



Ritualised Dung Kicking by White Rhino Males Amplifies Olfactory Signals but Reduces Odour Duration

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Abstract

Many mammals enhance their olfactory signals visually by depositing them in conspicuous locations such as well-travelled paths. It is also possible to enhance the odour itself through behaviours aimed at modifying odour emission rates. White rhinos defecate in communal middens. While defecating, territorial males kick sharply with their back feet which disperses their dung. Despite being a ubiquitous trait of territorial male white rhinos, the reason behind this behaviour is unclear. We hypothesised that the purpose of dung kicking was for olfactory signal amplification (OSA) in terms of an increased emission of volatile compounds (i.e. increased signal strength). Using dung collected from non-territorial adult males (because it is not possible to collect whole dung from territorial males), we show that the dispersal of male white rhino dung causes OSA by increasing the emission of hydrocarbon acids. The dung odour of territorial and non-territorial males differs only quantitatively, hence it is likely that the same emission patterns occur for territorial male dung odours following dung dispersal. The volatile compound indicating age of intact dung was toluene, but for dispersed dung it was acetophenone (similar to territorial male dung). Despite the benefits of OSA, dung dispersal carried a cost of decreased odour longevity. Thus, signal detectability is temporally reduced. However, territorial males likely counteract this by defecating in middens both before and during peak visitation times by other individuals (15:00–23:00). As a result, we suggest that dung kicking by territorial males amplifies signal strength, such that their dung odours are the most prominent and easily detectable by individuals visiting the middens. This would then better signal territorial ownership to both potential rivals and potential mates.

Keywords *Ceratotherium simum* · Dung dispersal · Odour amplification · Territoriality · Chemical communication

Introduction

Olfactory signals communicate a wide range of information including territory ownership, reproductive state, and group

membership (Archunan and Rajagopal 2013; Barja et al. 2005; Theis et al. 2012). Information within these signals is transmitted via volatile organic compounds (VOCs) emitted from urine, faeces, and/or specialised glandular secretions (Archunan and Rajagopal 2013; Cross et al. 2014; Karthikeyan et al. 2013). As VOCs disperse from their source, their concentration decreases with increased distance. Therefore, the active space of an olfactory signal is the area around an odour source where the VOCs are at sufficient concentration to produce a behavioural reaction from a receiver (Elkinton and Cardé 1984). As the dispersal of olfactory signals is influenced by environmental factors, which can be seasonally driven, such as heat and wind (Alberts 1992; Bossert 1968; Nimmermark and Gustafsson 2005), a key challenge for animals using olfactory communication is to increase the likelihood that individuals will detect these signals (Gosling and Roberts 2001). One way this can be achieved is through visual enhancement. For example, individuals can increase detectability by depositing scent marks in heavily utilised areas. Wolves (*Canis lupus*) do this by leaving faecal marks on regularly used paths

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and crossroads (Barja et al. 2004). In addition, the specific location of scent marks can also provide key information. Elevated scent marks provide visual enhancement, but this also provides information on the depositor's body size and thus competitive ability (Alberts 1992; Sharpe 2015; White et al. 2002). Further, placing scent marks at elevated locations may also effect odour dispersal by increasing the active space (Alberts 1992; Gorman and Mills 1984). For example, black backed jackals (*Canis mesomelas*) defecate on top of rocks and large herbivore dung piles (Hayward and Hayward 2010) which increases the height, and therefore the active space, of their faecal marks. Consequently, strategic placement of scent marks can offer olfactory signal amplification (OSA) as well as visual enhancement.

Increasing the duration of an odour is another form of OSA. Lipids found in the urine marks of lions (*Panthera leo*) and tigers (*Panthera tigris*) slow down the release of VOCs and therefore increase longevity of the odour (Andersen and Vulpius 1999; Asa 1993; Burger et al. 2008). However, many species do not possess physiological mechanisms that regulate odour release, and thus rely on adjusting their behaviour to increase signal detection (e.g. strategic placement of scent marks). Although behavioural OSA has been suggested, studies investigating the effect of specific behaviours on odour release are limited. Moreover, herbivores in particular remain understudied in this regard. For instance, beira (*Dorcotragus megalotis*) (Giotto et al. 2008), black wildebeests (*Connochaetes gnou*) (Skinner and Chimimba 2005), dik-diks (*Madoqua kirkii*) (Tinley 1969), oribi (*Ourebia ourebi*) (Monfort and Monfort 1974), sable (*Hippotragus niger*) (Estes 1991), springboks (*Antidorcas marsupialis*) (Estes 1991), steenboks (*Raphicerus campestris*) (Walther 1990), suni (*Neotragus moschatus*) (Somers et al. 1990), and Thompsons gazelles (*Eudorcas thomsonii*) (Estes 1991) all perform ground scraping as part of their defecation sequence, but the reason for such behaviour is largely unknown.

White rhino (*Ceratotherium simum*) olfactory signals are concentrated in communal middens (also called dung piles or latrines) by individuals of all ages and sex (Owen-Smith 1973), where age, sex, territorial and oestrous state are identifiable via dung odours (Marneweck et al. 2017a). These middens are located at strategic locations including territory boundaries, well-travelled rhino paths, and next to water sources (Owen-Smith 1973), which increases encounter probability. The primary function of middens is for territorial males to advertise territory ownership (male-male communication) and find reproductive females (female-male communication) (Marneweck et al. 2018). However, secondary functions likely include non-territorial males locating reproductive females for sneaky copulations, females to assess male quality (male-female communication), and potential challengers to assess both the territorial male and territory quality (Marneweck et al. 2018).

