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SHORT COMMUNICATION

Behavioral and adrenocortical responses of captive white rhino adolescents to the introduction of a new calf

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Abstract

Changes in group composition are not exclusive to zoos and conservation breeding centers. The recent increase in poaching of African rhino species has accelerated the arrival of orphan calves at rehabilitation centers. Introducing new members into an existing group is often stressful for many mammal species. However, when young animals are involved, such responses may be reduced or absent. The goal of the present study was to evaluate the effect of introducing orphan calves on the stress responses of young orphan rhino from existing groups. The behavior and fecal glucocorticoid metabolite (fGCM) concentrations of eight orphan southern white rhinoceros (*Ceratotherium simum simum*) were assessed 1 month before and after the introduction of a new calf. From the 10 variables measured, only the response to humans and submissive behaviors showed significant changes. Stereotypies were not observed during the course of the study, and adrenocortical activity, monitored by means of fGCM concentrations, did not increase after the introductions, showing values within the range observed in free-ranging white rhino. However, strong individual differences were evident in most variables. Our results suggest that the introduction of white rhino calves into an existing group of young rhinos caused minimal stress in existing group members. Although these findings should be treated with caution when generalizing to other captive populations due to the small and heterogeneous sample, our findings may have management implications for rhino orphanages as well as zoos and breeding centers where non-breeding pairs are frequently maintained.

Keywords Group formation · Stress · Captivity · Population management · Adrenocortical activity · White rhinoceros

Introduction

Management of captive populations for conservation purposes often requires relocation of animals between institutions (Burks et al. 2004; Foose and Wiese 2006). These relocations frequently involve the introduction of individuals into, or the removal from, an established group. Changes in group composition are not exclusive to zoos or conservation breeding centers. The continuing pressure by poaching of African rhino species has substantially increased the number of orphan calves arriving at rehabilitation centers. In South Africa, where poaching is more severe (769 rhinos poached in 2018: DEA, 2019), the arrival of orphans to rehabilitation centers can be as frequent as one calf per week (personal obs.). After the initial stabilization phase, calves are introduced into existing orphan groups of similar age, that sometimes include a surrogate mother (wild-caught young adult female 5–7 years of age).

With few exceptions (Doyle et al. 2008), the introduction of new members into a group elicits stress in many mammal species (e.g., cynomolgus macaques, *Macaca fascicularis*: Clarke et al. 1995, chimpanzees, *Pan troglodytes*: Brent et al. 1997, bottlenose dolphins, *Tursiops aduncus*: Waples and Gales 2002, African elephants, *Loxodonta africana*: Burks et al. 2004). Increased levels of aggression (e.g., Bernstein et al. 1974; Watts and Meder 1996), decreased affiliative interactions (Clarke et al. 1995), and increased

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adrenocortical activity (Levine 1993) are characteristic when individuals are introduced into an established group. However, different results have been observed when young animals are involved (Berman and Kapsalis 1999; Fàbregas and Guillén-Salazar 2007). Aggression and related injuries are infrequent (e.g. Bernstein and Draper 1964) and affiliative relationships are typically observed (Fàbregas and Guillén-Salazar 2007; Reinhardt et al. 1987).

Given the high probability for aggression between unfamiliar individuals to develop, careful consideration should be given to the planning and implementation of animal introductions (Burks et al. 2004). In this regard, caution is advised when introducing adult rhinos into new groups (Fouraker and Wagener 1996; Hutchins and Kreger 2006). However, whether the introduction of a young animal also prompts stress is yet to be established. We used the arrival of orphan white rhino calves (*Ceratotherium simum simum*) to a rehabilitation and release facility in South Africa to investigate whether the introduction of a new orphan elicits stress in existing group members. A total of 28 behaviors, the presence of facial injuries, and fecal glucocorticoid metabolite (fGCM) concentrations were studied (Carlstead and Brown 2005). We hypothesize that aggression, space claim, and fGCM concentrations will not significantly increase given the fluid composition of groups in young animals of this species (Owen-Smith 1973).

Methods

The study took place between September and December 2017 at the Wildlife Veterinary Services of Kruger National Park (South Africa). The studied animals consisted of eight (4:4) adolescent orphaned southern white rhinoceros, with estimated ages of 14 months to 4 years (Table 1). Rhinos were housed in four 50 × 25 m *bomas* (captive wildlife enclosure) with a surrogate mother. Alfalfa (*Medicago sativa*) and teff (*Eragrostis teff*) hay were provided daily ad libitum in equal amounts on concrete slabs. Water was supplied in concrete troughs ad libitum.

