Olfactory enrichment and visitor effects in lack rhinoceroses (*Diceros bicornis*) at two UK zoos

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Abstract

Black rhinoceroses are highly endangered in the wild and a successful captive breeding programme is therefore vitally important. However, the captive population is not self-sustaining and little is known about the effects of the zoo environment on rhinoceros welfare and breeding. Environmental enrichment is known to be a useful tool in relieving some of the constraints of captivity and it can improve reproductive success. Studies on visitor effects have shown that some zoo species, including black rhinoceroses, may experience visitors as stressful. The effect of visitor numbers and olfactory enrichment on black rhinoceros activity, investigation and communication behaviours were studied at Chester Zoo and Port Lympne Wild Animal Park. Two enrichment conditions were tested by adding dung piles from a conspecific and from another ungulate species to the enclosure. Ten rhinoceroses were observed over two days for baseline data, one day for each enrichment condition and one day post enrichments. Visitor numbers along the perimeter of the enclosure were recorded. No significant differences were found between baseline and enrichment conditions, or post-enrichment days in the main behavioural categories. However, there was a significant difference between frequency of faeces investigation on days with conspecific dung enrichment and baseline days. There was also a significant positive correlation between visitor numbers and pacing. The enrichment was ineffective in reducing stress related behaviours or increasing reproductive related behaviours, however, there are patterns in the data that require further studies. Pacing increased with rising visitor numbers. If pacing behaviour is an indicator of stress in black rhinoceroses, their exposure to visitors should be reduced in order to improve welfare and breeding.

Introduction

The black rhinoceros (*Diceros bicornis*) has undergone one of the most rapid population declines known for a large mammal, falling by 97% from 1970 to1995 (Foose 1996). This degeneration is largely due to the destruction of habitats and poaching of rhinoceros horn, and today there are only approximately 3,600 left in the wild, and approximately 200 in captivity (Foose 1996; Anon 2004). Due to their endangered status it is important that we are able to conserve and breed them in captivity. However, the captive population is not stable or self-sustaining (Carlstead *et al.* 1999a). The environmental constraints of captivity seem to be causing problems for breeding, as one third of the captive population has not reproduced;

there is also a delayed age of first reproduction, long inter-birth intervals and similar numbers of birth and deaths (Smith and Read 1992 cited in Carlstead *et al.* 1999b). Captive rhinoceroses also seem to face problems with unusual diseases not seen in the wild, reducing the chance of population growth (Carlstead *et al.* 1999b). Little is known about what is causing these adverse affects in zoos and further work is urgently needed to improve rhinoceros welfare and captive breeding programs.

Environmental enrichment

In the last 20 years there is increasing concern for the welfare of animals in zoos (Shepherdson 1999). Undesired behaviours such as stereotypies and apathetic states are sometimes generated by the zoo environment, as well as a lack of opportunity to forage, which usually take up most of an animal's day in the wild. Abnormal behaviours are not necessarily evidence that an animal is suffering (Mason 1991), however, they are generally taken as an indicator that an environment is sub-optimal for species-specific needs (Carlstead 1996). Studies have shown that environmental enrichment can reduce stereotypies and promote natural behaviour (Carlstead 1996; Carlstead and Seidensticker 1991; Grindod and Cleaver 2001). Enrichment can also improve reproductive success by providing an environment necessary for developing normal behaviour, reducing stress, and improving social stability. Enrichment is therefore important for captive breeding programmes of endangered species (Carlstead and Shepherdson 1994; Dahl 1982; Frisch 1987 cited in Shepherdson 1999). Captive animals do not have the same level of control over their environment as their wild counterparts and enrichment is a way to facilitate some control for the animal, which in turn can reduce stress (Carlstead 1996; Shepherdson 1998). Another benefit of environmental enrichment for animals concerns the perception of zoo visitors. Today, the public have higher expectations from zoos, and inactive or stereotyping animals give the impression that the animals are "bored" or "unhappy". Therefore, the greater the diversity of natural behaviour the more attractive and educational the visitors experience the zoo (Carlstead 1996; Shepherdson 1999).

