Spatial and seasonal patterns of communal latrine use by spotted hyenas (*Crocuta crocuta*) reflect a seasonal resource-defense strategy

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Abstract

Communal marking sites, or latrines, appear to play an important role in intraspecific communication and social dynamics in a wide range of mammal species. The spatial distribution of latrines can provide clues to their function, and has been well documented in a number of species. Latrine use may vary considerably through time, however, and a more comprehensive approach to their study that considers spatial and seasonal patterns of use is required to understand more fully the costs and benefits of latrine use, and hence their adaptive significance. This study investigated spatial and seasonal patterns of latrine use by spotted hyena (*Crocuta crocuta*) in northern Botswana, examining their potential role in resource defense. Latrine characteristics and hyena activity were monitored to test the influence of season and location (relative to clan territories and roads) on latrine use. We conducted monthly scat counts (at 78 latrines) and continuously recorded hyena visitation (to 50 latrines) in five clan home ranges, demonstrating clear seasonal patterns in latrine use. Latrines were smaller in the wet season (November-March), resulting from fewer visits by hyenas, reduced scat accumulation, and the seasonal activity of coprophagous beetles. We speculate that such a seasonal pattern may be driven by reduced competition for food during the wet season. Latrines located within core clan areas were no larger or more frequently used than those in home-range boundary areas, but hyenas did preferentially place latrines alongside roads, and were more likely to re-use road-side latrines in subsequent years. This pattern was not due to observer detection bias, and adds to the growing body of literature on the impact that roads and other anthropogenic features have on the communication and movement ecology of wild animals.

Keywords: scent-marking, olfactory behavior, large carnivore, hyena, Crocuta*, latrine*

Significance statement

Although most mammals use communal marking sites, very little is known about their function and detailed patterns of use in many species. We investigated latrine use in spotted hyena (*Crocuta crocuta*) and describe spatial and temporal marking patterns that are consistent with optimizing scent longevity and detection. Spatially, hyenas preferentially located latrines along the edge of man-made vehicle tracks, which may increase signal detection and transmission. Seasonal marking patterns suggest that hyenas optimize their communication by concentrating activity in the dry season, thereby avoiding the disruptive effects of coprophagous dung beetles and rain, and focusing activity during periods when food resources are expected to be scarcer. These results demonstrate seasonal and spatial optimization of communication, including in response to novel anthropogenic features in the environment such as roads, advancing our understanding of communication strategies in mammals more broadly.

Scent-marking, where animals actively deposit scents in the environment, is widespread in mammals (Bradbury and Vehrencamp 1998) and is utilized in territoriality, group identity, individual recognition, alarm signaling, and reproduction (Johnson 1973; Jordan et al. 2014; Gilfillan et al. 2017). Because scent signals remain in the environment for prolonged periods, communication can occur over longer time frames than visual or acoustic signals, and senders and receivers do not need to be in close proximity for communication to occur (Bradbury and Vehrencamp 1998). This makes scent an ideal medium for territorial advertisement (Gorman 1984), which may be particularly important where interactions with intruders or neighbors are associated with high risk of injuries, such as those among territorial carnivores (Gosling 1982; Packer et al. 1990; Cassidy et al. 2015).

Communal latrines are sites visited by multiple conspecifics that result in accumulations of scent-marks including feces, urine, and/or glandular secretions (Gorman and Trowbridge 1989). Several species of social mammals are known to use communal latrines, across a number of groups (for reviews see e.g., Primates: Irwin et al. 2004; Rodentia: Ferkin 2019; Lagomorphs: Sneddon 1991; ungulates: Müller-Schwarze 1987; Musteloidea: Buesching and Stankowich 2017; Carnivora: Buesching and Jordan 2021; marsupials: e.g. Rubial et al. 2011; Insectivora: Poduschka and Wemmer 1986). Communal latrines can function in social bonding, territorial advertisement by social groups, advertisement relating to mate defense, and potentially inter-specific communication (Jordan et al. 2007; Droscher and Kappeler 2014; Buesching and Jordan 2021; King et al. 2017). In determining the function of latrines, it is important to consider multiple aspects of scent marking behavior (Buesching and Jordan 2019).

The spatial distribution of communal latrines can reflect their adaptive function. While latrines placed peripherally may be more intuitively linked to a territorial function, latrines placed centrally can also function in territoriality, with the optimal spatial pattern of scent-marking depending on the economic costs of maintaining sites and their likelihood of intercepting intruders (Gosling and Roberts 2001). This limits the value of spatial data alone in studying latrine function, and information from other sources is required to allow a more complete functional understanding (Buesching and Jordan 2019).

Temporal variability in scent-marking is a potentially important indicator of latrine function, and has been shown to occur in some species (e.g. European water vole, *Arvicola terrestris*, Woodroffe et al. 1990; Swamp rabbit, *Sylvilagus aquaticus*, Zollner et al. 1996). Temporal patterns of scent-marking may reflect short-term and seasonal changes in breeding behavior, seasonal variation in biotic (e.g. food) and abiotic (e.g. weather) environmental conditions, and longer term changes in population size and demography (Rosell 2001); relating temporal patterns of latrine use to such external drivers allows a deeper understanding of latrine function.

