TANZANIA WILDLIFE RESEARCH INSTITUTE (TAWIRI)

PROCEEDINGS OF THE EIGHTH TAWIRI SCIENTIFIC CONFERENCE, 6th - 8th DECEMBER 2011, CORRIDOR SPRINGS HOTEL, ARUSHA, TANZANIA

Editors
Dr. Maurus Msuha
Dr. Stephen Nindi
Dr. Victor Kakengi
Dr. Janemary Ntalwila
Dr. Edward Kohi

Published by:
Tanzania Wildlife Research Institute
P. O. Box 661 Arusha, Tanzania

ISBN: 978-9987-9567-2-2

USING NON-INVASIVE METHODS TO INVESTIGATE FACTORS AFFECTING THE SUCCESS OF THE BLACK RHINOCEROS: EXAMPLE FROM ADDO ELEPHANT NATIONAL PARK, SOUTH AFRICA

R. Santymire[1], J. Meyer[1,4], J. Bird[2], B. Schulte [3] and E. Freeman[4]

[1]Conservation and Science Department, Lincoln Park Zoo, Chicago, IL, USA, [2]River Bend Lodge, Addo Elephant National Park, Addo, South Africa, [3]Western Kentucky University, Bowling Green, KY, USA and [4]George Mason University, Fairfax, VA, USA

Corresponding author: rsantymire@lpzoo.org

ABSTRACT

Non-invasive faecal hormone analysis is an effective method for monitoring and increasing our knowledge of the health and reproductive success of wild populations. For critically endangered and slow breeding species, it is important to understand the factors that limit population growth. Addo Elephant National Park (AENP) has 70% of South Africa's southwestern subspecies of black rhinoceros (Dicerosbicornisbicornis). For 3 years, we have been validating non-invasive field techniques for monitoring gonadal (progestin and androgen) and adrenocortical (glucocorticoid) activity in black rhinos from two sections of AENP. Camera traps were placed at rhino middens to identify individuals and facilitate collection of samples. Fresh faecal samples were collected, processed and analyzed for pregnancy on-site and sent to the United States for androgen (FAM) and glucocorticoid (FGM) metabolites analyses. Approximately, 250 samples were collected from known individuals (21 males; 22 females). Results demonstrate that males and females had similar ($P>0.05$) FGM concentrations; however, individuals in the section of AENP with the highest number of elephants and tourists had higher ($P<0.001$) FGM concentrations compared to the section with fewer elephant and tourist. Concentrations of FGM were lower ($P<0.01$) in the winter for both AENP sections. Pregnancy was accurately diagnosed in 100% ($n = 7$) of females with faecal progestin concentrations seven-fold higher ($P < 0.001$) in pregnant compared to non-pregnant rhinos. For males, FAM was higher ($P=0.006$) in summer and spring months and lowest in the winter. A similar pattern for progestin was observed in the females ($P=0.012$) with lowest faecal progestin concentrations in the winter. With validated, non-invasive monitoring methods, our long-term goal is to establish a multi-year, health monitoring program to investigate the relationship among black rhino hormonal activity, parasitic infection rates and varying abundance of mega-herbivores, predators and humans. Results will be used to facilitate management decisions for black rhino conservation.

INTRODUCTION

In the 1700s, the black rhinoceros (rhino; Dicerosbicornis) population in Africa was estimated at 850,000; however, its horn has been the target of poachers and by 1992 only approximately 2000 were left in the wild. Currently, the black rhino numbers have doubled to 4,880 individuals even though poaching has been on the rise (21.6% higher in 2011 compared to 2010; IUCN, 2011). Recently, the Western subspecies (Diceros bicornislongipes) was declared to be extinct, leaving only three subspecies on the continent (IUCN, 2011). With the escalation of poaching, understanding the needs of this elusive species is critical.

The majority of in situ research and management efforts are focused on recreating suitable habitat that will allow black rhino populations to increase (e.g. Birkett, 2002; Reid et al., 2007; Linklater and Swaisgood, 2008; Morgan et al., 2009). However, the impact of environmental conditions on population growth need to be further investigated with minimal disturbance. One method of studying these impacts on individual is to monitor biological factors including reproductive and stress physiology. Recently, hormonal analysis using samples collected by non-invasive methods, such as faecal or urine collection, have been developed. Non-invasive hormonal monitoring is advantageous because 1) hormones are concentrated versus continuous fluctuations found in the blood stream; 2) feces are easy to obtain without disturbing the subject through capture and handling and often provide large quantities of sample; 3) frequent collection can provide a longitudinal profile and better interpretation of the hormonal patterns; 4) hormone metabolites in faecal and urine samples are from several hours or days prior to collection and provide further comprehensive assessment (Monfort, 2003).

