The olfactory behaviour of spotted hyaenas (*Crocuta crocuta*) and sympatric mammals in the Okavango Delta, Botswana

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Abstract

Understanding the natural behaviour and coexistence of species is important for the conservation of intact ecosystems, and behavioural studies can enhance our knowledge of processes such as communication and competition. I investigated the social dynamics and scent-marking behaviour of spotted hyaenas (*Crocuta crocuta*), and the occurrence of interspecific olfactory eavesdropping among African mammals, within the Okavango Delta ecosystem of Botswana.

First, social network analysis found that the hyaena population was comprised of five main clans that maintained territories but whose home ranges overlapped considerably. Scavenging at large carcasses involved associations between individuals from different clans and resulted in relative tolerance toward territorial intruders. Second, observations of communal latrine use found that latrines are likely involved in territorial advertisement by hyaena clans, and cyclical patterns of latrine growth and decline were linked to annual rainfall. Third, a translocation experiment showed that hyaena scats appear to be less important for hyaena communication than other cues associated with latrines, especially as hyaenas did not differ in their behaviour towards scat originating from latrines located in resident versus non-resident clan home ranges. Heterospecific mammals investigated, scent-marked, and were vigilant at hyaena latrines, suggesting a potential function for hyaena latrines in interspecific eavesdropping and/or communication. Fourth, a urine presentation experiment found that the investigation of sympatric predator urine by African mammals stimulated vigilance behaviour, suggesting that olfactory signals provide information about predation risk. Hyaenas exhibited context-specific differences in behaviour towards predator urine: they were more likely to investigate urine samples

alongside indicators of predator activity (i.e. carrion odour, carcasses) and were most likely to exhibit vigilance at carcasses when exposed to urine from lions and wild dogs.

This study greatly enhanced our understanding of spotted hyaena behaviour within the Okavango Delta, an important ecosystem for the conservation of African large predators. Furthermore, this study provided the first experimental evidence of the role of hyaena scats in intraspecific communication and interspecific olfactory eavesdropping at latrines. My research findings regarding the population's clan structure and home ranges will form the foundation for subsequent investigation into human-wildlife conflict in the study area. Furthermore, the results from two scent experiments shed light on the potential importance of olfactory signals for mediating interspecific interactions among African mammals, particularly among large predators.

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Chapter 1 : General introduction

This study investigated the social dynamics and scent-marking behaviour of spotted hyaenas (*Crocuta crocuta*), and the occurrence and extent of interspecific olfactory eavesdropping, within the Okavango Delta ecosystem of northern Botswana. The study of these intra- and inter-specific interactions can help us to understand the behaviours that we observe in wild populations, and shed light on the functioning of intact ecosystems.

The thesis contains four data chapters, consisting of two observational studies and two experiments. In Chapter 2, I used social network analysis to determine the group structure of my study population and examine the effects of scavenging behaviour on intraspecific relationships. In Chapter 3, I investigated the influence of social and environmental factors on the use of communal marking sites ('latrines') by spotted hyaenas. In Chapter 4, two translocation experiments investigated the specific role of spotted hyaena faeces in intraspecific signalling and heterospecific eavesdropping at hyaena latrines. In Chapter 5, a scent presentation experiment examined the behaviour of spotted hyaenas and sympatric mammals when exposed to urine deposits from large predators.

In this introductory chapter, I detailed my study system, and outlined the use of remote cameras and scent presentation experiments for the study of animal behaviour.

1.1 Study system

1.1.1 African large predator guild

Groups of species within an ecosystem that compete over shared resources are termed 'guilds' (Polis and Myers, 1989; Ginsberg and Woodroffe, 1997). Within

much of sub-Saharan Africa, the large predator guild consists of six species: African lions (*Panthera leo*), leopards (*Panthera pardus*), African wild dogs (*Lycaon pictus*), cheetahs (*Acinonyx jubatus*), spotted hyaenas (*Crocuta crocuta*), and brown hyaenas (*Hyaena brunnea*). Competition within the guild can impact the distribution and abundance of guild member species, and these effects can vary significantly between populations (Creel, 2001). Large carnivores compete through dietary overlap (Hayward, 2006; Hayward and Kerley, 2008), interference competition (Kruuk, 1972; Cooper, 1991), and direct predation (Laurenson, 1994; Palomares and Caro, 1999). All members of the guild are at risk of interspecific killing (Palomares and Caro, 1999), but this risk is generally higher for juvenile individuals and subordinate predators such as cheetahs and wild dogs. For example, cheetahs in Tanzania suffer from 95 % cub mortality, with approximately 78 % of these deaths resulting from intraguild predation, mostly by lions (Laurenson, 1994). These intraguild interactions thus have important influence on the population dynamics of the species concerned, and hence the structure of the community. Knowledge of these interactions is also important to effectively conserve increasingly threatened ecosystems. Due to escalating habitat loss, competitors have been forced to inhabit smaller protected areas, thereby increasing the likelihood of encounters among predator species (Caro and Stoner, 2003).

Animals may benefit from encounters with conspecific and heterospecific competitors through acquisition of food resources (e.g. hyaenas scavenging from wild dog kills, Carbone et al., 2005), but also risk serious consequences such as injury or death (Polis and Myers, 1989). To maximize the potential benefits and minimise the costs of aggressive encounters, animals should assess risk before

engaging in such interactions, and one way to do so is through using environmental cues (Smith and Parker, 1976; Benson-Amram et al., 2011). Throughout their environment, large carnivores gather information through several sensory modalities, and this information can be used to exploit potential opportunities and avoid detrimental encounters with both conspecifics and heterospecifics (Dall et al., 2005; McComb and Reby, 2005; Webster et al., 2010). The African large predator guild provides an interesting case for examining the use of information to gauge risk and reward, because these species live in an environment in which their competitors are also potential predators or prey (Polis and Myers, 1989). Consequently, environmental signals may be attractive or aversive to receivers depending on the circumstances. Carnivores could be attracted to heterospecific signals (e.g. lions approaching wild dog vocalizations, Webster et al., 2010), or they could avoid these signals due to the threat of intraguild predation (e.g. wild dogs moving away from lion roars, Webster et al., 2012). Despite the significant amount of work examining competition among these large predators, research on potential eavesdropping and/or other forms of communication between these species has only recently gained importance. Where there is mutual benefit in assessing the likely outcome of an encounter, direct communication between species may be adaptive (e.g. snorting by ungulates as a 'pursuit-deterrent' signal; Caro et al., 2004), but indirect 'eavesdropping' may be favoured when unintended receivers are the only beneficiary (e.g. prey species avoiding areas containing predator odour; reviewed by Apfelbach et al., 2005).

I also examined the behaviour of mammals from two other guilds: herbivores and mesopredators. All of the herbivore species involved in this study are preyed upon

by spotted hyaenas (and sympatric large predators), and a majority of the studied species are ungulates (e.g. impala *Aepyceros melampus*, southern giraffe *Giraffa giraffa*, plains zebra *Equus quagga*). Mesopredators are medium-sized predators (e.g. black-backed jackals *Canis mesomelas*, caracals *Caracal caracal*, honey badger *Mellivora capensis*) which generally feed on smaller prey items such as rodents, birds, and reptiles (Begg et al., 2003; Kaunda and Skinner, 2003; Melville et al., 2004).

1.1.2 Study site: Okavango Delta, Botswana

Botswana is a landlocked country in southern Africa that is mostly covered by the sandy Kalahari Basin (Ramberg et al., 2006). In the north-western section of the country, rainfall from the Angolan highlands travels into Botswana via the Okavango River, where geological fault lines have created an inland wetland ecosystem totalling 28,000 km^2 called the Okavango Delta (Ramberg et al., 2006). Rainfall and flooding is highly seasonal, with rainfall occurring during summer months (November to March) and floodwaters arriving in June (Ramberg et al., 2006). The Okavango, a UNESCO World Heritage Site (as of 2014) and Ramsar Convention Wetland of International Importance (since 1997), hosts an enormous amount of biodiversity, including 86 species of fish, 64 reptiles, 122 mammals, and 444 birds (Ramberg et al., 2006). The delta ecosystem is home to stable populations of five of the six large predator species, as brown hyaenas mostly inhabit the more arid habitats of central Botswana (however, brown hyaenas were sighted a handful of times during my study in the southern section of the study area).

My study site was located at the eastern edge of the Okavango Delta ecosystem, within the Moremi Game Reserve and surrounding wildlife management areas

(Figure 1-A). The Moremi Game Reserve is a protected area managed by the Botswana Ministry of Environment, Wildlife, and Tourism, and the wildlife management areas (WMAs) are land concessions maintained by village community trusts through community-based natural resource management (Mbaiwa et al., 2011). This area was characterized by a mosaic of habitat types, ranging from rivers and floodplains to dry grasslands and woodlands (McNutt, 1996). Due to the presence of a permanent water source, the study area hosted a relatively permanent prey population throughout the year (Cozzi, 2012). The southern edge of the study area was demarcated by a veterinary cordon fence which was erected in 1982 to separate wild buffalo (*Syncerus caffer*) from domestic livestock (Ramberg et al., 2006). However, previous work in the area has shown that this fence is not impermeable to wildlife (Cozzi et al., 2013a), so many study animals travelled throughout the protected reserve, WMAs, and livestock areas.

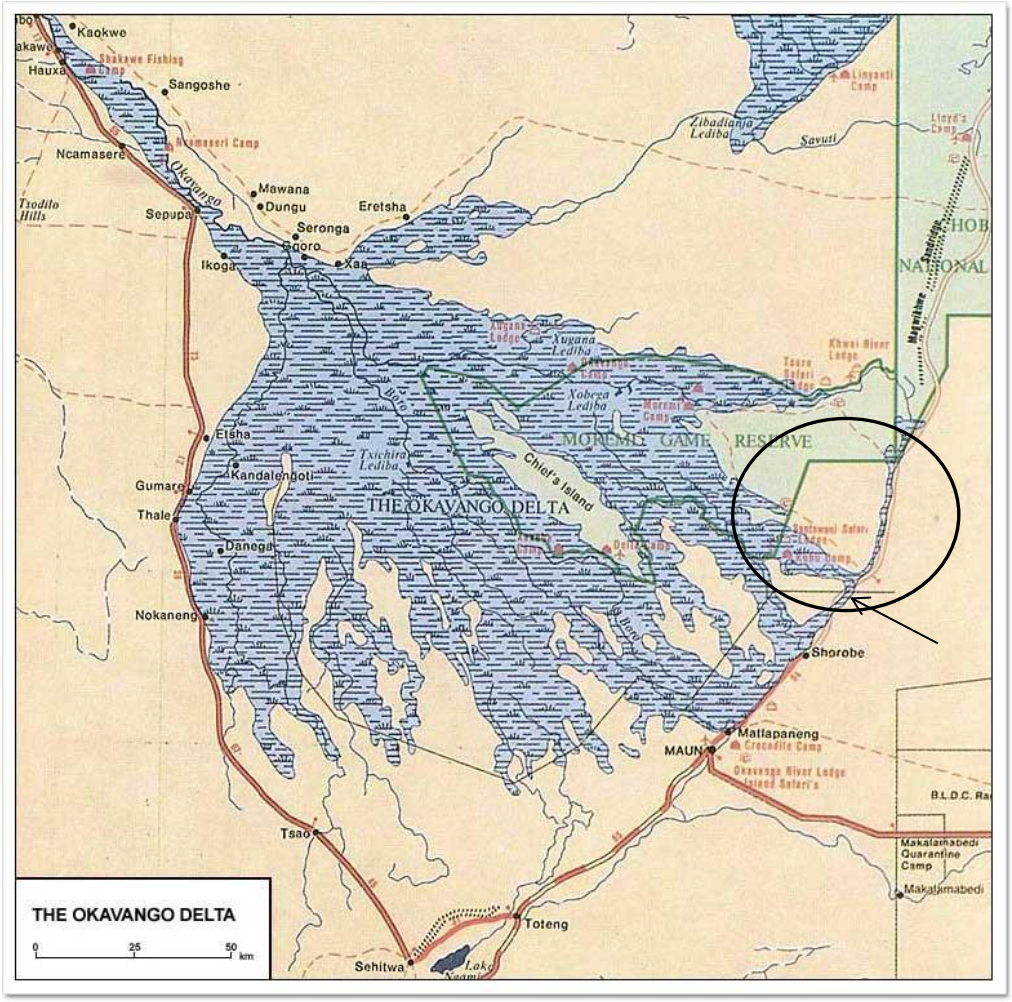


Figure 1-A. The location of the study area within the Okavango Delta ecosystem. *The study area (black circle) encompasses the eastern section of Moremi Game Reserve (delineated by green shading and solid green line). A veterinary cordon fence (solid black line) runs along the southern edge of the Delta, with a black arrow indicating the fence's location within the study area. Solid red lines represent paved roads, and black dots represent villages. Adapted from image copyrighted 2013 by Botswana Tourism Organization. (Retrieved online on August 15, 2017 at http://www.botswanatourism.us/experience_botswana/okavango_delta_map.html).*

While in many ecosystems around the world, top predators have been hunted and/or persecuted, often to extinction, the Okavango ecosystem contains an intact guild and thus provided a rare opportunity to study the functioning of a complete community. Fieldwork was conducted in association with the Botswana Predator Conservation Trust, an in-situ conservation research organization that has been

studying African wild dogs since 1989 and began monitoring all five large predator species in 2007. Therefore, this site allowed for the simultaneous study of individually-identified animals across five species within an intact predator guild.

1.1.3 Study species: the spotted hyaena

Of the four extant hyaena species (spotted hyaena, brown hyaena, striped hyaena *Hyaena hyaena*, and aardwolf *Proteles cristata*), the spotted hyaena has the largest body size and lives in the largest and most complex social groups (Watts and Holekamp, 2007) (Figure 1-B). The spotted hyaena, hereafter referred to as 'hyaena', is a nocturnal predator and keystone species within African ecosystems (Holekamp, 2006). Current assessments estimate the global wild hyaena population to be between 27,000 and 47,000 individuals, but populations are currently declining due to threats such as direct persecution by humans and habitat degradation (Bohm and Honer, 2015). To develop appropriate and effective conservation strategies, it is important to understand a species' natural behaviour and how they coexist with sympatric species.



Figure 1-B. An adult spotted hyaena feeding on a giraffe carcass. *Photo credit: Jessica Vitale.*

Hyaenas live in permanent groups called clans and exhibit individual-based fission-fusion sociality, in which individuals make decisions about joining or leaving subgroups several times a day (Kruuk, 1972; Smith et al., 2008). Despite spending much of their time alone or in small subgroups, hyaenas maintain strong relationships with clan members and recognize each other individually (Holekamp et al., 1999a), and all of a clan's offspring are raised together at a communal den (Kruuk, 1972). Female hyaenas are philopatric, but male hyaenas generally disperse from their natal clan to join a new clan (Henschel and Skinner, 1987). Hyaenas exhibit enormous behavioural flexibility across their range, including activity patterns, territoriality, foraging, and clan sizes (Holekamp and Dloniak, 2011). For example, hyaenas inhabiting the desert ecosystems of southern Africa live in small clans (< 10 individuals) that occupy large home ranges (500 – 1000 km^2) (Tilson and Henschel, 1986; Mills, 1990). On the other hand, the prey-rich ecosystems of East Africa have extremely dense hyaena populations, such as in the Ngorongoro Crater, Tanzania, where clans comprised of up to 70 members live in home ranges approximately 28 km^2 in size (Kruuk, 1972).

Hyaenas have evolved advanced cognitive abilities and intelligence in order to navigate the complex social life of the clan (Holekamp et al., 2007). Hyaenas use visual, acoustic, and olfactory information when communicating with conspecifics. There are a limited number of experimental and systematic studies examining the olfactory behaviour of wild hyaenas. Most published research on olfaction in hyaenas has involved observations of captive hyaenas or laboratory analysis of anal gland secretions (Woodmansee et al., 1991; Drea et al., 2002a; Drea et al., 2002b; Burgener et al., 2009; Theis et al., 2012; Theis et al., 2013), but some studies have examined natural scent-marking behaviour in the wild (e.g.

Kruuk, 1972; Bearder and Randall, 1978; Mills and Gorman, 1987; Boydston et al., 2001). Hyaenas regularly use scent in a variety of situations, such as ritualized greetings between clan members that involve mutual ano-genital sniffing and serve to reinforce social bonds (East et al., 1993; Smith et al., 2011). The use of communal latrines by hyaenas has been observed in several studies, specifically Bearder & Randall (1978) and Kruuk (1972), who described characteristics of latrines and suggested their potential role in territorial behaviour. Olfaction is not only important in hyaena social interactions but also for foraging behaviour, as they have been observed locating large carcasses using scent across several kilometres (Kruuk, 1972; Mills, 1990).

Due to their relative abundance and fission-fusion subgrouping patterns, spotted hyaenas are often observed interacting with heterospecific predators through direct competition for resources (i.e. dietary overlap, kleptoparasitism of carcasses) and intraguild predation (Polis and Myers, 1989; Hayward, 2006). Depending on the circumstances, hyaenas can act as aggressive foes or passive subordinates. The proportion of kills that hyaenas make themselves, as opposed to scavenging from competitors, ranges from 43 to 95 % depending on the ecosystem (Holekamp and Dloniak, 2011). Furthermore, aggressive interactions between hyaenas and other predators are highly varied and their outcomes depend on several factors, such as habitat, the presence of valuable resources (e.g. carcasses), and the ratio of hyaenas to heterospecific competitors (Cooper, 1991; Fanshawe and Fitzgibbon, 1993; Creel and Creel, 1996).

Given the extensive behavioural plasticity observed in hyaenas across multiple ecosystems, it is necessary to understand the details of hyaena behaviour in a given ecosystem for effective population management. I investigated the

behaviour of a previously under-studied population of hyaenas living within an intact large predator guild in northern Botswana. I used motion-triggered cameras and olfactory experiments to investigate the social, scent-marking, and olfactory eavesdropping behaviour of wild hyaenas.

1.2 Using remote cameras for behavioural studies

In an effort to limit disturbance to wildlife, researchers have adopted several 'non-invasive' methods for surveying animal populations, including hair snares, remote cameras, and track surveys (MacKay et al., 2008). Remote cameras, often termed 'camera traps', are useful tools for gathering photographic records of species and individuals within a population, and are commonly used to determine species occupancy within a specific ecosystem (Silveira et al., 2003; Kays and Slauson, 2008). When deployed in the environment for several days or even months, these cameras can provide a photographic database of individually-identifiable animals (i.e. with unique natural markings) in a population faster and more effectively than relying on direct observations alone. Hyaenas can be individually-identified through natural markings that are unique to each individual, such as body spot patterns and ear notches. During my study, I deployed camera traps at sites of interest (e.g. carcasses, dens, latrines) to supplement direct sightings of hyaenas, and I created an extensive database of photographed individuals from the population. I was responsible for the identification of all hyaenas photographed (via direct sightings and camera traps) by myself and other researchers at my field site. After my two years of fieldwork, the number of individuals identified in the study area rose from 183 to 496 individuals.

The development of video-recording technology has allowed for the use of remote camera traps in behavioural studies of a wide variety of species, such as river

otters (Lontra canadensis, Barocas et al., 2016), brown bears (Ursus arctos, Tattoni et al., 2015), red (*Cervus elaphus*) and roe (*Capreolus capreolus*) deer (Wikenros et al., 2015), and African carnivores (Apps et al., 2017). As a relatively inconspicuous form of observation (but see Meek et al., 2014), these cameras are particularly useful for studying the behaviour of species that are elusive, nocturnal, and/or nervous of human presence (Caravaggi et al., 2017). Camera trapping is becoming increasingly cost-effective, as relatively inexpensive cameras distributed throughout the environment can potentially record more observations simultaneously than direct observers. However, there are several limitations of remote observation that should be taken into consideration for behavioural studies. For example, technological failures due to battery exhaustion or destruction by wildlife can result in the loss of data and/or periods without active monitoring (Caravaggi et al., 2017). Furthermore, a camera's limited field of view means that some behaviours and/or information will not be observed; thus it is important to select specific (e.g. short-term) behaviours that can be accurately studied under these circumstances (Caravaggi et al., 2017).

1.3 Studying communication through scent presentation experiments

Communicating through chemical signals, also termed olfactory communication, is a widespread behaviour throughout the animal kingdom, and scent-marking is particularly common in mammals (Johnson, 1973). Olfactory signals are advantageous because scents are long-lasting and can be deposited in the environment, and therefore broadcast information longer than acoustic or visual modalities (Gorman and Trowbridge, 1989). Thus, signallers do not need to be present to communicate with receivers, and consequently communicating individuals can avoid agonistic encounters with competitors (Gosling, 1982).

However, although long-lasting compared to other modes of communication, scent-marks degrade over time and must be replenished, a costly activity due to the time and energy required to continuously produce and deposit marks (Gosling and Roberts, 2001).

Observations of direct intra- and inter-specific encounters between wild individuals are often rare, but we can learn about these relationships through the experimental presentation of acoustic and olfactory signals originating from individuals/species of interest (Durant, 2000). For instance, wild dogs did not consistently move away from playbacks of hyaena whoops (Webster et al., 2012), which could reflect the variable nature of interactions between these two species. In the case of scent-marking, the deposition of scents in the environment by animals results in copious scent-marks that any given individual may encounter each day (Eisenberg and Kleiman, 1972). Thus, the responses of animals to experimentally-presented scent-marks can provide insight into how they might behave during natural encounters with conspecific and heterospecific scents, and may also reflect relationships among individuals or species. For example, female lions are known to vigorously defend their territories from rival prides (Schaller, 1972), and female lions responded more often to experimentally-presented urine samples from females of another pride than to urine from their own pride members (Gilfillan et al., 2017).

A common practice in population monitoring studies using camera traps is to place attractants (e.g. bait and/or a scent lure) in front of the cameras to bring animals within detection range and to increase the likelihood of capturing photographs in which individuals can be identified (Schlexer, 2008). In fact, the use of baits and scent lures has been shown to attract or deter certain species. For

instance, prey species in the Central Brazilian Amazon avoided cameras baited with a carnivore attractant containing a sardine and egg mixture (Rocha et al., 2016), and mesocarnivore (but not large predator) detection rates were higher at sites containing cooking fat lures compared to a control (Satterfield et al., 2017). Although altering animal behaviour is generally an unintended consequence of camera monitoring surveys, these examples show that scents deployed in the environment can influence animal behaviour and that it is possible to detect these effects remotely using camera traps. In recent years, several studies have used remote cameras to record the natural scent-marking behaviour of wild species (e.g. Allen et al., 2015; Allen et al., 2017) or to monitor their responses to experimentally-presented olfactory stimuli (e.g. Andersen et al., 2016; Apps et al., 2017; Wikenros et al., 2017). These studies have used videos from camera traps to analyse the sniffing, scent-marking, and/or vigilance behaviours of several mammalian species, and experimental studies found that individuals responded more strongly to mammal olfactory stimuli than control treatments. For example, red foxes (*Vulpes vulpes*) investigated, scent-marked, and were vigilant more often near lynx (*Lynx lynx*) scat than control sites (Wikenros et al., 2017), and spotted-tailed quolls (*Dasyurus maculatus*) were more vigilant near Tasmanian devil (*Sarcophilus harrisii*) odour than a control (Andersen et al., 2016).

The data chapters in this thesis were written and prepared as manuscripts for publication in academic journals. As such, some information contained within the introduction and methodological sections is repeated. Although the preparation of these manuscripts involved assistance and input from several co-authors (and consequently the pronoun ‘we’ is used throughout the text rather than ‘I’), I led the study design, data collection, analysis, and writing of all chapters.

Chapter 2 : Clan membership and home ranges of a spotted hyaena (*Crocuta crocuta*) population in northern Botswana: the influence of scavenging behaviour on social network analysis

Abstract

Understanding the association and space-use patterns of individuals within a population is an important component of effective conservation. For elusive populations, and especially for group-living species with flexible association patterns, subjective determination of social groups may fail to capture subtleties in intraspecific relationships. The overall aim of this study was to determine the group composition and home ranges of a previously under-studied spotted hyaena (*Crocuta crocuta*) population, and to evaluate the efficacy of social network analysis in delineating clan membership from opportunistic observations. Throughout 2011-2016, we collected data on the geographical locations and dyadic associations of individually-identified hyaenas from remote cameras and opportunistic direct observations in a range of circumstances, including at sites with large carcasses. During data collection, the identity and membership of clans was inferred from repeated associations between identified individuals. Our goal was to evaluate the accuracy of these clan designations and the influence of scavenging behaviour on association patterns using formal network analysis. Clan membership was delineated based on the strength of dyadic associations, and utilization distributions were calculated to evaluate spatial overlap within dyads. Our analysis defined five main clans within the study area (1355 km^2), each comprising a mean of 20.6 individuals (including den-dwelling cubs) inhabiting a mean home range size of 239.28 km^2. The association and spatial overlap values for individual dyads within the same clan were significantly higher than those for

dyads from different clans. Importantly, scavenging behaviour was found to influence hyaena association patterns, as 90 % of associations between individuals of different clans occurred at carcass sites. The close correspondence between the clans detected by our analysis and those which were previously defined while in the field highlights the value of social network analysis in assessing population structure. However, the observed effects of large carcass scavenging on intraspecific associations elucidate the importance of considering a species' behavioural ecology and field observations when interpreting social network analyses.

2.1 Introduction

Knowledge of the social structure and space-use patterns of an animal population is important for its conservation and management. Social structure plays an important role in several biological processes such as mate choice (e.g. spotted hyaenas *Crocuta crocuta*, Szykman et al., 2001), survival (e.g. chacma baboons *Papio cynocephalus ursinus,* Silk et al., 2009), and disease transfer within a population (reviewed by Altizer et al., 2003). Space use patterns can influence the frequency and nature of human-wildlife conflict (e.g. pumas Puma concolor, Teichman et al., 2013), and the impacts of conflict-mitigation measures on animals are affected by a population's behavioural ecology (Conover, 2002). Population and behavioural monitoring of a species is an important component in the development of informed strategies for applied species management.

Determining a population's social structure can be a complex process for gregarious species that exhibit flexible association patterns such as fission-fusion sociality (Aureli et al., 2008). Spotted hyaenas (hereafter referred to as 'hyaenas') are social carnivores that live in permanent groups called clans and exhibit

individual-based fission-fusion sociality, in which individuals make decisions about joining or leaving subgroups, as opposed to group-based fission-fusion sociality in which stable subgroups join and leave other stable subgroups (Kruuk, 1972; Smith et al., 2008). Despite spending much of their time alone or in small subgroups, hyaenas maintain strong relationships with clan members and recognize each other individually (Holekamp et al., 1999a). Clan members reinforce social bonds through ritualized greetings involving mutual ano-genital sniffing (East et al., 1993; Smith et al., 2011), and all of a clan's offspring are raised together at a communal den, which serves as the clan's centre of social activity (Kruuk, 1972). Female hyaenas are philopatric, but male hyaenas generally disperse from their natal clan to join a new clan (Henschel and Skinner, 1987).

Structured group-living is an invariant aspect of hyaena behavioural ecology, but clan sizes and space use vary among ecosystems (Holekamp and Dloniak, 2011). For example, hyaenas inhabiting desert ecosystems in southern Africa live in small clans (< 10 individuals) occupying very large home ranges (500 – 1000 km^2), as observed in Namibia (Tilson and Henschel, 1986) and South Africa (Mills, 1990). In contrast, prey-rich ecosystems have extremely dense populations of hyaenas, such as in the Ngorongoro Crater, Tanzania, where clans comprised of up to 70 members live in home ranges approximately 28 km^2 in size (Kruuk, 1972). Territoriality also varies extensively across hyaena populations (Holekamp and Dloniak, 2011), with clans in some populations vigorously defending territories against non-residents (Kruuk, 1972; Smale et al., 1997), and those in other populations sometimes tolerating intruders (Hofer and East, 1993b).

Many studies of hyaena social behaviour benefit from direct observations (e.g. continuous den monitoring, overnight follows) of habituated hyaenas over multiple decades to unequivocally determine the identities, relatedness, and clan membership of all individuals in the population (as in Holekamp et al., 1997; Honer, 2005). However, determination of clan membership in previously under-studied and/or elusive populations presents a major challenge. The overall aim of this study was to determine the composition and home ranges of clans within a previously under-studied population of hyaenas. Furthermore, we aimed to investigate the influence of high-value, ephemeral resources (such as large carcasses) on intraspecific associations. We collected data on the geographical location and dyadic associations of 390 identified individuals from camera traps and opportunistic sightings in a range of circumstances, including at natural carcass sites. Throughout data collection in the field, the delineation and membership of clans was inferred through repeated associations among individuals. Thus, our aim was to investigate the accuracy of these clan determinations using social network analysis, which has been used to estimate group structure in a variety of species, such as Thornicroft's giraffes (Giraffa camelopardalis thornicrofti, Bercovitch and Berry, 2013), Bechstein's bats (Myotis bechsteinii, Kerth et al., 2011), and Asian elephants (Elephas maximus, De Silva et al., 2011). In network analysis, the relationships between individually-identifiable animals are measured as interactions or associations, and the analysis uses these repeated interactions to determine the population structure (Wey et al., 2008; Farine, 2014). For a population to have social structure, it must contain non-random dyadic associations (Whitehead et al., 2005). When stronger associations

exist within rather than between groups of individuals, the network can be divided into distinct ‘communities’ or social groups (Newman, 2006).

As group-living is an invariant aspect of hyaena behaviour throughout the species’ range in Africa, we predicted that our hyaena population would exhibit non-random dyadic associations patterns, such that individuals would associate more often with certain individuals (i.e. members of the same clan). Although hyaena territoriality varies among ecosystems, we predicted some degree of territoriality within our population, meaning that the strength of dyadic associations would be strongly correlated with overlap in the spatial distribution of individuals (i.e. clan members would share the same territory). The availability of food resources has the potential to influence association patterns and movement of individuals. For example, hyaenas in some populations have been shown to leave their usual territories and encounter non-clan members while following migrating prey (Hofer and East, 1993b). Furthermore, a previous study on our population found that the presence of elephant carcasses influenced the short-term ranging behaviour of radio-collared hyaenas, and resulted in multiple extra-territorial forays and inter-clan associations for one individual (Cozzi et al., 2015). Therefore, we predicted that hyaenas in our study would associate with individuals from outside their own clan while scavenging at large carcasses, and that the spatial overlap among clans would increase as a result of this scavenging behaviour.

2.2 Materials and Methods

2.2.1 Study site

This study was conducted within the Okavango Delta ecosystem of Botswana, a region characterized by a mosaic of habitat types and distinct wet and dry seasons influenced by annual rainfall and flooding patterns (Ramberg et al., 2006). Data were collected from 2011-2016 throughout an area of approximately 2193 km^2, located in the south-eastern section of Moremi Game Reserve and its surrounding Wildlife Management Areas (centre of study area: S19.50098, E23.61010). The southern boundary of the study area was demarcated by a veterinary fence that separates wildlife areas from domesticated livestock (for further details, see Cozzi et al., 2013a). The study area therefore serves as a buffer zone located between the eastern section of Moremi Game Reserve and human-dominated landscapes to the south.

2.2.2 Data collection and selection

Hyaenas were observed opportunistically from a vehicle or through motion-triggered camera traps (StealthCam G30 and Prowler models) located at sites of interest (i.e. hyaena latrines, hyaena dens, natural carcasses) and at randomized locations within grid transects (as part of concurrent research monitoring mammal occupancy, see Rich et al., 2016). Photographs of all individuals were taken during direct observations whenever possible, and all individuals that were photo-identified from direct and remote sightings were given a unique ID code for matching in subsequent observations. All identifications were done by eye using the hyaenas' natural markings such as body spot patterns and ear notches. Clan membership was initially estimated subjectively during data collection, based on

observations of repeated associations among individuals (especially at communal den sites).

