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Effects of immobilization on fertility in female black rhino (*Diceros bicornis*)

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Abstract

Forty-six female black rhino were immobilized 113 times in the Sinamatella Intensive Protection Zone, Zimbabwe, from August 1992 to October 1997. The effects of immobilization on inter-calving interval (ICI), calving rate (calves/female/year), conception and calves born/year were assessed. The mean ICI ($n = 17$) was 40.24 ± 4.96 months. There was a significant linear relationship between the number and interval of immobilizations in the preconception interval (PCI) and duration of ICI; further investigations were made to overcome possible effects of temporal autocorrelation. Both a non-linear model and a general linear model (with five independent effects) showed a significant relationship only between the ICI and mean immobilization interval in the pre-conception interval (PCI), but not the mean immobilization interval in the whole ICI (including gestation immobilizations). Both suggested that the relationship between immobilization and inter-calving interval was not the result of temporal autocorrelation and that the immobilization regime significantly affected the ICI. Using the calving rate as a response variable, five effects were tested in a general linear model. Only the immobilization rate in the PCI was significant. For conceptions per calendar month, we examined two effects in a general linear model: the number of mature females immobilized each month, and rainfall in the month of conception plus 2 previous months. Both effects were significant. For the number of calves born/year, the effect of the immobilization regime (the number of mature females immobilized/year) and rainfall were examined. Only immobilization was significant. We suggest that the unusually intensive immobilization regime undertaken at Sinamatella has negatively impacted on female fertility, and discuss possible mechanisms. We also suggest the need to adopt guidelines to minimize the impact of immobilization on fertility in female black rhino.

Key words: black rhino, *Diceros bicornis*, immobilization, fertility, inter-calving interval, foetal loss

INTRODUCTION

Black rhino *Diceros bicornis* numbers have decreased dramatically in the past 40 years because of the demand for rhino horn, mainly from Far Eastern markets (Martin, 1993). The current population is estimated at around 2500 with *c.* 375 animals in Zimbabwe. Protection from poachers and regular monitoring is essential for population recovery.

In the Sinamatella Intensive Protection Zone (IPZ) management plans by the Department of National Parks and Wildlife Management of Zimbabwe (DNP) have included regular immobilization of the population, for radio-collaring, de-horning, ear-notching and transponder fitting, all with the aim of protection and monitoring. This study was an opportunistic attempt, working within the confines of the management regime, to investigate whether immobilization was having an impact on the fertility of female black rhino.

The reproductive cycle of the female black rhino is well documented (Hitchins & Anderson, 1983; Smithers & Skinner, 1990; Owen-Smith, 1992; Bertschinger, 1994; Adcock, 1996). The age at first calving normally ranges from 6 to 9 years. The oestrous cycle occurs year-round and lasts *c.* 35 days. Gestation is 15 months, after which a single calf is born. After calving, the pre-conception interval (PCI) under optimal conditions is about 6–8 months, giving a minimum inter-calving interval (ICI) under natural conditions of about 21–23 months. ICI varies widely, and various factors have been proposed to explain this including density-dependence (Hitchins & Anderson, 1983), nutrition (Owen-Smith, 1992) and possibly female age (Adcock *et al.*, 1998). However, in

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populations at below carrying capacity, a mean ICI of 40 months or greater may be indicative of foetal loss or undetected post-natal calf mortality (Hitchins & Anderson, 1983; Adcock *et al.*, 1998). Calving seasonality is very variable in different range areas. Hitchins & Anderson (1983) noted births throughout the year in the Umfolozi/Hluhluwe population in South Africa, and noted that there did not seem to be a well-defined seasonality in East Africa.

The Sinamatella population is unusual in the intensity with which invasive management operations have been undertaken. This was mainly for repeated radio-collaring because of consistently high failure rates (Alibhai, Jewell & Towindo, 1999). In most other range states immobilization of rhino has been used primarily for essential translocation and one-off management procedures. Similarly, radio-collaring is usually undertaken only to monitor animals after translocation, and not repeated when the collar fails (K. Adcock, pers. comm.).

There is an acknowledged risk of mortality in the black rhino during or shortly after chemical immobilization (Veterinary Unit, 1995, 1996), and in association with translocation (Leader-Williams, 1993; Brett, 1998; Hofmeyr, 1998). Although direct anaesthetic mortalities have been significantly reduced in recent years, translocation mortalities (requiring immobilization) remain unacceptably high and it is likely that stress is an important component (Kock, 1991). No quantitative data seem to have been published on the effects of immobilization on fertility, but the Veterinary Unit of the DNP (Veterinary Unit, 1995) suggested that there were concerns about the potential of an immobilizing drug to cause abortion or premature birth, particularly in the first trimester.

This study was undertaken opportunistically; it was not possible to establish a conventional experimental approach under the management policy of the time. Nevertheless, analysis of the available data suggests that the fertility of female black rhino in the Sinamatella IPZ may have been significantly compromised by the immobilization regime.

METHODS

Study area and black rhino population

The Sinamatella Intensive Protection Zone (IPZ) for the black rhino was established in 1993 (Department of National Parks, 1993). It is an unfenced area of 1500 km^2 within Hwange National Park and the Deka Safari Area in north-western Zimbabwe, and holds an IUCN Key 1-rated population of black rhino of continental importance. The population currently exists at low density (estimated 0.04 rhino/km^2 extending beyond the IPZ boundaries). Reports of frequent sightings in the area about 50 years ago suggest that the species existed at much higher density (Tafangenyasha & Campbell, 1995). Since the designation of the IPZ in 1993, no poaching has been detected and armed scouts of the DNP continuously patrol the area. The woody vegetation in the IPZ is predominantly *Colophospermum mopane*, *Combretum* and *Acacia* species. The area lies at an altitude of *c.* 1000 m. Rainfall is seasonal, falling mainly from November to April, with a mean of 572 mm/year (Tafangenyasha & Campbell, 1995).

