapping leopard marking sites would impact the number of individual leopards detected at sites (reduction in individuals), detection rates (reduced total number of leopard detections) and widen the confidence intervals of the accompanying density estimates. If scents of lions and spotted hyenas showed no deterrent effect on leopards in chapter 2, then I hypothesized that camera trapping leopard marking sites would not significantly impact the accuracy and precision of leopard density estimates.

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CHAPTER 2

Characterizing scent marking by African leopards and interspecific interactions with two sympatric carnivores

Abstract

Solitary carnivores strongly rely on scent marking to communicate about reproductive status and territory ownership. A few studies also emphasized olfactory interactions between species, particularly between sympatric carnivore guild members (potential competitors). Carnivores tend to either be attracted to or repelled by scents of conspecifics or heterospecifics. This suggests the potential to use scent marking as a tool in wildlife management to manipulate carnivore movements and mitigate human-wildlife conflicts. The African leopard (*Panthera pardus*) is a solitary carnivore sympatric to competitors such as lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*). It is also a regular conflict species across its range, and is data-deficient on its scent-marking interactions at both the intraspecific and interspecific levels. These reasons motivated my choice to use leopards to investigate the role of scent marking in the species' interactions with conspecifics, lions and spotted hyenas, and the potential of such interactions to manipulate the spatial distribution of leopards. I gained knowledge on the leopard population and habits of individuals through five months of game drives. Then, using camera traps (1450 trap nights), I monitored 43 potential marking sites of leopards in the western sector of the Sabi Sands Game Reserve and further implemented treatments by adding freshly collected lions' or spotted hyenas' scats to seven sites, respectively, and 13 control sites with no scats. A total of eight independent male individuals (65 detections, ~7% of subadults) and seven independent female individuals of any reproductive status (57 detections) were

identified. Oestrus scent marking in females could not adequately be assessed. However, 40% of consecutive visits at marking sites were performed by individuals of different sexes. My data also revealed that females scent marked significantly more than males ($X^2=11.04$, $df=1$, $p<0.05$), suggesting a greater importance than previously reported for female territory advertising. Overall, as expected, the data supported the hypothesis that adult leopards scent mark for mating and territory advertising purposes. The scent-marking incidence was also significantly different across monitored plant species (Fisher exact test's two-tailed $p=0.058$) and leopards marked or investigated *Terminalia sericea* on half of the detections at such sites. Investigating, rubbing and spraying were the three most frequent behaviours at marking sites, but frequencies did not significantly differ between control and treatment sites (Fisher exact test's two-tailed $p>0.05$). The difference in the proportions of males and females detected was also insignificant across sites (Fisher exact test's two-tailed $p>0.05$). Finally, I used generalized linear mixed effect models to analyze whether lion and spotted hyena scents affected leopard visit interval, stay duration, and behaviour duration at marking sites. I expected a decreased visit frequency and shorter stays of leopards at treatment marking sites. Unexpectedly, leopard visit frequency did not vary significantly between treatment or control sites (*post hoc* Tukey tests $p>0.05$). Leopards also stayed significantly longer at sites with lion scats (*post hoc* Tukey test for lion-spotted hyena and lion-control comparisons: $p\text{-values}<0.05$) than control or spotted hyena scats sites. My results supported a strong dear enemy effect characterizing olfactory interactions of leopards: scent-marking sites were used by the species to gather information about the surrounding conspecifics and lions and reciprocally advertise their own presence. Such a strategy could facilitate the avoidance of risky encounters by the species. Evidence from my field trials did not provide significant support for the use of competitors' scents as effective tools for conflict mitigation through the manipulation of the spatial distribution of leopards.

Key words: Behavioural ecology, dear enemy effect, intraguild interactions, generalized linear mixed effect models, translocated scats.

Introduction

Scent marking is a core component of the social and spatial organizations of solitary carnivores (Wemmer and Scow 1977, Macdonald 1980, Rafiq et al. 2020). It usually delineates territory boundaries, prevents undesirable risky encounters with conspecifics (individual of the same species), and facilitates punctually desirable ones such as for mating (Wemmer and Scow 1977, Macdonald 1980, Apps 2013, Allen et al. 2016a). Scent marking takes various forms ranging from faeces and urine excretions to sprays of anal or other types of marking fluid secretions, rubbing of different body glands on a substrate, rolling, licking, scraping the ground, scratching trees, and sniffing or flehmening scents (Reiger 1971, Macdonald 1980, Asa 1993). Furthermore, scent marking can elicit both behavioural and physiological responses in a receiver (Garvey et al. 2016a, Soso and Koziel 2017, Rafiq et al. 2020). A few studies have thus suggested the high potential of scent marking as a wildlife management tool, particularly in manipulating carnivores' movements to reduce incidences of human-carnivore conflict (Table 1.1, Garvey et al. 2016b, Jones et al. 2016). However, detailed descriptions and knowledge on scent-marking behaviours and their implications for interacting individuals are lacking for many solitary carnivores, which are often relatively cryptic and roam over large territories (Macdonald 1980, Allen et al. 2016b). The African leopard (*Panthera pardus*) is one such example.

Leopards are solitary except during mating or when females raise cubs (Grimbeek 1992, Bothma 1998). The species tends to occur at low densities throughout its range, but this can vary with resource availability in an area (e.g., 5.41 individuals/100 km² (Allen et al. 2020) to 1 individual/29 km² (Bertram 1976) in the Serengeti National Park, Tanzania; 1 individual/13

km² in Tsavo National Park, Kenya (Hamilton 1976); 1 individual/53 km² in the Waterberg, South Africa (Grimbeek 1992)). In poor resource environments like the arid Kalahari Desert, leopard territories can span over 1 000 km² for females to over 2 000 km² for males, whereas rich-prey environments like the Kruger National Park support higher densities of leopards, with territories as small as 16.4 km² for males and 5.6 km² for females (Bothma 1998, Friedmann and Traylor-Holzer 2008, Bothma and Bothma 2012). Conservation efforts for the species mostly target populations in non-protected areas (e.g., farmlands), which are regularly in conflict with humans (Pitman 2012, Jacobson et al. 2016). Leopards sometimes depredate livestock in farms and villages and are occasionally killed in retaliation (Pitman 2012). However, the inefficacy of removing individuals has been shown in several studies (McManus et al. 2015, Lennox et al. 2018). Instead of solving the depredation issue, the lethal removal of leopards increases the turnover rate in the local leopard population, with potentially several nomadic individuals roaming around the vacant territory (Balme et al. 2009, Balme et al. 2010). Furthermore, new resident male leopards often kill cubs sired by other males (i.e., previous male resident) (Balme and Hunter 2013). Thus, the territorial instability and rapid turnover created by farmers' removal eventually place female leopards in a parenting dead-end (Friedmann and Traylor-Holzer 2008, Pitman 2012). The perceived short-term benefits of the removal strategy seem greater to local communities than other more passive or expensive solutions that have failed them (fencing, livestock guarding dogs, and different livestock husbandry practices) (Pitman 2012, McManus et al. 2015, Eklund et al. 2017, Lennox et al. 2018). Leopards already exhibit decreasing population trends and simultaneously face other human-related threats (e.g., shrinking habitat, poaching, hunting of natural prey species) (Pitman 2012, Jacobson et al. 2016). In the current Anthropocene crisis era, gaining insights into factors influencing or determining leopard distribution carries a double stake: providing knowledge to design better conservation plans and mitigating conflicts with humans (Jones et

al. 2016). An adapted use of scent marking to manipulate leopard movement and deter them from conflict areas could fulfil these goals (Garvey et al. 2016a, Jones et al. 2016, Table 1.1).

The functions of intraspecific olfactory communication in carnivores are generally similarly understood across the literature (Wemmer and Scow 1977, Macdonald 1980). However, comparing results of experimental studies emphasizes the knowledge gap concerning the effects of scent marking on interacting carnivores, and reveals an unclear and paradoxical effect of scent marking at the interspecific level (Table 1.1, Banks et al. 2016, Garvey et al. 2016b). For example, packs of wild dogs (*Lycaon pictus*) (Jackson et al. 2012) and wolves (*Canis lupus*) (Ausband et al. 2013) were respectively successfully redirected towards and maintained within desired protected areas using translocated foreign scent marks of the same species. In both studies, packs were repelled by the artificially deposited scent marks, creating an olfactory biofence that successfully prevented conflicts with humans. On the other hand, red foxes (*Vulpes vulpes*) spent more time in areas artificially scent marked with foreign red fox urine (Arnold et al. 2011). And in other scenarios still, wild stoats (*Mustela erminea*) were significantly attracted to scents of predators (ferrets—*Mustela furo* and domestic cats—*Felis catus*) (Garvey et al. 2016b); red foxes were attracted to scents of competitors (dogs—*Canis lupus domesticus* and cats) (Banks et al. 2016); cheetahs (*Acinonyx jubatus*) significantly avoided marking sites recently visited by lions (*Panthera leo*) and leopards (Cornhill and Kerley 2020a). Overall, whether intraspecific or interspecific scents attract or repel a species is inconsistent across studies. Before using scent marking in large-scale wildlife management plans, small-scale trials and observations are required.

Two main theories of olfactory interactions oppose each other: the landscape of fear and the dear enemy effect. Both concepts usually characterize interactions between heterospecifics (individuals of different species) but could be extended to a finer scale for conspecific interactions. The landscape of fear describes the aversive response of animals on a spatio-

temporal scale (Palomares and Caro 1999, Hayward and Slotow 2009). For example, cheetahs are mostly active during daylight and limit the use of dense vegetation areas to minimize risks of encountering other large carnivores (lions, spotted hyenas—*Crocuta crocuta*, leopards) (Hayward and Slotow 2009). On the other side, the dear enemy effect suggests that staying informed of the surrounding community activity, particularly of residents of high risks (predators and strong competitors capable of severe injuries) and reciprocally signalling its presence, are an anti-predator strategy that facilitates avoiding risky physical encounters (Banks et al. 2016, Garvey et al. 2016b). This strategy improves identification and reaction times of individuals when they detect scents of heterospecifics moving close-by, and minimizes costs of unnecessary flights or risks of lethal injuries (Banks et al. 2016, Garvey et al. 2016b). Consequently, under the landscape of fear, carnivores should avoid areas of intense predator or competitor activity (e.g., scent-marking sites). Whereas, under the dear enemy effect, carnivores should be attracted to areas accumulating cues on community members (e.g., scent-marking sites), particularly for predators and strong competitors.

To determine the potential of scent marking as a tool of wildlife management, we must precisely understand the effects of intra and interspecific olfactory interactions on leopards and weigh the relative importance of the landscape of fear and dear enemy effect on leopard dispersal. This study attempts to answer: 1) What are the characteristics of leopard scent marking and marking sites in the Sabi Sands? 2) What are the roles of intraspecific scent-marking interactions occurring at marking sites of African leopards? 3) Do interspecific olfactory interactions with lions and spotted hyenas influence the dynamics of leopards at scent-marking sites?

Regarding the characteristics of leopard scent-marking behaviours and marking sites, I expected leopards to rub and spray substrates, scrape the ground, scratch bark, roll, and investigate scents as described across the literature (Grimbeek 1992, Bailey 1993, Bothma and

le Riche 1995, Bothma and Coertze 2004). Based on reports of scent-marked objects (Table 1.1), I further expected a greater scent-marking incidence of some plant species across the sampled marking sites.

Regarding the types of intraspecific scent-marking interactions, I expected visits of leopards at marking sites for 1) mating purposes: 20 to 50-day cyclic peaks of non-pregnant females intensively scent marking over ~14 days corresponding to oestrus periods (Bothma and Coertze 2004), 2) territory advertising purposes: continuous scent-marking frequencies over time of leopards. Given the high infanticide risks in leopards (about 33% of cubs) (Balme and Hunter 2013), I expected females attending or raising cubs and nomadic subadult males to avoid areas of high conspecific activity such as scent-marking sites. On the contrary, dominant male leopards likely benefit from advertising their territory and knowing the strength of neighbouring males or the status of surrounding females. Therefore, I expected no females attending or raising cubs (Bothma and Coertze 2004), and a greater activity of dominant males than subadult males at scent-marking sites, as observed with Namibian cheetahs (Melzheimer et al. 2020).

Finally, African leopards strongly compete with other members of the large carnivore guild (Palomares and Caro 1999, Hayward and Slotow 2009). They are significantly smaller than lions and more likely to be killed or severely wounded during such encounters (Bothma 1998, Friedmann and Traylor-Holzer 2008). Encounters with spotted hyenas also often occur at kill sites where leopards have about a 50% chance of winning or of hyena stealing their kill (Balme et al. 2017), with potentially severe injuries in both cases (Palomares and Caro 1999, Friedmann and Traylor-Holzer 2008). Following these observations, I expected significant repelling effects of scents of lions and spotted hyenas on leopards, causing a significant reduction in leopard visits and marking activities at scent-marking sites.

Methods

Study area

The Sabi Sands Game Reserve (hereafter SSGR) is a protected area conglomerate formed of 20 private game reserves covering 625 km² (midpoint: 31°29' E, 24°49' S) in the Mpumalanga Lowveld, South Africa (Balme et al. 2013). The study focused on six properties (ca 100 km²) collectively forming the western sector of the SSGR: the Dulini/Exeter, Leopard Hills/Ululapa, Savanna, Inyati, Idube, and Ulusaba lodges (Figure 2.1).

