Non-Invasive Reproductive Monitoring of Black Rhinoceros Females in the Wild: Patterns of Fertility and the Influence of Environmental Factors

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Abstract

The black rhinoceros (*Diceros bicornis*) has suffered one of the most dramatic declines of all mammals in Africa and it is essential to acquire a better knowledge of its reproductive biology in order to develop successful conservation strategies.

The main objectives of this study were to monitor ovarian cycles and pregnancy in wild black rhinoceros females and to evaluate the influence of environmental factors on fertility, including management procedures such as translocation and captive holding. To this end, a non-invasive method of reproductive assessment based on faecal progestagen analysis was developed for black rhinoceros females.

The 15-months gestation period was characterised by an early phase of two to four months during which the life of the primary corpus luteum was extended to 40 to 60 days. A marked increase in faecal progestagen subsequently occurred and after the third month of gestation, 97.6% of the pregnant animals were correctly identified by the test from the analysis of one faecal sample only.

After parturition, wild black rhinoceros females exhibited a post-partum anoestrus of at least four months, followed by a period of oestrous cyclicity lasting four to eight months. Three quarters of oestrous cycles had a mean length of 26.8 ± 1 days, with a phase of low concentrations lasting an average of 9 ± 0.5 days and a phase of high concentrations lasting an average of 18 ± 1.1 days. Other oestrous cycles were on average twice as long and characterised by either a prolonged luteal phase or by an extended follicular phase.

Wild black rhinoceros females presented a period of higher fertility during the late spring/early summer in Zimbabwe, coinciding with the early rainy season, which resulted in a parturition peak during the late rainy season. Captive females in the

northern hemisphere also showed a higher incidence of conception during the summer.

In a wild population growing at an annual recruitment of 7.2%, females first conceived at a mean age of five years and produced calves every 2.5 years. By contrast, wild females recently translocated to the same area exhibited a lag of nearly four years before resuming normal breeding activity. In captivity, unproductive females were mainly nulliparous animals while multiparous females only produced calves every 3.5 years, due to low conception rates. The influence of the social environment, in which captive black rhinoceros are brought up and maintained, seems to be critical for successful breeding.

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List of acronyms

ATP	Adenosine Triphosphate
BW	Body Weight
CITES	Convention on Trade in Endangered Species of Wild Fauna and Flora
eCG	equine Chorionic Gonadotropin
EIA	Enzyme immunoassay
E1C	Estrone conjugates
GC-MS	Gas Chromatography- Mass Spectrophotometry
HPLC	High Performance Liquid Chromatography
20α-OHP	20α-dihydroprogesterone
PdG	Pregnanediol-3-glucuronide
SVC	Save Valley Conservancy
ZSL	Zoological Society of London

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Garnier, J.N., Green, D.I., Pickard, A.R., Shaw, H.J. and Holt, W.V. (1998). Non-invasive diagnosis of pregnancy in wild black rhinoceros (*Diceros bicornis minor*) by faecal steroid analysis. *Reproduction, Fertility and Develop*ment, **10**, 451-458.

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1 BACKGROUND

1.1 INTRODUCTION

The family Rhinocerotidae appeared during the early Eocene some 50 million years ago and represented one of the most ecologically diverse and widespread families of megaherbivores (Prothero, 1993). The black rhinoceros (*Diceros bicornis*), which was already present in Africa, southern Europe and the near East approximately 10 million years ago, is one of the five living rhinoceros species that survive today. The other African species is represented by the white rhinoceros (*Ceratotherium simum*), while the three Asian species include the Indian, or Greater one-horned rhinoceros (*Rhinoceros unicornis),* the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and the Javan rhinoceros (*Rhinoceros sondaicus*), which is the most primitive and has changed little since 10 million years ago (Emslie & Brooks, 1999).

The black rhinoceros has suffered a dramatic decline in numbers and the surviving populations are isolated and of small size, thus requiring intensive management. Apart from the *in situ* protection of these populations and their habitats, their long-term survival is dependent on the development of co-ordinated breeding programmes that will protect them against the genetic and demographic risks of extinction. However, the success of such programmes depends on an adequate knowledge of the reproductive biology of the species and on the ability to monitor the reproductive performances at an individual and at a population level.

Like all rhinoceros species, the black rhinoceros is characterised by a long generation time, but its specific solitary habits and preferences for dense habitats, as well as low densities, have rendered increasingly difficult the evaluation of

reproductive success in this species. The only information available on its reproductive activity in the wild is mainly anecdotal, having been gathered during ecological studies that were undertaken in the 1960s and 1970s. The situation is however worse for other rhinoceros species, such as the Sumatran, for which there has only been less than 60 minutes of total observations in the wild during the last 50 years (Foose & Van Strien, 1997).

The traditional approaches that have been used to monitor reproductive status in domestic species are inadequate for wildlife species, as they would require their very regular immobilisation over long-periods of time. For this reason, non-invasive techniques based on the measurement of steroid metabolites in excreta are much more appropriate and have been increasingly developed for endangered wildlife species. In particular, urinary steroid analysis was validated for monitoring reproductive status in captivity in both African rhinoceros species at the Institute of Zoology (Hindle, 1991). Although the present study was based on a similar technique, it had to be modified and adapted for use under free-ranging conditions, since it had been established that the regular collection of urine samples from these animals was too difficult in the wild (Brett, 1989).

The main objective of our study was to gain a better knowledge of black rhinoceros reproductive biology by monitoring fertility levels in individual females in their natural environment.

More particularly, the study aimed to:

1. validate faecal steroid analysis for monitoring the reproductive status of free-ranging black rhinoceros and evaluate its use as a pregnancy diagnosis technique;

2. characterise reproductive cycles and estimate the influence of environmental factors on reproductive activity in the wild;
3. evaluate the influence of management techniques, such as translocation and captive holding, on reproductive success in this species.

Ultimately, the long-term goal of this study is to optimise reproductive output in this endangered species.

The present chapter will review the demographic status of rhinoceroses and requirements for black rhinoceros conservation, followed by the existing information on rhinoceros reproductive biology. The importance of reproductive monitoring in wildlife conservation and management will subsequently be presented, as well as the techniques for reproductive assessment that are currently available, with an emphasis on faecal steroid analysis.

1.2 RHINOCEROS CONSERVATION STATUS

1.2.1 Causes of decline

The demand for rhinoceros horn has been the main cause of the dramatic decline suffered by all rhinoceros species, although habitat destruction and fragmentation have also contributed to the decline of the three Asian species (Foose & Van Strien, 1997).

Rhinoceros horn has been a commercially valuable commodity for centuries, used primarily in countries in the Middle East and the Far East (see Milliken *et al.*, 1993). In North Yemen, rhinoceros horn is the preferred substance for carving *jambyia* handles, which are traditional daggers representing an important status symbol in the cultural life of Yemeni men. The tremendous increase in rhinoceros

horn exports to Yemen during the 1970's coincided with a marked improvement of the standard of life of the Yemenis. Their revenues increased substantially after the oil boom, enabling them to afford *jambyias*, which could fetch $US 13 000 in 1982 (Milliken *et al.*, 1993).

The other market for rhinoceros horn is in the Far East, especially China, Taiwan, Japan, Hong Kong, South Korea and Thailand, where it has always been used as an ingredient in traditional medical prescriptions. Belief in these healing properties is also deeply rooted, since an early Chinese medical book dating to the 1st century BC, the Divine Peasant's Herbal, recommends rhinoceros horn (Milliken *et al.*, 1993). Although there have been revisions to the Chinese medical pharmacopeia since those times, modern medical and popular books still contain applications for rhinoceros horn, which is generally indicated for high fever associated with serious illness. Research on pharmaceutical properties of rhinoceros horn showed evidence of anti-pyretic properties when used in high doses (But *et al.*, 1991). Retail prices can fetch $US 10 000 per kilo but horns from Asian species are considered to be more valuable and can cost as much as $US 50 000 per kilo (Milliken *et al.*, 1993).

1.2.2 Indian rhinoceros

By the first decade of the 20th century, the Indian rhinoceros was nearly extinct from its original range in India and Nepal, where it used to inhabit riverine grasslands. Intensive protection measures and very strong governmental commitment to rhinoceros conservation, linked to its cultural value, has resulted in a spectacular reversal of such a negative trend. In 1998, the population amounted to approximately 2470 animals, with the majority being located in Kaziranga National Park in north-east India and in Chitwan National Park in Nepal (Foose & Van Strien, 1997; Wirz-Hlavacek, 1999).

In captivity, 136 Indian rhinoceros were kept in 51 institutions in 1998, originating from a stock of 33 founders (Wirz-Hlavacek, 1999). For the last 30 years, the number of captive births has remained just above the number of deaths (Rookmaker, 1998).

1.2.3 Sumatran rhinoceros

Although not as rare as the Javan rhinoceros, the Sumatran rhinoceros is considered to be the most critically endangered species of rhinoceros, as approximately 50% of its population has been lost through poaching over the last decade (Foose & Van Strien, 1997). Today, only about 300 individuals are estimated to survive world-wide, in highly scattered and fragmented populations in Sumatra, the Malay Peninsula and on the island of Borneo.

A major component of the conservation plan for this species has involved the development of breeding programmes since 1984, both *ex situ* and *in situ* in managed breeding centres in native habitats. Unfortunately, only 40% of the 40 rhinoceros that were captured in the wild and translocated to breeding facilities have survived, and none has reproduced yet (Foose & Van Strien, 1997). Conservation efforts have been hampered by various aspects of the ecology of this species, which inhabits very dense forests, occurs at very low densities and is by nature very solitary, secretive and elusive. Breeding success has also been seriously affected by the lack of knowledge of the reproductive biology of this species.

1.2.4 Javan rhinoceros

The Javan rhinoceros was formerly widespread on Java and from Malaysia to Vietnam but by 1937, the species was reduced to just 25 animals on Java. Since 1967, intensive conservation efforts have resulted in a steady increase of the population to

50-60 rhinoceros in 1989 (Foose & Van Strien, 1997). This population is located in a relatively well-protected area in Ujung Kulon national park and it has remained unchanged for the last decade. More recently, another population of 5-7 Javan rhinoceros has been discovered in southern Vietnam. With fewer than 70 individuals remaining, the Javan rhinoceros is considered as one of the rarest large mammals existing in the world today.

In captivity, 22 animals were recorded during the last four centuries, but no individual has recently been placed *ex situ* (Rookmaker, 1998).

1.2.5 White rhinoceros

1.2.5.1 Status in the wild

Historically, the white rhinoceros (*Ceratotherium simum*) occurred in two discrete populations separated by a gap of 2000 km (Owen-Smith, 1988). The northern subspecies (*C.s.cottoni*) occurred west of the Nile River, between Sudan and northern Uganda, numbering around 2000 animals in the early 1960s. There are now only around 25 individuals remaining in the Democratic Republic of Congo in Garamba National Park (Emslie & Brooks, 1999).

The southern subspecies (*C.s.simum*) occurred south of the Zambezi River and was largely exterminated during the late 19th century, except for around 20 individuals that survived in Natal. Through the subsequent enforcement of intensive protection measures, this species has achieved a remarkable recovery and numbered approximately 8440 animals in 1997 (Emslie & Brooks, 1999).

The same conservation measures described for the black rhinoceros apply to the white rhinoceros, except that the populations of southern white rhinoceros in South Africa are listed on Appendix II of CITES. International trade in this species is

therefore permitted if it is legal and sustainable. Sport hunting in South Africa involves 0.5-0.6% of the total population for the elimination of surplus males (Emslie & Brooks, 1999).

1.2.5.2 Status in captivity

In captivity, all southern white rhinoceros descend from animals that were translocated from Hluhluwe-Umfolozi to zoological institutions during the 1970s. There were 704 southern white rhinoceros in captivity in 1998, and only nine northern white in two institutions (Göltenboth, 1999).

Breeding output of the captive population has been dramatically poor, and it is estimated that it is declining by 3.5 % every year in North America (Emslie & Brooks, 1999). The situation is even worse for the northern white with only five births having occurred in captivity. Despite a number of surveys of captive breeding in African rhinoceroses which emphasised the alarming situation and which showed that single pairs of white rhinoceros were unsuccessful (Kock & Garnier, 1993; Lindemann, 1982), there has been a very slow response to improve reproductive management of this species. Only very recently has there been a co-ordinated effort to place these animals in breeding and to monitor reproductive activity in females which are not producing. Unfortunately, reproductive monitoring in a large number of captive females demonstrated that the majority of animals had no or erratic ovarian cycles (Schwarzenberger *et al.*, 1999b).

1.2.6 Black rhinoceros

1.2.6.1 Status in the wild

Originally, the black rhinoceros colonised a large variety of ecosystems, ranging from deserts to montane forests. Its distribution only avoided the equatorial

forest belt and some of the most arid desert regions of Africa (Figure 1.1.a), since it lived generally close to permanent water sources and has woodland savanna for primary habitat (see Skinner & Smithers, 1990).

Seven subspecies of black rhinoceros were initially described on the basis of anatomical parameters, of which only four have survived today (Groves, 1967). The validity of this classification is nevertheless uncertain as analysis of mitochondrial DNA revealed that there was a very close genetic relationship between the surviving subspecies (O'Ryan *et al.*, 1994). Rather than different subspecies, patterns of genetic variations suggest a west-to-east continuum with populations in Namibia and Natal being the extremes (Swart & Fergusson, 1997). These subspecies are therefore more considered as ecotypes, which include: 1) the south-western ecotype (*Diceros bicornis bicornis)*, which is more arid-adapted and larger than the others and occurs in Namibia; 2) the south-central ecotype (*D. b. minor)*, which is the most numerous of the black rhinoceros ecotypes; 3) the eastern ecotype (*D. b. michaeli)* found mainly in Kenya and 4) the north-western ecotype (*D. b. longipes)*, which is the most endangered and only remains in Cameroon, with 10-18 animals (see Emslie & Brooks, 1999).

Declines in black rhinoceros numbers started as soon as in the 19th century in western and southern Africa, but it is estimated that there were still some 100 000 black rhinoceros surviving in Africa in 1960. However, when the first pan-African census was completed in 1979, only 10 000 to 15 000 black rhinoceros were found to have survived on the continent. Although early census figures have to be taken cautiously considering the difficulty of obtaining reliable and accurate census data for black rhinoceros populations, they do however illustrate the dramatic decline suffered by this species. Successive waves of poaching eliminated or reduced black rhinoceros

populations in country after country to the north. The considerable increase in rhinoceros horn poaching observed in the 1970's coincided with the increased demand for rhinoceros horn in North Yemen and to the breakdown in law and order in East Africa, together with a marked increase in corruption (Milliken *et al.*, 1993).

Between 1984 and 1995, the population further decreased by 73% to reach the critical point of around 2400 animals (Figure 1.2.a). Wild populations mainly remained in South Africa, Namibia, Zimbabwe and Kenya at this time and the species had become extinct in a number of countries (Angola, Botswana, Central African Republic, Chad, Ethiopia, Malawi, Somalia, Sudan, Uganda). Since 1996, black rhinoceros numbers have, for the first time in the last few decades, showed a slight increase due to the consistent growth of populations in South Africa and Namibia. In 1997, it was estimated that there were approximately 2600 black rhinoceros that were mainly distributed in four countries (Figure 1.1.b). These included:

- South Africa, with 1043 animals (mainly *Diceros bicornis minor*) which are spread across 25 populations, of which the two largest are in Hluhluwe-Umfolozi Game reserve and in Kruger National Park;
- Namibia, where 707 black rhinoceros (only *D.b.bicornis*) are mainly distributed in the north of the country;
- Kenya, with 424 black rhinoceros (only *D.b. michaeli*) being maintained in highly protected and fenced small areas designed as "sanctuaries" ;
- Zimbabwe where 339 black rhinoceros (only *D.b.minor*) are kept in private "Conservancies", which are medium to large sized fenced areas, and in "Intensive Protection Zones" which are unfenced areas located in National Parks (Emslie & Brooks, 1999).

1.2.6.2 Status in captivity

The first black rhinoceros officially recorded in captivity since Roman days was captured in Nubia (Sudan) in 1868 and sold to London Zoo. The first captive birth was recorded in 1941 and a total of 775 black rhinoceros have been recorded in captivity up to the end of 1994, of which 62% were imported from the wild, mainly from East Africa (Rookmaker, 1998).

The captive black rhinoceros population numbered 235 animals in 1998 (170 were of the eastern ecotype and 65 of the southern ecotype) (Göltenboth, 1999).

Recent trends for this captive population indicates a much better breeding output compared to previous results. During the 1970s, black rhinoceros numbers in captivity decreased by around 7% per year, but these losses could be compensated by imports from the wild until the CITES ban on rhinoceros trade in 1977 (Figure 1.2.b) (Lindemann, 1982). Such a negative trend was due to a high mortality rate linked to specific diseases affecting captive black rhinoceros, as well as to the fact that 40% of the captive institutions did not put their animals in a breeding situation (Kock & Garnier, 1993). Such syndromes included hemolytic anaemia, which at one time was responsible of 40% of all adult deaths, mucocutaneous ulcer syndrome, cholestatic hepatopathy, encephalomalacia, fungal pneumonia, leptospirosis and tuberculosis (Miller, 1994). Some of these syndromes were initially thought to be related to the very particular energy metabolism of the erythrocytes of the black rhinoceros, which were found to have less than 5% of the ATP levels found in other mammals (Paglia *et al.*, 1986). However, recent comparative research has showed that although all rhinoceros species presented such particular metabolism, only the black rhinoceros red blood cells had a catalase deficiency, this enzyme being one of the most important in antioxidant metabolism (Paglia *et al.*, 1996).

Although exact causes for these specific pathologies still have to be determined, precise guidelines for managing black rhinoceros in captivity are progressively being established. In particular, the implementation of vaccination programmes and nutritional recommendations has certainly contributed to decrease mortality rates. As a result, the black rhinoceros population in captivity started to show a slight increase in the 1990s (Bride *et al.*, 1996). However, the specific susceptibility of the black rhinoceros to a large number of potentially fatal syndromes emphasizes the need to achieve optimum breeding performances in captivity.

1.2.6.3 Conservation objectives and strategies

1.2.6.3.1 Conservation objectives

The dramatic decline of the black rhinoceros has led not only to a considerable reduction in numbers of these animals, but also to the significant fragmentation in the species range and to genetic isolation. Only four black rhinoceros populations now number more than 100 animals, while only 14% have more than 50 animals (Emslie & Brooks, 1999). The black rhinoceros is classified as "critically endangered", meaning that there is a 50% probability of extinction within two generations, except for the south-western ecotype which is "vulnerable" (Mace & Lande, 1991).