New orphans were introduced directly into a group, without previous contact of any sort. Data were collected on resident orphans O2-O9 (Table 1) before and after the introduction of a new calf into a boma. A total of 60 h of behavioral data were collected during 1 month before and after the introduction of a new calf. During the study, five new orphan calves arrived at the facility. Three bomas received one orphan and one boma received two, on consecutive days (Table 1). Each study rhino was observed twice daily for 10 min, in the morning (05:00–07:00) and afternoon (16:30–18:30), to avoid human interference due to cleaning and feeding. These are also the periods during which free-ranging wild rhinos are more active during the summer months (Owen-Smith 1973). The order in which rhinos were observed each day was established by randomized lists to avoid biases due to time of day, environmental temperature, proximity to feeding time, or other unknown factors. Rhinos were individually identified through distinctive features such as body shape, horn shape, and ear notches. Focal sampling and continuous recording were used to record rhino behavior (Martin and Bateson 1993). Behaviors were grouped into seven categories: affiliative, discomfort, alertness, submission, space claim, stereotypies, and aggression (Table 2). Because fights also occur during the night (personal obs.), the presence or absence of superficial facial wounds was also recorded. A wound was considered any skin abrasion with visible flesh or blood. If the abrasion only involved superficial dermal layers, the wound was not noted. Additionally, we recorded the response of rhinos to humans (i.e., the researcher) to determine whether rhinos were more excitable after the introduction of a new calf. This variable indicated whether the focal rhino did not interrupt its behavior when the researcher (MF) arrived at the bomas (no response) or, alternatively, it showed alertness and fled (response). Facial injuries and response to humans were recorded before the morning session, when the researcher arrived at the bomas. To avoid observer effects (Martin and Bateson 1993), all other behavioral data were collected after the rhino had resumed the behavior performed when the researcher arrived. Data were collected using binoculars, spreadsheets, and a stopwatch.

To assess adrenocortical activity, we collected 72 fecal samples from all resident rhino but rhino O1 (Table 1) (median 8 samples/animal; range 4–14). Feces were collected between 7:00 and 9:00 and within 2 h of defecation. Approximately 50 g of fecal material was collected by removing pieces from the middle

Table 1 Age and sex structure of the rhino groups in this study. Data were collected on all resident orphan rhinos (O), except O1 due to an insufficient number of fecal samples. The S stands for "surrogate mother" (wild-caught young adult female 5–7 years of age). No data were collected on surrogate mothers or introduced rhino calves

Boma	Resident rhino	Introduced rhino calves
1	O1 (male calf)	O10 (male)
	O2 (male calf)	
	S1	
2	O3 (subadult female)	O11 (female)
	O4 (subadult male)	O12 (female)
	S2	
3	O5 (subadult male)	O13 (male)
	O6 (male calf)	
	S3	
4	O7 (female calf)	O14 (female)
	O8 (female calf)	
	O9 (female calf)	
	S4	

Table 2 List of behaviors within each behavioral category. Descriptions of specific behaviors can be found in Carlstead and Brown (2005), Carlstead et al. (1999), Metrione et al. (2007), and Owen-Smith (1971, 1973)

Category	Behaviors
Alertness	Alert, tail up, defense formation, flee, disturbed by humans
Affiliative	Rub, follow orphan, follow surrogate, follow group, naso-nasal contact
Discomfort	Whine, squeak
Submission	Yield, present side
Space claim	Snarl-chase, snort, charge
Agonistic	Horn wrestle, horn against horn stare, attack, fight
Stereotypies	Backing, bar biting, pacing, foot dragging, head swiping, mouthing

of 3–4 boli of a dropping. The sample was placed immediately on ice and frozen at − 20 °C within 1 h of collection. Frozen samples were lyophilized, pulverized, and sieved through a metal wire-mesh strainer to remove undigested material (Fieß et al. 1999). Between 0.10 and 0.11 g of fecal powder was then extracted with 80% ethanol in water (3 ml) according to the procedure described by Ganswindt et al. (2002). Extracts were analyzed for fGCM concentrations using an already established enzyme-immunoassay for white rhino (Badenhorst et al. 2016). Detailed assay characteristics, including full descriptions of the assay components and cross-reactivities, are provided by Touma et al. (2003). Sensitivity of the assay at 90% binding was 2.4 ng/g fecal dry weight. Intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low concentration controls, were 6.6% and 6.7%, and 7.9% and 8.9%, respectively. Extractions and analyses were performed at the Endocrine Research Laboratory, University of Pretoria (South Africa) as described by Ganswindt et al. (2002).