Immobilization procedures

Three principal management operations for black rhino were conducted in 1992, 1994 and 1995 and 2 of shorter duration in 1993 and 1997, by the Veterinary Unit of the DNP and the Veterinary Department of the Ministry of Lands, Agriculture and Water Resources with the DNP. These were for a combination of radio-collaring, transponder fitting, de-horning, ear-notching and nutrition trials (Kock & Atkinson, 1993; Veterinary Unit, 1995, 1996). The operations were conducted in the latter part of the dry season, lasting from a few weeks to *c.* 3 months. De-horning was undertaken in 1992 and 1994, and radio-collaring from 1994 onwards. Ear-notching and transponder fitting were undertaken throughout. In each operation individuals were effectively immobilized at random, usually being processed in the order in which they were located by scouts on the ground. Six females were immobilized for translocation from outlying areas into the IPZ in 1993, and 8 females, including those translocated, were kept in holding pens (bomas) for periods ranging from 2 to 6 months during 1993–95. During boma confinement, regular immobilizations were carried out for routine veterinary tests and management (Veterinary Unit, 1996). Other immobilization operations were undertaken on individuals during 1992–98 when necessary to remove and/or replace radio-collars.

Kock & Atkinson (1993) and the Veterinary Unit (1995, 1996) have described the details of the procedures and drugs used in detail. Details of the 1997 operation have not been reported, but it was short (*c.* 2 weeks) and procedures and drugs similar to the previous years were used (C. Foggin, pers. comm.).

Data collection from subsequent monitoring of immobilized animals

From August 1992 to October 1997, 83 black rhino (both sexes) were immobilized 205 times ($\bar{x} = 2.47$) in the Sinamatella IPZ. Of these, 37 males were immobilized 92 times ($\bar{x} = 2.49$) and 46 females were immobilized 113 times ($\bar{x} = 2.46$). Of the 46 females, 14 were not recorded again after 1992, 1 was not recorded after 1994 and 2 died in bomas. Other exclusions were made on the basis of translocation/boma confinement. Of the 46 females, 8 were confined in bomas during part of the study period (including the 2 that died). Of these 8, 6 had been translocated into the IPZ in 1993. These were excluded from the dataset where appropriate, as indicated. The remaining females were included in analyses as indicated. During July 1994 to December 1998, 561 aerial signals and/or sightings of radio-collared black rhino were recorded and 1905 ground-tracking observations of the radio-collared animals (signals and/or sightings) were made. Data gathered from August 1992 until December 1998 (77 months) are reported. Data gathered by the Veterinary Unit of the DNP during immobilization operations were made available to the authors by permission of the DNP.

The marking of animals during immobilization, either by radio-collaring and/or ear-notching, enabled them to be subsequently monitored and female reproductive performance assessed.

Data collection was by 3 independent means. First, aerial monitoring of collared animals was conducted by the DNP at a mean frequency of once every 1.2 months, from 1994 to 1998. Second, systematic ground monitoring was carried out at an approximate frequency of 3 times/week during the dry season (May–October) and 2–4 week intervals during the rainy season (November–April). Systematic ground transects were walked to ensure complete coverage of the IPZ and outlying areas in 1994 (Alibhai *et al.*, 1995) and subsequent monitoring used the same operational census blocks. Third, law-enforcement scouts maintained a presence within the IPZ and recorded rhino sightings (including new calves) and spoor almost every day during the study. All three methods were used to ensure an even sampling of animals, so that if a particular animal had not been seen for a month, a greater effort was made to try to locate it. Owing to high radio-collar failure rates (Alibhai *et al.*, 1999) many animals were tracked by spoor, and subsequently identified by ear-notches or individual features. New calves were also identified by finding small spoor and tracking to get a visual identification. The DNP Veterinary Unit attempted to age all adults which were immobilized either by dentition (Hitchins, 1978) or general visual assessment. Where there was uncertainty, they were classed as adult. Calves and sub-adults were assessed on size as described by Hitchins (1970).

Monthly rainfall data for the Sinamatella IPZ during the study were provided by the DNP (B. Russell, pers. comm.).

Data management and analysis

The possible impact of immobilization on fertility was examined by looking at the following fertility parameters: inter-calving interval (ICI), calving rate (defined as calves/female/year), conceptions/calendar month, and the numbers of calves born/year.

ICI

The ICI period was sub-divided for examination into a pre-conception interval (PCI) and gestation interval, since the PCI is variable and gestation is fixed at 15 months. The PCI was defined as the period from one calving to the conception leading to the next calving. The PCI and ICI were determined retrospectively from calving dates.

A preliminary analysis showed that there were significant linear relationships between 2 parameters of the immobilization regime (number of immobilizations and time from parturition to immobilization) against the duration of the ICI. However, this may have been partly or wholly owing to a temporal autocorrelation, i.e. a female with a ‘naturally’ long ICI would have a greater chance of being immobilized more often and at longer intervals. The possible effects of temporal autocorrelation were investigated in 2 ways.

First, the parameters used to assess the impact of the immobilization regime were investigated. As the number of immobilizations and the time from parturition to immobilization may be related to overall ICI length, 2 parameters that are more independent of the immobilization regime were used:

(1) *immobilization interval* (IMI), where the first IMI was the period from the previous parturition to the first immobilization, the second IMI was period from first immobilization to the second immobilization, etc.;

(2) *immobilization rate*, defined as the number of immobilizations/year.

Second, the following hypothesis was proposed. If the immobilization regime had no effect on the duration of the ICI, then there should be no difference in the relationship between the length of the ICI and immobilizations performed in the PCI period, and those performed in the whole ICI period (i.e. including gestation immobilizations). Gestation immobilizations were recorded as those undertaken on females who subsequently calved within 15 months, and whose pregnancies had therefore not been affected. However, if immobilization had affected other gestations, the females concerned would have been ‘put back’ into the PCI period, thus extending it. Any effect of immobilization would therefore be indicated in the observed PCI period.

The hypothesis was tested in 2 ways. First, the time to last immobilization (TLI) was plotted in each ICI against the length of ICI. TLI was used, as this was the measure of immobilization most likely to show a linear temporal autocorrelation. How this relationship compared with the relationships shown by the immobilization intervals (mean IMI in PCI and mean IMI in ICI) against length of ICI was then examined. Second, the effects of the immobilization rate, the mean IMI, and 3 other independent variables (i.e. mean monthly rainfall from previous parturition to following conception; annual rainfall in season following the previous parturition; the season of previous birth (October–December, January–March, April–June, July–September)) were tested in a general linear model (GLM) with ICI as the response variable.

The effects of immobilizations undertaken in PCI were examined in a separate GLM to those undertaken throughout ICI to avoid problems associated with multicollinearity. It was not possible to include female age as one of the independent variables because accurate ages were available for only a few females; the possible effect of age on ICI was investigated separately.