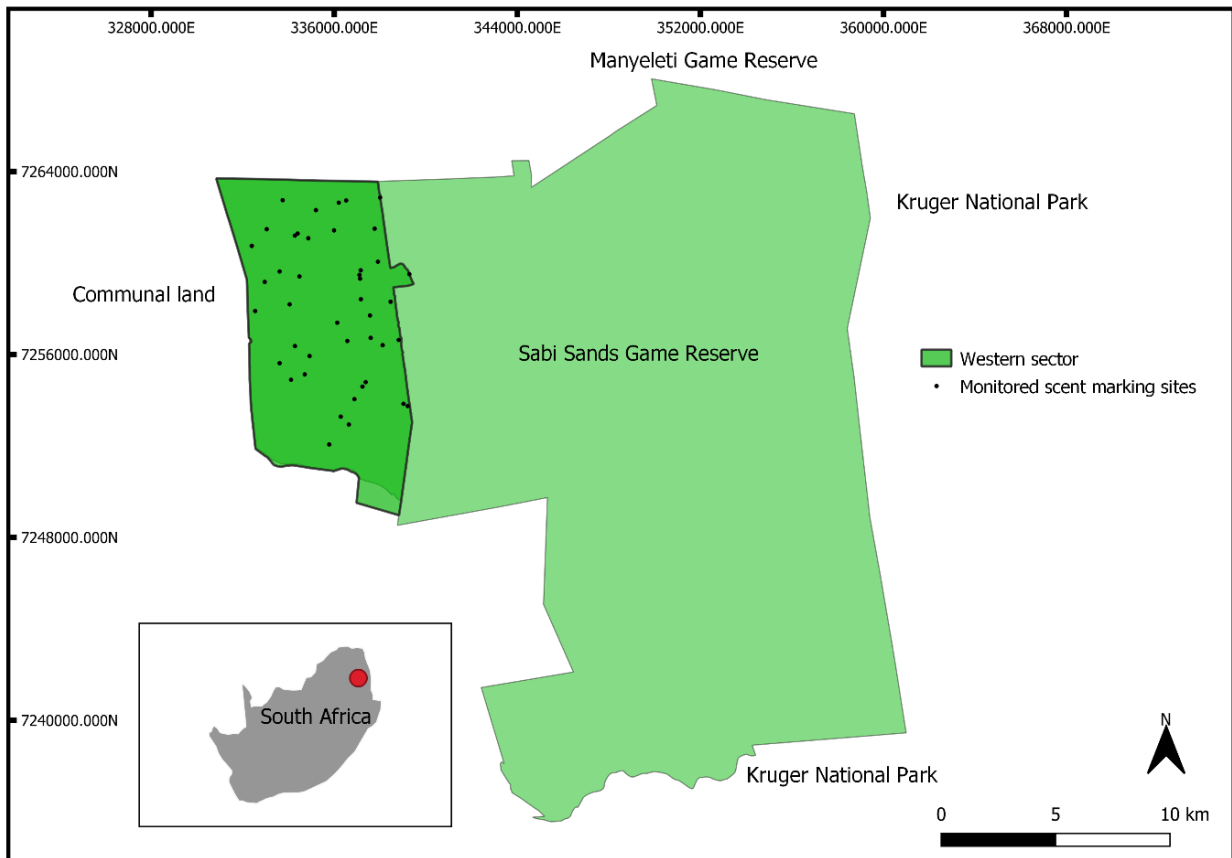


Figure 2.1. The western sector of the SSGR, along with the monitored scent-marking sites (characterized by the presence of one remote video camera trap) in the greater study area's surrounding.

Historically, the area was a mosaic of farms and ranches used for cattle production and game shooting activities (Fortescue 1997). The SSGR was established in 1948 when 14 cattle

farmers consolidated their ranches into one reserve (SSW 2017). It has been collectively managed to conserve wildlife since then (Balme et al. 2013). Formally abundant species of the ecosystem that became locally extinct like elephants (*Loxodonta africana*), cheetahs (*Acinonyx jubatus*), nyalas (*Tragelaphus angasii*), and white rhinos (*Ceratotherium simum*) were reintroduced into the area since the 1970's (Spenceley 2005). Waterholes from the remains of previous land use ensure perennial water supply for wildlife (Smith and Fitchett 2020). Finally, the removal of fences between internal properties of the reserve, East and South with the Kruger National Park (19 485 km²), and North with the Manyeleti Game Reserve (230 km²) provide sufficient land to support large-scale population dynamics of wildlife and facilitates animal dispersal (Fortescue 1997, Balme et al. 2013). The western sector remains separated from communal lands by an electric fence on its western boundary (Fortescue 1997).

The eco-tourism business of the SSGR is now well established and relies on 35-40 on-site lodges (Schmidtz and Willott 2012). Multiple dirt roads are also distributed throughout the reserve, and animals are used to the presence of game drive vehicles (Pirie et al. 2014). The SSGR is internationally renowned for its great diversity of mammal species, including the Big5 (lion, leopard, elephant, buffalo – *Syncerus caffer*, and black rhino – *Diceros bicornis*), as well as other charismatic African species like blue wildebeest (*Connochaetes taurinus*), zebra (*Equus quagga*), impala (*Aepyceros melampus*), spotted hyena, giraffe (*Giraffa camelopardalis*), and the endangered wild dog (*Lycaon pictus*) (Smith and Fitchett 2020). However, the SSGR is particularly famous for its high density of leopards (about 11 ind/100 km²) (Balme et al. 2019) and detailed knowledge of their populations (e.g., family trees, territories, diet) (Balme et al. 2013).

The western sector is characterized by relatively flat terrain with some rocky outcrops and is dominated by a savanna ecosystem interspersed with grassland and woodland patches (Rutherford et al. 2006, Smith and Fitchett 2020). Common vegetation includes *Diospyros*

mespiliformis and *Vachellia* spp. trees, *Combretum* spp. and *Euclea divinorum* shrubs, and *Themeda triandra*, *Panicum maximum*, and *Digitaria eriantha* grasses (Rutherford et al. 2006). Two rivers cross the SSGR: the Sabie River flowing year-round along the southern boundary, and the Sand River which may dry in winters and traverses the reserve diagonally before joining the Sabie River in the south-east part (Smith and Fitchett 2020). The local climate is semi-arid, with dry winters between April and September and warm rainy summers between October and March (Fortescue 1997). Mean annual temperatures range from 19°C to 33°C, and annual precipitation averages 650 mm (Balme et al. 2013).

Fieldwork: data collection

Over a five-month period (April 6th-August 23rd, 2021), leopards were observed during game drives (~6h/day). I collected information regarding the different individuals, their sex, relative age, kinship, territory, and flank photos to establish a database that served for further identification of leopards detected on camera traps. For any witnessed scent-marking behaviour, I recorded the location, the identity of the leopard, types of behaviour performed, and object targeted (i.e., tree, shrub). I then generated a QGIS (version 3.2, QGIS Development Team 2020) map to visualise intense leopard olfactory activity areas.

Simultaneously to recording data during daily game drives, I set up 32 camera traps (22 Bushnell Trophy Cam HD – Model 119537, 10 Browning Recon Force Advantage – Model BTC-7A) across 43 leopard scent-marking sites selected based on observations, the knowledge of rangers and trackers, and signs confirming leopard olfactory activity (tracks, scraping marks, and characteristic popcorn scent of leopard urine spray (Apps et al. 2014)). Except for three wider sites where camera traps were paired, all sites had a single camera trap. Cameras were positioned at about 40 cm high to capture leopard flanks and encompassed the marking area in the field of view. They were parameterized to take 1-minute-long videos per trigger with a 30-second interval between consecutive triggers. Finally, camera traps were checked every five

days to ensure that wildlife had not interfered with them (break, knock over, or move traps), replace SD cards and batteries. Camera traps were in the field for 61 days from May 3rd to July 2nd, 2021.

Based on the detection of leopards on camera traps during the first 20 days, camera traps were rotated until most traps detected leopards. Then, I randomly assigned sites to the control or treatment groups. I added either faeces of lions or spotted hyenas (collected freshly in the study area and frozen) to treatment marking sites. Scats were collected using latex gloves and stored in sealed plastic bags. The same species' scats were added to the same sites every 12 days while control sites remained free from the addition of any scats. The procedure was repeated three times (three additions occurring at 12 day intervals).

Data analyses

I first described data collected on leopards and their olfactory behaviours during game drives. Using CameraBase (Tobler 2015, version 1.7, www.atrium-biodiversity.org) software, previously taken flank photos and gathered information on leopard individuals, I sorted camera trap data, sexed leopards with conspicuous features (genitalia, swollen nipples, shoulder width, dewlap size, Balme et al. 2012), and identified all leopard individuals from rosettes of the pelage (Sunquist and Sunquist 2002). Then, I classified leopard behaviours as walking, olfactory activities (investigating, rubbing, spraying, scraping, rolling, urinating, defecating), or others. Based on the relatively short visits of leopards at scent-marking sites observed during game drives, and to gather the importance of marking sites in a high leopard density area, I used 20 min as an independent criterion for any individual detection at a trap.

All statistical analyses were performed in R (version 3.4.3, R Core Team 2017) using the lme4 package (Bates et al. 2015). Based on the sample size meeting the assumptions, a chi-square test first compared the frequencies of males and females simply walking past the marking bush

or performing a type of olfactory activity. I calculated the percentage of consecutive male-male, female-female, female-male or male-female visits at a site. Three exact fisher tests further checked for the selection of scent-marked plant species by leopards across the sampled marking sites, the difference in male and female proportions, and differences in frequencies of olfactory behaviour types between control, lion sites, and hyena sites. Lastly, a series of generalized linear models (GLM) and generalized linear mixed effect models (GLMEM) were computed to measure the impact of lions' and spotted hyenas' scents on the visit duration of leopards at marking sites, the duration of olfactory activities, and the visit interval of individual leopards at a marking site (Table 2.1). Models were fitted using a gamma family and a log link function (Zuur et al. 2009, Bates et al. 2015). Durations (defined as the number of seconds a leopard remained at a site) were taken as the response variable, treatments as fixed effects, and for GLMEM, identity (sex and adult or subadult) of previous and current leopard visitors as random effects, as done by Cornhill and Kerley (2020b). Model fit for data was checked looking at QQplots, residuals vs fitted plots and AIC values (Bolker et al. 2009). Finally, models were compared based on AIC values, and the most parsimonious models were selected. *Post hoc* Tukey tests were used for pairwise comparisons within models.

Lastly, I described the activity of lions and spotted hyenas only detected on camera traps scent marking (scraping, spraying, rolling, rubbing or flehmening) or investigating scents at leopard marking sites. I reported additional side observations of interspecific small carnivore activity occurring at leopard marking sites.

Table 2.1. Models computed. “TD” stands for total visit duration (in seconds), “OD” for olfactory activity duration (in seconds).

Model	Type	Response variable	Independent variable	Random effect
1a	Glmer	TD	Treatment	Current visitor, previous visitor
1b	Glm	TD	Treatment	
1c	Glmer	TD	Treatment	Current visitor
1d	Glmer	TD	Treatment	Previous visitor
2a	Glmer	OD	Treatment	Current visitor, previous visitor
2b	Glm	OD	Treatment	
3a	Glmer	Visit interval	Treatment	Previous visitor
3b	Glmer	Visit interval	Treatment	Current visitor, previous visitor

Results

Game drive observations

During the game drives, 15 leopards (7 females, 8 males) were seen at least once, and nine individuals (6 males, 3 females) were observed performing a type of olfactory activity on 140 occasions (Figure 2.2). Two females and four males scent marking were non-territorial subadults between 2 and 5 years old. Sexually mature territorial males (5 to 12 years old) were involved more often in scent-marking activities, whereas scent marking females constituted only about 18% of observations (Table 2.2). Importantly, five resident females were denning with cubs or pregnant at some point during the survey.

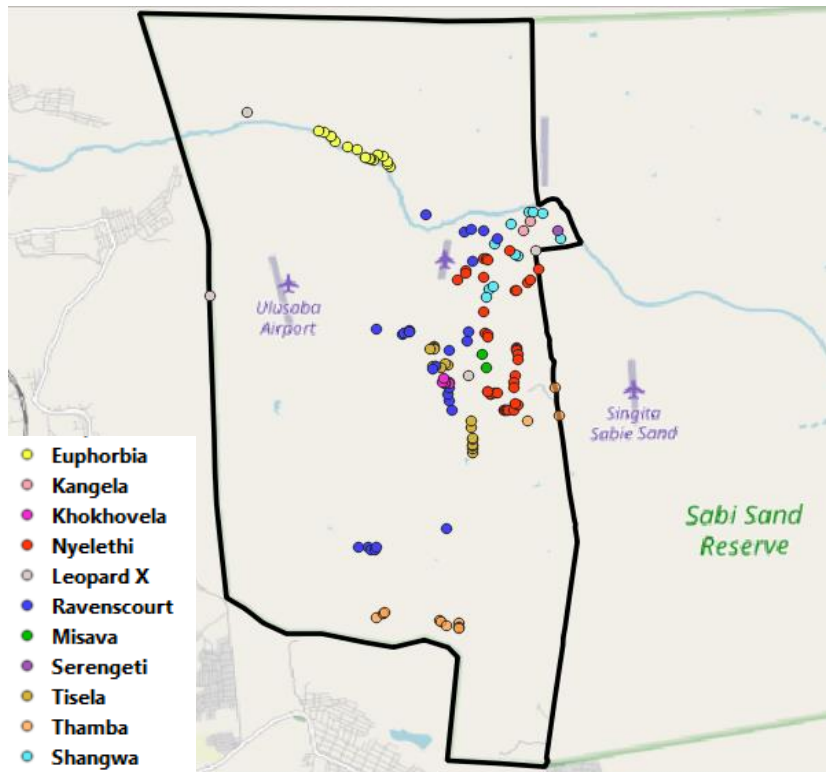


Figure 2.2. Leopards observed performing olfactory activities during game drives in the western sector of SSGR between April 6th and August 23rd, 2021. Each colour represents a different individual. Leopard X (grey dots) represents scats found and not identified to a specific leopard individual. Dull yellow, purple, and bright pink are for the three females, the remainder represents male leopards.

Scent-marking sites usually featured a prominent bush on a roadside or hanging branches in dry river beds. Plant species frequently selected by leopards included 26% grass, 25% magic guarri bush (*Euclea divinorum*), 13% bush willows (*Combretum* spp.), and 9% silver cluster leaf (*Terminalia sericea*). In decreasing order, the most frequent behaviours were spraying, scraping, rubbing, and investigating (Table 2.2). I estimated olfactory activities to be relatively short (less than 5 seconds) and to occur repeatedly over short distances during a leopard walk (e.g., sometimes as close as about 10 m between consecutive marked objects).

Table 2.2. Observed olfactory activities of leopards (25 events for females, 110 times for males) during game drives in the western sector of SSGR (April 6th -August 23rd, 2021). An individual sometimes performed more than one behaviour while being observed.

Behaviour	Females	Males	Total
Spray	18	50	68
Rub	4	23	27
Investigate	-	11	11
Scrape ground	2	31	33
Urinate	1	5	6
Defecate	2	5	7
Roll	1	3	4
Lick	-	1	1
Other	-	1 bark scratching	1
Total	28	130	158

Camera trapping survey

Camera traps were moved across 43 different locations while looking for the most frequented marking sites of leopards. A total of 16 individual leopards were detected over 122 independent events. Seven females, including a subadult and three denning individuals, were each detected one to 17 times, totalling 57 detections (Table 2.3). Eight males, including three subadults and a new individual for the area, were each detected one to 22 times, totalling 65 detections (Table 2.3). I could not identify six additional leopard detections (at least three males) due to bad image quality and recorded one cub of unknown sex.

Table 2.3. Total detections and independent recaptures (20 min interval) of independent leopard individuals (>18 months old) at 43 marking sites in the western sector of the SSGR between May 3rd and July 2nd, 2021.