The literature on ungulate enrichment is very restricted despite enrichment programmes for these species at most zoos. Black rhinoceroses are mostly solitary animals (Estes 1991) and adults are often kept individually to avoid aggression. Unpublished small-scale zoo studies provide increasing evidence that the lack of stimulation and reduced foraging times in captive Black rhinoceroses can lead to extreme inactivity or stereotypies such as pacing. In a study by Carlstead *et al.* (1999a) scores for stereotypic and other stress related behaviours were correlated with poor reproductive performance in female black rhinoceroses.

Rhinoceros olfactory communication

Black rhinoceroses have a well developed sense of smell (Massicot 2002). In the wild, both females and males use urination and defecation as a way of communication; they are

believed to be able to identify conspecifics through scent. Around the boundaries of their territory they leave dung in communal heaps known as middens. The rhinoceroses scrape the dung with their hind legs to make it more conspicuous and to pick up the scent on their feet in order to mark a trail (Carlstead *et al.* 1999b).

The American Zoo and Aquarium Association (AZA) suggest that presenting zoo rhinoceroses with dung of an unfamiliar conspecific can increase territory-marking behaviour and facilitate general stimulation (Fouraker and Wagener 1999). To my knowledge, no published scientific investigations verify this enrichment, although other olfactory studies have included using spices and other food scents, which have successfully increased activity (e.g. Hadley 2000).

Visitor effects

Visitors are an inevitable part of the daily lives of zoo animals and, as most aspects of the environment, can influence their behaviour (Thompson 1989). The majority of visitor effect studies have been conducted on primates and some concluded that visitors have a stressful effect on the animals, which has direct implications for animal welfare (review: Hosey 2000). It is uncertain, however, for the majority of non-primate species, whether visitors have any effect at all (Hosey 2000).

Male rhinoceroses defend territories and do not tolerate unfamiliar males within this area. Females also hold ranges but are more tolerant of others (Carlstead *et al.* 1999b). Carlstead *et al.* (1999b) thus suggests that males will "perceive the public as intrusive or in some way feel constrained in their movements or marking behaviour". They found a positive association between black rhinoceros mortality, percentage of public access, and fear scores, suggesting that high visitor numbers are stressful for black rhinoceroses. Experiments on primates and ungulates (other than rhinos) have shown that visitors affect vigilance behaviour and social spacing, locomotion, affiliative behaviour and/or social aggression (Thompson 1989; Carlstead *et al.* 1999b).

Study Aim

The aim of this study was to determine if olfactory enrichment in form of ungulate dung affects rhinoceros behaviour with emphasis on physical and psychological welfare and reproduction. Indicators chosen for improved welfare and reproductive activities were: an increase in general activity, a reduction in the time spent in stereotypic pacing, an increase in species typical sexual and territorial behaviours, and an increase in investigative behaviours. We also investigated whether visitor numbers affect rhinoceros behaviour indicating welfare.

Methods

Subjects

The subjects were four male and two female adult black rhinoceroses at Port Lympne Wild Animal Park and two females and two males at Chester Zoo. The rhinoceroses were aged from 11 to 32 years. All animals were individually housed except for the two females from Port Lympne, which both had a dependent calf with them.

The olfactory enrichment programme

The two types of olfactory enrichment used were dung from a male rhinoceros ("Rhino") and dung from a different ungulate species' ("Ungulate"). At Chester Zoo dung was taken from Persian Onagers (*Equus hemionus onager*) and at Port Lympne from Przewalski's horses (*Equus przewalskii*). In order to retain novelty, the rhinoceros dung was selected from a male that the subjects could not smell from their location. Fresh dung was collected immediately before being placed within the outside enclosure. Half a bucket of dung was placed in the enclosure of each rhinoceros at approximately 11:00 on enrichment days, and removed 24hrs later. The dung was placed away from any existing middens within the enclosure. Order of enrichment was alternated to account for any carry-over effects.

Data Collection

The study was conducted between 12th May and 24th July 2003. Baseline data was collected over two days followed by one day of "Rhino" enrichment, one-day post enrichment ("Post-Rhino"), one day with "Ungulate" enrichment, followed by a post enrichment day ("Post-Ungulate"). Each rhinoceros was observed for five 1hr periods per day between 08:00 and 17:00, randomly placed within four sessions: 08:00-10:00, 10:00-13:00, 13:00-15:00, and 15:00-17:00. Each rhinoceros was observed for an equal number of hours within each session. This gave a total of 102hrs observation for baseline recordings, 50hrs observation for "Rhino" enrichment, 48hrs observation for "Post-Rhino" enrichment, 46hrs observation for "Ungulate" enrichment, and 45hrs observation for "Post-Ungulate" enrichment. The ethogram was taken from a previous unpublished study at Chester Zoo and adapted forming broad categories for *Active*, *Inactive, Pacing*, *Sexual/Territorial*, and *Investigative* behaviours (Table 1). Instantaneous sampling was used to record *Active, Inactive* and *Pacing* at 5min intervals. All occurrence sampling was used to record frequency of *Sexual/Territorial*, and *Investigative* behaviours throughout each 1hr session.