Although it is considered that genetic threats to black rhinoceros populations have been over-emphasized due to the high degree of heterozygosity found in southern African black rhinoceros (Swart & Ferguson, 1997), the rapid growth of populations is essential to protect them from the consequences of poaching. In order to attain optimum growth rates in wild populations, it has been estimated that numbers had to be maintained at 75% of the ecological carrying capacity of the area. This level is considered to be the threshold equilibrium level at which negative feedback from density-dependant effects, food resources, social interactions and other

environmental factors significantly reduce the rate of population increase (Bride *et al.*, 1996). Due to the costs associated with each conservation strategy, sub-populations are prioritised following a rating system that considers their size ("key" populations have more than 50 animals), their significance in conserving the subspecies and the likelihood of conservation measures being effective (Emslie & Brooks, 1999). Field efforts and available resources can thus be concentrated on protecting and managing populations that are viable in the long-term.

1.2.6.3.2 Conservation strategies

1.2.6.3.2.1 Protection

1.2.6.3.2.1.1 Law enforcement

The first level of protection around black rhinoceros areas consists in the very regular patrolling of the area and sighting of the animals to detect poachers' incursions before too much damage has been inflicted. Intensive patrol coverage is, however, costly in financial terms as well as in manpower. It has been estimated that a minimum of approximately 1 man/20 km² and of $US 200-400/km² was necessary in order to protect an area with rhinoceros efficiently, although these figures vary with the area size and the density of animals (Leader-Williams *et al.*, 1990).

1.2.6.3.2.1.2 Community involvement

In situ conservation efforts need to be directed at preventing poachers entering a rhinoceros area, since poachers cannot operate without people who have got good knowledge of the area. Intelligence work is essential but the keystone for rhinoceros protection and wildlife conservation in general, is in the improvement of levels of economic activity in the surrounding local communities. When local people derived benefits from the presence of wildlife in an area (direct employment, creation of

community projects, etc.), this tends to reverse the conversion of wildlands to other land-uses and to reduce illegal activities such as poaching (C.Stockil, personal communication).

1.2.6.3.2.1.3 Dehorning

The dehorning of black rhinoceros has also been undertaken as a conservation strategy in Namibia and in Zimbabwe in order to reduce the poaching pressure (Kock & Atkinson, 1993). Such a drastic measure, which involves the removal of a portion (70-80%) of the front and rear horn while the animal is under anaesthesia, was adopted as an emergency procedure in response to the dramatic poaching crisis that was clearly leading the Zimbabwean black rhinoceros population to extinction. Between 1991 and 1993, 224 black rhinoceros were dehorned in Zimbabwe and the great expertise of the dehorning team as well as the improvement in technique and drug combinations, led to a mortality related to dehorning of less than 2% (Kock & Atkinson, 1994).

As a result, dehorning has certainly contributed to the survival of the remaining black rhinoceros in Zimbabwe. However, it needs to be associated with other protective measures, such as increased law enforcement and awareness campaign, to be fully effective (Milliken *et al.*, 1993). In addition, it needs to be repeated on the regular basis since black rhinoceros re-grow an average of 5 cm of horn length per year (Pienaar *et al.*, 1991).

1.2.6.3.2.2 Monitoring of populations

Detailed information is required on size and dynamics of each population in order to achieve conservation objectives stated in conservation plans (Brooks, 1989).

Accurate evaluations of population size of black rhinoceros are difficult to obtain. Census techniques used for black rhinoceros include the total count of individually known animals, which is suitable for small populations (< 100 animals). For larger populations or populations with a low density of animals, the mark-recapture method can provide an estimate of the population size based on the pattern of re-sighting of individually identified animals seen on successive surveys (Brooks, 1989). A computer program has been developed for that purpose (Bride *et al.*, 1996). Both methods require the individual identification of each animal through external anatomical features, naturally or artificially created, such as ear-notching. Such individual identification is increasingly difficult as animals are more wary of humans, live in dense habitats at low densities and have not always been ear-notched.

The evaluation of the dynamics and reproductive output of black rhinoceros populations is also essential to assess the population's breeding performances, which is key for future management decisions.

Various reproductive parameters, such as age at sexual maturity, calving intervals, calf:cow ratios and recruitment rates, can be used for the evaluation of breeding output in wild populations. However, their main disadvantage is that their evaluation can only be undertaken retrospectively, after a minimum of five years of observations, due to the long generation times of these animals. This implies that the negative trends in breeding output will only be detected after they have already affected population growth. A low population growth has the same consequence as poaching in term of animal numbers and needs to be avoided imperatively considering the current demographic status of the black rhinoceros (Bride *et al.*, 1996).

Another disadvantage of these parameters is that they are often based on the performances of only a few animals and do not reflect the great variations in reproductive success that occur between individuals. This may preclude the identification of sub-fertile animals and therefore any potential treatment. It may also lead to a great loss in animals and conservation efforts, especially when animals are translocated for breeding purposes.

The assessment of individual reproductive status of black rhinoceros would therefore greatly assist in the monitoring of populations, by providing an instantaneous evaluation of the current breeding output, at both individual and population levels.

1.2.6.3.2.3 Translocation

Translocation of animals is a key strategy used black rhinoceros conservation. It is used to move animals between sub-populations (1 or 2 unrelated rhinoceros into each population every generation) in order to maintain genetic diversity, as part of meta-population management (Foose *et al.*, 1993). Translocation is also frequently undertaken in order to remove animals from high-density areas, for example in certain Kenyan sanctuaries of small size. It can also be used to reintroduce the species where it previously occurred, as has been seen in the Lowveld in Zimbabwe or in Malawi. In South Africa, small breeding groups of black rhinoceros are also auctioned by Natal Parks Board to approved buyers, in order to re-establish or reinforce populations.

The determination of fertility levels of individual animals would greatly assist in optimising the efficiency of this management procedure. From a demographic point of view, it would ensure that translocated animals are chosen amongst those fulfilling exactly certain fertility criteria. This would avoid potential losses in animals and expenditures, since translocations are costly exercises that are also accompanied by a

certain mortality rate. After animals have been translocated, it would also be most useful to monitor their reproductive output, since breeding performances of translocated populations have been found to be extremely variable (Brett, 1998).

1.3 RHINOCEROS REPRODUCTION

1.3.1 Asian rhinoceros and white rhinoceros

1.3.1.1 Social structure

The Indian rhinoceros males are solitary, with cow-calf units being the only persistent associations (Laurie, 1982). The Javan rhinoceros and Sumatran rhinoceros are also solitary (Borner, 1979; Shenkel & Shenkel-Hulliger, 1969b).

By contrast, the social organisation of white rhinoceros has been described as being semi-social and territorial. Adult females live in overlapping home ranges and group with subadults, forming groups of up to six animals. Adult bulls are typically solitary but associate with females that are receptive (Owen-Smith, 1988).

1.3.1.2 Sexual maturity

Indian rhinoceros females had a mean age at first calving of between six and eight years of age in the wild (Dinerstein & Raj Jnawali, 1991; Laurie, 1982). In captivity, females start to reproduce at an average of five years and males at nine years (Wirz-Hlavacek, 1999).

White rhinoceros females have been observed to start cycling as early as 3.8 years of age in the wild but the youngest age recorded at first parturition was 6.5 years (Owen-Smith, 1988, Pienaar, 1994). Bulls are considered to reach sexual maturity around eight years, although they generally start holding a territory around 12 years of age (Owen-Smith, 1988).

1.3.1.3 Oestrous cycles

1.3.1.3.1 Indian rhinoceros

Inter-oestrous periods ranging from 36 to 58 days have been recorded in free-ranging Indian rhinoceros (Laurie, 1982). In captivity, mean cycle lengths of between 43 and 48 days were measured in females by using non-invasive reproductive assessment (Kassam & Lasley, 1981; Kasman *et al.*, 1986; Schwarzenberger *et al.*, 2000a).

Oestrous behaviour in the Indian rhinoceros is particularly notable, with frequent urination and a highly intensified respiration that is both audible and visible during expiration, like whistling (Buechner & Mackler, 1978; Fowler, 1986). Breeding males in the wild are easily identifiable as they possess extensive secondary neck folds and large procumbent mandibular incisors (Laurie, 1982).

1.3.1.3.2 Sumatran rhinoceros

Oestrous cycles in three females Sumatran rhinoceros have been reported to last between 25 and 30 days, based on behavioural observations (Bosi, 1996; Schaffer *et al.*, 1994), coinciding with results from non-invasive reproductive assessment and ultrasound examinations in captive females (Heistermann *et al.*, 1998; Roth *et al.*, 2001).

Signs of oestrus in the female included restless activity and whistling behaviour, increased interest in the male and urination frequency, a swollen and soft vulva with mucoid vaginal discharge (Bosi, 1996). Males are very aggressive towards females, sometimes fatally, except when females are in oestrus. Roth *et al.* (2001) provided the first evidence that Sumatran rhinoceroses were induced ovulators, with ovulation occurring within 46 hours of copulation.

1.3.1.3.3 White rhinoceros

In the wild, inter-oestrous intervals of approximately 30 days have been reported (Owen-Smith, 1988).

In captivity, behavioural observations of white rhinoceros females indicated oestrous cycle length ranging from 30-90 days (Lindemann, 1982). The combined measurement of urinary 20α-OHP and oestrogens resulted in cycle lengths of 25 and 32 days for northern and southern white rhinoceros, but these figures only referred to one animal of each species (Hindle *et al.*, 1992). By using both regular ultrasound examination and faecal pregnanc analysis, Radcliffe *et al.* (1997) found a cycle length of 31 and 35 days with a follicular phase of nine days. More recently, longitudinal studies using faecal pregnancs analysis have revealed the occurrence of longer cycle lengths in captive white rhinoceros, lasting up to 8-10 weeks, as well as of acyclic animals or "flatliners" (Hermes *et al.*, 2000; Patton *et al.*, 1999; Schwarzenberger *et al.*, 1998).

1.3.1.4 Gestation and lactation

The gestation length in both the Indian and white rhinoceros has been described to average 16 months (Hodges & Green, 1989; Nath *et al.*, 1993; Owen-Smith, 1988; Schwarzenberger *et al.*, 2000a). The birth weight in captivity has been determined to vary between 55 and 90 kg in the Indian rhinoceros and between 55 and 65 kg in the white rhinoceros (Fowler, 1986; Wirz-Hlavacek, 1999).

Lactation in a captive Indian rhinoceros female has been reported to last 20 months (Nath *et al.*, 1993). Weaning was observed to occur at around one year of age in wild white rhinoceroses (Owen-Smith, 1988).

The chemical composition of captive white rhinoceros' milk showed that mid-lactation composition did not differ between black and white species (Mathews, 1973).

No information is available for the Javan rhinoceros, nor for the Sumatran rhinoceros (Rookmaker, 1998).

1.3.1.5 Calving interval

In the wild, the mean observed calving interval for Indian rhinoceros females was 45.6 months (Dinerstein & Raj Jnawali, 1991) while in captivity, it averaged 36 months (Wirz-Hlavacek, 1999).

It is estimated that the Javan rhinoceros population of Ujong Kulon National Park, which has not increased in numbers over the last 20 years, presented a long calving interval of around eight years (Foose & Van Strien, 1997).

Birth intervals recorded for individually known white rhinoceros cows averaged 31 months in the wild and 24 months in captivity (Lindemann, 1982; Owen-Smith, 1988).

1.3.1.6 Recruitment

In the wild, successful protective measures have allowed the Indian rhinoceros population in Chitwan to grow at around 6% per annum (Lauric, 1982). In contrast, the extremely reduced Javan rhinoceros population has stabilised in numbers while the Sumatran has dramatically declined (Foose & Van Strien, 1997).

Free-ranging populations of white rhinoceros have been known to increase at an annual rate of 8-9% (Owen-Smith, 1988). By contrast, the recruitment of the white rhinoceros population in captivity has been catastrophic. A large proportion of founders has never produced any offspring, while over 50% of the captive population

presented no or erratic ovarian cycles (Lindemann, 1982; Schwarzenberger *et al.*, 1999b). White rhinoceros kept in pairs have rarely reproduced and they exhibited the best breeding output in institutions which managed them in large groups (Kock & Garnier, 1993; Lindemann, 1982). Management parameters like group size, the social hierarchy of the animals, a change in males, and enclosure size seem to be important factors of influence but their mechanisms of action on breeding success remains to be determined (see Schwarzenberger *et al.*, 1999b).

1.3.1.7 Seasonality

One study of the Indian rhinoceros population in Chitwan National Park detected birth peaks during the moosoon while another study did not find any seasonal pattern (Dinerstein & Raj Jnawali, 1991; Laurie, 1982).

Free-ranging white rhinoceros females exhibited conception peaks early in the rainy season in Natal (Owen-Smith, 1988). They have been found to cycle all year round in captivity (Schwarzenberger *et al.*, 1998) where they produce calves throughout the year (Rookmaker, 1998).

1.3.1.8 Reproductive pathology

Perinatal death accounted for 29% of all mortality reported in the captive Indian rhinoceros population maintained in European institutions in the 1970s and 1980s, with a majority of stillbirths and neonatal deaths being responsible (Kock & Garnier, 1993).

Reports of reproductive pathologies include leiomyomas in the uterus, pyometra, retained pregnancy and cystic ovaries in the Indian rhinoceros (Göltenboth *et al.*, 2000; Kock & Garnier, 1993); uterine cysts and tumours in the Sumatran rhinoceros (Roth & Brown, 1999; Schaffer *et al.*, 1994) and cystic endometrial

hyperplasia in old females, endometrial cysts, endometritis, vaginal haemangioma, ovarian tumours, follicle cysts and persistent corpus luteum in white rhinoceroses (Godfrey *et al.*, 1991; Hermes *et al.*, 2000; Radcliffe *et al.*, 1997; Reese *et al.*, 1992).

1.3.2 Black rhinoceros

1.3.2.1 Social structure

1.3.2.1.1 Social organisation

Originally, black rhinoceros were believed to be territorial, due to the fact that most observations involved solitary animals, that they mark their territory while defecating and urinating and that they are known to be aggressive amongst themselves (see Skinner & Smithers, 1990). However, some bulls have overlapping home ranges and detailed information is lacking concerning the relations of dominance between all males in a population (Adcock, 1994; Goddard, 1967; Joubert & Eloff, 1971; Shenkel & Shenkel-Hulliger, 1969a).

Adult males are generally solitary but will associate closely with receptive females. Aggressive behaviour occurs mainly between bulls when a female is in oestrus or following the arrival of new individuals in a resident population.

The only stable social group is the mother-calf unit, which persists until the mother gives birth again, at which stage she will reject her previous calf before parturition occurs.

1.3.2.1.2 Sex ratio

Most natural black rhinoceros populations show an adult sex ratio close to parity or slightly favouring males, and birth sex ratio has been found to be close to parity (see Berger & Cunningham, 1995). However, male births outnumbered female

births in new and small populations in South Africa, as well as in captivity (Bride *et al.*, 1996).

1.3.2.1.3 Mating system

The mating system of the black rhinoceros has been described to be both polygynous and polyandrous (Goddard, 1966; Ritchie, 1963).

1.3.2.2 Reproductive anatomy

1.3.2.2.1 Male

The black rhinoceros male reproductive tract has been described as comparable to both the stallion and the bull (Schaffer & Beelher, 1990; Schaffer *et al.*, 1991). The accessory glands lie within the pelvic canal. They include paired bulbourethral glands, a prostate with two trapezoidal lobes and bilateral vesicular glands. The testes are extra-abdominal and lie dorsolaterally to the penis in the same skin-fold.The penis is musculocavernous and the tip of the flaccid penis is directed caudally.

1.3.2.2.2 Female

The female anatomical structures have also been described as comparable to both the mare and cow (Godfrey *et al.*, 1991, Schaffer & Beelher, 1990; Schaffer *et al.*, 1991). In adult females, the total length of the reproductive tract varied between 70 cm and 100 cm. The vagina is smooth with large longitudinal folds that course between the urethral opening and the cervix. A hymen was found to block the vaginal canal in nulliparous females. Another characteristic of both the black and white rhinoceros reproductive tract is the very convoluted cervix with interdigitating folds that appear on ultrasound as dark and light swirls above the bladder. These unique anatomical features have hampered the development of successful AI in African

rhinoceroses, but a recent and innovative catheter system seems to have overcome these problems (Göltenboth *et al.*, 2000; Hermes *et al.*, 2000).

A short bifurcated body leads to a bicornuate uterus and uterine horns occupy more than 40% of the entire length of the tract. The ovaries consist of an outer cortex and a central medulla, similar to those seen in ruminants. They are flat and oval when quiescent and spheroid during active folliculogenesis. Unlike horses, rhinoceroses appear to ovulate on the surface of the ovary at several sites

1.3.2.3 Sexual maturity

1.3.2.3.1 Males

1.3.2.3.1.1 Wild

Little information is available on age at sexual maturity in free-ranging black rhinoceros males. The few records available indicate that males reach sexual maturity at around six years, start holding a territory around 8-10 years of age and subsequently begin breeding (Adcock, 1994; Shenkel & Shenkel-Hulliger, 1969a).

1.3.2.3.1.2 Captivity

A black rhinoceros calf was apparently born to a male 3½ years old but this seems exceptional (Jones, 1979). Data from captive animals suggest that males are not able to sire offspring until the age of 4½ at the earliest and that first conception generally occurs with males of approximately six years of age (Lindemann, 1982; Rookmaker, 1998).

1.3.2.3.2 Females

1.3.2.3.2.1 Wild

A wild black rhinoceros female has been described to conceive as early as 3.8 and other anecdotal reports indicate that some animals first bred at around five years

old (Goddard, 1967; 1970a, 1970b; Hitchins & Anderson, 1983;; Shenkel & Shenkel-Hulliger, 1969a). More recent estimations based on a larger sample size determined a mean age at first conception of 6-6.5 years for females *Diceros bicornis michaeli* and *D.b. minor* and of 8 years old for females *D.b.bicornis* (Bride *et al.*, 1996).

1.3.2.3.2.2 Captivity

A captive born female was able to produce a calf at the early age of 4½ years but studies involving a large number of animals determined a mean age at first calving of 8.3 and 9 years (Lindeman, 1982; Smith & Read, 1992).

1.3.2.4 Oestrous cycles

1.3.2.4.1 Oestrous behaviour

Descriptions of oestrous behaviour in wild black rhinoceros have been reported by Brett *et al.* (1989), Goddard (1966), Hitchins & Anderson (1983), Shenkel & Shenkel-Hulliger (1969a). The period from the first day of attendance by the bull to the time of copulation was described to last 6-7 days and the duration of receptivity only 1-3 days. During the initial approach of the female, the male adopts a variety of postures which include rushing, jabbing, horn-clubbing, puffing, nudging with horn and head, rubbing muzzle on female's sides and shoulders, resting chin on croup, while the female may threaten and attack, feigned or real, and squeal.

