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

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ACKNOWLEDGEMENTS

We thank the University of Pretoria and the National Research Foundation of South Africa (NRF) for funding the lead author in conducting this study. Financial support was provided by Epi-Use to cover all research-associated costs. Special thanks to SANParks for providing access to their animals as well as logistical support for data collection, and to the Endocrine Research Laboratory (University of Pretoria) for assisting in hormone extraction and analysis.

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 one 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.

Key words: 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 & 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 & Gales 2002, African elephants, *Loxodonta africana*: Burks et al. 2004). Increased levels of aggression (e.g. Berstein et al. 1974; Watts & Meder 1996), decreased affiliative interactions (Clarke et al. 1995) and increased 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 & Kapsalis 1999; Fàbregas & Salazar 2007). Aggression and related injuries are infrequent (e.g. Bernstein & Draper 1964) and affiliative relationships are typically observed (Fàbregas & 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 & Wagener 1996; Hutchins & 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 & 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 four years (Table 1). Rhinos were housed in four 50 x 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 one 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 & 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 as 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 & 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 of 3-4 boli of a dropping. The sample was placed immediately on ice and frozen at -20 °C within one hour 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 - 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 & 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 Sign-ranked tests, despite the overall decrease in mean percentage of change in all variables but space claim and facial injuries (Table 3). 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, with the exception of a five-fold increase in aggression, which coincided with a 32% increase in fGCM concentrations in one of its residents (orphan O3, Table 1). The fact that the other resident in that boma showed a 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 & Wagener 1996; Hutchins & Kreger 2006), we hypothesized that, due to the young age of group members, it would not apply to our study. White rhino are 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 & 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.

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.

Data availability

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

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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) O2 (male calf) S1	O10 (male)
2	O3 (subadult female) O4 (subadult male) S2	O11 (female) O12 (female)
3	O5 (subadult male) O6 (male calf) S3	O13 (male)
4	O7 (female calf) O8 (female calf) O9 (female calf) S4	O14 (female)

Table 2 List of behaviors within each behavioral category. Descriptions of specific behaviors can be found in Carlstead & 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

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 bold 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

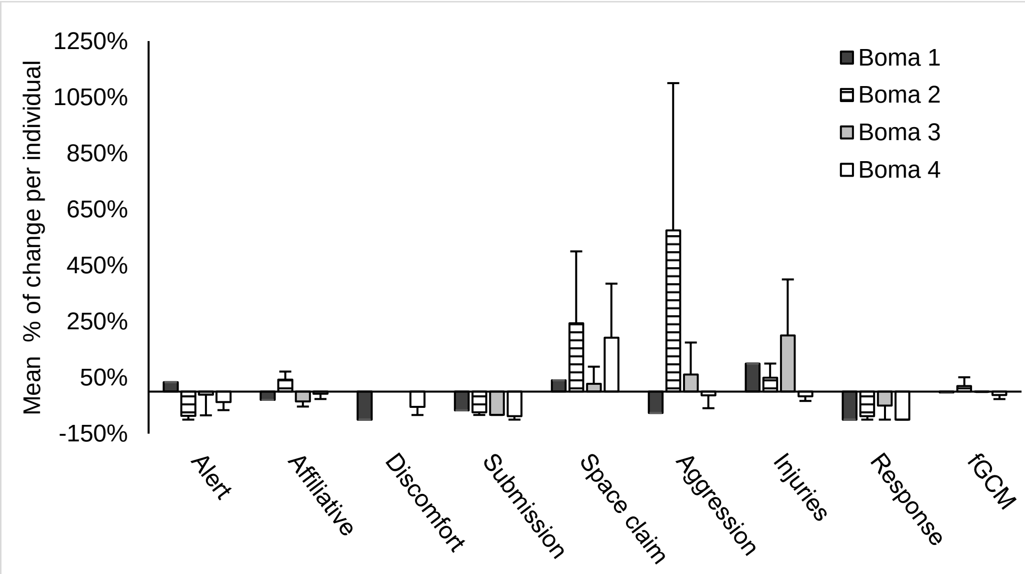

Fig 1 Mean percentage of change (+/- SEM) per resident rhino after the introduction of a new calf into each boma (i.e. enclosure). “Response” stands for the response of resident rhino to humans, “injuries” for the incidence of facial injuries, and fGCM for fecal glucocorticoid metabolite concentrations.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