Table 1 **Ethogram of recorded behaviours.**

Behaviour	Description
Active:	
Walking	Locomotion in a direct line from 'A' to 'B', with a purpose.
Exploring	Locomotion in no particular direction.
Trotting	Moving quickly usually picking feet up and with tail lifted. Normally occurs

	after being startled.
Running/Charging	Locomotion faster than trotting, usually with aggressive behaviour, head lowered.
Wallowing	Lies down and rolls on the ground, usually within mud wallow.
Standing active	Stationary, on feet but performing another *Investigative*, *Sexual/Territorial* or *Other* behaviour.
Lying active	Lying with body on ground, head up and eyes open, and performing any *Other* behaviour.
Inactive:	
Resting	Lying with body and head on ground, eyes usually closed.
Lying passive	Lying with body on ground, head up but not performing any *Other* behaviour.
Standing passive	Stationary on feet and not displaying any *Other* behaviour.
Pacing:	Walking repetitively and invariantly between two places without obvious goal or function, often in a figure of eight.
Sexual/Territorial:	
Hind Leg Scrape	Rapidly alternates hind feet against ground whilst remaining stationary: often associated with elimination (before and/or after urination/defecation).
Spray	Urine is projected in a strong spray or in distinct squirts.
Flehmen	Rhinoceros raises head and curls underside of upper lip upwards, often after smelling or licking urine.
	Elimination of faeces onto an existing pile of faeces
Midden Building	Elimination of faeces onto the added pile of faeces.
own midden	Elimination of faeces, not on top of a midden.
Enrichment midden	Release of urine in a stream.
Defecating	
Urinating	
Investigative behaviours:	
Lick Urine	Licking or wiping top lip in a pool of urine or area that has previously been sprayed with urine, often followed by *flehmen*.
Smell Urine	Smelling pool of urine or area that has previously been sprayed with urine, often followed by *flehmen*.
Faeces Investigation	
-own faeces	Sniffing existing faeces pile.
-enrichment faeces	Sniffing the added pile of faeces.
	Sniffing anything other than faeces or urine, including the air.

Smelling	Licking area of ground or wall (unknown as to whether this area has been
Lick object	urine sprayed).
	Spending time near to, looking directly at or olfactory investigation of
Interest rhino	neighbouring rhinoceros.
Other:	Any other behaviour not listed above.

The visitors standing along the surrounding barrier of the enclosure where the animals were situated were counted after each 5min instantaneous behaviour sampling point.

Statistical analysis

The percentage of time spent in *Active*, *Inactive* and *Pacing* behaviours was calculated for each day. For all other behaviours, the frequency per hour was calculated for each day and individual. Non-parametric tests were used due to small sample sizes. The medians of the two baseline days were compared with the single treatment days and post-treatment days. Any significant differences between baseline days, enrichment days and post-enrichment days were detected by the Friedman two-way non-parametric analysis of variance test. Significant differences were analysed post-hoc using the Wilcoxon signed rank test. Comparisons between sexes and zoos were carried out descriptively only due to the small sample size of females and number of rhinos studied at Chester Zoo.

Only the baseline data was used for investigating visitor effects. Visitor numbers were added and adjusted for sample number to calculate a daily total and compared with the percentage of intervals spent with *Active*, *Inactive* or *Pacing* behaviours and the frequency of *Sexual/Territorial* and *Investigative behaviours*. The Spearman's rank correlation coefficient was used to detect any relationship between visitor numbers and behaviours.

Results

Activity and Pacing

The median number of intervals spent with *Active* and *Inactive* behaviours for the baseline days was 39.0% and 53.9% respectively. These levels remained fairly constant for the "Rhino" day (38.4% *Active*, 55.2% *Inactive*) and "Post-Rhino" day (35.9% *Active*, 51.5% *Inactive*). The difference between the median percentage of intervals spent in *Active* and *Inactive* behaviours lessened on the "Ungulate" day (44.7% *Active*, 49.6% *Inactive*) and "Post-Ungulate" day (46.0% *Active*, 48.3% *Inactive*). None of the differences for *Active* or *Inactive* behaviours were significant.

