Grant recipient:

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Project title: Applying a new reliable and rapid field-based pregnancy test to the monitoring and management of endangered black rhinoceros.

Country for proposed grant: Republic of South Africa.

FINAL PERFORMANCE REPORT:

1, A-C. CURRENT PROJECT STATUS

A: Utility of new black rhino field based faecal assay

The projects aims were threefold (progress in achieving each aim is documented below):

(a) To test the utility of a new rapid field-based pregnancy assay for monitoring the reproductive performance of large free-ranging populations of rhinoceros.

We successfully installed horn implant transmitters into a total of 14 adult female black rhino (7 in 2007/08, 7 in 2008). This is 4 more than the 10 adult females originally proposed. Each of the 14 female black rhino have been regularly located and sampled, approximately once weekly over 12 months by Mr. Roan D. Plotz (RDP) and Zulu field ranger Mr. Bom E. Ndwandwe (BEN) (see Figure 3 & 5).
We collected a single blood sample and rectal faecal sample from each of the 7 adult female black rhino sedated during horn-implant installation in November 2008. In addition, we were able to collect blood and rectal faecal samples from 2 other adult females to provide a total of 16 blood and rectal faecal samples (including the 7 samples collected in 2007-08). This is 6 more than the 10 originally proposed. RDP and BEN also collected 115 faecal samples from all 14 adult females at approximately 1 to 2 month intervals for a period of at least 12-months (see Figure 5).

We extracted 6 hormone controls from black rhinoceros faecal samples of known pregnancy status (determined via blood test) that will be used as our pregnancy indicators (see MacDonald et al. 2008; submitted with this report) and used them to conduct an initial field-based pregnancy assay of 80 faecal samples (see Figure 4). Early assays resolved difficulties of obtaining assay ingredients at the field site and setting up a small laboratory. We plan to have all 16 blood and rectal faecal samples analyzed independently for pregnancy within labs at SBS-VUW within the next 6-months. We will use these results to determine the accuracy of free ranging black rhino faecal pregnancy analysis. After obtaining CITES permits all 16 blood and rectal faecal samples, and 115 faecal samples, were transported from our field site; Hluhluwe-iMfolozi Park (HiP), KwaZulu-Natal, South Africa, to the laboratories at our home organization; School of Biological Sciences at Victoria University of Wellington (SBS-VUW), New Zealand. We will shortly re-assay those samples there using the more accurate radioimmunoassay (RIA) techniques for comparison with the results of our field-based assays. These final laboratory-based assays will also allow us to make comparisons with previous field faecal pregnancy analyses (see Macdonald et al. 2008, included with this report).

RDP arrives back at SBS-VUW to complete faecal hormone assays, and collate and analyse all field data, towards the submission of his Ph.D. thesis, and related articles for peer-review, in 2010. The horn-implant radio transmitters were operational longer than expected such that five of the adult female black rhino still have working radio transmitters. Thus, RDP trained and employed Mr. Andrew Paul Stringer (APS) to collect additional faecal samples in his absence. In so doing we will maximize data from the rhinoceros with horn implant radio-transmitters and increase overall sample size.

The compilation of a detailed report for HiP management, currently in preparation and awaits the results from laboratory pregnancy analyses. In the interim and in order to inform HiP management of our progress, we submitted a detailed progress report (Plotz, Linklater & Kerley, 2008a). Furthermore, we have continued to highlight whenever possible the potential benefits of black rhino faecal pregnancy analysis and have published an article in *Aardvark*, the newsletter of the Zoological Society of Southern Africa (Plotz, Linklater & Kerley, 2008b; Appendix I). RDP also made oral presentations at four local forums; an African rhinoceros workshop (Plotz & Linklater, 2007), rhinoceros management group meeting (Plotz & Linklater, 2008b), Ezemvelo KwaZulu-Natal Wildlife (EKZNW) research conference (Plotz & Linklater, 2008a, Appendix II) and HiP research forum (2009b) in which he outlined our project and its potential implications. *Rufford Small Grants* was acknowledged in the *Aardvark* article (See Appendix I), African Zoology manuscript (Plotz & Linklater, 2009a, already submitted) and all oral presentations (RSG logo used).

