

News and Views from the BIAZA Research Committee

16th Annual Symposium on Zoo Research, 1st-2nd July 2014

This year's symposium will be hosted by Blair Drummond Safari Park, Stirling on 1st and 2nd July. The theme of this year symposium is "What the BIAZA Research Committee can do for you" to help raise the profile of the work that the Research Committee does within aquarium and zoo collections across BIAZA. As always the symposium hopes to showcase the work of researchers across BIAZA collections from zoo keepers, education staff to academic researchers. The call for abstracts for conference presentations and posters is currently ongoing and will close by 26th May. Oral presentations should be no longer than 15 minutes and posters no larger than A1. The closing date for delegate registration is 9th June.

Further information, registration and presentation/poster applications are available on the BIAZA website: <http://www.biaza.org.uk/news/1479/33/Research-Symposium-2014/d.events/>.

Journal of Zoo and Aquarium Research

The current issue of the EAZA journal 'Journal of Zoo and Aquarium Research' (JZAR) Volume 2: No. 1 (2014) is now available online: <http://www.jzar.org/jzar>. This issue includes research on 'Subspecies identification with mtDNA and morphometrics in captive palm cockatoos (*Probosciger aterrimus*)' carried out at EEP collections and 'Zoos as a context for reinforcing environmentally responsible behaviour: The dual challenges that zoo educators have set themselves' carried out at Chester Zoo. JZAR uses evidence-based research carried out at EAZA living collections presented via full research articles, reviews and technical notes. There is a particular interest for small scale studies and reports of evidence-based husbandry interventions, provided they are supported by data. Potential authors are encouraged to support this initiative by submitting manuscripts on any area of zoo or aquarium-based research. Please visit the website (www.jzar.org) for more details on scope and author guidelines.

BIAZA Awards 2013 – Best Research Project

Many congratulations to Miss Katie Edwards and Chester Zoo on winning the award for best research project 2013. The research project entitled 'Investigating population performance and factors that influence reproductive success in the eastern black rhinoceros (*Diceros bicornis michaeli*)' aimed to address current issues of breeding the critically endangered black rhinoceros in EAZA collections.

Commendation for best research project was awarded to Bristol Zoo Gardens (Interspecific semantic alarm call recognition in the solitary Sahamalaza sportive lemur).

BIAZA Awards 2013 – Small Collection Award

The Small Collection Award for best research project was won this year by Hawk Conservancy Trust for their research entitled 'Field identification of white-headed vultures using plumage patterns'. Many congratulations from the Research Committee.

A report of each of these projects is included in this issue. Well done to all these collections and others who submitted entries for the awards, it is very pleasing to see a good variety of research topics covered and the high standards achieved by all.

BIAZA Research Committee Letters of Support

There were 25 applications for Research Committee support for multi-institution studies in 2013. Of the applicants receiving support 3 were at PhD level, 5 were other postgraduate, 1 undergraduate application and others were zoo/academic institution projects. Applications for multi-institution support continue to be received with 1 MSc application already supported this year to date. For information on applying for Research Committee support please see: <http://www.biaza.org.uk/Research/general-guidance/>.

We believe this is a valuable service both for the researchers and for BIAZA members but we would appreciate any feedback from either point of view that may help us improve the system. Please send any comments to Fatemah at the BIAZA office (ga@biaza.org.uk).

Feature Article

BIAZA Research Awards 2013 – Winner

Investigating population performance and factors that influence reproductive success in the eastern black rhinoceros (*Diceros bicornis michaeli*)

Katie Edwards, Sue Walker and Susanne Shultz, North of England Zoological Society, Chester Zoo

Introduction

Ex situ populations play a vital role in the conservation of critically endangered species such as the black rhinoceros. However, for *ex situ* populations to reinforce *in situ* conservation efforts, it is important that they are self-sustaining, both demographically and genetically, to maximise their future viability. However, *ex situ* populations of black rhinoceros in North America have not previously been self-sustaining (Carlstead and Brown 2005; Carlstead et al. 1999a; Carlstead et al. 1999b; Smith and Read 1992), as high rates of mortality and inconsistent rates of reproduction have limited population growth. However, the cause of sub-optimal reproductive performance is not fully understood, and the performance of the European population of black rhinoceros was yet to be formally assessed.

The aim of this research was to determine the sustainability of European captive population of eastern black rhinoceros, and investigate factors that may influence population performance.