Trotting, Running/Charging, Wallowing, *Lying* and *Other* behaviours all occurred, but were infrequent or brief, so they were rarely recorded on the intervals. All of the behaviours within the *Active/Inactive* categories were tested for differences but none were found to be significant.

Three of the rhinoceroses did not perform pacing behaviour (Figure 1). However, there is great variation in the amount of pacing between individuals. For example, the median percentage of intervals spent pacing for the baseline days is 2.5% with one rhinoceros pacing for 30.9% of the time on baseline days (Figure 1). The median time spent pacing decreased on the "Rhino" day (0.7%) and "Post-Rhino" day (0.8%) and was 0% for both the "Ungulate" day and the "Post-Ungulate" day. However, none of these differences were significant.

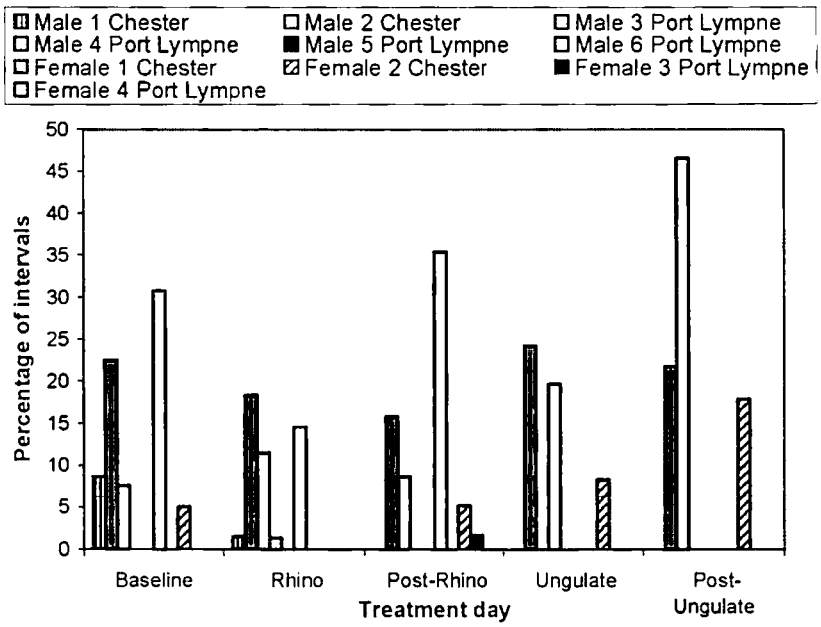

Figure 1 **Pacing in individual rhinoceroses.**

Investigative Behaviour

Frequency of *Investigative* behaviours was similar for most days (22.1/hr baseline, 23.0/hr "Rhino", 23.3/hr "Ungulate" and 24.1/hr "Post-Ungulate"). However, the median increased on the "Post-Rhino" day to 36.0/hr, mainly due to an increase in *Smelling*. The differences were not significant.

Only one of the behaviours within *Investigative* behaviours showed any significant differences across the treatment days, this was *Faeces Investigation* ($S=17.91$, $df=4$, $P<0.01$) (Figure 2). Post-hoc analysis showed that the difference lies between "Rhino" (5.5/hr) and baseline days (0.9/hr, $P<0.05$). There was also a trend of increased *Faeces Investigation* on the "Ungulate" day. Subsequent analysis of *Investigating their own Faeces* category, shows no significant differences, and therefore the significant increase in *Faeces investigation* is due to smelling the added dung. There was also a difference in *Faeces Investigation* between "Rhino" (4.9/hr) and "Ungulate" (2.6/hr, $P<0.05$) treatments.

Sexual/Territorial Behaviour

Sexual/Territorial behaviour did not change between baseline days (3.1/hr) and the "Rhino" day (3.3/hr), however it decreased slightly on the other three treatment days (2.3/hr "Post-Rhino", 2.5/hr "Ungulate", 2.4/hr Post-Ungulate"). These differences were not significant.