Association (subgrouping) data were recorded using the 'gambit of the group' method, which defines all individuals observed together at a given time as associating (Farine and Whitehead, 2015). Subgroups were defined as one or more hyaenas separated from other hyaenas by at least 200 m (Smith et al., 2008). For remote camera sightings, hyaenas recorded within two minutes of each other were designated as the same subgroup (hyaena traveling speed ranges from 4-10 km per hour, Mills, 1990). Each change in subgroup was entered into a database with the following information: date, time, identification codes of all identified subgroup members, and location coordinates (recorded using a handheld Garmin 72 GPS unit).

Prior to analysis, we excluded data from rarely-sighted individuals, and from repeated observations of the same animals made in quick succession. Subgrouping data were converted into a list of all pairs of individuals ('dyads') observed together at each date and time. Hyaenas change subgroups several times a day (Smith et al., 2008), but given that our observations were opportunistic and usually short in duration, we removed all repeated sightings of identical dyads within 24 hours to ensure independence of associations. The resultant dataset consisted of 4013 unique observations of hyaena subgroups in the field. We selected the median number of days (three) on which individuals were seen during the study (range = 1-91 days, mean ± SD = 7.95 ± 12.8 days) as a threshold for including individuals in the analysis (as in Mourier et al., 2012), and thus included all individuals that were recorded at least once on each of at least four days. Statistical analyses were then performed on all subgroups in which any

individuals that were sighted on at least four days were present. Consequently, our analyses included some individuals that were sighted on fewer than four days (if present in the selected subgroups), to avoid creating an artificially-closed network.

2.2.3 Statistical analyses

2.2.3a Network community structure and clan assignment

All statistical analyses were performed in R version 3.3.3 (R Core Team, 2016). The community structure of our network was investigated using the 'asnipe' (Farine, 2013), 'igraph' (Csardi and Nepusz, 2006), and 'assortnet' (Farine, 2014) packages. Dyadic association values were calculated using the Simple Ratio Index: the number of observations in which two individuals were recorded together, divided by the sum of the number of observations of the two individuals together and the number of observations of either/both individual(s) in separate subgroups (SRI; Cairns and Schwager, 1987). It was chosen given the following considerations about our data collection: every animal and every subgroup were not recorded in every sampling period, it was not possible to identify every animal in every subgroup observed, and we were not able to obtain calibration data (Hoppitt and Farine, 2017). In the context of social network analysis, 'communities' are groups of individuals which tend to associate with each other within the wider population (Croft et al., 2008); for our analysis, a community was equivalent to a hyaena clan. We assessed the strength of community structure within the network using 'modularity', where a value of one (in a range of zero to one) indicates strong community structure (Newman and Girvan, 2006). Communities within the network were determined using the Multilevel Community Detection algorithm, a hierarchical model in which each individual starts in its own community and is re-assigned to the community where it achieves

the highest contribution to modularity (Blondel et al., 2008). The multilevel algorithm was chosen because, during algorithm comparisons, it achieved the second highest overall modularity and, unlike the fast-greedy algorithm (which had the highest modularity), it did not separate den-dependent offspring from the rest of their clan (as determined from direct observations at communal dens). The robustness of community assignments was assessed using the method developed by Shizuka & Farine (2016), in which the network and its community membership were recalculated 100 times (via bootstrapping). The community assortativity value (ranging from zero to one) measures the degree to which pairs within the same community in the observed network were also assigned to the same community in replicate networks (Shizuka and Farine, 2016).

2.2.3b Testing for non-random associations

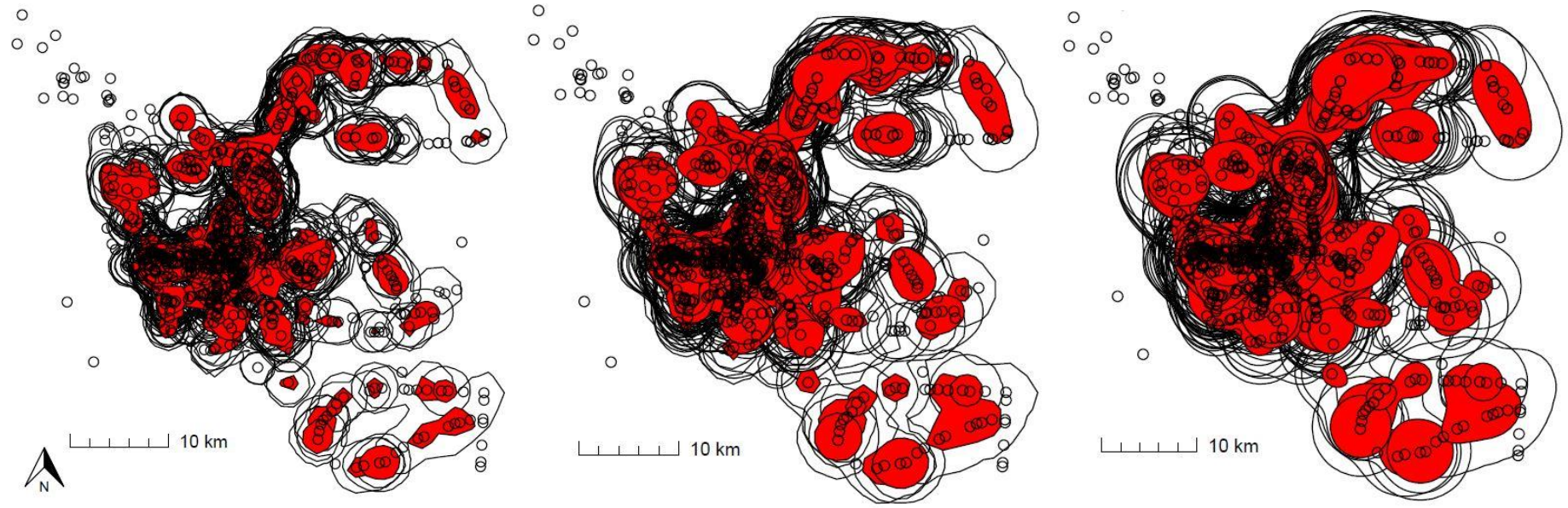
We tested the occurrence of non-random associations in our population by comparing the weighted degrees and coefficients of variation (CV) in our observed network with those of randomized networks. The degree of a network measures the number of relationships observed for each individual, and the weighted degree takes into account the strength of each relationship (Wey et al., 2008). In our case, weights were given by the number of times a given dyad was seen together. The weighted degrees were calculated for the observed network and 1000 network permutations that swapped individuals between subgroups using the 'sna' package (Butts, 2008); a significant *P* value indicated an observed weighted degree was significantly higher than the values from permutations (Farine, 2013). The CVs were calculated for the observed network and for 1000 randomized networks (with 100 swaps each) using the 'raster' package (Farine and Whitehead, 2015; Hijmans et al., 2016). A significantly higher CV of observed

SRI values compared to that of random data set would indicate the presence of preferred companions in population (Farine and Whitehead, 2015). The *P* value was calculated by dividing the number of times the CV of observed values is smaller than that of a randomized network by the number of randomizations (Farine and Whitehead, 2015).

2.2.3c Home range analysis

All spatial analyses were conducted using the 'adehabitatHR' package (Calenge, 2015). The total area occupied by an individual or social group is typically called their 'home range', which may overlap with those of other individuals or social groups (Gosling and Roberts, 2001). To determine the home ranges of individual hyaenas, we prepared a list of the sighting locations for each individual by removing any coordinates that were recurrent during 24-hour periods. Individuals were selected for home range analysis if they were observed at a minimum of seven unique locations. Locations for each observation were transformed from latitude and longitude to UTM coordinates (WGS 84) using the 'rgdal' package (Bivand et al., 2017). We then used kernel techniques to estimate the utilization distribution (UD; the distribution of probabilities that an animal is found at a location) for each individual (Worton, 1989). In choosing a smoothing parameter for UD estimation, the reference bandwidth is not reliable for data in which animal locations are clumped (as opposed to normally distributed) as it results in over-smoothing (Kie, 2013), and the least-squares crossed validation method (Silverman, 1986; Worton, 1989) failed to converge for several individuals in our analysis. Consequently, the smoothing parameter was selected by visualizing the effects of various values on individual home range polygons (95% kernel) relative to sections of the study area with low sampling effort or success (i.e. areas lacking

observations of any hyaenas). We selected a bandwidth that balanced over-smoothing into areas of low sampling effort with avoiding over-restriction of home range polygons: h = 1500, grid = 100, extent = 2.5 (Figure 2-A). We then used a fixed-kernel approach (Worton, 1989) to calculate the UD using this bandwidth for all individuals. The kernel overlap of all dyads at both the 95 and 50 contours was calculated using the Utilization Distribution Overlap Index (UDOI).



h = 1000, grid = 100, extent = 2.5

h = 1500, grid = 100, extent = 2.5

h = 2000, grid = 300, extent = 3

Figure 2-A. The smoothing parameter was selected by visualizing the effects of various values on individual home range polygons relative to sections of the study area with low sampling effort or success. *The plots show 95 % kernels (black lines) and 50 % kernels (red polygons) for 110 individuals with ≥ 7 unique relocations. Black open circles represent relocation coordinates for all hyaena sightings (i.e. sampling effort).*

2.2.3d Correlation between dyadic associations and home range overlap

A full Mantel test (Mantel, 1967) was performed to investigate the correlation between dyadic association indices (SRI) and spatial overlap (UDOI), using the 'vegan' package (Oksanen et al., 2017). The Mantel test can only compare two matrices of identical size (i.e. comprising the same individuals), so only the 110 individuals used in the UDOI analysis (i.e. those observed at ≥ 7 unique locations) were included in the association matrix for this analysis. The Mantel test was performed twice to test the matrix of SRI values against two different matrices of UDOI values (at 95 % and 50 % kernels).

2.2.3e Repeated analyses excluding large carcass data

To examine whether scavenging from large carcasses affects the associations and spatial overlap of hyaenas in our population, we re-analysed all of the above metrics using subgrouping data that excluded observations at large carcasses. Consequently, all direct and remote camera sightings that resulted from observations at carcasses large enough for prolonged scavenging (i.e. giraffe, elephant, and buffalo) were excluded from the data. Using this second set of data, we determined the network community structure, compared the observed and randomized networks, calculated individual UDs, and performed correlational analyses as described above.

2.2.3f Clan membership and home ranges

To estimate clan home range size and the density of resident hyaenas, we assigned individuals to a specific clan. For those individuals that were observed on at least four days, we compared their community assignments resulting from the Multilevel Community Detection algorithm when applied to data including and excluding observations at large carcases. Those individuals that did not change

assigned communities between the two sets of data were accordingly assigned a specific clan membership. Data for individuals which could not be assigned a clan in this way (i.e. changed communities between data sets) were excluded from subsequent analyses. We then used a Mann-Whitney U test to investigate whether SRI values for dyads of individuals from the same clan were significantly different than SRI values for dyads of individuals from different clans. The Mann-Whitney U test was also used to test UDOI values for dyads of individuals from the same clan or from different clans.

To estimate the size of each clan's home range, we collated all relocations (excluding carcass data) of assigned clan members observed during 2014 – 2015 (i.e. the period of greatest observer effort) and calculated the area of the 95 % kernel. The parameters used to calculate each clan's UD were the same as those used for individual home range analysis (h = 1500, grid = 100, extent = 2.5). To investigate the influence of scavenging behaviour on spatial overlap between clans, we then re-calculated these home ranges including locations from carcass observations, and compared the spatial overlap (UDOI) between pairs of clans when home ranges were calculated with and without carcass data using a Wilcoxon signed-rank test.

To determine the density of resident hyaenas, we calculated the overall area of the relocations of assigned clan members during 2014 – 2015 (including carcass observations) at the 95 % kernel, and divided this area from the number of individuals that contributed to these relocations. The relocations of den-dwelling cubs (known for only one clan) were removed from the home range and density analyses, as they were only ever observed at the den, and we were unable to monitor dens for all clans during 2014-2015.

2.3 Results

Hyaena observations were recorded over a total area of 2193 km^2, with the main study area (95% kernel minimum convex polygon) occupying 1355 km^2. The majority of observations occurred during 2014 and 2015 (Figure 2-B). We observed 4013 subgroups (excluding recurrent groupings within 24 hours) with the majority of sightings only having one individual (Table 2-A); the largest subgroup observed contained 18 individuals.

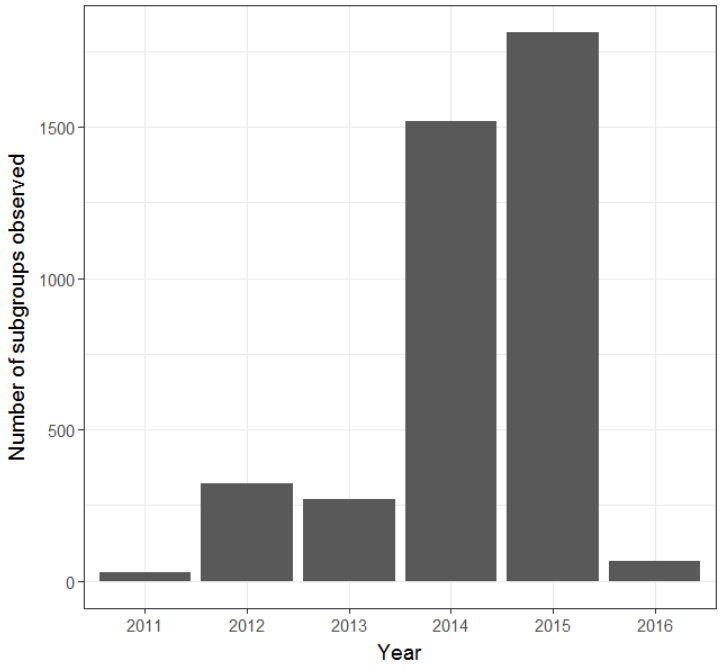


Figure 2-B. The number of subgroups observed during each year of the study.

Table 2-A. The subgroup sizes observed during the study.

Subgroup size	Number of times observed	Percentage of observations
1	2742	68.3
2	721	18.0
3	280	6.98
4	144	3.59
5	64	1.59
6	23	0.57
7	19	0.47
8	7	0.17
9	3	0.07
10 +	10	0.25

Over the course of our five-year study, 390 hyaenas were individually identified from photographs, and 184 of these individuals were observed on at least four days (170 individuals when excluding carcass observations). Since our network analysis included all subgroups that involved any individuals which were seen on at least four days, their companions that did not meet the sighting threshold were also included. Therefore, the number of individuals included in the analysis was 252 when including carcass observations and 225 when excluding them.

2.3.1 Network community structure

This population exhibited strong group structure and high community assortativity (Table 2-B), with approximately 60 % of individuals assigned to the same community regardless of the inclusion or exclusion of observations at large carcasses (Table 2-C). Two analyses showed that individuals were found to interact strongly with a small number of other individuals, in a pattern which was inconsistent with a null hypothesis that associations were random. First, the weighted degree of the observed network was significantly higher than the weighted degrees of 1000 randomized networks for data including ($p < 0.001$) and excluding ($p = 0.016$) observations at carcasses. Second, the CV of the observed network was significantly higher than the CVs of 1000 randomized networks for data including ($p < 0.001$) and excluding ($p < 0.001$) observations at carcasses. These results therefore suggest strong clan structuring in this population.

Table 2-B. The results of applying the Multilevel Community Detection algorithm to networks including and excluding observations at carcasses.

Carcass observations	**Number of communities**	**Community assortativity**	**Modularity**
Included	36	0.9075	0.8483
Excluded	46	0.8483	0.8394

Table 2-C. The result of including observations from large carcasses when determining the clan assignments of 252 individuals*(as compared to clan assignments that were determined using data that excluded these observations).*

Changes resulting from the inclusion of carcass observations	**% of individuals**
Did not change clans	59.9
Added to a clan	12.7
Added to analysis (only now seen on ≥ 4 days)	10.7
Only observed alone	7.14
Always part of small unknown subgroup	5.56
Removed from or changed clans	3.97

There were 112 individuals that were included in both analyses (i.e. including and excluding carcass data) and did not change community membership between them. These individuals were assigned to nine clans, eight of which corresponded with clans which had been subjectively defined based on observations in the field (Table 2-D). Clan members were seen together much more often than they were seen with members of other clans: SRI values for dyads from the same clan were significantly higher than those for dyads from different clans, whether carcass data were (Mann-Whitney $U = 18952000$, $n = 22651$, $p < 0.001$) or were not ($U = 18067000$, $n = 22651$, $p < 0.001$) included.

Table 2-D. Nine clans that were designated by community detection analysis.*The numbers of individuals per clan were assigned by the algorithm, but to consider demographic changes over time, we also determined the highest number of clan members observed concurrently (i.e. during the same year). The clan size for only one clan (Disney) included den-dwelling cubs. The home ranges of five clans were estimated at the 95 % kernel using data from 2014-2015; home ranges were not estimated for clans that contained ≤ 8 individuals and were thus deemed incomplete.*

Clan	Number of individuals per clan assigned by algorithm	Highest number of members observed concurrently	Home range area (km^2)
Spice	21	17	128.84
Westerosi	20	20	408.62
Disney	19	19	176.70
Athena	17	14	191.42
Ginger	17	17	290.82
Moremi	8	8	N/A
South	6	6	N/A
Fly	3	3	N/A
Unknown	1	1	N/A

Four of the nine clans designated by the algorithm were particularly small (≤ 8 individuals), which is likely due to low sampling effort in their home ranges, and we thus assume that our knowledge of their membership is incomplete. For the five full clans, clan size as selected by the community detection algorithm ranged from 17 to 21 individuals (mean ± SD = 18.8 ± 1.79 individuals). To consider demographic changes over time due to births, deaths, and dispersal, we evaluated the number of assigned clan members present in the population concurrently (i.e. during the same year; Table 2-D, Figure 2-C). The highest numbers of concurrent clan members were observed during the period of highest observer effort (2014-2015). We thus consider the highest number of members observed concurrently as the most accurate estimate of a given clan's size, which for the five main clans ranged from 14 to 20 individuals (mean ± SD = 17.4 ± 2.3 individuals).

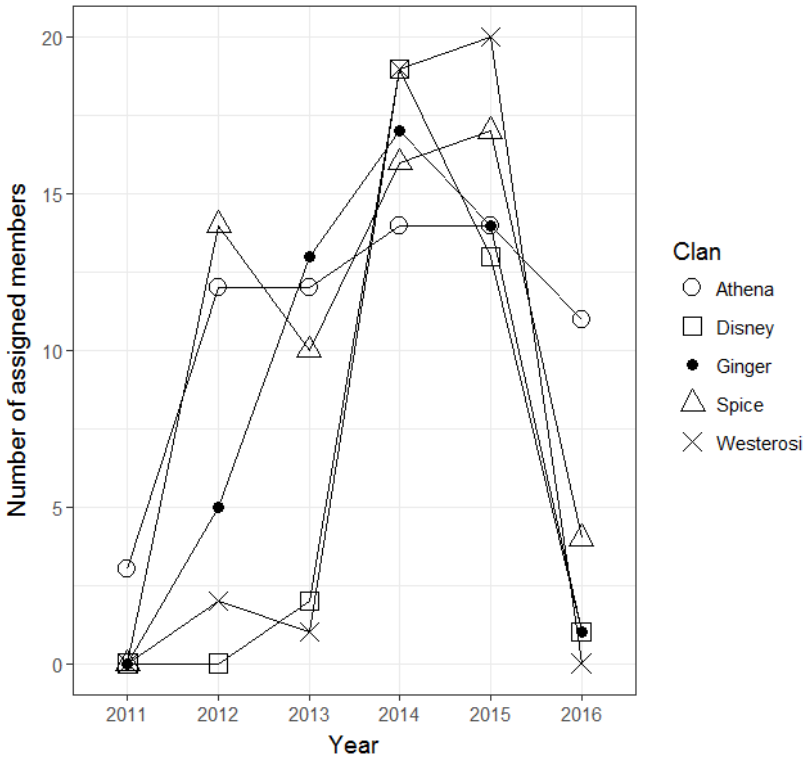


Figure 2-C. The number of assigned clan members that were observed during each year of our study. *Data for 2016 was only available through June and is thus an incomplete dataset.*

2.3.2 Home range size and overlap

We calculated the UDs for 110 individuals when including carcass observations, and again for 98 individuals when excluding carcass data. Dyads from the same clan tended to occupy the same geographic area: the levels of overlap (UDOI) for dyads of individuals from the same clan (Figure 2-D) were significantly higher than those for dyads of individuals from different clans (Figure 2-E), whether including (95 % kernel: Mann-Whitney U = 238060, n = 8282, $p < 0.001$; 50 % kernel: U = 559770, n = 8282, $p < 0.001$) or excluding (95 % kernel: Mann-Whitney U = 203230, n = 7141, $p < 0.001$; 50 % kernel: U = 480850, n = 7141, $p < 0.001$) carcass observations. Clan home range sizes ranged from 176.70 to 408.62 km^2 (mean ± SD = 239.28 ± 99.73 km^2; Table 2-D, Figure 2-F). The inclusion of carcass observations did not significantly change the amount of

spatial overlap between clans (Wilcoxon signed-rank test: V = 115, n = 20, p = 0.2064). Eighty-seven assigned clan members were observed during 2014-2015 over an area of 493.29 km^2, equalling a density of 0.176 resident hyaenas/km^2 excluding den-dwelling cubs.

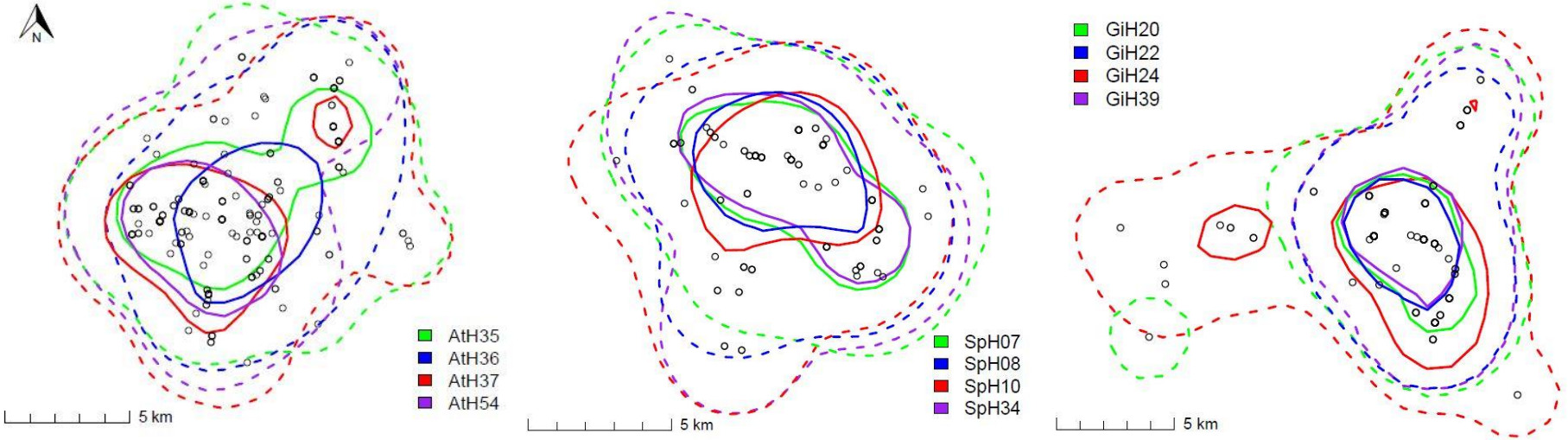


Figure 2-D. Home ranges of four individuals of the same clan, for three clans.*Each individual within a plot is represented by a different colour. Open circles represent relocation coordinates of individuals. The 95 % (dotted lines) and 50 % (solid lines) kernels are shown for each individual.*

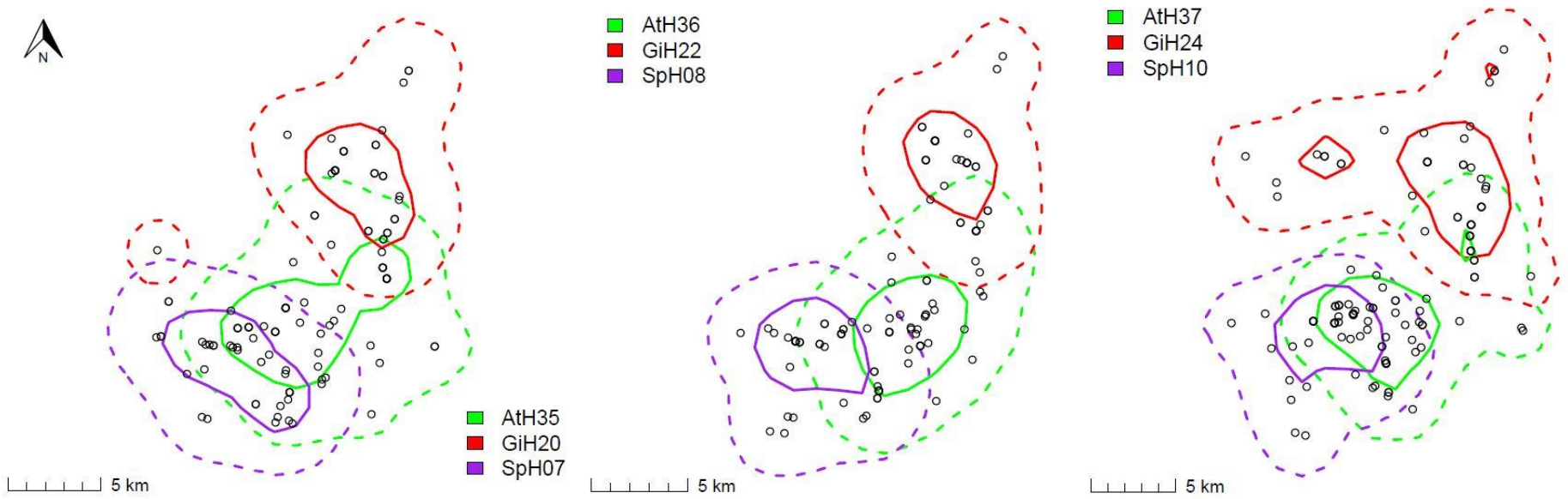


Figure 2-E. Home ranges of individuals from three clans, with each plot showing a different combination of individuals from the same three clans. *Each individual (and by extension, each clan) within a plot is represented by a different colour. Open circles represent relocation coordinates of individuals. The 95 % (dotted lines) and 50 % (solid lines) kernels are shown for each individual.*

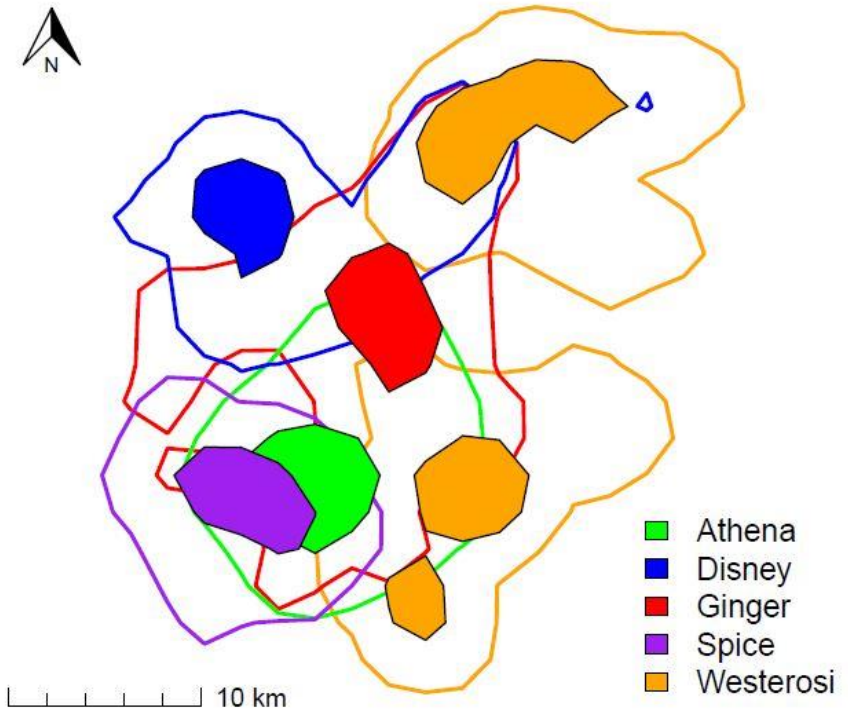


Figure 2-F. Home ranges of the five main clans that were delineated by our network analysis, which corresponded to our clan designations from the field.*Coloured lines indicate the 95 % kernel, and filled polygons indicate the 50 % kernel, for each clan. The relocation dataset used to create this map did not include observations at carcasses.*

2.3.3 Correlation between dyadic associations and spatial overlap

For data that included carcass observations, there was a strong correlation between the association strength between pairs of individuals, and the spatial overlap between their UDs, at both the 95 % (Mantel statistic = 0.552, n = 109, p = 0.001) and 50 % (Mantel statistic = 0.538, n = 109, p = 0.001) kernels. This was also the case for data that excluded carcass observations at the 95 % (Mantel statistic = 0.525, n = 97, p = 0.001) and 50 % (Mantel statistic = 0.514, n = 97, p = 0.001) kernels.

2.4 Discussion

This study determined the clan membership and estimated home range sizes of a previously under-studied population of hyaenas in northern Botswana. As expected, the population exhibited strong group structure with non-random association patterns, and dyadic association strength was strongly correlated with spatial overlap. Social network analysis delineated five main clans within the study area, each comprising 14 to 20 individuals (excluding den-dependent cubs) inhabiting home ranges of 176.70 to 408.62 km^2, whose compositions mostly corresponded with our expectations from the field. The dyadic association and spatial overlap values for individuals within the same clan were significantly different than for dyads of individuals from different clans, suggesting that the clans delineated by the network analysis were an accurate reflection of population social structure. Importantly, the inclusion of carcass data in the analysis showed that scavenging behaviour influences hyaena association patterns, and 90 % of inter-clan associations occurred at carcasses.

Despite the strong associations among frequently-sighted individuals in our population, 47 % of photo-identified hyaenas were observed on fewer than four days. This is potentially a result of the opportunistic nature of sightings data. Additionally, it is possible that our study area hosts a hyaena population comprised of permanent resident clans and a large number of transient individuals. Transient individuals observed during our study could have been dispersing males prospecting for a new clan (Van Horn et al., 2003) or commuters searching for prey (as in Hofer and East, 1993b). Although the southern edge of our study area is separated from domesticated livestock by a veterinary fence, this fence has been shown to be permeable to hyaena movements (Cozzi et al., 2013a).

Knowledge of a permanent and relatively defenceless food resource (i.e. cattle) could motivate hyaenas to commute from protected wildlife areas into the livestock area to hunt. Our study area, located in the buffer zone between the protected game reserve and the livestock area, may therefore serve as traveling route for hyaenas preying on livestock. For example, two individuals from known clans with home ranges in Moremi Game Reserve were also sighted in the livestock area south of the fence (approximately 30 km from their typical range; L. Rich and J. Vitale, unpublished data). Additionally, 50 % of 44 identified individuals sighted south of the fence were also observed north of the fence in the WMAs (L. Rich and J. Vitale, unpublished data).