At a fine scale, individual white rhinos defecate in specific locations within a midden. Territorial males defecate in the centre, while non-territorial males, adult females, and younger animals primarily defecate around the edge (Owen-Smith 1973; Marneweck et al. 2018). This spatial distribution of dung may increase the detectability of the signals from these different individuals (Marneweck et al. 2018). Yet, within these middens, territorial males are the only individuals to scatter their dung, which they do with backward kicking motions of their hind feet (Owen-Smith 1971) (Supplementary video). To date, it is unclear why they do this. As one of the main functions of middens is communication from territorial males (i.e. male-male and male-female communication; Marneweck et al. (2018)), it is possible that these males kick their dung to enhance their olfactory signals (i.e. OSA). To explore this, we tested whether the increase in dung surface area caused by kicking resulted in an increase in VOC emission, and thus facilitated OSA. However, odour amplification could lead to a decrease in odour longevity. Plants produce VOCs (Lerdau et al. 1997), so their VOC emission is limitless. However, odour sources such as scent marks do not produce VOCs, rather, they emit them as the result of the organic matter and bacterial breakdown (Archie and Theis 2011). This results in VOC emission being finite. Therefore, if an individual were to increase the emission of their scent mark via OSA, the overall longevity would likely be reduced. If this is the case, then dung kicking by territorial males could constitute a strategy of increasing VOC emission at the expense of odour duration. To counteract the reduced longevity, territorial males could adjust their behaviour such that they deposit dung at optimal times to ensure detection before odour depletion (i.e. during times of high midden use by other individuals). To investigate this, we tested the following hypotheses: (1) the dispersal of male dung will cause an increase in VOC emission, (2) the increase in VOC emission will ultimately decrease odour longevity, and (3) territorial males will defecate during periods of high visitation by other individuals to increase olfactory signal detectability.

Methods and Materials

Collection of Dung Odours We conducted this study between May and August 2014 (i.e. dry season) in the 896 km² Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. Here, we collected a fresh (<5 min) dung sample (one dung bolus) from a total of 19 different non-territorial (subordinate) adult male white rhinos. We identified individuals by variations in horn shape, skin folds, and other distinguishing characteristics, with adults defined as individuals >7 years of age (Hillman-Smith et al. 1986). There are no physical differences between territorial and non-territorial males (Owen-Smith 1971, 1973) and as such, we identified territorial status from

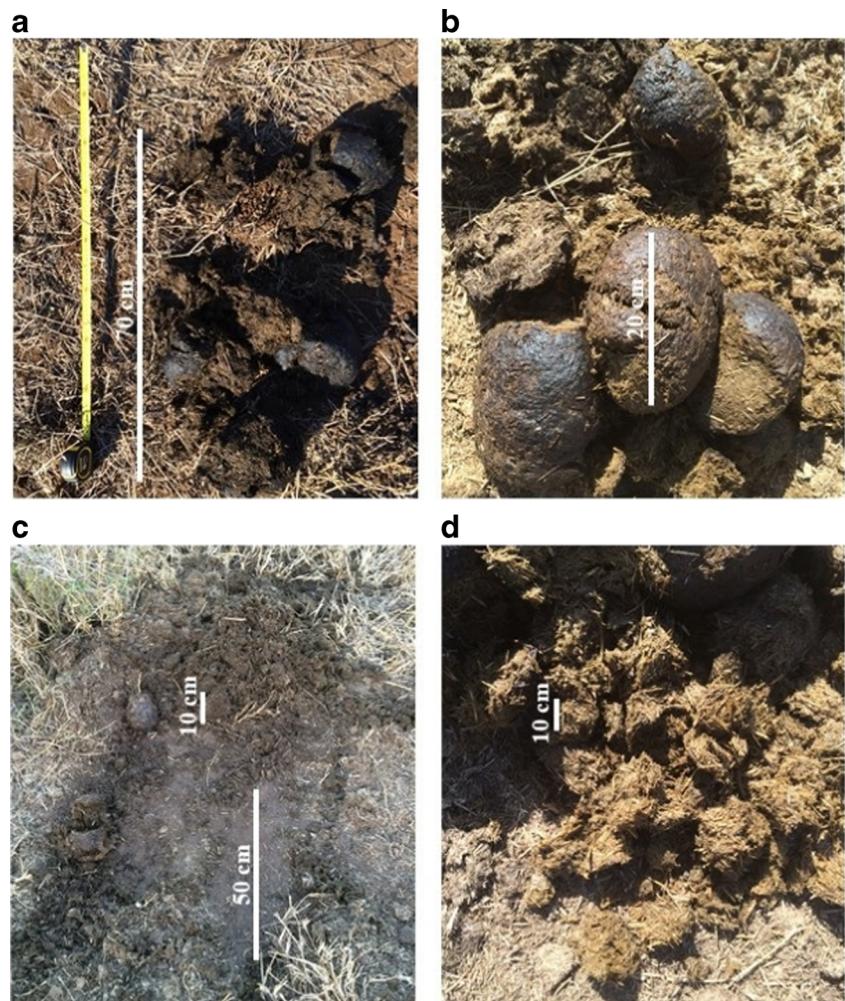
behaviour. We defined territorial males as adult males performing territorial-specific behaviours of dung kicking and spray urination, and non-territorial males as adult males not performing these territorial-specific behaviours (Owen-Smith 1971, 1973).

We used dung from non-territorial adult males as a surrogate for territorial males, as it was not possible to obtain intact (i.e. whole and un-kicked) dung boluses from territorial males (see Fig. 1). This is because territorial males kick before, during, and after defecating, which breaks up the boluses (Owen-Smith 1973). We felt confident in using non-territorial dung as a surrogate because the VOCs emitted from territorial and non-territorial male dung differ only quantitatively, with territorial males emitting higher proportions of hydrocarbon acids (Marneweck et al. 2017a). This is likely due to higher concentrations of faecal testosterone found in territorial male dung (Rachlow and Berkeley 1998). Specifically, the proportion of acids emitted is 69% higher in territorial dung compared to what is emitted from non-territorial dung (Marneweck et al. unpublished data). Therefore, we expected the same pattern of emission in territorial dung odours, just on a larger scale.

Specifically, we would expect the dispersal of dung to cause a larger increase in acid emission from territorial dung compared to that emitted from dispersed non-territorial dung. Moreover, the classification algorithm used in Marneweck et al. (2017a) had fairly low success in identifying the territorial state of males from dung odours, highlighting the similarities. Consequently, we are confident that non-territorial male dung provides a suitable surrogate with which to explore OSA patterns of territorial male dung.