Individual behaviours within the *Sexual/Territorial* category did not show significant differences. None of the rhinoceroses were observed defecating onto the added middens, and defecating on any area other than their own middens was extremely rare. The reduction in *Sexual/Territorial* behaviour over the post-enrichment days and the "Ungulate" day appeared to be caused by a slight reduction in *Spraying*.

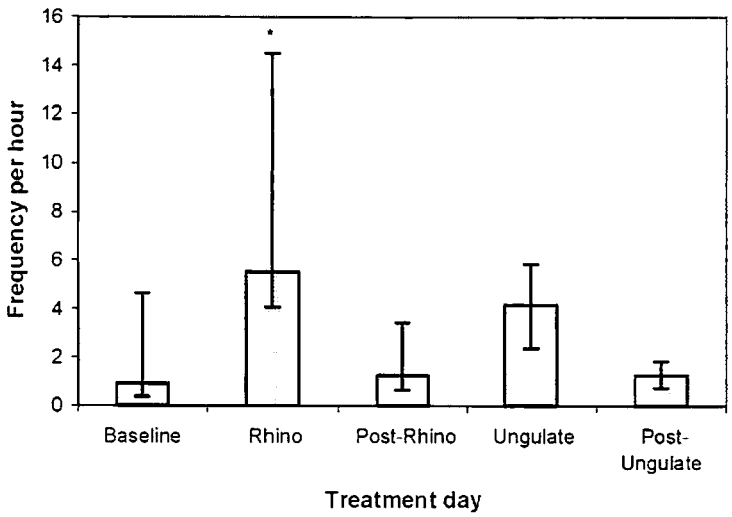

Figure 2 **Faeces investigation over five different treatments. Bars show median frequency, error bars show interquartile ranges. (* = P<0.05)**

Visitor Effects

There is a trend towards a negative correlation between visitor numbers and *Active* behaviours for both male and female rhinoceroses (Figure 3). For the combined data, this relationship is not significant.

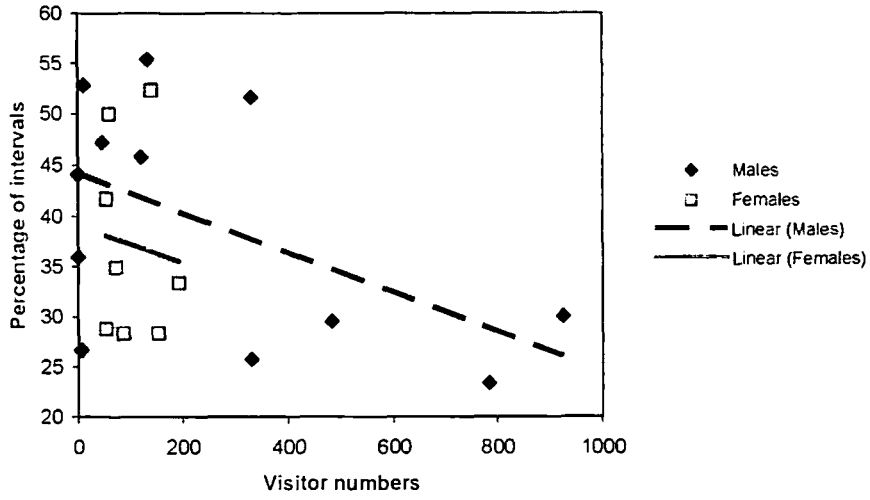

Figure 3 **Relationship between *Active* behaviours and the number of visitors (females n=4, males n=6).**

There is no relationship between percentage of time spent in *Inactive* behaviours and visitor numbers. However, *Pacing* has a significant positive correlation with visitor numbers (r_s= 0.68, P<0.01) for all rhinoceroses (Figure 4).