Calving rate

The calving rate was calculated as the number of calves/female/year. The period from the first immobilization to 15 months after the last immobilization was assessed for each female. The effects of the immobilization rate (number of immobilizations over the same period of time), the mean IMI and 3 other independent variables (mean monthly rainfall over the same period; the period itself from first immobilization to 15 months after the last; the period from previous parturition to first immobilization) were examined with calving rate as the response variable in a GLM. Mean monthly rainfall was taken as the most appropriate measure of precipitation owing to large variation in calving periods. The calving period itself was used as an independent variable to see if the calving rate was simply related to it (i.e. the longer the calving period, the higher the calving rate). The third independent variable, period from previous parturition to first immobilization, allowed for birth-lag effects (i.e. the longer that period the higher the chance for an earlier birth in the measured calving period). Data were analysed for immobilizations only in the PCI, and immobilizations throughout the ICI were analysed separately for the reasons mentioned earlier.

Conceptions per month

It was possible to establish conception dates of calves born in the IPZ from subsequent calving dates. The conception frequency (number of calves born each calendar month) was calculated and this was treated as the response variable in a GLM, testing against 2 independent variables: the number of mature females immobilized in each calendar month, and the total 3-monthly rainfall (rainfall in the same month plus 2 previous months). This 3-month period is suggested as a measure of possible precipitation effect on conception for a browsing species such as the black rhino (R. Emslie, pers. comm.).

Annual calving rate

The relationship between the immobilization regime and fertility for the numbers of mature females immobilized and the numbers of calves born each year (annual calving rate) was examined. Immobilizations were performed in the latter part of the year and gestation is 15 months. If a female had been in early pregnancy at the time of immobilization (year 1) her calf would be expected in year 3. If a female had been in later gestation at the time of immobilization, her calf would be expected in year 2. We therefore examined the relationship between the numbers of mature females immobilized each year and the mean annual calving rate for the following 2 years. To calculate the annual calving rate, the following procedure was used. From 1992 to 1995 veterinary operations were carried out annually (the 1993 operation was of short duration). This effectively provided a 'sample' of the population, and the calving rate was calculated for the number of calves born in the sample that year. In 1994, a systematic ground census of black rhino in the IPZ (Alibhai *et al.*, 1995) was undertaken. From 1994 to 1998 we were able to establish accurately the numbers of calves that were born in the IPZ, which allowed calculation of the annual calving rate as a function of the estimated 'population'. For this reason it was not possible to calculate the annual calving rate in its conventional form (calves/female/year) for the latter part of the study. Instead we used calves/individual/year for the whole study, on the assumption that the sex ratio of the population did not change significantly during this period.

We also examined the possible effect of rainfall on the annual calving rate. If the onset of the rains affected conceptions, the effect would be apparent in the number of births in the following year. The response variable, annual calving rate, therefore had to be measured differently for each effect (annually for the rainfall effect and mean of 2 years for the immobilization effect). The 2 effects were therefore examined independently in a linear regression model.

Data transformation

A $\log_{10}$ transformation of the variables in ANOVA and the y variable in regression analyses was used where appropriate to normalize the data and reduce residual heteroscedasticity.

RESULTS

Inter-calving interval (ICI)

Of 18 ICIs recorded, three came from two females translocated and confined in bomas in 1993. One of these was a female who had conceived before being translocated, was then brought into the boma, and gave birth after release. The second was from a female which gave birth 2 years after boma release and whose ICI was measured from this birth to a subsequent birth. These two were included in the analysis. The third ICI encompassed a boma-confinement period of 4 months and this was excluded from the analysis because this confinement may have delayed conception.

For 17 ICIs from 13 females (see Table 1), the mean ICI was 40.24 ± 4.96 months, the shortest ICI was 23 months and the longest 90 months; 35.3% of ICIs

Table 1. Female age (where known), inter-calving interval (ICI) duration, numbers of immobilizations and mean immobilization interval (IMI), during both the preconception interval (PCI) and the whole ICI period, of 18 ICIs for 13 females. F, first calf of a female; S, second calf of same female; B, excluded ICI due to boma confinement

			No. of immobilizations		Mean IMI (months)	
Female	Female age (years)	ICI (months)	In ICI (gestation)	In PCI	In PCI	In ICI
1	12	50	5 (1)	4	7.25	8.60
2F	–	48	3 (1)	2	15.5	15
2S	–	26	1 (1)	0	0	19
3	–	36	3 (1)	2	7	12
4F	–	40	1 (1)	0	0	38
4S	–	33	1 (1)	0	0	24
5	10	35	3 (2)	1	7	9.33
6F	–	66	2 (1)	1	31	28
6S	–	23	0 (0)	0	0	0
7	–	80	3 (1)	2	31	25
8F	7	27	2 (1)	1	2	13
8S	9	30	1 (0)	1	13	13
9	17	23	1 (0)	1	3	3
10	7	24	1 (1)	0	0	15
11	11	90	2 (0)	2	30.5	30.5
12	–	26	4 (3)	1	6	4.75
13F (B)	–	49	3 (1)	2	12	16
13S	–	27	0 (0)	0	0	0

were 40 months or longer. The distribution of ICIs by number of immobilizations in the PCI showed that for six animals not immobilized, the mean ICI was 23.83 ± 2.65. For six animals immobilized once, the mean ICI was 34.50 ± 6.52. For five animals immobilized more than once, the mean ICI was 60.80 ± 10.29 (the ICI excluded from the analysis was 49 months when the female had been immobilized twice in PCI). The difference between the means (using a log transformation) was highly significant ($F_{2,14} = 6.92$, $P < 0.01$) and pairwise comparison using the Tukey test showed that 0 *vs* 1 immobilization was not significant but 0 *vs* 2 and 1 *vs* 2 were significant ($P < 0.05$). (Since four females provided two ICIs each, we tested for possible bias in variation within and between individual ICI values. Using log transformed ICI values, ANOVA showed that there was no significant difference in ICI duration between females contributing one ICI each ($n = 9$) and those contributing two ICIs each ($n = 8$) ($F_{1,15} = 0.23$, $P > 0.05$).