Our clan sizes are likely to be conservative estimates given our criteria for assigning clan membership, which required individuals to be present in and assigned to the same community in both analyses (including and excluding carcass observations). Younger individuals (e.g. juveniles or den-dependent cubs) that were never observed feeding at carcasses may have been disregarded by our clan assignment procedure, resulting in smaller estimated clan sizes. We were unable to monitor den activity consistently, so our observations for some clans and time periods lacked den-dependent offspring. During the period of highest observer effort, we only observed one communal den, whose four cubs were included in concurrent clan size. Therefore, given the similarity of overall group size for the five main clans, we can expect the four clans without den monitoring to have had a similar number of den-dwelling cubs in addition to their highest concurrent clan size. This results in a final clan size estimate ranging from 18 to 24 total individuals, with a mean of 20.6 individuals per clan.

Although we found significant correlation between the strength of associations and spatial overlap among individuals, the home ranges occupied by the five clans overlapped considerably at the 95 % kernel. Additionally, our home range map shows partial overlap between two clans at the 50 % kernel, which is generally reported as an individual's or social group's exclusive territory (Laver and Kelly, 2008). Thus, for our population, clan membership should not be assumed based solely on the geographic location of opportunistic sightings, especially given the occurrence of extra-territorial forays by several individuals (J. Vitale, unpublished data; Cozzi et al., 2015). The observed variation in home range sizes among our clans could be due to the differing resources supported across the mosaic of habitat types of our study area, as space use by hyaenas is influenced by resource availability (Trinkel et al., 2004). Within the Okavango Delta ecosystem, grassland and floodplain habitats exhibit the highest species richness (Rich et al., 2016), and prey abundance is lower in habitat dominated by mopane (*Colophospermum mopane*) woodland (Bartlam, 2010; Broekhuis, 2012). Clans inhabiting areas of low prey abundance likely occupy larger home ranges than clans inhabiting prey-rich habitats, and much of the largest clan home range in our study (Westerosi clan, 408.62 km^2) is characterized by mopane habitat.

For the years 2014- 2015, we calculated a conservative density estimate of 0.176 resident hyaenas/km^2 excluding den-dwelling cubs. This result is relatively consistent with results from two previous surveys at our study site: a 2014-2015 mammal-occupancy camera trap study estimated overall hyaena density to be 0.127 individuals/km^2 (95 % CI = 0.088 – 0.190; Rich, 2016), and a calling station survey conducted between 2007-2010 estimated density to be 0.144 adults/km^2 (Cozzi et al., 2013b). Therefore, our study site hosts a medium-sized

density of hyaenas compared to the range of reported densities across ecosystems (Holekamp and Dloniak, 2011), from the densely-packed habitats of East Africa (1.65 hyaenas/km2, Amboseli National Park; Watts and Holekamp, 2008) to the sparsely-populated deserts of Namibia (0.0085 hyaenas/km2, Naukluft National Park; Tilson and Henschel, 1986) and South Africa (0.009 hyaenas/km^2, Kalahari Desert; Mills, 1990). Our population appears most similar to hyaenas in South Africa's Kruger National Park, which had a mean clan size of 17 individuals and a mean clan home range of 130 km^2 (Henschel and Skinner, 1987; Holekamp and Dloniak, 2011).

Scavenging opportunities at large carcasses brought together individuals from different clans. We observed 28 pairs of associating hyaenas that were assigned to different clans by our analysis (excluding redundant dyads within 24 hours), and 90 % of these inter-clan interactions occurred at carcass sites. Observations of inter-clan interactions during this study (either directly or via remote camera video) often involved submissive behaviour by the non-resident individual towards the hyaena(s) resident to that territory (seen in 48 % of 25 inter-clan pairs with photo/video records; J. Vitale, unpublished data). This is consistent with observations from the Serengeti ecosystem, in which hyaenas commuting to areas with migratory prey located outside their clan territory showed clear submissive behaviour to resident individuals that they encountered (Hofer and East, 1993b). On the other hand, our results differed in that non-resident individuals in the Serengeti typically waited to feed until after resident individuals had abandoned the carcass (Hofer and East, 1993b), but in our study, 77 % of 22 inter-clan scavenging pairs with photo/video records were observed feeding (or attempting to feed) alongside each other (J. Vitale, unpublished data). This relative tolerance

towards intruders seemed to occur when the intruding hyaenas were alone or in pairs, as opposed to behaviours observed during one observation session in which visits by subgroups from two neighbouring clans to an elephant carcass (located within their home range overlap zone) resulted in prolonged clashes and whooping bouts (J. Vitale, unpublished data). Although scavenging influenced associations among individuals from different clans, we found that the inclusion of carcass observations did not significantly alter the amount of spatial overlap between pairs of clans. This result is likely a reflection of previous findings that carcass availability altered hyaena ranging behaviour for only 10-12 days (Cozzi et al., 2015), and thus our analysis of long-term spatial overlap did not capture these short-term changes.

Including observations at large carcasses had several notable effects on the community assignments that resulted from our network analysis. First, the Multilevel Community Detection Algorithm detected fewer communities with the inclusion of carcass observations, probably because feeding together at large carcasses for multiple days increased the number of interactions between individuals that might not otherwise be strongly associated. Second, including carcass observations resulted in the separation of one clan's den-dependent or juvenile offspring from the rest of the clan (i.e. a female and the cub she was observed nursing were assigned to separate communities). Third, in the analysis including carcass observations, approximately 11 % of individuals were added to the dataset (i.e. were seen on less than four days when carcass observations were excluded), and approximately 12 % of individuals were re-assigned to clans, having been designated as lone individuals or belonging to extraneous communities when excluding carcass observations. Consequently, some

individuals appear to be erroneously assigned to clans by the community detection algorithm when including carcass observations. For example, one individual was observed feeding at an elephant carcass on three occasions alongside individuals with which it was grouped into a clan by our analysis using carcass data. This individual was later fitted with a GPS collar and its typical home range (monitored for 14 months) was found to be located over 20 km away, and it was never observed interacting with these clan members again (J. Vitale, unpublished data). Thus, the inclusion of carcass observations in the social network analysis resulted in individuals assigned to the same community that were not truly part of the same clan.

Our study is the first to use social networking analysis of opportunistic observations to determine the membership and home ranges of clans within a hyaena population. The apparent accuracy of our population density estimates, and the close correspondence between the clans detected by our analysis and those which were previously defined while in the field, highlights the value of social network analysis in assessment of population structure. However, the observed effects of large carcass scavenging on hyaena association patterns emphasise the importance of considering a species' behavioural ecology and field observations when interpreting social network analyses and the resultant community structure of a population.

Chapter 3 : Communal latrine use by spotted hyaenas (*Crocuta crocuta*) reflects a seasonal territorial marking strategy

Abstract

The creation and maintenance of communal latrines is widespread among mammals, and latrine use is influenced by spatial and temporal variation in the costs and benefits of maintaining such marking sites. By studying scent-marking frequencies and the spatial distribution of latrines, this study aimed to systematically investigate latrine use by spotted hyaenas (*Crocuta crocuta*) in northern Botswana, and to examine the potential role of latrines in territorial advertisement by hyaena clans. Latrine characteristics and hyaena activity were monitored to test the influence of season and a latrine's location (relative to clan territories and human-made roads) on latrine use. The number of hyaena scats was recorded at 78 latrine sites in five clan home ranges on a monthly basis to investigate changes in the number of deposits ('latrine size'), and 50 latrines were each monitored continuously for one month with remote cameras to record hyaena visitation and scat deposition rates. Variation in latrine use showed patterns directly related to season: the wet (rainy) season (November-March) was correlated with decreases in latrine size, hyaena visitation, and scat deposition. In testing for the effects of spatial distribution on latrines, the number of scats per latrine was significantly higher at latrines located within clan territories (< 50% utilization) than at latrines in areas of overlap among neighbouring clan home ranges (> 50% utilization). Hyaenas preferentially placed latrines along the edge of roads (rather than further off-road), and were more likely to re-use road-side latrines in subsequent years. This pattern of latrine distribution at the edges of

passable roads highlights the potential for hyaena latrines to be used for non-invasive population monitoring.

3.1 Introduction

Chemical signalling is a mode of communication utilised by a wide range of animals in a variety of behavioural contexts (Bradbury and Vehrencamp, 1998). Scent-marking, a behaviour that involves animals depositing scents in the environment (onto various substrates including other animals, vegetation, objects), can serve important functions in territoriality, group identity, individual recognition, alarm signalling, and reproduction (Johnson, 1973; Jordan et al., 2014; Gilfillan et al., 2017). Scent-marks can have temporal stability, which enables information to be broadcast for longer periods than visual or acoustic signals, and thus provide a record of an individual's presence in space and time (Gorman and Trowbridge, 1989). As a result, scent-marking is often an important mode of communication for species in which individuals or groups defend a territory (Gorman, 1984). Scent-marks can be used to demarcate and advertise the ownership of a territory through 'scent-matching': non-residents can identify territory owners by making associations between the scent of an individual and any scent-marks they have encountered in the vicinity (Gosling, 1982). Signals broadcast over protracted periods are an effective means of communicating the presence of territorial residents, and particularly those for which territorial interactions with intruders or neighbours are associated with high risk of injuries, such as those among territorial carnivore species (Gosling, 1982; Packer et al., 1990; Cassidy et al., 2015).

A frequently-occurring scent-marking behaviour is the creation and maintenance of 'communal latrines', sites visited by multiple conspecifics that result in

accumulations of scent-marks such as faeces, urine, and/or glandular secretions (Gorman and Trowbridge, 1989). Several species of social mammals are known to use communal latrines, including white-footed sportive lemurs (Lepilemur leucopus, Droscher and Kappeler, 2014), swift foxes (Vulpes velox, Darden et al., 2008), meerkats (Suricata suricatta, Jordan et al., 2007), European badgers (*Meles meles*, Pigozzi, 1990), and spotted hyaenas (Crocuta crocuta, Bearder and Randall, 1978). Communal latrines can function in social bonding, territorial advertisement by social groups, and advertisement relating to mate defence (Jordan et al., 2007; Droscher and Kappeler, 2014).

Spotted hyaenas are nocturnal predators for which olfactory communication appears to be particularly important. Hyaenas live in permanent social groups called clans but exhibit fission-fusion sociality, in which individuals frequently join and depart subgroups that may forage independently, and thus the reaffirmation of social bonds among clan members may be particularly important (Kolowski et al., 2007; Smith et al., 2008). Ritualized 'greeting ceremonies', in which pairs of individuals engage in mutual ano-genital sniffing, are thought to serve this purpose and demonstrate the investment in olfactory communication in hyaena society (Kruuk, 1972; Glickman et al., 1997; Smith et al., 2011). Olfactory communication among hyaenas may also involve the maintenance of communal latrines, a behaviour that has previously been observed for hyaenas in many ecosystems (Kruuk, 1972; Bearder and Randall, 1978). Despite widespread observations of latrine use by hyaenas, much of the published information concerning hyaena latrine use comes from anecdotal reports made as part of studies of other behavioural phenomena (with the exception of Bearder and Randall, 1978, and Mills and Gorman, 1987).

Although scent-marks are long-lasting signals, they degrade in the environment over time and consequently animals must expend time and energy replenishing signals (Gosling and Roberts, 2001). Animals may therefore invest in scent-marking at different times and/or locations as part of an economical marking strategy. For example, the spatial distribution of latrines within a territory has been shown to vary depending on territory size and resource use, and has been explained by social group size and the energetic costs associated with scent-marking (Mills and Gorman, 1987). Animals may invest in latrines to mark territorial boundaries (e.g. European badgers, Buesching et al., 2016) while others do so in the core areas of their territory (also called 'hinterland' marking; e.g. brown hyaenas Hyaena brunnea, Gorman and Mills, 1984). In describing hyaena latrines, Bearder & Randall (1978) distinguished between 'temporary latrines,' which develop near short-term sites of interest such as carcasses, and 'long-term latrines,' which are usually associated with environmental landmarks and visited regularly over a long period of time. Like many species, hyaenas are known to prefer moving along frequently-travelled routes such as roads rather than through thick bush (Bearder and Randall, 1978), and hyaena latrines in the Serengeti (Kruuk, 1972) and Transvaal Lowveld ecosystems (Bearder and Randall, 1978) were found primarily along roads and game trails (although this could have been the result of sampling bias). If latrines are part of a deliberate scent-marking strategy for intraspecific communication among hyaenas, and are consequently constrained by the costs associated with scent-marking as described elsewhere (Roberts and Gosling, 2001), we would expect hyaenas to preferentially build and maintain latrines in locations that facilitate increased detectability and visitation rates (e.g. along roads).

The maintenance of communal latrines is likely important in territorial defence by hyaena clans, particularly since there is evidence for individual- and group-specific odours in anal gland secretions (called 'paste') that are often deposited at latrines (Burgener et al., 2009; Theis et al., 2012). Furthermore, in certain hyaena populations, clan subgroups were observed frequently visiting latrines located along territorial boundaries to investigate and deposit scent-marks (as in East African ecosystems: Kruuk, 1972; Hofer and East, 1993a). However, the role of latrines in hyaena territoriality has not yet been formally tested. If latrines function in territorial advertisement and defence by hyaena clans, we would expect hyaenas to invest more (i.e. through greater numbers of scats, higher visitation and scat deposition rates) in latrines located inside clan territories (i.e. in exclusive areas) than in peripheral areas of the home range overlapping with neighbouring clans.

Patterns of scent-marking and latrine use may also vary seasonally. For instance, the physical effects of seasonal rainfall on deposited marks could contribute to variation in scent-marking behaviour throughout the year. Rainfall appears to degrade (i.e. wash away) scent-marks from other species, such as giant pandas (*Ailuropoda melanoleuca*, Nie et al., 2012) and river otters (*Lontra canadensis*, Torgerson, 2014). Additionally, seasonal scent-marking patterns could be related to seasonal variation in intrusion pressure and/or territoriality among social groups. For example, latrine use by meerkats was more likely during the peak breeding season and was also correlated with monthly rates of encounters with intruding males (Jordan et al., 2007). Given that our study ecosystem, the Okavango Delta of Botswana, exhibits distinct wet and dry seasons, patterns of latrine use by hyaenas may vary throughout the year. Rainfall in the Delta is

highly seasonal, occurring between November and March, and the commencement of the rainy season coincides with synchronized calving and altered space use for many herbivores species, which could alter resource distribution among hyaena clan territories (Wilson and Dincer, 1976; Owen-Smith and Ogutu, 2013). Therefore, due to seasonal rainfall, we would expect scats deposited at latrines to be degraded more quickly during the rainy season. Furthermore, if latrine use is a reflection of territorial behaviour by hyaena clans, and territories are defended year-round, we would expect hyaenas to visit and maintain latrines at equivalent rates throughout the year.

Here, we present a systematic investigation of natural latrine use by a hyaena population to test these hypotheses about the function of latrines in hyaena behaviour. Through direct observation and remote cameras, we monitored latrine characteristics and hyaena activity at latrines in the Okavango Delta ecosystem over five years. We examine whether hyaenas preferentially place latrines alongside roads versus further off-road, and if this placement affected whether or not hyaenas continued to use a latrine in subsequent years. We also test the influence of season and a latrine's location relative to clan territories (i.e. within exclusive clan territory or areas of overlap between clan home ranges) on latrine size (i.e. number of scats), hyaena visitation, and scat deposition rates.

3.2 Materials and Methods

3.2.1 Study site

This study was conducted within the Okavango Delta ecosystem of northern Botswana, specifically in the south-eastern section of the Moremi Game Reserve and surrounding Wildlife Management Areas (centre of study area: S19.50098,

E23.61010). The habitat was characterized by a heterogeneous mixture of grasslands, acacia scrubland, and mopane woodland (for further details, see McNutt, 1996). Precipitation was highly seasonal, with an annual rainy season occurring from November to March (Wilson and Dincer, 1976).

3.2.2 Data collection

3.2.2a Latrine characteristics

Latrines were found opportunistically from 2012 - 2016 during daily movements throughout the study area (approximately 2193 km^2) in a vehicle. Characteristics of latrines were recorded by observers while on foot. For this study, a latrine was defined as a site containing at least two distinguishable piles of faeces (i.e. faecal deposits called 'scats'). For each latrine, the following characteristics were recorded: date, time, geographical coordinates, total number of hyaena scats, the presence or absence of necrophagous beetles on scats, and whether the latrine was located road-side or further off-road. Geographical coordinates were recorded using a handheld Garmin 72H unit from the approximate centre of each latrine's expanse. At our study site, 'roads' were established, unpaved vehicle tracks formed in the sand. Latrines were defined as 'road-side' if they were within 20 m of a road, whereas latrines further than 20 m from the road were designated as 'off-road' (determined using the distance between the geographical coordinates of latrines and roads in Garmin MapSource). 'Single scats', defined as a single distinguishable pile of faeces that was not deposited in a communal latrine, were also recorded opportunistically. Latrines located in the main study area were checked on a monthly basis to document changes in the number of scats over time, but for data analysis we only used the data from 78 latrines (interval between latrine checks: range = 0 – 192, mean ± SD = 29.85 ± 16.21 days).

Latrines were selected if they had at least four records of scat counts during 2014-2016 and were located within the home range of at least one of the five study clans; a latrine's data were included until it was no longer considered to be active (i.e. if there was no deposition of new scats for several months after the end of a rainy season). The amount of rainfall in millimetres was recorded manually each day, using a graduated cylinder rain gauge placed at the field research station (the approximate centre of the study area).

3.2.2b Road-side and off-road transects

To investigate whether hyaenas placed latrines preferentially road-side or off-road, we conducted 18 transects from a vehicle to search for latrines and single scats. Three 5 x 5 km blocks encompassing the centre of the study area (i.e. surrounding the field station) were drawn in Garmin MapSource (version 6.16.3). For each block, the starting coordinates and a 360º bearing were randomly-generated in Microsoft Excel for each of three road-side and three off-road transects. Each road-side transect commenced at the closest location on a road to the starting coordinates generated by MapSource. The road was driven along in the most similar direction to the 360º bearing until the vehicle's odometer reached three kilometres. At road junctions, the road which followed the bearing more closely was selected. Off-road transects commenced at the coordinates generated in MapSource, and following the bearing as closely as possible (while avoiding scrub impenetrable to the vehicle) until the odometer reached three kilometres. If the vehicle reached the edge of the 5 x 5 km block before driving three kilometres during either transect type, the transect was paused and restarted at the same latitude or longitude along the opposite edge of the block, along the same 360-degree bearing. Care was taken to drive both types of transect at the same low

speed (approximately five kilometres per hour) to ensure equal effectiveness of latrine spotting. The characteristics of all latrines or scats encountered during transect searches were recorded in the manner outlined above.

3.2.2c Visitation monitoring

One motion-triggered camera was placed at each of 50 latrines to monitor visitation rates by hyaenas throughout 2014 and 2015. Infrared StealthCam Prowler and StealthCam G30 cameras (StealthCam LLC, Grand Prairie, TX, USA) were affixed to a pole that was driven into the ground and angled towards the latrine to capture a five-photo burst or 30 sec video without a scheduled trigger delay (actual recovery period 5 to 10 sec depending on the camera). Cameras were placed in such a way to encompass as much of the latrine as possible but we were unable to monitor the entire expanse of most latrines; if a latrine was located along a road (as in 94% of 50 monitored latrines), the camera was positioned to capture scats as well as any movement along the road. The characteristics of the focal latrine were recorded at the start and end of monitoring. The total monitoring time at each site was calculated by adding the length of time the camera was turned on and facing the experimental site over the course of the camera's deployment. Cameras were checked every 3-5 days to ensure that they were still functioning. If a camera was found to not be recording the site (due to disturbance by wildlife or an exhausted battery), the monitoring period was calculated up to the time of the disturbance (if captured on camera) or the last video recorded before the disturbance, and the camera was reactivated/repositioned (mean number of inactive camera days per site = 1.88 ± 2.30 days, range 0 – 8.04 days). As such, recording effort may have been underestimated.

Photos and videos from each latrine were checked for visits from hyaenas. The following information was recorded for each visit: date, time, and the identities of all hyaenas observed. A 'visit' was defined as a lone hyaena or group of hyaenas recorded by the camera, with recordings over five minutes apart designated as separate visits. Individual hyaenas were identified by eye using their unique spot patterns, and all photo-identified individuals were given a unique ID code for subsequent matching of sighted individuals.

3.2.3 Clan assignments and territory classifications

As part of a larger study (see Chapter 2), hyaenas were observed opportunistically through direct sightings or motion-triggered cameras placed at sites of interest (e.g. latrines, dens, carcasses). Association (subgrouping) data were recorded using the 'gambit of the group' method, which defines all individuals observed together at a given time as associating (Farine and Whitehead, 2015). Subgroups were defined as one or more hyaenas separated from other individuals by at least 200 m in direct sightings (Smith et al., 2008), and as one or more hyaenas recorded within two minutes of each other by remote cameras (hyaena traveling speed between 4-10 km per hour, Mills, 1990). Because we were interested in long-term associations, we removed all duplicate sightings of identical dyads within 24 hours. Statistical analyses were performed on all subgroups in which any individuals that were sighted on at least four days were present.

In Chapter 2, the structure of the hyaena population was investigated through social network analysis (Wey et al., 2008) using dyadic association values calculated as the Simple Ratio Index (Cairns and Schwager, 1987). In the context of network analysis, 'communities' are groups of individuals which tend to associate with each other within the wider population. We used the 'modularity'

of the observed community structure to assess its quality, in which a value of one (in a range of zero to one) indicates strong community structure (Newman and Girvan, 2006). Communities within the social network were determined using the Multilevel Community Detection algorithm, a hierarchical model in which each individual starts in its own community and is re-assigned to the community where it achieves the highest contribution to modularity (Blondel et al., 2008). We examined whether scavenging behaviour affected associations among individuals by comparing the community assignments resulting from the Multilevel Community Detection algorithm when including and excluding observations at large carcasses from the dataset. Those individuals that did not change assigned communities between the two sets of data were considered to belong to an identifiable clan, and their data were used for spatial analysis of clan home ranges. The individuals that were not assigned to a clan were thus excluded from this process.

The total area occupied by an individual or social group is typically called their 'home range', which may overlap with those of other individuals or groups, whereas the exclusive space defended by an individual or group is designated as their 'territory'(Gosling and Roberts, 2001). Five clans were delineated by the social network analysis, comprised of 18 to 24 individuals per clan (mean = 20.6 individuals). To determine the home range of each clan, we first collated all relocations during 2014-2015 of assigned clan members, removing all duplicate relocations from a given individual within one day. We then used kernel techniques to estimate the utilization distribution, a probability distribution of the areas used by an animal, for each clan (Worton, 1989). We defined each clan's home range at the 95 % kernel contour, which correlates with the 95 %

probability of an animal being found within the defined area (Worton, 1989). To determine the territory boundaries for each clan, we calculated the percentage of home range overlap for each pair of neighbouring clans (n = 20 pairs, n = 5 clans) at multiple kernel contours, starting at 95 % and decreasing by 5 % for each recalculation (as in Jordan et al., 2007). The kernel contour at which most clans no longer overlapped was taken as the estimated clan territory boundaries, which in the case of our hyaena population constituted the 50 % kernel (Table 3-A, Figure 3-A).

Table 3-A: The range, mean, and standard deviation of the percentage of overlap between 20 pairs of 5 clans at the 95%, 80%, 70%, 60%, and 50% kernels.

Kernel	**Percentage of overlap between clans**			
	Minimum	*Maximum*	*Mean*	*Standard deviation*
95%	0	76.6	32.0	21.8
80%	0	44.4	13.6	14.2
70%	0	35.7	8.4	10.3
60%	0	36.4	4.02	10.1
50%	0	12.5	1.13	3.49

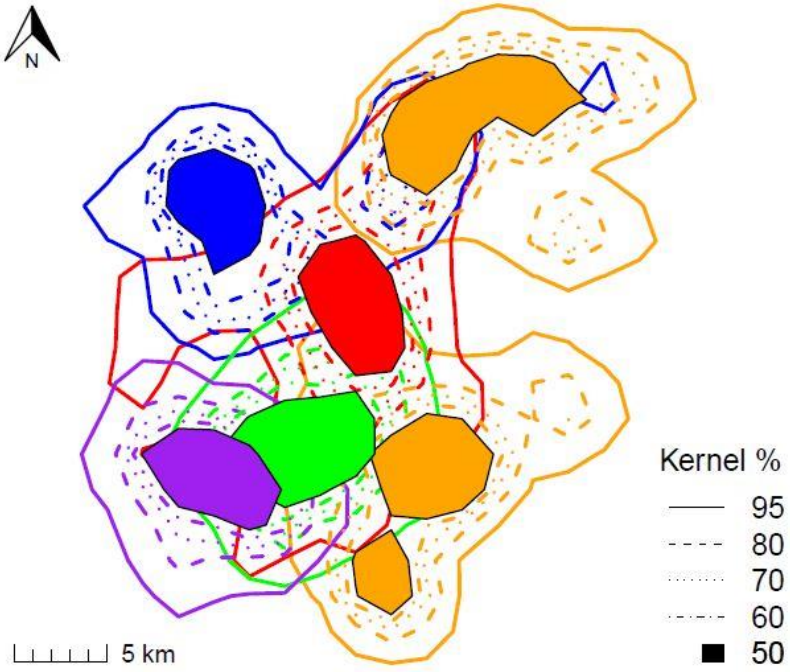


Figure 3-A: Visualizing the variation in home range overlap among clans at different kernels of the utilization distribution.*Each clan is represented by a different colour. The solid line indicates the 95% kernel, generally reported as a clan's home range, and other line types indicate 80, 70, and 60 kernel contours. The kernel contour at which most clans no longer overlapped (50% kernel, filled polygons) was taken as the estimated boundary of clan territories. Utilization distributions were calculated using smoothing parameter of h = 1500, grid = 100, extent = 2.5.*

The coordinates of all latrines were then overlaid onto the clan territory and home range map to designate each latrine as either within a clan's territory or within the areas of home range overlap among clans. In one of our analyses, we wanted to look for an association between hyaena visitation rate and latrine location (inside territory versus in areas of overlap). However, the initial territory and home range estimates were generated from the same observations which were used to produce estimates of visitation rates, and hence were not statistically independent. Thus, for this analysis only, we recalculated clan home ranges and territories from a separate dataset that excluded sightings from the monitored latrines, and used these to re-assign the location designations for the monitored latrines.

3.2.4 Statistical analyses

We performed all analyses using R (version 3.3.3; R Core Team, 2016). To investigate whether hyaenas placed latrines and single scats preferentially road-side or off-road, we performed Mann-Whitney U tests on the number of latrines (and single scats) encountered during transect searches. To investigate whether there was any difference in the likelihood of finding latrines and single scats among the three transect blocks, we used Kruskal-Wallis tests using the number of latrines and single scats encountered. To determine whether hyaenas were more likely to re-use latrines in subsequent years if they were located road-side or off-road, we performed a chi-squared test of independence using latrines that were checked during at least two years.

A series of linear models were fitted to investigate variation in the number of scats, hyaena visitation rate, and scat deposition rate among latrines. For all models, fixed effects included the number of days since first rainfall, territory designation (overlap versus territory), and year. The continuous variable 'days since first rainfall' was created by calculating the number of days between the date of each latrine record and the date of the first rainfall of the most recent rainy season. This term was included as both a linear and a quadratic variable in all models, to test for a non-linear relationship between season and the response variables. Saturated global models included all single terms and their two-way interactions, unless data were insufficient for model fitting or if the inclusion of certain interactions resulted in multicollinearity; the final terms included in each global model are listed in the legends of model output tables. The best model for each analysis was determined through backwards stepwise deletion of terms from the saturated global model using the 'drop1' function of the 'lme4' package

(Bates et al., 2016). The validity of the best model was assessed based on normality and homogeneity of variance of the residuals.

Variation in latrine characteristics were analysed using generalized linear mixed-effects models (GLMMs): a Poisson distribution was assumed when investigating the factors influencing latrine size (i.e. number of scats), and a binomial distribution was assumed when investigating the presence or absence of necrophagous beetles feeding on hyaena scats. Since each latrine had multiple records of scat counts, latrine identity was included as a random effect in both models.

Fifty latrines were monitored over 30 days using motion-triggered cameras. The number of scats was recorded at the start and end of this monitoring period, in order to calculate a scat deposition rate for each latrine. The visitation rate was calculated by dividing the number of hyaena visits by the total amount of time that the camera was actively recording the latrine. We performed two linear regression models to investigate the factors influencing hyaena visitation rate and scat deposition rate. Visitation rates were cube-root transformed to meet the assumptions of homogeneity of variance and normality of residuals. For these analyses, 'days since first rainfall' was calculated as the number of days between the middle date of each latrine's 30-day monitoring period and the date of the first rainfall of the most recent rainy season. To test whether camera disturbance (i.e. lack of monitoring due to exhausted battery or interference by wildlife) influenced our observed visitation rates, we also included the number of days of camera inactivity in the global model for visitation rate.

3.3 Results

During 2012 – 2016, 194 latrines and 272 additional single scats were found in the study area. Hyaenas were observed depositing faeces, urine, interdigital secretions (by scraping the ground with forepaws), and anal gland secretions at latrines. Cumulative rainfall per wet season, which runs from approximately November through March, was as follows: 708.5 mm during 2013-2014, 428.2 mm during 2014-2015, and 446.6 mm during 2015-2016.

3.3.1 Latrine placement

Latrines were distributed throughout the study area and located within multiple clan home ranges (Figure 3-B, Figure 3-C). Approximately 77% of 184 latrines and 37 % of 246 single scats that had known road-side or off-road designations were located along a road. We conducted 18 transects during October 2014 (two blocks of six transects each) and September 2015 (one block of six transects) to investigate whether hyaenas preferentially place latrines and scats road-side or off-road (Table 3-B). Latrines were located significantly more often road-side than off-road (Mann-Whitney U = 16, n = 18, p = 0.0154), and there was no significant difference in latrine frequency among the three transect blocks (Kruskal-Wallis $\chi^2 = 2.77$, df = 2, p = 0.251). There was no significant difference in the likelihood of single scats being found during road-side or off-road transects (Mann-Whitney U = 49.5, n = 18, p = 0.359), and no significant difference among transect blocks (Kruskal-Wallis $\chi^2 = 1.42$, df = 2, p = 0.493).