When in oestrus, the female's behaviour is characterised by the frequent squirting of small amount of urine which forms a white streaky deposit around the swollen vulva and which leave scent trails on the ground. A bull detects a female approaching oestrus by sniffing the female's urine and performing of flehmen response (Plate 3). He then begins a series of preliminary mounts before copulation takes place, performs mate guarding, a high frequency of spray urination and

redirected activity in the form of bush destruction, as well as fighting with other males (Plate 3).

In the wild, the direct observation of 47 copulations showed that they last between 12 and 43 minutes, and that between two and nine ejaculations occurred per copulation (Hitchins & Anderson, 1983).

1.3.2.4.2 Oestrous cycle length

1.3.2.4.2.1 Wild

Behavioural studies in wild black rhinoceros females reported an average inter-oestrous interval of 35 days (range, 26-46 days; n=10), with more erratic intervals during puberty and before first conception (Hitchins & Anderson, 1983).

1.3.2.4.2.2 Captivity

Behavioural records involving a larger number of females suggested that oestrous cycle lengths averaged 24 to 32 days, with individual cycle lengths ranging from 20 to 83 days (Lindemann, 1982). Using non-invasive reproductive assessment, the mean cycle length has been determined to lie between 21 and 26.5 days (Brown *et al.*, 1997; Hindle *et al.*, 1992; Schwarzenberger *et al.*, 1993).

1.3.2.5 Gestation

Isolated records on captive females indicate a gestation length varying from 419 to 480 days and an average of 458 days (n=30), based on behavioural observations (Lindemann, 1982). By using faecal and urinary steroid analysis, gestation lengths ranging from 442 to 494 days were determined (Berkeley *et al*, 1997; Hindle *et al.*, 1992; Schwarzenberger *et al.*, 1993, 1996b).

Anecdotal observations in the wild report a gestation period lasting between 446 and 478 days (Goddard, 1967; Hall-Martin & Penzhorn, 1977; Hitchins & Anderson, 1983).

In the wild, the only published observation of a birth reports that the calf was dropped within ten minutes of becoming visible, while the mother stood; females have been described as particularly irritable before and after calving (Kingdon, 1979).

At birth, black rhinoceros calves have been reported to weigh an average of 40 kg in the wild (Skinner & Smithers, 1990).

1.3.2.6 Lactation

Lactation has been described as persisting for up to 15 months but some calves were observed to be still suckling at 17.5 months, 19 months and even 24 months (Goddard, 1967; Hall-Martin & Penzhorn, 1977; Hitchins & Anderson, 1983; Joubert & Eloff, 1971; Ritchie, 1963). Calves have been observed to start eating solid food at five weeks (Shenkel & Shenkel-Hulliger, 1969a).

The information available on the composition of milk in the black rhinoceros indicates that it is comparable to cow's milk but more concentrated in lactose and less concentrated in energy, proteins, fat and calcium (Fowler, 1986; Robbins, 1993).

Early attempts to hand-rear black rhinoceros calves were rare and often unsuccessful until more specific milk formulas were developed (Kirkwood *et al.*, 1989).

1.3.2.7 Calving interval

1.3.2.7.1 Wild

Mean calving intervals of 24 to 48 months have been reported (Bride *et al.*, 1996; Goddard, 1967, 1970b; Hall-Martin & Penzhorn, 1977; Hitchins & Anderson, 1983; Shenkel & Shenkel-Hulliger, 1969a; Western & Sindiyo, 1972).

Black rhinoceros females in the wild are considered to be able to produce at least seven and at most 12 calves during their reproductive life, which has been estimated to end at approximately 30-35 years of age (Shenkel & Shenkel-Hulliger, 1969a).

1.3.2.7.2 Captivity

In studies involving a large number of black rhinoceros births in captivity, calving intervals varied between 17 months and 112 months, with means of 35 and 38 months (Lindemann, 1982; Smith & Read, 1992).

1.3.2.8 Recruitment

1.3.2.8.1 Wild

Wild natural populations showed fecundity rates varying between 5.3 and 11% (Goddard, 1967, 1970a; Hitchins & Anderson, 1983; Western & Sindiyo, 1972), but a fecundity rate of 7% seems to be the average for black rhinoceros populations (Bride *et al.*, 1996; Goddard, 1967).

1.3.2.8.2 Captivity

In the 1970s, it was estimated that 40% of the collections were not contributing to reproductive output in captivity and in the early 1990s, there were still 50% of the adult females which were not breeding (Kock & Garnier, 1993; Lindemann, 1982). In the mid-1990s, a notable improvement was observed in the captive population in North America, with a core group of zoos achieving annual recruitment rates of 4% (Emslie & Brooks, 1999).

1.3.2.9 Seasonality

1.3.2.9.1 Wild

Black rhinoceros have been described as being able to mate at any time of the year (Hall-Martin, 1986; Hitchins & Anderson, 1983, Mukinya, 1973; Ritchie, 1963, Shenkel & Shenkel-Hulliger, 1969a). However, a bimodal distribution of births has been observed in black rhinoceros populations monitored in Natal over a 13-year period, with parturition peaks occurring in mid-summer and in mid-winter (Hitchins & Anderson, 1983). Other populations in the southern part of the African continent only showed parturition peaks in the late summer (Hall-Martin & Penzhorn, 1977; Joubert & Eloff, 1971).

In East Africa, a parturition peak was described in June/July in Tsavo (Shenkel & Shenkel-Hulliger, 1969a) but could not be identified in the Ngorongoro (Goddard, 1967).

1.3.2.9.2 Captivity

A study of the distribution of all births recorded in captivity (n=292) indicated that young captive black rhinoceros are born throughout the year, although a slight increase in birth numbers has been noticed during the months of October to December (Rookmaker, 1998).

1.3.2.10 Reproductive pathologies

1.3.2.10.1 Abortion

In 1980, a survey in zoological institutions revealed that 12% of all captive breeding females had had abortions (Lindemann, 1982). More than half of these females had never produced live offspring. In a subsequent survey, abortion was

found to be responsible for around 4% of all cases of mortality reported (Kock & Garnier, 1993).

1.3.2.10.2 Perinatal death

In the wild, calves which are less than three months of age are known to be vulnerable to predation by hyaenas, lions and even marauding dogs (Berger, 1994; Elliot, 1987; Goddard, 1967; Hall-Martin & Penzhorn, 1977).

In captivity, loss of calves during their first year has been reported to vary between 12 and 20%, with more than half of these deaths occurring within the first week (Lindemann, 1982; Noble & Ryder, 1992). Apart from stillbirth, causes of perinatal death have included the failure to obtain maternal antibodies from colostrum, as well as a congenital heart failure caused by persistent ductus arteriosus (Reese & Edwards, 1996; Stickle *et al.*, 1992).

1.3.2.10.3 Post-partum pathology

Three cases of uterine prolapse have been reported, one of which having been caused by a uterine tear following parturition (Kock & Garnier, 1993).

1.3.2.10.4 Subfertility and other reproductive pathologies

In captivity, Lindemann (1982) reported that 20% of adult females had never bred, although they had been exposed to breeding situations. The same author concluded that this lack in breeding success was mainly due to incompatibility between animals. There are however a number of reproductive pathologies which have been reported in captive black rhinoceros, having been diagnosed with ultrasound examination or identified at post-mortem examination. These include ovarian and/or endometrial cysts and tumors, uterine torsion, vestibulo-vaginitis, while endometrial hyperplasia, leiomyofibromas and ovarian fibromas were identified

in older animals at necropsy (Godfrey *et al.*, 1991; Göltenboth *et al.*, 2000; Schaffer *et al.*, 1991).

1.4 WILDLIFE CONSERVATION AND REPRODUCTIVE ASSESSMENT

1.4.1 General conservation issues

Most endangered wildlife species now only exist in small and isolated populations that are vulnerable to stochastic events (Soulé, 1986). Environmental risks, such as epidemic diseases and natural catastrophes, are increasingly recognised as severe threats to small populations, while demographic threats (biased sex ratio, random fluctuation in individual reproduction) are a challenge for populations smaller than 20 animals (Foose *et al.*, 1993). Small populations may also lose genetic variability, which is necessary for individual fitness and for adapting to ever-changing environmental conditions, through drift and inbreeding depression which in turn may lead to decreased fertility, increased neonatal and juvenile mortality (Foose *et al.*, 1993). Under a certain population size or Minimum Viable Population, these genetic and demographic processes feed back on each other to create an "extinction vortex".

Conservation strategies for captive populations of endangered species usually aim at ensuring that 90% of a species' genetic diversity is maintained for the next 200 years (Soulé, 1986). In order to preserve populations of endangered species from the short-term genetic risks (5-10 generations), it has been established that a minimum effective population size of 50 individuals is necessary, while 500 animals is required for maintaining long-term adaptability (Soulé, 1986). Because the effective population size only represents a portion (20-60%) of the total population (Mace, 1986), populations need to be managed as a single larger meta-population, which

entails moving animals between sub-populations in order to correct genetic and demographic problems (Foose *et al.*, 1993).

Although most captive breeding programmes for endangered species are based on such genetic considerations and management techniques, they also apply to an increasing number of wild populations of small size. Population simulation models for black rhinoceros populations maintained in Kenyan sanctuaries have shown that they were non-viable without intensive management, requiring the continuous monitoring of population size and structure and the periodic introduction of unrelated individuals (Foose *et al.*, 1993). Both wild and captive populations need therefore to be considered as complementary components of an endangered species' conservation strategy.

The success of such conservation strategies would be greatly optimised by the ability to monitor reproductive potential and success of individuals in a population, as well as by an accurate knowledge of the species reproductive characteristics.

1.4.2 Importance of reproductive assessment

1.4.2.1 Assessment of ovarian activity

Assessing the reproductive status of wild animals is necessary in order to determine the reproductive potential of individuals and to accurately evaluate the breeding output of individuals and of populations.

At an individual animal level, such information is essential for the effective breeding management of endangered wildlife in captivity, by determining which animals to pair together or move to another zoo, and by detecting reproductive disorders (Hodges, 1996; Holt, 1994). In the wild, the assessment of individual

reproductive status is also important since translocation and re-introduction processes, which represent costly and risky exercises, are becoming more frequent.

At a population level, assessment of ovarian or testicular activity allows for a more accurate determination of reproductive parameters such as age at sexual maturity, patterns of ovarian cyclic activity and seasonality, gestation period and calving intervals (Lasley & Kirkpatrick, 1991). Such parameters reflect the general health and stability of a population and provide a basis for important management decisions. In addition, an accurate evaluation of the impact of various management procedures, such as translocation or dehorning, needs to be based on a precise assessment of reproductive parameters.

At a species level, being able to monitor reproduction is the starting point for obtaining basic information on the reproductive biology of the species. This is presently missing for most mammalian species (see Monfort, 2000). The fundamental reproductive physiological mechanisms that are used in mammals (polyoestrus, induced ovulation, delayed implantation, sperm storage, etc.) imply the occurrence of numerous differences in gamete or hormonal function between species (Holt, 1994). The use of non-invasive reproductive assessment has enabled the characterisation of reproductive activity in a number of wildlife species in captivity. However, since reproductive success in captivity is influenced by a number of environmental and non-environmental factors, the study of captive specimens does not represent a true reflection of their natural reproductive pattern, which need to be evaluated under free-ranging conditions.

1.4.2.2 Assessment of pregnancy status

In captivity, the diagnosis of pregnancy is also essential for the evaluation of the outcome of breeding or insemination attempts, for monitoring foetal well-being and for predicting parturition. It allows ahead of time for the animal to be provided with the most suitable environment during pregnancy, thereby reducing pregnancy loss and increasing neonatal survival (Hodges, 1996).

In the wild, the determination of pregnancy has been useful for providing more accurate demographic data by assessing foetal loss rates. This was undertaken in elk (Stoops *et al.*, 1999), caribou (Messier *et al.*, 1990), and moose (Berger *et al.*, 1999). It has also enabled the evaluation of foetal loss associated with infectious diseases, such as brucellosis in bison (Lasley & Kirkpatrick, 1991).

1.4.2.3 Assessment of reproductive failure

The ability to monitor reproductive status and to determine the normal reproductive function in wildlife species provides the starting point for addressing the problem of reproductive failure in endangered species (Hodges, 1996). Reproductive failure is not uncommon in captivity, but the contributing factors, which can be anatomical or functional, are poorly understood. The epidemiological analysis of sub-fertility or infertility conditions, which often have a multi-factorial origin, is also an area demanding increasing attention.

Another area where non-invasive assessment of reproductive failure can be useful is for evaluating the efficiency of contraceptive methods in wildlife populations. Contraception is important in regulating numbers under captive situations, where limited space is available (Kleiman *et al.*, 1996). It is also necessary for some species under natural situations, when free-ranging populations exceed their

carrying capacity in a certain habitat or when introduced species start having deleterious effects on the ecosystem balance (see Cowan *et al.*, 2000).

1.4.2.4 Reproductive technologies

Reproductive technologies refer to procedures designed to overcome natural reproductive constraints through the use of gametes and/or embryos and include techniques such as artificial insemination (AI), *in vitro* fertilization (IVF) and embryo transfer (ET) (Hodges, 1996; Holt, 1994). At the individual level, these techniques can overcome infertility problems, while, at the population level, artificial breeding techniques can greatly contribute to the genetic management of metapopulations. However, the number of non-domesticated species in which reproductive technologies have been applied remains very small and their development relies upon a good understanding of the basic female physiology (Holt, 1994).

Indeed, information on the timing of ovulation is indispensable for effective use of timed mating and assisted reproduction procedures such as artificial insemination and embryo transfer (Hodges, 1996; Holt, 1994). The success of AI or IVF also depends on the ability to collect and cryopreserve semen. Semen was successfully collected from Indian and African rhinoceroses by electroejaculation from anaesthetized animals (Göltenboth *et al.*, 2000; Platz *et al.*, 1979; Schaffer *et al.*, 1998), by penile massage in unanaesthetized animals after extensive periods of training (O'Brien & Roth, 2000; Schaffer & Beelher, 1988; Schaffer *et al.*, 1991) and post-mortem by recovery of epididymal sperm (Schaffer *et al.*, 1991; O'Brien & Roth, 2000). Post-ejaculatory semen could be collected from a Sumatran rhinoceros although samples contained numerous abnormal spermatozoa (O'Brien & Roth,

2000). The same authors also successfully developed a sperm cryopreservation protocol in this species.

1.4.3 Invasive methods of reproductive monitoring

1.4.3.1 Blood sampling

Since all aspects of reproduction are dependent on characteristic changes in hormone production, the analysis of endocrine status is the most effective method for monitoring reproductive function. However, due to their intractable nature, wild animals need to be immobilised for blood sampling.

Chemical immobilisation of wild animals is required for most management operations, thus providing a good opportunity for sampling free-ranging animals. The recent and dramatic improvement in the drug combinations, delivery systems and experience of the immobilisation teams has considerably reduced mortality linked with chemical immobilisation of free-ranging wildlife (Kock, 2000). Nevertheless, there is always a risk associated with the chemical immobilisation of any wild animal, whether in the wild (Kock, 2000) or in captivity (Fowler, 1986). The regular chemical restraint of individual wild animals for long-term reproductive studies, in which animals need to be sampled repeatedly and regularly over periods of reproductive activity, is therefore unrealistic.

In addition, chemical immobilisation can alter the quality of samples collected for reproductive hormone analysis due to the potential stress and subsequent interference between reproductive hormones and stress hormones (Godfrey *et al.*, 1991). This particularly applies to some cervids, in which adrenals are known to secrete a significant amount of progesterone when exposed to stressful situations (Asher *et al.*, 1989).

Finally, the impact of chemical immobilisation on reproductive events is unknown for most species, simply because the tools for monitoring such impact, such as non-invasive reproductive assessment, were previously not available. Only recently was such a study undertaken, which showed that neonate production in moose (*Alces alces*) was not decreased by the handling of animals (Berger *et al.*, 1999).

Captive individuals can sometimes be rendered more tractable and be trained to stand in a restraint chute for regular sampling, as has been undertaken with black and white rhinoceros (Berkeley *et al.*, 1997; Radcliffe *et al.*, 1996, 1997; Schaffer *et al.*, 1991). In certain zoological institutions such as Port Lympne Zoo in the UK, all rhinoceros are used to being handled very regularly at a very early age and blood sampling, as well as routine veterinary care, can be achieved without chemical immobilisation (B. White, personal communication).

In species which can be handled through intensive training, such as captive elephants, regular blood sampling without chemical immobilisation has permitted the acquiring of considerable knowledge on their reproductive physiology (see Hodges, 1998).

1.4.3.2 Laparotomy and laparoscopy

Laparotomy and laparoscopy are invasive methods that can be used for reproductive status assessment, but which cannot be performed without chemical restraint. These techniques have increasingly been replaced by non-invasive procedures, except for sexing birds (Fowler, 1986).

1.4.4 Non-invasive methods of reproductive assessment

1.4.4.1 Behavioural observations

Traditionally, reproductive assessments of wild animals have used behavioural observations to establish reproductive parameters such as age at sexual maturity, oestrous cycle and gestation lengths, calving interval. Some species exhibit typical signs during oestrus, such as the increased activity and vocalisations in female felids, the acceptance of males in other carnivores and ungulates, and the genital swelling of primates (Fowler, 1986).

However, the observation of oestrous behaviour is not always accompanied by ovulation in some ungulates and it can occur in certain cases of ovarian malfunction (Holt, 1994). In addition, certain species, such as elephants, do not exhibit consistent signs of oestrous behaviour, except for occasional mounting behaviour (Fowler, 1986). There are also pronounced differences in behaviour between individuals of certain species, for example the black rhinoceros (Carlstead, 1999b, Ritchie, 1963). This renders the interpretation of reproductive behaviour for assessing reproductive status increasingly unreliable.

In the wild, ecological studies of endangered species such as black rhinoceros were undertaken in the late 1960's and 1970's (Goddard, 1967, 1970a; Joubert & Eloff, 1971; Ritchie, 1963; Shenkel & Shenkel-Hulliger, 1969a). These studies were facilitated by the fact that some black rhinoceros lived in open habitats and were not too wary of humans at that time, while their density was still high. However, observations of their reproductive behaviour were sparse and opportunistically made. In other species, behavioural studies that led to an increase knowledge of reproduction included those covering a great span of time and involving the monitoring of

recognisable animals, such as that which have been undertaken with elephants (Moss, 1988).