Leopard ID	Detections	Recaptures
M1	3	2
M2	1	0
M3	5	4
M4	12	11
M5	22	21
M6	15	14
M7	6	5
M8	1	0
F9	9	8
F10	4	3
F11	7	6
F12	9	8
F13	1	0
F14	17	16
F15	10	9

On average, all traps were within the territorial reach of five individuals (adults and subadults). Seventeen camera trapping sites did not detect any leopards. Nine sites detected only one individual; all other sites detected two, three or more individuals (Appendix 1). Overall, 38% of the sites were shared by individuals of both sex, 35% by males only, and 27% by females only. Twenty-eight percent of consecutive visits were female-female interactions (meaning that after a female leopard visited the site, the next individual was also a female, either self or a different individual), 32% were male-male interactions (either self or with a different individual), and 40% were male-female or female-male interactions. In decreasing order and accounting for the sex and territorial/sexual status of visitors, most consecutive visits at a

marking site were performed by mature males when the previous visitor was a mature male (or self), mature females when the previous visitor was a mature female (or self), mature males when the previous visitor was a mature female, and mature females when the previous visitor was a mature male (Figure 2.3).

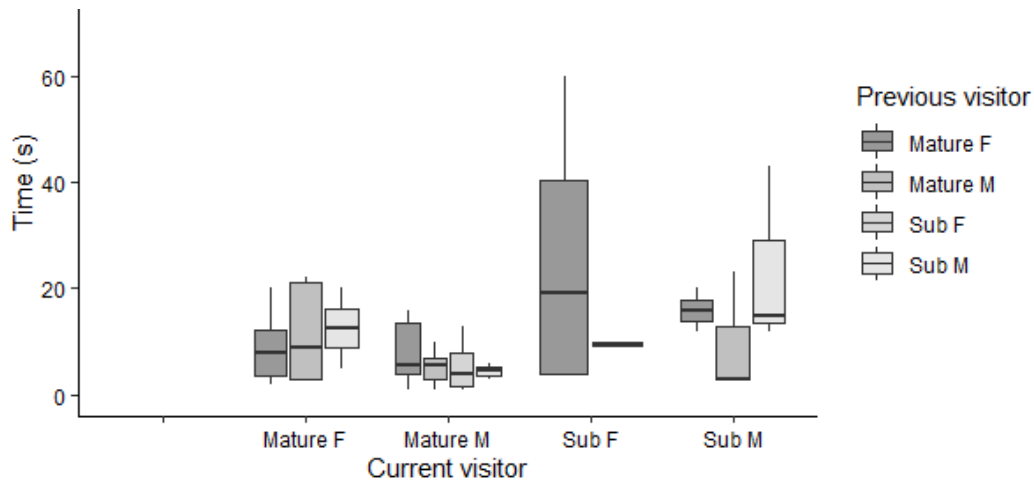


Figure 2.3. Time spent at marking sites by leopards based on the sex (F stands for females, M for males) and status (sexually mature and territorial adults or non-sexually mature and non-territorial subadults) of the current and previous visitors. Data are pooled across groups and outliers do not appear on the graph. Only pair sample sizes > 1 are represented ($N_{\text{MatureF-F}}=20$, $N_{\text{MatureF-M}}=13$, $N_{\text{MatureF-SubM}}=2$, $N_{\text{MatureM-F}}=14$, $N_{\text{MatureM-M}}=22$, $N_{\text{MatureM-SubF}}=4$, $N_{\text{MatureM-SubM}}=2$, $N_{\text{SubF-MatureF}}=4$, $N_{\text{SubF-MatureM}}=2$, $N_{\text{SubM-MatureF}}=2$, $N_{\text{SubM-MatureM}}=3$, $N_{\text{SubM-M}}=3$).

The majority of the marking bushes at visited sites were *E. divinorum*, *D. mespiliformis*., *Combretum* spp., *T. sericea*, or a mix of species (Figure 2.4). Bush selection by leopards and whether they walked-by or scent marked was significant at 90% confidence level (Fisher exact test's two-tailed $p=0.058$). Leopards visited sites with *T. sericea* and *Combretum* spp. more than relatively available but ignored *Combretum* spp. as marking objects, walking past every time, and only scent marked *T. sericea* half of the time (Figure 2.4). Leopards also scent marked grasses and mixed bushes more than proportionally available across monitored sites (Figure 2.4).

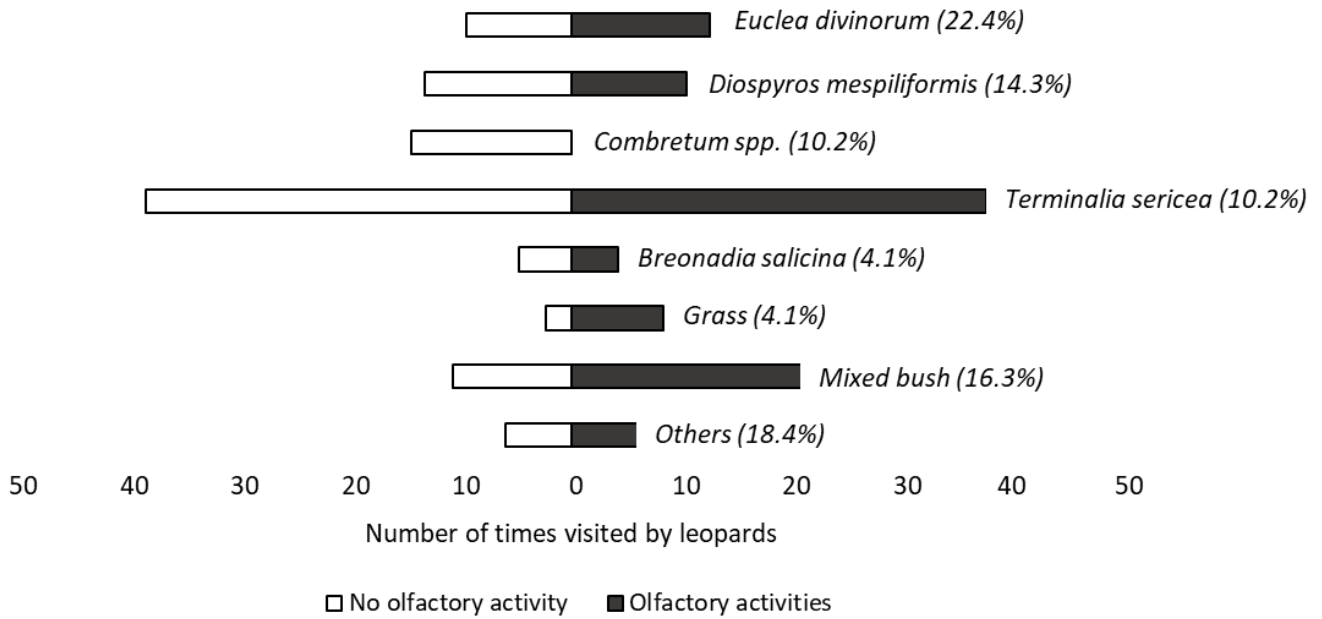


Figure 2.4. The number of olfactory and non-olfactory behaviours of leopards observed at monitored scent-marking sites. Target plant species of each marking sites are given with their relative availability (%) across monitored marking sites.

Overall, leopards simply walked past the sites on 64% of the detection events and performed olfactory activities on 36% of the detections (Table 2.4), but there was a significant difference in leopards' behaviours between sexes ($X^2=11.04$, $df=1$, $p<0.05$). When detected, females were involved about half of the time in olfactory activities, whereas males walked past the bush about 77% of the time (Table 2.4).

Table 2.4. Leopard behaviour category per detection and detailed olfactory activities per sex before and after treatment at camera trap sites in the western sector of SSGR between May 3rd and July 2nd, 2021. More than one olfactory activity could occur during one detection event. “Control” refers to sites without scats, “hyena” refers to sites with hyena scat, “lion” refers to sites with lion scats, “olf. activities” to olfactory activities. Three unsexed individuals do not figure in the table. “Others” groups non-olfactory activities such as rasping and grooming.

	Control		Hyena				Lion				TOTAL		
	Before		After		Before		After		Before			After	
	F	M	F	M	F	M	F	M	F	M		F	M
Walk	5	10	12	14	3	4	6	12	1	4	1	9	80
Olf. activity	4	1	7	1	2	2	3	5	5	3	6	5	46
TOTAL	9	11	19	15	5	6	9	17	6	7	7	14	125
Detailed activities													
Spray	2	1	6	-	1	1	2	-	3	2	3	1	22
Rub	1	-	-	-	-	-	-	2	2	-	1	-	29
Investigate	-	-	1	1	1	1	1	2	1	1	2	4	17
Defecate, Urinate,	1	-	-	-	-	-	-	1	1	-	-	-	7
Scrape, Roll													
Others	-	-	-	-	-	-	1	1	2	-	1	1	4

Leopards may visit a marking site once a week, on two consecutive days, or more punctually, except for two sites respectively visited every one to five and one to six days interval max (Figure 2.7, Appendix 1). In terms of age and sex related activity, adults visited sites and performed olfactory behaviours more frequently than subadults which tended to simply walk by. Overall, males had shorter visiting cycles than females to sites (1-2 times/week for males vs 1 time/ 1-2 weeks for females) (Appendix 2). Regardless of the behaviours, both males and females had a modal stay duration of 3 seconds at marking sites (mean_{Female}=8.9s, mean_{Male}=6.0s) (Figure 2.5). There is more variation in the visit durations of subadult individuals than territorial adults, but the average visit duration was generally higher in subadults than in adults (Figure 2.3). The most frequently recorded olfactory activities were spraying, rubbing, and investigating bushes (Table 2.4). There was no significant difference in the frequency of these behaviours between control and treatments sites (Fisher exact test's two-tailed p=0.2). Average olfactory activity duration was 12.7s for females (Mean_{investigating}=8.8s, Mean_{rubbing}=4.4s, Mean_{spraying}=2.2s, Mean_{others}=4.9s) and 17.2s for males (Mean_{investigating}=8.2s, Mean_{rubbing}=4.0s, Mean_{spraying}=2.9s, Mean_{others}=38.8s) (Figure 2.6).

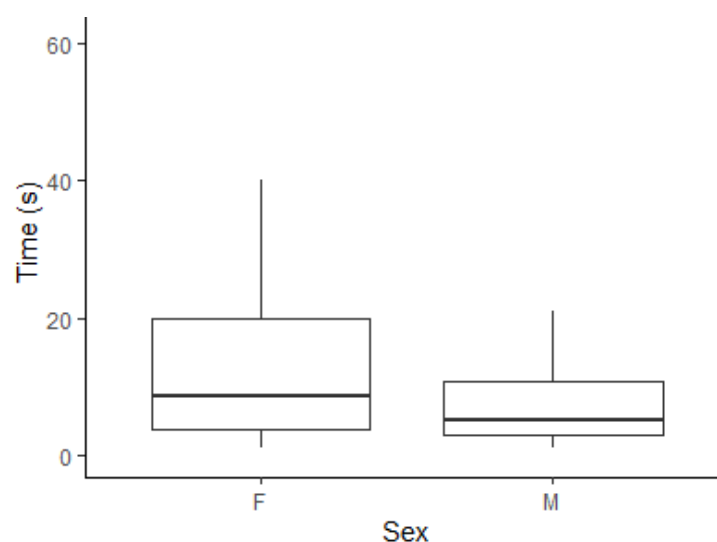


Figure 2.5. Time spent by female (N=57) and male (N=68) leopards visiting 43 marking sites between May 3rd -July 2nd, 2021, in the SSGR. Data are pooled across groups, and outliers do not appear on the graph.

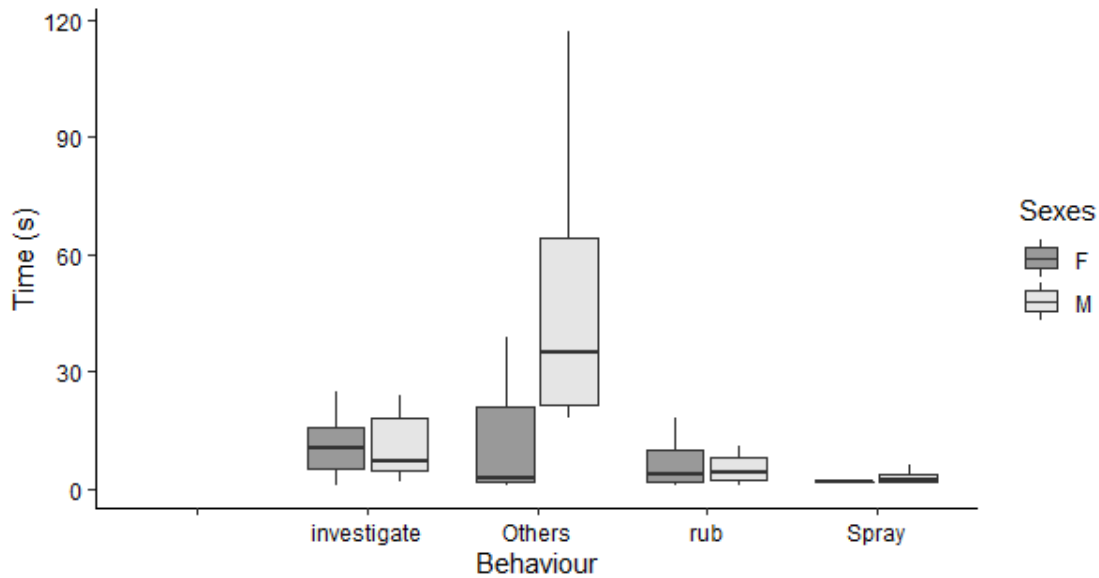


Figure 2.6. Time spent by leopards in the SSGR investigating ($N_{\text{Female}}=10$, $N_{\text{Male}}=9$), spraying ($N_{\text{Female}}=18$, $N_{\text{Male}}=4$), rubbing ($N_{\text{Female}}=20$, $N_{\text{Male}}=4$), and rolling, scraping, defecating and urinating grouped under the “others” category ($N_{\text{Female}}=3$, $N_{\text{Male}}=4$). Data are pooled across groups, and outliers do not appear on the graph.

Lion and hyena scats were added to seven sites, respectively, and 13 sites served as a control. Overall, control sites detected 80 leopard visits (7 females detected 41 times, 7 males detected 36 times, one cub and 2 unidentified leopard photos), hyena sites detected 27 visits (5 females detected 9 times, 5 males detected 16 times and 2 unidentified leopard photos), and lion sites detected 22 visits (3 females detected 7 times, 5 males detected 13 times and 2 unidentified leopard photos) (Figure 2.7). The difference in the proportions of males and females was not significant between the control and treatment groups (Fisher exact two-tailed $p>0.05$).