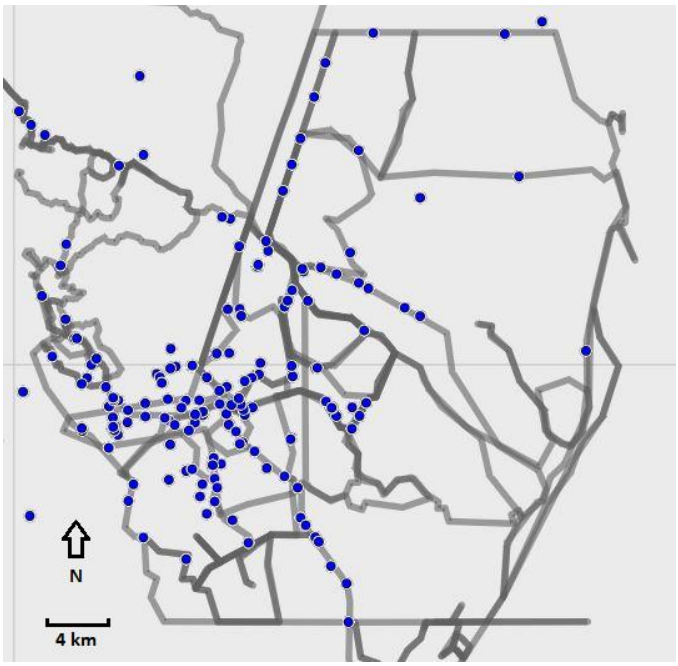


Figure 3-B. The spatial distribution of 166 latrines throughout the main study area.*Latrines are represented by blue dots, and roads indicated by grey lines. A total of 194 latrines were found during 2012-2016, but 28 latrines are located outside the main study area and thus not shown on this map.*

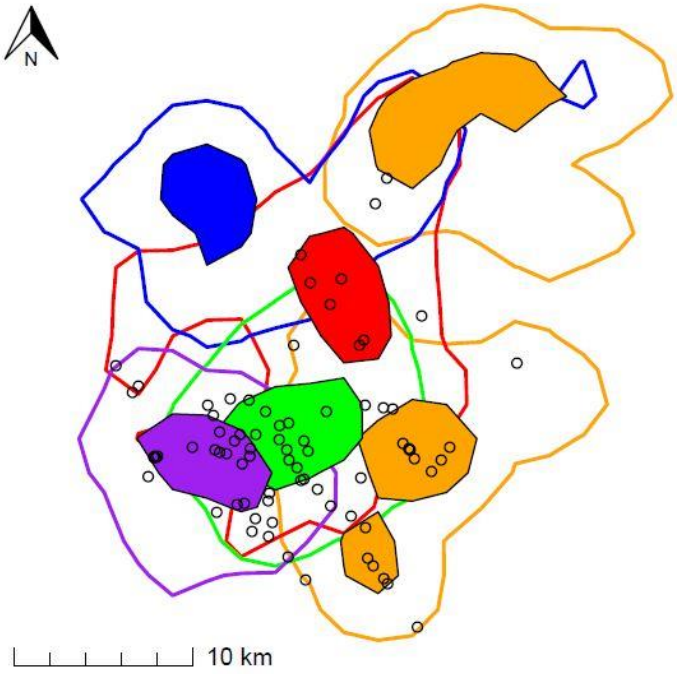


Figure 3-C. The locations of the 78 latrines selected for analysis of latrine size, in relation to five clan home ranges and territories.*Different colours indicate different clan home ranges (95% kernel, solid lines) and territories (50% kernel, filled polygons).*

Table 3-B. The number of latrines found during 18 transects driven in three blocks of the study area (three road and three off-road transects were driven in each block).

Transect type	**Number of marking sites found**	**Block** *1*	*2*	*3*
Road	Latrines	6	2	3
	Single scats	1	1	0
Off-road	Latrines	1	0	0
	Single scats	2	1	1

One hundred and forty-two latrines were checked for activity during more than one year, and approximately 70 % of these latrines were used in multiple years. Hyaenas were significantly more likely to reuse latrines in subsequent years if the latrines were located road-side rather than off-road ($\chi^2 = 18.80$, df = 1, $p < 0.001$).

3.3.2 Latrine size

After initial discovery, latrines were revisited repeatedly from 2014-2016 to record the number of scats present, resulting in 1,128 records from 78 latrines. Scat accumulation exhibited a seasonal pattern in which the mean number of scats per latrine decreased after the annual onset of rain (Figure 3-D).

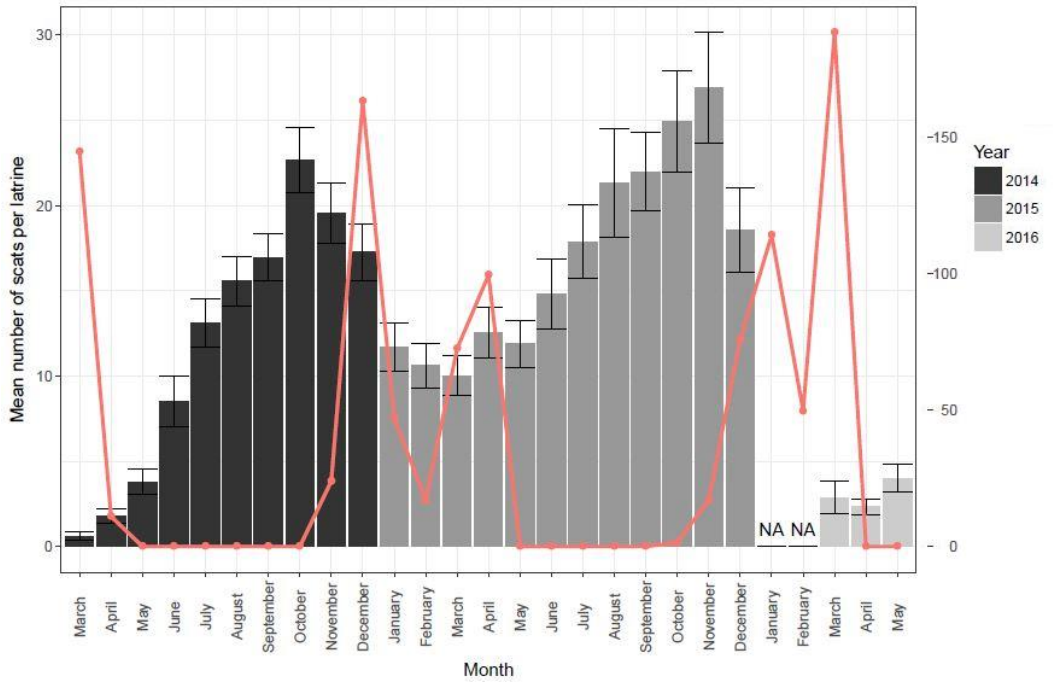


Figure 3-D. The variation in the mean number of scats per latrine for 78 latrines, and the amount of rainfall, per month over a period of 27 months. *The red line indicates total rainfall per month. Error bars indicate ± one standard error of the mean. Latrine data were not available for the months of January and February 2016 and thus have been marked 'NA' on the graph.*

The number of days since first rainfall, territory designation, and year significantly influenced the size of latrines (Table 3-C). The mean number of scats per latrine decreased soon after the start of the rainy season, and increased after the end of the rainy season, with the last rainfall occurring 189 and 160 days after the start of rainfall during 2014 and 2015, respectively (Figure 3-E). Latrines located within clan territories accumulated more scats than those located in overlap areas, and latrines accumulated more scats during 2015 than during 2014 (Table 3-C).

Table 3-C. Factors influencing hyaena latrine size (i.e. the number of scats per latrine). *Backwards selection using the 'drop1' function was used to determine the best model from a saturated global model: Number of scats ~ Days since first rainfall + I(Days since first rainfall^2) + Territory designation + Year + Days since first rainfall x Territory designation + I(Days since first rainfall^2) x Territory designation + Days since first rainfall x Year + I(Days since first rainfall^2) x Year + Territory designation x Year + (1|Latrine). CI = confidence interval, * = CI did not overlap zero.*

Term	Level	Estimate	95% CI
(Intercept)	-	1.6076	1.26, 1.96*
Days since 1st rainfall (linear)	-	-0.0145	-0.0335, 0.00447
Days since 1st rainfall (quadratic)	-	0.4904	0.446, 0.535*
Territory designation	Overlap	-	-
	Territory	-0.1618	-0.602, 0.278
Year	2014	-	
	2015	0.4121	0.324, 0.500*
Days since 1st rainfall (linear) x Year	Days x 2014	-	
	Days x 2015	0.0907	0.0637, 0.118*
Days since 1st rainfall (quadratic) x Year	Days x 2014	-	
	Days x 2015	-0.1734	-0.213, -0.134*
Days since 1st rainfall (quadratic) x Territory designation	Days x Overlap	-	
	Days x Territory	0.0894	0.0440, 0.135*
Territory designation x Year	Overlap x 2014	-	
	Territory x 2015	0.2335	0.150, 0.317*

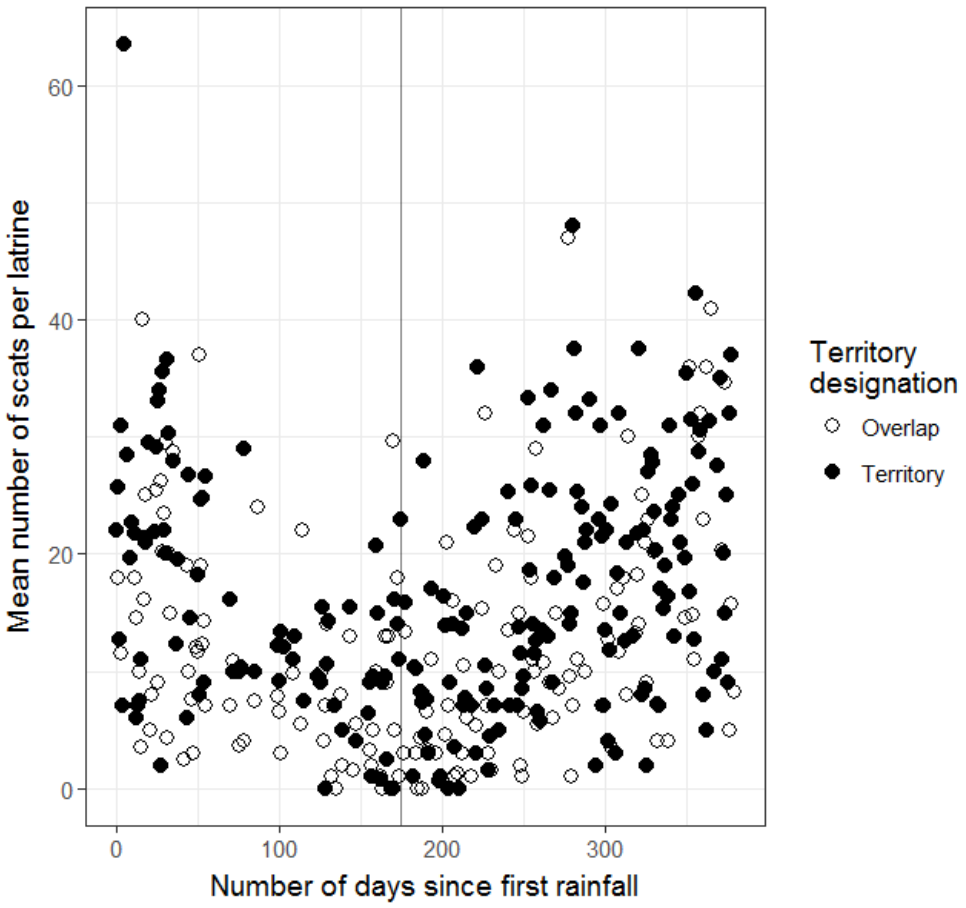


Figure 3-E. The seasonal variation in the mean number of scats per latrine based on territory designation.*The vertical grey line indicates the mean last day of rainfall over the two wet seasons (2013-2014 and 2014-2015).*

The presence or absence of necrophagous beetles (Family: Trogidae) on hyaena scats was significantly predicted by the number of days since first rainfall (Table 3-D). Beetles were more likely to be present on hyaena scats during or just after the start of the rainy season (Figure 3-F).

Table 3-D. Factors influencing the likelihood of necrophagous beetles feeding on hyaena scats at 78 latrines.*Backwards selection using the 'drop1' function was used to determine the best model from a saturated global model: Beetles (present or absent) ~ Year + Days since first rainfall + Latrine size + Territory designation + Year x Latrine size + Year x Territory designation + Latrine size x Territory designation + (1|Latrine). CI = confidence interval, * = CI did not overlap zero.*

Term	Estimate	95% CI
(Intercept)	-6.3716	-7.67, -5.08*
Days since 1st rainfall	-4.7725	-5.78, -3.77*

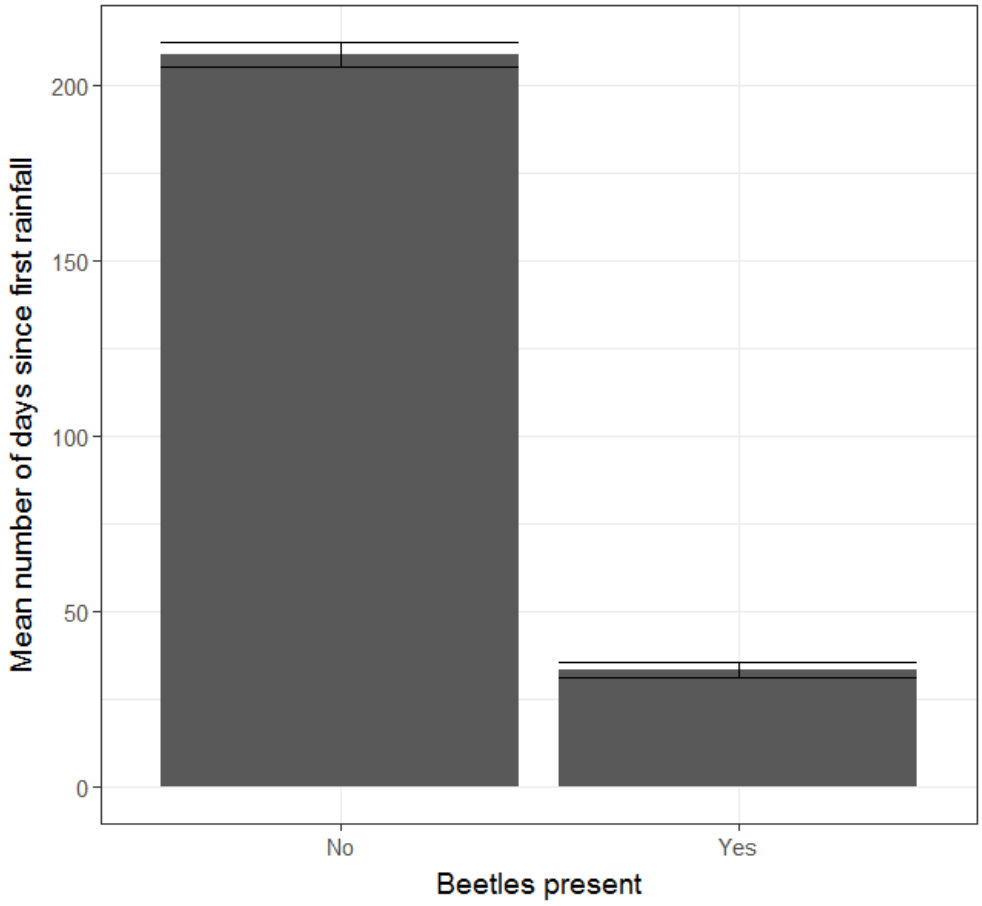


Figure 3-F. The influence of the number of days since first rainfall on whether necrophagous beetles were present on hyaena scats at 78 latrines.*Beetles were less likely to be present as the number of days since the first rainfall increased. Error bars indicate ± one standard error of the mean.*

3.3.3 Hyaena visitation and scat deposition

Fifty latrines were monitored using camera traps over a period of approximately 30 days to evaluate the rate of visitation by hyaenas and the change in the number of scats over time (i.e. scat deposition rate). Latrines were actively recorded by cameras (i.e. turned on and facing latrine) for 17.63 to 36.90 days (mean ± SD = 28.43 ± 3.49 days). Out of the 34 latrines for which at least 50 % of visiting hyaenas could be photo-identified, 19 latrines (55.9 %) were visited by multiple known clans during the monitoring period.

Hyaena visitation rates ranged from 0.033 to 0.936 visits per day (mean ± SD = 0.335 ± 0.225 visits/day), which is equivalent to one visit every 25.63 to 720.72 hours (average ± SD = 129.71 ± 128.90 hours). The monitored latrines were visited by 0.033 to 1.672 hyaenas per day (mean ± SD = 0.466 ± 0.366 hyaenas/day). Similar to its effect on latrine size, the number of days since first rainfall had a significant quadratic relationship with visitation rate (Table 3-E, Figure 3-G).

Table 3-E. Factors influencing hyaena visitation rates at latrines. *Backwards selection using the 'drop1' function was used to determine the best model from a saturated global GLM: Visitation rate ~ Days since first rainfall + I(Days since first rainfall^2) + Territory designation + Year + Days since first rainfall x Territory designation + Days since first rainfall x Year + I(Days since first rainfall^2) x Territory designation + I(Days since first rainfall^2) x Year + Territory designation x Year + Days inactive. CI = confidence interval, * = CI did not overlap zero.*

Term	**Estimate**	**95% CI**
(Intercept)	0.7465	0.684, 0.809*
Days since 1st rainfall (linear)	0.0469	0.00736, 0.0865*
Days since 1st rainfall (quadratic)	-0.0905	-0.140, -0.0408*

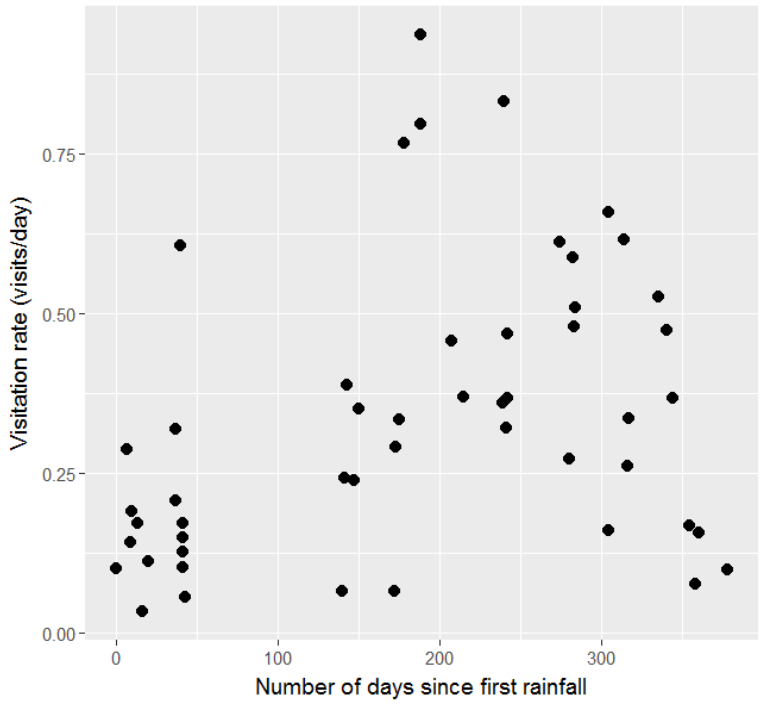


Figure 3-G. The variation in hyaena visitation rate at 50 latrines based on the number of days since first rainfall.

The change in the number of scats (scat deposition rate) was analysed for 49 of the 50 monitored latrines, as one latrine did not have scat count data on the exact start and end dates of monitoring. Scat deposition rate was positively correlated with hyaena visitation rate (Pearson's correlation coefficient = 0.603, df = 47, $p < 0.001$; Figure 3-H). Scat deposition rate significantly increased as the number of days since first rainfall increased (Table 3-F).

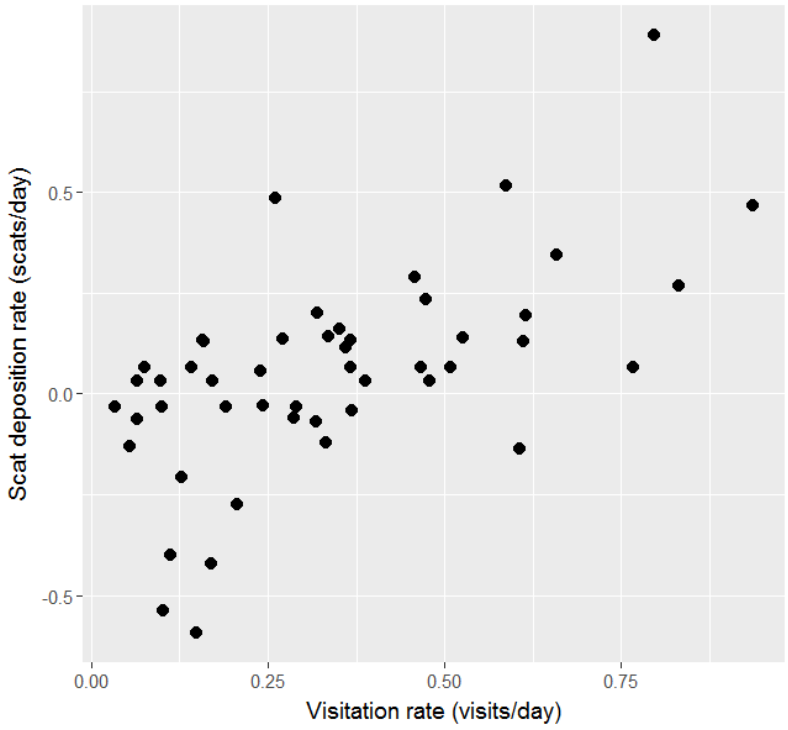


Figure 3-H. The correlation between hyaena visitation rate and scat deposition rate at 49 latrines.

Table 3-F. Factors influencing hyaena scat deposition rate at 49 latrines.*Backwards selection using the 'drop1' function was used to determine the best model from a saturated global GLM: Deposition rate ~ Days since 1st rainfall + I(Days since 1st rainfall^2) + Territory designation + Year + Days since 1st rainfall x Territory designation + Days since 1st rainfall x Year + I(Days since 1st rainfall^2) x Territory designation + I(Days since 1st rainfall^2) x Year + Territory designation x Year. CI = confidence interval, * = CI did not overlap zero.*

Term	Estimate	95% CI
(Intercept)	0.0509	-0.0108, 0.113
Days since 1st rainfall (linear)	0.1436	0.0813, 0.206*

3.4 Discussion

This study was the first to systematically and simultaneously investigate the effects of social and environmental factors on latrine use by spotted hyaenas. Latrines were used extensively by hyaenas within this ecosystem, and the patterns

observed in this study indicate a seasonal territorial marking strategy: hyaenas visited latrines and defecated more frequently during the dry season, maintained larger latrines within clan territories, and placed latrines preferentially along roads as a likely mechanism to maximize latrine discovery. The rainy season was linked to decreases in latrine sizes, with rainfall and necrophagous beetles apparently contributing to the degradation of scats, which were already less abundant due to reduced hyaena visitation and scat deposition during this period. Latrines also appeared to be involved in territorial behaviour by hyaena clans, since latrine size (i.e. number of scats) was significantly higher at latrines located within clan territories than at latrines in areas of overlap among clan home ranges. The presence of human-made roads also influenced hyaena latrine use: hyaenas preferentially placed latrines alongside roads (rather than further off-road), and were more likely to re-use latrines in subsequent years if they were located along roads.

We observed an annual cycle of hyaena latrine use in relation to seasonal rainfall in which scats largely disappeared from known latrine sites during the rainy season and accumulated throughout the dry season. The lack of scats during the rainy season at monitored latrines was not simply the result of permanent latrine abandonment by hyaenas, since approximately 74 % of latrines were used again by hyaenas in subsequent years. Neither of the two previous studies focusing on hyaena latrine behaviour found significant differences in latrine size between wet and dry seasons: seasonal effects were not mentioned in the Kalahari study (Mills and Gorman, 1987), whereas Bearder and Randall (1978) concluded that there was no significant difference in latrine size between seasons in the Transvaal Lowveld of South Africa. Since scent-marking can function in mate defence and

reproductive behaviour, seasonal changes in latrine use could be linked to a species' breeding season, as observed in meerkats (Jordan et al., 2007) and genets (*Genetta genetta*, Barrientos, 2006). However, since hyaenas breed throughout the year (Holekamp et al., 1999b), reproductive behaviour is unlikely to account for the observed seasonal peak and trough in latrine use. Therefore, within our ecosystem, the rapid disappearance of latrines during the rainy season was likely in part a result of seasonal consumption of scats by necrophagous beetles and the degradation of scats by heavy rainfall. Previous investigation of beetle fauna at hyaena latrines found that soft and/or fresh hyaena scats were more attractive to scarab beetles (Krell et al., 2003), and this is a likely explanation for the seasonal presence of these beetles at hyaena latrines during our study. Furthermore, reduced rates of hyaena visitation and scat deposition throughout the rainy season also contributed to this decline in scat number.

Seasonal variation in hyaena visitation rate could have been the result of several factors. First, the production and maintenance of scent-marking sites is costly in both the production of marking substances and the amount of time spent marking and commuting to latrine sites (Gosling and Roberts, 2001). Thus, hyaenas should not waste time or energy scent-marking during a period in which their marks are degraded relatively quickly by necrophagous beetles and rainfall and consequently provide less signalling benefit. Second, given that most of the monitored latrines were located alongside roads, it is possible that hyaenas are using roads less often during the rainy season. However, this is unlikely given observed movement patterns in African wild dogs (*Lycaon pictus*) within our study area, which used roads more often during the rainy season as efficient paths through dense vegetation (Abrahms et al., 2015). There is no evidence to suggest

that hyaenas use roads less during the wet season, especially as the occupancy probabilities for hyaenas calculated from a concurrent camera survey (in which all cameras were placed along roads) did not differ between seasons (Rich et al., 2017). Third, hyaenas have been shown to alter their space use patterns with shifts in seasonal abundance of prey in ecosystems such as the Serengeti (Kruuk, 1972; Hofer and East, 1993a) and Etosha National Park in Namibia (Trinkel et al., 2004). However, calling station surveys conducted in our study area throughout 2007-2010 found that hyaenas did not exhibit seasonal changes in densities (Cozzi et al., 2013b), and a concurrent camera survey found no seasonal difference in occupancy probabilities (Rich et al., 2017). Consequently, it seems most likely that the observed decrease in hyaena visitation rates during the rainy season was related to the reduction in signalling benefit provided by latrines (as scats were degraded by rainfall and necrophagous beetles). Although the reduction in scats during the rainy season was likely driven in part by the interfering impacts of necrophagous beetles, other scent-marks used in hyaena communication (e.g. paste) would presumably be effective even in the presence of beetles. Therefore, the observed changes in hyaena visitation rates may be related to other factors such as any potential variation in clan territoriality between seasons.

Consistent with our predictions, hyaenas appeared to preferentially maintain latrines located within exclusive clan territories: latrine sizes (i.e. the number of scats) were significantly higher at latrines located within territories than in areas of home range overlap. On the other hand, hyaena visitation and scat deposition rates were not significantly influenced by a latrine's territory designation. This could be a result of our visitation analysis including visits by all hyaenas (regardless of clan membership), and territorial intruders visiting latrines within

clan territories may only investigate scent-marks and refrain from depositing their own marks (unless challenging for territory ownership, Gosling, 1982). Furthermore, the apparent transient nature of many individuals observed within the study population (see Chapter 2) means that many hyaenas encountering these latrines were likely territorial intruders.

Given the likely territorial function of latrines, and the observed seasonal patterns of latrine use, it is possible that reduced latrine use during the rainy season was a reflection of decreased territoriality among clans. Although hyaena scats appeared to be degraded by beetles and rainfall, paste contains substantial amounts of fatty acids (Burgener et al., 2009) and thus is unlikely to be as severely affected by rainfall. Territorial advertisement at latrines could therefore occur year-round through the use of paste, but decreased visitation at latrines during the rainy season suggests that this is not the case. Therefore, Okavango hyaenas may exhibit decreased territoriality (and/or territorial boundaries are less aggressively defended) during the rainy season, potentially as a reflection of seasonal changes in prey availability and spatial distribution. During the dry season, the distribution of several large herbivore species appears to be concentrated near permanent water sources (Rich et al., 2016) and thus prey availability may vary among clan territories. On the other hand, the rainy season coincides with synchronized calving and a consequent population boom in many African herbivore species (Owen-Smith and Ogutu, 2013), and abundant water results in a more even distribution of several species throughout the landscape (Rich et al., 2016). The increased availability and more even spatial distribution of prey during the wet season may thus result in decreased territoriality among clans.

Given the complex fission-fusion sociality and hierarchical social rank structure of hyaena clans, latrines could also serve as a mechanism for social bonding and information transfer among clan members. There is evidence for individual- and group-specific scents in hyaena paste (Burgener et al., 2009), and thus depositing paste at communal latrines within their clan's territory could indicate an individual's continued presence in the clan as well as provide information about their movements. Furthermore, hyaenas consistently investigate the ano-genital regions of clan members during greeting ceremonies (Smith et al., 2011), an activity which could serve to confirm the scent profile of their partner (Burgener et al., 2009). Other social mammals that exhibit flexible grouping patterns have also been observed using latrines in such a way that suggests a function in intragroup communication, such as river otters (Barocas et al., 2016) and European badgers (Stewart et al., 2002). For instance, badgers were observed sniffing and marking at latrines which were only used by individuals of their own social group (i.e. unlikely to be territorial behaviour), and these behaviours could function in advertising or assessing the reproductive statuses of group females (Stewart et al., 2002).

Consistent with our prediction that latrine use by hyaenas reflects an economical marking strategy, we found that latrines in this ecosystem were frequently located along human-made roads. Latrines were significantly more likely to be used by hyaenas in subsequent years if located along a road, and transect searches showed that latrines were significantly more likely to be located road-side than further off-road. Efficient scent-marking behaviour requires the maximization of the likelihood that conspecifics will encounter deposited scents (Mills and Gorman, 1987), and thus placing latrines along frequently-used routes serves as an

economical marking strategy. Hyaenas are known to prefer traveling along roads rather than moving through thick vegetation (Bearder and Randall, 1978), and hyaenas that move long distances outside regular territories, such as the 'commuting' population of the Serengeti (Hofer and East, 1993a), may have a particular affinity for placing latrines on roads (Kruuk, 1972). Given the multiple observations of individuals outside their clan territories in our study system (see Chapter 2), it is possible that hyaenas within the Okavango ecosystem display a similar pattern. Given the significant influence of roads on latrine placement and re-use by hyaenas, the creation of roads by humans appears to influence hyaena scent-marking behaviour. Furthermore, since scent-marking at latrines appears to serve an important role in hyaena movement and territorial advertisement by clans, there are potential conservation implications for human road use patterns in habitats which support hyaena populations. Communal marking sites used by other species, particularly frequently-used sites, have also been found to be located along human-made trails and roads (e.g. brown bear Ursus arctos horribilis rubbing trees, McTavish and Gibeau, 2010). Thus, road use by humans has the potential to not only modify animal behaviours such as movement patterns and space use (reviewed by Trombulak and Frissell, 2000), but also the scent-marking behaviour of a wide range of species, and should be evaluated in the conservation management of any ecosystem.

There is potential for latrine surveys to be used as a non-invasive technique for population monitoring, especially when long-term observational studies and/or individual identification of individuals are not feasible. Latrine use has been evaluated as a method for estimating population abundance in several species such as European badgers (Tuyttens et al., 2001), water voles (Arvicola terrestris,

Woodroffe et al., 1990), and river otters (Mowry et al., 2011). For example, the size of a river otter population in Missouri, USA, was best predicted by the number of scats per latrine and latrine density within the study area (Mowry et al., 2011). However, previous studies urge caution when interpreting latrine data for this purpose and suggest further research to validate methods across populations, habitats, and various temporal scales (Tuyttens et al., 2001; Gallant et al., 2007). The results from our study suggest that, for hyaenas living within similar ecosystems, the presence of larger latrines likely indicates the locations of clan territories. Additionally, our results showed that environmental factors such as season and the spatial distribution of roads should also be taken into consideration when evaluating latrine use in a novel hyaena population.

In conclusion, this study greatly enhanced our understanding of latrine use by hyaenas and demonstrated the role of latrines in territorial advertisement by clans. These findings provide a foundation for further investigation of Okavango hyaena behaviour such as potential seasonal variation in both clan territoriality and the availability of olfactory signals in the environment. Furthermore, our findings have implications for wildlife conservation and management given the observed influence of human-made roads on hyaena scent-marking behaviour and the potential for latrine studies to be used as a non-invasive population monitoring tool.