1.4.4.2 Examination of the reproductive tract

Just as in domestic large animals, transrectal examination of the reproductive tract can also be used for assessing ovarian activity or the presence of a foetus, but it still requires the immobilisation of the animal. Pregnancy diagnosis can nevertheless be made through transrectal palpation in medium sized ungulates. Large mammals with long reproductive tracts, such as rhinoceroses, are obviously difficult to palpate, since the gravid uterus of a mature black rhinoceros drops into the abdominal cavity during late gestation (Godfrey *et al.*, 1991).

In the wild, examinations of vaginal histology can be performed on immobilised animals, but in the case of black rhinoceros they did not yield significant results (Kock *et al.*, 1989). Post-mortem examinations of reproductive tracts are difficult since the carcasses in the wild are usually found a few days, or even weeks, after death occurred, when decomposition processes have usually started. However, in species for which destructive sampling of a population is possible, such as elephants, examinations of reproductive tracts have allowed important information to be gathered (see Hodges, 1998)

1.4.4.3 Ultrasound examination

Real-time ultrasonography is increasingly used in the case of exotic species. The general advantages of this procedure are that: 1) it provides real-time imaging; 2) it produces high-resolution characterisation of tissues and organs; 3) it enables morphometric measurements of structure *in situ*; 4) it facilitates documentation and storage of primary data and 5) it is portable and compatible with field studies. Some

limitations include the need for chemical or physical restraint and the high costs of ultrasound equipment (Hildebrandt & Göritz, 1995, 2000). In addition, there is a slight risk of foetal loss or damage associated with chemical immobilisation (Holt, 1994)

In rhinoceroses, ultrasound examination have been performed successfully on animals restrained in a free-stall chute (Berkeley *et al.*, 1997; Radcliffe *et al.*, 1996, 1997; Schaffer & Beelher, 1990; Schaffer *et al.*, 1994). This process nevertheless necessitated the training of the animals for around six months.

One of the main applications of ultrasound examination is pregnancy diagnosis and foetal monitoring (Hildebrandt & Göritz, 1995). By using this examination, early pregnancy was diagnosed in black rhinoceros as soon as 28-day post-conception and early embryo loss was also diagnosed at a similar stage of pregnancy in a white rhinoceros (Radcliffe *et al.*, 1996, 1997).

Ultrasound examination is also used for describing the normal anatomical and physiological features of the female reproductive tract and for identifying pathological conditions, for example in the African and Sumatran rhinoceroses (Hermes *et al.*, 2000; Schaffer *et al.*, 1991, 1994).

1.4.4.4 Steroid analysis

1.4.4.4.1 Steroid metabolism

1.4.4.4.1.1 General considerations

In general, steroid hormones are metabolised by the liver and excreted in the urine, or with an enterohepatic circulation in the bile into faeces, with also some excretion in milk and saliva (Gower & Honour, 1984; Herricks, 1991). The excretion of steroid metabolites in faeces has a lag time which depends mainly on the time necessary for the intestinal passage of bile to the rectum. This lag has been estimated

to be 12-24 h in ruminants, 1-2 days in non-ruminants (see Schwarzenberger *et al.*, 1996a).

Measurement of hormone metabolites in excreta in each species requires the preliminary identification of the metabolites excreted, since steroid metabolism and route of excretion is species-specific. In faeces, oestrogens are mainly excreted in their native form and/or as free oestrone and 17α-oestradiol. Faecal oestrogen evaluations can therefore use specific oestrogen antibodies or antibodies against total unconjugated oestrogens.

In contrast, recent studies using chromatographic techniques in combination with immuno-assays and radio-metabolism studies indicated that progesterone is extensively metabolised before excretion. Almost all progesterone is reduced to 5α- or 5β-reduced pregnanes which have either a 20-oxo, a 20α-OH or a 20β-OH group (pregnanediones, mono- and dihydroxylated pregnanes). Luteal activity is therefore best described using group-specific antisera that cross-react with 5-reduced pregnanes compared to antisera that are highly specific for progesterone.

1.4.4.4.1.2 Steroid metabolism in rhinoceros

Radio-metabolism studies performed on the white rhinoceros showed that the majority of progesterone metabolites were excreted into urine while oestradiol was mainly excreted via the bile into faeces (Hindle & Hodges, 1990). In contrast, a similar study in Sumatran rhinoceros showed that progesterone metabolites were almost exclusively excreted into faeces (>99%), while oestrogen metabolites were mainly excreted in urine (>70%) in the form of oestradiol-17β-glucuronide (Heistermann *et al.*, 1998).

Different metabolites are also excreted by different species, even within the same taxa. The Indian rhinoceros mainly excretes oestrone sulphate and pregnanediol-3α-glucuronide in urine (Kasman *et al.*, 1986) while in faeces, pregnanes of the 5β-series were identified as being abundant faecal metabolites of progesterone (Schwarzenberger *et al.*, 2000). The Sumatran predominantly excretes oestradiol glucuronide in urine while the most abundant faecal progesterone metabolites were identified as pregnanes of the 5β-series (Heistermann *et al.*, 1998).

The white rhinoceros predominantly excretes oestradiol glucuronide and 20α-hydroxyprogesterone (20α-OHP) conjugates in urine (Hindle *et al.*, 1992; Hindle & Hodges, 1990). In this species, the main faecal progesterone metabolites appear to be pregnanes containing a 20-oxo-group (Schwarzenberger *et al.*, 1998).

The black rhinoceros mainly excretes oestrone glucuronide and 20α-OHP conjugates in urine (Hindle *et al.*, 1992). However, the authors found that different metabolites were present in relation to the stage of reproductive activity. No urinary pregnanediol-3α-glucuronide could be measured during ovarian cycles in captive black rhinoceros, but this metabolite was detected in urine after the third month of pregnancy.

In the black rhinoceros, the first information on the nature of faecal progesterone metabolites was given by Schwarzenberger *et al.*, (1993), who used HPLC separation and analysis with EIA of faecal extracts from captive females. The authors found small quantities of immunoreactive substances co-eluting with progesterone and 20α-OHP. By using HPLC and GC-MS. Patton *et al.* (1996) were able to identify that the main progesterone metabolites in faecal samples collected from both wild and captive black rhinoceros. They consisted principally of a mixture of 5α- and 5β- reduced pregnanes, with a predominance of the 5α-series which

included, in descending order of abundance: 5α-pregnane-3β-ol-20-one, 5β-pregnane-3α-ol-20-one, 5α-pregnane-3β,20α-diol, 5β-pregnane-3,20-dione, 5α-pregnane-3α,20α-diol, 5β-pregnane-3α,20α-diol, 5β-pregnane-3β,-ol-20-one.

1.4.4.4.2 Urinary steroid analysis

Initially, urinary steroid analysis was developed for monitoring ovarian function in a large number of wildlife species. Measurement of oestrone conjugates (E1C) and pregnanediol-3-glucuronide (PdG) has indeed allowed the characterisation of ovarian activity in a large number of species for the first time, including giraffids, cervids, equids, bovids, carnivores, Old and New World monkeys (see Heistermann *et al.*, 1995; Hodges, 1996; Lasley & Kirkpatrick, 1991).

In other free-ranging wildlife, urine-soaked soil or snow was found to be collected easily from feral horses in order to monitor pregnancy (Kirkpatrick *et al.*, 1988, 1990; 1993) and from bison to characterise oestrous cycles (Kirkpatrick *et al.*, 1992). Collection of urine samples was also successful from wild male mountain gorillas (*Gorilla gorilla beringei*) in order to measure testosterone and cortisol (Robbins & Czekala, 1997). Moreover, non-invasive studies using urinary steroid analysis were undertaken in free-ranging dwarf mongooses (*Helogale parvula*) and wild dogs (*Lycaon pictus*) (Creel *et al.*, 1992, 1997, 1998) in order to define reproductive phenomena such as spontaneous lactation, biased sex ratio at birth, and reproductive suppression.

The measurement of urinary oestrogens and progesterone metabolites has enabled the characterisation of oestrous cycles for the first time in the Indian rhinoceros (Hodges & Green, 1989; Kassam & Lasley, 1981; Kasman *et al.*, 1986) and in both African species (Hindle & Hodges, 1990; Hindle *et al.*, 1992).

Measurement of urinary PdG was useful to detect pregnancy from mid- to late-pregnancy in captive (Hindle *et al.*, 1992; Ramsay *et al.*, 1987) as well as in wild black rhinoceros females (Brett *et al.*, 1989). However, the urine sample collection from wild females was difficult as fresh urine dried quickly in such hot climates.

1.4.4.4.3 Faecal steroid analysis

More recently, the development of analysis of faecal steroid metabolites has proved to be much more practical than urinary steroid analysis and has been used in an increasing number of species.

1.4.4.4.3.1 Advantages and disadvantages

Faecal steroid analysis is considered to be one of the most effective tools available in zoo research today, since the practicality of the sampling procedure allows its use for long-term monitoring of reproductive function in individual animals, both in captivity and under natural conditions ((Brown & Wildt, 1997; Monfort, 2000). In addition, faecal steroid analysis also presents the advantage of providing a representative, "pooled" value of hormonal concentrations (Brown & Wildt, 1997). Hormonal circulating levels can vary markedly and a single blood sample may produce a value that only represents one aspect of a pulsed secretion. In contrast, a faecal sample represents hormonal metabolites over a period of hours.

Nevertheless, faecal steroid analysis presents the disadvantage of requiring an extensive processing time required for drying samples. This step is necessary to overcome problems such as the variability in diets and water contents that has previously been noted to affect hormonal results (Wasser *et al.*, 1993). In addition, it is important to collect and process a large quantity of each sample in order to avoid potential variations in hormonal results linked to an uneven distribution of steroids in

faecal balls. This problem was identified in baboon and elephant (Wasser *et al.*, 1993, 1996), but not in sheep and scimitar-horned oryx (Palme *et al.*, 1996; Morrow & Monfort, 1998).

Another limitation of this technique is its limited use for predicting ovulation when developing assisted reproductive technologies, since there is a lag between the presence of steroids in the serum and their excretion in faeces (Gower & Honour, 1984).

1.4.4.4.3.2 Primates

Faecal oestrogen measurement was found to be useful for the detection of pregnancy in gorilla (*Gorilla gorilla*), orang-utan (*Pongo pygmaeus abeli* - Bamberg *et al.*, 1991) and pygmy lori (*Nycticetus pygmaeus* - Jurke *et al.*, 1997). It also permitted the assessment of luteal activity in primates that included gorilla, orang-utan (Bamberg *et al.*, 1991), cynomologus monkey (*Macaca fascicularis* - Shideler *et al.*, 1993) and Goeldi's monkey (*Callimico goeldii* - Pryce *et al.*, 1994).

In the wild, faecal steroid analysis permitted the characterisation of ovarian cycles in muriqui monkeys (*Brachyteles arachnoides* - Ziegler *et al.*, 1997), baboons (*Papio cynocephalus* - Wasser, 1996) and cotton-top tamarins (*Saguinus oedipus* - Savage *et al.*, 1997).

1.4.4.4.3.3 Carnivores

In felids, oestrogens are almost exclusively excreted in faeces (Brown & Wildt, 1997). Faecal oestrogen analysis has enabled the characterisation of cyclic ovarian activity and was found to be a reliable indicator of ovulation in leopard cat (*Felis bengalensis*), snow leopard (*Panthera uncia*), clouded leopard (*Neofelis nebulosa*), cheetah (*Acinonyx jubatus*), ocelot (*Leopardus pardalis*), Margay

(*Leopardus wiedii*), Pallas cat (*Otocolobus manul* - see Brown & Wildt, 1997; Brown *et al.*, 1994; Graham *et al.*, 1995).

Progesterone metabolites are excreted almost exclusively in faeces in felids, in the form of non-enzyme-hydrolysable conjugates, while in most other species progesterone metabolites are unconjugated in faeces (Brown & Wildt, 1997). Progestagen analysis has been useful for monitoring pregnancy and pseudopregnancy in the tiger (*Panthera tigris*), lion (*Panthera leo*) and caracal (*Caracal caracal*), cheetah, clouded leopard and snow leopard (Brown *et al.*, 1996; 1997; Graham *et al.*, 1995) and mink (Möstl *et al.*, 1993).

In other carnivores, ovarian activity and seasonal patterns of reproductive activity were identified and characterised in maned wolves (*Chrysocion brachyurus*), African wild dog (*Lycaon pictus*) and black footed ferret (*Mustela nigripes* - Brown *et al.*, 1997; Monfort *et al*, 1997; Wasser *et al.*, 1995).

1.4.4.4.3.4 Artiodactyls

Faecal steroid analysis was initially undertaken in domestic cows (*Bos taurus*), to evaluate oestrous cyclicity and to diagnose pregnancy (Desaulnier *et al.*, 1989; Möstl *et al.*, 1984). These studies showed that 17α-oestradiol was the primary faecal oestrogen during pregnancy and that its measurement enabled the detection of pregnancy 90 days post-conception. Faecal oestrogen evaluations were subsequently used to diagnose pregnancy in captive wildlife species that included red buffalo (*Syncerus caffer nanus*), yak (*Bos mutus*), Nubian ibex (*Capra ibex nubiana* - Safer-Hermann *et al.*, 1987), muskox (*Ovibos moschatus* - Desaulnier *et al.*, 1989), mhoor gazelle (*Gazella dama mhorr*), sable antelope (*Hippotragus niger* - Chapeau *et al.*, 1993). They also permitted the diagnosis of pregnancy in free-ranging bison

(Kirkpatrick *et al.*, 1992, 1993), caribou (Messier *et al.*, 1990) and elk (Stoops *et al.*, 1999).

Faecal progestagen measurement has been used to monitor ovarian cyclicity in captive muskox (*Ovibos moschatus* - Desaulnier *et al.*, 1989), scimitar-horned oryx (*Oryx dammah* - Morrow & Monfort, 1998; Shaw *et al.*, 1995), sable antelope (*Hippotragus niger* - Brown *et al.*, 1997; Thompson, 1998), okapi (*Okapia johnstoni* - Schwarzenberger *et al.*, 1999a), vicuna (*Vicuna vicuna* - Schwarzenberger *et al.*, 1995), anoa (*Bubalus depressicornis* - Frank *et al.*, 1997), as well as in semi-captive moose (*Alces alces* - Schwartz *et al.*, 1995) and free-ranging bison (*Bison bison* - Kirkpatrick *et al.*, 1992). The analysis of faecal progestagen immunoreactivity also allowed the diagnosis of pregnancy in caribou (Messier *et al.*, 1990), moose (Monfort *et al.*, 1993), okapi (Schwarzenberger *et al.*, 1999a), free-ranging bison (Kirkpatrick *et al.*, 1993) and free-ranging elk (Stoops *et al.*, 1999), as well as bighorn sheep (*Ovis canadensis* - Borjesson *et al.*, 1996).

Seasonality could be assessed by using faecal steroid analysis and a seasonal pattern of reproduction was established in scimitar-horned oryx but not in sable antelope (Brown *et al.*, 1997; Thompson *et al.*, 1998).

1.4.4.4.3.5 Proboscids

In the African elephant, the corpus luteum is unusual in that it secretes principally 5α-reduced compounds (5α-dihydro-progesterone and 5α-pregnan-3-ol-20-one) and very little progesterone (see Hodges, 1998). Although pregnanolones have been identified as the predominant progestin metabolites in both urine and faeces of the African elephant (Heistermann *et al.*, 1997), profiles of faecal immunoreactive progesterone were indicative of ovarian cyclic activity in the African elephant (Wasser *et al.*, 1996).

By contrast, the measurement of faecal oestrogens was of little significance in the African elephant (Wasser *et al.*, 1996).

1.4.4.4.3.6 Perissodactyls

1.4.4.4.3.6.1 Equids

The development of faecal steroid analysis in wild equids was greatly facilitated by existing work on domestic equids. Early reports of faecal measurement of oestrogens in domestic mares showed that pregnancy diagnosis was possible after three months of gestation, and that 17β-oestradiol and oestrone were the major faecal oestrogens (Bamberg *et al.*, 1984). Although subsequent studies showed that oestrogen metabolites were mainly excreted in urine in horses (see Schwarzenberger *et al.*, 1996a), faecal oestrogen evaluations have permitted the successful diagnosis of pregnancy in captive Equids, including Grevy's zebra (*Equus grevy* - Safer-Harmann *et al.*, 1987), Przewalski horse (*Equus przewalski* - Bamberg *et al.*, 1991; Chapeau *et al.*, 1993), as well as in feral horses (*Equus caballus* - Kirkpatrick *et al.*, 1991). Faecal oestrogen evaluations have also been used to evaluate reproductive disorders such as foetal loss among feral mares (Lucas *et al.*, 1990) or cryptorchidism in horses (Palme *et al.*, 1994).

In Equids, seventy five percent of progesterone metabolites are excreted in the faeces and faecal 20α-progestagen analysis proved useful for detecting pregnancy and for monitoring cyclic ovarian activity in domestic mares, Prezwalski horses and feral horse (Kirkpatrick *et al.*, 1991; Schwarzenberger *et al.*, 1991, 1992).

1.4.4.4.3.6.2 Tapirids

In Malaysian tapir (*Tapirus indicus*), faecal oestrogen concentrations were higher in pregnant than in non-pregnant animals (Bamberg *et al.*, 1991).

1.4.4.4.3.6.3 Rhinocerotids

In both captive African rhinoceros species, the measurement of faecal progestagens has enabled the diagnosis of pregnancy 3-4 months after fertilisation has occurred, as well as the monitoring of cyclic ovarian activity (Berkeley *et al.*, 1997; Brown *et al.*, 1997; Patton *et al.*, 1999, Radcliffe *et al.*, 1997; Schwarzenberger *et al.*, 1993, 1996b, 1998, 2000b). The assays utilised. were radioimmunoassays for progesterone (Patton *et al.*, 1999; Radcliffe *et al.*, 1997) or enzymeimmunoassays for either 20-oxo-pregnanes (Schwarzenberger *et al.*, 1996b, 1998) or progesterone (Berkeley *et al.*, 1997) or 20α-progestagens and pregnanediol (Schwarzenberger *et al.*, 1993).

In the Sumatran rhinoceros, faecal progestagens were measured by using an enzyme immunoassays for 5β-pregnane-3α,20α-diol and 5α-pregnane-3α-ol-20-one and this, combined with urinary oestrogen analysis, allowed the determination of cycle length for the first time in this species (Heistermann *et al.*, 1998). Faecal progestagen analysis using an EIA for 20-oxo-pregnanes and 20α-OH-pregnanes were also useful for evaluation of luteal activity and detection of pregnancy after the third month in the Indian rhinoceros (Schwarzenberger *et al.*, 2000a).