In summary, objectives are almost complete in that all faecal samples have been collected but await laboratory confirmation of field-based assay results. The time and effort needed for collecting 115 faecal samples, as well as the ongoing monitoring of body condition scores, vegetation and location data in order to meet all goals delayed final pregnancy analysis. Nevertheless, we now have all the necessary data and samples to enable us to achieve all objectives in early 2010 when RDP returns to SBS-VUW from the field site.

(b) To train local Zulu field-rangers and management how to incorporate pregnancy testing into long-term monitoring of the strategic Hluhluwe-iMfolozi (HiP) donor population of black rhinoceros

We have trained a Zulu field ranger, Mr. Bom E. Ndwandwe (BEN) (see Figure 3 & 5) in the correct procedures for collection and storage of black rhino faecal samples. As a consequence of this training BEN has recently gained promotion to the newly created and ongoing position of HiP black rhino monitor. HiP-Research Centre at Hilltop (see Figure 4) has acted as our field laboratory where 80 faecal samples were analysed in the presence of two members of HiP research management (i.e. Section Rangers, Conservation Managers). This can be repeated as the technique is perfected (see Figure 4). The closable plastic faecal storage containers were distributed by us to the various Section Rangers earlier in the year and have brought us several additional faecal samples in return. A written training and protocol manual to be used and incorporated by HiP management as part of an ongoing monitoring technique for black rhinoceros in HiP will follow after the completion of our report. Extra glass jars for pregnancy analysis have been contributed to HiP-Research and additional equipment will be provided once our project is completed to assist in the transfer of this technique to field staff. Reports and peer-reviewed manuscripts have already been submitted and the training manual is in progress and scheduled for completion as RDP completes his Ph.D. thesis in 2010. It was delayed by the initial difficulties encountered in the first trials of the technique at the field site.

(c) *Determine whether the poor breeding performance of the HiP population is attributable most to poor conception rates, pregnancy termination, or depredation of young calves.*

We will shortly (within 6 months) be completing the laboratory assays of all of the 115 faecal samples (Goal a) that we have collected. We will than collate our results to determine the pregnancy rates and pre-natal calf losses of our 14 female black rhino. Four out of 11 females of breeding age were followed through to the birth of their subsequent calf (see Table 1: 3 of the 14 were sub-adults). In addition, our monitoring shows that every female of breeding age in our cohort has had a calf (see Table 1). Once the faecal samples are analyzed we will combine these results with our field observations of calf birth and survival to more accurately determine pregnancy rates. To accurately estimate population pregnancy rates it is particularly important to pregnancy test the female rhinoceros that had dependent calves at the start of the study but did not produce another calf during our observations. Whether or not they were pregnant prior to calf independence and the end of field observations has a large influence on population fecundity rates. APS is currently monitoring the remaining five female black rhinoceros with working radio-transmitters in the hope of detecting any new calf births. We will marry our results from pregnancy analysis with observations of calving and calf survival to ascertain which life stage may be limiting population performance in HiP (Plotz, Linklater & Kerley, 2008b). At this time, conception and birth rates are high in this strategic source population and higher than supposed previously, which suggests that low fecundity is unlikely to be limiting population performance. Managers should probably look elsewhere for the cause of poor population growth.

Our observation of a black rhinoceros calf that succumbed to its injuries after a lion predation attempt confirms that predation may at least in part account for the longer inter-calving intervals, low numbers of calves per adult female, and poorer population growth observed previously (Plotz & Linklater, 2009a, already submitted). We have focused on the potential implications this may have for conservation managers of black rhinoceros. Predation is rarely considered when managing a black rhinoceros population for improved productivity, although predation’s role in HiP’s black rhinoceros population has been debated before (Fanayo et al. 2006).