Chapter 4 : Quantifying intraspecific communication and interspecific eavesdropping at spotted hyaena (*Crocuta crocuta*) latrines

Abstract

Spotted hyaena latrines are comprised of scent-marks with various multi-modal characteristics, including hyaena scats' conspicuous white colour. Little is known about the communication function or signalling behaviour within latrines, including the occurrence and extent of heterospecific eavesdropping. We conducted experiments to investigate intraspecific functions of hyaena scats and interspecific eavesdropping by mammals from three guilds (herbivores, mesopredators, and large predators). First, we conducted a translocation experiment in which scats from latrines (from within and outside a resident clan's home range) were translocated 200 metres from a resident latrine cleared of scats. Second, a multimodal experiment investigated receiver behaviour towards artificial and natural white scats, and scat-derived scent which lacked the visual signal. We used motion-triggered cameras to record responses, and analysed mammal encounter rates, investigation behaviour, vigilance, and scent-marking. Our results showed variation in the responses of species from different guilds to the visual and olfactory characteristics of signals at hyaena latrines. Hyaenas investigated and scent-marked original latrines more frequently than translocated sites, and did not differ in behaviour towards resident versus non-resident translocated scats, suggesting that scats may not signal social group and/or are less important than other scents in intraspecific signalling. Herbivores were similarly likely to investigate and be vigilant when exposed to artificial scats as they were olfactory cues, suggesting that African herbivores use multiple

environmental characteristics when assessing risk. Large and meso-predators investigated and scent-marked original latrine sites more often than translocated scats, demonstrating that hyaena latrines may be important for both intra- and inter-specific communication among wild African predators.

4.1 Introduction

Animals communicate using acoustic, chemical, and visual modalities (Bradbury and Vehrencamp, 1998). Chemical signalling is particularly widespread among mammals, and plays a vital role in territory defence, alarm signalling, reproduction, and other social behaviours (Johnson, 1973; Bradbury and Vehrencamp, 1998). Chemical signals that are deposited in the environment as scent-marks are advantageous because they are long-lasting compared to visual or acoustic cues, and signallers and receivers can transfer information without direct encounters (Gorman and Trowbridge, 1989). Thus, the deposition of scent-marks is widely used by mammals for territorial advertisement, in which resident individuals mark throughout their territory and/or along territory borders (Gorman, 1984).

Given the costs of territorial scent-marking in time and energy (to produce and deposit marks), signallers should employ an economical marking strategy that depends on the sizes of their social group and territory (Gorman and Trowbridge, 1989). A signaller can increase signal detectability by depositing scents on conspicuous landmarks and/or along pathways frequently used by conspecifics (Gorman and Trowbridge, 1989). Visually-conspicuous objects can be used as scent-marking substrates as a form of signal amplification to increase detectability of scent-marks (as observed in black-backed jackals Canis mesomelas, Hayward and Hayward, 2010). Additionally, depositing scent-marks at established marking

sites can facilitate signal discovery by receivers (Gorman and Trowbridge, 1989). The formation of communal marking sites (called 'communal latrines') at which multiple individuals deposit scent-marks, is a behaviour exhibited by a variety of species, including white-footed sportive lemurs *Lepilemur leucopus* (Droscher and Kappeler, 2014); European badgers *Meles meles* (Roper et al., 1993); meerkats *Suricata suricatta* (Jordan et al., 2007) and spotted hyaenas *Crocuta crocuta* (Kruuk, 1972).

Scent-marks deposited in the environment broadcast information for varying lengths of time, and these marks can therefore be used by unintended receivers called eavesdroppers (Bradbury and Vehrencamp, 1998). 'Eavesdropping' by conspecifics and heterospecifics refers to the use of information from signals in the environment which does not benefit the signaller, and has been shown to occur in a variety of taxa (Bradbury and Vehrencamp, 1998). Much of the previous work on olfactory eavesdropping has been conducted in laboratory or controlled settings, but recent studies have recorded behavioural responses of wild mammals to experimentally-presented predator faeces (van der Meer et al., 2012; Kuijper et al., 2014; Wikenros et al., 2015; Andersen et al., 2016; Wikenros et al., 2017). Predators may use olfactory signals to locate prey (Hughes et al., 2010b), and prey species often shift their activity patterns or habitat use when exposed to predator scents (reviewed by Apfelbach et al., 2005). Olfactory eavesdropping might be particularly useful to large predators, since sympatric competitors are also potential predators and/or prey (Polis and Myers, 1989), and individuals could gather information about the proximity of these competitors through attending to their scent-marks. In addition, medium-sized carnivores, or 'mesopredators', are particularly vulnerable to injury or death during encounters with larger predators

(Palomares and Caro, 1999), and may use olfactory cues to avoid agonistic encounters (Andersen et al., 2016). On the other hand, large and meso-predators may eavesdrop on competitor odours in order to seek out an encounter and any associated hunting and scavenging opportunities (Wikenros et al., 2017). Predators may also over-mark olfactory signals (i.e. deposit their scent-marks on top of others) from heterospecific competitors to communicate their presence or dominance. Thus, olfactory eavesdropping could potentially have a large impact on predator-prey dynamics and both intra- and inter-specific competition, but our understanding of its significance in wild populations is limited. In particular, only recently have studies started to examine the occurrence of interspecific over-marking and/or olfactory communication among wild mammalian carnivores (e.g. Andersen et al., 2016; Banks et al., 2016; Allen et al., 2017; Apps et al., 2017; Wikenros et al., 2017).

Within sub-Saharan Africa, the large predator guild consists of six species: African lions (*Panthera leo*), leopards (*Panthera pardus*), cheetahs (*Acinonyx jubatus*), African wild dogs (*Lycaon pictus*), brown hyaenas (*Hyaena brunnea*), and spotted hyaenas (Hayward and Slotow, 2009). Spotted hyaenas (hereafter referred to as 'hyaenas') are social, nocturnal predators that live in stable matrilineal social groups called clans but form and disband subgroups of varying size several times a day (Kruuk, 1972; Smith et al., 2008). Scent signalling plays a fundamental role in the hyaena's intricate communication system (Kruuk, 1972; Frank, 1986; Glickman et al., 1997). Hyaenas create and maintain communal latrines that are visited repeatedly by multiple individuals, resulting in accumulations of faeces ('scat'), urine, and glandular secretions (Kruuk, 1972; Bearder and Randall, 1978). While hyaena urine deposits and glandular secretions

are not visually conspicuous, their scats are rich in calcium and consequently have an atypical white colour, giving latrines a distinctive visual appearance and multi-modal signalling potential (Kruuk, 1972; Bearder and Randall, 1978). Latrines are thought to be involved in territory defence by clans (Kruuk, 1972; Gorman and Mills, 1984), particularly as there is evidence that hyaena 'paste' (anal gland secretion) conveys group-specific odours (Theis et al., 2012) and hyaenas have been observed maintaining latrines along clan territory boundaries (Kruuk, 1972; East and Hofer, 1991; Hofer and East, 1993a). Despite knowledge of broad patterns of hyaena latrine use, and speculation on their function from previous studies in different parts of their range, little is known about the specifics of latrine formation and hyaena signalling behaviour within them.

In contrast to intraspecific signalling, relatively few studies have examined heterospecific responses by free-living mammals to communal marking sites (but see Hayward and Hayward, 2010; McTavish and Gibeau, 2010; Allen et al., 2015; Tattoni et al., 2015; Allen et al., 2017). Hyaena latrines are an advantageous system in which to study heterospecific eavesdropping due to the conspicuous nature of latrines and the varying multi-modal characteristics of the different types of hyaena scent-marks. Furthermore, owing to the abundance and diversity of mammals in African savanna ecosystems (Fattebert et al., 2016), hyaena latrines provide an opportunity to simultaneously examine heterospecific eavesdropping within a trophic level (i.e. by large predators) as well as among trophic levels (i.e. by mesopredator and herbivores).

We investigated the role of hyaena scat in latrine use by hyaenas and in potential heterospecific eavesdropping through two experiments. First, we conducted a scat translocation experiment by clearing natural latrines of all hyaena scats and

creating two artificial sites nearby (using scats translocated from the original resident latrines and from latrines located in non-resident clan home ranges). Second, we conducted a multimodal experiment to test responses to visually conspicuous (i.e. natural and artificial white scats) and inconspicuous (i.e. scat-derived scent and control soil) treatments. Conspecific and heterospecific behavioural responses were monitored using camera traps during both experiments. We formulated several hypotheses and predictions which are not mutually exclusive: the translocation experiment addresses hypotheses #1–6, and the multimodal experiment addresses hypothesis #7 (Table 4-A).

Table 4-A. Hypotheses and predictions addressed by the scat translocation experiment (hypotheses #1-6) and multimodal experiment (hypothesis #7). *These hypotheses are not mutually exclusive.*

Category	Hypothesis	Predictions
Conspecific	1. Latrines are important long-term scent-marking sites used for intra-specific communication	Hyaenas will: • visit latrines repeatedly; • encounter latrines more than translocated scats; • investigate and scent-mark at latrines.
	2. Scats contain important olfactory signals for hyaena communication	• investigate translocated scats; • scent-mark in response to translocated scats; • form new latrines at translocated sites.
	3. Scats are used to distinguish between resident and foreign hyaenas	• be more likely to investigate, exhibit vigilance, and/or scent-mark foreign scats.
Heterospecific	4. Cues associated with hyaena latrines and/or translocated scats are used by heterospecifics to assess the likelihood of direct encounters	Heterospecifics: • that could benefit from encounters with hyaenas (e.g. scavenging large and meso-predators) will be more likely to encounter original latrines than those vulnerable to predation (i.e. herbivores); • will be more likely to investigate original latrines due to the greater number of scent-marks (i.e. information) present; • that investigate marking sites will be more likely to exhibit vigilance.
	5. Heterospecifics scent-mark at hyaena marking sites for signal amplification (i.e. increasing detectability of their own deposits) and/or interspecific communication	• will scent-mark both treatments; • that are hyaena's main competitors (i.e. large predators, jackals) will be most likely to scent-mark.
Both	6. Site age influences behavioural responses to hyaena marking sites	Individuals will: • be more likely to investigate both treatments over time due to the deposition of new scent-marks, as fresher marks are more relevant to receivers
	7. Conspicuous visual cues (e.g. white colour) are more important than scent cues in locating hyaena scats, but not in eliciting scent-marking or vigilance	• investigate visual treatments (i.e. natural and artificial scats) more frequently than non-visual treatments (i.e. scat-derived scent or control); • scent-mark and/or display vigilance more frequently when exposed to hyaena olfactory signals (i.e. natural scats, scat-derived scent) than artificial scats.

4.2 Materials and Methods

4.2.1 Study site

This study was conducted in northern Botswana, specifically within the south-eastern section of the Moremi Game Reserve and surrounding Wildlife Management Areas (centre of study area: S19.50098, E23.61010). The habitat was characterized by a heterogeneous mixture of grasslands, acacia scrubland, and mopane woodland. All six species of the African large predator guild inhabited in the study area, five of which (excluding brown hyaenas) have been monitored simultaneously since 2007.

4.2.2. Translocation experiment

Hyaena latrines have been systematically recorded in the study area since 2012, with detailed data collection occurring between March 2014 and May 2016. Individual latrines were located opportunistically during movements throughout the study area in a vehicle, and their locations were recorded using a handheld Garmin 72 unit. We selected 10 latrines that contained scats for at least two consecutive years for experimental manipulation, and conducted these translocations between May to October 2015. All of these 'original' latrines were located adjacent to unpaved roads and had at least 18 intact (i.e. not crushed) white hyaena scats at the start of the experiment (mean ± SD = 33.9 ± 17.74 scats; range 18 – 67 scats). 'Donor' latrines were located within the home range of a non-neighbouring hyaena clan (determined by repeated sightings of known clan members) at a geodesic distance of at least 14 km from their paired original latrine, and contained at least 10 intact white scats for collection.

For each translocation, 10 scats were collected from both the original and donor latrines to create two 'translocated' sites: scats were placed within a 4 x 3 m area, 200 m along the road in either direction (randomly selected) from the original latrine. The original latrine was cleared of all scats by hand, including white remnants of crushed scats. All handling of scats (collection, clearing) was conducted while wearing powder-free nitrile gloves. Experimental sites were monitored for one month without further anthropogenic alteration, using one motion-triggered infrared camera at each site to monitor encounter rates and responses of all species. StealthCam G30 (30 sec videos) and Prowler (60 sec videos during daylight, 30 sec videos in darkness) infrared cameras (StealthCam LLC, Grand Prairie, Texas, USA) were set to record videos without any trigger delay (actual recovery periods were 5 - 10 sec depending on the camera). Cameras were fixed onto vertical poles approximately 1.5 m above the ground, and placed on the opposite side of the road from the sites, tilted at an angle to capture the entire width of the road as well as the experimental area (Figure 4-A).



Figure 4-A. Video screenshot showing camera placement in relation to the road and experimental stimuli.*The road is shown by horizontal vehicle tracks, and translocated scats (white piles) are located on the opposite side of the road. In this case, the camera was triggered by a southern giraffe (*Giraffa giraffa*).*

The total monitoring time at each site was calculated by adding the lengths of time that the camera was turned on and facing the experimental site during the camera's deployment. Cameras were checked every 3-5 days to ensure that they were still functioning. If a camera was found to not be recording the site (e.g. due to disturbance by wildlife or exhausted battery), the monitoring period was calculated up to the time of the disturbance (if captured on camera) or the last video recorded before the disturbance, and the camera was reactivated/repositioned (mean number of inactive camera days per site = 2.48 ± 2.17 days; range 0 – 8.35 days). To investigate whether hyaenas replenished scats at cleared latrines and/or deposited new scats at translocated sites, the number of hyaena scats at each site was recorded every few days.

4.2.3 Multimodal experiment

A parallel experiment was conducted between March to June 2016 to investigate the relative importance of the visual and olfactory cues of hyaena scats. This experiment included four treatments ('control', 'artificial scat', 'natural scat', and 'scent') that were located, in randomized order, 200 m apart along an unpaved road. The control treatment consisted of sand collected from vacant ground and deposited in ten visible piles at the experimental site. The 'artificial scat' treatment consisted of 10 wood pieces similar in size to an average hyaena scat (approximately 10 cm), painted with white water-based acrylic paint, and dried in the sun for seven days. The 'natural scat' treatment consisted of the halves of 10 intact hyaena scats collected from active latrines. The scat-derived 'scent' treatment was created by crushing the remaining halves of these 10 scats and mixing the resultant piles with sand to mask the white colour. Like the translocation experiment, all stimuli were placed within a 4 x 3 m area to be

monitored by motion-triggered infrared cameras for one month. Six replicates (each consisting of the four treatments placed 200 m apart) were conducted along six different 800 m sections of road within which no natural hyaena latrines were present.

4.2.4 Video scoring

Videos were analysed for encounters and behaviours of all mammals at experimental sites using Avidemux Multi-platform Video Editor (version 2.6.8). An 'encounter' was defined as an individual or social group triggering the camera and generating an image which was sufficient to identify the species. Any videos triggered by a given herbivore species within ten minutes were recorded as the same encounter, since grazing or browsing herds could remain in close proximity to the experimental area while out of view of the camera. Separate sequential videos of a single encounter were pooled (as in Tattoni et al., 2015) to score the following information for each individual: date, time, species, minimum distance to stimuli, and the presence or absence of selected behaviours. The following behaviours were recorded when observed anywhere in the camera's field of view: investigate (head and nose pointed downward within 0.5 m of the ground, or nose touching vegetation, in the absence of mastication), scent-mark (any excretion deposited onto the soil/vegetation, and/or rolling/rubbing the ground/vegetation), and vigilant (standing stationary with head erect and ears alert, without mastication). Rubbing behaviour was included as scent-marking behaviour due to the potential for the deposition of secretions from sebaceous and/or apocrine glands (as in grey wolves Canis lupus, Harrington and Asa, 2003).

4.2.5 Statistical analyses

Nineteen species from three guilds were selected for analyses of encounter rates and behaviours observed during the translocation experiment (Table 4-B). We selected species had at least 10 observations overall and that encountered each site type (original latrine and two translocated treatments) at least once over the course of all 10 translocation trials. Mammal species in the large and meso-predator guilds were further grouped based on taxonomic family (i.e. Felidae) for intraguild analyses, as models did not converge when each species was included separately. We performed a series of statistical analyses that grouped recipients based on guild, large predator taxa, and mesopredator taxa; we were not interested in specific intraguild trends concerning herbivores.

Table 4-B. Nineteen species from three guilds were selected for statistical analyses of data from the translocation experiment.*Species in the large predator and mesopredator guilds were further grouped for intraguild analyses.*

Species	**Guild**	**Intraguild grouping**	**Number of observations**	**% of observations with investigatory behaviour**
Spotted hyaena (*Crocuta crocuta*)	Large predator	Hyaena	249	52.2
African wild dog (*Lycaon pictus*)	Large predator	Wild dog	72	73.6
Lion (*Panthera leo*)	Large predator	Big cat	75	4.0
Leopard (*Panthera pardus pardus*)	Large predator	Big cat	48	2.1
Black-backed jackal (*Canis mesomelas*)	Mesopredator	Jackal	92	53.3
Wild cat (*Felis silvestris lybica*)	Mesopredator	Small cat	36	2.8
African civet (*Civettictis civetta*)	Mesopredator	Civet	35	3.1
Honey badger (*Mellivora capensis)*	Mesopredator	Badger	31	9.7
Serval (*Leptailurus serval*)	Mesopredator	Small cat	18	11.1
Caracal (*Caracal caracal*)	Mesopredator	Small cat	14	0.0
Impala (*Aepyceros melampus*)	Herbivore	N/A	1997	17.6
African elephant (*Loxodonta africana)*	Herbivore	N/A	773	29.9
Plains zebra (*Equus quagga*)	Herbivore	N/A	192	20.8
Southern giraffe (*Giraffa giraffa*)	Herbivore	N/A	115	5.2
Greater kudu (*Tragelaphus strepsiceros)*	Herbivore	N/A	52	19.2
Common warthog (*Phacochoerus africanus*)	Herbivore	N/A	27	40.7
Steenbok (*Raphicerus campestris*)	Herbivore	N/A	26	34.6
Common tsessebe (*Damaliscus lunatus lunatus*)	Herbivore	N/A	21	52.4
Cape buffalo (*Syncerus caffer caffer)*	Herbivore	N/A	16	31.3

Generalized linear mixed-effects models ('GLMMs'; lme4 package, Bates et al., 2016) and non-parametric tests were performed in R (version 3.3.1; R Core Team, 2016) to analyse encounter rates and behavioural responses. Candidate models for all GLMMs were created from a saturated global model using the 'dredge' function ('MuMIn' package, Barton, 2016). To account for model uncertainty, model-averaging was used when multiple candidate models were within two corrected Akaike's information criterion (AICc; Burnham and Anderson, 2004) units of the best model (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). All terms selected by model-averaging were significant predictors of a response variable, and the model output tables indicate the relative importance (weighted probability that a term was part of the best model) of each term. We used the conditional average of the parameter estimates, which averages the estimates from only the top models in which the parameter is involved (Barton, 2016). The experimental site (i.e. 10 translocations or six multimodal replicates) was included as a random effect in all GLMMs. Camera location was included as a random effect in models of investigatory and vigilance behaviour, but not in GLMMs of encounter rate and scent-marking behaviour (for which response variables were summed for each camera). Ordinal date (for encounter rate models) and site age (for investigation models) were rescaled and centred in all analyses to improve model fitting. Whenever possible, all two-way interactions of fixed effects were included in global models, but certain interactions were removed when data proved insufficient for model fitting; the terms used in each global model are listed in the legends of output tables.

For all analyses except those concerning solely hyaenas, we combined data concerning foreign and resident scats into one 'translocated' treatment. This is

because we did not expect heterospecifics to differentiate between hyaena scats based on clan origin, and were not interested in this distinction. To investigate any influence of scat origin on hyaena behaviour, we performed a two-tailed Mann-Whitney U test on hyaena encounter rates and chi-square tests on behavioural responses, using a treatment variable differentiating between foreign and resident scats.

To investigate the factors influencing encounter rate in the translocation experiment, we performed GLMMs on a response variable of the number of encounters per site and used an offset of the log-transformed total monitoring time (in days) per site (Zuur et al., 2009). Fixed effects of the global model included treatment and guild (or taxon, for intraguild analyses). We included ordinal date in the model to control for seasonal variations in species abundance or movements, as all translocations were conducted within one calendar year (May to October 2015). Encounter rates for recipients grouped by guild and mesopredator taxa were modelled using the negative binomial distribution, but large predator data were modelled using the Poisson distribution. We were not interested in, and did not predict differences in, encounter rates for the multimodal experiment, since all treatments were placed at vacant sites without preconceived biological significance.

To investigate the probabilities that individuals would investigate, scent-mark, and/or display vigilance during the translocation and multimodal experiments, we performed GLMMs with a binomial error distribution. All behavioural analyses were performed on data limited to encounters in which an individual came within 2 m of the stimuli area. This threshold was chosen to ensure that individuals had the opportunity to perceive the stimuli, and because animals traveling on roads

were within 2 m of the adjacent experimental sites. The same species selected for analyses of translocation experiment data were used in the multimodal experiment analyses, with the exception of wild dogs as they did not encounter any of the multimodal treatments. Within the mesopredator guild, badgers and small cats were never observed marking during the translocation experiment and were not included in the intraguild scent-marking analysis. We excluded elephants and giraffe from all vigilance analyses due to the difficulty of observing vigilance in these species from the camera's field of view. Fixed effects in the global model for investigatory behaviour included guild (or taxon), treatment, and site age (in days). Fixed effects in the global models for vigilance and scent-marking behaviour included guild (or taxon) and treatment; the global model for vigilance also included whether or not individuals investigated the experimental site during their encounter. Analyses of mesopredator vigilance and scent-marking during the multimodal experiment were performed using Fisher's exact tests, as the data were insufficient for fitting GLMMs.

4.3 Results

Thirty experimental sites (comprising 10 original latrines and 20 translocated sites) were observed during the translocation experiment for a total of 899.01 days (mean length of time per site ± SD = 29.97 ± 4.22 days; range = 24.29 – 42.30 days) and were encountered by 37 species. Behavioural analyses examined the factors affecting whether or not individuals from 19 species displayed investigatory, scent-marking, or vigilance behaviour during each encounter. While the number of hyaena scats at translocated sites remained constant throughout each trial, original latrines were replenished with scats; consequently, the effective strength of the treatment may have declined over time (Figure 4-B).

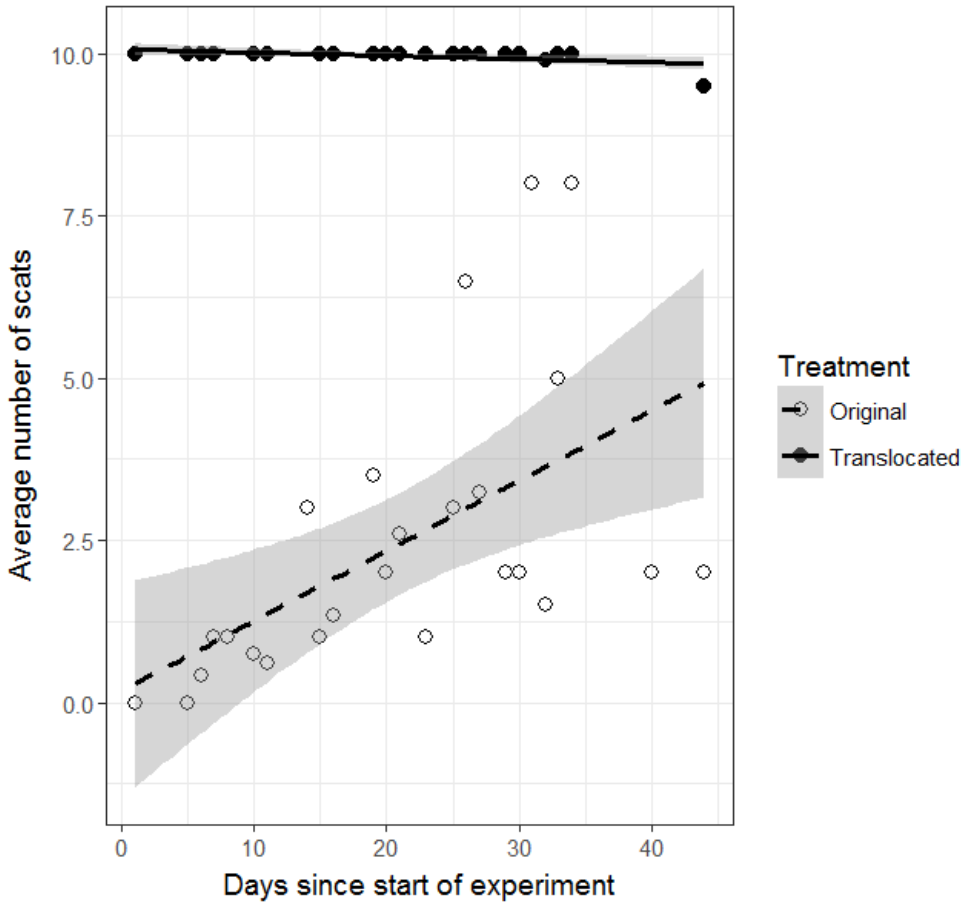


Figure 4-B. The number of scats present at each treatment was assessed every few days during each trial's month-long monitoring. *Grey shading indicates 95% confidence intervals for the fitted regression lines.*

4.3.1 Hyaena behaviour

Our results are consistent with the prediction that hyaenas would continue to encounter, investigate, and scent-mark original latrines. Hyaenas encountered original latrines significantly more often than translocated scats, despite the sites being located only 200 m apart (Table 4-C, Figure 4-C).

Table 4-C. Factors influencing the number of large predator encounters during the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: No. of encounters ~ (Treatment + Taxon + Date)^2 + (1|Latrine) + offset(Time monitored). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-1.2596	-1.53, -0.993*	
Taxon	Hyaena	-		1.00
	Big cats	-1.4561	-1.96, -0.957*	
	Wild dog	-2.0641	-2.75, -1.38*	
Date	-	-0.2490	-0.486, -0.0122*	1.00
Treatment	Original	-		1.00
	Translocated	-1.0069	-1.33, -0.683*	
Taxon x Treatment	Hyaena x Original	-		1.00
	Big cats x Translocated	1.2247	0.619, 1.83*	
	Wild dog x Translocated	-0.6344	-1.75, 0.480	
Taxon x Date	Hyaena x Date	-		0.40
	Big cats x Date	-0.2600	-0.570, 0.0502	
	Wild dog x Date	-0.4907	-1.10, 0.122	

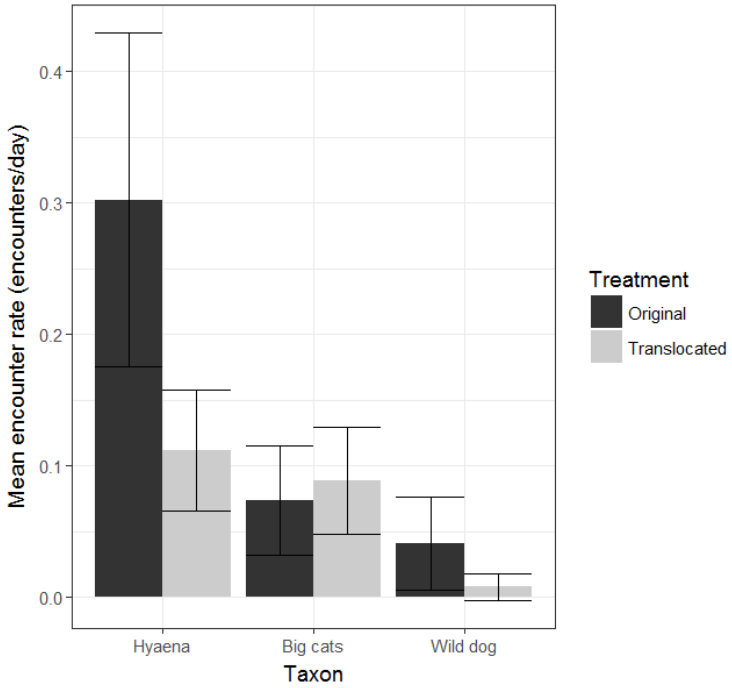


Figure 4-C. Variation in large predator encounter rates during the translocation experiment based on taxon and treatment, using raw data.*Error bars indicate 95% confidence intervals.*

Furthermore, hyaenas were more likely to investigate and scent-mark original latrines than translocated scats (Table 4-D, Table 4-E, Figure 4-D). Contrary to our predictions, translocated scats rarely elicited behavioural responses from hyaenas. The presence of scats alone was not sufficient to initiate a new latrine: hyaenas never marked at sites with translocated scats (Figure 4-D) and investigated these sites in only 18 % of encounters. Furthermore, there was no difference between translocated sites with scats originating from latrines in foreign versus resident home ranges in hyaena encounter rates (Mann-Whitney test: U = 74, n = 20, p = 0.075) or the likelihood of investigation (χ^2 = 0.0164, df = 1, p = 0.898) and vigilance (Fisher's exact test: n = 103, p = 0.518).

Table 4-D. Factors influencing whether large predators displayed investigatory (i.e. sniffing) behaviour during an encounter in the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Investigate (yes or no) ~ (Taxon + Treatment + Site age)^2+ (1|Latrine) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		1.1656	0.624, 1.71*	
Taxon	Hyaena	-		1.00
	Big cats	-3.6192	-4.77, -2.47*	
	Wild dog	0.3375	-0.439, 1.11	
Treatment	Original	-		1.00
	Translocated	-2.6952	-3.44, -1.95*	
Site age	Site age	-0.0905	-0.394, 0.214	0.30

Table 4-E. Factors influencing whether large predators scent-marked during an encounter in the translocation experiment. *Model parameters were generated from one top model, as no other candidate models were within two AICc of the best model. Global model for large predator comparison: Mark (yes or no) ~ Treatment + Taxon + (1|Latrine). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI
(Intercept)		-0.9288	-1.52, -0.341*
Taxon	Hyaena	-	
	Big cats	-3.1506	-5.15, -1.15*
	Wild dog	-0.6006	-1.34, 0.135
Treatment	Original	-	
	Translocated	-1.7617	-2.52, -1.00*

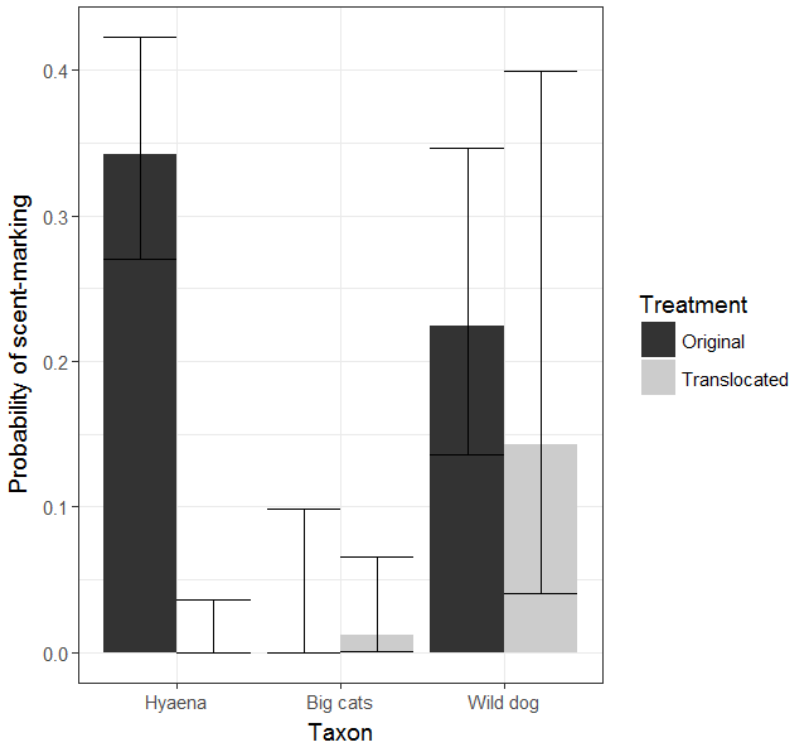


Figure 4-D. Variation in the likelihood that large predator species scent-marked during an encounter in the translocation experiment based on treatment, using raw data.*Binomial error bars indicate 95% confidence intervals.*

4.3.2 Heterospecific behaviour

As predicted, we found guild- and taxon-specific differences in heterospecific encounter rates and behavioural responses. Large and meso-predators were more

likely to encounter and investigate original latrines than translocated scats, whereas herbivores were less affected by treatment (Table 4-F, Table 4-G, Figure 4-E, Figure 4-F). For large predators, this pattern was driven by hyaenas and wild dogs; big cats were least likely to investigate stimuli in general (Table 4-D), and their encounter rates did not differ based on treatment (Figure 4-C). Mesopredators overall were more likely to encounter and investigate original latrines than translocated scats, and jackals were most likely to investigate stimuli (Table 4-H, Table 4-I).