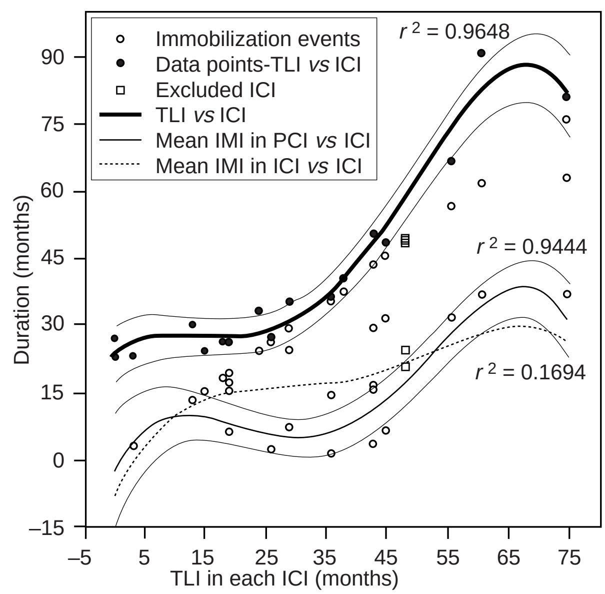

Fig. 1. Time (months) to last immobilization (TLI) in each of 18 inter-calving intervals (ICIs) against duration (months). The three slopes are fourth degree polynomial fits with 95% confidence intervals (in plot of TLI *vs* mean IMI in ICI, 95% confidence intervals are too wide to show). Open circles, chronological history of immobilization events for each ICI on the *y* axis. One excluded ICI (open boxes, immobilization events; closed circles, plot of TLI *vs* ICI). See also Table 1.

The relationship between ICI and time to last immobilization (TLI)

Figure 1 shows the relationship between the period to last immobilization in ICI and the duration of the 17 ICIs. Since the relationship did not fit a simple linear regression model, we examined the polynomial regression model, and following a stepwise procedure (Zar, 1974) we found that the best fit was obtained with a fourth-degree polynomial ($F_{4,12} = 82.11$, $P < 0.0001$, $r^2 = 0.9648$). The polynomial regression fit to the ICI data raised two interesting points. First, the relationship between the period to last immobilization and the length of ICI was not linear, which would have been expected if there was temporal autocorrelation. Second, because of the statistically significant fit to the polynomial regression model, it was possible to examine the extent of any possible effect in different parts of the ICI.

To examine effects of immobilization on the length of ICI, we calculated the mean immobilization interval

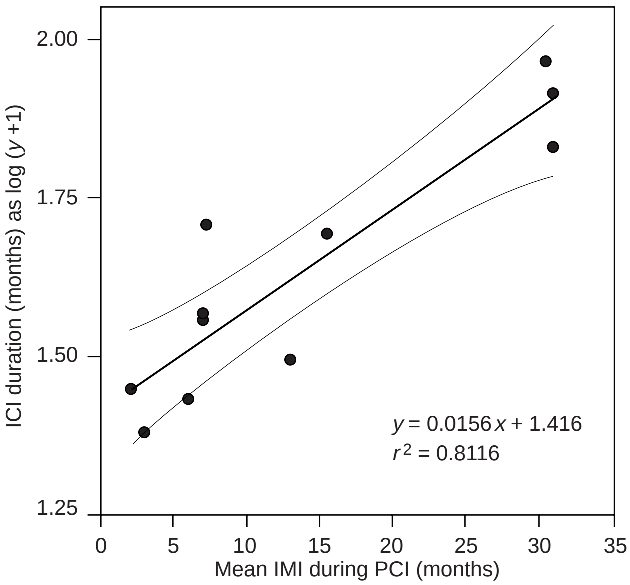

Fig. 2. Mean immobilization interval (IMI) (months) during the preconception interval (PCI) against inter-calving interval duration (ICI) (months).

(IMI) for immobilizations undertaken in the entire ICI (including gestation) and those in PCI only. As Fig. 1 shows, the mean IMI for immobilizations in the ICI gave a poor fit, ($F_{4,7} = 0.36$, $P > 0.05$, $r^2 = 0.1694$), whereas the mean IMI for immobilizations in the PCI gave a highly significant fit to the fourth-degree polynomial ($F_{4,6} = 25.47$, $P < 0.001$, $r^2 = 0.9444$). Indirectly this indicated the relationship between the mean immobilization interval in PCI and the length of ICI as an alternative to linear regression. We had hypothesized that a simple temporal autocorrelation would have resulted in the same relationship being shown by immobilization regime in PCI and ICI, which was not the case. Figure 1 also gives the chronological history of immobilizations for each ICI, on the *y* axis and also shows the ICI which was excluded from the analysis. Untransformed data were used in the above analysis, as we were simply examining the pattern of ICI distribution.

The relationship between ICI and the immobilization regime

ICI (log transformed) was treated as the response variable and the effects of the five independent variables in a general linear model (GLM) were tested. Of the 17 ICIs, six had no immobilizations in the PCI period (see Table 1), these were excluded giving $n = 11$. For the PCI period, the whole model was significant ($P < 0.05$) and accounted for 97.71% of the variation in the length of ICI ($r^2 = 0.9771$). A stepwise regression showed that only two effects, the immobilization rate ($t = 2.87$, $P < 0.05$) and mean IMI ($t = 8.37$, $P < 0.0001$) contributed significantly, accounting for 90.72% of the variation ($r^2 = 0.9072$). Linear regression analysis showed that only the mean IMI in PCI was significant ($F_{1,9} = 38.77$, $P < 0.001$), accounting for 81.16% of the variation in ICI ($r^2 = 0.8116$) (Fig. 2).

For the ICI period, of the 17 ICIs, two had zero immobilizations, and three were not immobilized in the gestation period (see Table 1). These were excluded giving $n = 12$ for the ICI period. The whole model including the same independent variables was not significant ($P > 0.05$, $r^2 = 0.4735$). A stepwise regression showed that none of the effects were significant.

Repeating the GLM analyses including zero immobilizations (and therefore, zero IMI) gave very similar results. For PCI, the whole model with all five effects was significant ($P < 0.01$, $r^2 = 0.8671$) and once again stepwise regression showed that only the mean IMI was significant ($t = 51.52$, $P < 0.0001$, $r^2 = 0.7783$). For ICI, the whole model was not significant and neither were any of the effects.

Investigation of possible effect of female age on ICI

Of the 13 females (providing 17 ICIs) the ages for only six females (providing seven ICIs) were reliably estimated (Table 1). Analysing the data in a GLM (albeit with this small sample) using ICI (log transformed) as the response variable and the mean IMI in PCI, immobilization rate and age as the independent effects, showed that the length of ICI was significantly related to the mean IMI in PCI ($t = 3.03$, $P < 0.05$, $r^2 = 0.7325$) but not to the immobilization rate or age (the analysis excluded female number 10 with zero immobilizations in the PCI; including this female, the analysis gave $t = 3.72$, $P < 0.05$, $r^2 = 0.7343$).