Female body condition also indicates that range condition is not limiting calving rates and recruitment. We regularly measured the body condition of all 14 females over a 15-month period using Reuter and Adcock’s (1998) body condition scoring system (see Table 2). ASP continues to score the condition of the 5 new females that still have working radio-transmitters. In addition, other than our cohort of females and calves, we have sighted and recorded the body condition of more than 60 other identifiable male and female black rhino in Masinda, Nqumeni and Mbhuzane sections of HiP. This equates to 45% of all known black rhino in these sections and 27% of the entire known HiP black rhino population of

270. A mean body condition score of 3.0 (average, 1.0 is very poor and 5.0 is excellent, Reuter & Adcock, 1998) from a subset of 7 female black rhino during winter 2007 (see Table 2) suggests that food is not significantly impacting on their fecundity and will help, we hope, together with our data from faecal pregnancy rates and vegetation sampling (see Figure 1 & 2), resolve the debate about how best to explain and manage the poor performance of source populations such as HiP.

We have collected over 300 GPS locations, over 15 months, from our 14 females with radio transmitters in the Masinda and Nqumeni sections of HiP. Our team has also collected an additional 100+ GPS locations of black rhino in the Mbhuzane section (n=10). From these locations we have calculated average mean winter home range sizes of 8.53 km^2 for the Masinda section (n=6) and annual mean home range size of 7.20 km^2 in the Mbhuzane Section of HiP (n=10; Plotz, Linklater & Kerley, 2009). These average home range sizes are 3-4 times smaller than the average annual home range size of 23 km^2 published by Reid *et al.* (2007). Based on the fact that home range size has been used as a proxy for range condition, where larger home ranges indicate poor resource conditions, Reid et al. (2007) concluded that range condition is deteriorating and carrying capacity is reduced for black rhinoceros in HiP. Our measures confirm that Reid et al. (2007) estimates are inflated as they base their findings on a small number of locations recorded over ten years (i.e. 1-3 sightings per year by field rangers) but then compare their estimates to historical studies with sighting frequencies over shorter periods.

Based on our analyses of HiP home range sizes above, we recently published an article in the international conservation journal *Oryx*, where we highlight the problem of using historical home ranges that are collected in different ways to argue that habitat has deteriorated (Linklater et al., 2010; submitted with this report). This additional work is a direct consequence of *Rufford Small Grant* funding investigations. Please also note that the authorship on this article indicates that it is a consensus of opinion of leading rhinoceros researchers from several institutions. Slotow et al. (2010) responded to our concerns (also submitted with this report). We remain concerned, however, with the use of poor quality data to infer habitat deterioration in HiP. Consequently, Linklater et al. (2010) and Plotz & Linklater (2008a-Appendix II & b; 2009b) has been our research groups attempt to highlight these problems for local population managers as a precursor to a manuscript in preparation where we will publish the home range data we have collected and report average annual home ranges mentioned above and in Plotz, Linklater, Kerley (2009).

We have recorded 181 browse abundance surveys for 10 of our adult females every time we sighted them and this will help to determine what preferred black rhino browse is. We have completed 128-vegetation sampling points along transect lines over a 126 km^2 area within HiP (see Figure 2). We have also recently completed a further 241 vegetation sampling points within individual black rhino home ranges that we have calculated (see Figure 1). The information from our extensive vegetation sampling will enable us to determine what browse black rhino prefer, and then compare how much of that preferred browse is available within as well as outside their preferred habitats (i.e. home ranges). Ultimately, we will be able to assess whether HiP black rhino are resource limited and the abundance of preferred black rhino browse in an area that is one ninth of the entire Park (126-km^2 out of 960 km^2).
In summary, most objectives have been achieved. The data set is complete and analyses will be completed during 2010 as RDP writes his Ph.D. thesis. The large number of body condition scores, home range locations and vegetation surveys that

we have collected will allow us to determine whether HiP's black rhino are resource limited. We have already discovered that lion and spotted hyaena predation of black rhino calves is occurring and that it may, at least in part, account for poor population growth (Plotz & Linklater, 2009a) and we will use all findings to write a report and additional manuscripts that outlines what may be limiting this and other populations of black rhino (*Obj. e.*). Preliminary results have already been communicated to EKZNW population managers as an interim report (Plotz, Linklater & Kerley, 2008a) and oral presentations (Plotz & Linklater, 2008a & b; 2009b).