Table 4-F. Factors influencing the number of mammal encounters during the translocation experiment. *Model parameters were generated from one top model, as no other candidate models were within two AICc of the best model. Global model: No. of encounters ~ Treatment + Guild + Date + Treatment x Guild + Date x Guild + (1|Latrine) + offset(Time monitored). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI
(Intercept)	-	-0.1210	-0.410, 0.169
Guild	Herbivore	-	
	Mesopredator	-1.2107	-1.60, -0.823*
	Large predator	-0.8193	-1.19, -0.450*
Date	-	0.1921	-0.0254, 0.410
Treatment	Original	-	
	Translocated	0.2491	-0.0759, 0.574
Guild x Treatment	Herbivore x Original	-	
	Mesopredator x Translocated	-0.6458	-1.17, -0.121*
	Large predator x Translocated	-0.9423	-1.45, -0.435*
Guild x Date	Herbivore x Date	-	
	Mesopredator x Date	-0.3841	-0.638, -0.130*
	Large predator x Date	-0.4640	-0.719, -0.209*

Table 4-G. Factors influencing whether mammals displayed investigatory (i.e. sniffing) behaviour during an encounter in the translocation experiment.*Model parameters were generated from one top model, as no other candidate models were within two AICc of the best model. Global model: Investigate (yes or no) ~ (Guild + Treatment + Site age)^2 + (1|Latrine) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI
(Intercept)	-	-1.0066	-1.48, -0.532*
Guild	Herbivore	-	
	Mesopredator	0.6588	0.171, 1.15*
	Large predator	1.5407	1.20, 1.88*
Treatment	Original	-	
	Translocated	-0.0242	-0.469, 0.421
Site age	-	-0.1016	-0.250, 0.0473
Guild x Treatment	Herbivore x Original	-	
	Mesopredator x Translocated	-1.0166	-1.67, -0.360*
	Large predator x Translocated	-2.7692	-3.34, -2.20*
Site age x Treatment	Site age x Original	-	
	Site age x Translocated	-0.2384	-0.425, -0.0517*

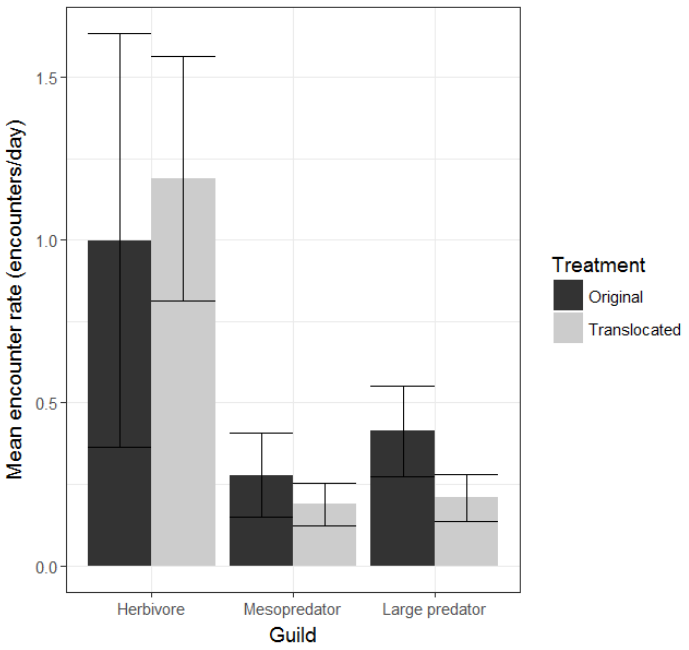


Figure 4-E. Variation in the mean encounter rates of mammals during the translocation experiment based on guild and treatment, using raw data.*Large predator data included hyaenas. Error bars indicate 95% confidence intervals.*

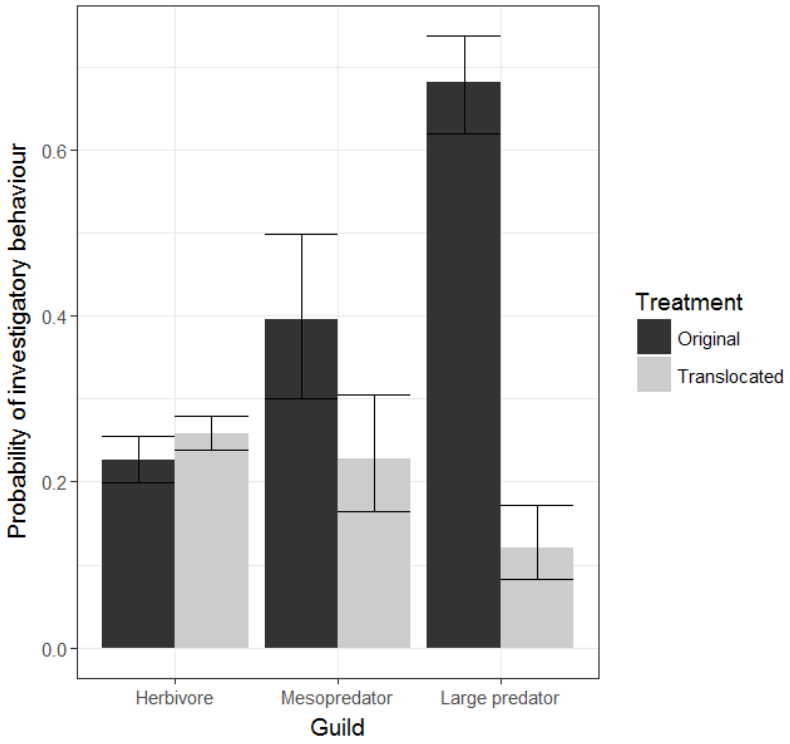


Figure 4-F. Variation in the likelihood of investigatory behaviour by mammals during an encounter in the translocation experiment based on guild and treatment, using raw data.*Large predator data included hyaenas. Binomial error bars indicate 95% confidence intervals.*

Table 4-H. Factors influencing mesopredator encounter rates during the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: No. of encounters ~ (Treatment + Taxon + Date)^2 + (1|Latrine) + offset(Time monitored). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	-2.4531	-2.90, -2.00*	
Taxon	Jackal	-		1.00
	Badger	-1.1389	-1.65, -0.627*	
	Civet	-0.6974	-1.17, -0.227*	
	Small cats	-0.0780	-0.497, 0.341	
Treatment	Original	-		0.68
	Translocated	-0.3614	-0.680, -0.0428*	
Date	-	-0.1573	-0.460, 0.145	0.53
Date x Treatment	Date x Original	-		0.19
	Date x Translocated	-0.2932	-0.676, 0.0896	

Table 4-I. Factors influencing whether mesopredators displayed investigatory behaviour during an encounter in the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Investigate (yes or no) ~ (Taxon + Treatment + Site age)^2 + (1|Latrine) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	0.6683	-0.0878, 1.42	
Taxon	Jackal	-		1.00
	Badger	-2.6514	-4.20, -1.10*	
	Civet	-1.1080	-2.14, -0.0804*	
	Small cats	-3.3253	-4.61, -2.04*	
Treatment	Original	-		1.00
	Translocated	-0.9789	-1.74, -0.217*	
Site age	-	0.1405	-0.526, 0.807	0.79
Taxon x Site age	Jackal x Site age	-		0.79
	Badger x Site age	-0.9501	-2.49, 0.593	
	Civet x Site age	1.4836	0.147, 2.82*	
	Small cats x Site age	-0.1830	-1.55, 1.18	
Site age x Treatment	Site age x Original	-		0.38
	Site age x Translocated	-0.6061	-1.43, 0.222	

Heterospecifics of all three guilds were more likely to be vigilant if they investigated the sites, and were more likely to be vigilant at original latrines overall (Table 4-J, Table 4-K, Table 4-L). Taxon was also a strong predictor of vigilance among large and meso-predators: wild dogs were the large predator most likely to be vigilant (Table 4-K), and jackals were the only mesopredator that were more likely to be vigilant when encountering original latrines than translocated scats (Table 4-L, Figure 4-G).

Table 4-J. Factors influencing whether mammals were vigilant during an encounter in the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ (Guild + Treatment + Investigation)^2 + (1|Latrine) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-2.0668	-2.63, -1.51*	
Investigation	No	-		1.00
	Yes	1.7533	1.43, 2.08*	
Treatment	Original	-		0.75
	Translocated	-0.3728	-0.888, 0.143	
Investigation x Treatment	No x Original	-		0.32
	Yes x Translocated	-0.2924	-0.774, 0.189	

Table 4-K. Factors influencing whether large predators were vigilant during an encounter in the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ Taxon + Treatment + Investigation + Taxon x Treatment + Treatment x Investigation + (1|Latrine) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-1.7626	-2.57, -0.951*	
Taxon	Hyaena	-		1.00
	Big cats	-0.1903	-0.977, 0.597	
	Wild dog	1.3451	0.707, 1.98*	
Investigation	No	-		1.00
	Yes	1.2152	0.495, 1.94*	
Treatment	Original	-		0.77
	Translocated	-0.8260	-1.68, 0.0293	
Investigation x Treatment	No x Original			0.30
	Yes x Translocated	0.7176	-0.550, 1.99	

Table 4-L. Factors influencing whether mesopredators were vigilant during an encounter in the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ Taxon + Treatment + Investigation + Treatment x Investigation + (1|Latrine) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-1.5337	-2.60, -0.470*	
Species	Jackal	-		1.00
	Badger	-2.1724	-4.38, 0.0382	
	Civet	-2.4211	-4.11, -0.732*	
	Small cats	0.5640	-0.485, 1.61	
Sniff	No	-		1.00
	Yes	2.4321	1.22, 3.64*	
Treatment	Original	-		1.00
	Translocated	-0.7673	-1.83, 0.296	
Sniff x Treatment	No x Original	-		0.35
	Yes x Translocated	-0.8293	-2.49, 0.836	

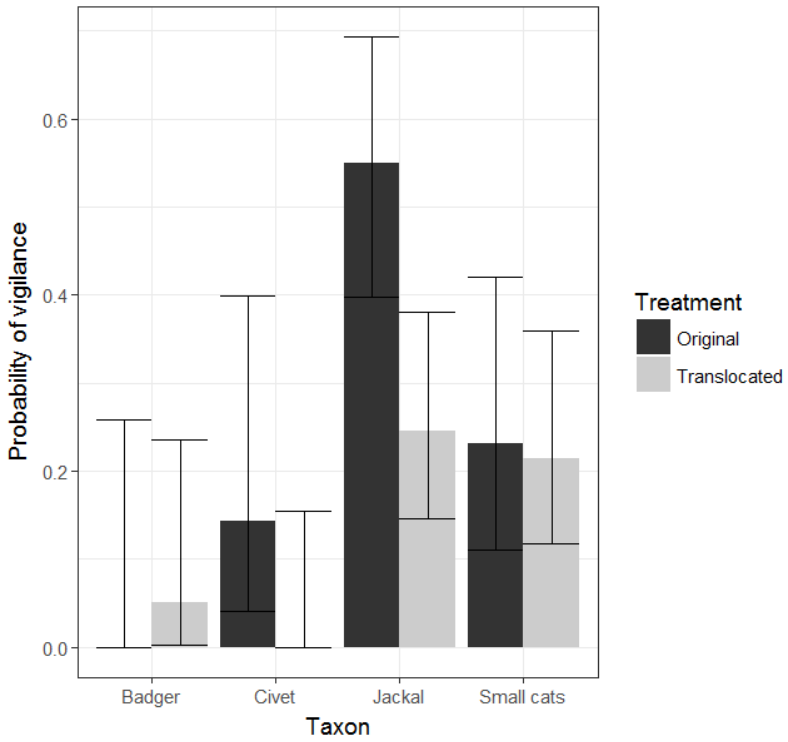


Figure 4-G. Variation in the likelihood of mesopredators being vigilant during an encounter in the translocation experiment by treatment and taxon, using raw data.*Binomial error bars indicate 95% confidence intervals.*

Overall, 8 of the 19 species (42 %) were observed scent-marking during the translocation experiment, including 5 of the 10 meso- and large predator species: civets (marked in 11 % of 35 encounters), wild dogs (21 % of 72 encounters), hyaenas (20 % of 249 encounters), jackals (28 % of 89 encounters), and leopards (2 % of 47 encounters). Visiting large predators (15 % of 439 encounters) and mesopredators (13 % of 223 encounters) were more likely to scent-mark than herbivores, which marked in only 0.835 % of 2635 encounters (Table 4-M).

Table 4-M. Factors influencing whether mammals scent-marked during an encounter in the translocation experiment.*Model parameters were generated from one top model, as no other candidate models were within two AICc of the best model. Global model: Mark (yes or no) ~ Treatment + Guild + Treatment x Guild + (1|Latrine). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI
(Intercept)	-	-4.6541	-5.40, -3.90*
Guild	Herbivore	-	
	Mesopredator	3.1917	2.38, 4.00*
	Large predator	3.4440	2.75, 4.14*
Treatment	Original	-	
	Translocated	-0.6947	-1.53, 0.144
Guild x Treatment	Herbivore x Original	-	
	Mesopredator x Translocated	-0.6358	-1.81, 0.543
	Large predator x Translocated	-2.5334	-3.98, -1.09*

Among large predators, big cats rarely scent-marked in general, and wild dogs scent-marked both sites (Table 4-E, Figure 4-D). Concerning mesopredators, small cats and badgers were never observed scent-marking, jackals marked both types of treatments, and civets only marked original latrines (Table 4-N, Figure 4-H).

Table 4-N. Factors influencing whether mesopredators scent-marked during an encounter in the translocation experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Mark (yes or no) ~ Treatment + Taxon + (1|Latrine). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	-1.3526	-2.70, -0.002*	
Taxon	Civet	-		1.00
	Jackal	1.1454	-0.102, 2.39	
Treatment	Original	-		0.68
	Translocated	-1.0920	-2.06, -0.128*	

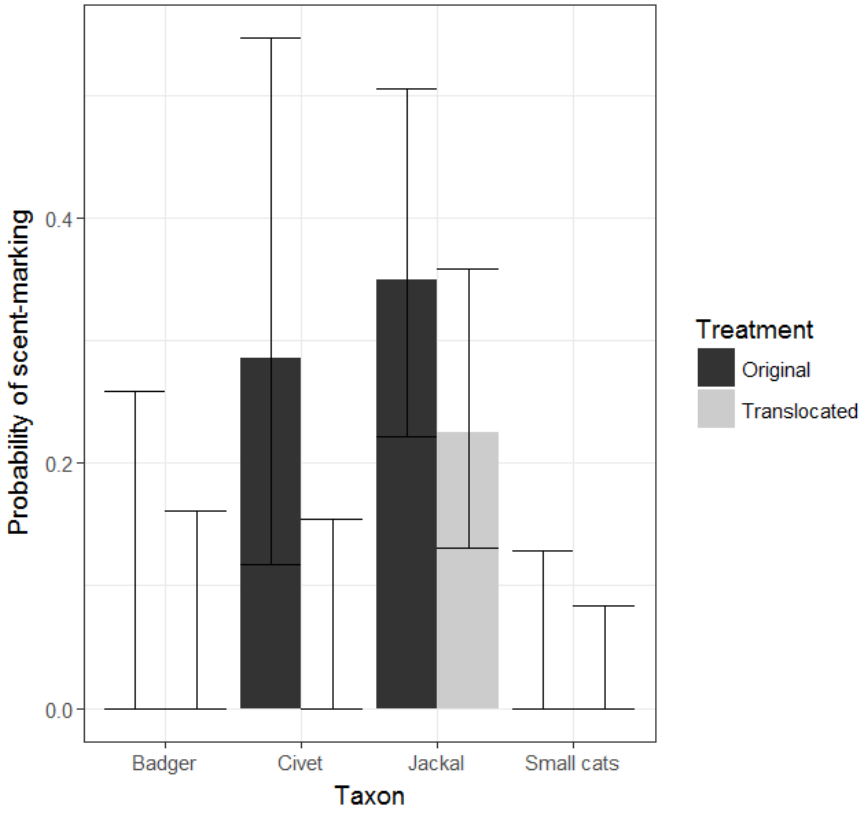


Figure 4-H. Variation in the probability of mesopredators scent-marking during an encounter in the translocation experiment based on treatment and taxon, using raw data. *Binomial error bars indicate 95% confidence intervals.*

4.3.3 Conspecific and heterospecific behaviour

Contrary to our predictions, mammals overall were more likely to investigate original latrines, but less likely to investigate translocated scats, with increasing site age (Table 4-G, Figure 4-I). Furthermore, site age was the least important predictor of investigatory behaviour among large predators (Table 4-D). Concerning mesopredators, civets were more likely to investigate stimuli as site age increased, but other species were not influenced by site age (Table 4-I).

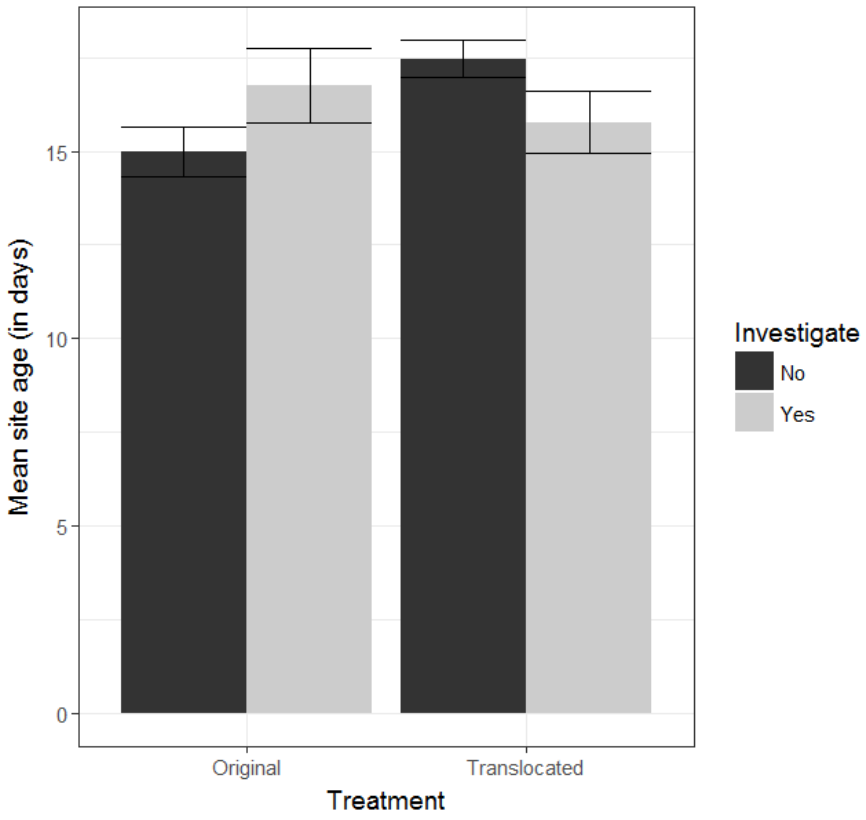


Figure 4-I. The influence of site age and treatment on mammal investigatory behaviour during the translocation experiment, using raw data.*Animals were more likely to investigate original latrines, and less likely to investigate translocated sites, with increasing site age. Error bars indicate 95% confidence intervals.*

Observations at 22 experiment sites during the multimodal experiment revealed guild- and taxon-specific effects of treatment on investigatory behaviour.

Herbivores and large predators were more likely to investigate sites with conspicuous visual cues (artificial and natural scats) than non-visual treatments (scent and control), but mesopredators were most likely to investigate sites with hyaena-related olfactory cues (Table 4-O, Figure 4-J). Concerning large predators, hyaenas were more likely to investigate artificial scats than control or scent treatments, whereas big cats rarely responded and were not influenced by treatment (Table 4-P). The most important predictor of response for mesopredators was taxon, as badgers and small cats were less likely to investigate experimental sites than jackals or civets (Table 4-Q).

Table 4-O. Factors influencing whether mammals displayed investigatory behaviour during an encounter in the multimodal experiment. *Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Investigate (yes or no) ~ (Guild + Treatment + Site age)^2 + (1|Experiment) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	-2.5990	-3.02, -2.18*	
Treatment	Control	-	-	1.00
	Artificial scat	1.7842	1.39, 2.18*	
	Natural scat	1.4990	1.05, 1.94*	
	Scent	0.4542	-0.0157, 0.924	
Guild	Herbivore	-		1.00
	Mesopredator	0.6455	-0.283, 1.57	
	Large predator	-0.4571	-1.93, 1.02	
Site age	-	-0.2732	-0.625, 0.0782	1.00
Guild x Treatment	Herbivore x Control	-		1.00
	Mesopredator x Artificial scat	-1.5118	-2.77, -0.252*	
	Large predator x Artificial scat	0.1855	-1.47, 1.84	
	Mesopredator x Natural scat	-0.0344	-1.20, 1.13	
	Large predator x Natural scat	-0.1663	-2.13, 1.80	
	Mesopredator x Scent	0.4507	-0.810, 1.71	
	Large predator x Scent	-1.2034	-3.70, 1.29	
Site age x Treatment	Site age x Control	-		0.43
	Site age x Artificial scat	0.2720	-0.0852, 0.629	
	Site age x Natural scat	0.3300	-0.0986, 0.758	
	Site age x Scent	0.4716	0.0682, 0.875*	
Guild x Site age	Herbivore x Site age	-		0.37
	Mesopredator x Site age	-0.1061	-0.575, 0.363	
	Large predator x Site age	-0.5842	-1.30, 0.130	

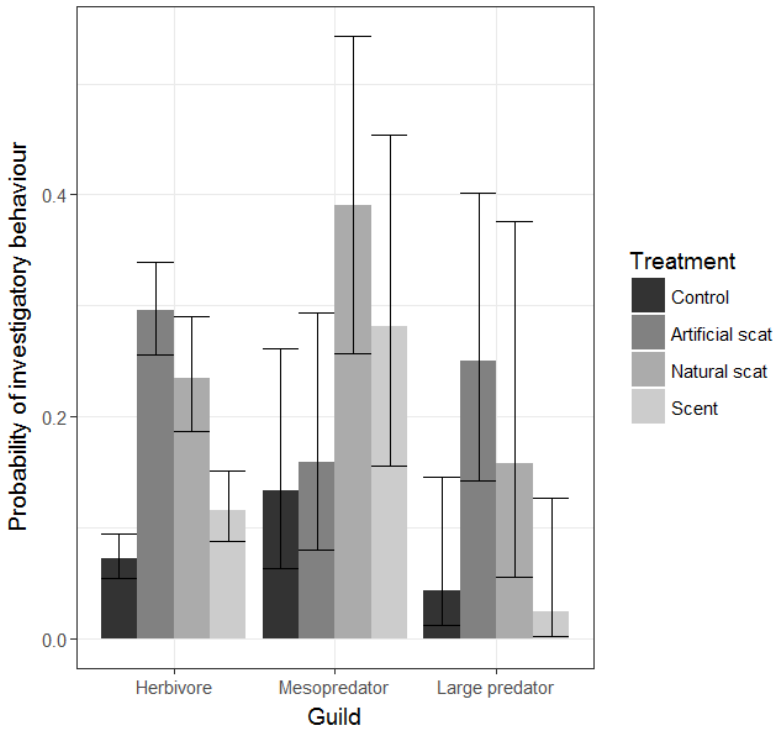


Figure 4-J. Variation in the likelihood of investigatory behaviour by mammals during an encounter in the multimodal experiment based on treatment and guild, using raw data.*Large predator data included hyaenas. Binomial error bars indicate 95% confidence intervals.*

Table 4-P. Factors influencing whether large predators displayed investigatory behaviour during an encounter in the multimodal experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Investigate (yes or no) ~ Taxon + Treatment + Site age + Taxon x Site age + (1|Experiment) + (1|Camera). CI = Confidence interval, * Confidence intervals do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	-2.4135	-3.97, -0.855*	
Treatment	Control	-	-	1.00
	Artificial scat	2.1014	0.396, 3.81*	
	Natural scat	1.3145	-0.673, 3.30	
	Scent	-0.7526	-3.26, 1.76	
Taxon	Hyaena	-		1.00
	Big cats	-2.0076	-3.46, -0.559*	
Site age	-	-0.6908	-1.58, 0.196	0.64
Taxon x Site age	Hyaena x Site age	-		0.25
	Big cats x Site age	0.9369	-0.692, 2.56	

Table 4-Q. Factors influencing whether mesopredators displayed investigatory behaviour during an encounter in the multimodal experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Investigate (yes or no) ~ Taxon + Treatment + Site age + Treatment x Site age + Taxon x Site age + (1|Experiment) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	0.0720	-1.10, 1.24	
Taxon	Jackal	-		1.00
	Badger	-3.3242	-4.90, -1.75*	
	Civet	-0.4084	-1.54, 0.720	
	Small cats	-2.3104	-3.31, -1.31*	
Treatment	Control	-		0.23
	Artificial scat	0.0572	-1.34, 1.45	
	Natural scat	1.0814	-0.192, 2.35	
	Scent	1.0738	-0.246, 2.39	
Site age	-	-0.1648	-0.731, 0.402	0.22

Similar to our results on investigatory behaviour, the effect of treatment on scent-marking behaviour during the multimodal experiment was guild-specific: scent-marking by large predators (0.68 % of 146 encounters) and herbivores (0.58 % of 1723 encounters) was rare and not influenced by treatment (Table 4-R, Figure 4-K). On the other hand, mesopredators were observed scent-marking in 7.4 % of 162 encounters, with jackals in particular driving this pattern (39.1 % of 23 encounters by jackals involved marking). Furthermore, scent-marking by mesopredators was strongly influenced by treatment (Fisher's exact test: $n = 162$, $p < 0.001$) as they only marked treatments with hyaena-related olfactory cues.

Table 4-R. Factors influencing whether mammals scent-marked during an encounter in the multimodal experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Mark (yes or no) ~ Treatment + Guild + (1|Experiment). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	-5.5661	-6.79, -4.34*	
Guild	Herbivore	-		1.00
	Mesopredator	2.2022	1.25, 3.15*	
	Large predator	0.1095	-2.01, 2.23	
Treatment	Control	-		0.65
	Artificial scat	-0.0317	-1.59, 1.52	
	Natural scat	1.4867	0.257, 2.72*	
	Scent	0.6213	-0.760, 2.00	

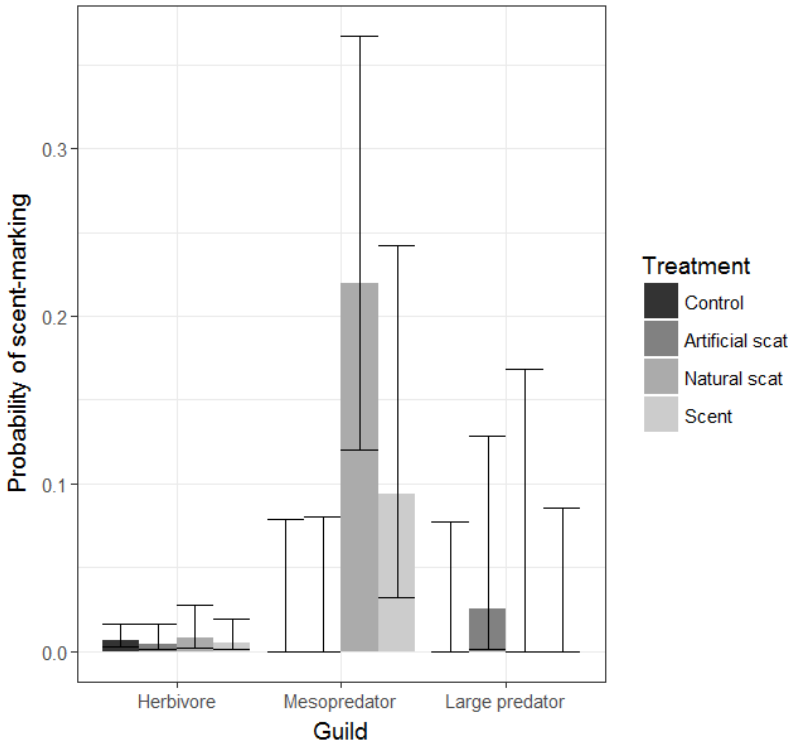


Figure 4-K. Variation in the likelihood that mammals scent-marked during an encounter in the multimodal experiment based on treatment and guild, using raw data.*Large predator data included hyaenas. Binomial error bars indicate 95% confidence intervals.*

Treatment did not influence mammal vigilance overall or within the large predator guild, as investigatory behaviour was the strongest predictor of vigilance in both analyses (Table 4-S, Table 4-T). However, among mesopredators, vigilance was not only significantly influenced by investigatory behaviour (χ^2 = 12.48, df = 1, p = 0.004) but was also most likely to occur at treatments with hyaena-related olfactory cues (χ^2 = 9.16, df = 3, p = 0.027) (Figure 4-L). Furthermore, jackals were the mesopredator most likely to exhibit vigilance (Fisher's exact test: n = 162, p < 0.001).

Table 4-S. Factors influencing whether mammals were vigilant during an encounter in the multimodal experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ (Guild + Treatment + Investigation)^2 + (1|Experiment) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	-1.7456	-1.97, -1.52*	
Guild	Herbivore	-		1.00
	Mesopredator	0.4434	0.000382, 0.886*	
	Predator	-0.2925	-0.836, 0.251	
Investigation	No	-		1.00
	Yes	1.0145	0.694, 1.33*	
Guild x Investigation	Herbivore x No	-		0.30
	Mesopredator x Yes	0.4678	-0.386, 1.32	
	Large predator x Yes	0.7377	-0.475, 1.95	

Table 4-T. Factors influencing whether large predators were vigilant during an encounter in the multimodal experiment.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ Taxon + Treatment + Investigation + Taxon x Investigation + (1|Experiment) + (1|Camera). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)	-	-1.8869	-2.69, -1.08*	
Investigation	No	-		1.00
	Yes	1.3002	-0.131, 2.73	
Taxon	Hyaena	-		0.57
	Big cats	-0.7324	-1.89, 0.422	
Taxon x Investigation	Hyaena x No	-		0.33
	Big cats x Yes	2.3957	-0.530, 5.32	

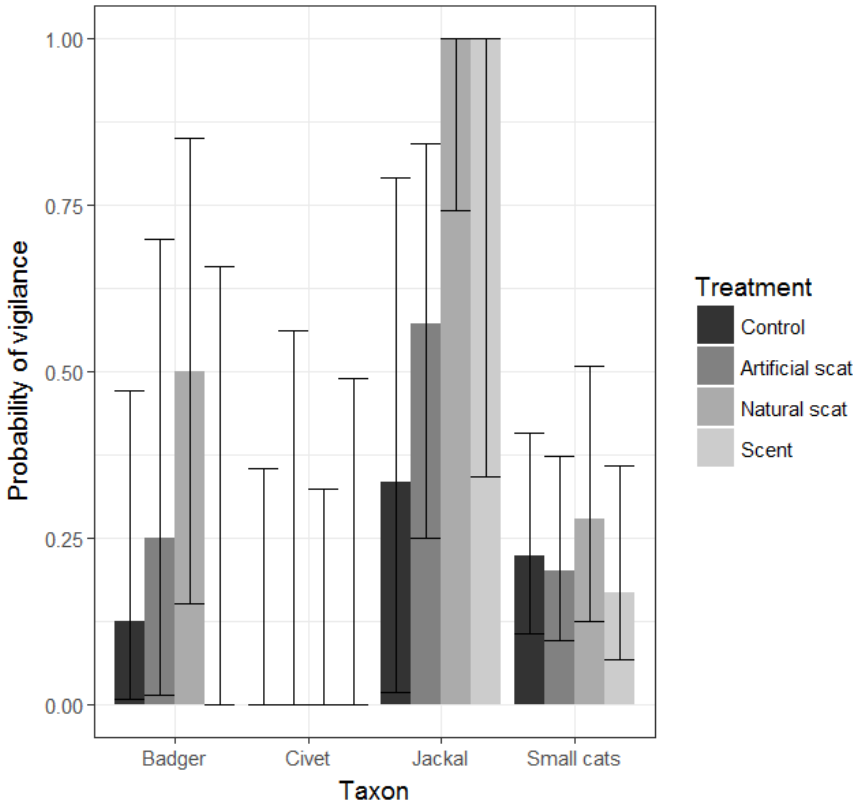


Figure 4-L. Variation in the likelihood of mesopredator species being vigilant during an encounter in the multimodal experiment based on treatment, using raw data*. Binomial error bars indicate 95% confidence intervals.*

4.4 Discussion

The visual and olfactory signals associated with hyaena latrines had variable effects on the behaviour of mammals from different guilds and species. Hyaenas responded similarly to translocated scats originating from resident and non-resident clan home ranges, and individuals consistently investigated and scent-marked original latrines more frequently than translocated scats. Among heterospecifics, herbivores were similarly likely to investigate white visual cues as hyaena olfactory signals, and investigatory behaviour was the strongest predictor of vigilance behaviour, suggesting that African herbivores use multiple environmental characteristics to inform a general state of vigilance. On the other hand, the investigatory and scent-marking behaviours of carnivores, specifically canids, suggest that olfactory cues present at hyaena latrines are particularly salient for interspecific eavesdropping among carnivores and are potentially involved in interspecific communication.