To collect dung samples, we followed individual white rhinos on foot until they defecated. To ensure that the rhinos were unaware of our presence, and thus not disturb natural behaviour, we remained downwind and followed individuals at a distance of ~50 m. Moreover, once a rhino had defecated, we did not approach the midden until the individual had moved >50 m away. After they had defecated, we removed a fresh bolus from the midden and collected an odour sample using headspace extraction (Amirav and Dagan 1997). We collected an odour sample by collecting the air for 25 min from dung enclosed in a polyacetate bag using a micro-air sampler (Supelco PAS-500) with a realized flow rate of

Fig. 1 Photos **a** and **b** represent a non-territorial male dung pile, **c** a territorial male dung pile with noted scrape marks from kicking, and **d** an artificially dispersed non-territorial male dung pile



150 ml/min. VOCs emitted from the dung were captured in a small thermodesorption trap filled with 1 mg of Tenax® and 1 mg of Carbotrap®. We collected an initial odour sample (<5 min after defecation, representing dung at 0 h) in the field and then removed the bolus from the midden to ensure that the odour sample was restricted to the specific individual. We then relocated the bolus to the research camp where we collected subsequent odour samples at 6, 12, 24, and 48 h from time of defecation. We chose these times based on Marneweck et al. (2017a, b), where changes occur rapidly during the first 12 h and persist for approximately 48 h. In between sampling intervals, we left the dung outside on natural substrate (short grass), unprotected, to allow natural degradation to occur. We left dung either as one solid bolus or as scattered pieces (see below) during this time. After 48 h, we returned dung to the environment and stored the thermodesorption traps for later laboratory analysis.

To determine if breaking up of the dung bolus resulted in OSA, we first collected odour samples from ten intact boluses. To mimic the kicking action of territorial males, we then broke up the remaining boluses ($n = 9$) into similar sized pieces to what is found in the wild ($3 \times 3 \times 6$ cm – $5 \times 5 \times 10$ cm; Marneweck pers. obs., Fig. 1). We did this by hand, using a polyacetate bag as a glove to prevent any odour contamination. Once the bolus was broken apart, we collected odour samples from the combined separated pieces of the bolus. In total, we collected 92 odour samples; intact boluses $n = 49$ (10 at 0 h, 10 at 6 h, 10 at 12 h, 10 at 24 h, and 9 at 48 h), dispersed boluses $n = 43$ (9 at 0 h, 9 at 6 h, 9 at 12 h, 9 at 24 h, and 7 at 48 h). Some dung samples were lost to the environment (due to removal by birds, dung beetles, and wind) and as a result, some of the samples sizes are lower at 48 h from defecation.

Dung Odour Analysis We carried out gas chromatography-mass spectrometry (GC-MS) analysis of the odour samples on a Bruker 450 GC with a $30 \text{ m} \times 0.25 \text{ mm}$ internal diameter Varian VF-5 ms column, connected to a Varian VF-1 ms column coupled to a Bruker 300 quadrupole mass spectrometer in electron-impact ionization mode at 70 eV. Thermodesorption traps were placed in a Varian 1079 injector equipped with a chromatoprobe thermal desorption device. We identified VOCs using Varian Workstation software with the NIST 2011 mass spectral library (NIST/EPA/NIH Mass Spectral Library, data version: NIST 2011; MS search software version 2.0 d). We verified the identification of VOCs with retention times of authentic standards and published Kovats indices wherever possible (Supplementary material).

Quantification of the VOC emission rates were carried out using injections of standardised volumes of a set of commercially (Sigma-Aldrich) available standards. One calibration compound per VOC functional group was injected as a placeholder for all VOCs in the group. We selected six synthetic

standards (acetic acid, benzaldehyde, limonene, methyl salicylate, (Z)-hex-3-en-1-ol, (E)- β -caryophyllene) that covered a range of chemical structures found in the dung odour samples, e.g. acetic acid was selected as a standard for hydrocarbon acids, limonene was selected as a standard for monoterpenes, and (E)- β -caryophyllene acted as the placeholder for all sesquiterpenes. Each compound was injected three times, following the same methods as for the dung odour samples. The peak areas in the chromatograms were averaged per injected standard, resulting in a conversion factor per VOC functional group. All VOCs in the functional group were subsequently standardised with this conversion factor. Finally, all samples were multiplied by the factor 2.4 to convert the emission rates in the sampling time of 25 min to the emission rate per hour. In the following, all emission rates are scaled to mg per hour.

Collection of Behavioural Data We collected behavioural data between April and September 2015 (i.e. dry season) to correlate with the odour data collection period. We set up motion-triggered video recording camera traps at ten middens, each frequented by a different resident territorial male (identified via differences in horn shape and size). An average territory is 1.65 km^2 (Owen-Smith 1975), therefore, focal middens were separated by at least 2 km to help ensure separation. Further, video recordings confirmed that the middens were utilised by different territorial males. To record behavioural data, we used infrared camera traps (either a Cuddeback Black Flash® E3 or Cuddeback Attack Black Flash® 1194 model). We used these cameras as they do not emit visible light or have a flash, creating minimal disturbance at the midden and therefore allowing us to capture natural behaviour. We placed cameras approximately 3 m from the edge of the midden to allow for sufficient field of view. We programmed the cameras to record 30-s videos at each trigger with a 1-s delay before becoming active again. From this, we created a profile for each adult white rhino so that we could record individual visitation and defecation times.

From the video footage, we recorded data on all adults (identified as individuals >7 years based on body size and horn development; Hillman-Smith et al. (1986), identified territorial males as adult males performing territorial behaviours (i.e. dung kicking, spray urinating), and non-territorial as adult males not performing these behaviours (Owen-Smith 1971, 1973). Although the oestrous state of female white rhinos can be determined by observing the behaviours of territorial males (i.e. following, mounting etc. Owen-Smith (1973)), we were unable to record these behaviours on the cameras and thus unable to identify female oestrous state. As a result, we grouped all adult females together for analysis.