2. PROJECT IMPACT

The project was targeted at testing the utility of a new rapid field-based pregnancy test to better monitor and manage black rhino. We collected 115 faecal samples (see Figure 5) and our trial and demonstration analyses of these samples (see Figure 4) to HiP management has already highlighted but also resolved some of the potential difficulties in conducting pregnancy assays in a field setting (e.g., incorrect mixing and proper storage of chemicals) and time constraints of ongoing monitoring of free-ranging black rhinoceros. However, we will continue to collect and analyze all our samples to determine the utility of this technique for monitoring and managing black rhinoceros. Preliminary results also indicate that calf mortality (e.g. Plotz & Linklater, 2009a), not poor range condition (Linklater et al., 2010) and fecundity (e.g., see Table 1), are the most likely causes of historically poor population performance.

We have endeavoured to increase the impact of our findings by publishing our results to a wider audience, through oral presentations attended by HiP management (Plotz & Linklater, 2007, 2008 a, b & 2009), reports (Plotz, Linklater & Kerley, 2008a) and peer reviewed manuscripts in journals (Plotz & Linklater, 2009a, submitted with this report; Linklater et al. 2010, submitted with this report) and we will continue in this way as our analysis progresses. Furthermore, our project continues to gain media publicity (e.g., Wild Touch, *South African Broadcast Channel 1*, 2008; Morris, 2009 *Wild Magazine*, submitted with this report), enabling us to distribute this growing knowledge to an even wider audience. Most importantly, we are having a significant impact on HiP managements understanding of the performance of this strategically important black rhino population. As such, our project continues to have important implications for the better management and improved productivity of this critically endangered species in HiP and elsewhere.

TABLE 1: Summary of calving history (2007-2009) of 14 female black rhino in HiP fitted with radio-transmitters from 2007-09. Note that all adult females (F) of breeding age (n=11) were sighted with a calf and 4 were followed through to a subsequent calf. * = Radio-transmitter not installed in that year. ⊕ - new calf observed. Age classes: **A** = 0 to 6 months, **B** = 6 to 12 months, **C** = 1 to 2 years, **D** = 2 to 3.5 years, **E** = 3.5 to 7 years, and **F** = 7 years+ (breeding age) *(From Plotz & Linklater, 2009b).* *(From Plotz & Linklater, 2009b)*

ID	Age	Age of calf in 2007	Age of calf in 2008	Age of calf in 2009
950	**F**	**C/D calf**	**A calf (Sep) ⊕**	**Translocated**
210	**F**	**D calf**	**A calf ⊕**	**Last seen Jan. 09**
450	**F**	**D/E calf**	**B calf ⊕**	**C calf**
250	**F**	**C calf**	**C/D calf**	**D calf**
960	**F**	**D calf**	**D/E calf**	**E calf**
281	**E**	**Not of breeding age**	**Not of breeding age**	**Not of breeding age**
970	**F**	**C/D calf**	**A calf (May) ⊕**	**B calf**
420	**F**	**---------***	**B calf**	**Trans. fail**
360	**E**	**---------***	**Not of breeding age**	**Not of breeding age**
290	**F**	**---------***	**C calf**	**C calf**
880	**E**	**---------***	**Not of breeding age**	**Not of breeding age**
410	**F**	**---------***	**C calf**	**C calf**
840	**F**	**---------***	**B/C calf**	**C calf**
530	**F**	**---------***	**B/C calf**	**C calf**
Total: 14				**11F/3 E**

TABLE 2: Mean winter (April to October) 2007 body condition scores of 7 female black rhino in Hluhluwe-iMfolozi Park. Note: resource conditions are generally poorer in winter (low rainfall, cold) than summer (high rainfall and hot). Note, 1.0 = very poor condition, 5.0 = excellent body condition; Reuter & Adcock, 1998).