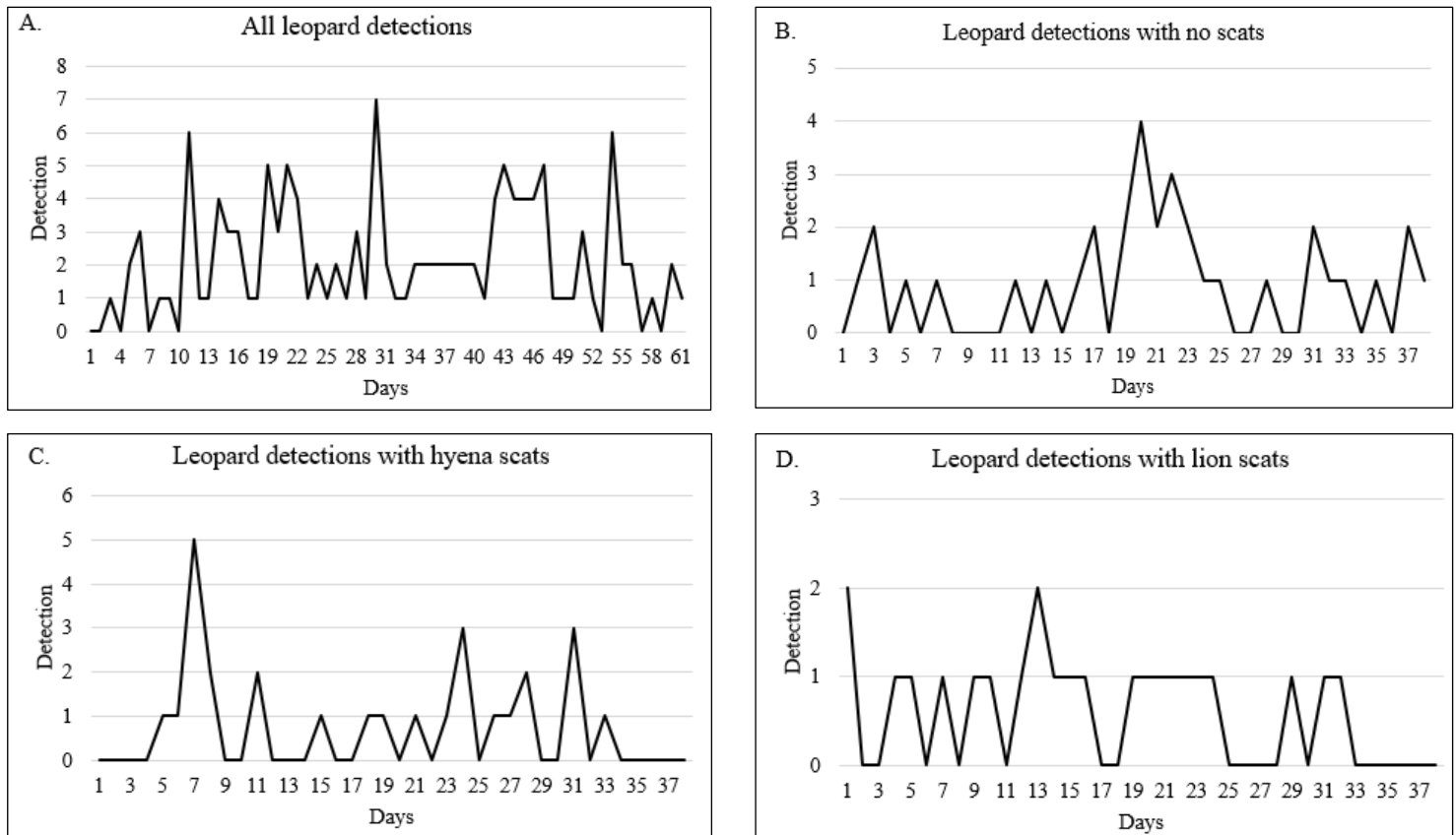


Figure 2.7. The number of independent photographic detections of leopard across sites during the 61-day survey period (A) and during the 38-day treatment period across control (B), hyena (C) and lion (D) sites respectively added no scats, hyena scats, or lion scats.

Overall, GLMEM accounting for visitor identity provided a better fit for data than GLM and other GLMEM (Tables 2.1 & 2.4). For the three selected models, relatively little variation exists between individuals (random effects < 1) and models displayed narrow confidence intervals around estimates (Table 2.5). Model 3a first showed that leopards came back more often where lion scats were added (coef=13.94) and less often where hyena scats or no scats were added (both coef=13.70) (Table 2.5). However, the relationship was non-significant ($p > 0.05$, $df = 47$) (Table 2.5) and *post hoc* Tukey test confirmed the insignificant difference in visit frequency for control-hyena ($z = 0.01$, $p = 1.00$), control-lion ($z = -0.85$, $p = 0.67$), and lion-hyena ($z = -0.78$, $p = 0.71$) pairs. Secondly, model 1a revealed a significant relationship between leopard stay duration at marking sites and the addition of lion scats ($p = 0.011$, $df = 119$). Leopards tended to

stay longer at marking sites with lion scats (coef=3.18s) than at control sites (coef=2.53s, $z=-2.54$, $p=0.030$) and sites with hyena scats (coef=2.89s, $z=-3.31$, $p=0.0027$) (Table 2.5). Visit stay was not significantly different between control and hyena groups ($z=1.47$, $p=0.31$). Last, model 2a indicated a significant relationship between olfactory activity duration with regards to the identity of current and previous visitors and treatment groups ($p<0.05$, $df=66$) (Table 2.5). Leopards performed olfactory activities for a longer time at sites with lion scats (coef=2.67s) than at control sites (coef=2.00s, $z=-2.50$, $p=0.03$) or hyena sites (coef=1.38s, $z=-3.15$, $p=0.005$) (Table 2.5). Leopards also performed shorter olfactory activities at hyena sites than control sites, but the difference was not significant ($z=1.68$, $p=0.21$) (Table 2.5).

Other carnivore observations

During game drives, large carnivore activity and competitive interactions were frequently witnessed. Two main prides of lions occupied the south and central part of the study area. The dominant lion male in the south got killed by another coalition during the survey, attracting nomadic lion males in the area. Smaller prides and nomadic lion coalitions of two also punctually moved through the study area. Hyena activity was also relatively intense throughout the study area, although density was not known. At least eight main hyena latrines were found. Kleptoparasitism of leopards by lions and hyenas as well as leopard infanticide by other leopard males, were observed multiple times. Camera traps further recorded multiple spotted hyenas alone or in a group moving past the monitored scent-marking sites without showing interest into the targeted objects or translocated scats. Lions were detected on five occasions walking past the marking sites (either alone or in a group, both males and females, and a cub). They did not show interest in the marking site or scats except for one male that investigated the targeted bush, scraped over the translocated lion scat, and sprayed the bush.

Table 2.5. Best models outputs displaying model reference (Ref), Akaike Index Criterion (AIC), degree of freedom (DF), model deviance (Dev), random effect's standard deviation (SD), coefficient estimates of fixed effects (control, hyena scats, lion scats groups) and their respective lower and upper bounds (LCI, UCI) for 95% confidence interval and t-value and p-value. Model 1a describes the time spent at marking sites by leopards based on both current and previous visitors' identity. Model 2a describes the duration of leopard olfactory activity given the identity of both current and previous visitors. Model 3a describes the visit interval of leopards at a marking site given the identity of the previous visitor.

Ref	AIC	DF	Dev.	SD random effect	Fixed effect coef.	LCI	UCI	t-value	p-value
1a	901	119	889	Previous visitor = 0.39	Control = 2.53	2.14	2.92	12.68	
				Current visitor = 0.25	Hyena = 2.89	1.31	3.04	-1.47	0.14
					Lion = 3.18	2.29	4.06	2.54	0.01
2a	443.7	66	431.7	Previous visitor = $2.8e^{-5}$	Control = 2.00	1.5	2.50	7.79	
				Current visitor = 0.41	Hyena = 1.38	0.16	2.60	0.93	0.09
					Lion = 2.67	1.65	3.69	0.01	0.01
3a	1543.7	47	1533.7	Previous visitor = 0.10	Control = 13.70	13.33	14.08	$2e^{-16}$	
					Hyena = 13.70	12.83	14.58	0.99	0.99
					Lion = 13.94	13.03	14.85	0.39	0.39

Finally, almost all camera trap sites recorded intense nocturnal small carnivore scent-marking activity during the control phase (small-spotted genet—*Genetta genetta*, white-tailed mongoose—*Ichneumia albicauda*, African wildcat—*Felis lybica*, side-striped jackal—*Canis adustus*). An apparent decrease in small carnivore activity seemingly occurred after the addition of lion scats at treatment sites.

Discussion

This study aimed at gaining insights into the olfactory interactions of leopards. To my knowledge, this is the first study monitoring scent-marking sites of African leopards in a mixed woodland savanna system in Africa (compared to most existing detailed observations in the Kalahari Desert, Table 1.2). I report strengthening, contrasting, and novel observations to previous studies (Eisenberg 1970, Schaller 1972, Hamilton 1976, Bailey 1993, Bothma and le Riche 1984, 1995). Firstly, most monitored marking sites that detected leopards were visited and marked on multiple occasions by at least two individuals. This reinforces the intraspecific communication role hypothesis of scent marking in leopards and supports the collective use of specific marking sites by leopards, as initially suggested by Eisenberg (1970) in the Wilpattu National Park, Sri Lanka and later re-observed by Bailey (1993) in the Kruger National Park, South Africa. However, these results contradict the conclusions of Bothma and le Riche (1984, 1995) in South Africa, Hamilton (1976) in Kenya, and Schaller (1972) in Tanzania, who found no consistency in the use of specific marking posts by leopards. Secondly, a cross-study comparison suggests a geographic variation of scent-marking behaviours in leopards between environment types (e.g., arid vs dense vegetation habitats) and is supported on multiple aspects. For instance, I recorded olfactory behaviours similar to those previously reported (Table 1.2), with rubbing, spraying, scraping and investigating scents being the most frequently observed, as in Rafiq et al. (2020). Nonetheless, studies in the Kalahari only reported trees as marking

posts of leopards (Table 1.2, Bothma 1998) and described high frequencies of tree clawing by leopards (Bothma and le Riche 1984, 1995, Bothma and Coertze 2004). Across game drive observations and camera trapping, tree clawing was only witnessed once in a subadult male. Observed marking sites of leopards were predominantly characterized by prominent deciduous thorn-free bushes or lower branches of trees, small shrubs, and grass on a gentle slope or side of termite mounds. Bailey (1993) also noticed a seasonal change in marking behaviours with more scrapes encountered during winters and more spraying events observed during summers. My study occurred at the end of winter; scraping was observed mostly during game drives but not as often as spraying behaviours. Thirdly, I found a significant relationship (90% confidence level) between scent-marked plant species across the sampled marking sites and whether leopards performed olfactory activities or walked past the bush. In particular, *Terminalia sericea* was marked or investigated on half of the leopard visits at such marking sites. Although observed plant species across game drives and monitored marking sites might not be representative of the entire vegetation of the study area, leopards did not exhibit a marking trend for aromatic plant species as observed by Grimbeek (1992) in the Waterberg. Other purposes than scent marking (e.g., shade, preferred area of prey species, good cover for ambush) could explain high detection rates of non-scent marking leopards at sites comprising, for example, *Combretum* spp. This explanation has also been suggested by Melzheimer et al. (2020) for non-scent marking cheetahs detected at marking sites in Namibia. Finally, Bothma and Coertze (2004) mostly reported male leopards scent marking in the Kalahari and rarely observed pregnant females and non-oestrus females at scent-marking sites. I recorded both male and female visits at marking sites relatively frequently in the SSGR. Additionally, females detected included both females in oestrus and females known to be denning scent marking or walking past the sites. It was difficult to evaluate oestrus status in females, and only four cases were confirmed: females rasped and/or scent marked more intensively, grabbing branches with their

forelegs, and assiduously rubbing their face, neck and back against the bush as described by Sadleir (1966), and/or were sighted soon after mating with males in the area (personal communications). These differences in the status and identity of marking individuals suggest that leopard scent-marking strategies vary with their density. Overall, comparing my observations to previous studies emphasizes a high plasticity in the scent-marking behaviours of leopards across their range, but this could also be owed to inconsistencies in monitoring techniques resulting in different conclusions.

Secondly, the study aimed at evaluating the effects of intra- and interspecific olfactory interactions on leopards. In particular, my study was interested in evaluating the relative importance of an olfactory landscape of fear and a dear enemy effect to assess the potential of scent marking in manipulating leopard movements. To my knowledge, this is the first *in situ* experimental study to investigate this question with leopards. My results first emphasized interesting patterns at the intraspecific level. The high visitation rate and scent-marking activity of individuals of any reproductive status suggest a greater importance of marking sites for territory advertising purposes than expected in females. Competition for territories is greater in an area of high leopard density, which explains why some marking sites were shared by individuals of the same sex and hence had no apparent reproductive function. Females occupy high-quality territories, whereas males distribute based on access to females (Bothma 1998). Thus, in high leopard density habitats, I hypothesize that not advertising an active presence and territory ownership exposes females to territory invasion from neighbouring individuals, or to take-overs by new immigrants. Additionally, in the western sector of SSGR, the male:female ratio was skewed towards males, contributing to the greater pressure exerted on females in the area (Balme and Hunter 2013). For example, during the study, males committed at least two infanticide events (personal observations). Thus, the greater risks associated with not advertising their territory in the SSGR would explain why females frequently visited marking

sites and scent marked proportionally more often than males, contrarily to those in the Kalahari (Bothma 1998).

On the other hand, males occupy larger territories. Advertising their dominant status and patrolling boundaries is energetically costly (Rafiq et al. 2020). I suggest that patrolling males pass through highly frequented leopard paths and marking sites but mainly react to females or intrusive male presence. This would explain why males did not engage in olfactory activities proportionally as often as females, although they visited sites more times than females. The higher frequencies of male-male (32%) and male-female/female-male (40%) olfactory interactions recorded, and the greater number of sites shared by both sexes (38%) further support this theory. Finally, a few sub-adults (~18% of detections) were detected walking by marking sites or only investigating bushes. I suggest that these individuals used scent-marking sites to gather information about the surrounding residents, their strengths and territories. This concurs with the small home range size and conspecific avoidance behaviour of sub-adult male leopards observed by Fattebert et al. (2013) on Phinda Private Game Reserve in South Africa. Similarly, this has been noted in sub-adult cheetahs at cheetah communication hubs in Phinda Private Game Reserve and Hluhluwe-iMfolozi Park, South Africa (Cornhill and Kerley 2020b). In essence, contrary to my second hypothesis, females strongly used marking sites for territory advertising, and males had a lower olfactory activity than expected.

The implication of marking sites in mating interactions of leopards was mostly suggested by the high proportions of consecutive male/female or female/male visits at marking sites. Oestrus scent marking was observed in females but could not be adequately quantified. Male leopards appeared to mainly use marking sites for asserting their dominant status and for reproductive purposes, as initially hypothesized. This behavioural strategy correlates the use of marking sites by other felids like male cheetahs in two South African game reserves (Cornhill and Kerley 2020b), male pumas (*Puma concolor*) in the USA (Allen et al. 2015), and male

tigers (*Panthera tigris*) in Nepal (Smith et al. 1989). Sub-adult male leopards visited marking sites less often than adults, as expected, and exhibited similar behaviours as sub-adult cheetahs, investigating sites to gather information. Additionally, more mature nomadic individuals or sub-adult male leopards investigating scents may also seek opportunistic mating chances by locating surrounding females approaching oestrus. Overall, my observations correlate a strong intraspecific dear enemy effect, whereby leopard individuals keep track of one another, and advertise their presence to facilitate reciprocal avoidance or availability for mating.