Statistical Analysis As absolute concentration is subject to variability across samples, we used relative abundance of a VOC within a sample (i.e. proportion) for statistical analysis. To

assess if the odours of intact and dispersed dung were different over the 48-h sampling period, we conducted a perMANOVA based on Bray-Curtis similarities of square-root transformed proportions with posthoc pairwise comparisons.

To investigate if time (i.e. hours from defecation) or treatment (i.e. intact or dispersed) had an effect on the contribution of each VOC functional group to the odour, we created five candidate linear mixed-effects models. We set the contribution of each VOC functional group as the response variable, time and treatment, plus interactions between time, treatment, and VOC functional group as explanatory variables. We controlled for individual rhino identity as a random factor in all candidate models. We did not include VOC functional group alone because it is uninformative as each functional group contribution varies considerably (Marneweck et al. 2017b).

Using a random forest classification algorithm, we interpreted the best predictors (i.e. VOCs) of dung age using the metric mean decrease in accuracy (MDA). The MDA is the increase in the percentage of times the outcome is misclassified when the variable is randomised. A higher MDA means less misclassification, and thus greater accuracy, and ultimately indicates higher importance to the classification.

To investigate if time or treatment had an effect on the number of VOCs emitted from non-territorial male dung (a surrogate for VOC emission), we created three candidate linear mixed-effects models. We set the number of VOCs emitted as the response variable, time and treatment, plus their interaction, as explanatory variables, and controlled for individual rhino identity as a random factor.

To investigate if time or treatment had an effect on the absolute emission of VOCs from non-territorial male dung, we created a further three candidate linear mixed-effects models. We set the log of the absolute emission as the response variable, time and treatment, plus their interaction, as explanatory variables, and controlled for individual rhino identity as a random factor.

We recorded the time of each visit and defecation for all adults and categorised them into one of six four-hour time periods (11:00–14:59, 15:00–18:59, 19:00–22:59, 23:00–02:59, 03:00–06:59 or 07:00–10:59). These time periods were based on the active periods recorded by Owen-Smith (1973), where white rhinos are often found resting during the heat of the day. We calculated the number of visits and defecations per individual during each time period as a proportion of their total number of visit and defecations. To assess the factors affecting the time of midden visit or defecation from individuals of different adult state (i.e. territorial male, non-territorial male, and female) we created three candidate linear mixed-effects models. We set the proportion of visits per time period as the response variable, state and time period, plus their interaction, as explanatory variables, and controlled for individual rhino identity as a random factor. We repeated this analysis for the proportion of defecations per time period.

We assessed collinearity between independent explanatory variables prior to analysis using variance inflation factors (VIF) and Spearman rank correlation tests, ensuring that all variables had VIF values below 2 in the final statistical models. To identify the best model(s), we used model selection based on Akaike Information Criterion (AIC_c), where $\Delta AIC_c \leq 2$ indicates most important predictors (Burnham and Anderson 1998). We performed all statistical analyses and created all figures in RStudio v 1.0.136 (Team 2016) for Windows, using functions in the packages nlme (Pinheiro et al. 2015), MuMIn (Bartoń 2013), randomForest (Liaw and Wiener 2012), and vegan (Oksanen et al. 2015).

Results

Odour

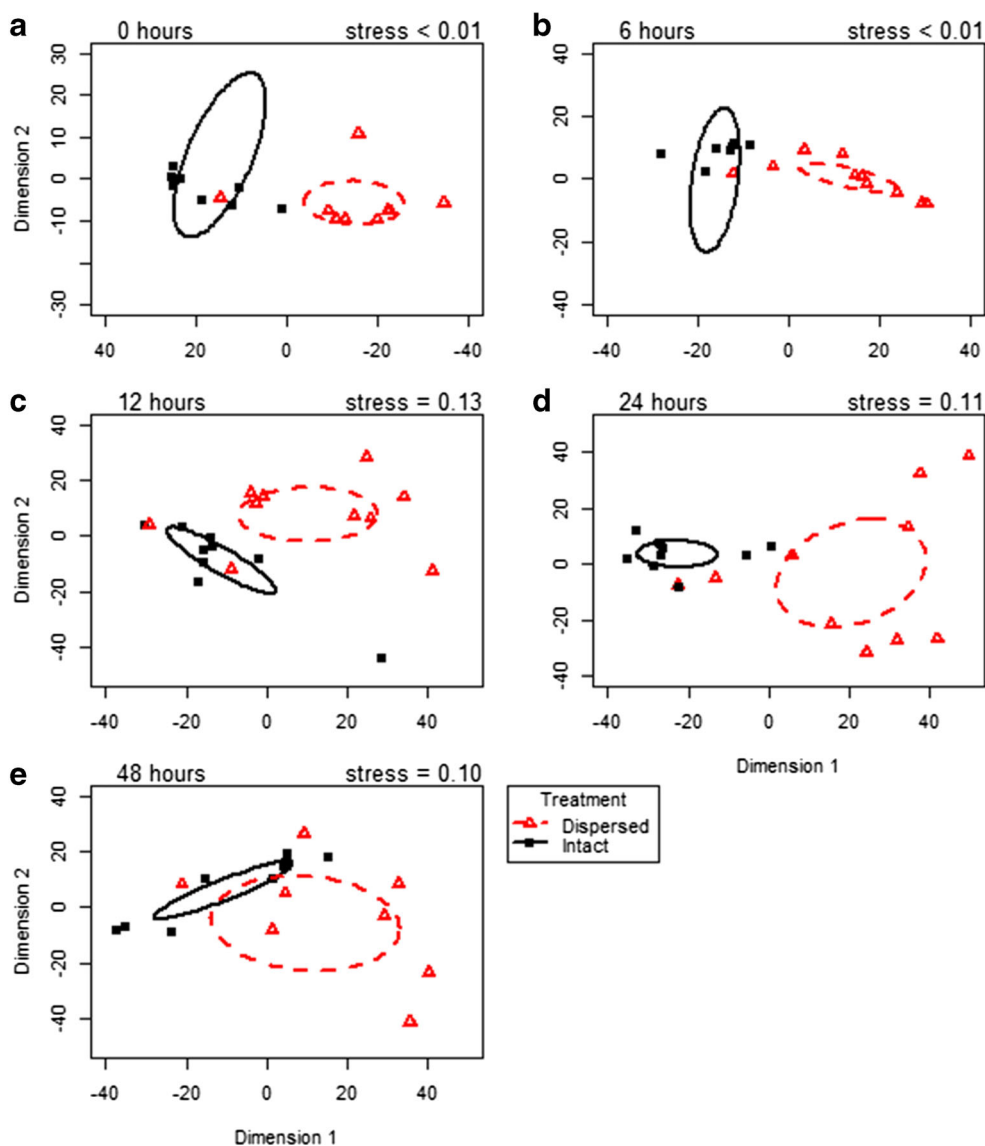
An interaction between time (i.e. hours from defecation) and treatment (i.e. intact or dispersed) had a significant effect on overall dung odour ($F_4 = 2.142$, $P = 0.002$). At 0, 6, 12, and 24 h from defecation, the odour of dispersed dung was significantly different to the odour of intact dung (0 h: $t = 2.1244$, $P = 0.001$, Fig. 2a; 6 h: $t = 1.9496$, $P = 0.001$, Fig. 2b; 12 h: $t = 1.8279$, $P = 0.008$, Fig. 2c; 24 h: $t = 2.4154$, $P = 0.001$; Fig. 2d). However, at 48 h from defecation, the odour of dispersed and intact dung was similar ($t = 1.4941$, $P = 0.059$, Fig. 2e).