Faecal oestrogens were found to be elevated during the follicular phase in black rhinoceros (Berkeley *et al.*, 1997; Brown *et al.*, 1997) although they did not show any significant pattern in another attempt to evaluate cyclic activity in African rhinoceroses (Schwarzenberger *et al.*, 1996b). In contrast, patterns of faecal oestrogen concentrations in the Indian rhinoceros were related to oestrous behaviour (Schwarzenberger *et al.*, 2000a). Faecal oestrogen concentrations in a black rhinoceros female showed low levels throughout pregnancy, except in peaks occurring one month after mating and two months before parturition, as well as one

day before parturition (Berkeley *et al.*, 1997). Low oestrogen concentrations were also observed during pregnancy of the Indian rhinoceros (Schwarzenberger *et al.*, 2000a).

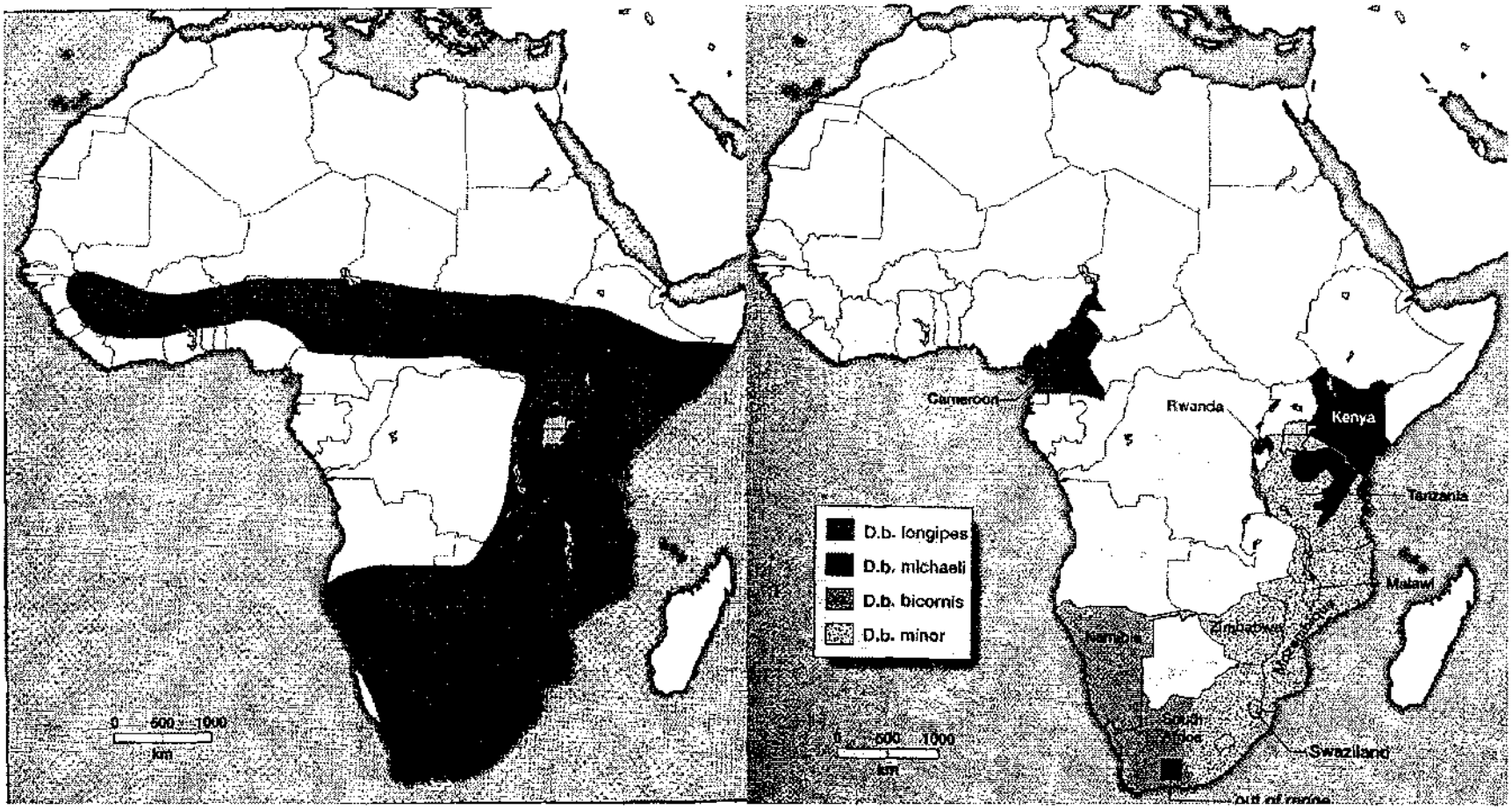
1.4.4.5 Salivary steroid analysis

In captive black rhinoceros, pregnancy was diagnosed two months after conception by measuring immunoreactive 20α-progestagens in saliva (Czekala & Callison, 1996).

1.4.4.6 Others

Recently, infra-red thermography, which measures the heat-radiation a body reflects in the environment, allowed the diagnosis of pregnancy in black rhinoceros females which were 6, 9 and 11 months pregnant (Hilsberg & Eulenberger, 1997). This technique allows an animal to be investigated from a distance of 1-20 m without chemical immobilisation.

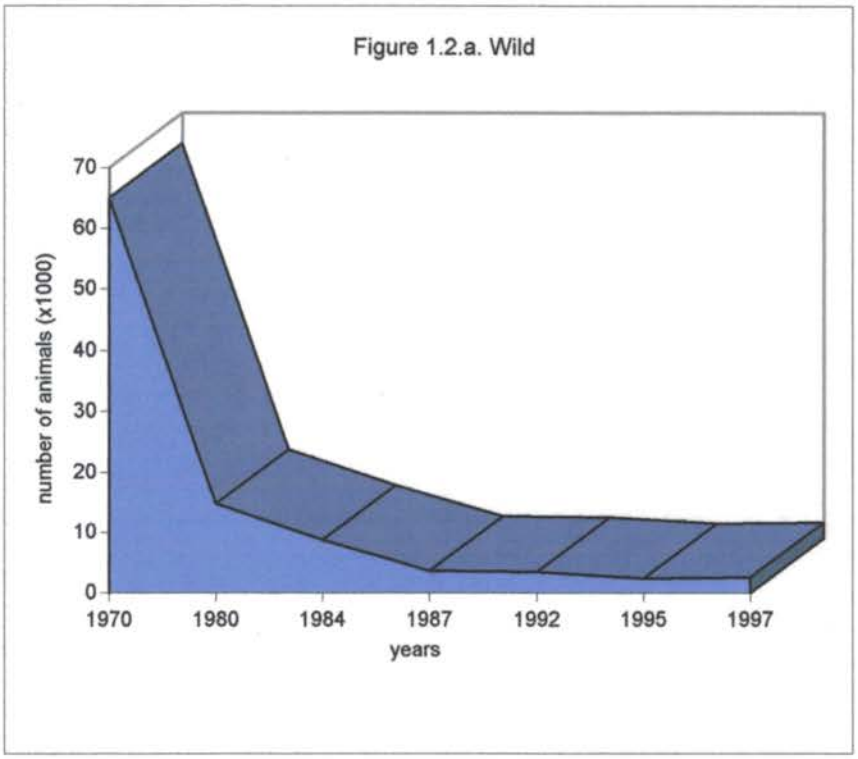
In conclusion, non-invasive techniques for monitoring reproduction, especially faecal steroid analysis, provide the most suitable tools for reproductive studies. The initial step of the present study was to develop such a technique for wild black rhinoceros females, in order to acquire a better knowledge of their basic reproductive biology and to evaluate the influence of environmental factors on their reproductive success. Such knowledge is essential for the success of future conservation strategies of this endangered species.



a) Past distribution (ca. 1700)

b) Present distribution

Figure 1.1. Past and present distribution of the black rhinoceros in Africa (from Emslie & Brooks, 1999).



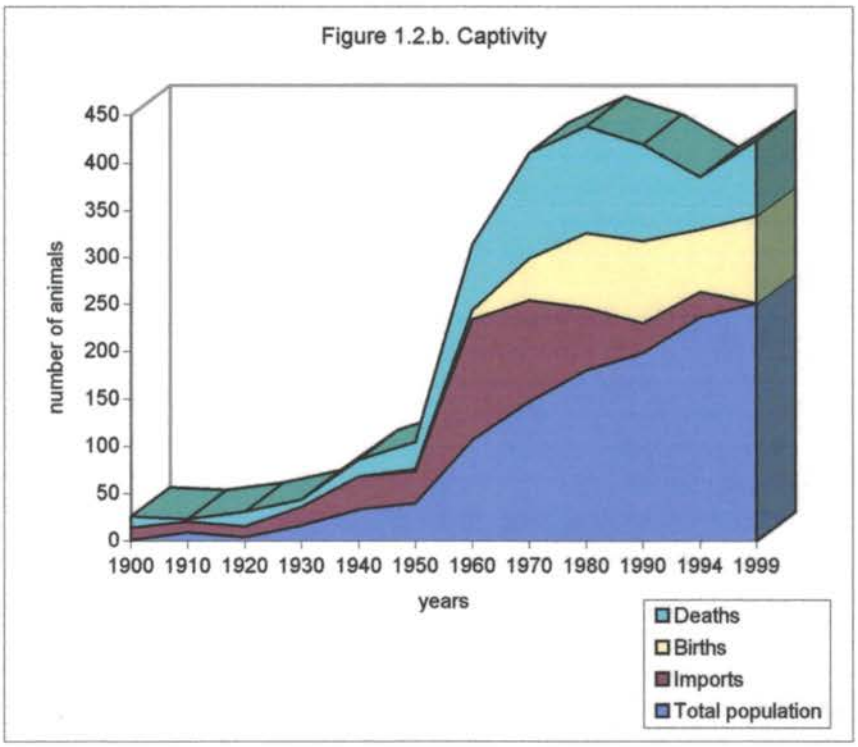
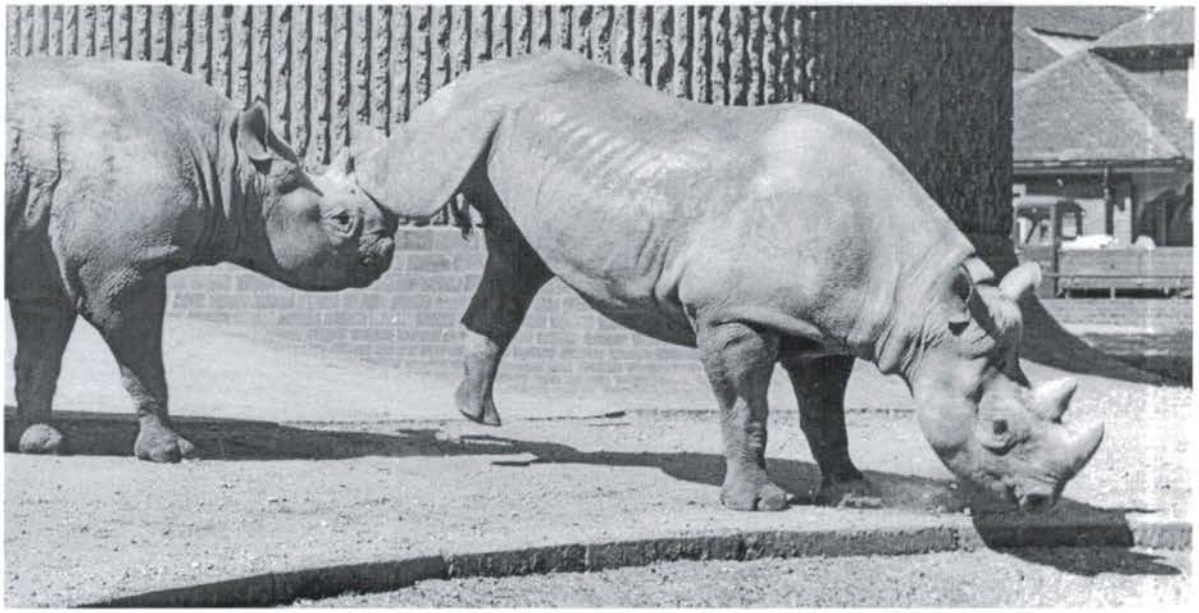
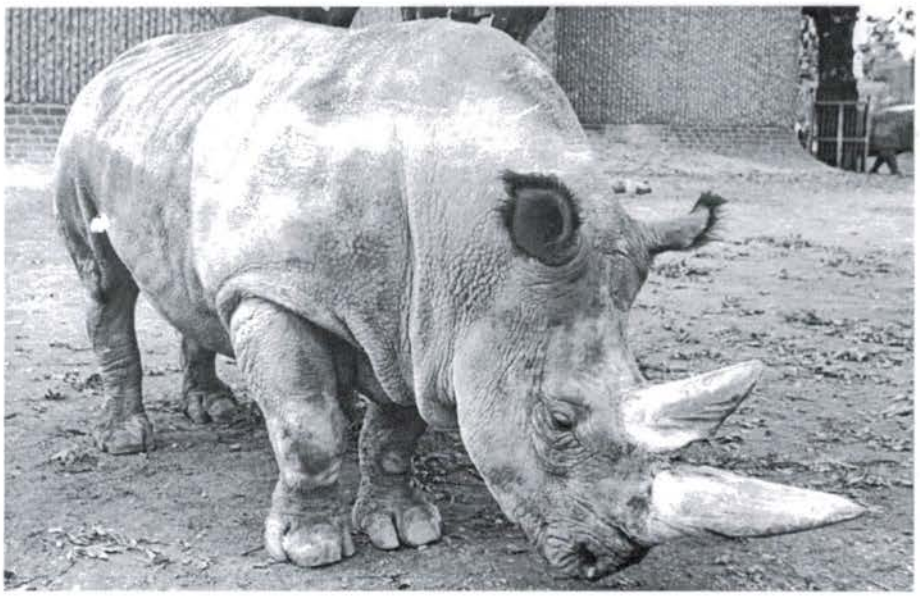


Figure 1.2. Numbers of black rhinoceros in the wild and in captivity (from Emslie & Brooks, 1999; Goltenboth, 1999; Rookmaker, 1998)



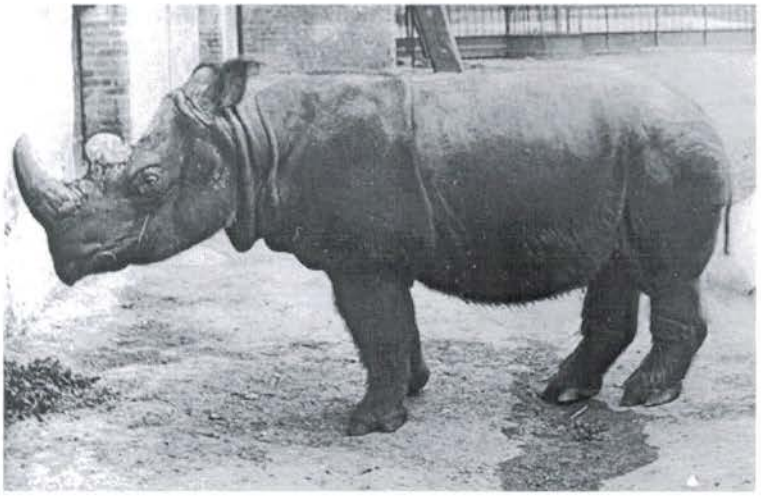
a) Black rhinoceros



b) White rhinoceros

Plate 1. African rhinoceros species exhibited at the Zoological Society of London (from archives ZSL)

a) Indian rhinoceros



b) Sumatran rhinoceros

Plate 2. Asian rhinoceros species exhibited at the Zoological Society of London. The Sumatran rhinoceros was exhibited in 1898 (from archives ZSL)

a) Flehmen performed by a rhinoceros male

b) Mounting behaviour

c) Fighting between males

Plate 3. Reproductive behaviour and activity in the black rhinoceros

2 MATERIALS AND METHODS

2.1 INTRODUCTION

The study included wild, semi-wild and captive populations of black rhinoceros. A wild population was studied in the Save Valley Conservancy (SVC) in Zimbabwe. "Wild" breeding applies to situations where rhinoceroses are in areas of large to medium size (>10 km²) with a natural breeding system (Bride *et al.*, 1996). This population was divided into two sub-populations. One sub-population was located in the southern part of the SVC and mainly included black rhinoceroses which had been translocated there in 1986/88 and could therefore be considered as resident in the area. This sub-population will subsequently be referred as the "wild" population. Another sub-population was located in the northern section of SVC and consisted in animals which had been translocated there in 1993. This sub-population will be referred as the "translocated" population.

A semi-wild population was also monitored in Imire Game ranch in Zimbabwe. The semi-wild status applies to black rhinoceroses living in small areas (<10 km²) with a natural breeding system (Bride *et al.*, 1996).

A captive black rhinoceros population was studied in the UK. Captive breeding refers to situations where rhinoceroses live in small (< 10 km²) to very small areas with a manipulated breeding system (Bride *et al.*, 1996).

2.2 STUDY IN WILD BLACK RHINOCEROSES

2.2.1 Study area and animals

2.2.1.1 Background to the Save Valley Conservancy

Located on the West bank of the Save River in the south-east Lowveld in Zimbabwe, SVC (20E, 32S) is made up of 24 neighbouring properties which are amalgamated into a single complex covering approximately 3387 km². The original motivation for the formation of the Conservancy was to provide sufficiently large areas to facilitate black rhinoceros breeding. After the first black rhinoceroses were translocated to the area in 1986/88, the need for a co-ordinated monitoring and anti-poaching programmes catalysed the formation of the Conservancy, which was largely supported by WWF. With a maximum stocking rate of one black rhinoceros/10 km², the SVC has the potential to harbour a population of more than 300 black rhinoceroses, which is important in order to maintain long-term genetic diversity (Du Toit, 1994; Price Waterhouse, 1994).

2.2.1.2 Main ecological features

SVC is located in Natural Region V of Zimbabwe, characterised by low and unreliable rainfall and inherent poor soil fertility (Price Waterhouse, 1994). The climate is characterised by low and erratic rainfall between November and April (300-500 mm per annum), coinciding with the summer months. Mean daily temperatures are 32-35°C during the summer.