Rhino ID	# Number of body cond. scores collected	Mean body cond. score
950	14	3.0
970	16	3.0
960	21	3.0
210	19	2.5
250	25	2.5
450	18	3.5
281	8	4.0
Mean (n=7):	**17**	**3.0**

Figure 1: Section of Hluhluwe-iMfolozi Park illustrating 241 GPS points (e.g., green dots) randomly placed within the home ranges of 10 female black rhino (e.g., coloured polygons). A vegetation survey was conducted at each GPS location in order to determine the abundance of preferred black rhino browse within and outside the home ranges of our cohort of black rhino. Note: The red polygon is a 126 km^2 universal home range surrounding all 10 adult female black rhino

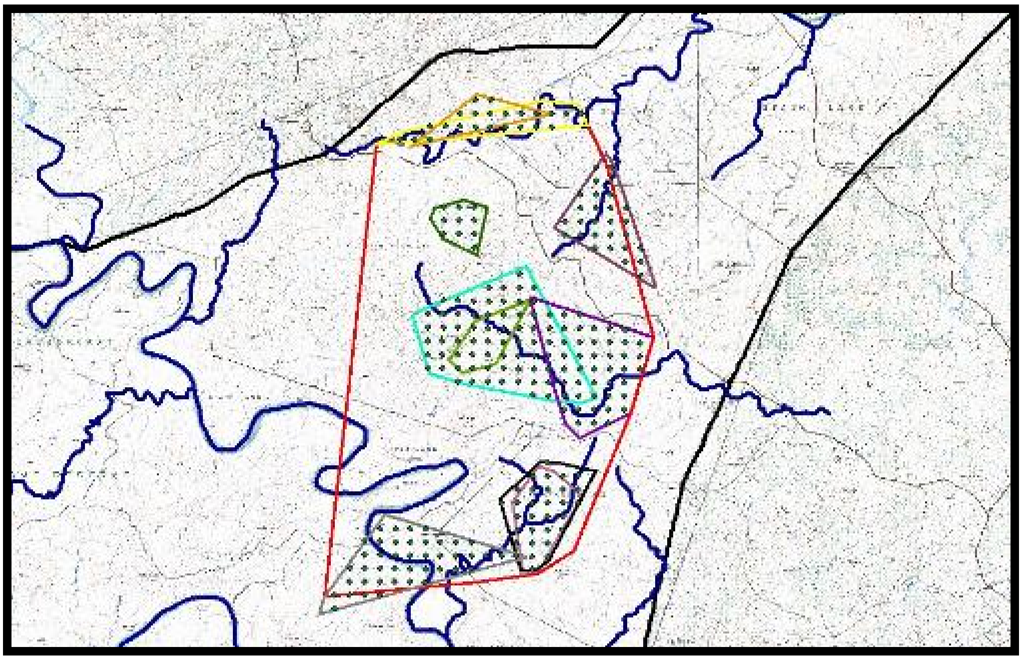

Figure 2: Section of Hluhluwe-iMfolozi Park illustrating 128 GPS points (e.g., green dots) randomly placed within the universal home range of 10 female black rhino (e.g., red polygon). A vegetation survey was conducted at each GPS location in order to determine the abundance of preferred black rhino browse within and outside the home ranges of our cohort of black rhino. Note: The red polygon is the 126 km^2 universal home range surrounding all 10 adult female black rhino.