Four females for whom two consecutive ICIs were available, but not all of whose ages were available, were also investigated. A superficial examination of this small dataset indicated that there was no obvious increase in the duration of the second ICI, which would be expected if older females had longer ICIs (Table 1).

Calving rate

It was possible to calculate calving rate for 20 females during the study. However, five of these had been translocated and/or confined in a boma at some stage (three in 1993, one twice in 1993 and 1995, and one in 1995) and so were excluded. Also excluded were females that did not calve at all during the defined calving period and females that did not have a known calf before the first immobilization (i.e. females for whom birth-lag effects could not be accounted). Although this reduced the dataset, it provided consistency. For the remaining females ($n = 10$) we tested the effects of the five factors on the calving rate in a GLM. For the PCI period, after excluding the calving period, the model was significant ($P < 0.05$) with the four effects accounting for 82.37% of the variation in the calving rate.

Table 2. The number of calves/female/year, the calving period (from first immobilization to 15 months after the last immobilization), the number of immobilizations/female/year and the mean immobilization interval (IMI) for both the preconception interval (PCI) and the whole inter-calving interval (ICI) for females. Female numbers from 1–10 the same as for Table 1

			No. of immobilizations/year		Mean IMI (months)	
Female	Calves/year	Calving period (months)	In ICI	In PCI	In ICI	In PCI
1	0.22	55	1.09	0.87	8.6	7.25
2	0.32	76	0.63	0.32	16	15.5
3	0.24	50	0.72	0.48	11.33	7
4	0.44	54	0.67	0.22	22	4
5	0.33	36	1	0.33	9.33	7
6	0.30	40	0.60	0.30	28	31
7	0.22	54	0.67	0.44	25	31
8	0.32	75	0.64	0.48	11	6.7
9	0.23	53	0.68	0.68	3	3
10	0.23	53	0.68	0.45	13	13

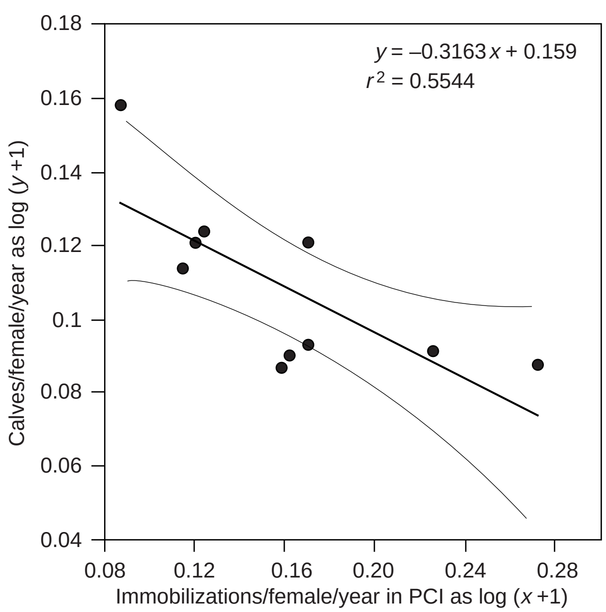

Fig. 3. Immobilizations/female/year in the preconception interval (PCI) against calves/female/year.

However, only one effect, the immobilization rate, was significant ($t = -2.88$, $P < 0.05$, $r^2 = 0.5009$). For the ICI period, none of the effects were significant. Figure 3 shows the relationship between the calving rate and the immobilization rates in PCI. Table 2 shows the calving rates for the 10 females.

Conception

Accurate calving dates were available for 23 calves born from the end of 1993 to the end of 1998. The monthly immobilization pattern was plotted excluding boma immobilizations and those carried out on pregnant females (whose gestation ended in calving) who could not have conceived. We then examined the effect of the immobilization regime on the number of conceptions per month, for the numbers of mature females immobilized each month, and the rainfall (rainfall in the month of conception plus 2 previous months) in GLM. With a double log transformation, GLM analysis of the data showed that the whole model was highly significant ($P < 0.001$) and accounted for 79.22% of the variation, both effects being significant (mature females, $t = -2.96$, $P < 0.05$; rainfall, $t = 2.82$, $P < 0.05$). An interaction effect between the two independent variables was not significant ($t = -0.54$, $P > 0.05$; see Fig. 4a, b). Table 3 shows monthly conceptions, births and immobilizations.

Table 3. The numbers of births, conceptions and mature females immobilized in the Sinamatella IPZ. Conceptions (determined retrospectively) from August 1992 to births at December 1998

Month	Births	Conceptions	No. of mature females immobilized
January	1	7	0
February	1	4	1
March	1	2	0
April	7	3	0
May	4	1	0
June	2	2	0
July	3	1	3
August	1	0	18
September	2	0	20
October	1	1	8
November	0	1	8
December	0	1	2

Annual calving rate

We examined the effect of the immobilization regime (the number of mature females immobilized/year) and rainfall, on the number of calves born/year. Since the response was calculated differently for the two levels, the effects could not be analysed in a single multi-factorial model. With a double log transformation, a linear regression analysis (Fig. 5) showed that the relationship was statistically significant ($F_{1,4} = 9.56$, $P < 0.05$, $r^2 = 0.7050$). Rainfall in 1 year, on the other