Few endocrine studies have been conducted on wild black rhino, including a field pregnancy test (MacDonald et al., 2008) and evaluation of estrous cyclicity and the influence of season on reproduction (Garnier et al., 2002). Additionally, faecal hormone concentrations and scent-marks have been evaluated relative to distance traveled post-release in translocated black rhino (Linklater et al. 2006). Extensive endocrine studies have been carried out in zoos (Brown et al., 2001; Carlsteadand Brown, 2005; Carlstead et al., 1999; Wasser et al., 2000). Because zoo-housed individuals are managed under different environmental and dietary conditions than wild black rhinos, we cannot assume that hormonal patterns are similar between wild and captive populations. Therefore, our goal is to establish a health monitoring program that will investigate the relationship among hormonal activity and ecological factors of a population of south-western black rhino subspecies (Dicerosbicornisbi cornis) found in two sections (Addo and Nyathi) of Addo Elephant National Park (AENP) in Addo, South Africa. Our specific objectives are to investigate the impact of resource availability (competition with elephants), predation pressures (lions and hyenas) and eco-tourism on the rhinos' health and reproduction using non-invasive field methods. Our hypotheses are that: 1) high densities of elephants, predators and tourists are associated with a suppression of gonadal (reproductive) activity in black rhino; and 2) adrenocortical activity is positively associated with high anthropogenic activity and negatively affects reproduction in the black rhino.

METHOD

Study Area

The AENP is located in the Eastern Cape of South Africa and was originally founded in 1931 to protect the remaining 11 elephants in the area (Whitehouse and Hall, 2000). In 1995, indigenous black rhinos of this ecotype (D. b. bicornis) were reintroduced into the AENP and the eastern black rhino subspecies (D. b. michaeli) were removed. Our study focused on two sections, Addo's Main Camp and Nyathi, which have varying biotic and abiotic characteristics (Table 1).

Table 1. The varying abiotic and biotic factors of our study area, which includes two sections in AENP (Addo and Nyathi) that are separated by a highway and fence.

Factors	Addo	Nyathi
Sex ratio	Female-biased	Male-biased
Elephant density	High (~300)	Moderate (~100)
Predators	Present	Absent
Vegetation	Limited	Abundant
Tourism	High	Low
Size of section	11,500 ha	14,000 ha

Rhino Population

There were 44 black rhinos managed in the two sections of AENP, Addo: n = 18 (9 females, 8 males, 1 unsexed) and Nyathi: n = 26 (11 females, 13 males, 2 unsexed). Each rhino is immobilized at approximately 2 to 4 years of age and given a specific pattern of ear notches that can be used to identify individuals. Prior to notching, rhinos can be positively identified by other anatomical features, such as their size, horn and body scars, and by association with their mother.

Camera Traps

The camera traps were placed in areas based on black rhino sightings by AENP staff and at active latrines called middens, which are areas where black rhinos defecate and then scrape the feces with their hind legs to spread odors advertising their presence (Hutchins and Kreger, 2006). A high-quality digital photo was quietly taken when the camera was activated through a passive infrared motion detector. Each morning that fresh feces were found in view of a camera, a sample was collected and stored in a cooler. The images stored on the camera were checked to identify the individual that defecated and assess body condition; time of defecation also was determined from the photo.

Faecal Hormone Monitoring

Faecal samples were monitored daily by two methods: camera traps and direct observation of individuals defecating. Faecal hormones were extracted from feces using a field technique (Santymireand Armstrong, 2010). Faecal progestin metabolite concentrations analysis was conducted in the field to test for pregnancy (Freeman et al.,2010; MacDonald et al., 2008). However, these results were verified at Lincoln Park Zoo's (LPZ's) endocrinology laboratory along with additional analyses for faecal androgen and corticosterone (for stress analysis) metabolite concentrations. An USDA importing permit (# 107647) was obtained.

Data Analysis

Data analysis was done using Systat 12 and Sigma Plot v.11 (SPSS, Inc.). A Kolmogorov-Smirnov test was used for normality assumption testing and the Levene median test for equal variance assumption testing. Non-parametric tests were used when data were not normal. Analysis of Variance (ANOVA) was used to compare among individuals, seasons and reproductive state. For all analyses, $P < 0.05$ was considered significant.