To evaluate the landscape of fear and dear enemy phenomena at the interspecific level, I simulated olfactory interactions with strong competitors using translocated lion and spotted hyena scats to scent-marking sites of leopards. I expected a delay in leopard return to treatment sites, but neither of the hyena scats or lion scats impacted the visit frequency of leopards. The experiments, therefore, did not verify the hypothesized deterrence effect of strong competitors (landscape of fear) on leopards. The behavioural response of leopards to lion scents differed from the repelled one of cheetahs in Cornhill and Kerley (2020a). Furthermore, lion scats caused leopards to stay significantly longer at marking sites and performed olfactory activity for a longer time. This again went contrary to my hypothesis. In the cheetah study of Cornhill and Kerley (2020a), cheetah behaviour remained the same at marking sites irrespective of the previous visitor species. However, the pattern observed with leopards matches behavioural responses of smaller carnivores like wild stoats (Banks et al. 2016) and red foxes (Garvey et al. 2016a) exposed to predator and strong competitor scents. Leopards displayed typical behaviours of a dear enemy reaction to lion scents, spending more time gathering information and reciprocally signalling their own presence. These findings concur with the absence of spatial displacement of leopards by lions observed in du Preez et al. (2014) and Miller et al. (2018). Hyena scats and control sites had no significant effects on leopards, meaning that the landscape of fear created by spotted hyenas' scents was insignificant on leopards under these

experimental settings. Overall, the data from my experimental outcomes did not validate any of my hypotheses at the interspecific level. The notion that sympatric, larger carnivores displace leopards spatially and instil fear in them is not supported by my data.

Finally, a few logistical constraints are worth mentioning. First, the behavioural response of leopards was only recorded when caught by camera traps. The use of GPS collared leopards would strongly facilitate monitoring the effects of specific scent marks on leopards and the use of their territory (Hamilton 1976). Measuring glucocorticoid levels in faeces of leopards before and after trials would also evaluate at a finer scale whether olfactory interactions with lions and spotted hyenas generate stress in leopards (Ganswind et al. 2018). Secondly, faeces were collected when available, but there was no means of controlling the identity (sex, reproductive status, social rank, group membership) of lions or hyenas. Further studies should investigate whether scents of a male and female lion or alpha and subdominant spotted hyena elicit the same behavioural responses in species like leopards. Inconsistencies in translocated scent marks of foreign wolves are given as a main hypothesis for wolves trespassing an olfactory biofence in the USA (Ausband et al. 2013). The synthesised scent of lion scats successfully deterred elephants (Valenta et al. 2020), and fresh scats of lions successfully repelled cheetahs from marking sites (Cornhill and Kerley 2020a). However, my experiments faced two main issues: African civets (*Civettictis civetta*) sometimes ate freshly added lion scats; hence some sites remained scat-free until I found and added some more; consistency of lion scats content and probably their chemical composition varied with the diet of lions. Scats containing a large amount of hair seemed to impact less leopards and be less attractive to civets. Finally, I used scats for the ease of collection, but spotted hyenas also strongly rely on anal pasting and lions on urine and sprays to mark their territories (Macdonald 1980). Ferrero et al. (2011) found that 2-phenylethylamine in urine of lions elicits avoidance in

prey species. More significant results could perhaps be obtained with artificial scent dispensers or means to translocate anal pastings of hyenas and marking fluids sprayed by lions.

To conclude, experiments revealed unexpected interaction patterns in the olfactory communication of leopards in the SSGR. At the intraspecific level, the study emphasized the essential social roles of scent-marking sites for leopards in high leopard density and dense vegetation habitats. Territory advertising appeared as an unprecedentedly recorded important purpose of scent marking in females. The oestrus signalling role of scent marking was also observed but could not be quantified. Males displayed an optimal use of marking sites for information gathering, reproduction and asserting dominance. Observed patterns were similar to those found by Bailey (1993) in the Kruger National Park. At the interspecific level, my study did not confirm the potential of olfactory interactions with lions and spotted hyenas to manipulate movements of leopards or mitigate human-leopard conflicts. Further trials under different leopard densities and experimental settings are needed. Natural sympatric intraguild olfactory interactions constitute interesting tools in wildlife management due to the low risks for species to habituate to these chemical compounds. Other repulsive sprays with capsaicin and other plant-based compounds, like those used against black bears (*Ursus americanus*) in North America (Rogers 1984), could also be explored in the form of scent-dispensers for farmer-carnivore conflict mitigation in Africa.

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CHAPTER 3

The density of leopards in the western sector of South Africa's Sabi Sands: does the presence of sympatric carnivore scents impact estimates?

Abstract

Monitoring large solitary carnivores is challenging, but accurate results are essential to conservation and management, especially when target species are subjected to persecution and hunting. Field biologists face time and cost constraints and must make optimal sampling decisions. Given the important role of scent marking and olfactory communication in the ecology of solitary carnivores, I suggested monitoring scent-marking sites of such a species and accounting for olfactory interactions with close competitors to estimate its density. I used the African leopard (*Panthera pardus*) as a model species and contrasted the number of detections, individuals recorded, and estimates of density (and associated levels of precision) between two surveys in the western sector of the Sabi Sands Game Reserve, South Africa. One survey monitored 43 scent-marking sites of leopards (scent survey, 1450 trap nights), and the other followed a standard camera trapping procedure with paired camera traps placed at 15 road junctions (control survey, 672 trap nights). During the scent survey, I simulated an active olfactory presence of lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) by artificially adding the species' scats to leopard scent-marking sites and recorded them as covariates. In the control survey, habitat types were recorded as covariates. Based on camera trap data, I estimated density using Spatially-Explicit-Capture-Recapture (SECR) models under both Bayesian and

maximum likelihood frameworks. Relative to the camera trapping effort, results across surveys and frameworks were similarly close. The control survey detected 14 individuals (8 males, 6 females) over 68 independent detections, and the scent survey detected 15 individuals (8 males, 7 females) over 119 independent detections. Five individuals detected differed between surveys, but overall proportions of male and female detections were not significantly different (Fisher exact two-tailed $p > 0.05$). Density estimates and precision of top models were also relatively close (12.81 ind/100 km² SE= +/-0.07, SD=2.93, for the control survey vs 11.89 ind/100 km², SE= +/-0.05, SD=2.46, for the scent survey). The null model (homogeneous detection probability and encounter frequency across sexes) was ranked as the best fit or one of the best fits for both surveys under both analytical approaches. Sex-specific encounter frequencies ranked amongst the best fits in the scent survey only, and neither the habitat types or olfactory interactions with lions and spotted hyenas influenced leopard densities in the study area. Overall, the density estimate from the scent survey indicated that the sampling design provided enough data for SECR analyses and precise results. Density estimates were the highest ever recorded in South Africa with SECR methods but parameters and results generally concorded with previous studies. However, the sex ratio outputs revealed that the scent survey mostly described the reproductive potential of the resident leopard population and detected fewer subadults (18% vs 38% in the control survey). The Bayesian approach also seemed more robust, and the control survey appeared more reliable and cost effective. I advise future studies to use standard camera trapping procedures to monitor a leopard population.

Key words: Bayesian analysis framework, camera trapping, cost-effective sampling design, intraguild olfactory interactions, maximum likelihood approach, SECR modelling.

Introduction

The monitoring of key regulating components in ecosystems is of particular importance in the Anthropocene crisis (Svenning et al. 2016, Munguía-Carrara 2020). In particular, large apex predators are of conservation concern and are drawing an increasing amount of research to estimate their population status and resilience to human disturbance (Svenning et al. 2016, Rich et al. 2017, Abade et al. 2018, Munguía-Carrara 2020). Global decreasing population trends are acknowledged, but a general lack of information is observed on the applied behavioural ecology of solitary carnivores worldwide (Gese 2001, Balme et al. 2014, Svenning et al. 2016, Rich et al. 2017). The African carnivore guild is considered one of the most functionally diverse and the last functionally intact sympatric predator assemblage left on Earth (Dalerum et al. 2009). The African leopard (*Panthera pardus*) is a member of this guild and is a technically challenging species to monitor and manage in many parts of its global range (Jacobson et al. 2016). It occurs at varying densities (maximum recorded in South Africa is 12.7 individuals/100 km², Kruger National Park, Maputla et al. 2013) and roams on large territories with significant populations living outside of protected habitats (Pitman 2012, Swanepoel et al. 2013). Leopards in these areas sometimes prey upon livestock and are shot by farmers in retaliation as well as for the perceived risks that they represent (Pitman 2012). Hence, leopards are central to socio-ecological conflicts in the region. Additionally, the species occupies an important place in the local beliefs and economy (e.g., cultural usage of body parts for ceremonies or medicinal purposes, fur trade, game viewing, trophy hunting, poaching) (Pitman 2012, Jacobson et al. 2016, Trouwborst et al. 2020). Therefore, obtaining accurate density estimates and understanding the distribution of leopards enable the design of adequate management strategies and facilitate the response to conflicts.

An overview of the diversity of methods available to estimate animal density reveals the difficulty of selecting cost-effective sampling methods and making management decisions

based on inter-technical reports comparison. Multiple field sampling methods have been tested. These include spoor and tracking census (Stander 1998, Thorn et al. 2010), faecal sampling for DNA analyses (Sugimoto et al. 2014), interviews of local communities (Taubmann et al. 2016), population counts with playbacks (Thorn et al. 2010), standard paired camera trapping procedures at road junctions (Balme et al. 2009), camera trapping with baits (du Preez et al. 2014), and camera trapping with scent lures (Braczkowski et al. 2016). Depending on data collection methods, statistical analyses result in density estimates that vary in their accuracy and degree of confidence (Balme et al. 2009, Thorn et al. 2010). Multiple candidate models further combine different assumptions, simulating realistic detection parameters to ensure adequate model fit and improve estimate precision (Burton et al. 2015, Meek et al. 2016). For example, Singh et al. (2014) accounted for sex-specific detection probabilities in the density analyses of tigers (*Panthera tigris*) in India. Havmøller et al. (2019) accounted for habitat types, prey abundance, and distances to human settlements and protected area boundaries to estimate leopard density in Tanzania. Gerber et al. (2010) incorporated, amongst others, the behavioural response of individuals (trap happy vs trap shy) to camera traps to improve density estimates of four carnivore species in Madagascar. Finally, Devens et al. (2021) accounted for spatial variations to explain leopard density across different land uses in South Africa. Variations of Capture-Mark-Recapture (CMR) and Spatially-Explicit-Capture-Recapture (SECR) models using camera trap data are the most frequently adopted analyses for cryptic species occurring at low densities like leopards and whose individuals are identifiable from patterns of the pelage (Gopaldaswamy 2006). These models rely on the ratio of capture rates (detection frequency of individuals triggering traps for the first time) to recapture rates (detection frequency of individuals already recorded at least once) over a given period to extrapolate the total number of individuals in an area (Efford 2011). SECR models incorporate spatial covariates such as land use or vegetation cover to account for heterogeneous species distribution across an area,

and best explain its density (Gopaldaswamy 2006). Moreover, the maximum likelihood and Bayesian frameworks are common ecological modelling approaches (Ellison 1996, Ward 2008). Based on observed probabilities of events, the maximum likelihood approach infers population parameters that maximise data fit (Ellison 1996). In contrast, the Bayesian approach incorporates both the observed probabilities and information already known about the analysed data (e.g., probability distribution) to compute the best fit for the data (Ellison 1996). Furthermore, criteria assessing the relative fit of models (e.g., AIC, loglikelihood) also change between studies (Ward 2008), and some methods provide additional information about the studied population. For example, tracking and the use of GPS radio-collars inform us about the distribution of individuals and territory usage (Hamilton 1976, Bothma and le Riche 1984, Bothma and Bothma 2012). Overall, technical considerations and options for estimating density can be overwhelming to field biologists and challenging to decision-makers. Field biologists also often experience time and cost constraints. They try to optimize data collection using ecologically meaningful methods for the species of interest but often neglect potentially significant effects of its interactions with community members (e.g., competitors) (Winterbach et al. 2014).

Using leopards as a model species, this study is motivated by investigating the potential of scent-marking sites to monitor the density of cryptic large carnivores. Olfactory communication holds a central place in the ecology of solitary carnivores (Wemmer and Scow 1977, Macdonald 1980, Rafiq et al. 2020). It serves both intraspecific interactions (mating, territory demarcation) (Macdonald 1980, Rafiq et al. 2020) and interspecific interactions (advertising its presence, facilitating avoidance) (Banks et al. 2016, Cornhill and Kerley 2020) and thus likely shapes the distribution and movements of leopards. In wildlife management, scents may increase the detection probability of cryptic and/or solitary species (Ferrerias et al. 2018), increasing their time in front of the camera traps (e.g., mustelids in Randler et al. 2020), and thereby improving

image quality and individual identification. In North America, puma (*Felis concolor*) communal scrape sites are also monitored to gain knowledge on the species' local population and emphasized conspicuous intra and interspecific effects of olfactory interactions on the carnivore community (Allen et al. 2015). Finally, intraspecific scent marks are successfully used to manipulate some carnivore species' movements (e.g., grey wolves—*Canis lupus*—in Ausband et al. (2013), and wild dogs—*Lycaon pictus*—in Jackson et al. (2012)). Since repulsive interspecific interactions have been observed between large African guild members (Palomares and Caro 1999, Rafiq 2016, Ramesh et al. 2017), this study was interested in whether their interspecific olfactory interactions had an impact on leopard density estimates, and whether monitoring leopard marking sites generated a different level of estimate precision compared to standard sampling procedures.

Therefore, this study aimed to determine: 1) the density of leopards in the western sector of the Sabi Sands Game Reserve, 2) if the presence of lions' and spotted hyenas' scents at leopard scent-marking sites affected the density estimates of leopards (the number of leopard detections overall, the individuals recorded, and the sex of leopards detected on camera traps).

Given the high leopard density (11.8 individual/100 km²) recorded in the central sector of the Sabi Sands Game Reserve (Balme et al. 2019), I expected similarly high densities of leopards in my study area too, mainly because of the spatial proximity (and lack of fences) and habitat similarities between the western and central sectors of the reserve.

Regarding the impact of intraguild competitors' scents on leopard density estimates, my hypothesis is based on the results of chapter 2. Since the scents of lions and spotted hyenas did not appear to repel leopards in chapter 2, then I expected the monitoring of scent-marking sites to provide relatively similar density estimate accuracy and precision compared to density results of a standard road survey.