The SVC falls within an arid eutrophic savanna community with woodland that has long been recognised for high diversity and its biomass of large wild herbivores. Vegetation types include *Colophospermum mopane* woodland and thicket, *Acacia-Combretum* open woodland, *Acacia tortilis* savanna and thicket, alluvial

vegetation, rivulet vegetation, granite kopje vegetation, *Brachystegia glaucescens* groves and sandveld open woodland (Price Waterhouse, 1994). Herbivore and large carnivore communities that are traditionally associated with these habitats are present in the Conservancy, although at low densities.

Seasonal streams and a few important rivers provide water sources in the Conservancy and a number of artificially-created watering points are also available on all properties.

2.2.1.3 Black rhinoceros population and study animals

Black rhinoceroses were first reintroduced to the SVC between 1986 and 1988 from the Zambezi Valley, with 20 animals being translocated in the southern section of the SVC. In 1993, an additional 14 black rhinoceroses were translocated to the SVC. Twelve of these animals originated from the Midlands region and adults were in poor body condition at that time (R. Du Toit, personal communication). Among these animals, ten (two males and four female-calf units) settled down in the northern section of SVC while a female with her calf settled down in the southern section of SVC. In November 1993, two more black rhinoceroses (one male, one female) were translocated to the northern population while in September 1994, another two males were translocated onto Senuko ranch in the southern section of SVC.

The composition of the population determined on 1.3.99 for the southern and northern populations of SVC is given in Table 2.1. Individual reproductive monitoring was undertaken in some females of the southern population (Table 2.2). In the northern section of SVC, only births and deaths were recorded in order to compare fertility parameters between the two sub-populations.

2.2.2 Data collection in SVC

2.2.2.1 Locating and identifying of the animals

During management operations such as translocations and dehorning, black rhinoceroses were systematically ear notched in order to be individually recognisable (Brooks, 1989). Transponder microchips were also inserted subcutaneously in the animals but they only allowed for the identification of an animal at very close range.

Radio-collaring of some animals took place in December 1994 and March 1995. The radio-collars used a cylindrical transmitter with a collar made of cylindrical reinforced hose pipe, in order to be adapted to the unusual black rhinoceros neck shape, triangular and continuous with the top of the skull. Most animals lost their radio-collars after a few weeks, except a few black rhinoceroses that included a study animal (Bulawayo) which kept her collar for 33 months. Subsequently the collar design was revised and prototypes prepared but rhinoceros management operations were officially suspended in the country in May 1995, thus precluding another radio-collaring operation.

All study animals in the SVC, except for the radio-collared female, could only be found by tracking with local game scouts. Since black rhinoceros need to drink on a daily basis, the task consisted of identifying the animal's characteristic footprint at dawn around water holes within her home range. Each individual female was identified by the size and markings of her footprint, as well as by the features of her calf's footprint. The size of each footprint was calculated by measuring the distance between the marks left by the lateral and the medial toe nail of each foot, while specific markings consisted in the spoor left by different lines and cracks present on the sole of each foot.

Tracking of the animal followed until the animal's freshest dung pile could be found and sampled, which could take up to six hours. When conditions were favourable so that the animal would remain undisturbed (animal upwind, vegetation not too dense), tracking would then continue until the female was sighted.

The study animal that had been radio-collared was located using triangulation methods, by which the origin of the signal was located by finding the intersection of two directions. Once the animal had been found, its dung-pile was usually found by back tracking the female's spoor. The rapidity and success in locating an animal and its dung pile was greatly enhanced in the radio-collared female, which could always be located when needed, in a time rarely exceeding two hours.

2.2.2.2 Faecal sample collection

Hormonal data was collected from wild females identified in Table 2.2. A dung pile was considered to be fresh when the superficial layer of faecal pellets was still wet and no insect contamination had occurred. Footprint identification near the dung pile and direct observation of the animal confirmed that the excreta originated from that animal. The faecal sample could not always be collected from the freshest dungpile, for example when the animals had scattered their droppings or defecated in communal middens, or when the animal had remained close to its dung-pile. When this occurred, either another fresh dungpile was sampled or the sample collection would be postponed to the following day.

The comparison in faecal progestagen content between the two freshest dung piles dropped by a female showed that no significant differences existed between the two dungpiles (n=19, df=18, P=0.1) (Figure 2.1). This indicated that progestagens were not readily altered after a dung pile had been exposed to ambient conditions, such as natural heat, and that either of the two freshest dung piles deposited by a wild

black rhinoceros cow could be sampled for faecal steroid analysis. This also suggested that enough quantity of each sample had been collected so as to avoid potential differences in steroid metabolites concentrations that may be linked to an uneven distribution of steroids in faecal balls.

Faecal samples were collected by breaking apart a faecal pellet and placing an amount equivalent to a handful into polythene bags using a wooden stick. Samples were then placed in a thermos flask that had been stored at 4°C until they could be stored in an electrical cool box in the car, which occurred between 30 minutes and four hours after the sample collection. Faecal samples were transferred to permanent freezing facilities and stored at -18°C after returning to the research base.

2.2.2.3 Behavioural data collection

Monitoring of reproductive activity in wild animals was undertaken during approximately three years in the Save Valley Conservancy (Table 2.2). Male-female consortship was considered as reproductive behaviour because previous studies identified that a male started to consort with a female after he had detected that she was approaching oestrus (Goddard, 1966; Hitchins & Anderson, 1983). Consortship with both adult and sub-adult males was recorded since records from captive animals indicate that males can sire as early as 4½ years (Rookmaker, 1998), although males are usually considered to be adults when above eight years of age in the wild (Hitchins & Anderson, 1983; Owen-Smith, 1988).

The assessment of consortship was undertaken by recording the presence of a male with the female, through direct observations of the animals. The observation of one male consorting with a female represented one unit, and the relative proportions

of interactions with different males were evaluated for each female, during a period between parturition and the time when faecal progestagen levels indicated pregnancy.

Other signs indicative of reproductive behaviour that were exhibited by wild females in the Save Valley Conservancy, and that could be recorded, included the observation, during tracking, that a female had frequently dropped small amounts urine, which is characteristic of oestrous behaviour in black rhinoceros (sec 1.3.2.4.1). The direct or indirect observation of the chasing of a female by a male, fighting between males over a female, as well as mating activity, were also recorded.

2.2.2.4 Fertility data collection

Population numbers, as well as the number of births and deaths were recorded in the SVC during a five-year period (Table 2.2), in order to determine various fertility parameters that are described in 4.2.2. The cause of death was also determined through a post-mortem examination of the carcass.

Animals were aged in order to be classified into age classes and to determine the population composition and the sex ratio in different age classes. The age of founder animals had been estimated during immobilisation procedures by evaluating the toothwear pattern (Du Toit, 1986). For animals born in SVC after 1994, most dates of birth were known within a week since a very regular monitoring of females had been initiated after that date. For animals born before 1994, only the approximate time of the year was known. In order to classify these animals into age classes, animals born during the early, mid or late part of the year were considered to be born on 1/3, 1/7 and 1/11 of that year respectively.

2.2.2.5 Collection of other samples in wild black rhinoceroses

Paired serum and faccal samples were collected from 15 wild females, including heifers and multiparous females, which were immobilised during management operations in the Save Valley Conservancy and Hwange National Park in Zimbabwe in 1994 and 1995. The reproductive status of these females was established retrospectively after observation of the birth of a calf as well as through results of faecal progestagen analysis.

2.3 STUDY IN SEMI-WILD ANIMALS

2.3.1 Study area and animals

2.3.1.1 Main ecological features

Located 130 km south-east of Harare, Imire game ranch (18E, 31S) is a small scale farm (7500 acres) combining game ranching, cattle and tobacco farming. Precipitation ranges from 800 to 1000 mm per annum. The vegetation consists mainly of sourveld, characterised by tall fibrous perennial grasses and a paucity of palatable browse. The major vegetation types include open grassland with scattered woodlands (*Brachystegia, Julbernardia Acacia karoo,* riverine) with termite mounds on which a variety of shrubs and trees grow.

2.3.1.2 Black rhinoceros population

Initially, seven black rhinoceroses were translocated to Imire in June 1987, having been orphaned through poaching in the Zambezi Valley. They included three males (Noddy, Fumbi and Sprinter) and four females (Cuckoo, Amber, DJ, Mvu), which were all estimated to be approximately three months at capture and were subsequently hand-reared.

Black rhinoceroses in Imire are let out in the bush during the day, with armed guards following them and "herding" them to feed in suitable vegetation, but are brought back to an enclosure at night. Supplementary food (50kg game cubes/day/seven rhinoceroses) is given to them twice a day, with a vitamin and mineral supplement.

After a female had given birth, the calf was separated from her at approximately 6-7 months of age, after which time he was hand-reared without further contact with the other black rhinoceroses. Some of these calves were then translocated to wild areas such as Matusadona National Park.

2.3.2 Data collection

Due to the reduced dispersion of the animals, good visibility in the open habitat, and relative tolerance of these animals to humans, faecal samples could be collected off the ground after observation of defaecation. These animals were under constant surveillance, facilitating the daily recording of the occurrence of reproductive behaviour (Table 2.2).

Signs of reproductive activity that could be easily observed at Imire were the chasing of a female by one male or more, the fighting between males over a female, during periods which lasted between two days and two weeks until mating was observed. Mating could take place while the female was still amongst the other black rhinoceroses, in which case successive mating by two males could sometimes be recorded. Alternatively, mating could also take place after the pair had separated from the other animals. However, the animals were not left isolated for more than one day for security reasons.

2.4 STUDY IN CAPTIVITY

2.4.1 Study areas and animals

The captive black rhinoceros population maintained in U.K institutions was monitored between 1995 and 2000 and its composition is presented in Table 2.3. In the first three institutions, animals are managed as pairs which are only put together during periods of sexual receptivity. They are kept in separate indoor stalls and have access to an outer paddock.

2.4.1.1 London Zoo

A pair has been managed together since the male (Jos) was 1.5 years and the female (Rosie), which was hand-reared, two years old. When the faecal sample collection started (Figure 2.5), the female was 7.5 years old and the male was 7 years old. Animals are fed with browse given *ad libitum*, 4-6 kg browser cube/animal/day, vegetables and some vitamin E supplementation.

2.4.1.2 Whipsnade Wild Animal Park

Initially, the pair consisted of a hand-reared female (Emma) and a male (Katakata), which had been together since both animals were around 3 years old. The male was subsequently replaced by another one (Quinto), which was of the same age and arrived from Chester Zoo in July 1998.

2.4.1.3 Chester Zoo

One adult pair was initially managed there (Parky & Esther). The male was sent to Port Lympne in October 1994 and came back to Chester in September 1996, until September 1998 when he was moved to Zurich Zoo. A younger pair (Quinto & Pangani), which have the same father, had also been together since 1992, at which

time the male was two and the female 3.5 years. The male Quinto was replaced by Katakata of Whipsnade in July 1998.

Their diets consists of lucerne and browse, grass and vegetables available, supplemented with concentrates (browser breeder pellet: 2 kg/animal/day; horse & pony cube: 4 kg/animal/day) and a mineral and vitamin supplementation.

2.4.1.4 Port Lympne

The Port Lympne situation is different from other captive situations as black rhinoceroses are managed in an extensive system of large paddocks. These paddocks are separated from each other by artificial or natural fences. Each paddock has a rhinoceros house with 3-4 pens into which the animals are brought back at night in winter. Black rhinoceroses are run as a pair or more often as a trio during the day, with pairing of the animals being based on age, sex, relatedness and friendship (B. White, personal communication). When a cow continues to cycle after several mating, she is then introduced to another bull. Adult males are not managed in contiguous paddocks, but a sub-adult male can be managed in a paddock adjacent to an adult male. After a female has given birth, the calf is left with its mother until around 1.5 years old.

Black rhinoceroses in Port Lympne are fed daily with browse given *ad libitum* throughout the day, lucerne (and grass in the summer), a variety of vegetables and potatoes. Concentrates (2 kg South African browser pellet and 1 kg ungulate pellet per animal) and a mineral and vitamin supplement are also given to them, especially during pregnancy and lactation.

In 1995, Port Lympne had nine adult animals (five males and four females) and five more females were subsequently moved to that institution. One male (Kingo)

was sent to Chester between July 1995 and July 1996, while the male from Chester (Parky) was at Port Lympne between October 1994 and September 1996.

2.4.2 Data collection

Faecal and urine samples were collected daily in the morning from a captive female (Rosie). The occurrence of reproductive behaviour (chasing, mounting and mating) was also noted daily for this female, as well as for females identified in Table 2.2. All births and deaths were recorded in these four institutions during a five-year period in order to evaluate fertility parameters.

2.5 HORMONAL ANALYSIS

2.5.1 Faecal progestagen analysis

2.5.1.1 Sample preparation

Samples collected from wild animals were thawed and dried at 65°C for 18 hours in an oven (Labotec, Johannesburg, South Africa) before being shipped to London. This time was initially estimated to be sufficient to remove the moisture content from faecal samples on the basis of visual and tactile examination of samples. A subsequent trial involving the regular weighing of samples through the drying process showed that the weight of samples became stable after 12 hours of drying.

Samples from captive animals were dried at the Institute of Zoology at 40°C for 12 hours. A comparison between faecal progestagen levels in paired sub-samples dried at 65°C for 18 hours or at 40°C for 12 hours showed that their faecal progestagen concentrations were not significantly different ($P<0.05$).

Faecal samples were subsequently sifted to remove coarse vegetation debris and stored at +4°C until extraction.

2.5.1.2 Faecal extraction

Methanol extraction was used to remove steroids from solid faecal matter, by combining 0.1g dry faeces with 0.2 g aluminium oxide, 0.6 ml methanol and 0.5 ml distilled water (Möstl *et al.*, 1993). Aluminium oxide was used to reduce colorimetric interference (Schwarzenberger *et al.*, 1996b). The suspension was vortexed for 10 minutes and centrifuged at 1,720*g* for 30 minutes at 4°C. The supernatant was diluted with 0.02M Tris buffer (pH 7.5), 1:50 for non-pregnant animals and 1:100 or 1:200 for pregnant females.

2.5.1.3 Enzyme immunoassay (EIA)

Faecal progestagens were measured using the EIA validated and described by Hindle *et al.* (1992) for measuring 4-pregnen-20α-ol-3-one (20α-dihydroprogesterone, 20α-OHP) in African rhinoceros urine. The EIAs were performed in microtitre plates coated with an antibody against IgG, using a double antibody technique. The antibody was raised in rabbits immunized against 20α-OHP conjugated to BSA through carboxymethyloxime (donated by Dr M.J. Peddie, Department of Physics and Pharmacy, University of Southampton). The enzyme label was biotin conjugated to 20α-OHP (donated by Dr E. Möstl, Institut für Biochemie, Veterinärmedizinische Universität, Vienna, Austria). Antibody and enzyme label dilutions were 1:150,000 and 1:800,000 respectively.

The validation of the assay included evaluations of: the parallelism to the standard curve, the specificity, the accuracy, the precision and the sensitivity of the assay.

The standard curve was prepared with 20α-OHP (Sigma P6288, Sigma Chemical Co., Poole, Dorset, U.K.) and ranged from 3.125 to 800 pg per well. Serial

dilutions (1/5 to 1/320) of faecal extracts originating from two pregnant and two non-pregnant black rhinoceroses gave a displacement curve parallel to that obtained with 20α-OHP standards for dilutions comprised between 1/20 and 1/320 (Figure 2.2).

Cross-reactions of other steroids with the 20α-OHP antiserum were determined to evaluate the specificity of the assay. The antiserum showed cross-reactivity with 5β-pregnan-20α-ol-3-one (47.2%), 5α-pregnan-3β,20α-diol (11.1%), 5α-pregnan-3α,20α-diol (1.4%), 5α-pregnan-3,20-dione (0.31%), 5β-pregnan-3,20-dione (0.26%), 5β-pregnan-3α,20α-diol (0.15%), 5β-pregnan-3β,20α-diol (0.11%), 5β-pregnan-3β-ol-20-one (0.11%), 5α-pregnan-3α-ol-20-one (0.1%), 5α-pregnan-3β-ol-20-one (0.05%), 5β-pregnan-3α-ol-20-one (0.013%).

The accuracy of the assay, defined as closeness to the "true" value, was determined by adding different amounts (range, 25 to 200 pg per well, n=5) of unlabelled 20α-OHP to faecal samples containing low concentrations of endogenous hormone. Recovery of exogenous 20α-OHP was 87.9 ± 4.2%.

The precision of the assay is defined as the spread of replicate observations about the mean and is expressed as the coefficient of variation. The intra-assay coefficients of variation were 12% and 5.2% for a high and low concentration buffer quality control samples respectively while it was 12.4% for a pooled black rhinoceros faecal sample. The inter-assay coefficient of variation was determined to be 18.4%.

The sensitivity of the assay was determined as 90% binding and was 62.5 pg/well.

Since the antiserum cross-reacted with 20α-hydroxylated progestagens, other than 20α-dihydroprogesterone, immunoreactivity measured in faecal extracts will be referred to as immunoreactive 20α-progestagens. Hormone data were expressed as ng / g of faeces (dry weight).

2.5.2 Urinary progestagen analysis

2.5.2.1 Sample preparation and extraction

Prior to being extracted, urine samples were analysed for creatinine concentrations by the method of Hodges & Green (1989), to help compensate for variations in fluid intake and output. Urine samples were afterwards hydrolysed with beta-glucuronidase-aryl-sulfatase and extracted with diethyl ether by the method of Hindle *et al.* (1992). Hydrolysis efficiency was determined by adding tracer amounts of [3H] oestrone sulphate (E1S) and [3H] pregnanediol glucuronide (PdG) to a urine pool before hydrolysis and results varied from 53 to 75% for E1S and from 19 to 63.8% for PdG. After having tested whether such variability was due to the enzyme used, the incubation time, the reconstitution time or the extraction, it could only be concluded that such variability could only be attributed to differences in urine samples.

2.5.2.2 Enzyme immunoassay

Immunoreactive 20α-OHP in urine samples was measured by using the same EIA as described above, which had been validated for urinary 20α-OHP analysis in both black and white rhinoceros by Hindle *et al.* (1992).

2.5.3 Serum progesterone analysis

Blood samples were collected via venipuncture in the ear during management procedures. The serum was recovered and stored at -20°C until analysis.

Progesterone was measured in serum samples by radioimmunoassay (RIA), as described by Shaw *et al.* (1989), which utilised a sheep anti-progesterone antibody cross-reacting with 11α-hydroxy-4-pregnen-3,20-dione (29.8%), 11β-hydroxy-4-pregnen-3,20-dione(16.5%), 5β-pregnan-3α,20α-dione (16.1%) and 5α-pregnan-3α,20α-dione (2.63%). The sensitivity of the assay at 90% binding was 20 pg/ml.