While broad spatial patterns of latrine placement and temporal patterns of use are well studied in many species (see Buesching and Jordan 2019), relatively little is known regarding the effects of anthropogenic structures and landscape features on terrestrial mammal communication, particularly with regards to scent communication. For example, artificial structures (e.g. buildings, fences) and landscape features (e.g. roads, boundaries) may funnel movements and consequently influence scent-marking (e.g. Krofel et al. 2017; Rafiq et al. 2020) and latrine placement (Barja et al. 2004). Furthermore, in landscapes modified by human activities, anthropogenic noise may disrupt acoustic communication in a range of species and environments (reviewed in Brumm 2013). As human populations and influences expand into ever more remote ecosystems, it is increasingly important to understand the impact of anthropogenic structures and activities on animal movement and behavior (van Dyck 2012).

In this study, we investigated the effects of spatial and temporal factors on latrine use by a population of spotted hyenas in the Okavango Delta ecosystem of Botswana, including their placement in relation to vehicle tracks - semi-permanent anthropogenic modifications in this landscape. Spotted hyenas (hereafter referred to as 'hyenas') are an ideal study system to investigate latrine use due to the conspicuous nature of their latrines; hyena feces are distinctively white in color as a result of high calcium content and thus their latrines are easily recognizable to human observers. Furthermore, hyenas live in permanent social groups called clans but exhibit fission-fusion sociality, in which individuals frequently join and depart subgroups that can 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 importance of olfactory communication in hyena society (Kruuk 1972; Glickman et al. 1997; Smith et al. 2011). Olfactory communication among hyenas also involves the maintenance of communal latrines, a behavior that has previously been observed for hyenas in different ecosystems (Kruuk 1972; Bearder and Randall 1978). Despite widespread observations of latrine use by hyenas, much of the published information concerning hyena latrine use comes from anecdotal reports made as part of studies of other behavioral phenomena (with the exception of Bearder and Randall 1978; Mills and Gorman 1987).

Studying the spatial and temporal distribution of hyena latrines provides important insights into hyena social behavior, their interaction with the physical environment, and the potential impact of human structures on their behavior. In describing hyena latrines, Bearder and 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 repeatedly over a long period of time. Like many species (e.g. African wild dogs, *Lycaon pictus*, Abrahms et al. 2016), hyenas are known to prefer moving along low resistance routes such as roads rather than through thick bush (Bearder and Randall 1978). This may explain why hyena latrines in the Serengeti (Kruuk 1972) and Transvaal Lowveld ecosystems (Bearder and Randall 1978) have been reported primarily along roads and game trails: hyenas may preferentially situate latrines on roads in order to facilitate increased detectability and visitation (c.f. Iberian wolf, *Canis lupus signatus*, Barja et al. 2004). However, studies of hyena latrine placement have been largely opportunistic, and since researchers are disproportionately likely to make observations on roads, the reported preference for the placement of latrines on roads may be an artefact of sampling bias.

As well as being influenced by the abiotic environment, the distribution of hyena latrines may reflect social behavior, and in particular intra-specific spacing or territoriality, but the role of latrines in hyena territoriality has not yet been formally evaluated. The maintenance of communal latrines is likely important in territorial advertisement by hyena clans, particularly since there is evidence for individual- and group-specific odors in anal gland secretions (called 'paste') that are often deposited at latrines (Burgener et al. 2009; Theis et al. 2012). There are several possible patterns of latrine placement and temporal use which could result from territoriality. First, latrines may be preferentially placed along territorial boundaries, to minimize the risk of costly transgressions and aggressive encounters. Indeed, in certain hyena 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 1993). Alternatively we may expect hyenas 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 neighboring clans (Kilshaw et al. 2009). As described, however, spatial patterns alone offer limited insight into latrine function, as the most economical territorial signaling strategies depend on site-specific costs of latrine maintenance (e.g. Gorman and Mills 1984).

In multi-purpose territories, resources include mates and food, and it can be difficult to decipher which resources shape communication strategies. Where access to these various resources are not closely tied spatially or temporally, seasonal patterns of latrine use may also provide important insights into latrine function. For example, latrine use by many species is more likely during the peak breeding season (e.g. meerkats, water voles, swamp rabbits), and in meerkats this is also correlated with monthly rates of encounters with intruding males (Jordan et al. 2007). As spotted hyenas appear to display no clear breeding season across their range (see Holekamp and Dloniak 2010), or in southern Africa more specifically (Lindeque and Skinner 1982), no such peaks would be expected, with latrines expected to be visited year-round. In contrast to the year-round need to defend mates, prey availability varies seasonally, and so therefore may resource defense strategies. For example, in the Serengeti, the annual migration affects prey availability, and hyena territorial boundaries break down during this time as a result (Hofer and East 1993). In our study ecosystem, the Okavango Delta, the commencement of the rainy season (November to March) coincides with synchronized calving and altered space use for many herbivores species, which could alter resource distribution among hyena clan territories (Wilson and Dincer 1976; Owen-Smith and Ogutu 2013). If latrine use is specifically related to the defense of food resources (as opposed to other resources such as mates), we might expect reduced activity during the wet season when food is more plentiful. In contrast, if latrine use reflects mate defense, hyenas should visit and maintain latrines consistently throughout the year. Indeed, we might even expect an increase in latrine activity during the wet season to compensate for the likely reduced longevity of signals as a result of rainfall.