Methods

Study area

The study was conducted in the Sabi Sands Game Reserve (hereafter SSGR), a protected area composed of 20 private properties and spanning over 625 km² (midpoint: 31°29' E, 24°49' S) in the Mpumalanga Lowveld, South Africa (Balme et al. 2013). A first survey encompassed the six properties (*ca* 100 km²) of SSGR western sector (Dulini/Exeter, Leopard Hills/Ululapa, Savanna, Inyati, Idube, and Ulusaba), and a second survey only covered about 67 km² of the western sector (excluding Inyati and Ulusaba) (Figure 3.1).

The area formally served game shooting, ranching, and farming activities (Fortescue 1997). Since 1948, it has been an established game reserve and managed for the conservation of wildlife (Balme et al. 2013, SSW 2017). Internal fences were removed, and remnants of previous exploitations (e.g., waterholes) now contribute to wildlife management (Smith and Fitchett 2020). Locally extinct characteristic species of the ecosystem like cheetahs (*Acinonyx jubatus*), nyalas (*Tragelaphus angasii*), white rhinos (*Ceratotherium simum*) and elephants (*Loxodonta africana*) were further reintroduced since the 1970s (Spenceley 2005). SSGR has 35-40 on-site lodges, multiple dust roads cross the reserve for eco-tourism purposes (Schmidtz and Willott 2012), and animals are relatively accustomed to the presence of game drive vehicles (Pirie et al. 2014). Additionally, the northern fences with the Manyeleti Game Reserve (230 km²) and eastern and southern fences with the Kruger National Park (19 485 km²) were removed, enabling large-scale dispersal and population dynamics of wildlife (Fortescue 1997, Balme et al. 2013, Figure 3.1). Only an electric fence bounds the western sector from communal lands on the western side (Fortescue 1997).

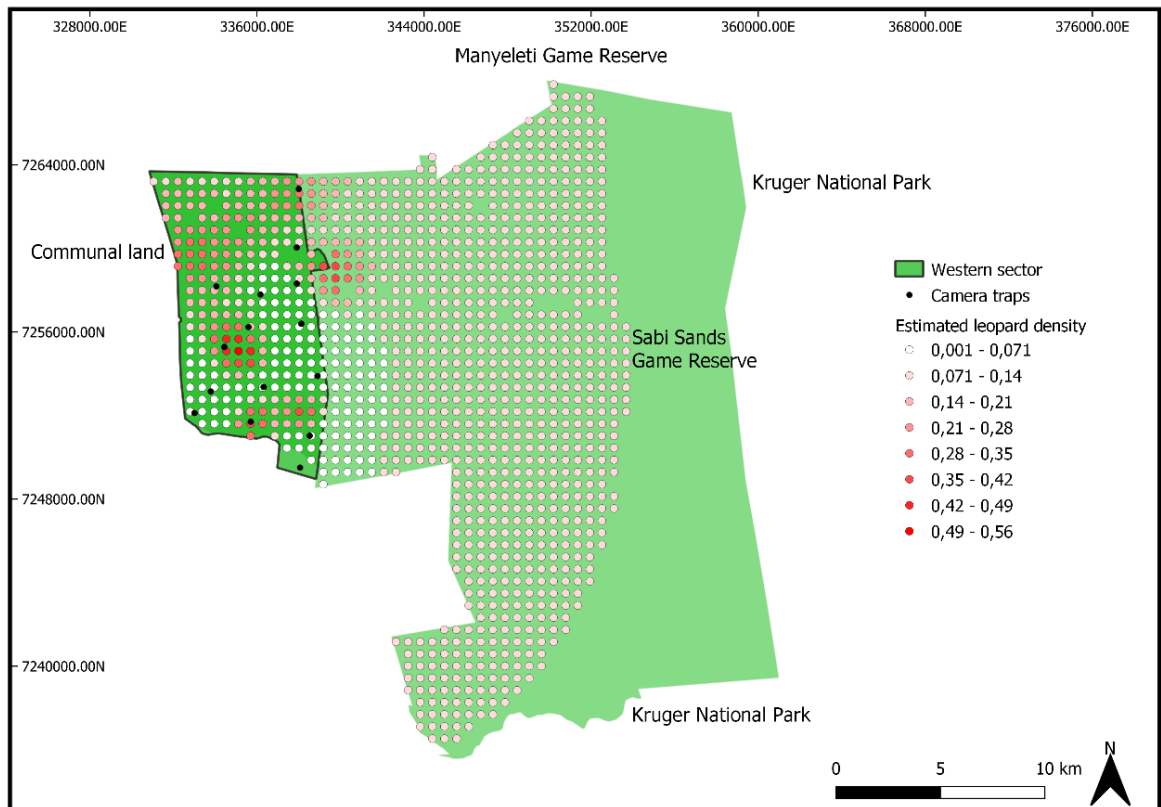
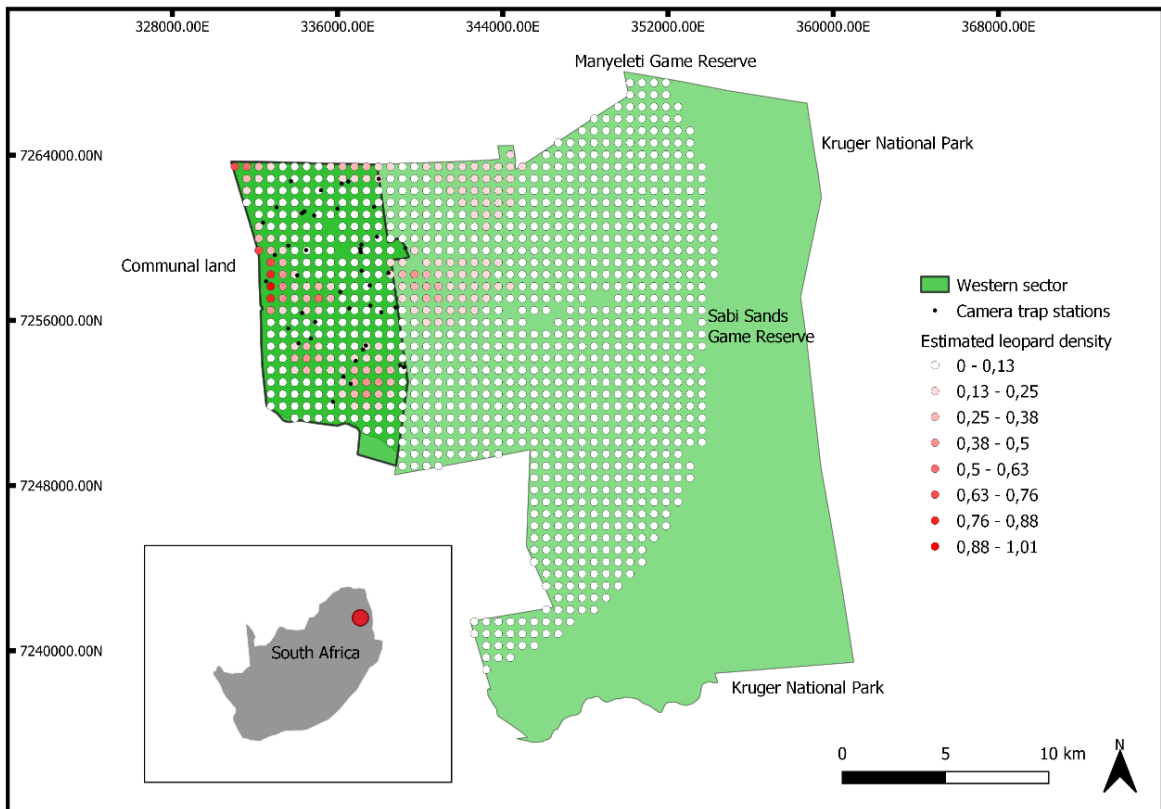


Figure 3.1. The western sector of the Sabi Sands Game Reserve, showing camera trap deployment and pixelated leopard density for the scent survey (top map) and control survey (bottom map), and surrounding areas.

The region is dominated by a savanna ecosystem with relatively flat terrains and is scattered with rocky outcrops and patches of woodland and grassland (Rutherford et al. 2006, Smith and Fitchett 2020). The Sabie River flows year-round along the SSGR southern boundary. The Sand River flows diagonally across the reserve, joins the Sabie River in the southeast area, and may dry in winter (Smith and Fitchett 2020). The local climate is semi-arid; winters are dry (April-September), and summers are warm and rainy (October-March) (Fortescue 1997). Mean annual temperatures vary between 19°C and 33°C, and annual precipitation averages 650 mm (Balme et al. 2013). Common vegetation includes *Panicum maximum* and *Digitaria eriantha* grasses, *Vachellia* spp. trees and *Diospyros mespiliformis*, and *Euclea divinorum* and *Combretum* spp. shrubs (Rutherford et al. 2006). Mammal diversity is particularly high and includes charismatic African species like zebra (*Equus quagga*), giraffe (*Giraffa camelopardalis*), spotted hyena, impala (*Aepyceros melampus*), and the endangered wild dog (*Lycaon pictus*) as well as the Big5 (lion, leopard, elephant, buffalo – *Syncerus caffer*, and black rhino – *Diceros bicornis*) (Smith and Fitchett 2020). SSGR is also famous for its detailed knowledge and high density of leopards (11.8 individuals/100 km²) (Balme et al. 2013, Balme et al. 2019).

Data collection

I implemented two consecutive surveys to estimate leopard density in the western sector of the SSGR. An olfactory survey ran over 61 days (May 3rd to July 2nd, 2021) with 32 camera traps (10 Browning Recon Force Advantage – Model BTC-7A and 22 Bushnell Trophy Cam HD – Model 119537) moved between 43 leopard scent-marking sites and covering *ca* 100 km² (Figure 3.1). Lion scats, spotted hyena scats, or no scats were respectively added to camera trapping sites over three periods of 12 days. Scats were freshly collected in the study area and frozen in hermetically sealed plastic bags until deposited at sites. The second survey lasted 46 days (July 6th to August 20th, 2021) and followed a standard density estimate protocol with 15 paired camera traps located on road junctions and spread across *ca* 67 km² using a 5-km² grid

(Figure 3.1). I acknowledge the relatively small sample size. However, given the absence of internal fences within the study area and based on leopard observations during game drives, I estimated that the trap layout was sufficient to extend the density results to the whole western sector (100 km²). Depending on the survey, camera traps were set to record 1-minute videos or three rapid shot-fire photos per trigger with 30 s between consecutive triggers. In both surveys, cameras were positioned at about 40 cm height to capture leopards' flanks, and checked every five days to ensure that wildlife had not interfered with them (break, knock over or move traps), collect SD cards, and replace batteries.

Simultaneously as I ran the surveys, I gathered information on leopards during game drives and knowledge of the rangers and trackers in the study area that would help with further identification of camera trap images (sex, relative age, territory, particularities, and flank photos of the different individuals).

Data analyses

Using CameraBase software (Tobler 2015, version 1.7, www.atrium-biodiversity.org) and previously collected flank photos and information, I sorted camera trap data, sexed individuals (conspicuous genitalia, swollen nipples, dewlap size, shoulder width, Balme et al. 2012) and identified all leopard individuals from rosettes of the pelage and specific traits (Sunquist and Sunquist 2002). I set the independence criterion at 24h for consecutive detections of an individual at the same camera trap site (one detection per calendar day) and only accounted for identified and independent individuals. Given the short-time periods of surveys relative to a leopard's lifespan, I performed statistical analyses assuming a closed population. Fisher exact tests compared the proportions of males and females between surveys.

Spatially-Explicit-Capture-Recapture (SECR) analyses require three input files: detection history (leopard ID, sex, sampling occasion, and camera trap station per detection), camera trap

deployment history, and suitable home range centres for leopards in the study area. I used QGIS software (version 3.2, QGIS Development Team 2020) to model all potential leopard home range centres per survey. To do so, I added a 15-km buffer around the camera trapping grids as recommended for large felids (Gopaldaswamy et al. 2012). Then, I created a raster displaying all home range centre points spaced at 578 m intervals (0.336 km²) to ensure that the analyses accounted for a maximum of suitable leopard areas and did not exclude individuals occupying small territories (Athreya et al. 2013, Braczkowski et al. 2016). Finally, following Athreya et al. (2013), I deleted home range centres that fell into non-suitable habitats for leopards (communal land, water bodies and fence line).

Secondly, I computed SECR models on R (version 3.4.3, R Core Team 2017) and used both Bayesian and maximum likelihood methods to compare the results of each survey. Following Hedges et al. (2015) for Bayesian analyses, I built four models, each using four Markov Chain Monte Carlo (MCMC) simulations, a sampling approach that approximates the posterior distribution for the unknown probabilities (Gopaldaswamy et al. 2012). Variations in three parameters (density of leopards per hectare (D), encounter frequency of leopards at home range centres (g_0), decreasing leopard detection probability with increasing distance from home range centres (σ)) enable to simulate heterogeneous detection probabilities (Efford 2015)). Models respectively simulated 1) constant encounter frequency of leopards and constant probability of detection throughout the area and across sexes; 2) constant encounter frequency of leopards across sexes but sex-specific probability of detection as the distance from home range centres increased; 3) constant probability of detection across sexes given increasing distance from home range centres but sex-specific encounter frequency of leopards at home range centres; 4) sex-specific probability of detection given increasing distance from home range centres and sex-specific encounter frequency of leopards at home range centres. Model fits were first assessed looking at Bayesian p-values (neither too close to 0 or 1), Geweke parameters (should range

between [-1.64;1.64]), and Gelman-Rubin parameters (should be close to 1) (Gelman and Rubin 1992, Geweke 1992). Finally, I selected the best model based on estimated marginal maximum likelihood values (Table 3.2). Density estimates were obtained by dividing abundance numbers by the effective sampled area respectively for control and scent surveys.

For maximum-likelihood estimations, I used the secr package from R (Efford 2015) and ran the same four models as for the Bayesian analyses. Additionally, I included a fifth model that accounted for varying leopard detection frequencies with either broad habitat type around camera traps (savanna, guarri thicket, *Terminalia sericea* thicket, *Combretum* spp. and *Terminalia* thicket, open woodland, dense river bed, grassland-riverine ecotone) or scats added (treatment groups) depending on the survey, and homogeneous detection probability across habitat type or treatment groups. Models were evaluated and compared with Akaike Index Criterion corrected for small sample size (AICc). The model with lowest AICc was chosen as top model and models with $\Delta AIC > 2$ were considered to be significantly different.