RESULTS

Over 250 faecal samples from known individuals(21 males; 22 females)were collected for over 3 years. Pregnancy was accurately diagnosed in 100% (n = 7 females) of females. Progestin metabolite values were higher (Mann-Whitney: $T_{12,33} = 461.0$, $P < 0.001$) in pregnant (533.4 ± 106.0 ng/g feces) versus non-pregnant (78.6 ± 8.5 ng/g feces) females. The inter-calf interval was 33.5 ± 6.1 months (n=7) in Addo versus 25.5 ± 1.6 months (n=12) in Nyathi. Faecal progestin metabolites were lowest (Kruskal-Wallis: $H_3 = 11.001$, $P = 0.012$) in the winter (103.8 ± 31.4 ng/g) compared to fall (166.9 ± 34.6 ng/g), but both were similar to spring (149.8 ± 47.3 ng/g) and summer (177.4 ± 71.8 ng/g). For males, faecal androgen metaboliteswerehigher (Kruskal-Wallis: $H_3 = 12.543$, $P = 0.006$) in summer (110.5 ± 10.3 ng/g) and spring (139.6 ± 16.1 ng/g) months and lowest in the winter(71.8 ± 7.0 ng/g), but similar ($P>0.05$) to fall

(108.9 ± 10.1 ng/g). Faecal glucocorticoid metabolites were similar ($P>0.05$) between sexes and age groups; however, Addo (160.5 ± 14.8 ng/g) had higher (Mann-Whitney: $T_{58,164} = 8125.0$, $P < 0.001$) concentrations compared to Nyathi (112.9 ± 8.6 ng/g). In Addo, concentrations of faecal glucocorticoid metabolites were lower (Kruskal-Wallis: $H_3 = 13.459$, $P = 0.004$) in the winter (94.1 ± 17.5 ng/g) than summer (232.1 ± 30.2 ng/g), but fall (111.5 ± 13.0 ng/g) and spring (145.9 ± 22.6 ng/g) were similar ($P>0.05$) to both seasons. Similarly, faecal glucocorticoid metabolites in Nyathiwere higher (Kruskal-Wallis: $H_3 = 23.406$, $P < 0.001$) in fall (109.3 ± 9.2 ng/g) and spring (186.3 ± 26.4 ng/g) than winter (59.3 ± 7.5 ng/g), but summer (96.7 ± 14.4 ng/g) was similar ($P>0.05$) to the other seasons.

DISCUSSION

We overcame the challenges of studying this elusive species by using camera traps on middens to identify the individual faecal sample. Using non-invasive sampling allows for the investigation of environmental pressures on the black rhino with minimal disturbance. Additionally, by processing the samples in the field (Santymire and Armstrong, 2010) we could analyze the sample immediately for pregnancy (Freeman et al., 2010) and have a safe and reliable method for shipping the samples back to the laboratory for analysis.

Our results are the first steps in understanding how varying environmental pressures can impact the success of the wild black rhino in AENP. Overall, we observed some effects of season on reproductive hormones with lower faecal androgen (in males) and progestin (in female) metabolites in the winter. In future analyses we will compare wet versus dry seasons, as precipitation rates may be more of the driving factor of reproduction. We also observed a year longer calving interval in Addo compared to the Nyathi section indicating that there may be factors that were impacting reproduction in the Addo section black rhinos. The recent literature indicates that it is important to monitor how reproduction is linked to adrenocortical activity (Linklater et al., 2010) in black rhinos. Some of the possible pressures in the Addo section include more elephants, presence of predators and the majority of tourist vehicles on roads than the Nyathi section. Black rhinos select their habitat based on a variety of factors, including distance to water, roads and fences, not simply on the quality of available browse (Morgan et al., 2009). Additionally, population models predict that competition with other browsers (e.g. elephants) and low precipitation rates could negatively impact reproductive success of black rhinos (Birkett, 2002). Decreases in food, water and shelter availability along with increased human disturbance results in larger home ranges and lower black rhino reproductive success (Reid et al., 2007). Here we determined that the Addo black rhinos had higher faecal glucocorticoid metabolite concentrations than Nyathi, which reinforces that one or all of these factors are impacting the population. Similarly, differences in competition for food, water and shelter between the AENP sections could affect hormonal activity and ultimately lead to differences in reproductive success and health among individuals.