Here, we present a systematic investigation of spatial and seasonal patterns in natural latrine use by a hyena population over five years in the Okavango Delta ecosystem. We observed latrine characteristics (i.e. number of scats) directly, and used remote camera traps to monitor hyena activity at latrines, with two objectives. First, we describe latrine spatial distribution, predicting that Okavango hyenas will exhibit a core marking strategy, as this may be optimal in extensive home-ranges that are more similar in size (~250km^2; Cozzi et al. 2015) to the large (~1000km^2)

ranges of the Kgalagadi desert where hyenas mark the core (Mills and Gorman 1987) than to the small ranges of boundary-marking (30km^2) Ngorongoro crater hyenas (Kruuk 1972). Second, we predicted that, in common with other species such as the Iberian wolf (Barja et al. 2004), latrines will be more common along roads than away from roads, reflecting the impact of human activity on hyena movement and communication. Finally, we sought predictors of temporal change in latrine use, testing the hypothesis that latrines are seasonally variable. We predicted that if latrines are involved in mate defense they would be visited throughout the year, due to aseasonal breeding in southern African hyenas (Lindeque and Skinner 1982). Alternatively, if hyena latrine function is primarily related to the defense of prey resources, we would expect reduced latrine use in the wet season, when prey is more abundant due to synchronized calving events (Wilson and Dincer 1976; Owen-Smith and Ogutu 2013).

METHODOLOGY

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 (center 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).

Data collection

It was not possible to record data blind because our study involved focal animals in the field.

Latrine characteristics

Latrines were primarily found opportunistically from 2012 - 2016 during daily movements throughout the study area (approximately 2193 km^2) in a vehicle, which was not limited to on-road travel. For this study, a latrine was defined as a site containing at least two distinguishable piles of feces (i.e. fecal deposits called 'scats'). For each latrine, the following characteristics were recorded: date and time of discovery, geographical coordinates, total number of hyena scats, presence or absence of coprophagous 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 center of each latrine's expanse. At our study site, 'roads' were established, unsealed vehicle tracks formed in the sand/substrate. The nearest distance of each latrine to any road was determined using the geographical coordinates of latrines and road tracks in Garmin MapSource. Latrines were defined as 'road-side' if they were within 20 m of such a road and confirmed by an observer, whereas latrines further than 20 m from the road were designated as 'off-road'. 'Single scats', defined as a single distinguishable pile of feces that was not deposited in a communal latrine, were also recorded opportunistically.

Of 194 latrines discovered during the field study, a subset of 78 latrines were checked approximately monthly (mean ± SD = 29.85 ± 16.21 [range, 0 – 192] days between checks) to count scats. This subset was selected for analyses as 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 three consecutive months after the end of a rainy season). Rainfall (mm/day) was recorded using a graduated cylinder rain gauge at the field research station (the approximate center of the study area).

Latrine transects

To investigate whether hyenas placed latrines preferentially road-side or off-road, we searched 18 transects (12 in October 2014 and six in September 2015) from a vehicle to record latrines and single scats. Three 5 x 5 km blocks encompassing the center 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 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 in the direction most similar to the 360º bearing until the vehicle's odometer reached three kilometers. At road junctions, the road which followed the bearing more closely was selected. Off-road transects commenced at the coordinates generated in MapSource, and followed the bearing as closely as possible (while avoiding scrub impenetrable to the vehicle) until the odometer reached three kilometers. If the vehicle reached the edge of the 5 x 5 km block before driving three kilometers 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 kilometers per hour) to ensure equal likelihood of latrine spotting. The characteristics of all latrines or scats encountered during transect searches were recorded as outlined above.

Visitation monitoring

To monitor visitation rates by hyenas, one motion-triggered camera trap was placed and maintained at each of 50 randomly selected latrines throughout 2014 and 2015. Infrared StealthCam Prowler and StealthCam G30 camera traps (StealthCam LLC, Grand Prairie, TX, USA) were affixed to poles that were 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). While it is possible that the presence of camera-traps may have affected the behavior or visitation of hyenas, this species is frequently captured on camera traps (e.g. Rich et al. 2016) and there is no reason to expect that any such effects would be specific to any spatial designation of latrine or visitation period. We placed cameras so that the field of view encompassed 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 (94% of monitored latrines), we positioned the camera to capture the greatest number of existing scats as well as any movement along the road. We recorded the characteristics of the focal latrine at the start and end of monitoring, and calculated the total recorded monitoring time at each site by adding the length of time the camera was turned on and facing the experimental site over the course of the camera’s deployment. We checked cameras every three to five 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 end of the last video recorded before the disturbance, and we reactivated/repositioned the camera (mean number of inactive camera days per site = 1.88 ± 2.30 days, range 0 – 8.04 days). Recording effort may therefore have been underestimated, as the camera may have continued functioning for an unknown duration following the last video that was recorded.