Our results do not support the hypothesis that hyaena scats have important biological or communicatory significance for hyaenas when located outside of established latrines: translocated scats did not elicit encounter rates or behavioural responses equivalent to those at original latrines, and scats alone were not sufficient to initiate new latrines over the study's time frame. Hyaenas largely ignored translocated scats and were never observed scent-marking these sites, yet a significant majority of encounters at original latrines resulted in extensive investigation and/or scent-marking. Additionally, hyaenas encountered original latrines more often than translocated sites, suggesting that hyaenas may have previous knowledge of latrines' geographical locations (i.e. hyaenas did not happen upon them aimlessly), and/or that latrines are situated on frequently-used

routes. It is also possible that passing hyaenas are attracted to odours associated with latrines, rather than seeking them out explicitly. However, 50% of original latrines had repeated visits by identified individuals. This suggests that individuals who have previously encountered a given latrine may remember this locational information for future visits. Since latrine locations are likely known by resident hyaenas, it is also possible that manipulated random sites (i.e. translocated scats) were not recognized. Since translocated scats did not stimulate the creation of new latrines, our results support the assertion that latrine locations are selected by hyaenas due to geographical features or landmarks (Bearder and Randall, 1978). In addition to having previous knowledge of latrine locations, hyaenas may have encountered latrines more often than translocated sites if latrines were located at important intersections (e.g. between roads and/or between roads and game trails) and translocated sites were not.

Contrary to our hypothesis that hyaenas use scats to distinguish between the presence of resident and foreign conspecifics, their responses did not differ based on scat origin, suggesting that scats alone may not signal hyaena social group. Scats may still serve an important function at latrines that is unrelated to clan territoriality, such as communication among members of the same clan, but this was not tested by our experiment. Given the evidence for group-specific odours in hyaena paste, and the explicit posturing involved in its deposition (Theis et al., 2012), paste is likely the most important conspecific signal found in latrines and this signal is only salient in the olfactory modality. To establish the relative importance of existing scent deposits and geographical cues in defining the location of hyaena latrines, experimental manipulation of all faecal, urinary, and glandular scent-marks would be required. However, removing all scent from

latrines would be extremely challenging and would also create considerable disturbance at the site (i.e. removing all surface soil and vegetation).

Our results support the hypothesis that site age influences mammal responses to hyaena marking sites. The increasing probability that mammals would investigate original latrines over time was potentially a result of the continued scent-marking at original latrines throughout the monitoring period, including the reappearance of scats. Through investigating fresher scents, eavesdroppers could gather more accurate information relating to the proximity of conspecifics (e.g. group members and/or territorial intruders) and heterospecifics (e.g. competitors, predators, and/or prey). The decline in response to translocated scats over time may have been due to reduced novelty of the new marking sites, gradual decay of scats, and/or fewer scents at these sites, since large and meso-predators largely favoured scent-marking original latrines.

Although herbivores responded similarly to original latrines, translocated natural scats, and artificial scats, they were more likely to investigate these stimuli than control sites, suggesting that the visual and olfactory signals in our experiments stimulated the observed investigatory behaviour. Herbivores were more likely to be vigilant during an encounter if they also investigated the site during that encounter, but treatment was not an important predictor of vigilance behaviour. This differs from studies that found heightened vigilance in ungulates following exposure to scat from both cursorial and ambush predators (compared to a control) in a dense forest ecosystem (Kuijper et al., 2014; Wikenros et al., 2015). Our results suggest that African herbivores investigate multiple visual and olfactory cues in the environment to inform a general state of vigilance.

On the other hand, carnivores showed clear differences in their responses to experimental stimuli: large and meso-predators were more likely to investigate and scent-mark original latrines than translocated scats. Our study demonstrated that latrines host several types of scent-marks, not only from hyaenas but from five other predator species that were observed marking, and thus latrines may be used in both intra- and inter-specific communication. As odorous, ritualised, and highly-frequented locations that also contain visually-conspicuous elements, hyaena latrines could be used as important communication sites by sympatric species. Additionally, since investigatory behaviour was associated with increased vigilance, scent-marks at latrines could mediate interspecific relationships by informing carnivores about the proximity of heterospecific competitors and intraguild predation risk. Large predator latrines have recently been shown to be multi-species marking sites in other carnivore communities (e.g. North American carnivores, Allen et al., 2017), but such studies are rare and thus we still have limited understanding of this behaviour. In our study, it was impossible to determine to which particular scent(s) mammals reacted, as it is possible that these naturally-occurring scats contained previous over-marks from multiple species before they were translocated. For example, where individuals urinated and/or rolled directly on top of hyaena scats (e.g. jackals, wild dogs) these responses may have been stimulated by conspecific signals located on the hyaena scats rather than, or in addition to, the hyaena scent itself. Future studies could use hyaena scats that were collected immediately after the defecation event as treatment stimuli (i.e. scats collected during observational follows before sympatric species could over-mark the hyaena scats), in order to test eavesdropper responses to ‘pure’ hyaena scat. Furthermore, we were unable to test the specific

mechanisms behind the observed differences in response to the multimodal experiment treatments. In particular, mammals could have investigated artificial scats because they were novel items, resembled hyaena scats, and/or were visually conspicuous in the environment. Ideally, the inclusion of an additional treatment, such as actual white hyaena scats removed of their odour, could have helped to elucidate the specific role of visual characteristics in eliciting variable responses by receivers, but we were unable to develop such a treatment. Additionally, if species-specific compounds could be isolated from hyaena scats, future presentation experiments could investigate mammal responses to solely hyaena-related olfactory signals (e.g. the method employed by Apps et al., 2017, to investigate responses to compounds in leopard urine).

Somewhat contrary to our prediction that hyaenas' heterospecific competitors would be most likely to scent-mark, canids were often observed investigating and scent-marking olfactory stimuli, but felids rarely reacted throughout our study. Although hyaenas frequently interact with lions and leopards (Cooper, 1991; Hayward and Kerley, 2008), these large cats generally ignored all of the olfactory stimuli. Instead, hyaena scent-marks appear to most influence canid behaviour, specifically wild dogs and jackals. Wild dogs and hyaenas frequently interact in our study area, and since wild dogs communicate primarily through olfactory means (Creel and Creel, 2002; Jordan et al., 2013), they may use hyaena latrines for both intraspecific territorial advertisement and interspecific communication with hyaenas. Similarly, as frequent scavengers, jackals are the only mesopredator that regularly compete directly with hyaenas (Kruuk, 1972). Interestingly, jackals appeared to discriminate between natural and artificial scats, as they were more likely to investigate, scent-mark, and be vigilant in response to natural scats than

artificial scats. Since jackals did not scent-mark artificial scats, this suggests that they did not over-mark natural scats solely as a platform for visual signal amplification inspired by the scats' conspicuous white colour. The odours in hyaena scats appeared to be particularly salient to jackals, and as frequent scavengers alongside hyaenas, jackals may be more likely than other species to benefit from information indicating hyaena activity in the area. Civets were observed spraying urine onto vegetation at hyaena latrines on several occasions, but never marked translocated sites. As civets have previously been recorded marking in 'civetries' (Bearder and Randall, 1978), the behaviours shown during our experiment suggest that they may also use hyaena latrines as sites for intraspecific communication.

In conclusion, our study is the first to demonstrate that hyaena latrines are multi-species marking sites to which several sympatric species respond. Our findings provide evidence of olfactory eavesdropping among African mammals, and demonstrate the potential for interspecific olfactory communication among hyaenas and canids.

Chapter 5 : Interspecific eavesdropping on African large predator urine by spotted hyaenas (*Crocuta crocuta*) and sympatric mammals

Abstract

Scent-marks are used in intraspecific communication by numerous species, and due to their persistence in the environment, these signals are available to unintended receivers called 'eavesdroppers'. The information available to eavesdroppers varies among species and environmental contexts, but the adaptive value of eavesdropping on predator cues in different situations is poorly understood. We conducted a scent presentation experiment to investigate species-specific and context-dependent olfactory eavesdropping by spotted hyaenas and 15 other mammal species in 1054 encounters with urine deposits from three sympatric predators (African wild dog *Lycaon pictus*, N = 15 deposits; leopard *Panthera pardus*, N = 14; African lion *Panthera leo*, N = 11) and one herbivore (African elephant *Loxodonta africana*, N = 8), collectively called 'donor species'. Urine deposits were presented at 18 hyaena latrines in the presence and absence of 'carrion odour' (an indicator of carcass proximity) and at seven natural carcasses. We video-recorded responses to each sample for five days using motion-triggered cameras, and analysed the likelihood that recipients investigated or were vigilant near urine samples. The presence of carrion odour had a guild-specific effect on sample investigation by mammals: mesopredators and large predators were significantly more likely to investigate urine samples when carrion was also present. In general, mammals were less likely to be vigilant when exposed to elephant urine than to predator urine. Hyaenas exhibited context-specific differences in behaviour towards presented urine. First, they were more likely to

investigate urine samples alongside indicators of predator activity (i.e. carrion odour, carcasses). Second, donor species only influenced vigilance behaviour at carcasses, where hyaenas were most likely to be vigilant when exposed to lion and wild dog urine. Overall, our results suggest that African mammals investigate heterospecific olfactory signals to assess risk and reward within the context of other environmental cues.

5.1 Introduction

Scent-marking is a multifaceted behaviour and mode of communication employed by most species of mammals, and functions variously in individual identification, reproduction, territoriality, and social cohesion (Gorman and Trowbridge, 1989). Olfactory communication is advantageous because deposited scent-marks are long-lasting and spatially-specific signals in the environment that allow for communication between widely-spaced individuals, and can be employed in situations for which visual and auditory signals are ineffective (Gorman and Trowbridge, 1989). Since scent-marks persist in the environment, they are vulnerable to detection and investigation by unintended receivers, in a phenomenon termed 'eavesdropping' (Bradbury and Vehrencamp, 1998). The information contained in scent-marks is readily available to interspecific eavesdroppers for assessing predation risk and/or resource availability (reviewed by Apfelbach et al., 2005). Experimental investigation of interspecific olfactory eavesdropping in wild mammal populations is scarce, but studies have emerged in recent years showing that various prey and carnivore species investigate, and/or be vigilant near, experimentally-presented heterospecific scents (Eads et al., 2015; Wikenros et al., 2015; Andersen et al., 2016; Apps et al., 2017; Wikenros et al., 2017).

Given the prevalence of scent-marking behaviour among mammals, an animal's environment contains an abundance of conspecific and heterospecific scent-marks of many types (Johnson, 1973). Thus, it would be adaptive for animals to attend to specific scent-marks of importance, and/or to those encountered in certain behavioural contexts, since it would be nearly impossible and futile to investigate every single scent encountered. Most studies to this effect have investigated how the presence of predator odours influences eavesdropping on competitor signals. For example, certain species have been shown to limit eavesdropping on heterospecific competitor scent when in the presence of predator olfactory cues, such as field voles (*Microtus agrestis*) exposed to least weasel (*Mustela nivalis nivalis*) scent (Hughes et al., 2010a), and great barred frogs (*Mixophes fasciolatus*) exposed to red-bellied black snake (*Pseudechis porphyriacus*) odour (Hamer et al., 2011). Environmental cues other than those derived from predators themselves can also be indicative of predator proximity, such as heterospecific alarm calls (Magrath et al., 2015) or, in the case of large predators, the presence of prey carcasses. Feeding on, or spending time nearby, carcasses is risky because the presence of large carcasses can attract multiple species of scavengers which are potential competitors and/or predators (Cozzi et al., 2015). On the other hand, carrion also provides a potential scavenging opportunity, and thus receivers may benefit from further investigating the circumstances. While many studies have investigated the influence of predator scent on receiver activities, few studies have examined the influence of additional environmental cues on animal responses to predator scents themselves. Sites with decomposing carrion elicit a strong smell even after carcass removal (DeVault and Rhodes, 2002), and thus animals could use such cues to assess risk and determine the relative benefits of eavesdropping

on predator signals under those circumstances. Therefore, we hypothesized that the likelihood of mammals attending to predator olfactory signals varies based on behavioural context and/or additional environmental cues. We predicted that mammals would be more likely to investigate and be vigilant near urine samples when odour from animal remains (i.e. carrion), an indicator of predator activity and thus heightened risk, was also present. Furthermore, as fresher urine deposits indicate the more recent presence of the depositor (and therefore greater associated risk) than older deposits, we predicted that the likelihood of sample investigation and vigilance by recipients would decrease with increasing sample age.

For large predators in particular, interspecific eavesdropping may be important in a wide variety of ecological interactions, since their sympatric competitors are also both potential predators and prey (Polis and Myers, 1989). In southern Africa, the large predator guild comprises six species: cheetahs (*Acinonyx jubatus*), African wild dogs (*Lycaon pictus*), leopards (*Panthera pardus*), African lions (*Panthera leo*), brown hyaenas (*Hyaena brunnea*), and spotted hyaenas (Crocuta crocuta; Hayward and Slotow, 2009). These large predators compete over available resources such as space and prey, with varying dietary overlap between species (K. Golabek & J.W. McNutt, unpublished data; Hayward and Kerley, 2008). Spotted hyaenas (hereafter referred to as 'hyaenas'), the focus of this study, are gregarious large predators living in social groups called clans (Kruuk, 1972). The fission-fusion nature of their clans, in which smaller subgroups continuously form and disband (Smith et al., 2008), and their flexible and opportunistic foraging behaviour (Holekamp and Dloniak, 2011), result in frequent interactions between hyaenas and heterospecific competitors (Kruuk,

1972). The interspecific dominance relationships and outcomes of large predator interactions involving hyaenas are variable and depend on multiple factors such as motivation (e.g. food, protecting offspring) and the number of individuals of each species involved (Cooper, 1991; Carbone et al., 2005). Hyaenas are generally considered to be the only species with the social group size, body size, and body mass to challenge lions directly (Cooper, 1991; Hayward and Kerley, 2008), but lions are also the primary cause of hyaena mortality in many ecosystems (Frank et al., 1995; Pangle and Holekamp, 2010a). Although hyaenas have been reported scavenging at up to 85 % of wild dog kills in one ecosystem in East Africa (Fanshawe and Fitzgibbon, 1993), predation and kleptoparasitism by hyaenas is generally inversely related to African wild dog pack size (Carbone et al., 2005). The relationship between leopards and hyaenas is potentially the most unpredictable, as male leopards have a body mass most similar to that of hyaenas (Caro and Stoner, 2003) but have also developed prey hoisting behaviour as an anti-theft strategy in areas with high densities of sympatric competitors (Stein et al., 2015).

All members of the African large predator guild use olfactory signals in intraspecific communication (Eaton, 1970; Gorman and Mills, 1984; Bothma and Coertze, 2004; Jordan et al., 2013; Gilfillan et al., 2017), and thus individuals frequently encounter both conspecific and heterospecific scent-marks in their environment. Since urine appears to be the more salient scent-mark for intraspecific communication in several of these species (e.g. wild dogs, Jordan et al., 2013), we chose to examine et heterospecific responses to urine scent-marks. Specifically, we hypothesized that mammals investigate large predator urine to assess both the threat of predation or competition, and the opportunity for

kleptoparasitism, predation, or scavenging. We predicted that mammals would be more likely to investigate large predator urine than a control treatment. We also predicted that mammals would be more likely to be vigilant if they had investigated large predator urine than if they had not, and that they would be more likely to be vigilant near large predator urine than a control. Furthermore, we predicted that hyaenas' responses to large predator urine would vary and reflect their particular interspecific relationships: given the significant threat posed by lions, we expected that hyaenas would be most likely to investigate and be vigilant near lion urine.

Hyaenas conduct much of their scent-marking at communal latrines, which are marking sites visited by multiple individuals that investigate and deposit scent-marks (Kruuk, 1972; Bearder and Randall, 1978). Latrine use is a conspicuous behaviour by hyaenas in our study system, particularly as latrines are most often located along road tracks (J. Vitale, Chapter 3). Due to the frequent nature of hyaena visitation at latrines (as often as one visit every 25 hours; J. Vitale, Chapter 3), and the ease at which we could locate them, latrines provided an advantageous location to investigate hyaena eavesdropping behaviour.

Our study site's simultaneous monitoring of all members of the large predator guild at the individual level (excluding brown hyaenas) provides a unique opportunity to investigate interspecific eavesdropping using naturally-deposited scent-marks collected from known individuals of species exhibiting spatial overlap within an intact guild. We investigated the responses of 16 mammal species to urine samples collected from three large predators (lions, leopards, and wild dogs) and one herbivore (elephant, to serve as a control), which were placed at hyaena latrines in the presence or absence of 'carrion odour,' a second

treatment created to simulate carcass proximity. Additionally, we conducted a parallel experiment in which urine samples from these same donor species were presented alongside actual herbivore carcasses.

5.2 Materials and Methods

5.2.1 Study site

This study was conducted in Botswana's Okavango Delta ecosystem, specifically within the south-eastern section of the Moremi Game Reserve and its surrounding Wildlife Management Areas (centre of study area: S19.50098, E23.61010). The habitat is characterized by a heterogeneous mixture of grasslands, acacia scrubland, and mopane woodland. Annual precipitation is highly seasonal (November-March), and annual floodwater arrives in the Delta during the peak dry season (August-September; Wilson and Dincer, 1976). All six species of the African large predator guild occur in the study area, five of which (excluding brown hyaenas) have been monitored simultaneously since 2007.

5.2.2 Urine collection and preservation

Latrine presentations were conducted using 40 urine deposits from African wild dogs (N = 15), leopards (N = 14), and African lions (N = 11) that were collected within 1 – 91 minutes (mean ± SD = 14.86 ± 25.22 min) of excretion onto sand. Presentations at carcasses were conducted using 11 urine deposits from leopards (N = 2), lions (N = 3), wild dogs (N = 4), and elephants (N = 2); large predator deposits were collected within 0 - 60 minutes of deposition (mean ± SD = 11.87 ± 20.02 min), and eight of the 11 deposits presented at carcasses were also used in latrine presentations. We were unable to collect samples from cheetahs or brown hyaenas for our experiments. Eight urine deposits from elephants, used as a non-

predator ‘control’ treatment, were collected within a few hours of deposition at which point the sand was still damp and smelt strongly of urine to the human nose. All urine-soaked sand was collected in cleaned glass jars (double-rinsed and baked at 220ºC for 24 hours) with foil-lined lids and stored at -20ºC. A second type of treatment, ‘carrion odour’, was created by mixing plain sand (collected from multiple random locations) with liquid from decomposing carcasses (of mixed-herbivore species, collected opportunistically throughout the study), to match the consistency of urine-soaked sand, and stored at -20ºC in cleaned glass jars (double-rinsed and baked at 220ºC for 24 hours).

5.2.3 Experimental protocol

We presented the collected urine deposits at 18 latrines and seven carcasses. The locations of hyaena latrines have been systematically recorded in the study area since 2012. Latrines were found opportunistically during daily movements throughout the study area in a vehicle, and all presentations occurred at latrines located adjacent to unpaved road tracks (with the exception of one latrine that was located approximately 30 m off-road). Abandoned carcasses were found opportunistically throughout the study area, and urine presentations were conducted after any original ‘owner’ of the carcass had finished feeding and left the site open to scavenging.

Urine samples from the four ‘donor species’ (lion, leopard, wild dog, and elephant) were presented in a randomized and balanced design. Each ‘deposit’ (i.e. urine-soaked soil from one excretion event) provided enough soil for one to four separate ‘samples’, each used in a different trial. A clean metal spoon was used to place (and later remove) each sample (approximately 30 g) onto bare sand

or short grass. A given sample was monitored for five nights using one motion-triggered video camera (see below). If no hyaenas were observed during this period, the sample was removed and replaced with a new sample from the same donor species for another five nights of observation. This process was repeated until at least one hyaena was exposed to the sample within a five-day period (time until the first hyaena encounter after a treatment's first deployment: range = 0.047 – 13.4 days, mean ± SD = 3.28 ± 3.15 days). After completion of a urine donor treatment, any remaining sample was removed from the ground, and urine from the subsequent donor species was deposited two metres away, with the camera angle shifted to encompass the new presentation area. For trials involving carrion odour (presented at eight of 18 latrines), each carrion sample (approximately 30 g) was placed 1.5 m to the right or left (randomized and balanced) of the urine sample from the camera's viewpoint.

The same protocol from latrine presentations was followed at carcasses, and urine samples were placed 1 – 2 m from the carcass. Urine samples from each donor species were presented in randomized order, observed for five nights, and repeated if necessary until a hyaena encountered the site (time until the first hyaena encounter after a treatment's first deployment: range = 0.217 – 36.3 hours, mean ± SD = 7.41 ± 10.89 hours). However, it was sometimes not possible to present all four urine treatments at each carcass if the carcass was rapidly finished or removed from the area by scavengers. Carrion samples were not presented alongside urine samples at carcasses.

At each experimental site, one StealthCam G30 or StealthCam Prowler (StealthCam LLC, Grand Prairie, Texas, USA) infrared camera was set to record videos during all hours of the day without any scheduled trigger delay (G30: 60

sec during daylight and 30 sec in darkness; Prowler: 60 sec at all hours); actual recovery delays were 5 to 10 sec depending on the camera. Cameras were fixed onto poles at a height of approximately 1.5 m and placed six metres from the presented samples, tilted at an angle to capture the sample area and, in the case of latrine presentations, the entire width of the road. The exact locations of the urine and carrion samples were pointed out on camera by the observer for later reference during video scoring.

5.2.4 Video analysis

We recorded the following information for each individual mammal captured on video: date, time, species, estimated minimum distance(s) to the presented sample(s), and the presence or absence of selected behaviours. The following behaviours were recorded: sample investigation (defined as head and nose pointed downward within 0.5 m of the sample) and vigilance within one metre of the urine sample (defined as standing stationary with head erect and ears alert, without mastication). Vigilance was recorded within one metre of the sample (as opposed to throughout the entire field of view of the camera) in an effort to distinguish between vigilance in response to the presented urine and vigilance resulting from extraneous variables. During presentations at carcasses, the carcass was sometimes dragged by feeding animals from its original location and the distance of the urine sample to the carcass changed over time. Therefore we also scored the distance of the sample to the carcass during each encounter. Videos were analysed using Avidemux Multi-platform Video Editor software (version 2.6.8).

5.2.5 Statistical analyses

Sixteen species from three guilds (herbivore, mesopredator, and large predator) were selected for analysis of behaviours observed at the latrine presentations (Table 5-A). Species were chosen if they had encountered each general category of urine donor (predator and control) and both carrion treatments (present and absent) at least once during latrine presentations.

Table 5-A. The sixteen species selected for behavioural analysis.

Species	**Guild**	**# of individual encounters**	**% of encounters involving sample investigation**
Spotted hyaena (*Crocuta crocuta*)	Large predator	160	11.3
African wild dog (*Lycaon pictus*)	Large predator	55	21.8
Leopard (*Panthera pardus pardus*)	Large predator	14	7.1
African lion (*Panthera leo*)	Large predator	13	0.0
African civet (*Civettictis civetta*)	Mesopredator	33	3.0
Black-backed jackal (*Canis mesomelas*)	Mesopredator	24	20.8
Wild cat (*Felis silvestris lybica*)	Mesopredator	22	0.0
Honey badger (*Mellivora capensis)*	Mesopredator	16	31.3
Aardwolf *(Proteles cristata)*	Mesopredator	6	33.3
Serval (*Leptailurus serval*)	Mesopredator	5	20.0
Impala (*Aepyceros melampus*)	Herbivore	349	8.9
African elephant (*Loxodonta africana)*	Herbivore	281	17.8
Southern giraffe (*Giraffa giraffa*)	Herbivore	35	0.0
Plains zebra (*Equus quagga*)	Herbivore	29	6.9
Greater kudu (*Tragelaphus strepsiceros)*	Herbivore	7	14.3
Common warthog (*Phacochoerus africanus*)	Herbivore	4	0.0

We examined the probabilities that individuals would investigate urine samples or be vigilant within one metre of the sample. Analyses of investigatory behaviour were limited to encounters in which individuals came within two metres of the

urine sample, to ensure that individuals had an opportunity to perceive the experimental stimuli, and because animals traveling on roads were up to two metres from the sample(s). Analyses of vigilance behaviour used data from encounters during which individuals came within one metre of the urine sample and thus had the opportunity to respond. We excluded elephants and giraffe from our analyses of vigilance behaviour due to the difficulty of observing vigilance in these species from remote cameras.

The probability that individuals would display these behaviours was analysed in R (version 3.3.1; R Core Team, 2016) by performing generalized linear mixed-effects models (GLMMs) with a binomial error distribution and logit link function, using the 'lme4' package (Bates et al., 2016). We performed multiple GLMMs for two categories of recipients: all mammals (classified by guild) and hyaenas. Latrine and deposit identities were included as random effects in all models. Hyaena ID was included as a random effect in all models that analysed only hyaena data (with the exception of the GLMM of hyaena vigilance behaviour at latrines, as this model failed to converge when ID was included). To improve model fitting, sample age (the time in days since the sample was deployed) was rescaled and centred in all analyses using the 'scale' function. We created candidate models from a saturated global model using the 'dredge' function in the MuMIn package (Barton, 2016), after which we obtained model-averaged estimates of parameters if there were multiple models within two units of the corrected Akaike's information criterion (AICc; Burnham and Anderson, 2004) of the best model (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). If model-averaging was used, all terms in the output from model-averaging were significant predictors of a response variable, and the output tables indicate the

relative importance (the weighted probability that a term was part of the best model) of each term. Fixed effects for models testing sample investigation by mammals included recipient guild, urine donor species, carrion odour (present or absent), and sample age. Fixed effects for models testing mammal vigilance behaviour included the same terms, with an additional term of whether or not individuals had directly investigated the sample ('sample investigation'). GLMMs for analyses of hyaena data alone included all of the same terms, with the exception of guild classification. Global models included all two-way interactions of fixed effects unless data proved insufficient for model fitting or if the inclusion of certain interactions resulted in multicollinearity; the final terms included in each global model are listed in the legends of output tables.

Analyses of urine presentations at carcasses were restricted to hyaena data, since encounters by other species were infrequent. GLMMs were performed in the same manner as previously outlined. Random effects for all models of carcass data included carcass, deposit, and hyaena identities. Fixed effects in the global model of sample investigation included urine donor species, sample age, and sample distance to the carcass. The same fixed effects were included in the global model for vigilance, with an additional 'sample investigation' term.

5.3 Results

5.3.1 Responses to urine presentations at latrines

Twelve of the 16 mammal species were observed investigating urine samples in 12.3 % of 1053 encounters within two metres, but donor species was not a predictor of mammal investigatory behaviour (Table 5-B). Separate analysis of hyaena responses showed that donor species was a weak predictor of hyaena

investigatory behaviour, but the differences between treatment levels were not significant (Table 5-C).