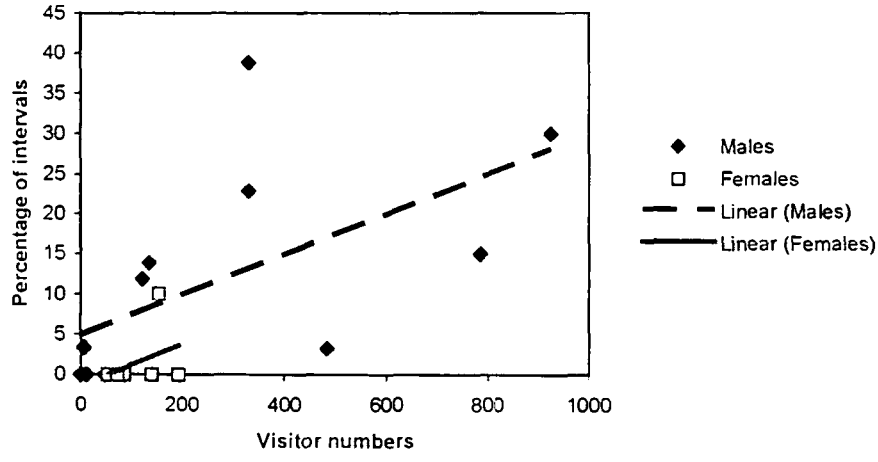

Figure 4 **Relationship between pacing and the number of visitors (females n=4, males n=6).**

As zoos differed in the time their animals spent pacing and in their visitor numbers, we also investigated the relationship between behaviour and visitor numbers within zoos to ensure the change in pacing was not confounded by variations between the two zoos (Figure 5). At Port Lympne, there was a significant positive correlation between pacing and visitor numbers (r_s=0.79, P<0.01). At Chester Zoo, there was a similar positive relationship.

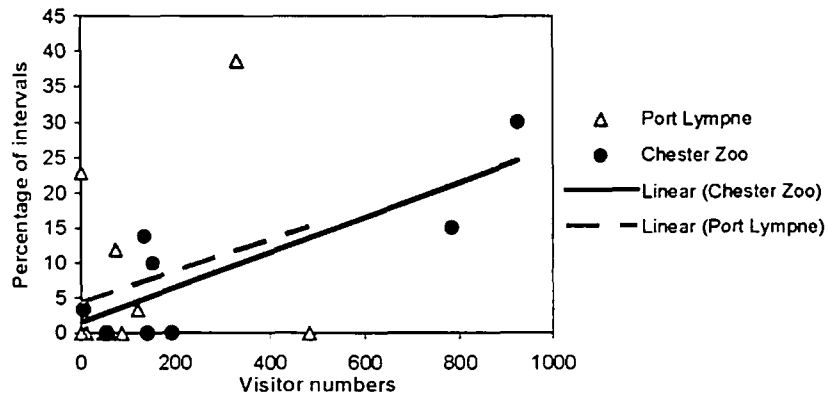

Figure 5 **Relationship between pacing and the number of visitors for the two zoos (Port Lympne: n=6, Chester: n=4).**

Investigative did not significantly correlate with visitor numbers for all the rhinoceroses combined. However, as illustrated in Figure 6, females showed a positive relationship between *Investigative* and visitor numbers and males showed a negative relationship, but this was statistically not significant.

For all rhinoceroses, the relationship between the frequency of *Sexual/Territorial* behaviour and visitor numbers was not significant. However, as illustrated in Figure 7, *Sexual/Territorial* behaviours seemed to have a positive relationship with visitor numbers for females, but males did not seem to be affected.

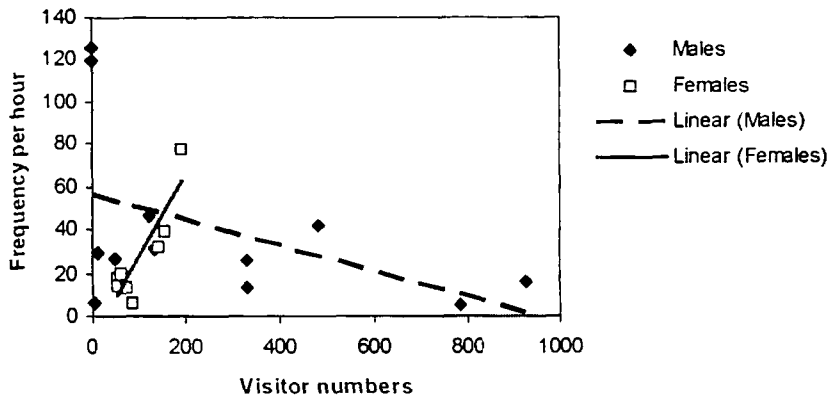

Figure 6 Relationship between *Investigative* behaviours and visitor numbers for the sexes.