2.5.4 *Validation of the EIA for progestagen measurement in black rhinoceros*

2.5.4.1 Validation in samples collected during pregnancy

Serum progesterone concentrations and faecal 20α-progestagen concentrations measured in paired serum and faecal samples collected from wild black rhinoceros females were almost positively correlated (r_s=0.8, n=4, P<0.1) when samples from the first four females presented in Figure 2.3.a) were considered. This relationship however weakened when samples collected from females 5 and 6 were included (r_s=0.42, n=6, P>0.05).

Paired serum and faecal samples were also collected from non-pregnant wild females and the differences between mean faecal 20α-progestagens concentrations from pregnant (mean=5413 ± 1183 ng/g, n=6) and non-pregnant (mean= 201 ± 47 ng/g, n=10) black rhinoceros females were significant (P<0.01).

2.5.4.2 Validation in samples collected from non-pregnant females

The measurement of serum progesterone and faecal 20α-progestagen concentrations in paired serum and faecal samples collected from non-pregnant females are presented in Figure 2.3.b. They showed a poor relationship between levels of circulating progesterone and of faecal progesterone metabolites (r_s=0.48, n=9, P>0.05).

Urine 20α-OHP and faecal 20α-progestagens were measured in paired urine and faecal samples collected daily from a captive non-pregnant black rhinoceros female (Rosie) in July/August 1995, when she was showing regular signs of oestrous behaviour. The profile of immunoreactive progestagen excretion is presented in Figure 2.4. It shows that behavioural oestrus coincided with low faecal 20α-progestagen concentrations and preceded an increase in concentrations. There is also a good

temporal relationship between concentrations of 20α-OHP in urine and faeces (r_s=0,52, n=38, P<0.01). However, these results have to be considered with caution because of the great variability in hydrolysis efficiency of urine samples that was occurring at that time.

Faecal 20α-progestagens were also measured in faecal samples collected daily from the same female between May 1996 and February 1997 and the resulting profile is presented in Figure 2.5. During this time, the female was in daily contact with a male, except between days 236 and 271 of the profile when the female had to be isolated from the male after they fought aggressively. Regular oestrous behaviour was observed between days 1 and 236 of the profile, with a mean inter-oestrous interval of 31.7 ± 4.3 days (range, 19-55 days, n=7).

The hormonal profile shows cyclic fluctuations of faecal progesterone metabolites concentrations. Periods of high concentrations, lasting an average of 15 ± 1.5 days (range, 9-26 days, n=11) gave way to periods of low concentrations, which lasted an average of 11.6 ± 2.1 days (range, 5-29 days, n=11) (Table 2.4). Reproductive activity was observed to last up to four days in this female and was detected to occur either just before or during periods of low concentrations of faecal 20α-progestagens. Faecal 20α-progestagens levels showed a significant increase to high concentrations between five and 12 days after the first signs of reproductive activity were observed. The only time when behavioural observations of oestrus and hormonal variations were not related is represented by the absence of oestrous behaviour around day 160, although a marked decline in faecal 20α-progestagens occurred.

2.5.4.3 Discussion

The degree of association between serum and faecal 20α-progestagen concentrations in wild black rhinoceros females was generally poor. Such a result may originate from the fact that the measurement of faecal progesterone metabolites using the 20α-OHP assay does not provide a reflection of serum progesterone concentrations in wild black rhinoceroses. However, this is not supported by the fact that the measurement of faecal 20α-progestagens had previously been found to be useful for monitoring pregnancy and cyclic ovarian activity in captive black rhinoceros females (Schwarzenberger *et al.*, 1993). In addition, some pregnant wild females presented a relationship approaching significance between serum progesterone and faecal progesterone metabolites and the differences in faecal progesterone metabolite concentrations between pregnant and non-pregnant wild females were significant. Finally, there was also a good temporal relationship between oestrous behaviour and cyclic variations of faecal 20α-progestagen concentrations in non-pregnant females. These observations therefore tend to indicate that the 20α-OHP assay provides a measurement of faecal progesterone metabolites which could be used for assessing reproductive status in wild black rhinoceros.

The poor correlation that was observed between serum progesterone and faecal progesterone metabolites concentrations is likely to be associated with the low number of wild females in which paired samples could be collected. In addition, pregnant females were not sampled at the same time during the gestation period and differences in the degree of association between serum and faecal concentrations in pregnant animals may suggest that a shift in progesterone metabolism occurs during pregnancy. This is corroborated by observations that urinary or faecal progesterone metabolites concentrations showed significant variations during the gestation period

in captive black rhinoceros females (Berkeley *et al.*, 1997; Hindle, 1991; Schwarzenberger *et al.*, 1993, 1996b).

In non-pregnant females, another factor contributing to the weak correlation between serum progesterone and faecal progesterone metabolites concentrations is the lag time of up to 12 days that was detected between the first signs of reproductive activity and a significant increase in faecal 20α-progestagen concentrations. Such a lag had already been noted in previous studies on captive black rhinoceros (Schwarzenberger *et al.*, 1993). Part of this lag may be attributable to the delay that coincides with the intestinal passage of bile to the rectum since steroids are excreted through the entero-hepatic system (Gower & Honour, 1984).

This lag time has not been precisely measured in the black rhinoceros, but radio-metabolism studies in the Sumatran rhinoceros, which is also a browser, found that progestagen excretion in faeces peaked two days after the administration of radio-labelled progesterone (Heistermann *et al.*, 1998). Similar studies on the white rhinoceros and the African elephant found that most radioactivity was recovered in faeces between two and three days and two days respectively after administration of radio-labelled progesterone (Hindle & Hodges, 1990; Wasser *et al.*, 1996). It can therefore be concluded that this lag is of a minimum of two days in the black rhinoceros.

However, the lag time between reproductive behaviour and a significant rise in faecal progestagen concentrations is longer than the delay associated with the gastrointestinal time. It is therefore probable that there is also a delay between ovulation and a significant rise in faecal progesterone metabolites, as had been previously observed in the white rhinoceros (Radcliffe *et al.*, 1997). Similarly, a lag

of three to five days between mating and a significant increase in serum progesterone has also recently been identified in the Sumatran rhinoceros (Roth *et al.*, 2001).

The weak correlation that was obtained between serum progesterone and faecal 20α-progestagen concentrations was probably associated with the low number of paired samples that could be collected from wild females, as well as with the time lag that exists between a change in the functional state of the ovary and variations in faecal progesterone metabolites concentrations.

The measurement of faecal progesterone metabolites using the 20α-OHP assay therefore has the potential to be used for assessing pregnancy and for monitoring ovarian cycles in wild black rhinoceros females.

Table 2.1. Black rhinoceros population in the Save Valley Conservancy on 1.3.99

SOUTH (WILD)

Males					Females					Calves		
	ID	Name	Birth	Introduction	ID	Name	Birth	Introduction		ID	Name	Birth
1	85/Z/S	Penga	1957	1986/88	1 92/Z/S	Pukwani	1960	1986/88	1	92.03/S	Betty	Jan-97
3	91/Z/S	Guy	1960/62	1986/88	2 89/Z/S	Bulawayo	1962	1986/88	2	89.03/S	Chando	Apr-98
4	97/Z/S	Mukachana	?	1986/88	3 88/Z/S	Sirica	1962	1986/88	3	88.02/S	Alice	Dec-96
5	93/Z/S	Pumula	1972	1986/88	4 84/Z/S	Netsai	1967/72	1986/88	4	84.03/S	Boy	Feb-97
6	90/Z/S	Shava	1960/62	1986/88	5 86/Z/S	Mazyan	1962	1986/88				
7	100/Z/S	Nhamo	?	1986/88	6 26/Z/M/S	Disco	?	1993	5	26.03/S	Chiyedza	Apr-98
8	87/Z/S	Buttom	1962	1986/88	7 26.01/M/S	Harare	late 91	born SVC				
9	95/Z/S	Noname	1977	1986/88	8 00.15/S	Sara	1988/89	born SVC	6	84.01/01/S	Atalia	Oct-96
10	00.8/G/S	Magnum	1988/89	1994	9 84.01/S	Jete	1990	born SVC	7	26.01/02/S	?	Aug-98
11	51/Z/G/S	Goliath	1974/79	1994	10 89.01/S	Mupunga	Nov-93	born SVC				
12	00.13/S	Dundwen	1988/89	born SVC	11 88.01/S	Increase	1994	born SVC				
13	94.01/S	Muzaman	1989	born SVC	12 92.02/S	Julie	Nov-94	born SVC				
14	86.01/S	Handboy	1989	born SVC	13 26.02/S	CD	May-94	born SVC				
15	00.16/S	Sun	1990/91	born SVC	14 89.02/S	Kumalo	May-96	born SVC				
16	98.02/S	Mafupi	early91	born SVC								
17	00.17/S	Jaggers	1991/92	born SVC								
18	92.01/S	Lety	Apr-91	born SVC								
19	94.02/S	Pikinin	Feb-94	born SVC								
20	84.02/S	Bonus	Mar-95	born SVC								
21	00.15/01/S	Monarch	Mar-95	born SVC								
22	94.03/S	Salary	Jul-96	born SVC								
23	26.01/01/S	Rufaro	Jul-96	born SVC								

NORTH (TRANSLOCATED)

1	24/Z/M/S	Jeffrey	1978	1993	1 22/Z/M/S	Jenny	1978	1993				
2	96/Z/S	Obert	1972/77	1993	2 23/Z/M/S	Susan	1963-65	1993	1	23.04/M/S	Cosmos	Apr-98
3	22.02/M/S	Johnson	mid90	1993	3 28/Z/M/S	Mary	1968	1993	2	28.02/S	?	Jul-98
4	28.01/M/S	Maxwell	mid90	1993	4 27/Z/M/S	Mudenga	1963	1993	3	27.03/M/S	Ben	Apr-97
5	23.03/M/S	Petras	Mar-94	1993	5 00.12/G/S	Rumbidzai	1988/89	1993	4	00.12/01/S	?	May-98
6	22.03/M/S	Maduma	Mar-95	1993	6 23.02/M/S	Maria	1991	1993	5	23.02/01/S	Brenda	Jan-97
					7 27.02/M/S	Clever	early 1993	1993	6	27.02/01/S	?	Aug-98
					8 28.01/S	Thomasine	Dec-94	born SVC				

Table 2.2. Types and periods of data collection in each study area. Dates indicate periods during which each type of data was collected.

Study areas		Data collected		
Status	Animals	Hormonal Data	Behavioural data	Fertility data
Wild[1] (SVC)	Bulawayo	5/96-4/98	5/96-4/99	3/94-3/99
	Pukwani	5/96-2/98	5/96-4/99	3/94-3/99
	Netsai	5/96-3/98	5/96-4/99	3/94-3/99
	Jete	5/96-4/98	5/96-4/99	3/94-3/99
	Sirica	5/96-7/98	5/96-4/99	3/94-3/99
	Sara	1/97-11/98	5/96-4/99	3/94-3/99
	Other females	None	None	3/94-3/99
Semi-wild[2] (Imire)	DJ	9/96-3/98	9/94-3/98	None
	Other females	None	9/94-3/98	None
Captive[3] (UK)	Rosie	5/96-2/97	1/94-1/98	1/95-1/00
	Arusha	None	1/95-1/99	1/95-1/00
	Vuyu	None	1/95-1/99	1/95-1/00
	Naivasha	None	1/95-1/99	1/95-1/00
	Rukwa	None	1/95-1/99	1/95-1/00
	Esther	None	1/95-1/99	1/95-1/00
	Other females	None	None	1/95-1/00

[1] Situations where rhinoceroses live in areas of large to medium size (>10 km²) with a natural breeding system

[2] Situations where rhinoceroses live in small areas (<10 km²) with a natural breeding system.

[3] Situations where rhinoceroses live in small (< 10 km²) to very small areas with a manipulated breeding system.

Table 2.3. Black rhinoceros population in captivity in the U.K on 1.1.00

LONDON ZOO

Males						Females						Calves		
	ID	Name	Birth	Introduction	Origin		ID	Name	Birth	Introduction	Origin	ID	Name	Birth
1	391	Jos	May-89	Nov-90	captivity	1	384	Rosie	Nov-88	Nov-88	captivity			

WHIPSNADE WILD ANIMAL PARK

1	430	Quinto	Sep-90	Jul-98	captivity	1	451	Emma	Feb-91	Oct-93	captivity			

CHESTER ZOO

1	453	Katakata	Oct-90	Jul-98	captivity	1	312	Esther	May-82	May-84	captivity	1	696	Manyara	Aug-98
						2	422	Pangani	Mar-89	Nov-92	captivity	2	680	Kitani	Jun-97

PORT LYMPNE

1	164	Jaspa	Feb-71	Jan-90	captivity	1	195	Rukwa	1970	Jan-73	Kenya	1		Galana	Nov-99
2	341	Kingo	Oct-83	Oct-83	captivity	2	194	Naivasha	1970	Jan-73	Kenya	2	714	Magadill	Feb-99
3	534	Addo	1975	Oct-94	S.Africa	3	408	Nakuru	Oct-89	Oct-89	captivity	3		Zambezi	Oct-99
4	483	Baringo II	Dec-92	Dec-92	captivity	4	342	Arusha	Nov-83	Nov-83	captivity	4		Rufiji	Jun-99
5	659	Mweru	Sep-96	Sep-96	captivity	5	455	Etna	Dec-92	Nov-95	captivity	5	698	Tana	Sep-98
						6	558	Vuyu	Aug-91	Aug-95	S.Africa	6		Kivu	Dec-98
						7	165	Lucia	Oct-71	Oct-98	captivity				
						8	456	Jaga	Dec-92	Nov-99	captivity				
						9	663	Ruaha	Dec-96	Dec-96	captivity				

Table 2.4. Relationship between ovarian cycle parameters and reproductive behaviour in a captive nulliparous black rhinoceros female (Rosie) showing regular oestrous behaviour during a 10-month period (Figure 2.5).

Cycle parameters				Reproductive behaviour	
Periods of high concentrations[1]		Periods of low concentrations[1]		Periods of reproductive behaviour[2]	
Days on profile	Faecal 20α-progestagens (ng/g) Mean ± SE	Days on profile	Faecal 20α-progestagens (ng/g) Mean ± SE	Days on profile	Behaviour
1-17	680.3 ± 71.7	18-22	326.8 ± 18.4	18-19	C
23-48	639.7 ± 54.6	49-53	231.6 ± 42.7	*45-47*	C
54-68	356.6 ± 39.7	70-74	106.4 ± 5.4	*69-71*	C
75-95	259.9 ± 43.9	96-124	87.3 ± 12.1	100-102	C, F
125-136	567.3 ± 116.3	137-145	233.6 ± 13.2	*134-136*	C
146-157	382.5 ± 18	158-169	204.2 ± 19.4		
170-178	482.3 ± 36.2	179-194	151.3 ± 20.8	183-186	m
195-208	579.2 ± 71.9	209-219	157.1 ± 17.6	215	C, F
220-229	468.6 ± 59.8	230-244	164.3 ± 18.8	234-235	m, F
245-261	759.6 ± 79	262-269	305 ± 27.8	Separated	
270-281	650.2 ± 34.5	282-295	287.8 ± 15.2	284-285	m, M

[1] The profile was divided into distinct periods, the definition of each period corresponding to the part of the profile that presented similar background concentrations, although the distinction between one period to the next was subjectively determined. Mean background concentrations for each period were subsequently calculated by removing progressively higher and lower values from the original data set until the coefficient of skewness was no longer significant as well as by calculating the mean of the remaining concentrations.

Periods of high concentrations corresponded to periods of at least two of three consecutive values above mean background concentration.

Periods of low concentrations corresponded to periods of at least two consecutive values below mean background concentration.

[2] Reproductive behaviour consisted in the male chasing the female with an erection (C), fighting between male and female (F), mounting (m), mating (M). Days in italic indicate that behavioural observations coincided with periods of HC.

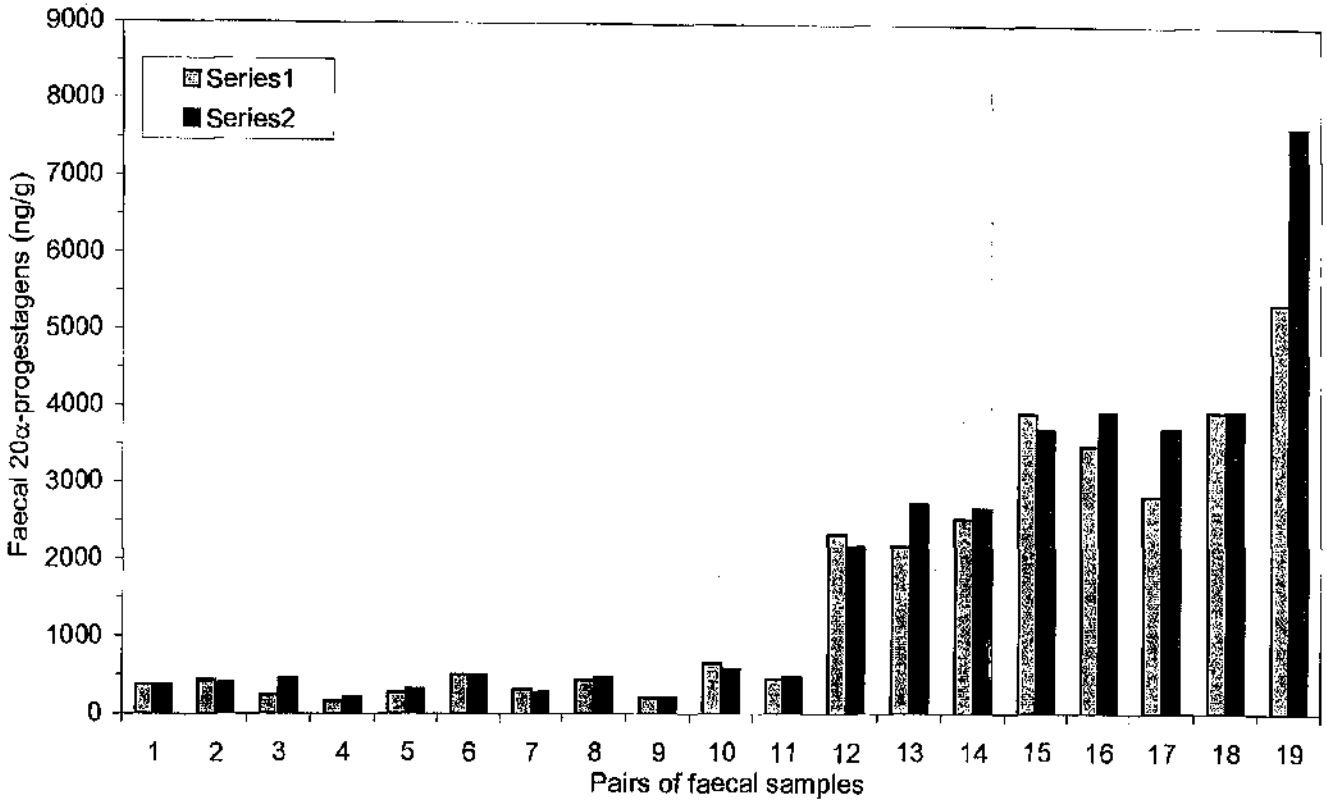


Figure 2.1. Comparison of faecal steroid concentrations in paired samples collected successively from the two freshest dung piles (series 1 and 2) deposited by black rhinoceros female (series 1 vs. series 2, P=0.1). The first 11 pairs were collected from non-pregnant females (n=2) and the last 7 pairs from pregnant females (n=2).