An interaction between VOC functional group and treatment was the most important predictor of VOC functional group contribution to dung odour (Table 1), explaining almost all of the variation. Dispersal caused an increase in acids and benzenoids, and a decrease in monoterpenes (Fig. 3). The most important VOCs for indicating the age of intact and dispersed dung were the benzenoids toluene (Fig. 4a) and acetophenone (Fig. 4b), respectively.

An interaction between time and treatment was the most important predictor for the number of VOCs emitted from non-territorial dung (Table 2), explaining almost all of the variation. The dispersal of dung caused an increase in the number of VOCs emitted at 0 and 24 h, had no effect between 6 and 12 h, and caused a decrease in the number of VOCs emitted at 48 h (Fig. 5).

Although the mean absolute VOC emission from non-territorial dung was higher from dispersed dung compared to intact dung directly after defecation (19.8 ± 35.5 and 13.4 ± 20.7 mg/h, respectively; Fig. 6), and lower from dispersed dung for later periods, treatment did not affect absolute emission due to high variation (Table 3; Fig. 6). Time was the most important predictor for the absolute emission of VOCs from non-territorial dung (Table 3), explaining almost all of the variation, where absolute emission decreased over time (Fig. 6).

Fig. 2 Multidimensional scaling (MDS) plot based on Bray-Curtis similarities of the variation of VOCs emitted from male dung, intact and dispersed, at **a** 0 h, **b** 6 h, **c** 12 h, **d** 24 h, and **e** 48 h from defecation. Each point represents one dung sample odour. Encompassing circles represent 95% confidence intervals



Behaviour

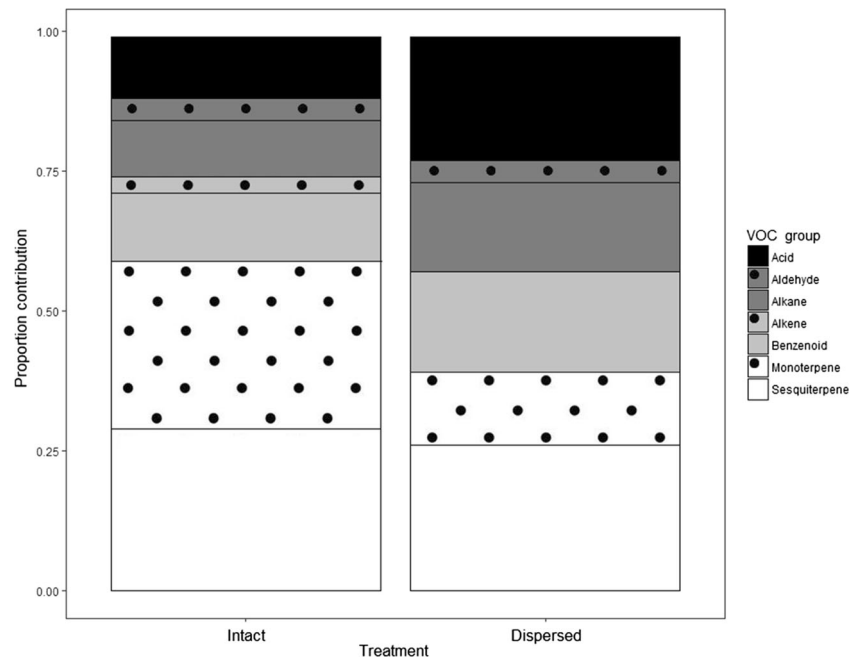
An interaction between adult state (i.e. territorial male, non-territorial male, or female) and time was the most important predictor of both midden visit and defecation (Table 4), explaining almost all of the variation. Females visited middens more often during 15:00–18:59 and less often during

23:00–02:59 compared to both territorial and non-territorial males (Fig. 7a). Territorial males visited more often during 11:00–14:59 and 23:00–02:59 compared to non-territorial males and females (Fig. 7a). When defecating, territorial males defecated more often than non-territorial males and females during 11:00–14:59 and 19:00–22:59.