We checked photos and videos from each latrine for visits from hyenas, recording the following information at each visit: date, time, and the identities of all hyenas observed. A ‘visit’ was defined as a lone hyena or group of hyenas recorded by the camera, with recordings over five minutes apart designated as separate visits. We identified individual hyenas by comparing their unique spot patterns to a reference database, within which all photo-identified individuals were given a unique ID code for subsequent matching of sighted individuals.

Clan assignments and territory classifications

As part of a larger study (Vitale 2018), we determined clan membership and home-range extents for the local hyena population. Hyenas were detected opportunistically through direct sightings or on camera traps placed at sites of interest, and photographed individuals were identified by their unique spot patterns. The structure of the population was designated using social network analysis and the Multilevel Community Detection algorithm (Blondel et al. 2008; Wey et al. 2008), which assigned 112 individuals to ‘communities’ (i.e. clans). We then used the spatial data of assigned clan members (the geographic locations of direct observations and camera trap sightings of individuals; mean ± SD number of locations per individual used in analysis = 23.9 ± 22.4 locations) to determine clan home ranges and territories. 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. Within these home ranges, the space utilized exclusively and defended by an individual or group is designated as their ‘territory’ (Gosling and Roberts 2001). We collated the geographic locations of assigned clan members during 2014-2015 and used kernel techniques to estimate each clan’s utilization distribution (i.e. the probability distribution of the area used by the group; Worton 1989). Each clan’s home range was defined at the 95% kernel contour, and the clan territory was estimated at the 50% kernel (see Electronic Supplementary Material for further information on the social network and spatial analyses). The coordinates of all latrines were then overlaid onto the clan territory and home range map to classify each latrine as occurring either within a clan’s territory or an area of home range overlap. We classified latrines into two home-range zones rather than finer scale incremental isopleths due to sample size limitations, and the problem that many latrines fell within multiple isopleths within overlapping ranges in this contiguous population. Nevertheless, by utilizing the territory/overlap dichotomy, our approach is consistent with existing multi-group studies in the literature (e.g. Stewart et al. 2001; Jordan et al. 2007).

As initial territory and home range estimates were generated from the same observations which were used to produce estimates of visitation rates, a lack of independence meant that we were unable to test for an association between latrine location and visitation rate using these data. Hence, to ensure statistical independence in this particular analysis, we recalculated clan home ranges and territories from a separate dataset that excluded sightings from monitored latrines, and used these to re-assign the location designations for the monitored latrines.

Statistical analyses

We performed analyses using R (version 3.3.3 and 3.6.1; R Core Team 2019). To investigate whether hyenas 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 hyenas 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 data from latrines that were checked during at least two years.

To evaluate the factors affecting latrine size (i.e. number of scats present), a generalized linear mixed-effect model (GLMM) with Poisson distribution was fitted to data collected during monthly latrine monitoring surveys. Terms included in the model set were days since rain (i.e. since the onset of the current rainy season), home-range zone (Core/Territory = within 50% Kernel Utility Distribution [KUD], Overlap = beyond 50% KUD and within 95% KUD), year and the interaction term days since rain x home-range zone. A GLMM with binomial distribution was used to investigate the factors affecting the presence or absence of coprophagous beetles, which feed on hyena scats. Terms included in the global model were days since rain, year, scat count and the interaction term days since rain x scat count. For both GLMMs, data were collected in 2014 and 2015 during 1128 visits to 78 unique latrine sites, and latrine identity was included as a random effect to control for repeated measures.

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 hyena visits recorded on the camera by the total amount of time that the camera was actively recording the latrine. Two General Linear Model sets (GLMs) with Gaussian distribution were used to investigate latrine visitation rates (visits per day), and latrine growth rates (average daily increase or decrease in scats over the monitoring period) respectively. Both models included season (Wet=Nov-Mar, Dry=Apr-Oct) and home-range zone (Core/Territory, Overlap), and their two-way interaction, as fixed effects. In addition, the latrine growth rate model included the term visitation (hyenas/day) and all two-way interactions.

All GLMs and GLMMs were fitted using the lme4 package (Bates et al. 2016). Continuous variables were scales and centered. In each case, candidate models were created from the saturated global model using the ‘dredge’ function in the MuMIn package (Barton 2019). As the Akaike weight of the best model in all model sets above was less than 0.9 and several models had AICs within seven units of the best model (Burnham et al. 2011; Grueber et al. 2011), we conducted model averaging using the MuMIn package (Barton 2019). We selected the top models whose cumulative AIC weights were more than 0.95 to construct model-averaged estimates of the parameters (Burnham et al. 2011). Model diagnostics were performed by inspection using the DHARMa package (Hartig 2019), which uses a simulation-based approach to create readily interpretable scaled residuals from fitted models.