Results

Detections and sampling effort

A total of 68 independent leopard detection events (8 males detected 39 times with 31 recaptures, 6 females detected 29 times with 23 recaptures) were recorded over a sampling effort of 672 camera trapping nights for the control survey, and 119 independent leopard detection events (8 males detected 63 times with 55 recaptures, 7 females detected 56 times with 49 recaptures) were recorded over a sampling effort of 1450 nights for the scent survey (Table 3.1). The proportions of males and females were not significantly different between surveys (Fisher exact two-tailed $p > 0.05$). Subadults represented ~18% of detections in the scent survey (~7% for subadult males) and ~38% in the control survey (~18% for subadult males). Both surveys detected six male and six female individuals, two males were only detected in the

control survey, and two males and one female were only detected in the scent survey. Additionally, 11 and 7 leopard images could not be identified in the control and scent surveys, respectively, and one cub was detected in each survey.

Assessing Bayesian and likelihood models

Across both surveys, all models reached convergence and showed a measure of acceptable fit. Gelman potential shrink factor estimates were < 1.5 , Geweke convergence parameters ranged between -1.64 and 1.64 , and p-values were all between 0.1 and 0.9 (Table 3.2). Covariance plots also showed no significant correlation between parameters. Maximum likelihood analyses showed that demographic closure was met during the control survey ($p > 0.05$) but was unexpectedly violated for the scent survey ($p < 0.05$). Finally, both Bayesian and maximum likelihood frameworks indicated that model 1 (null model) for the control survey (marginal likelihood = $-52\,967.096$, AICc = 208.298) and model 3 (heterogeneous encounter frequency between males and females but homogeneous detection probability) for the scent survey (marginal likelihood = $-78\,299.784$, AICc = 471.889) provided the best fit for the respective data sets (Table 3.2). Additionally, model 3 was the second best fit for the control survey (marginal likelihood = $-52\,992.312$, $\Delta\text{AIC} = 1.934$) under both Bayesian and likelihood approaches (Tables 3.3 & 3.4). For the scent survey, the second-best fit was model 4 (sex-specific detection frequency and encounter probability) with the likelihood analysis ($\Delta\text{AIC} = 0.532$) (Table 3.4) and model 1 with the Bayesian analysis (marginal likelihood = $-78\,330.409$) (Table 3.2). Therefore, I reported both top and second-best model estimates when they differed significantly. Finally, both control and scent surveys gave the fifth model (encounter frequency as a function of habitat type or treatment groups) as the least fitting for the respective data sets.

Table 3.1. Total detections and independent recaptures (1 calendar day) of independent leopard individuals (>18 months old) during the control survey and scent survey, respectively. Control survey used 15 paired camera traps located at road junctions in the western sector of the SSGR between July 6th and August 20th, 2021. Scent survey used 32 camera traps located at 43 leopard marking sites in the western sector of the SSGR between May 3rd and July 2nd, 2021. Individual IDs are different between survey.

Survey	Leopard ID	Detections	Recaptures
Control	M1	3	2
	M2	5	4
	M3	9	8
	M4	2	1
	M5	4	3
	M6	8	7
	M7	6	5
	M8	2	1
	F9	3	2
	F10	5	4
	F11	9	8
	F12	8	7
	F13	3	2
	F14	1	0
Scent	M1	15	14
	M2	1	0
	M3	6	5
	M4	5	4
	M5	21	20
	M6	3	2
	M7	11	10
	M8	1	0
	F9	7	6
	F10	16	15
	F11	9	8
	F12	4	3
	F13	1	0
	F14	10	9
	F15	9	8

Table 3.2. Bayesian SECR parameter outputs for leopard density estimation in the SSGR.

Survey	Model	Bayesian p	Ln (marginal likelihood)	Total iterations	Burn-in to converge
Control	1	0.455	-52967.096	40000	5000
	2	0.504	-57061.176	100000	5000
	3	0.500	-52992.312	40000	5000
	4	0.520	-57928.4	100000	5000
Scent	1	0.757	-78330.409	40000	5000
	2	0.840	-102628.23	40000	5000
	3	0.784	-78299.784	100000	5000
	4	0.834	-100928.73	40000	5000

Leopard estimates – Bayesian framework

The regional leopard abundance for the study area and its 15-km buffer was 49.57 leopards (SD=11.39) for the control survey, and 44.78 (SD=9.45) for the scent survey. Pixel density estimates at home range centres ranged from 0.0007 to 1.008, accounting for the 15 km buffered area and both surveys (Figure 3.1). The best fitting models estimated 12.81 leopards (+/-0.07) per 100 km² (posterior SD=2.93; Highest Posterior Density (HPD) 95% confidence interval=[7.72;18.79]) for the control survey and 11.89 individuals (+/- 0.05) per 100 km² (posterior SD=2.52; HPD 95% confidence interval=[6.92;16.50]) for the scent survey (Table 3.3).

The selected models did not discern male from female detection probabilities. Hence, the movement index (σ) remained the same across sexes, 1.80 km (posterior SD=0.18; HPD 95% confidence interval=[1.45;2.17]) for the control survey, and 2.03 km (posterior SD=0.16; HPD 95% confidence interval=[1.73;2.33]) for the scent survey (Table 3.3). Less fitted models

showed a maximal range of 0.74 km and 1.47 km between male and female movements for the control and scent surveys, respectively.

The encounter probability was 0.05 (+/-0.00 SE) for the control survey and 0.05 (+/- 0.00 SE) and 0.03 (+/- 0.00 SE) for models 3 and 1 respectively of the scent survey (Table 3.3). Finally, the female to male ratio was 0.75:1 for the control survey, and 6.14:1 for model 3 and 0.89:1 for the null model of the scent survey.

Leopard estimates – Maximum likelihood framework

The regional leopard abundance for the study area and its 15-km buffer was 47.23 leopards (+/- 10.90) for the control survey, and 42.79 (+/-9.06) for the scent survey. The control survey resulted in 12.16 leopards (+/-3.32) per 100 km² (95% CI=[7.19;20.55]) and 11.98 leopards (+/- 3.20) per 100 km² (95% CI=[7.16;20.05]) for the top model and 12.05 leopards (+/-0.05) per 100 km² (95% CI=[7.20;20.17]) for the second-best model of the scent survey (Table 3.4).

The movement parameter was 1.78 km (+/-0.17) and 1.60 km (+/-0.19) for top and second-best models respectively of the control survey. Sigma was 1.27 km (+/-0.12) for the top model and 1.15 km (+/-0.11) for the second-best model of the scent survey. The capture probability was 0.05 (+/- 0.01) for the control survey and 0.4 (+/-0.01) or 0.5 (+/- 0.01) depending on best or second-best models, respectively, for the scent survey.

Table 3.3. Parameter outputs for the posterior distribution of leopard SECR density estimates in the SSGR. Sigma and Sigma2 are expressed in km and density in individuals/100 km².

Survey	#	Sigma		Sigma2		Lam0		Beta sex		Psi		Psi sex		Nsuper		Theta (θ)		Density	
		Coef	SD	Coef	SD	Coef	SD	Coef	SD	Coef	SD	Coef	SD	Coef	SD	Coef	SD	Coef	SD
Control	1	1.80	0.18	1.80	0.18	0.05	0.01	0.00	0.00	0.07	0.02	0.43	0.15	49.78	11.38	1.00	0.00	12.81	2.93
	2	2.14	0.58	1.74	0.20	0.05	0.01	0.00	0.00	0.07	0.02	0.88	0.11	50.77	11.68	1.00	0.00	13.06	3.01
	3	1.81	0.18	1.81	0.18	0.05	0.01	-0.09	0.32	0.07	0.02	0.43	0.15	50.23	11.39	1.00	0.00	12.93	2.93
	4	2.45	0.82	1.74	0.21	0.04	0.03	0.37	0.63	0.07	0.02	0.88	0.11	50.86	11.87	1.00	0.00	13.09	3.06
Scent	1	2.00	0.14	2.00	0.14	0.03	0.00	0.00	0.00	0.06	0.02	0.47	0.14	44.65	9.26	1.00	0.00	11.88	2.46
	2	2.51	0.29	1.26	0.11	0.04	0.01	0.00	0.00	0.08	0.02	0.61	0.13	55.87	12.31	1.00	0.00	14.87	3.28
	3	2.03	0.16	2.03	0.16	0.05	0.02	-0.53	0.34	0.06	0.02	0.86	0.10	44.69	9.48	1.00	0.00	11.89	2.52
	4	2.61	0.31	1.14	0.11	0.03	0.01	0.53	0.29	0.08	0.02	0.61	0.13	55.81	12.44	1.00	0.00	14.85	3.31

Table 3.4. Maximum likelihood outputs including density of leopards/100 km², encounter probability $g(0)$, sigma in km (parameter of decaying detection with increased distance from activity centres), AICc and ΔAIC .

Survey	Model	Density (SE)	$g(0)$ (SE)	Sigma (SE)	AICc	ΔAIC
Control	1	12.16 (3.32)	0.05 (0.01)	1.78 (0.17)	208.298	0.000
	2	12.15 (3.31)	0.05 (0.01)	1.78 (0.17)	211.507	3.209
	3	12.19 (3.33)	0.05 (0.01)	1.60 (0.19)	210.232	1.934
	4	12.20 (3.33)	0.06 (0.02)	1.53 (0.21)	213.968	5.670
	5	12.01 (3.28)	0.01 (0.01)	1.76 (0.17)	252.267	43.969
Scent	1	11.39 (2.97)	0.03 (0.01)	1.99 (0.14)	493.844	21.955
	2	11.43 (2.99)	0.03 (0.01)	1.98 (0.1)	495.236	23.347
	3	11.98 (3.20)	0.04 (0.01)	1.27 (0.12)	471.889	0.000
	4	12.05 (3.22)	0.05 (0.01)	1.15 (0.11)	472.241	0.352
	5	11.40 (2.97)	0.02 (0.01)	1.99 (0.14)	497.495	25.606

Discussion

The study aimed to estimate the density of leopards in the Sabi Sands Game Reserve's western sector and assess the effect of intraguild olfactory interactions on estimates and their precision. I compared a standard paired camera trapping survey at road junctions to a survey monitoring scent-marking sites of leopards through Bayesian and maximum likelihood frameworks. To my knowledge, this is the first study to test scent-marking sites as camera trapping locations to estimate leopard density. Despite different sampling procedures, final estimates were almost identical, as were the measures of precision. The scent survey resulted in 11.89 leopards/100 km² (+/- 0.07), and the control survey stabilised at 12.81 leopards/100 km² (+/- 0.05) under the Bayesian approach. Estimates were almost equivalent under the maximum likelihood approach. This is the highest density of leopards with SECR methods ever reported across a diversity of habitats in South Africa. For comparison, other SECR estimates

include 4.56/100 km² (+/- 1.35) in Lapalala and Welgevonden protected areas, Limpopo (Swanepoel et al. 2015), 0.35/100 km² (+/- 1.2) across 30 000 km² in the Western Cape (Devens et al. 2021), and 11.11/100 km² (+/-1.31) in the Phinda-Mkhuze Complex, KwaZulu-Natal (Balme et al. 2010). Maputla et al. (2013) reported 12.7 leopards/100 km² in the Kruger National Park but only relied on capture-recapture models. Other census reports exist on leopards in the SSGR (Panthera 2021), but this is the first one modelling leopard density using Bayesian and maximum likelihood frameworks for the western sector. The NGO Panthera frequently monitors and updates on-site lodges on population counts of large carnivores in the SSGR. They reported 78 individuals with a mean density of 12 individuals/100 km² in winter 2021 across the reserve (Panthera 2021). My results also closely compare to 11.8 leopards/100 km² (+/-2.6) found by Balme et al. (2019) in the central sector (adjacent to the western sector) of the SSGR using both Bayesian and maximum likelihood approaches. Furthermore, Balme et al. (2019) concluded that the sex and age of leopards did not significantly impact detection rates, which supports the selection of null models here. Finally, other than the closeness of my estimates together and with Balme et al.'s (2019), I am relatively confident in these results given the high number of recaptures in both surveys (104 in the scent survey and 54 in the control survey). Thus, I support Balme et al.'s (2019) thoughts on the SSGR combining particularly suitable conditions for leopards (e.g., low human disturbance, high prey abundance, suitable vegetation cover) to explain the contrasting densities observed between the study area and those found across leopard's range in South Africa. Estimate-wise, both scent and control survey methods appeared equivalent under both Bayesian and maximum likelihood frameworks. Using road junctions might be more cost effective because of the ease of locating these areas compared to scent-marking sites of leopards, and it provided a close estimate for a shorter sampling period (46 days vs 61 days).

Looking at parameters and assumptions underlying models helps to assess the relative suitability of survey types. First, the control survey analyses simulated the effect of habitat type on encounter rates of leopards, whereas the scent survey simulated the effect of sympatric strong competitors' scents (lions and spotted hyenas) on leopards. Both simulations ranked last in terms of model fit, indicating no significant relationships between leopard detection and these covariates. This concurs with Miller et al. (2018), who reported no significant effects of lions on the distribution of leopards across ten study sites in South Africa. Balme et al. (2019) also concluded that leopards of the SSGR do not spatially avoid lions. Havmøller et al. (2019) further found no support for habitat effect on the leopard density in the Udzungwa Mountains, Tanzania. However, contrastingly, Mann et al. (2020) found significant support for the distribution of leopards along habitat and vegetation (NDVI) gradients in the Little Karoo, South Africa. The study reported high leopard concentrations in subtropical thicket habitat and lower concentrations away from riverine areas. Balme et al. (2019) recorded a preference of leopards for dense vegetation areas in the SSGR and a positive relationship between spotted hyena and leopard detections. Ramesh et al. (2017) and Comley (2021) also reported a negative relationship between leopard and lion densities in KwaZulu-Natal, South Africa. Overall, although there is support in the literature for my results and model rejection, a finer spatial sampling scale with more camera traps would have perhaps improved the fit and accuracy of my models. Secondly, the computed sex ratios differ significantly between and within surveys. In the control survey, the top model gave a female to male ratio of 0.75:1, whereas the least fitting models indicated a ratio of 7.33:1. In the scent survey, the top model gave a female to male ratio of 6.14:1, whereas the second-best model gave 0.89:1, and the least fitting one gives 1.56:1. This may be due to model assumptions. The top model for the control survey and the second-best model for the scent survey (model 1) assumed homogeneous capture and encounter rates between leopards, whereas the top model for the scent survey (model 3) assumed a

difference in encounter rates between males and females. However, it could also be representative of differences in the sampling methods. Males generally move over greater distances (Bailey 1993) and more along roads (Balme et al. 2009) than females. Thus, camera traps placed at marking sites are less likely to capture males than females, whereas traps at road junctions are more likely to capture males and underestimate females' presence. Additionally, scent-marking sites serve territory advertising and mating purposes and hence are mostly visited by sexually mature individuals. Observations during game drives revealed an important proportion of male subadults that were captured relatively more often during the control survey than the scent survey (~18% vs ~7% of the detections). In sum, the high female to male sex ratio displayed in the scent survey might be an artefact of model 3 assumptions or due to the survey methods. The control survey seemed to generate a more accurate and representative sex ratio of the resident leopard population. Irrespectively of the model's approximations, this ratio appeared relatively low (0.75:1). Characteristic female:male ratio of leopards ranges around 1-1.8 females per male (Friedmann and Traylor-Holzer 2008). Nonetheless, lower and higher ratios were also recorded in Welgevonden (2.60:1) and Lapalala (0.70:1) protected areas (Swanepoel et al. 2015). In the SSGR, it is also possible that the population is in a transition phase between old male territory owners and the many younger nomadic males observed opportunistically waiting to take over a territory. Finally, the index of movement (σ) described typical home range patterns for solitary felids, with males travelling about twice the distance moved by females (Bailey 1993). Overall, as expected, olfactory interactions with guild members did not strongly impact leopards' density estimates, and the precision of estimates was similar to the control survey. However, marking sites seemed to provide more information on sexually mature territorial individuals than the overall population of independent leopards. For future studies interested in leopard density of an area, I advise

monitoring road junctions as they are more cost-effective and representative of the entire population.