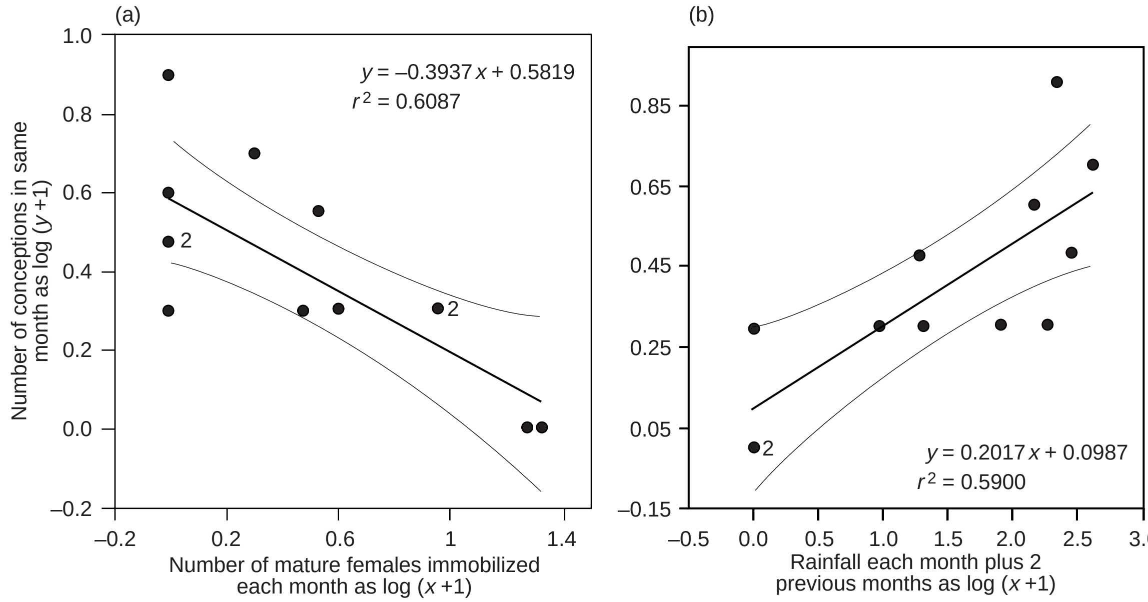

Fig. 4 (a) Number of mature females immobilized per month against number of conceptions in same month; (b) rainfall each month plus 2 previous months, against number of conceptions in same month.

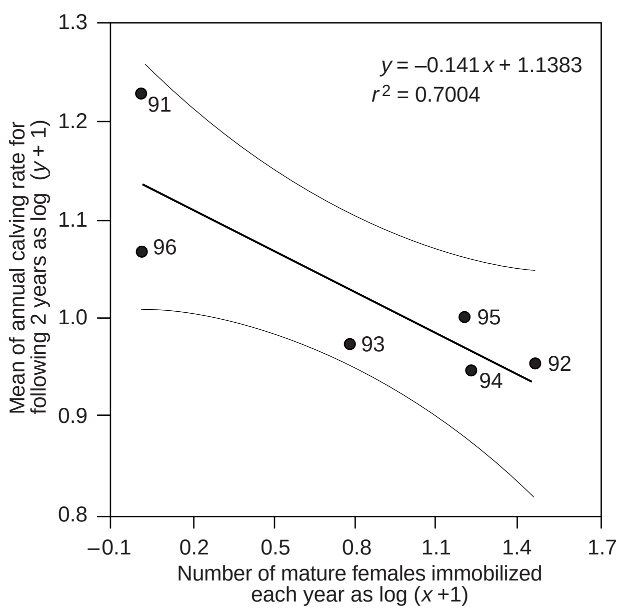

Fig. 5. Number of mature females immobilized each year against mean of annual calving rate for following 2 years.

hand, did not seem to effect the number of calves born the following year ($F_{1,5} = 0.10$, $P > 0.05$, $r^2 = 0.0198$).

DISCUSSION

The impact of immobilization on reproductive parameters

The possible effects of immobilization on four different reproductive parameters (ICI, calving rate, conceptions and calves born/year) were demonstrated independently. That all parameters seemed to have been affected is not surprising given the inter-relationship between them.

Our previous attempts to test the relationship between measures of the immobilization regime and ICI had been confounded by possible effects of temporal autocorrelation. Thus, although we found a highly significant relationship between mean ICI and immobilization regime, this was not sufficient evidence of a causal relationship. However, we were able to further investigate and subsequently clarify this in three ways:

(1) by using measures of immobilization which were not themselves temporally autocorrelated, e.g. immobilization interval and immobilization rate;

(2) by showing that the relationship between time to last immobilization in ICI against length of ICI was not linear;

(3) by showing a consistent difference in effects of immobilization in PCI and ICI.

In some respects this last point was even more surprising, because in those ICIs where gestation immobilizations occurred, the measures of immobilization (number and interval) had also included all the previous PCI immobilizations. Since the calving rate is a function of ICI duration, it was not surprising to find that the calving rate was also significantly related to the immobilization rate in PCI but not in ICI.

We therefore concluded that it was probable that the immobilization regime was having a real impact on the fertility parameters investigated, and that the relationships shown were not simply a result of temporal autocorrelation.

To get more accurate measures of calving rate, data recorded over a longer time are needed. Some of the calving periods in the present study were short, and the sample size was reduced to 10 females to make the data more consistent. However, when we repeated the analysis excluding only females who did not produce any calves in the calving period ($n = 15$) the relationship between the immobilization rate and calving rate was again significant in PCI ($F_{1,13} = 7.31$, $P < 0.05$, $r^2 = 0.3600$) but not in ICI ($F_{1,13} = 0.64$, $P > 0.05$, $r^2 = 0.0467$). Similarly including all females ($n = 20$), in PCI the regression was significant ($F_{1,18} = 29.83$, $P < 0.0001$, $r^2 = 0.6226$) but not in ICI ($F_{1,18} = 2.25$, $P > 0.05$, $r^2 = 0.0.1112$).

There seemed to be a relationship between the number of immobilizations and annual calving rate, but not with rainfall. This may have been manifest as foetal loss, or indeed reduced conception/post-natal calf mortality. However, the result should be interpreted with caution given the small sample size and the possibility of other contributory effects. Population growth rates of black rhino can fluctuate widely between successive years as a result of lag effects, and it is possible that such effects coincidentally correlated with the immobilization regime.

The timing of immobilization may be critical in determining the overall impact on ICI/calving rate. Examination of the slope of the polynomial in Fig. 1 suggests that immobilization in the earlier part of pregnancy may have less impact on the resulting length of ICI than immobilization late in pregnancy; an early resorption/abortion would 'cost' less to the female in terms of lost productivity. Note that the slope of the polynomial increases when the rhino is immobilized around 25–35 months after last calving. This period might be towards the end of a normal gestation, and if immobilization during this period caused foetal loss, female productivity would be substantially reduced.

Possible mechanisms of disturbance to fertility

There are three mechanisms through which immobilization impacts on fertility: delayed or disturbed conception; foetal loss (abortion or resorption); post-natal calf mortality.

Foetal loss (abortion or resorption)

The possibility of foetal loss was first considered on finding that the mean ICI at 40.24 ± 4.96 months (with 35.3% of ICIs ≥ 40 months) was longer than would normally be expected for a population existing at low density. The distribution of ICIs by number of immobilizations in the PCI also suggested that immobilization may be having an effect. Although initially it was thought that the relationship may have been owing to a temporal autocorrelation (many immobilizations simply taking place in naturally longer ICIs), it was still difficult to explain why the six animals not immobilized had all exhibited short ICIs.