Wilcoxon signed-rank tests were used to identify potential changes after the introduction of a new calf in all the variables. Prior to analysis, mean rates (behavior/h) per animal before and after the introduction were calculated for all continuous variables (i.e., aggression, space claim, submission, discomfort, alertness, affiliative, stereotypic behaviors, fGCM concentrations). For facial injuries and response to humans, a sum of days was used instead, as they were dichotomous variables (presence/absence) measured once a day. All tests were performed using SPSS 25 software (IBM Corp 2017) and statistical significance set at 0.05.

Results and discussion

Only submission and response to humans showed significant changes after the introduction of a rhino calf (Table 3). Frequent alertness and increased responsiveness are typical behavioral stress indicators (Carlstead and Shepherdson 2000; Cook et al. 2000). However, the decreased response to humans (in this case, the researcher) could have been due to habituation, the decreased responsiveness of individuals caused by repeated exposure to a stimulus (McFarland 1993). The remaining variables did not show significant differences in the Wilcoxon signed-rank tests, despite the overall decrease in mean percentage of change in all variables but space claim and facial injuries (Table 3), Fig. 1. This lack of statistical significance could have been due to the strong individual differences observed in these variables (Table 3) and the small and heterogenous sample size. Finally, stereotypies were never observed during the course of the study.

Boma 2, where two calves were introduced to the group (as opposed to one in the other bomas), showed similar changes to the other bomas (Fig. 1), with the exception of a fivefold increase in aggression, which coincided with a 32% increase in fGCM concentrations in one of its residents (orphan O3, Table 1). The

Table 3 Results of Wilcoxon signed-rank tests ($n = 8$) and total mean percentage of change observed after the introduction of a new calf into a group of rhino adolescents. Significant differences are indicated in italics font

Variable	# of rhinos showing increase	# of rhinos showing decrease	Mean change (%)	Z	p value
Alert	3	5	− 47	− 1.402	0.161
Affiliative	3	5	− 17	− 1.120	0.263
Discomfort	0	3	− 64	− 1.604	0.109
Submission	0	8	− 77	− 2.521	*0.012*
Space claim	5	3	39	1.260	0.208
Aggression	4	4	− 23	− 0.070	0.944
Facial injuries	2	1	56	1.134	0.257
Response to humans	0	7	− 92	− 2.401	*0.016*
fGCM	1	6	− 1	− 1.400	0.161

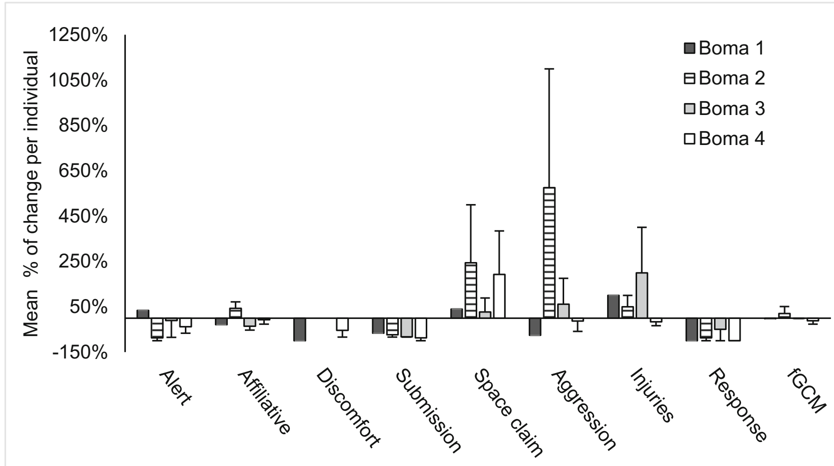

Fig. 1 Mean percentage of change (± SEM) per resident rhino after the introduction of a new calf into each boma (i.e., enclosure). "Response" stands for the response of resident rhino to humans, "injuries" for the incidence of facial injuries, and fGCM for fecal glucocorticoid metabolite concentrations. Boma 2 (represented with stripped bars) received two calves in consecutive days, whereas all other bomas received only one calf

fact that the other resident in that boma showed an 11% reduction in fGCM concentrations suggests that the increase in aggressive behavior in Boma 2 could be due to individual differences. However, given the small and heterogenous sample size, the possibility that the number of rhino calves introduced affects aggressive behavior in resident rhino cannot be excluded.