Table 1 The five candidate models used to investigate factors affecting VOC contribution to dung odour. The top model is indicated in bold, where $\Delta AIC_c \leq 2$

| Rank | Model | df | loglikelihood | AIC _c | ΔAIC_c | w_i |
|----------|--|-----------|-----------------|------------------|----------------|--------------|
| 1 | Group + Treatment + Group × Treatment | 28 | 1282.765 | −2508.624 | 0.000 | 0.999 |
| 2 | Group + Time + Group × Time | 67 | 1281.831 | −2424.462 | 84.162 | <0.001 |
| 3 | Treatment | 4 | 914.045 | −1820.068 | 688.556 | <0.001 |
| 4 | Time | 7 | 903.024 | −1791.987 | 716.637 | <0.001 |
| 5 | Time + Treatment + Time × Treatment | 12 | 887.133 | −1750.093 | 758.531 | <0.001 |

Fig. 3 The composition of odour from intact and dispersed non-territorial male dung



Discussion

Olfactory signal amplification (OSA) increases signal detectability by either increasing the VOC emission (signal strength) or extending VOC emission (signal longevity) (Alberts 1992; Burger et al. 2008; Piñeiro and Barja 2012). When defecating in communal middens, territorial male white rhinos are the only individuals that kick their dung (Owen-Smith 1973). It is possible that by doing this, these males amplify the olfactory signals released from their dung. Using non-territorial male dung as a surrogate, due to its similar VOC profile to territorial male dung coupled with the difficulty in obtaining un-kicked territorial male dung, we found that dispersal of the dung increased VOC emission (signal strength), but reduced signal duration (signal longevity). We found that the mean

absolute emission rate of dispersed dung directly after defecation was higher than that of intact dung. For later time periods we found that dispersed dung had lower absolute emission rates than intact dung. However, these differences were not significant. The interpretation of absolute emission rates, as an indicator for signal intensity, is difficult for two reasons. Firstly, not all VOCs may play a role as an olfactory signal. Secondly, since there are no data on the olfactory sensitivity of white rhinos, we do not know at which threshold levels the animals start, or cease, to respond. A behavioural effect might be linked to a certain combination of compounds that together reach a given threshold level. Furthermore, it is likely that a reduction in emission strength does not affect signal detectability as territorial males defecated in middens before peak visitation times of other individuals.

Fig. 4 The importance of volatile organic compounds (VOCs) for distinguishing the age of dung when **a** intact and **b** dispersed. Importance was based on mean decrease in accuracy (MDA). Only the top ten compounds are presented in the figure

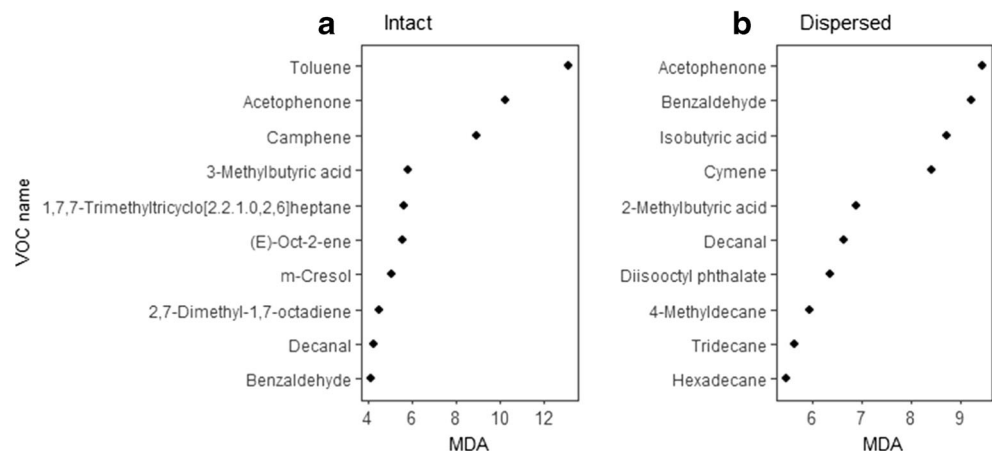


Table 2 The three candidate models used to investigate factors affecting the number of VOCs emitted. The top model is indicated in bold, where $\Delta AICc \leq 2$

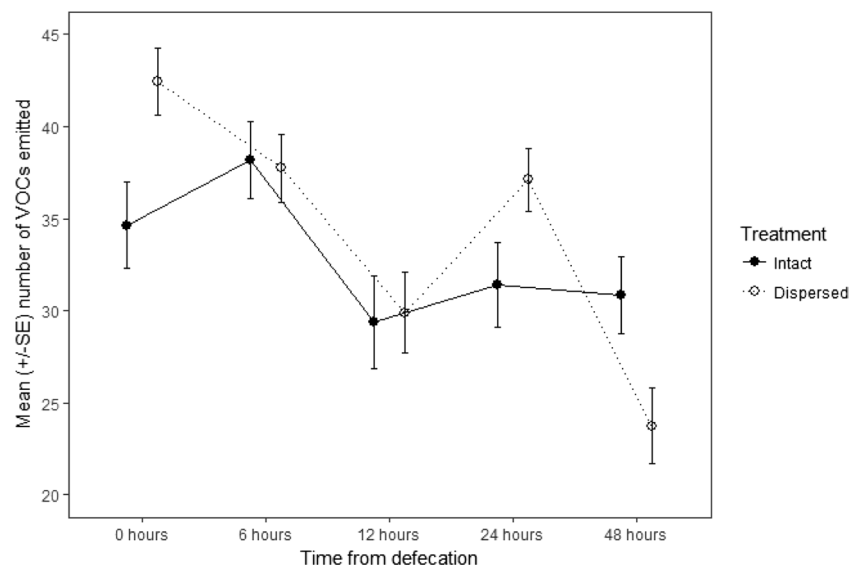
| Rank | Model | df | loglikelihood | AICc | $\Delta AICc$ | w_i |
|----------|--|-----------|-----------------|----------------|---------------|--------------|
| 1 | Time + Treatment + Time \times Treatment | 12 | -293.370 | 614.689 | 0.000 | 0.999 |
| 2 | Time | 7 | -307.315 | 629.962 | 15.274 | <0.001 |
| 3 | Treatment | 4 | -335.337 | 679.133 | 64.445 | <0.001 |