Several authors (Veterinary Unit, 1995; Radcliffe, Bommarito & Osofsky, 1996) have suggested that foetal loss (abortion/resorption) may occur in chemically immobilized black rhino. Mammalian foetuses in the first trimester of gestation, and particularly during the period of organogenesis, are more susceptible to insult. Radcliffe, Czekala & Osofsky (1997) reported that foetal resorption occurred in two pregnancies in a female white rhino at 28 days post-ovulation, pregnancy having been confirmed initially by trans-rectal ultrasonography.

Other factors may also lead to foetal loss resulting in lengthened ICIs and reduced calving rates, such as rhino density (Hitchins & Anderson, 1983) and possibly female age, although there had been no indication that female age affected ICI in Pilanesburg (Adcock *et al.*, 1998). As mentioned earlier, the Sinamatella density was low (estimated 0.04 km^2) and available data suggested that age was not a significant factor. Neither did female condition seem to be related to fertility. Those females for whom condition scores were available at the time of immobilization ($n = 10$) were all estimated to be in condition 4–5 (on a scale of 1 = very poor, 5 = very good).

Another observation made in the present study also suggested that early foetal loss might have occurred. We identified 15 females that had been immobilized during gestation and went on to produce calves. We grouped these females according to the 'trimester' (5-month period) of gestation at which they were immobilized. The observed distribution was highly skewed, with only one female in the first trimester category (6.7%), eight in the second trimester (53.5%) and six in the final trimester category (40%). However, most mature females were immobilized from August to November (90%), when the percentage of females in the first trimester over this period was low probably because of seasonal effects on conception. Using a known conception pattern in the present study, we calculated the probability of females being in each of the three trimesters. For example, all females who had conceived from March to October could potentially have been in the first trimester during immobilizations taking place from August to November. From our data, 10 (27%) females were predicted for the first trimester, 20 (54%) for the second, and seven (19%) for the third. Using the raw data, there was no significant difference between the two ratios. However, if the immobilization regime had affected the conception frequency, as suggested in the present study, the projected ratio (10 : 20 : 7) is already biased by immobilization. In the absence of a control it is not possible to draw any objective inference. However, the observation is interesting, especially as we found that none of the 15 females were in the first 4 months of the first trimester. The direct effects of immobilization on foetal loss need to be investigated.

Post-natal calf mortality

Post-natal calf mortality occurs in black rhino under natural conditions, particularly in the first 3 months, when the calves are susceptible to predation (Goddard, 1967; Berger, 1994). However, Brett (1998) recently showed that post-natal mortality was very high (50%) in calves born to females that had been translocated whilst pregnant. It is, of course, possible that undetected post-natal calf mortality may have been a factor in some females with very long ICIs. However, our data suggest that a combination of a high number of immobilizations and immobilization during gestation may also affect post-natal calf survival, although sample sizes were too small to draw a firm conclusion. This has interesting parallels with Brett (1998) where there may also have been an effect as a result of immobilization. From our data, of the 15 dams who had been immobilized during gestation, three dams lost their calves within the first 3 months. These three females had a high number of immobilizations/year (mean $= 1.17 \pm 0.40$) compared with the other 12 females also immobilized during gestation, all of whose calves survived (mean immobilizations/year of 0.65 ± 0.10). Calves of the nine females not immobilized during gestation all survived to more than 3 months (mean immobilizations/year of 0.24 ± 0.11).

Delayed/disturbed conception

There seems to be no consistent relationship between annual precipitation and conception patterns in populations of black rhino. Some seasonality of calving in black rhino in South Africa has been reported by Hitchins & Anderson (1983), who noted births throughout the year but a bimodal seasonal reproduction with peaks in mid-summer and mid-winter. Most conceptions ($n = 128$) occurred in October–November and April–July, with parturition peaks in January–February (19% of those observed) and June–August (41% of those observed), respectively. Hitchins & Anderson (1983) suggested that it was unlikely that rainfall (and therefore browse availability) determined this distribution, since one season is wet and the other dry. Also remarkably, in that study the lowest numbers of conceptions were recorded in December and January when browse conditions are probably favourable. In contrast, Owen-Smith (1992) suggested that the rainfall pattern impacting on nutrition was the main mechanism by which megaherbivores cue their reproductive activity. Other Southern African populations of black rhino showed a parturition peak in the late summer (Joubert & Eloff, 1971; Hall-Martin & Penzhorn, 1977). In East Africa, there does not seem to be any well-defined seasonality in births for the black rhino (Goddard, 1967; Hitchins & Anderson, 1983). In the present study, the conception peak was in January and conceptions occurred throughout the year except in August and September. Our analysis showed that the conception pattern was related to both the rainfall (rainfall in same month plus 2 previous months) and the frequency of immobilizations. Since most of the immobilizations were performed in the dry season when conception frequency is probably low, it is not surprising that there was a significant relationship. However, this assumes that rainfall is a critical factor and if it is, then it is surprising that rainfall was not significant factor when we examined the other reproductive parameters. Since stress can impact on conception in mammals by inducing anoestrus (Breazlie, 1987), we believe it is possible that in the present study both rainfall and immobilization frequency had an effect on conceptions.

Possible causative components of the immobilization procedure: drugs and stress

If the immobilization procedure has impacted on fertility, as we suggest, it is important to identify the causative components so that these can be reduced when immobilization is essential. The two main components responsible are drugs and stress.

Similar combinations of standard chemical immobilants were used for all operations. Large animal Immobilon (manufactured by C-Vet, containing etorphine hydrochloride (M99) with acepromazine maleate) was used with either xylazine (Bayer plc, as Rompun) / detomidine (Pfizer Ltd as Domosedan) or azaperone (Janssen Animal Health, as Stresnil) for immobilizing the animal. Naltrexone or Naloxone (du Pont pharmaceuticals) was used as a reversal agent. Manufacturers data sheets list contraindications and warnings for use of these drugs in species for which they are licensed (NOAH, 1998). None of the drugs used was licensed for rhino, but the contraindications listed on the datasheets for another perissodactyl, the horse, provide some insight into problems that may be related to their use in the black rhino. Large animal Immobilon is contraindicated in combination with other substances, and 'animals *must* be kept protected from extremes of temperature and under close supervision for at least 24 hours'. Domosedan is contraindicated in the last month of pregnancy, whereas Rompun is contraindicated in the latter stages of pregnancy, and the datasheet states 'as the safety of xylazine use during organogenesis has not been fully demonstrated by current methods it should be used with caution during the first month of pregnancy'. Azaperone, which was used as an alternative to xylazine or detomidine in some animals in 1995, is licensed for use in pigs, and no specific contraindications are made for pregnant animals. Azaperone was used for females 'suspected of being pregnant' in the 1995 veterinary operations (Veterinary Unit, 1995), but data for this year do not differ markedly from the trends shown for other years.