Table 5-B. Factors influencing whether mammals of three guilds investigated urine samples presented at latrines.*Model parameters were generated from one best GLMM selected using AICc. Global model: Investigate (yes or no) ~ Recipient guild + Urine donor + Carrion + Sample age + Recipient guild x Carrion + (1|Latrine) + (1|Deposit). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI
(Intercept)	-	-2.3637	-2.99, -1.74*
Carrion odour	Not present	-	
	Present	-0.0227	-0.695, 0.649
Recipient guild	Herbivore	-	
	Mesopredator	-1.9605	-4.05, 0.132
	Large predator	-1.3777	-2.51, -0.245*
Sample age	-	-0.3690	-0.595, -0.143*
Recipient guild x Carrion odour	Herbivore x Not present	-	
	Mesopredator x Present	2.2154	-0.0118, 4.44
	Large predator x Present	2.1283	0.834, 3.42*

Table 5-C. Factors influencing whether hyaenas investigated urine samples presented at latrines.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Investigate (yes or no) ~ Urine donor + Carrion + Sample age + (1|Latrine) + (1|Deposit) + (1|Hyaena ID). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-3.2673	-4.90, -1.63*	
Carrion odour	Not present	-		1.00
	Present	2.0056	0.464, 3.55*	
Urine donor	Lion	-		0.35
	Leopard	-1.1776	-2.61, 0.258	
	Elephant	-1.4142	-3.11, 0.285	
	Wild dog	-1.4496	-3.35, 0.455	

The results of the guild-level analysis were consistent with our prediction that mammals would be more likely to be vigilant when exposed to predator urine (rather than elephant urine) and if they investigated the sample during the encounter (Table 5-D, Figure 5-A). However, for separate analysis of hyaena data, vigilance behaviour was most influenced by whether or not individuals investigated the sample, and donor species was not a predictor (Table 5-E)

Table 5-D. Factors influencing whether mammals were vigilant within one metre of the urine samples presented at latrines.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ Recipient guild + Urine donor + Carrion + Sample investigation + Sample age + Recipient guild x Carrion + Recipient guild x Sample investigation + Recipient guild x Sample age + Carrion x Sample investigation + Carrion x Sample age + Sample investigation x Sample age + (1| Latrine) + (1|Deposit). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-2.1242	-3.24, -1.01*	
Urine donor	Wild dog	-		1.00
	Elephant	-2.5649	-4.72, -0.414*	
	Leopard	-0.5898	-1.60, 0.425	
	Lion	-0.0179	-1.02, 0.986	
Sample investigation	No	-		1.00
	Yes	2.5220	1.35, 3.69*	
Recipient guild	Herbivore	-		0.93
	Mesopredator	-0.7558	-2.65, 1.14	
	Large predator	-0.9701	-2.63, 0.686	
Carrion odour	Not present	-		0.69
	Present	-0.9770	-2.30, 0.343	
Recipient guild x Sample investigation	Herbivore x No	-		0.51
	Mesopredator x Yes	-1.6000	-4.95, 1.75	
	Large predator x Yes	-1.8063	-3.75, 0.138	
Recipient guild x Carrion odour	Herbivore x Not present	-		0.28
	Mesopredator x Present	-1.5484	-5.00, 1.90	
	Large predator x Present	1.3964	-0.808, 3.60	
Carrion odour x Sample investigation	Not present x No	-		0.15
	Present x Yes	1.2410	-0.759, 3.24	

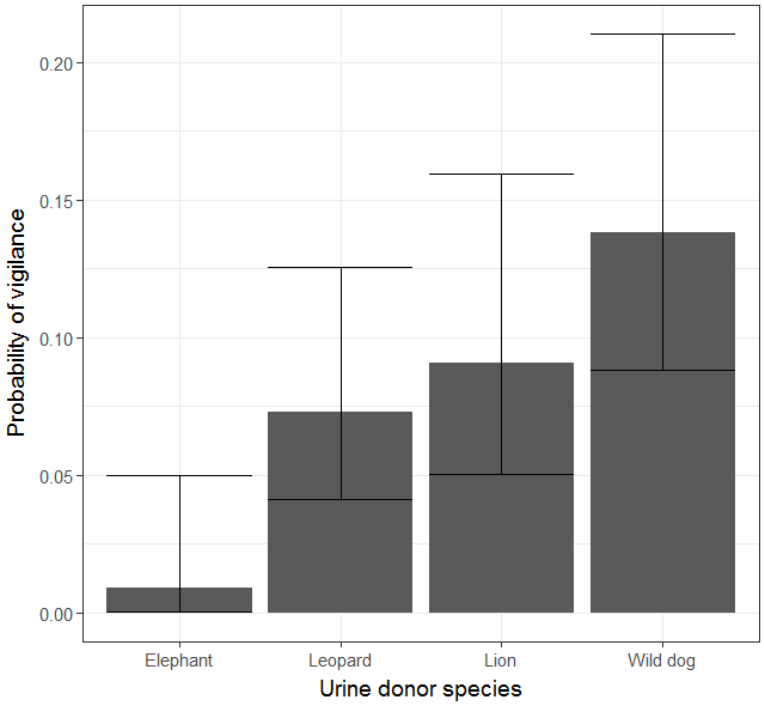


Figure 5-A. The variation in the likelihood that mammals were vigilant based on the species of urine donor, using raw data.*Binomial error bars indicate 95% confidence intervals.*

Table 5-E. Factors influencing whether hyaenas were vigilant within one metre of the urine samples presented at latrines.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ Urine donor + Carrion + Sample age + Sample investigation + Sample investigation x Sample age + (1| Latrine) + (1|Deposit). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-3.7848	-5.33, -2.24*	
Sample investigation	No			1.00
	Yes	1.8613	-0.000835, 3.72	
Sample age	-	0.7352	-0.360, 1.83	0.74
Carrion odour	Not present			0.17
	Present	0.7540	-1.55, 3.06	
Sample age x Sample investigation	Sample age x No			0.16
	Sample age x Yes	0.6122	-1.52, 2.74	

Sample age influenced the likelihood of sample investigation, since mammals overall were less likely to investigate urine with increasing sample age (Table 5-B), but it was not a predictor of vigilance (Table 5-D). Considering hyaenas separately, sample age was not a reliable predictor of their investigatory (Table 5-C) or vigilance (Table 5-E) behaviour.

Our results showed a guild-specific effect of carrion odour on investigatory behaviour: mesopredators and large predators were more likely to investigate urine samples in the presence of carrion, whereas herbivores were not influenced by carrion treatment (Table 5-B, Figure 5-B). This could have been due to mesopredators and large predators investigating the sample of carrion odour more often than herbivores, and consequently appearing to investigate the urine sample while determining the exact location of the carrion odour. However, herbivores and large predators were equally likely to investigate the carrion sample itself (Figure 5-C). Hyaenas were significantly more likely to investigate urine samples if carrion odour was also present (Table 5-C, Figure 5-D). Regarding vigilance behaviour, the presence of carrion odour did not significantly influence the likelihood that mammals in general (Table 5-D) or hyaenas (Table 5-E) were vigilant during an encounter.

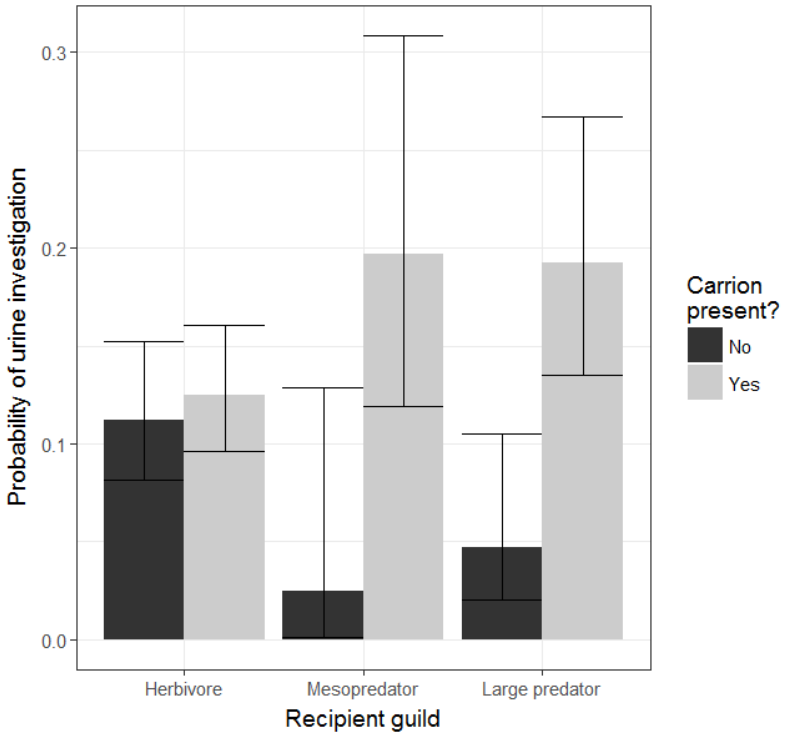


Figure 5-B. The variation in the probability that mammals from three guilds investigated urine samples with and without the presence of carrion odour, using raw data.*Large predator data includes hyaenas. Binomial error bars indicate 95% confidence intervals.*

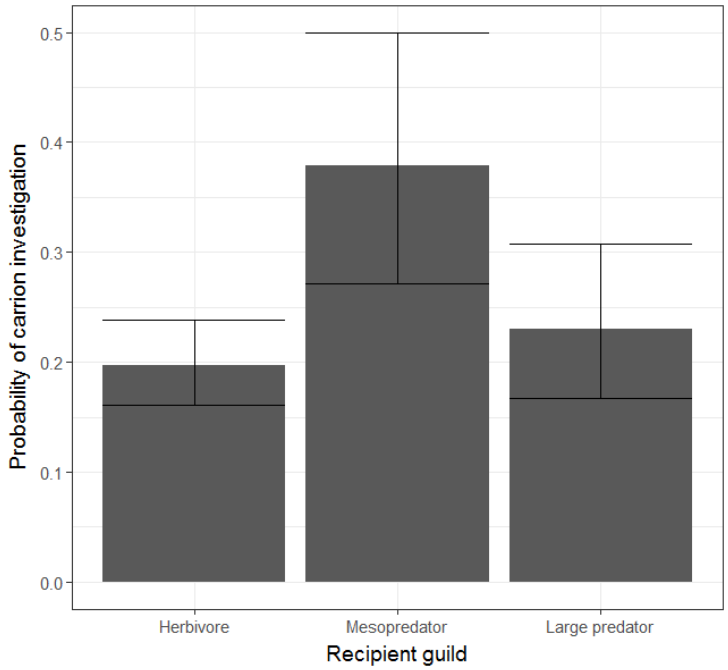


Figure 5-C. The variation in the probability that mammals from three guilds investigated the carrion odour itself, using raw data. *Large predator data includes hyaenas. Binomial error bars indicate 95% confidence intervals.*

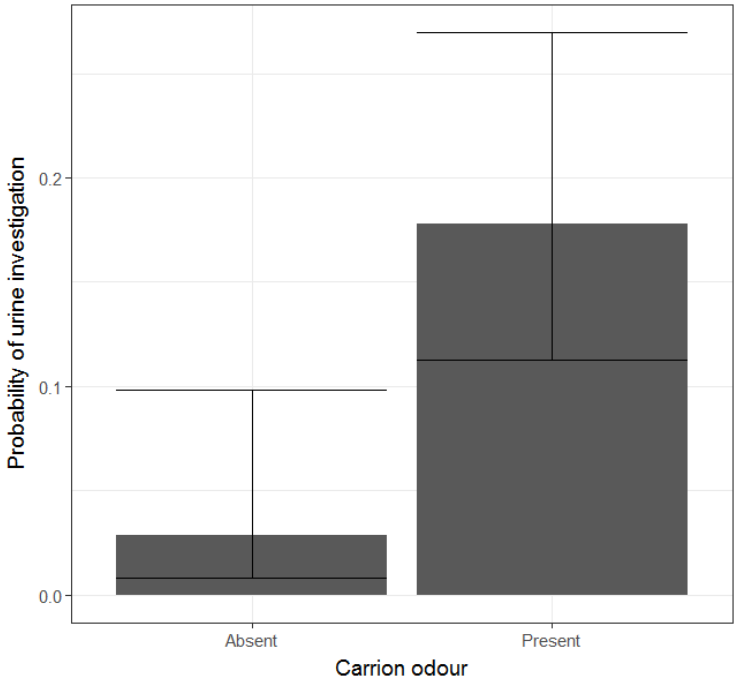


Figure 5-D. The variation in the likelihood that hyaenas investigated urine samples based on the presence of carrion odour, using raw data.*Binomial error bars indicate 95% confidence intervals.*

5.3.2 Hyaena behaviour at carcasses

Hyaenas were similarly likely to investigate urine samples presented at carcasses (mean probability of investigation = 0.158, 95% CI [0.116, 0.211]) as they were at latrines in the presence of carrion odour, and more likely to investigate these than urine samples presented without carrion odour (cf. Figure 5-D). Donor species was only a weak predictor of hyaena investigatory behaviour at carcasses (Table 5-F), but their vigilance behaviour was influenced by both sample investigation and donor species (Table 5-G). Hyaenas were more likely to be vigilant if they had investigated the urine sample, and were more likely to be vigilant within one metre of lion or wild dog urine than elephant urine (Table 5-G, Figure 5-E). Hyaenas were more likely to investigate urine samples with decreasing sample distance to the carcass, but sample distance was only a weak predictor of vigilance behaviour (Table 5-F, Table 5-G).

Table 5-F. Factors influencing whether hyaenas investigated urine samples presented at large carcasses.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Investigate (yes or no) ~ Urine donor + Sample age + Sample distance + Urine donor x Sample age + (1|Carcass) + (1|Deposit) + (1|Hyaena ID). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-1.1827	-2.36, -0.00681*	
Sample distance	-	-0.7353	-1.52, 0.0475	0.77
Urine donor	Elephant	-		0.19
	Leopard	0.3528	-1.02, 1.73	
	Lion	0.2347	-0.881, 1.35	
	Wild dog	1.0592	0.00364, 2.11*	
Sample age	-	-0.0698	-0.476, 0.336	0.16

Table 5-G. Factors influencing whether hyaenas were vigilant within one metre of urine samples presented at large carcasses.*Model parameters were generated using model-averaging as there were multiple candidate GLMMs within two AICc of the best model. Global model: Vigilant (yes or no) ~ Urine donor + Sample investigation + Sample age + Sample distance + Sample age x Sample investigation + (1|Carcass) + (1|Deposit) + (1|Hyaena ID). CI = Confidence interval, * Confidence intervals that do not cross zero.*

Variable	Level	Estimate	95% CI	Relative importance
(Intercept)		-2.5427	-4.44, -0.647*	
Sample investigation	No	-		0.80
	Yes	0.9311	-0.00604, 1.87	
Urine donor	Elephant	-		0.80
	Leopard	0.3897	-0.987, 1.77	
	Lion	1.7926	0.667, 2.92*	
	Wild dog	1.4019	0.0356, 2.77*	
Sample distance	-	0.3638	-0.576, 1.30	0.18

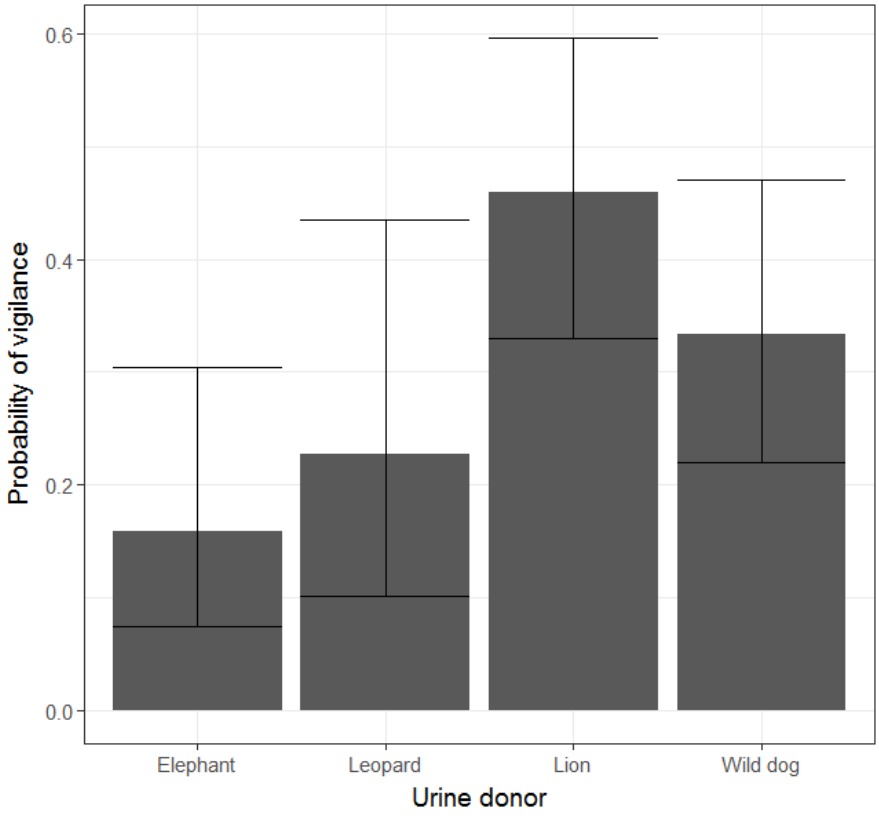


Figure 5-E. The variation in the likelihood that hyaenas were vigilant within one metre of a urine sample presented at carcasses based on urine donor species, using raw data.*Binomial error bars indicate 95% confidence intervals.*

5.4 Discussion

Our urine presentation experiment examined the influence of depositor species and carrion presence on the eavesdropping behaviour of wild African mammals. Mammals appeared to investigate sympatric predator urine in an effort to gain information about their environment, such as the proximity of heterospecifics, foraging opportunities, and/or predation risk. The presence or absence of carrion odour had a guild-specific effect on urine investigation: large and meso-predators (potential scavengers and thus beneficiaries of a carrion discovery) were more likely to investigate urine samples if carrion odour was also present, but herbivore investigatory behaviour was unaffected. Mammals in general were more likely to be vigilant when exposed to large predator urine than elephant urine, and thus

appear to have the ability to discriminate between the olfactory signals of these species. Hyaenas exhibited context-specific differences in behaviour: they were more likely to investigate urine samples presented alongside carrion (i.e. carrion odour, carcasses), and donor species only influenced vigilance behaviour at carcasses, where hyaenas were most likely to be vigilant when exposed to lion and wild dog urine. Our results appear to reflect the relationships between spotted hyaenas and their sympatric competitors, and highlight the potential role of olfactory signals in mediating interactions between them.

Contrary to our predictions, the species from which urine samples originated had relatively little influence on mammal investigatory behaviour. Instead, the most important predictors of investigatory behaviour by mammals in general were sample age and the presence of carrion cues (i.e. carrion odour). As scent-marks age, they may be less readily detected, or less informative, to eavesdroppers. This is consistent with previous studies of eavesdropping behaviour in European forests, which found that red deer (*Cervus elaphus*) and red fox (*Vulpes vulpes*) responses to lynx (*Lynx lynx*) scat decreased over time (Wikenros et al., 2015; Wikenros et al., 2017). Additionally, mesopredators and large predators were more likely to investigate a urine sample in the presence of carrion odour, but herbivores were not affected by this treatment. The presence of carrion odour could serve as an indicator of a nearby food resource, and this potential feeding opportunity would be of greater interest to carnivores than herbivores. Consequently, carnivores may be more attentive to olfactory signals when food is nearby, in order to gather further information about the circumstances such as the presence of social group members, heterospecific and conspecific competitors, and/or predators.

Vigilance behaviour by mammals was most influenced by the species of urine donor and whether or not individuals investigated the urine sample during the same encounter. Overall, mammals were least likely to be vigilant near elephant urine, and as elephants do not pose a predatory threat, this suggests that African mammals may use information from olfactory signals to inform predation risk. This finding is consistent with studies of anti-predator behaviour in other ecosystems, in which mammals were less vigilant when exposed to control treatments than to olfactory signals from predators (e.g. red deer exposed to wolf *Canis lupus* urine, Kuijper et al., 2014) and/or dominant interspecific competitors (e.g. spotted-tail quolls *Dasyurus maculatus* exposed to Tasmanian devil *Sarcophilus harrisii* urine, Andersen et al., 2016). Mammals may also be more vigilant in response to predator urine not because of any direct threat from the urine depositor, but because of the likelihood that the predator odour will attract other eavesdropping predators (Banks et al., 2016). Thus, in this study we were unable to determine what information in the olfactory signal may be responsible for eliciting the observed vigilance by eavesdroppers. Sample investigation was also a significant predictor of vigilance behaviour by mammals in general, which suggests that close proximity to the sample was either necessary or an aid for acquiring sufficient information to trigger vigilance. Alternatively, animals in a heightened state of awareness may have been more likely to investigate samples and/or more likely to be vigilant near the samples.

Our specific analyses of hyaena responses revealed the influence of context on their eavesdropping behaviour. Hyaenas were more likely to investigate urine samples when they were presented alongside carrion odour or actual carcasses than when presented alone. In the context of hyaena latrines, heterospecific urine

by itself may not be interesting enough to stimulate direct investigation by hyaenas (only 2.9 % of 70 encounters) compared to the myriad of intraspecific signals present at latrines. The complex social hierarchy of hyaena clans, and the spatial and temporal variability of social interactions associated with a fission-fusion society, require an intricate intraspecific communication system (Smith et al., 2011) within which latrines appear to play an important role (Kruuk, 1972; J. Vitale, Chapters 3 and 4). Thus, hyaenas may spend a majority of their time at latrines investigating conspecific marks and depositing their own scents rather than attending to heterospecific signals. However, the presence of carrion odour, potentially a more conspicuous indicator of carnivore activity, increased the likelihood of hyaenas investigating interspecific cues at latrines (17.8 % of 90 encounters). Similarly, hyaenas investigated urine samples at carcasses in 15.7 % of 217 encounters, possibly due to the increased likelihood of encountering other predators near carcasses. Thus, context was the most important predictor of hyaena investigatory behaviour, and contrary to our predictions, the probability that they would investigate a sample was not reliably predicted by the donor species. Hyaenas were more likely to investigate samples that were located closer to the carcasses, which could be a result of hyaenas generally being more likely to investigate the ground next to carcasses, and/or a heightened interest in olfactory signals located nearer to carcasses.

While hyaena vigilance behaviour was not reliably predicted by donor species at latrines, hyaenas were more likely to be vigilant at carcasses while within one metre of lion or wild dog urine than that of elephants. This is consistent with previous studies on natural vigilance behaviour in hyaenas, which showed that vigilance is context-specific (Pangle and Holekamp, 2010b). Large carcasses are

carnivore hot-spots where scavengers need to be wary of challenges by both conspecific and heterospecific competitors (Cozzi et al., 2015), and thus scavengers may regularly use environmental cues to gather information on the proximity of both conspecific and heterospecific competitors and predators. As previously mentioned, lions are a significant source of hyaena mortality in many ecosystems (Kruuk, 1972; Trinkel and Kastberger, 2005), and thus lion-related signals may stimulate anti-predator behaviour in hyaenas. On the other hand, interactions between hyaenas and wild dogs are variable and outcomes can be influenced by the number of each species involved (Carbone et al., 2005). Therefore, hyaenas may have exhibited vigilance when exposed to wild dog urine in order to assess risk and/or opportunities from a potential encounter with a dog pack.

Scent-marks are not the only heterospecific cues available to eavesdroppers, and previous studies have examined hyaena responses to experimental playbacks of sympatric predator vocalizations (Pangle and Holekamp, 2010a; Watts et al., 2010; Webster et al., 2010). Given the differences in the measured variables, and the nature of olfactory and acoustic signals, we are unable to make direct comparisons between hyaenas' responses to these sensory modalities. Specifically, acoustic signals reveal the location of the signaller at the time the receiver detects the signal, whereas olfactory signals indicate that the signaller was present at some point in the past (Bradbury and Vehrencamp, 1998). Nevertheless, these studies are similar to the current study in that they demonstrated that hyaenas detected the acoustic signals of sympatric predators and often altered their behaviour accordingly. For example, hyaenas pricked their ears and turned in the direction of the loudspeaker after playbacks of wild dog

vocalizations, but were inconsistent in whether or not they approached and investigated the source of the calls (Webster et al., 2010). Playbacks of lion roars often elicited avoidance behaviour by hyaenas, but much like the study involving wild dog vocalizations, responses to lion roars varied greatly among individuals (Pangle and Holekamp, 2010a; Watts et al., 2010). Thus, the evidence from our experiment and previous acoustic experiments suggest that hyaenas gain information about their environment by attending to heterospecific signals, but resultant behaviours may vary greatly among individuals and circumstances.

It is possible that the process of freezing urine deposits for later use in our experiments affected the chemical compounds and thus communicatory signals of the urine. For example, male house mice (*Mus musculus musculus*) were unable to distinguish between the scent of familiar versus unfamiliar females when presented with thawed urine, but could do so in response to fresh urine that had not been frozen (Hoffmann et al., 2009). On the other hand, captive male African elephants were able to distinguish between the urine of females in different reproductive states despite the freezing of samples (Bagley et al., 2006), and frozen grey wolf urine elicited a fear response in laboratory mice (Osada et al., 2013). Thus it is possible that freezing has varying effects on urinary compounds signalling different traits such as familiarity, reproductive state, and species. Presenting fresh urine samples would likely have resulted in stronger responses during our experiment (as in Hoffmann et al., 2009). However, as the required samples could not be reliably collected on the same days as their scheduled presentations, and previous studies have cited meaningful responses to thawed urine, we deemed freeze-storing urine to be the best protocol.

The rates of urine sample investigation in our experiment may partially be a result of the manner in which they were presented. Many carnivore species employ specific body postures while urinating, such as leg-lifts by canids (e.g. African wild dogs, Jordan et al., 2013) or scraping of hind paws by felids (e.g. African lions, Schaller, 1972). The use of posturing during marking events increases the likelihood that present group members will investigate the associated marks (Jordan et al., 2013). Furthermore, postures such as paw-scraping leave visible marks on the soil alongside the urine, and may serve as a visual signal to enhance detection (Allen et al., 2014). A study on scent-marking in pumas (*Puma concolor*) found that experimentally-presented puma urine accompanied by simulated scrape marks were more likely to be investigated by pumas than urine presented alone (Allen et al., 2014). Since our experiment presented only the olfactory modality of scent-marking, the urine samples may not have been as readily detected by, or were not as salient to, receivers as they would be when deposited naturally.

In conclusion, our study provides evidence that interspecific eavesdropping by wild African mammals on sympatric predator urine varies based on the presence of additional environmental cues such as carrion odour and actual carcasses. Specifically, these results reflect the relationship between spotted hyaenas and their sympatric competitors, and the potential role of olfactory signals in mediating interactions between them. Our findings provide additional insight into predator avoidance by herbivores, information gathering by opportunistic scavengers, and potential mechanisms for reducing interactions among predators. Future research should investigate the influence of heterospecific scent-marks on

mammal behaviour over larger temporal and spatial scales, and include an assessment of movement patterns post-investigation.

Chapter 6 : General discussion

The overall aim of this research was to investigate the behavioural ecology of spotted hyaenas in the Okavango Delta, and specifically the role of olfactory signals in the behaviour of spotted hyaenas and sympatric mammals. Analysis of hyaena sightings and association patterns (lChapter 2) determined that there were five main clans within the population, with hyaenas associating with individuals from different clans while scavenging at large carcasses. The substantial number of individuals seen only on a few occasions during the five-year study suggested that this population may have included a large number of transient individuals traveling between the protected reserve and livestock areas. Regarding scent-marking behaviour, I found that hyaenas' extensive use of latrines in this ecosystem followed a seasonal pattern and likely functioned in territorial advertisement by clans (Chapter 3). Scat seemed to be the least important olfactory signal for hyaenas, as the translocation experiment found that hyaenas generally ignored translocated scats and did not distinguish between those originating from latrines located in resident versus non-resident clan home ranges (Chapter 4). Not only did latrines appear to serve an important role in hyaena communication, but several sympatric mammals were also observed investigating and scent-marking these sites. These observations of interspecific responses to olfactory signals at latrines extended to the urine deposits of large predators, as hyaenas and other mammals appeared to use these scents in their assessment of predation risk (Chapter 5).

It is well established that the survival and behaviour of wild animals is influenced by relationships with sympatric species, particularly between predators and prey (e.g. wolves *Canis lupu*s and elk *Cervus canadensis*, Fortin et al., 2005) or among

intraguild competitors (e.g. African large predators, Cozzi et al., 2012). However, the specific mechanisms that determine long-term spatial or temporal partitioning among species can be difficult to discern (Hayward and Slotow, 2009). Avoidance behaviours by prey or subordinate competitors could be reactive (based on real-time risk) or predictive (based on knowledge of potential risk) in response to predators and dominant competitors (Broekhuis et al., 2013). Evidence provided by my study showed that olfactory signals may play an important role in mediating interactions among African mammals. Specifically, observations of eavesdropping and scent-marking at hyaena latrines (Chapter 4), and of investigatory and vigilance behaviour in response to sympatric predator urine (Chapter 5), suggested that African mammals used the scent-marks to assess the likelihood of competition and predation. As long-lasting signals in the environment, scent-marks could serve as information for reactive or predictive avoidance depending on the age of the mark. Fresh scents could indicate the current or recent presence of a predator or competitor, whereas the accumulation of scents over time could have indicated preferential use of an area (Wemmer et al., 1996). The fact that the animals in my study were more likely to be vigilant when exposed to predator odours than to a control treatment suggested a role for scent-marks in reactive avoidance behaviour. However, since I did not examine long-term effects of experimental stimuli on behaviour, the potential for predictive use of those same cues remains unexplored.

In communities containing multiple competing predators and many prey species, the behaviour of one species should be examined with consideration of the activities of sympatric heterospecifics. Several carnivore species were observed scent-marking at hyaena latrines, and, in the case of African wild dogs and black-

backed jackals, specifically over-marking hyaena scats. Without considering the relationships among these animals, additional interspecific functions of scent-marking (which is generally assumed to serve a purpose in intraspecific communication) for hyaenas or other mammals could be overlooked. The extensive marking behaviours by wild dogs and jackals during the translocation experiment, while likely serving an intraspecific purpose, may have also been a direct response to the presence of hyaena scent and thus indicative of potential olfactory communication among predator species. Ideally, future experiments aimed at determining whether these animals were responding to purely conspecific or heterospecific signals would exploit species-specific chemical cues. My colleagues at the Botswana Predator Conservation Trust (BPCT) have begun examining this approach by testing mammal responses to 3-mercapto-3-methylbutanol, a compound specific to wild cat and leopard urine (Apps et al., 2017). If similar species-specific compounds can be isolated from hyaena excretions, it may be possible to further investigate which particular signals elicited over-marking by sympatric predators.

For highly productive ecosystems with multiple species of animals, interspecific olfactory interactions could be particularly widespread. Evidence of olfactory investigation by multiple species has been observed at other communal marking sites, such as brown bear (*Ursus arctos horribilis*) rubbing trees (McTavish and Gibeau, 2010) and puma (*Puma concolor*) 'community scrapes' (Allen et al., 2017). Furthermore, the availability of olfactory information would be greater in species-dense ecosystems, and thus it may be more important for animals inhabiting such ecosystems to employ context-dependent olfactory eavesdropping (such as that observed during the urine presentation experiment, Chapter 4).

Given the annual patterns of latrine use by hyaenas (Chapter 3), there appeared to be a seasonal change in the availability of olfactory information via latrines, not only for hyaenas for intraspecific communication but also for any heterospecific eavesdroppers investigating these sites (Chapter 4). Heterospecifics that scent-marked latrines frequently during the translocation experiment, such as wild dogs and black-backed jackals, may have also decreased their marking behaviour during the rainy season (though this was not tested by my study). If they marked at hyaena latrines as a form of signal amplification, the relative decrease in hyaena scents during the rainy season (particularly white scats) may have resulted in less conspicuous latrines that were consequently less effective platforms for signal amplification. If heterospecifics scent-marked latrines as a form of interspecific communication, this behaviour may have similarly decreased during the rainy season due to the reduction in hyaena scents at latrines. Thus, decreased marking by hyaenas (and the potential decrease by heterospecific predators) could also have resulted in a reduced reliance on olfactory signals for risk assessment by prey species or subdominant predators (i.e. those at greatest risk of injury or death by larger predators) during the rainy season. To examine whether multiple mammal species exhibit decreased investigatory, scent-marking, and/or vigilance behaviour at latrines during the rainy season, future research should video-monitor known latrine sites year-round using camera traps.

The apparent significance of seasonality for latrine use by hyaenas highlights the general importance of the physical environment in shaping the adaptive evolution of olfactory communication. This influence has been shown for acoustic communication, where the nature of the medium (e.g. water, as in marine mammals, Janik, 2005) can influence the kinds of strategies which evolve.

Additionally, light and visibility can influence the relative roles of contrast and colour in visual communication (e.g. display behaviour by lekking birds in variable light environments, Endler and Thery, 1996). The physical environment has been shown to influence scent-marking in other species, such as the giant panda (*Ailuropoda melanoleuca*), which select marking trees based on characteristics such as bark roughness that enhance signal persistence over time (Nie et al., 2012). The behaviours observed at hyaena latrines (such as decreased visitation during the rainy season, Chapter 3) pointed to other subtle and perhaps overlooked equivalents for olfactory communication, such as the effects of seasonal rainfall on deposited scent-marks.

The results from my study could provide important information for the conservation management of this hyaena population. The delineation of hyaena clan membership and home ranges by this study forms an important foundation for continued population monitoring by my colleagues in the field. Specifically, the conclusion that a substantial portion of my study population were likely transient individuals that travelled among landscapes of varying protected status (i.e. Moremi Game Reserve, wildlife management areas, livestock areas) has implications for human-hyaena conflict in the region. For instance, several individuals that normally occupied home ranges within Moremi Game Reserve were observed near or south of the veterinary cordon fence (i.e. within the livestock area). The movement of animals outside protected areas is a common occurrence for unfenced reserves in Africa (e.g. lions, Packer et al., 2013); indeed, one purpose of forgoing the use of fences is to protect the natural movements of species (Hayward and Kerley, 2009). In order to mitigate conflicts over livestock in my study area, it is important to understand the source population for such

‘problem’ hyaenas. Current and future research by my partner organization (BPCT) will use the clan identities and home range information produced by my research to further understand the nature and frequency of hyaena predation on livestock in this area.

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