RESULTS

Between 2012 – 2016, 194 latrines and 272 additional single scats were found in the study area. Hyenas were observed depositing feces, urine, interdigital secretions (by scraping the ground with forepaws; cf. Tilson and Henschel 1986; East et al. 1989), 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.

Latrine placement

Latrines were distributed throughout the study area and located within at least five known clan home ranges. Approximately 77% of 184 latrines and 37 % of 246 single scats were located along a road. We searched 18 transects during October 2014 (two blocks of six transects each) and September 2015 (one block of six transects) to investigate whether hyenas preferentially place latrines and scats road-side or off-road (Table 1). Latrines were located significantly more often road-side than off-road (Mann-Whitney U = 16, n = 18, p = 0.015), and there was no significant difference in latrine frequency among the three transect blocks (Kruskal-Wallis χ^2 = 2.77, df = 2, p = 0.25). In contrast to latrines, 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 = 0.36), and no significant difference among transect blocks (Kruskal-Wallis χ^2 = 1.42, df = 2, p = 0.49).

One hundred and thirty-eight latrines (71.1% of all latrines discovered) were monitored in two study years during 2014-2016, and 63 latrines (32.5% of all latrines discovered) were monitored in all three study years. Ninety-six (69.6%) of the 138 latrines monitored in two years were used by hyenas in both years, and 49 (77.78%) of the 63 latrines monitored in all three study years were active in all three years. Hyenas 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, $p < 0.001$).

Latrine size

After initial discovery, latrines were revisited repeatedly from 2014-2016 to record the number of scats present, resulting in 1128 records from the 78 latrines used in this analysis. Scat accumulation exhibited a seasonal pattern in which the mean number of scats per latrine decreased after the annual onset of rain (Fig. 1).

After controlling for a significant effect of year (more scats were found in 2015 compared to 2014), the number of days since first rainfall was significantly related to the size of latrines (Table 2). The mean number of scats per latrine decreased soon after the onset 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 (Fig. 2). There was no clear effect of the location of the latrine within clan territories (Table 2, Fig. 2).

The presence or absence of coprophagous beetles (*Trogidae* spp.) on hyena scats was significantly predicted by the number of days since first rainfall (Table 3). Beetles were more likely to be present on hyena scats during or immediately following the onset of the rainy season.

Latrine visitation and scat deposition by hyenas

Fifty latrines were monitored using camera traps, each over a period of approximately 30 days, to evaluate the rate of visitation by hyenas 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 hyenas could be photo-identified, 19 latrines (55.9 %) were visited by more than one clan during the monitoring period, with a maximum of four known clans visiting a given latrine.

Latrine visitation rates by hyenas 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 1.07 to 30.03 days (average ± SD = 5.4 ± 5.37 days). The monitored latrines were visited by a maximum of seven hyenas per visit (mode = 1, median = 1). Season was the strongest predictor of latrine visitation rate, with visitation rates being lower in the wet season (Table 4, Fig. 3).

The growth of latrines - or mean daily change in the number of scats present (a proxy for scat deposition rate) - was analyzed 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. Visitation rate had a strong positive effect on latrine growth, with more visits increasing the number of scats at the site, while latrines tended to decrease in size during monitoring periods undertaken in the wet season (Table 5, Fig. 3).

DISCUSSION

This study is the first to systematically and simultaneously investigate the effects of social and environmental factors on latrine use by spotted hyenas. Latrines were used extensively by hyenas within this ecosystem, and the patterns observed in this study indicate a seasonal scent-marking strategy. Multiple factors contributed to reduced latrine size in the rainy season, but this may be driven by reduced inter-clan competition for prey during this period. In common with other species, human-made tracks/roads also influenced hyena latrine use. Overall, this study enhances our understanding of latrine use, especially the need to consider seasonal driver and anthropogenic influences on animal communication networks.

Maximizing the likelihood of detection likely drives scent-mark placement in many species. Indeed, previous work on spotted hyenas has shown that the marking strategy that a population adopts is related to the economics of latrine maintenance, which in turn depends on home-range size (Gorman and Mills 1984; Mills and Gorman 1987). In the Serengeti ecosystem, where clan home ranges are very small (~30 km^2), hyenas situate most latrines in the border regions (Kruuk 1972). By contrast, the enormous home ranges that hyenas occupy in the southern Kalahari (1381-1840 km^2) necessitate a different strategy: there, latrines were concentrated along the dry riverbed in the home-range core (Gorman and Mills 1984). Our Okavango study population has home range sizes intermediate to these two systems (176.70 to 408.62 km^2; Vitale 2018), and we observed the adoption of an intermediate marking strategy, whereby latrines were located throughout the clan's range, with latrines in the core and the overlap zones of the home-range apparently visited and maintained at similar rates. Such optimal distribution of scent-marks is probably widespread, and has already been shown to occur in a variety of other species including klipspringer (*Oreotragus oreotragus*, Roberts and Lowen 1997) and Iberian wolf (Barja et al. 2004).