Although increased aggression is expected when adult rhinos are introduced to other conspecifics (Fouraker and Wagener 1996; Hutchins and Kreger 2006), we hypothesized that, due to the young age of group members, it would not apply to our study. White rhino is considered the most social of all rhino species (Owen-Smith 1975). As adolescents, which comprises the period from leaving their mothers to reaching sexual and social maturity (Owen-Smith 1973), they join mother-calf units or other rhinos of similar age. These relationships last a few days or, if a stronger "bond" is formed, several months or even years (Owen-Smith 1971; Shrader and Owen-Smith 2002). The natural disposition to form groups or change companionship at this stage of life would explain a general lack of aggression to a newcomer. Similar fGCM concentrations before (overall mean 0.62 μg/DW, range 0.49–0.70 μg/DW) and after the introductions (mean 0.61 μg/DW, range 0.40–0.94 μg/DW), and within the range observed in free-ranging white rhino (Badenhorst et al. 2016), suggest that the studied animals adapted to the introduced individuals and the new social environment quickly. Additionally, if there was any increase in adrenocortical activity immediately after the introduction, it was short-lived.

In conclusion, our results suggest that the introduction of white rhino calves into an existing group of young rhinos caused minimal stress in existing group members. However, given our small and heterogeneous sample, our findings should be considered with caution when extrapolating to other captive white rhino populations. Further studies replicating our methods would be of value for captive management. Nevertheless, we believe that our results address an important knowledge gap given the number of orphan calves arriving at rehabilitation facilities, and the frequent movement of rhinos between zoos and other conservation centers. In these cases, although introducing rhino to each other at a young age is not a possibility for breeding pairs (Bertschinger 1994; Brown et al. 2001), those institutions that lack facilities or infrastructures necessary for breeding, or for non-breeding animals (e.g., surplus males, over-represented animals in the metapopulation gene pool), group formation before adulthood would be preferable for the welfare of the animals.

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

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed in this study were in accordance with the ethical standards of the University of Pretoria Animal Ethics Committee (V030-16).

Conflict of interest The authors declare that they have no conflict of interest.

References

Badenhorst M, Otto M, Goot AC, Ganswindt A (2016) Stress steroid levels and the short-term impact of routine dehorning in female southern white rhinoceroses (*Ceratotherium simum simum*). Afr Zool 51(4):211–215