Finally, neither *in situ* fieldwork nor ecological modelling are infallible; they only provide approximations of natural patterns. A few aspects should thus be kept in mind while interpreting my results. First, sampling efforts and procedures differed between surveys. Only two thirds of the western sector could be sampled during the control survey. The number of traps deployed, their layouts and time deployed were also different between surveys. The overall smaller sampling effort of the control survey potentially resulted in a slight overestimation of leopards. I do not think that the addition of scats during the scent survey significantly impacted the results, but it might have caused leopards to stay longer in front of the traps to investigate the scents and enabled a greater number of image identification than during the control survey. Secondly, maximum likelihood analyses unexpectedly indicated the violation of the closed population assumption during the scent survey. Although estimates looked realistic, results for this survey are to be interpreted with caution. Given the short time span of the survey, that no deaths of independent adults occurred during the study time, and that only one new individual was captured once at the border of the study area, other factors may be responsible for violating the closure assumption. I predict that either a fewer amounts of recaptures, especially of subadult males, occurred at marking sites, or that some individuals remained undetected for longer periods than expected by the model simulations. For instance, some individuals may have stayed several days at a kill site, hence not triggering any traps, which can significantly impact a survey of a short time length (Otis et al. 1978). Bayesian analyses accounted for an edge effect using a 15 km buffer. Thus, the framework should be relatively robust to the violation assumption of the population closure (Chandler et al. 2011, Athreya et al. 2013), and I am relatively confident that Bayesian estimations were not too significantly impacted. Finally, during modelling, I did not account for the behavioural response

of individuals to traps. It is possible that some effects have been neglected in the density analyses. Potential home range centres are also approximations based on best guess. For example, areas considered non-suitable home range centres were deleted, such as those falling in the fence line or dams in the southern tip of the western sector. In appearance, it created an area of non-suitable habitat, but a few leopards were recorded by the trap close-by.

To conclude, this study was the first SECR modelling attempt for the density of leopards in the western sector of the Sabi Sands. Averaged density across the surveys' top models is 12.35 leopards/100 km². It is the highest reported in South Africa with SECR methods but concurs with estimates obtained with similar analytical frameworks in the adjacent sector of the SSGR. The difference between control and scent survey estimates was neglectable and confirmed my hypotheses. The control survey appeared more reliable than the scent survey, although it may suffer a slight overestimation. The scent survey constituted a better tool for monitoring the reproductive potential of the leopard population rather than the entire population of independent individuals. A few discrepancies between models and survey parameters could be attributed to differences in model assumptions, sampling efforts, and camera trapping procedures. Finally, my results indicated no significant impacts of habitat types or of leopards' olfactory interactions with lions or spotted hyenas on leopard density estimates. This study is important to biologists and ecologists working on big cats, as camera traps are often used to monitor such species. Deciding where to set up traps is not always evident or immediate, although fieldwork time must be cost effective. My study helps to optimize and improve data collection. A uniform methodology, a finer spatial scale, and the use of GPS collars could, however, significantly improve the accuracy of my results.

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CHAPTER 4

Conclusions

Overall, this thesis was motivated by characterizing two aspects of the leopard population of the western sector of the Sabi Sands Game Reserve: its olfactory behavioural ecology and its density (and the relationship between the two).

First, chapter 1 provided a detailed background on olfactory communication in animals, carnivores, and leopards. It detailed case studies and resulting theories on scent marking that served to build up hypotheses for chapter 2. As the first study to monitor leopard scent-marking sites with camera traps, chapter 2 brought support to the previous scent-marking observations and hypothesized roles of scent marking reported in the literature of leopards. Leopards mostly rubbed and sprayed prominent thorn-free bushes and trees (especially *T. sericea* in my study), scraped grass patches and investigated scents. Adults constituted the majority of scent-marking individuals. I interpreted scent marking as facilitating mating encounters and avoiding risky encounters through territory advertising. This interpretation matches past reports' understanding of leopard olfactory communication (e.g., Bailey 1993, Bothma and le Riche 1984, Bothma and Coertze 2004, Rafiq et al. 2020). However, I provided the first evidence for the important role of olfactory territory advertising in females of any reproductive status. So far, only reports of females in oestrus scent marking existed. I recorded females in oestrus and females with cubs or known to be denning, frequently scent marking. Moreover, comparing chapter 2 results with chapter 1 literature review suggested a geographical and potentially demographic variation in the olfactory behaviours of leopards. Most extant reports originated from Kalahari studies, where large carnivores' environmental conditions and density are remarkably different from the Sabi Sands area. In comparison to those reports, leopards of the

Sabi Sands behaved more like those of the Kruger National Park observed by Bailey (1993). They rarely used scratching trees to interact, and preferentially rubbed and sprayed bushes, scraped the ground, and investigated scents. Thus, ethologically, this research reported a more diverse range of olfactory behaviours exhibited by leopards of the Sabi Sands Game Reserve compared to leopards of the Kalahari Desert.

Secondly, I implemented the first *in situ* manipulative experiments of leopards with lions' and spotted hyenas' scats. As detailed in chapter 1, similar experiments on cheetahs with lion and leopard scats (Cornhill and Kerley 2020), on elephants with lion scats (Valenta et al. 2020), on wolves with foreign wolves' scent marks (Ausbands et al. 2013), and on wild dogs with foreign wild dogs' scent marks (Jackson et al. 2012) successfully manipulated the movements of the target carnivore species. Chapter 2 and 3 partly aimed at testing the deterrent effect of lions' and spotted hyenas' scents on leopards, as hypothesized in chapter 1. My experiments failed at confirming this hypothesis. Both chapters 2 and 3 indicated that leopards did not behave significantly differently in the presence or absence of fresh intraguild competitors' scents. Chapter 2 specifically showed no significant difference in the behavioural response of leopards between sites with no scats and sites with scats of spotted hyenas. Nonetheless, the trials provided important insights into the dynamics and place of leopards amongst the top predators of the Sabi Sands. In chapter 2, although leopards did not modify their visit frequency to sites with lion scats, they spent significantly more time at sites with lion scats. This pattern concurs with observations of wild stoats, coyotes and red foxes being attracted to areas with olfactory cues of sympatric predators or strong competitors (ferrets, cats, dogs, red foxes, coyotes) (Shivik et al. 2011, Arnold et al. 2011, Banks et al. 2016, Garvey et al. 2016). Based on these similarities, I concluded chapter 2 with the suggestion that leopards, too, use olfactory cues to gather information about the presence of potentially dangerous competitors (lions and other leopards) and reciprocally signal their own presence. Such

behaviour constitutes an antipredator strategy that facilitates reciprocal avoidance of risky encounters by guild members as per the dear enemy principle.

Finally, chapter 3 was motivated by assessing the impact of intraguild olfactory interactions on the precision of density estimates and helping field biologists determine the most optimal sampling design for leopard density analyses. I thus opportunistically used the sampling data of chapter 2 to investigate the feasibility of monitoring leopard scent-marking sites to estimate leopard density. I compared the results to a consecutive control survey under both Bayesian and maximum likelihood approaches and provided, to my knowledge, the first SECR leopard density estimate for the western sector of the SSGR. Data showed no differences in the detection rates and density estimates of leopards between surveys with and without lions' and spotted hyenas' scents, confirming the results of chapter 2. Both surveys generated similarly realistic estimates of similarly acceptable precision. Averaged density across top models was 12.35 ind/100km² (+/-0.06). Thereby, field biologists could theoretically use any of the two sampling methods (roads or marking sites) to estimate leopard density. However, comparing output parameters showed that the sampling designs provided different information on the structure of the leopard population. In particular, the sex ratio varied drastically between surveys. The control survey captured a population sample more representative of the entire leopard population in the study area, whereas the scent survey mostly characterized the sexually mature leopard population. Additionally, the Bayesian framework accounted for an edge effect and therefore appeared more robust and accurate than maximum likelihood analyses, although results were almost equivalent. Thereby, the selected model (null model of the control survey) revealed a relatively low female to male ratio (0.75:1) in the study area compared to the accepted reference range 1-1.5 female per male (Friedmann and Taylor-Holzer 2008). Based on estimates accuracy, model assumptions, and cost-effective protocol, I advised future field studies to account for the location of their traps rather than surrounding competitor activity

when estimating leopard density. Following a standard camera trapping procedure, road junctions appeared as the optimal sampling strategy as they are easier to locate and provide, conservation-wise, a better overview of the leopard population status.

Chapter 1 described the current state of the literature and theories on olfactory communication, particularly on carnivores and leopards. It provided the foundations to design ecologically meaningful experiments and hypotheses in chapter 2. Chapter 2 was central to this thesis and composed a significant part of the conclusive wildlife management implications. The reasoning of chapter 3 and its importance to wildlife management were both motivated by chapter 1 but hypotheses mostly built up from findings of chapter 2. Finally, chapter 3 provided practical recommendations to field biologists and numerical benchmarks for reserve managers attentive to their leopard population.

Wildlife management implications

From a wildlife management perspective, locating scent-marking sites of leopards can constitute useful indicators for the spatial use and distribution of leopards in a reserve. Most monitored scent-marking sites were shared by multiple individuals, including same sex individuals. Leopards partially overlap territories of both sexes (Stander et al. 1997). The locations of marking sites could thus be used to emphasize areas of high leopard activity and approximate territory boundaries. The western sector of the Sabi Sands Game Reserve already has a good knowledge of its leopard population, distribution, and activities. However, other reserves aiming to improve their knowledge of their leopard population could look for conspicuous signs of scent marking (popcorn scents) and map these locations. Additionally, leopard scent-marking sites in the study area seemed to serve as interaction centres for many other smaller carnivore species. Being familiar with or monitoring leopards' scent-marking sites could help determine a common factor to the carnivore distribution of a reserve (e.g., habitat

suitability, proximity to water). It could also be the baseline for further investigation of carnivore dynamics, and may benefit game viewing and eco-tourism activity of a reserve. Finally, based on observations in the western sector, leopards only began to scent mark once they reached sexual maturity. Thus, scent-marking behaviours could be a useful indicator for assessing a small leopard population's reproductive potential and viability.

On non-protected areas and particularly on farmed landscapes, the identification of surrounding leopard scent-marking sites would help to adapt the spatial arrangement of the land use (e.g., livestock husbandry) to minimize conflicts with leopards. For example, livestock vulnerable to leopard depredation (calves, goats) should be kept further away from areas of high leopard activity (i.e., marking sites). Awareness and adapted livestock husbandry to the spatial distribution of cheetah scent-marking sites in Namibia reduced depredation rates by 86% (Melzheimer et al. 2020).

This research further provided benchmarks for comparisons with other leopard populations and ecosystems. As suggested by Balme et al. (2019), the leopard population of the Sabi Sands is likely at or very close to carrying capacity. Thus, managers and ecologists could compare other leopard densities to 12.81 ind/100 km² (+/-0.07) and the biotic and abiotic conditions of the Sabi Sands area to determine the state and growth potential of a population of interest. Additionally, my results characterized interactions between the three apex carnivores of the studied ecosystem. Negative competitive interactions between leopards, lions and spotted hyenas have been witnessed on multiple occasions throughout the study. The failure of trials to manipulate the movements of leopards with lion and spotted hyena scents may, therefore, reflect the high density and uniform distributions of the two sympatric competitors throughout the study area, rather than the absence of intraguild competition. In particular, my results imply that leopards do not rely on olfactory cues for spatial partitioning as a main avoidance means of spotted hyenas and lions in the Sabi Sands. Given the apparent absence of spatial partitioning

in my study, high rates of leopard kleptoparasitism may thus be observed in the Sabi Sands, especially by spotted hyenas (Balme et al. 2019). The high intensity of these competitive interactions is likely indicative of a self-regulating ecosystem close to carrying capacity. Leopards perhaps rely on temporal partitioning or visual cues for spatial partitioning from lions and spotted hyenas in the Sabi Sands area. Such knowledge is particularly important to understand the impact of human activities on carnivore interactions. Sévêque et al. (2020) reviewed the evidence for human disturbance impacting niche partitioning amongst carnivores. The study showed that human activities could impede, unbalance, or facilitate niche partitioning. Urbanization, habitat fragmentation, and modified landscapes (vegetation density, habitat type) could potentially modify and limit partitioning options of leopards, lions, and spotted hyenas. A tighter niche breadth and a greater competitive pressure could lead to declining apex predator populations outside protected areas. A comparison reference for apex predator interactions in a healthy self-regulating ecosystem helps assess the degree of disturbance undergone by other ecosystems. Finally, the absence of the predicted olfactory landscape of fear suggests that the three apex predators of the ecosystem are more equivalent in strength than initially thought and that at high densities, the top-down effect amongst them is weaker than expected. Bottom-up forces and competition for space are likely the factors shaping leopard, lion, and spotted hyena distributions.

Finally, two management recommendations are specific to the western sector of the SSGR. Given the low female to male sex ratio observed, I first advise monitoring of infanticide rates. Males compete for females and potentially kill cubs up to 15 months old if sired by other males to increase their own mating chances (Balme and Hunter 2013, Balme et al. 2013). The high number of males in the Sabi Sands exerts a greater pressure on females and makes females more susceptible to infanticides. An unbalanced sex ratio can eventually cause extreme infanticide rates, put females in a parenting dead end and strongly reduce the population.

However, before getting to this extreme scenario, the growing intraspecific pressure should *a priori* drive individuals (males) to disperse. The absence of fences also facilitates individual dispersal and natural self-regulation of the leopard sex ratio. Secondly, managers of the western sector should pay attention to the gene flow of the next few generations of leopards. Females do not disperse as far as males, and several young subadult males were seen opportunistically waiting to take over their father's territory during the study. With a greater number of males than females, dominant males risk mating with related females (mother, daughter, sister), which would weaken the genetic diversity of the leopard population. On all other ecological management aspects, the reserve appeared to provide optimal conditions for the long-term establishment and development of its local leopard population. The eco-tourism activity taking place in the western sector also seemed adapted and generated no visible disturbance on the behavioural ecology of leopards.

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