The broader effects of acute and chronic stressors resulting from immobilization may have been responsible for the reduction in fertility. Immobilization inevitably entailed stress, and was usually undertaken during the latter part of the dry season when temperatures were often above 35 °C and occasionally above 40 °C in the shade. Rhino were darted from a helicopter, usually necessitating a chase over rough terrain. Kock, *et al.*, (1990) reported finding significant elevations in serum cortisol and glucose in black rhino they had categorized as 'stressed' at capture (for translocation) over those categorized as 'normal' at capture, although it was not possible to determine resting serum cortisol before capture. In the same study, significant differences were reported in biochemical parameters, over capture, transport and boma confinement periods, which were attributed to the physiological response of the black rhinoceros to acute and chronic stressors. Recommendations were subsequently made (Kock, 1991) to reduce stressors using modified management techniques in an attempt to reduce unacceptably high translocation mortality rates. Concerning the specific effects of stress on fertility, it is known that increased corticosteriod production can inhibit the activity of the hypothalamo-pituitary-gonadal/adrenal axes, leading to reduction of ACTH, GnRH/FSH and LH, impairing all aspects of fertility (Ganong, 1997). Interference with fertility, implantation or attachment of the fertilized ovum, and growth and development of the embryo or foetus are recognized components of the stress response in animals and humans (Breazlie, 1987). Radcliffe *et al.* (1996) made the general statement that stresses associated with immobilization, transport and boma confinement could result in abortion in a wide variety of species.

If stress were a prime component of an immobilization effect on fertility, one would expect a more significant effect in animals held in bomas. Observations were made during this study on six female black rhino confined in bomas for various periods from 2 to 6 months. Since the females confined in a boma were also immobilized more times (1.73/female/year) than those not confined in a boma (0.69/female/year), it was not possible to distinguish the effects related to drugs and stress from those related to stress alone. However, the calving rate seemed to be lower in females confined in a boma after release (0.12 calves/female/year) than for females who were also immobilized but not confined to bomas (0.23/female/year). In addition, two females died in bomas as a result of management-related problems, one with a full-term foetus. A premature birth of a live calf (which died after 2 days) to a female in a boma has also been reported in South Africa by Hofmeyr (1998).

Adoption of guidelines for minimizing the impact of immobilization on fertility

As far as we are aware, there are no commonly adopted guidelines relating to immobilization of female black rhino, and the following may be worth consideration.

(1) *Minimize the frequency of immobilization.* Routine and/or repeated chemical immobilization may be inadvisable, particularly where it is used for radio-collaring when failure rates are high (Alibhai *et al.*, 1999). Similarly it may be beneficial to reassess meta-population management (Foose, 1993) in view of these data. The usefulness of this concept has been questioned on the grounds that it is not cost-effective (Leader-Williams, 1993), and has made no contribution to *in situ* conservation (Rabinowitz, 1995).

(2) *Avoid immobilizing/translocating pregnant females.* Our data suggest that the safest time to immobilize a female is before sexual maturity, or in the pre-conception period after calving. Given that it may be preferable to avoid immobilization of females with very young calves, where stress could be an important component, we suggest the safest period is between 6 and 8 months after calving. It is possible to identify females who are > 4–5 months pregnant, before immobilization, with a non-invasive faecal steroid test (Schwarzenberger *et al.*, 1996). Females accompanied by a calf of > 1 year, for example, could be routinely tested before immobilization. Females in the earlier stages of gestation can be identified by trans-rectal ultrasonography (Radcliffe, 1996), but this is only possible in the field *after* immobilization. However, it may be useful for deferring proposed translocations or boma-confinements. Brett (1998) also questions the wisdom of routinely translocating pregnant females because of an observed 50% subsequent neonatal mortality.

(3) *Minimize possible stressors during immobilization.* It would seem, from consistently high translocation mortality rates (Leader-Williams, 1993; Brett, 1998), that black rhino are very susceptible to stress. Where possible, animals should be immobilized from the ground (Galli & Flamand, 1995) to avoid the stress induced by a helicopter chase, and to avoid other possible stressors, e.g. operating during high temperatures, which may impact on female fertility.

(4) *Review boma management if immobilization is for translocation.* As discussed, translocation and boma management involves chronic stressors, which are probably partly responsible for high post-translocation mortality rates in the black rhino. Our preliminary observations suggest that they may also affect fertility. Boma confinement is used as a standard technique to 'settle' an animal before or after translocation, and is described in detail by Rogers (1994). It may require more frequent immobilization and is probably more stressful to the animal than routine *in situ* immobilization. It has been thought necessary for translocated animals to be confined in a boma for a period of 'settling-in' either at the capture site and/or at the release site, but there seem to be no data to substantiate either option. Geldenhuys (1991) and Kock (1991) reported that black rhino should be held in bomas close to the capture site for a minimum of 3 weeks and preferably a month, but did not provide supporting data. Rogers (1994) maintains that animals can be either taken straight from the point of capture to the new site, or alternatively that they can be kept confined in a boma near the capture site for 'at least 6 weeks'. He also states that they should be boma held at the release site for a few days. Again, no supporting data are given. Numerous anecdotal reports exist of animals 'bomb-shelling' or dispersing far and wide, after free-release, but we have also noted animals confined in

bomas for 6 months doing the same on release (Alibhai *et al*., 1996). In contrast, Emslie (1996) has noted fighting occurring after boma release as a result of poor dispersal. If boma confinement is considered a necessity, our data suggest that the period of confinement should be as short as possible (days rather than weeks) with minimal invasive management and minimal disturbance to the rhino.

Summary

Because this study was undertaken opportunistically, data collection did not conform to a classical experimental design. Nevertheless, we believe that the analyses provide sufficient evidence to suggest that the intensive immobilization regime negatively impacted on black rhino fertility in the Sinamatella IPZ. Black rhino are still very endangered and from the data presented here, and unacceptably high radio-collar failure rates (Alibhai *et al*., 1999), we emphasize the need for common guidelines relating to the immobilization of female black rhino in range states.

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