Berman CM, Kapsalis E (1999) Development of kin bias among rhesus monkeys: maternal transmission or individual learning?. Animal Behaviour 58 (4):883–894
Bernstein IS, Draper WA (1964) The behaviour of juvenile rhesus monkeys in groups. Anim Behav 12:28–31
Bernstein IS, Gordon TP, Rose RM (1974) Factors influencing the expression of aggression during introductions to rhesus monkey groups. In: Holloway RL (ed) Primate aggression, territoriality, and xenophobia. Academic Press, New York, pp 211–240
Bertschinger HJ (1994) Reproduction in black and white rhinos: a review. In: Penzhorn BL, Kriek NJ (eds) Proceedings of the symposium “rhinos as game ranch animals”. South African veterinary association, Onderstepoort, South Africa, pp 115–161
Brent L, Kessel AL, Barrera H (1997) Evaluation of introduction procedures in captive chimpanzees. Zoo Biol 16(4):335–342
Brown JL, Bellem AC, Fouraker M, Wildt DE, Roth TL (2001) Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in North America by noninvasive endocrine monitoring. Zoo Biology 20 (6):463–486
Burks KD, Mellen JD, Miller GW, Lehnhardt J, Weiss A, Figueredo AJ, Maple TL (2004) Comparison of two introduction methods for African elephants (*Loxodonta africana*). Zoo Biol 23(2):109–126
Carlstead K, Brown JL (2005) Relationships between patterns of fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. Zoo Biol 24:215–232
Carlstead K, Mellen J, Kleiman DG (1999) Black rhinoceros (*Diceros bicornis*) in U.S. zoos: I. individual behavior profiles and their relationship to breeding success. Zoo Biol 18:17–34
Carlstead K, Shepherdson DJ (2000) Alleviating stress in zoo animals with environmental enrichment. In: Moberg GP, Mench JA (eds) The biology of animal stress: basic principles and implications for animal welfare. CAB International, Wallingford, pp 337–354
Clarke AS, Czekala NM, Lindburg DG (1995) Behavioral and adrenocortical responses of male cynomolgus and lion-tailed macaques to social stimulation and group formation. Primates 36(1):41–56
Cook CJ, Mellor DJ, Harris PJ, Ingram J, Mathews LR (2000) Hands-on and hands-off measurement of stress. In: Moberg GP, Mench JA (eds) The biology of animal stress: basic principles and implications for animal welfare. CAB International, Wallingford, pp 123–146
DEA. Department of Environmental Affairs of the Republic of South Africa (2019) Minister of environmental affairs highlights progress on the implementation of the integrated strategic management of rhinoceros. https://www.environment.gov.za/progressonimplementationofintegratedstrategicmanagementofrhinoceros. Accessed on 13th March 2019
Doyle LA, Baker KC, Cox LD (2008) Physiological and behavioral effects of social introduction on adult male rhesus macaques. Am J Primatol 70(6):542–550
Fàbregas M, Guillén-Salazar F (2007) Social compatibility in a newly formed all-male group of white crowned mangabeys (*Cercocebus atys lunulatus*). Zoo Biol 26(1):63–69
Fieß M, Heistermann M, Hodges JK (1999) Patterns of urinary and fecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). Gen Comp Endocrinol 115:76–89
Foose T, Wiese R (2006) Population management of rhinoceros in captivity. Int Zoo Yearb 40:174–196
Fouraker M, Wagener T (1996) AZA rhinoceros husbandry manual. Fort Worth Zoological Park, Fort Worth, TX
Ganswindt A, Heistermann M, Borragan S, Hodges JK (2002) Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. Zoo Biol 21:27–36
Hutchins M, Kreger MD (2006) Rhinoceros behaviour: implications for captive management and conservation. Int Zoo Yearb 40(1):150–173
Corp IBM (2017) IBM SPSS statistics for windows, version 25. IBM Corp, Armonk, p 0
Levine S (1993) The influence of social factors on the response to stress. Psychother Psychosom 60:33–38
Martin P, Bateson P (1993) Measuring behaviour: an introductory guide, 2nd edn. Cambridge University Press, Cambridge
McFarland D (1993) Animal behaviour: psychobiology. In: Ecology and evolution, 2nd edn. Longman Scientific and Technical, Oxford
Metrione LC, Penfold LM, Waring GH (2007) Social and spatial relationships in captive southern white rhinoceros (*Ceratotherium simum simum*). Zoo Biol 26(6):487–502
Owen-Smith N (1971) Territoriality in the white rhinoceros (*Ceratotherium simum*) Burchell. Nature 231(5301):294–296
Owen-Smith N (1973) The behavioural ecology of the white rhinoceros. PhD dissertation. University of Wisconsin
Owen-Smith N (1975) The social ethology of the white rhinoceros *Ceratotherium simum* (Burchell 1817). Z Tierpsychol 38:337–384
Reinhardt V, Houser WD, Eisele SG, Champoux M (1987) Social enrichment with infants of the environment for singly caged adult rhesus monkeys. Zoo Biol 5:365–371
Shrader AM, Owen-Smith N (2002) The role of companionship in the dispersal of white rhinoceroses (*Ceratotherium simum*). Behav Ecol Sociobiol 52(3):255–261
Touma C, Sachser N, Möstl E, Palme R (2003) Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 130:267–278
Waples KA, Gales NJ (2002) Evaluating and minimizing social stress in the care of captive bottlenose dolphins (*Tursiops aduncus*). Zoo Biol 21:5–26
Watts E, Meder A (1996) Introduction and socialization techniques for primates. In: Kleiman DG, Allen ME, Thompson KV, Lumpkin S, Harris H (eds) Wild mammals in captivity: principles and techniques. University of Chicago Press, Chicago, pp 66–77