A number of objectives were established to guide this research:

1. *To determine in situ demographic parameters in this species and establish indicators of population performance.*
2. *To determine the viability of the European captive population of eastern black rhinoceros, and ways in which performance could potentially be improved.*
3. *To investigate whether differences in reproductive hormones are related to reproductive success.*
4. *To investigate whether social or environmental factors are related to adrenal activity.*
5. *To investigate whether social or environmental factors are related to reproductive success.*
6. *To investigate differences in hormone metabolite concentration between regular and irregular oestrous cycles.*

Methods

Demography and population viability analysis

Demographic data compiled on 1) *in situ* populations of eastern black rhinoceros in eight Kenyan reserves and 2) the *ex situ* European endangered species breeding programme (EEP) studbook, were used to calculate age-specific mortality and fecundity. Simulated population projections based on these data were used to compare projected growth rates between populations, to assess demographic sustainability. Sensitivity analysis was also performed to determine which aspects of population demography had the greatest potential to influence population growth rate. Key performance indicators relating to mortality, reproduction and population structure were compared between *in situ* reserves, between *in situ* and *ex situ* populations, and within the *ex situ* population over time (last 10 years versus last 25 years), to identify where there may be the most potential for improvement. Furthermore, the genetic viability of the EEP population was also assessed using studbook pedigrees to determine historical founder representation and genetic diversity within the current population.

Factors influencing reproductive success: subjects and faecal hormone analysis

This study included 62 eastern black rhinos situated at 13 zoological institutions across Europe, (23 males and 39 females), representing around 90% of the EEP population that had been at or approaching reproductive age during the study period. A total of 11,222 faecal samples (9,743 female, 1,479 male samples) were collected over a collection period lasting between 4 months and 6 years. Hormone metabolites were extracted from faecal samples using a wet-weight shaking extraction method. Enzyme immunoassays (EIAs) were validated and used to measure testosterone (males), progesterone and oestradiol (females), and glucocorticoid (males and females) metabolite concentration.

Intrinsic factors and reproductive success

Study subjects were characterised according to whether they had previously produced (or sired in the case of males) a calf (proven vs. non-proven) and whether or not they had bred during the last seven years. Hormone metabolite concentration was compared between reproductive categories. In females, faecal progesterone metabolite concentration was used to characterise oestrous cycles, according to a pre-established method (Brown et al. 2001). Oestrous cycles were categorised by length, and the relative proportion of different cycle types observed among reproductive categories were compared using cross-tabulation with Pearson's chi-squared. In males, faecal testosterone metabolite concentration was compared between males according to reproductive categories, using generalised linear mixed models (GLMMs) to control for repeated sampling within multiple individuals. Other intrinsic factors investigated included the expression and regularity of oestrous behaviours during the study period (females only; based on keeper observations), and the body condition score (BCS) of all study subjects, determined using a combination of direct observation and assessment of photographs, and following a previously established scoring system (Reuter and Adcock 1998).

Extrinsic factors, adrenal activity and reproductive success

Faecal glucocorticoid metabolite (fGCM) concentration was used to investigate the impact of social and environmental factors on adrenal activity, and ultimately on reproductive success. A questionnaire sent to each institution was used to collect information on each individual, relating to their captive environment, including social and environmental factors, and keeper ratings on various aspects of individual behaviour. Differences in fGCM concentration and reproductive success were investigated according to these extrinsic variables.

Hormonal correlates of irregular oestrous cyclicity

18 non-pregnant adult females were used to investigate hormone differences between regular and irregular oestrous cycles and between breeding and non-breeding females. Reproductive (progesterone and oestradiol) and adrenal hormones were compared using GLMM.

Results

Demography and population viability analysis

In situ populations of eastern black rhinoceros in eight reserves in Kenya were projected to grow at rates between 2.26% and 7.04% per annum (mean 5.09%). In comparison, the *ex situ* population was projected to grow at between 0.52% and 2.12% per annum, based on demographic data compiled over the last 10 and 25 years respectively. This indicates that although self-sustaining, the growth of the European captive population is below the rate of *in situ* populations and below that of a proposed target of 5% growth per annum. Furthermore, predicted growth rates based on the last 10 year period are below that of the last 25 years, suggesting that growth may have slowed over the last decade.

Performance indicators were compared within the *ex situ* population over time and to *in situ* populations in Kenya, which revealed that *ex situ* females were starting to reproduce later, with longer inter-birth intervals, resulting in a lower proportion of females breeding per annum. Furthermore, when comparing the two time-frames of the *ex situ* population, a lower proportion of breeding-age females produced offspring during the 10-year period from 2001-2010 than between 1986-1995 (40.7% versus 52.9% of females age 5-32), and those females that did breed produced fewer calves (mean 1.54 versus 2.11 calves produced in a 10-year period).

Pedigree analysis of studbook data revealed that this reproductive skew has been apparent throughout the studbook, with founder representation ranging from 0.26% to 10.24% and 50.8% of males and 44.4% of females died or left the population without first producing offspring. This unequal contribution has resulted in a founder genome equivalent of 13.39 wild-caught founders had they all reproduced equally. The reproductive skew is still apparent, with 42.1% of mature males and 48.6% of mature females in the current population yet to reproduce.

The current population has retained an estimated 96.27% of the genetic diversity of the founder population, but slow rates of growth and continued unequal representation could mean that this level of diversity may not be retained over the next 100 years.