Factors affecting the volatility of a VOC include its molecular weight and vapour pressure (Stoddart 1976). Further, a key factor determining VOC emission is the surface area from which the VOC is emitted (Alberts 1992). In this study, hydrocarbon acids were larger contributors to the odour when the dung was dispersed, compared to when the bolus remained intact. These compounds have low molecular weight and high vapour pressure, making them more susceptible to emission. Moreover, these VOCs are associated with territory ownership in white rhinos, where territorial male dung emits higher proportions of hydrocarbon acids than non-territorial male dung (Marneweck et al. 2017a). With the predicted pattern of 69% larger proportion acid emission for territorial male dung, this suggests that the hydrocarbon acids would be even larger contributors to territorial male odour and potentially dominate the odour after dung dispersal. As these VOCs are important for signalling territorial status, and their increased emission means quicker depletion, we would expect that the odour of territorial signals would be very strong, but the longevity even further reduced as a result of dung kicking. As increased testosterone levels are associated with greater competitive ability (Zielinski and Vandenberg 1993), dispersal of the dung could create a signal of a larger and more competitive male, which has reproductive and intimidation benefits for females and non-territorial males respectively.

Acetophenone is the most important VOC for indicating the age of territorial male dung during the dry season (Marneweck et al. 2017b). Here, we identified a change in the most important VOC predictor for dung age of non-territorial male during the dry season. When intact, toluene is the most important VOC, as described in Marneweck et al. (2017b). However, when dispersed, acetophenone is the most important VOC. Thus, dispersal causes changes leading to odour similar to that from territorial male dung. Toluene is a benzenoid compound, and benzenoids are common VOCs emitted from plants and thus represent undigested plant material in white rhino dung (Osborn and Lanzotti 2009; Marneweck et al. 2017b). Acetophenone is a ketone, a VOC also highlighted as important in beaver (*Castor canadensis*) castoreum scent-marks (Müller-Schwarze and Houlihan 1991). This suggests that the dispersal of dung allows for the release of a VOC important for indicating male territorial status, which is otherwise dominated by plant odours when the bolus is intact.

The dispersal of dung increases the surface area, which consequently increases the area available for the emission of VOCs. The VOCs available for emission are limited as, unlike plants, scent marks do not produce VOCs (Archie and Theis 2011; Lerdau et al. 1997). So, increased odour emission should lead to reduced longevity, which is what we found in our study. In the case of dispersed male white rhino dung, a

Fig. 5 The mean number of VOCs emitted from non-territorial male dung, intact and dispersed, over 48 h after defecation



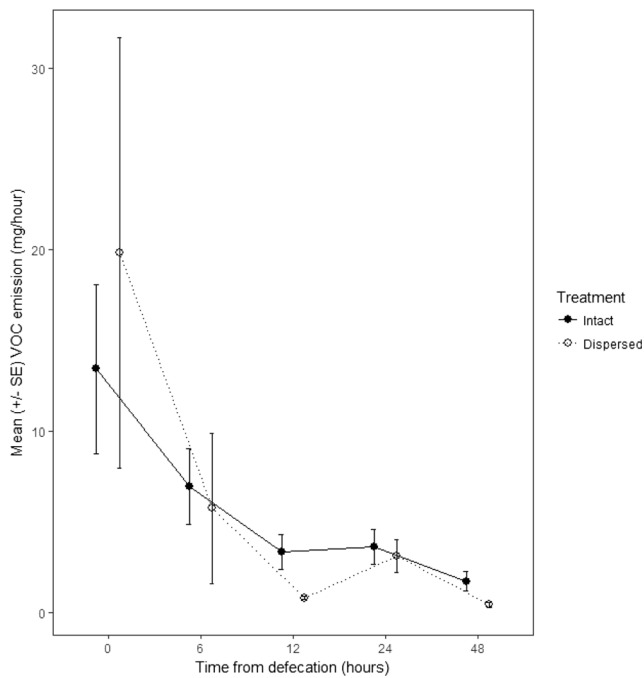


Fig. 6 Mean absolute emission of volatile compounds from non-territorial male white rhino dung both intact and dispersed

greater number of VOCs were emitted earlier, followed by a greater overall depletion over the 48 h, compared to the more stable/less dramatic release of VOCs from intact dung. Therefore, there seems to be a trade-off between the intensity of the signal and its duration. These results suggest that the kicking action associated with scent marking in territorial white rhinos is for OSA, but carries the cost of reduced signal longevity. However, the fact that all territorial males kick their dung suggests that the benefits of OSA outweigh the costs of reduced longevity. Moreover, behavioural adjustments by territorial white rhinos, such as timing when they defecate, may help counteract reduced signal longevity.

White rhino territories tend to be relatively small (on average 1.65 km²; Owen-Smith (1975)), which would suggest that regular re-marking of middens could be achieved over a short period of time. Owen-Smith (1975) supports this by reporting territorial males to defecate in each of their middens on average every second day. If this were the case, then the impact of reduced signal longevity could be minimised, and the probability of individuals visiting the midden and detecting the

olfactory signals increased. However, more recent evidence indicates that territorial males only defecate in the same midden on average once every ten days (Marneweck et al. 2018). Compounded with this, white rhino dung odours only last for a couple of days (Marneweck et al. 2017b). Although signals change over time, they are still recognisable as territorial male odours (i.e. still different to non-territorial odours after 48 h; Marneweck et al. (2017b)). Thus, it would seem that at any one midden within a male’s territory, there are long periods where the territorial male’s signal would be present, but very weak. As dung is a limited resource, the likely reason that territorial males are not increasing the frequency at which they remark middens is that they are already defecating at the maximum frequency permitted by their physiology. Moreover, it seems that males attempt to maximise the spatial distribution of their dung across the >30 middens they can have in their territories (Owen-Smith 1973) by limiting how much they deposit at any one midden (Marneweck et al. 2018).