Conservation implications

With validated, non-invasive monitoring methods, our long-term goal is to establish a multi-year, health monitoring program to investigate the relationship among black rhino hormonal activity, parasitic infection rates and varying abundance of mega-herbivores, predators and humans. Results will be used to facilitate management decisions for black rhino conservation. Investigating the impact of the biotic and abiotic differences between Addo and Nyathi will lead to a greater understanding of black rhino ecology and reproduction. This knowledge will be able to enhance SAN Parks commitment to conservation efforts of the critically endangered black rhino by improving decision making based upon science and leading to the reproductive success of black rhinoceros within other national parks where they occur.

REFERENCES

Birkett, A (2002). The impact of giraffe, rhino and elephant on the habitat of a black rhino sanctuary in Kenya. African Journal of Ecology 40, 276-282.

Brown, J.L., Bellem, A.C., Fouraker, M., Wildt, D.E., Roth, T.L (2001). Comparative analysis of gonadal and adrenal activity in the Black and White rhinoceros in North America by noninvasive endocrine monitoring. Zoo Biology 20, 463-486.

Carlstead, K., Brown, J.L (2005). Relationships between patterns of faecal corticoid excretion and behavior, reproduction, and environmental factors in captive Black (Dicerosbicornis) and White (Ceratotheriumsimum) rhinoceros. Zoo Biology 24, 215-232.

Carlstead, K., Fraser, J., Bennett, C., and Kleiman, D.G (1999). Black rhinoceros (Dicerosbicornis) in U.S. Zoos: II. Behavior, breeding success, and mortality in relation to housing facilities.Zoo Biology, 18, 35-52.

Freeman, E.W., Abbondanza, F.N., Meyer, J., Schulte, B.A., Brown, J.L (2010). A simplified method for monitoring progestagens in African elephants under field conditions. Methods in Ecology and Evolution 1, 86-91.

Garnier, J., Holt, W., Watson, P (2002). Non-invasive assessment of oestrous cycles and evaluation of reproductive seasonality in the female wild black rhinoceros (Dicerosbicornis minor). Reproduction 123, 877-889.

Hutchins, M., Kreger, M.D (2006). Rhinoceros behavior: Implications for captive management and conservation. International Zoo Yearbook 40, 150-173.

IUCN (2011). Dicerosbicornis. In: SSC African Rhino Specialist Group 2010. IUCN Red List of Threatened Species. Version 2010.2. <www.iucnredlist.org>. Downloaded on 17 November 2011.

Linklater, W.L., Flamand, J., Rochat, Q., Zekela, N., MacDonald, E.A., Swaisgood, R.R., Airton, D.F., Kelly, C.P., Bond, K., Schmidt, I., Morgan, S (2006). Preliminary analyses of the free-release and scent-broadcasting strategies for black rhinoceros reintroduction. Ecological Journal 7, 26-34.

Linklater, W., MacDonald, E., Flamand, J., &Czekala, N (2010). Declining and low faecal corticois are associated with distress, not acclimation to stress, during the translocation of African rhinoceros. Animal Conservation 13, 104-111.

Linklater, W.L., Swaisgood, R.R (2008). Reserve size, conspecific density, and translocation success for black rhinoceros. Journal of Wildlife Management 72, 1059-1068.

MacDonald, E.A., Linklater, W.L., Steinman, K.J., Czekala, N.M (2008). Rapid colour-change pregnancy test for rhinoceros using faeces. Endangered Species Research 4, 277-281.

Monfort, S.L (2003). Non-invasive endocrine measures of reproduction and stress in wild populations. Conservation biology: Reproductive science and integrated conservation (eds W.V. Holt, A.R. Pickard, J.C. Rodger & D.E. Wildt), pp. 147-165. Cambridge University Press, Cambridge.

Morgan, S., Mackey, R.L., Slotow, R (2009). A priori valuation of land use for the conservation of black rhinoceros (Dicerosbicornis). Biological Conservation 142, 384-393.

Reid, C., Slotow, R., Howison, O., Balfour, D (2007). Habitat changes reduce the carrying capacity of Hluhluwe-Umfolozi Park, South Africa, for critically endangered black rhinoceros Dicerosbicornis. Oryx 41, 247-254.

Santymire, R.M., Armstrong, D (2010). Development of a field-friendly technique for faecal steroid extraction and storage using the African wild dog (Lycaonpictus). Zoo Biology 29, 289-302.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L (2000). A generalized faecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. General and Comparative Endocrinology 120, 260-275.