Intrinsic factors and reproductive success

A high incidence of irregular cyclicity was observed across the study period. A total of 411 oestrous cycles were characterised within females of reproductive age (5-32; 14 proven and 15 non-proven) during the study period. These were categorised as short (<20 days), normal (20-40 days) or long (>40 days); periods of acyclicity were also observed, characterised by progesterone metabolite concentration remaining at baseline for over 10 days. Although all four cycle types were observed among proven and non-proven females, the relative proportion of different cycle

types varied between reproductive categories ($\chi^2=9.116$, $df=3$, $P=0.028$). Specifically, long cycles were observed more often in non-proven females ($P<0.05$), and acyclic periods were more common in proven females that had not bred for over seven years ($P<0.05$).

Non-proven females were less likely to exhibit regular signs of oestrus as reported by their keepers, and were scored as having higher body condition scores than proven females ((Mann Whitney $U=52.500$, $P=0.004$)); this effect was not apparent in males. However, among males, faecal testosterone metabolite concentration (Tt) was significantly lower in non-proven than proven males (GLMM $\chi^2=7.730$, $df=1$, $P=0.005$). Males that had not sired for at least seven years tended to exhibit intermediate testosterone compared to both non-proven males and those that had bred more recently, but this was not significant.

Extrinsic factors, adrenal activity and reproductive success

A number of social and environmental factors were correlated with faecal glucocorticoid concentration. However, these extrinsic factors were not correlated with reproductive success. However, the temperament of individual rhinos may be of relevance, as in both male ($\chi^2=4.022$, $df=1$, $P=0.045$) and female ($\chi^2=20.252$, $df=1$, $P<0.001$) black rhinos, individuals that were scored as more unpredictable in their behaviour exhibited higher fGCM concentration than those that almost always behaved the same. Furthermore, non-proven females tended to be scored as more unpredictable (Mann Whitney $U=71.0$, $P=0.093$). This may indicate that individuals that are more susceptible to changes in their environment may have increased adrenal reactivity to potential challenges, which could be one factor involved in differential reproductive success.

Hormonal correlates of irregular oestrous cyclicity

Within subject females, fGCM concentration was higher during periods of irregular cyclicity compared to during periods of regular cyclicity ($\chi^2=5.052$, $df=1$, $P=0.025$). Furthermore, fGCM were higher during longer cycles (>40 days) than during 20-40d cycles ($\chi^2=16.905$, $df=1$, $P<0.001$), short cycles (<20d; $\chi^2=5.461$, $df=1$, $P=0.019$) and periods of acyclicity ($\chi^2=17.505$, $df=1$, $P<0.001$). Faecal oestradiol metabolite (fE2) concentration was lower during periods of irregular cyclicity ($\chi^2=6.269$, $df=1$, $P=0.012$), and furthermore was significantly lower during acyclic periods ($\chi^2=38.394$, $df=1$, $P<0.001$), which could indicate impaired follicular development, resulting in reduced secretion of oestradiol.

Summary

Sub-optimal rates of reproduction and an unequal contribution of offspring across individuals could have important consequences for the future viability of this population. Demographic viability may be limited through reduced growth rates and genetic viability through unequal founder representation and loss of genetic diversity. This study has highlighted intrinsic differences in reproductive hormones between breeding and non-breeding males and females that could be related to differential reproductive success. However, this does not appear to be due to chronic adrenal activity, or to social or environmental aspects of the captive environment. However, behavioural differences in the way in which individuals respond to their environment may warrant further investigation. Additionally, differences in hormone concentration within females prior to and during periods of irregular cyclicity require further investigation, to determine whether irregular oestrous cycles are the result of disruption of normal oestrous cyclicity, and what factors may lead to their occurrence.

Implications of this research

This research improves our understanding of factors that may influence population performance in this species, which could be beneficial to population management both *in situ* and *ex situ*. These techniques would also be beneficial if applied to other species, particularly other *ex situ* breeding programmes where sustainability is vital to long-term success. The use of hormone analysis has proven to be a useful tool in breeding management of black rhinoceros at Chester Zoo, giving keepers additional information about their animals to help guide introductions. Previous to this study no rhinos had been born at the zoo for 10 years, the application of this research has played a role in the successful birth of five black rhino calves at Chester Zoo in the last 5 years.

References

- Brown J.L. *et al.* 2001 *Zoo Biology* 20, 463-486.
- Carlstead K. and Brown J.L. 2005 *Zoo Biology* 24, 215-232.
- Carlstead K. *et al.* 1999a *Zoo Biology* 18, 17-34.
- Carlstead K. *et al.* 1999b *Zoo Biology* 18, 35-52.
- Reuter H.O. and Adcock K. 1998 *Pachyderm* 26, 116-121.
- Smith R.L. and Read B. 1992 *Zoo Biology* 11, 375-383.