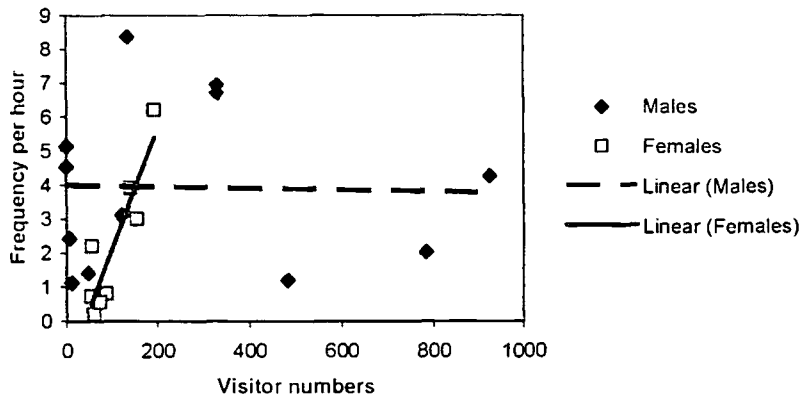

Figure 7 Relationship between *Sexual/Territorial* behaviours and visitor numbers.

Discussion

Activity and Pacing

Kiwia (1986) report that, depending on the time of year, in their natural habitat black rhinoceroses spend between 37.9% and 45.9% in active behaviours between 06:00 and 19:00. They also reported that the animals spend between 52.7% and 61.3% in inactive behaviours. The rhinoceroses of this study spent very similar times in *Active* and *Inactive* behaviours. However, when the categories are broken down into individual behaviours, the captive animals spent nearly twice as much time standing (20.9%) as opposed to their conspecifics in Kiwia's study (12.6-13.8%). The wild rhinoceroses also spent more time walking, between 14.2% and 22.1%, whereas with walking and exploring combined (Kiwia did not discriminate the two behaviours), the captive rhinoceroses spent 8.6% of the time in these behaviours. Rhinoceroses in the wild have home ranges of between 3.9km^2 and 133.0km^2 (Estes 1991), whereas the enclosures of the captive animals ranged from 0.9km^2 to 24.3km^2.

A difference in locomotion can therefore be caused by the space limitations. The captive animals also have no need to search for water and food as both are provided. If an animal's behaviour in captivity differs from its wild counterparts, this does not necessarily mean the captive animal is suffering (Veasey *et al.* 1996), but it may indicate that the animals are under-stimulated if the animal is not required to utilise mind and body in an alternative way.

The enrichment had little influence on time spent in *Active* and *Inactive* behaviours. The slight increase of *Activity* and decrease in *Inactivity* on "Ungulate" days requires further study to determine which type of dung influenced behaviour. Activity could also be influenced by visitors, since a slight negative relationship between visitor numbers and time spent in active behaviours was identified.

Individuals differed greatly in the time they spent pacing. Animals have differing coping mechanisms for stressful situations and this largely depends on individual characteristics such as temperament and past experience. Black rhinoceroses are well known for having individual differences in behaviour and temperament (Carlstead *et al.* 1999). The enrichment successfully reduced stereotyping in some individuals, but due to the large variation, differences were statistically not significant. With rising visitor numbers the time spent pacing increased, which supports the findings by Carlstead *et al.* (1999b) that male rhinoceroses find high visitor exposure stressful. This study identified the same trend for female rhinoceroses.

Investigative behaviour

The significant increase in *Faeces Investigation* demonstrates an interest by the animals in the dung, even though the dung did not significantly change any of their other behaviours. This may suggest that the enrichment needs to remain in the enclosure for longer periods or more than one midden should be used at a time. Scent trails could also be used to increase activity and enclosure use. In the wild, scent trails leading from a midden are a common feature, as rhinoceros' scrape their back feet through it after defecation. Goddard (1967) found that 60% of the wild rhinoceroses in his study followed a scent trail from their own dung, 70% followed a scent trail from another rhinoceros that shared their home range, and only 30% followed scent trails from distant rhinoceroses. This suggests that the rhinoceroses may be more interested in familiar scents than in those from unfamiliar animals, but further studies are necessary to investigate the use of faeces of familiar and unfamiliar rhinoceroses as enrichment in zoos. The animals in this study showed a slight interest in the "Ungulate" dung, but they sniffed the "Rhino" dung significantly more frequently than the "Ungulate" dung, suggesting that they can distinguish the difference between the dung from conspecifics and other species, and that conspecific dung was of more interest to them. They also seem to be able to identify their own dung, as there was not a significant increase in sniffing their own dung over any of the treatment days. Other biologically relevant types of dung could be tested as enrichment items, for instance lion dung, as lions are a predator of young rhinoceroses in

the wild. However, the effect would depend on the diet of the captive lions as diet composition is important for the repellence of predator odours (Nolte *et al.* 1994). The increase in *Smelling* on the "Post-Rhino" day could be a carry-over effect, indicating that the animals are searching for the dung that had been removed.