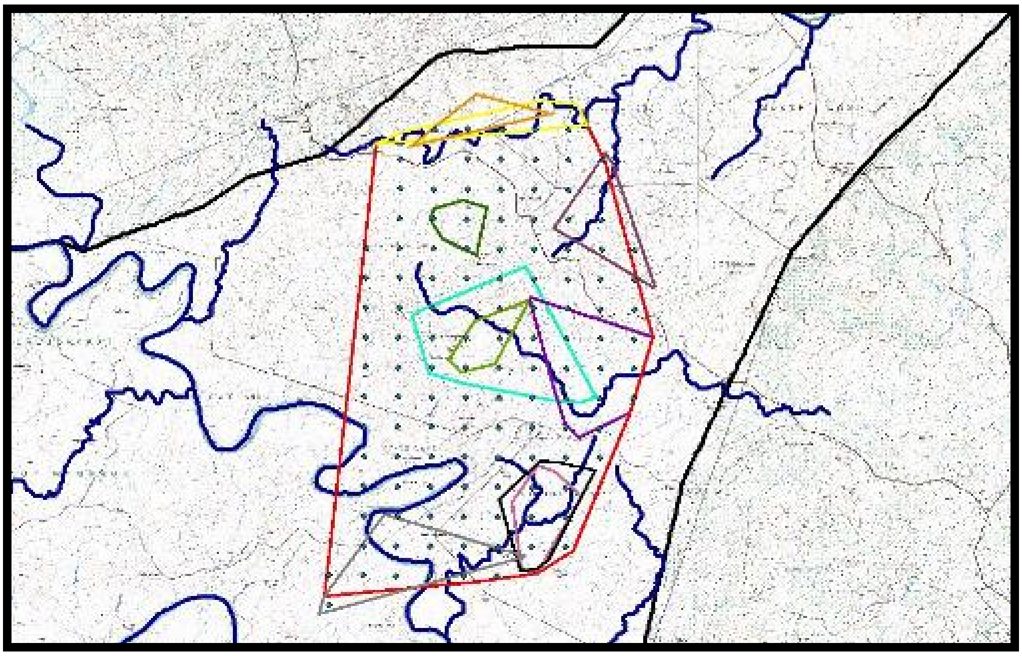

Photo: Dale R. Morris

Figure 3: Searching for black rhinoceros in Hluhluwe-iMfolozi Park, Roan Plotz uses radio-telemetry while Bom Ndwandwe scans the horizon.

Photo: Rosalynn Anderson-Lederer

Figure 4: Roan Plotz and Rosalynn Anderson-Lederer run black rhinoceros faecal pregnancy assays in field laboratory at cabin at Hluhluwe-iMfolozi Park-Research Centre, Hilltop.

Photo: Wayne L. Linklater

Figure 5: Roan Plotz (front) and Bom E. Ndwandwe (back) collecting black rhinoceros faecal samples in Hluhluwe-iMfolozi Park for pregnancy analysis.

3. FINANCIAL STATUS REPORT Equipment for faecal pregnancy assay		RS PROPOSED	RSG ACTUAL
VMR Plates 1		204	**204**
TMB Tablets 2		211	**211**
Tubes & rack 3		161	**161**
Test tubes and tops 4		70	**73**
Total Lab 20 MicroL pipette 5		98	**130**
Bursary/salary			
Trained Zulu Game Guard for fieldwork 6	GBP 179/ month x 8 months/ year (ZAR 2, 500/ month):	1, 432	**1, 432**
3 x Trainee Zulu field rangers for 2 month 7	Trained 2 trainee field ranger (GBP214 each)	642	**428**
Transport/ travel			
Vehicle running (including fuel) & maintenance 8	± 800km/ month calculated @ ZAR 3 per km for 5 months = ZAR 12, 000/14=: Large increases in fuel costs from May '08(800km x ZAR 4 per km for 3 months = ZAR9, 600 = R21,600/14=GBP171 x 5 months = GPB),	1, 371	**1, 543**
Operating expenses			
6 x Radio- transmitters for radio-telemetry tracking 9	Purchased 6 radio-transmitters (ZAR 1, 830 each = GBP 130 each)	780	**780**
TOTAL:		4, 969	**4, 962**

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APPENDIX I.

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Accessible online: http://www.zssa.co.za/images/aardvark/AARDVARK%20APRIL%202008.pdf

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The reproductive performance and ecology of black rhinoceros

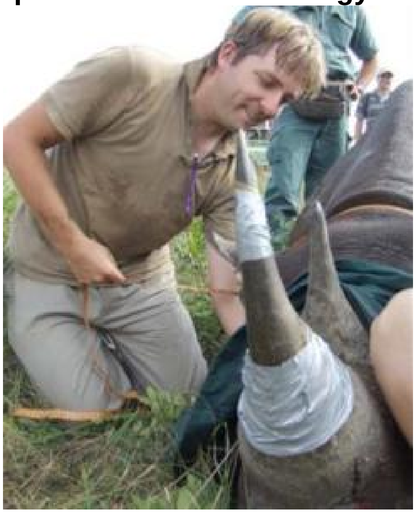

Photo: Liana S. Cahill

Roan Plotz returned to Hluhluwe-iMfolozi Park (HiP) recently to begin a second field season investigating the causes of apparently poor breeding performance amongst the Park's black rhinoceros (*Diceros bicornis*) population. HiP is a strategically important population because it serves as a major source of individuals for ongoing meta-population management and range expansion and so maintaining or improving calving and survival rates is important to the recovery of this critically endangered species. Concerns have been raised that apparently long inter-calving intervals, increases in home range size, and a decline in population size might indicate deteriorating breeding rates. The reasons for poor breeding performance are not well understood but might be attributed to predation of young calves, particularly by spotted hyena and lion, or poor resource conditions for females such that pregnancy rates are poor.