At finer spatial scales, hyena latrine use was also consistent with our prediction of an economical marking strategy. Specifically, we found that latrines in this ecosystem were frequently located along human-made unsealed roads, which is consistent with other species in this environment (leopard, *Panthera pardus*, Rafiq et al. 2020), and elsewhere (e.g. Iberian wolf, Barja et al. 2004). Road-side latrines were significantly more likely than off-road latrines to be maintained in consecutive years, and transect searches showed that latrines were significantly more likely to be located (by humans) if they were at the road-side as opposed to further off-road. Efficient scent-marking behavior 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. Hyenas are known to prefer traveling along roads rather than moving through thick vegetation (Bearder and Randall 1978), and hyenas that move long distances outside regular territories, such as the 'commuting' population of the Serengeti (Hofer and East 1993), may also preferentially place latrines on roads (Kruuk 1972), though it is not possible to control for detection bias by observers in that case. As we made multiple observations of individuals outside their clan territories (Vitale 2018), it is possible that hyenas within the Okavango ecosystem display a similar pattern. In any case, given the significant influence of roads on latrine placement and re-use by hyenas, the creation of roads by humans appears to influence hyena scent-marking behavior. Furthermore, since scent-marking at latrines appears to serve an important role in hyena movement and resource defense by clans, there are potential conservation implications for human road use patterns in habitats which support hyena 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 creation may not only influence animal patterns and space use (reviewed by Trombulak and Frissell 2000), but may also impact animal communication systems (Krofel et al. 2017; Rafiq et al. 2020). In this context however, it worth considering the possible origin of road-related marking behavior in this and other species. The reasons why animals may choose to mark along roads - ease of locomotion and likelihood of detection - also apply to natural animal trails, such as elephant pathways. While we do not currently have data to support or refute this suggestion, it is conceivable that road-based marking patterns reflect potential natural preferences to utilize these natural highways. This, and the potential that many vehicle tracks and roads may also be established along such natural trails in the first place, would be fruitful areas of future study.

We also observed an annual cycle of hyena 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. Within the Okavango ecosystem, the rapid disappearance of latrines during the rainy season was likely in part a result of the degradation of scats by heavy rainfall. Although there is no direct evidence of this from hyenas, 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), and there is no reason to expect hyena feces to be any more rain-resilient. Seasonal consumption of scats by coprophagous beetles may also play a role in reduced scat numbers at latrines. Indeed, dung beetles consumed hyena scats within three days during the rainy season in South Africa (Bearder and Randall 1978), and previous investigation of beetle fauna at hyena latrines found that soft and/or fresh hyena scats were more attractive to scarab beetles (Krell et al. 2003). Furthermore, some scent-mark types may persist longer than scats in rainy conditions. Paste in particular contains substantial amounts of fatty acids (Burgener et al. 2009) which are likely to improve its persistence through rainfall. Therefore, while it is possible that the observed decrease in visitation rates by hyenas during the rainy season was related to the reduction in signaling benefit resulting from rainfall and coprophagous beetles, if territorial communication had remained important during the rainy season, more resilient scent-marks such as paste could be utilized for this purpose. However, as we found that latrine visitation – not just scat counts – was lower during the rainy season, it is likely that territoriality is reduced among clans at this time.

Since scent-marking can function in mate defense and reproductive behavior, 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). Unlike those species, however, hyenas breed throughout the year (Lindeque and Skinner 1982; Holekamp et al. 1999), and so mate defense or reproductive behavior more broadly are unlikely to account for the observed seasonal differences in latrine use. Interestingly, neither of the two previous studies focusing on hyena latrine behavior reported 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, and as these populations are also aseasonal breeders (Lindeque and Skinner 1982) seasonal effects in marking are not related to mate-defense.

Hyenas alter their space use patterns with shifts in seasonal abundance of prey in ecosystems such as the Serengeti (Kruuk 1972; Hofer and East 1993) and Etosha National Parks (Trinkel et al. 2004). Calling station surveys (Cozzi et al. 2013) and a comprehensive camera-trap survey concurrent with our study (Rich et al. 2016) found no seasonal difference in densities or occupancy probabilities respectively. As prey abundance in the Okavango Delta is likely to be greater in the rainy season as synchronized calving and a consequent population boom in many African herbivore species (Owen-Smith and Ogutu 2013), it is possible that this reduction in scent-signalling at latrines during this period results from reduced competition for food resources during this period. Indeed, although data on such effects are rare, some species (e.g. greater hog badgers, *Arctonyx collaris*, Zhou et al. 2015a, b; European badger, *Meles meles*, Pigozzi 1990) have been shown to scent-mark most when resources are either less abundant or more energetically expensive to acquire. Zhou and colleagues (2015a, b) attribute this result to the scarce factor paradox (Valavanis-Vail 1954), where latrine use was inversely related to food abundance (e.g. Lynn 1991). Additionally, unlike in the dry season when the distribution of several large herbivore species appears to be concentrated near permanent water sources (Rich et al. 2017), rain-filled pans and puddles throughout the landscape result in a more even distribution of water and prey species during the rainy season (Rich et al. 2017). We suggest that it is likely that the increased availability and more even spatial distribution of water and prey during the rainy season may result in relaxation in territorial behavior among clans during this period.