Sexual/Territorial Behaviour

The rhinoceroses were never observed defecating on the added rhinoceros midden, a behaviour, which has been observed frequently in the wild, even when middens were artificially added to an area (Goddard 1967). Goddard (1967) found that the rhinoceroses would be more likely to defecate on a pile of their own faeces than on that from another rhinoceros. All the captive rhinoceroses had a choice between their own and the added midden and they too chose to defecate on their own middens. However, at Port Lympne the rhinoceroses are often rotated between paddocks where other rhinoceroses have defecated and are seen to use these middens rather than create their own. In future studies, the dung could be left for longer, or a greater amount can be added to the enclosure. Using dung from females may have also had an effect. If the donating female was in oestrus, this is very likely to produce increased sexual behaviour in the males. Sexual behaviour was rarely observed in the females, but two of the females had dependent calves and it is unlikely that the females were cycling, as they were both still lactating.

Visitor effects

Only the behaviour category *Pacing* was significantly correlated with visitor numbers, but the data for this study was limited as only the baseline data was used to assess visitor effects. If *Pacing* is an indicator of stress in black rhinoceroses, then the behavioural results show that black rhinoceroses find increasing visitor numbers more stressful. In future studies, the behavioural results could be verified by measuring stress hormones non-invasively in urine, faeces or saliva. A further investigation could identify which aspects of visitor presence the animals perceive as stressful.

There are other trends in the data, which require further studies. Carlstead *et al.* (1999b) suggests that males may perceive the public as a threat and feel constrained in their movements. This study showed a negative trend between visitor numbers and time spent in active behaviours, although findings were statistically not significant. This suggests that higher visitor numbers could have a negative effect on animal welfare if inactivity is a concern. The rhinoceroses may perceive the public as a threat and therefore behave more vigilantly and stand still for longer periods. Carlstead *et al.* (1999b) also suggests that male rhinoceroses may feel constrained in their scent-marking behaviour. Our results give a conflicting picture, as the *Sexual/Territorial* behaviour of the males was unaffected by visitor numbers, but for females this behaviour increased with rising visitor numbers. This was also the case with *Investigative* behaviours; they increased in females and decreased in males

with rising visitor numbers. The datasets of the two sexes do not have the same spread of visitor numbers and further investigations are needed. If the increase in pacing of both sexes is due to the rhinoceroses perceiving higher numbers of visitors as being more stressful, the stimulation or inhibition of other behaviours could represent different ways in which the sexes cope with this stressor, or that they perceive visitors in different ways.

Conclusions and animal welfare implications

Although the enrichment did not change the animals' *Active*, *Inactive*, *Pacing*, *Sexual/Territorial* and overall *Investigative* behaviours, the rhinoceros dung in particular did instil a high degree of interest by the rhinoceroses. There are great individual differences between rhinoceroses. Therefore, further studies are needed to determine if dung can be used as an effective form of enrichment. Our results suggest that high visitor numbers may have a negative effect on rhinoceros welfare. This should be considered when designing rhinoceros enclosures or when modifying existing exhibits to maximise welfare. The influence of visitors may not only concern animal welfare but is also likely to affect the results of zoo research, and may have confounded the enrichment results of this study. Our study highlights the importance of further enrichment and visitor effect research, which may be of importance for rhinoceros psychological and physiological wellbeing and captive breeding. Further investigations are needed to identify how to increase activity and to clarify the causes of pacing behaviour, so that rhinoceroses' needs can be understood and met.

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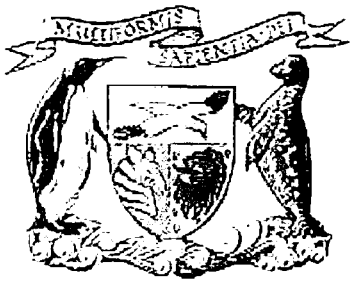

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