We began Roan's study last year by installing horn-implant transmitters in 7 adult female rhino with the help of Ezemvelo KwaZulu-Natal Wildlife's Game Capture unit, and hope to install up to 13 more this year. Using radio telemetry, Roan is able to make frequent observations of each female to track her reproductive state, body condition, habitat use and quality, and interaction with other rhino. The ability to monitor the reproductive status of rhino in the field has been enhanced by the development of a rapid, inexpensive, and non-technical colour-change pregnancy test that uses dung, just like the modern human colour-change pregnancy test uses urine. The combination of radio telemetry and pregnancy testing technologies mean that regular samples of fresh dung can be used to measure pregnancy, pregnancy loss, and calving rates across the population and between seasons for the same females.

If pregnancy rates are poor and there is evidence for pregnancy loss, and these correspond to poor maternal body and range condition, then the population's breeding performance is probably resource limited. However, if pregnancy rates are high but there is significant calf loss that is unrelated to maternal condition and home range quality, then the evidence implicates other factors, perhaps predation, as limiting breeding performance. Whatever the result the outcome will assist in better managing the population because there is uncertainty about whether greater removals of black rhinoceros for translocation to other reserves will improve performance by reducing density. Field work for this study will be completed before the middle of 2009.

Roan Plotz*: Ph.D. candidate in the Centre for Biodiversity and Restoration Biology at Victoria University of Wellington and advised by Wayne Linklater and Graham Kerley. Roan's work is currently supported by the US Fish & Wildlife Service, ****Rufford Small Grants****, Australian Geographic Society, and Enkosini Conservation Trust.*

Wayne Linklater*: Centre for Biodiversity and Restoration Ecology, Victoria University of Wellington, New Zealand.*

Graham Kerley*: Centre for African Conservation Ecology, Nelson Mandela Metropolitan University, South Africa.*

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APPENDIX II

Plotz, R.D. & Linklater, W.L. (2008). *Are black rhinoceros Diceros bicornis home range sizes in Hluhluwe-iMfolozi Park increasing in response to deteriorating range condition?* Ezemvelo KwaZulu-Natal Wildlife Conservation Symposium (Conservation in Practice), 25th to 27th November. Queen Elizabeth Park, Pietermaritzburg, KwaZulu-Natal, South Africa.

Conservation Symposium Abstract

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Home range size in black rhinoceros is used as a proxy for range condition and to detect deterioration of ranges through time. Since Hluhluwe-iMfolozi Park (HiP) serves as a strategic source population for black rhinoceros range expansion, an accurate estimate of home range size is critical for management of the species. Reid *et al*. (2007) report a mean home range size of 23.07 km^2 for HiP, 54% larger than historical estimates (Emslie, 1999). Based on their findings they conclude deteriorating range condition and reduced carrying capacity for black rhinoceros in HiP. Unfortunately, Reid *et al*. (2007) estimates are based on a small number of locations recorded over ten years meaning their estimates may be inflated. Home ranges calculated using data gathered over 10 years where the sighting rate is extremely low (i.e. 1-3 sightings per year) but then compared to estimates over shorter periods with higher sighting frequencies (Emslie 1999) will inevitably lead to different sized home ranges. Lent and Fike (2003) have warned about the dangers of comparing black rhinoceros home range estimates using different sighting rates over varying periods of time and appropriately advocate for annual home ranges for inter-study comparison. We present preliminary data on a cohort of black rhinoceros (n = 10) with VHF radio transmitters that were regularly located in random stratified fashion to estimate annual home range size in HiP. Our findings from years 2004 – 2007 show annual mean home range sizes of 6.40 km^2, which are 72% smaller than the 23.07 km^2 estimates of Reid *et al*. (2007). Therefore, based on our findings, there is no evidence for deteriorating range condition.