Regardless of season, and in contrast to our predictions, hyenas did not preferentially maintain latrines located within exclusive clan territories. Latrines within territories contained a similar number of scats to those in areas of home-range overlap, and visitation and scat deposition rates by hyenas were similar across the two latrine types. Rather than indicating a lack of territoriality, we suggest that this pattern could result from different individuals visiting latrines in different home-range zones. While many resident clan members may visit and contribute to latrines in territory cores, latrines in the overlap zone may be visited by hyenas from multiple clans, resulting in similar overall visitation and deposition rates in the two zones, even if each clan deposits more in its own core. Furthermore, the apparent transient nature of many individuals observed within the study population (Vitale 2018) supports the possibility that many hyenas encountering these latrines were territorial intruders.

It is theoretically possible that sampling bias may explain the observed seasonal patterns of latrine use, as most of the monitored latrines were located alongside roads, and it is conceivable that hyenas utilized roads less often during the rainy season. However, there is no evidence for seasonality in road use: the occupancy probabilities for hyenas calculated from a concurrent camera survey (in which all cameras were placed along roads) did not differ between seasons (Rich et al. 2016). Indeed, sympatric African wild dogs actually increased their road-use during the rainy season, as roads represent efficient paths through seasonally dense vegetation (Abrahms et al. 2015).

Finally, given the concentration of latrines along roads, our results also suggest that latrine surveys along roads may be developed as a cost-effective 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 (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). 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 hyenas.

In conclusion, this study enhances our understanding of latrine use by hyenas, suggesting that seasonal patterns of scent-marking behavior may be linked to seasonal fluctuations in prey availability. Furthermore, our findings have implications for wildlife conservation and management given the observed influence of human-made roads on hyena scent-marking behavior and the potential for latrine studies to be used as a non-invasive population monitoring tool.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethical statement

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Conflict of interest: The authors declare no conflicts of interest.

Ethical approval: This research was conducted under research permit EWT 8/36/4 XXV (15) issued by the Botswana Ministry of Environment, Wildlife, and Tourism, and with ethical approval from the University of Nottingham School of Life Sciences without requiring formal review by the ethics committee due to the observational and non-invasive nature of the study. Guidelines for the use of animals in ethological research, as outlined in Sherwin et al 2003, were followed throughout this study.

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Tables

Table 1 The number of latrines found during 18 transects driven in three blocks of the study area. Three road-side and three off-road transects were driven in each block)

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

Table 2 Model averaged outputs from a Generalized Linear Mixed-effects Model (GLMM) with Poisson distribution investigating latrine size (number of scats) during 1123 visits to 78 latrines. Outputs are from all models whose cumulative AIC weights were > 0.95 showing: a) Effect sizes and confidence intervals and b) AICc model weights for all models in the model set. Terms included in the model set were: Days since rain (i.e. onset of the current rainy season), Home range zone (Core/Territory = within 50% KUD, Overlap= beyond 50% KUD and within 95% KUD), and year. 2014 and home-range overlap zone were the reference categories

(a)

Term name		**Estimate**	**SE**	**z**	**CI (2.5-97.5%)**	**P**	
(Intercept)		2.353	0.137	17.217	(2.085, 2.621)	<0.0001	***
Year	2014	0	0	0	0	0	
	2015	0.0406	0.0196	2.071	(0.011, 0.076)	0.0384	*
		0.0317	0.00905	3.495	(0.014, 0.049)	0.000473	***

Days since rain + Home-range zone	Overlap	0	0	0	0	0
	Territory	0.0397	0.140	0.283	(-0.313, 0.528)	0.777
Days since rain x Home-range zone	Territory	-0.00097	0.00633	0.154	(-0.043, 0.025)	0.878

Significance codes: *** P<0.001; * P<0.05

(b)

Model	**df**	**AICc**	**delta**	**weight**
Year+ Days since rain + Home-range zone	5	10837.42	1.77	0.24
Year + Days since rain + Home-range zone + Days since rain x Home-range zone	6	10839.17	3.52	0.1
Days since rain	3	10840.71	5.05	0.05
Days since rain + Home-range zone	4	10842.48	6.83	0.02
Days since rain + Home-range zone + Days since rain x Home-range zone	5	10844.22	8.56	0.01

Year	3	10849.89	14.23	0
Year + Home-range zone	4	10851.65	16	0
(Null)	2	10852.82	17.16	0

Table 3 Model averaged outputs from a Generalized Linear Mixed-effects Model (GLMM) with binomial distribution investigating factors affecting the presence or absence of coprophagous beetles during 1123 visits to 78 latrines. Outputs are from all models whose cumulative AIC weights were > 0.95 showing: a) Effect sizes and confidence intervals and b) AICc model weights for all models in the model set. Terms included in the model set were: Days since rain (i.e. onset of the current rainy season), year, and scat count. 2014 was the reference category

(a)