One way that territorial males could increase the probability of their signals being detected by other rhinos is to defecate strategically, so that the amplified signal strength is highest when the majority of individuals visit the midden. This would increase detectability and counteract the issue of reduced signal duration. As a number of non-resident rhinos move through a male’s territory every day, defecating in any midden prior to peak visitation times would increase the probability of detection by these individuals. Yet, due to the size of its territory and the limitation of dung production, some visiting rhinos are inevitably going to visit middens where the territorial male’s dung odour is weak. However, if key individuals are present in a male’s territory (e.g. oestrous females, rival males) then a territorial male could likely increase the defecation frequency in the middens in that portion of its territory. For example, Owen-Smith (1973) observed a territorial male remain within a small portion of its territory after a rival male had intruded. While remaining in this area, the territorial male increased the frequency with which it defecated in the middens along that territory boundary, such that it defecated in them every two days. Thus, by defecating prior to or during peak visitation times, the probability of detection by these key individuals is likely increased. In line with this, we found that territorial males did in fact defecate both before

Table 3 The three candidate models used to investigate factors affecting the absolute emission rate of VOCs. The top model is indicated in bold, where $\Delta AICc \leq 2$

| Rank | Model | df | loglikelihood | AICc | $\Delta AICc$ | w_i |
|----------|-------------------------------------|----------|-----------------|----------------|---------------|--------------|
| 1 | Time | 4 | -127.115 | 262.528 | 0.000 | 0.999 |
| 2 | Time + Treatment + Time × Treatment | 6 | -131.729 | 276.095 | 13.567 | <0.001 |
| 3 | Treatment | 4 | -137.769 | 283.837 | 21.308 | <0.001 |

Table 4 The six candidate models used to investigate factors affecting the visitation and defecation time of adult white rhinos. The top models are indicated in bold, where $\Delta AICc \leq 2$

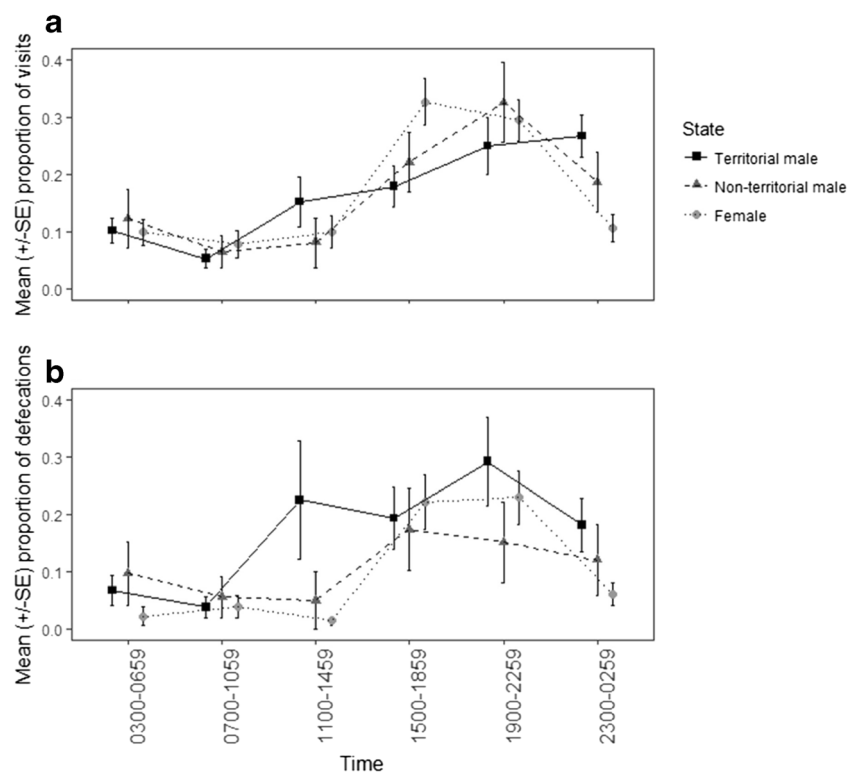
| Rank | Model | df | loglikelihood | AICc | $\Delta AICc$ | w_i |
|------------|--|-----------|------------------|-----------------|---------------|--------------|
| Visitation | | | | | | |
| 1 | State + Time + State \times Time | 20 | -1403.976 | 2849.465 | 0.000 | 0.998 |
| 2 | Time | 8 | -1423.049 | 2862.351 | 12.886 | 0.002 |
| 3 | State | 5 | -1466.128 | 2942.361 | 92.896 | <0.001 |
| Defecation | | | | | | |
| 1 | State + Time + State \times Time | 20 | -1395.547 | 2832.607 | 0.000 | 0.999 |
| 2 | Time | 8 | -1418.394 | 2853.042 | 20.435 | <0.001 |
| 3 | State | 5 | -1434.415 | 2878.936 | 46.328 | <0.001 |

and during times of high visitation from other individuals. This suggests that territorial males cope with the reduced signal duration after their OSA by responding with a strategy to increase detectability.

Many species using dung as a scent marking source also perform over-marking strategies, with regard to both territorial defence and mate guarding (Brachares and Arcese 1999; Jordan et al. 2011; Vogt et al. 2014). With this in mind, it is possible that the function of dung kicking by territorial male white rhinos may be for both OSA and over-marking. However, with other individuals defecating mostly around the edge of the midden, and territorial males defecating exclusively in the centre (Marneweck et al. 2018), we suggest that this is unlikely behaviour for white rhinos.

The results of our study identify the purpose of dung kicking in territorial male white rhinos. Specifically, territorial males use both OSA and strategic placement of signals both temporally (utilising middens at specific times) as well as spatially (placement within a midden, Marneweck et al. (2018)). Ultimately, by kicking dung, males increase detection of their odours by other individuals visiting the middens by increasing signal strength. Moreover, these signals may also provide distinctive and unambiguous signals to both intimidate males (potential rivals) and show competitive ability to females (potential mates). Finally, our results provide insight into the possible function of scraping behaviours observed from other communally defecating ungulates, such as sable and suni.

Fig. 7 Mean proportion of midden **a** visits and **b** defecations by adults per time period



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