Term name		**Estimate**	**SE**	**Z**	**CI (2.5-97.5%)**	**P**	
(Intercept)		-5.9291	0.8491	6.977	(-7.595, -4.263)	<0.0001	***
Year	2014	0	0	0	0	0	
	2015	-0.2379	0.3056	0.778	(-1.009, 0.099)	0.437	
Days since rain		-4.4699	0.6017	7.422	(-5.650, -3.290)	<0.0001	***

Scat count	-0.3354	0.8222	0.408	(-2.036, 1.305)	0.684
Days since rain x Scat count	-0.4482	0.627	0.714	(-2.114, 0.317)	0.475

Significance codes: *** P<0.001

(b)

Model (incl. term codes)	**df**	**logLik**	**AICc**	**delta**	**weight**
Year + Days since rain + Scat count	5	-213.65	437.36	0.23	0.25
Days since rain + Scat count + Days since rain x Scat count	5	-213.75	437.56	0.43	0.22
Days since rain + Scat count	4	-215.01	438.06	0.93	0.17
Days since rain	3	-216.77	439.56	2.43	0.08

Table 4 Model averaged outputs from a General Linear Model (GLM) with Gaussian distribution investigating latrine visitation rates (visits per day). Outputs are from all models whose cumulative AIC weights were > 0.95 showing: a) Effect sizes and confidence intervals and b) AICc model weights for all models in the model set. Terms included in the model set were: Season (Wet=Nov-Mar, Dry=Apr-Oct), Home range zone (Core/Territory= within 50% KUD, Overlap= beyond 50% KUD and within 95% KUD). Dry season and home-range overlap were the reference categories. N=51 latrines

(a)

Term name		**Estimate**	**SE**	**z**	**CI (2.5-97.5%)**	**P**	
(Intercept)		0.42086	0.0452	9.12		<0.0001	***
Season	Dry		0	0		0	
	Wet	-0.2324	0.0547	4.142	(-0.342, -0.122)	0.0001	***

Home-range zone	Overlap		0	0		0
	Core/territory	0.02061	0.0431	0.471	(-0.053, 0.169)	0.637

Significance codes: *** P<0.001

(b)

Model **(incl. term codes)**	**df**	**AICc**	**delta**	**weight**
Season (Dry)	3	-19.75	0	0.65
Home-range zone (Overlap) + Season (Dry)	4	-18.55	1.2	0.35

Table 5 Model averaged outputs from a General Linear Model (GLM) with Gaussian distribution investigating latrine growth rates (average daily increase or decrease in scats over the monitoring period). Outputs are from all models whose cumulative AIC weights were > 0.95 showing: a) Effect sizes and confidence intervals and b) AICc model weights for all models in the model set. Terms included in the model set were: Season (Wet=Nov-Mar, Dry=Apr-Oct), Home range zone (Core/Territory= within 50% KUD, Overlap= beyond 50% KUD and within 95% KUD), and Visitation (hyena visits/day). Dry season and home-range overlap were the reference categories. N=51 latrines

(a)

Term name		**Estimate**	**SE**	**z**	**CI (2.5-97.5%)**	**P**	
(Intercept)		-0.0047	0.0859	0.054	(-0.177, 0.167)	0.95729	
Season	Dry	0	0	0	0		
	Wet	-0.233	0.0818	2.785	(-0.398, -0.069)	0.00535	**
		0.359	0.175	2.008	(0.069, 0.694)	0.0446	*

VisitationRate						
Home-range zone	Overlap	0	0	0	0	
	Territory	0.0243	0.0541	0.44	(-0.085, 0.201)	0.65982
Season x VisitationRate	Dry	0	0	0	0	
	Wet	0.0136	0.162	0.082	(-0.654, 0.791)	0.93493
Home-range zone x Season	Territory/Wet	-0.0069	0.0405	0.167	(-0.309, 0.151)	0.86713
Home-range zone x VisitationRate	Overlap	0	0	0	0	

Territory	0.00406	0.0636	0.062	(-0.450, 0.596)	0.95038

Significance codes: ** P<0.01; * P<0.05

(b)

Model (incl. term codes)	**df**	**AICc**	**delta**	**weight**
Season + VisitationRate	4	-17.95	0	0.42
Home-range zone + Season + VisitationRate	5	-16.4	1.55	0.19
Season + VisitationRate + Season x VisitationRate	5	-15.5	2.45	0.12
Home-range zone + Season + VisitationRate + Home-range zone x Season	6	-14.33	3.62	0.07
Home-range zone + Season + VisitationRate + Zone x VisitationRate	6	-13.9	4.05	0.06

Home-range zone + Season + VisitationRate + Season x VisitationRate	6	-13.87	4.08	0.06
Season^2	3	-12.93	5.02	0.03
Home-range zone + Season	4	-12.22	5.73	0.02
Home-range zone + Season + VisitationRate + Home-range zone x Season + Season x VisitationRate	7	-11.71	6.24	0.02

Figure Legends

Fig. 1 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 are marked ‘NA’

Fig. 2 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)

Fig. 3 Violin plot showing median visitation by hyenas to 50 latrines in each season between 2013-2015. Box boundaries show the upper and lower quartiles, whiskers represent the minimum and maximum values, and dots represent raw data points