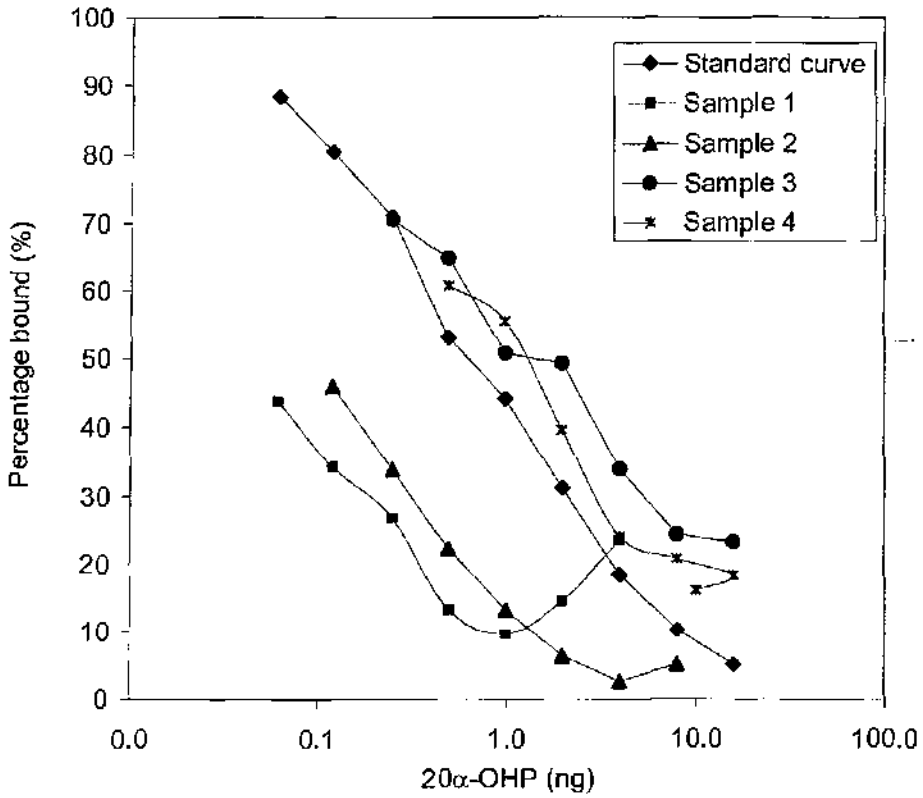
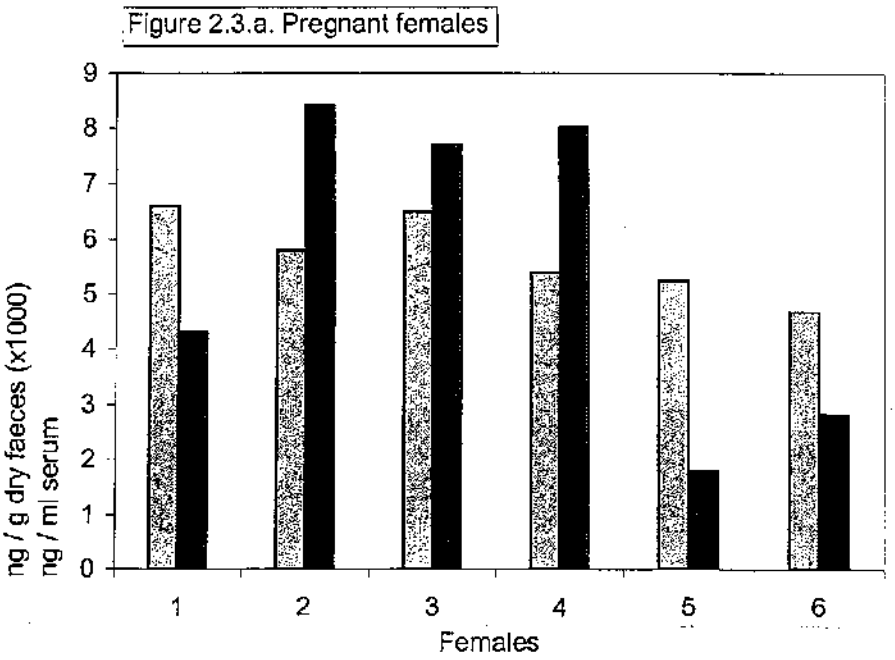


Figure 2.2. Standard curve of the 20α-OHP assay and serial dilutions of black rhinoceros faecal samples. Each sample is shown by a different symbol. A log scale is used for the x axis.



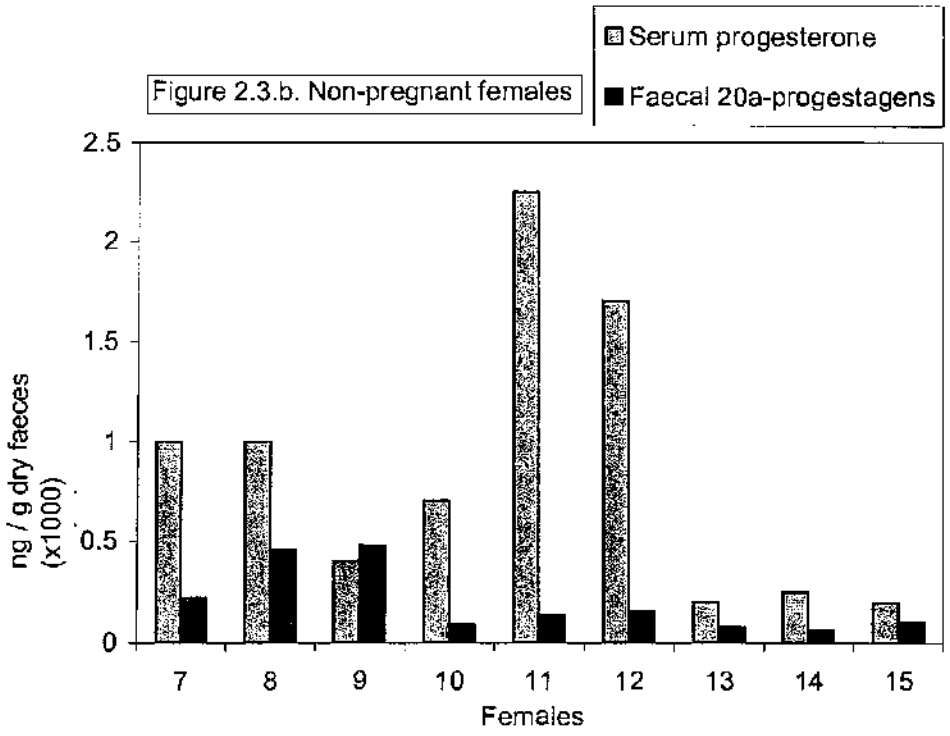


Figure 2.3. Relationship between faecal 20α-progestagens and serum progesterone concentrations in paired samples. Faecal and serum samples were collected from wild black rhinoceros females that were pregnant (a) and non-pregnant (b).

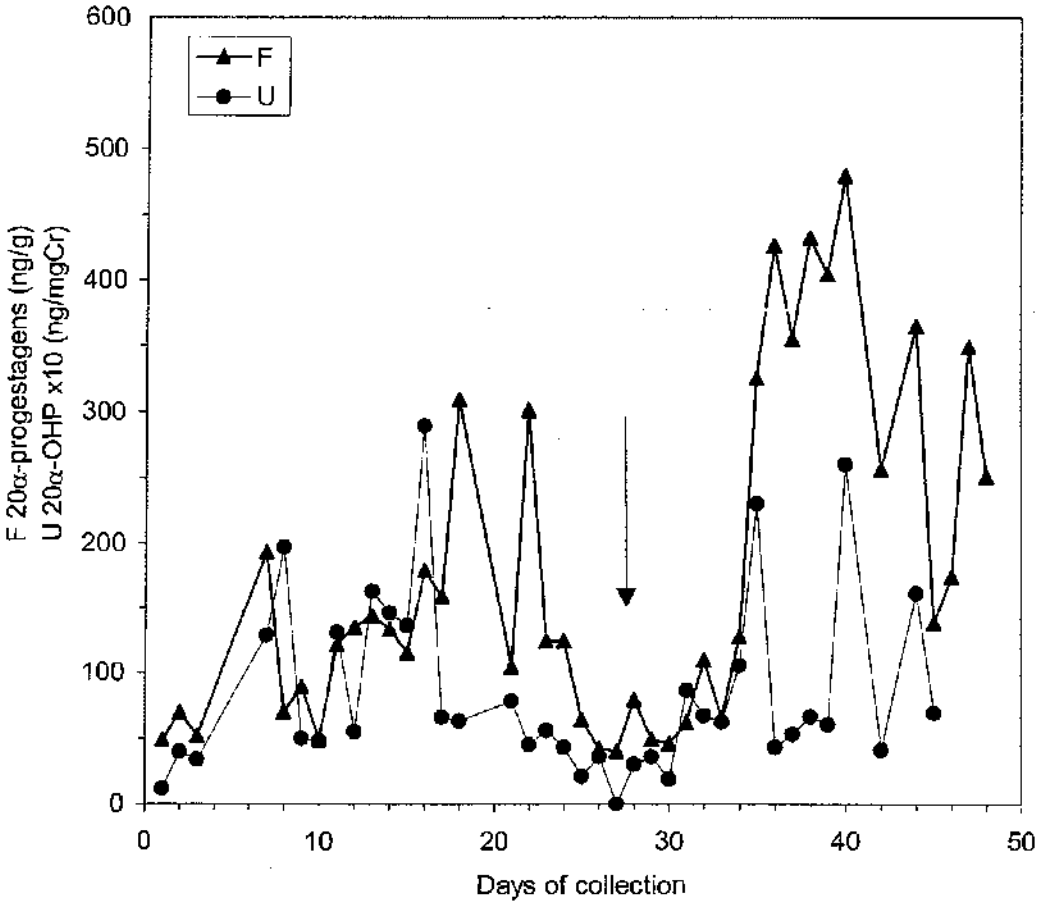


Figure 2.4. Relationship between faecal 20α-progestagens and urinary 20α-OHP concentrations in paired samples collected from a captive black rhinoceros female (n=48). The arrow indicate the time of observed oestrus.

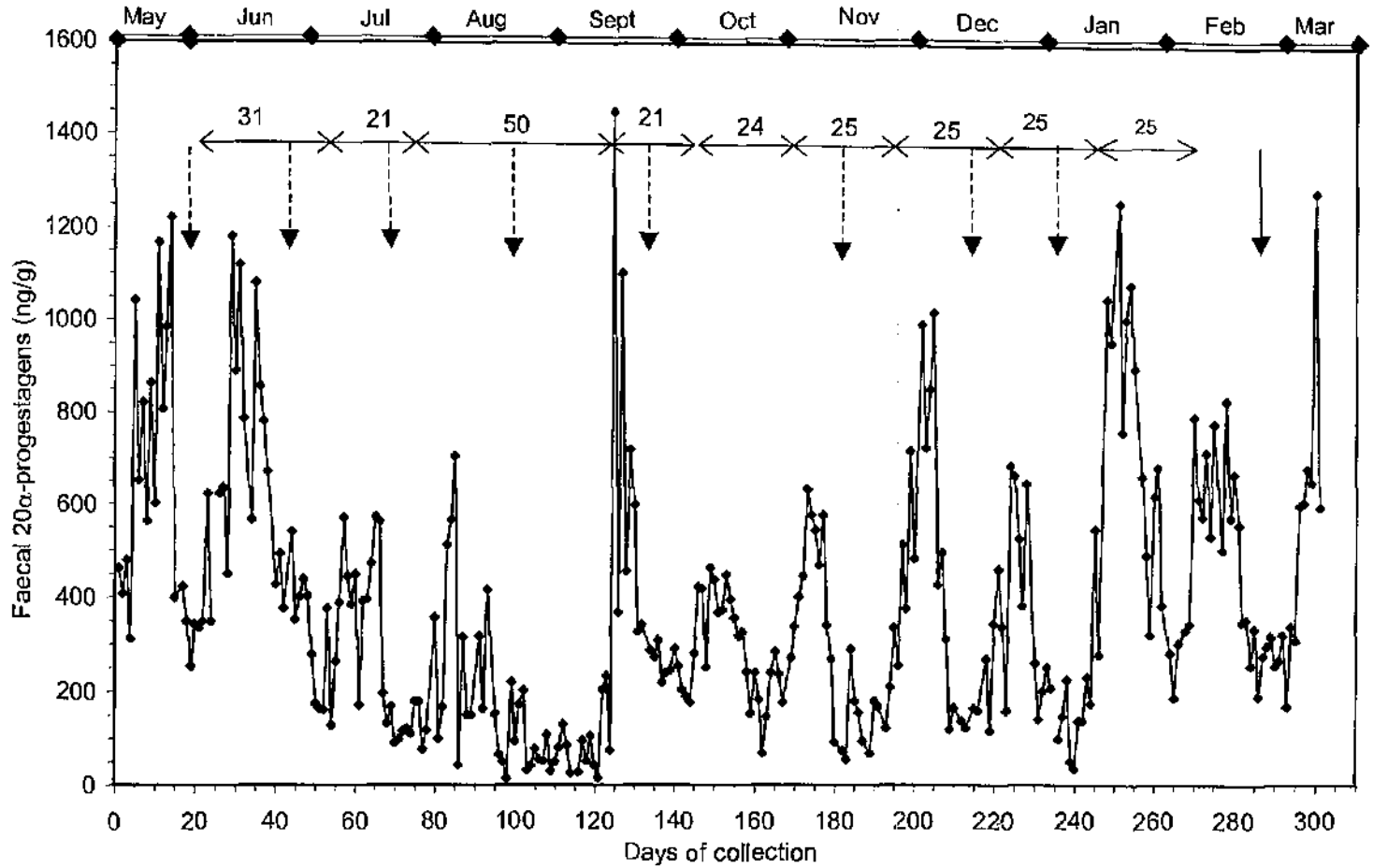


Figure 2.5. Concentrations of faecal 20α-progestagens in a captive nulliparous black rhinoceros female (Rosie) from May 1996 to February 1997. Vertical arrows indicate reproductive activity (full line indicate mating and dotted line indicate chasing with an erection, fighting and mounting). Horizontal arrows indicate the cycle length (in days), defined as the time interval between successive periods of sustained elevation of faecal progestagen concentrations .

3 NON-INVASIVE MONITORING OF PREGNANCY AND REPRODUCTIVE CYCLES

3.1 INTRODUCTION

The results presented in Chapter 2 indicate that the measurement of faecal 20α-progestagens provided a valid indirect indicator of ovarian activity and had the potential to be used for pregnancy diagnosis in wild black rhinoceros cows.

Although pregnancy profiles had been characterised through faecal progestagen measurement in captive black rhinoceros (Berkeley *et al.*, 1997; Schwarzenberger *et al.*, 1993, 1996b), the first objective of this study was to investigate the potential use of faecal 20α-progestagen measurement for pregnancy diagnosis in free-ranging females of this species, so that it might become an effective management tool. The other objective was to monitor the intergestation period and to characterise patterns of cyclic ovarian activity in wild females, in order to acquire a better knowledge of the normal reproductive cycle in the species.

3.2 MATERIALS AND METHODS

3.2.1 Pregnancy

3.2.1.1 Animals and data collection

Six female black rhinoceros, including five wild (Pukwani, Netsai, Bulawayo, Sara and Sirica) and one semi-wild (DJ) were monitored during the study (Table 2.1).

Two wild females (Sara and Bulawayo) were monitored throughout the entire gestation period in 1997/98 while three others (Pukwani, Netsai, Sirica) were monitored during six or seven months within a period covering the last nine months of gestation in 1996. Sample collection occurred once or twice per week during the

first and last three months of pregnancy and every week during mid-pregnancy. Sample collection frequency was initially planned using knowledge of previous calving intervals. Frequency was then adjusted in line with environmental conditions and was also governed by the on-going results of hormonal analysis. Samples from non-pregnant animals were collected two or three times per week, during the three months before the onset of pregnancy (n=3) or between four and seven months following parturition (n=3), since some black rhinoceros females on the Conservancy conceived as soon as eight months after parturition.

On the Imire Game ranch, faecal samples were collected off the ground every three to five days for two months before pregnancy, and for the first six months and the last two months of pregnancy from the female DJ. Between the eighth and thirteen month of pregnancy, one sample was collected every five to ten days.

The occurrence of mating and other reproductive behaviour was recorded as described in the preceding chapter.

3.2.1.2 Statistical analysis

Data were aligned to the day of parturition on the individual profiles for the three females that could be monitored throughout the whole gestation period. A composite profile was established after aligning data to the day of parturition in the six females and grouping it in monthly intervals. Data for the composite profile were presented as means ± SE, using pooled standard deviation, and then presented from month one to 15 of gestation since the gestation period was of 15 months. The Shapiro-Wilk W test was used to determine whether data sets for each animal and for each phase of pregnancy were normally distributed. Analysis of variance was used to evaluate differences in mean 20α-progestagen concentrations during each phase of

gestation and between animals. Student's *t*-test was used to investigate differences between mean hormone concentrations in non-pregnant animals and those sampled at different phases of gestation. The probability distribution of the sample mean (i.e., ± 1.96 x SE) was used to calculate 95% confidence interval.

In order to evaluate whether the measurement of faecal progesterone metabolites could be used as a pregnancy test in wild black rhinoceros females, all individual faecal samples that had been collected as described in 3.2.1.1. were tested retrospectively. The probability distribution of the original data (i.e., ± 1.96 x SD) was used to calculate the 95% upper tolerance limit of 20α-progestagen concentrations for non-pregnant females (Messier *et al.*, 1990). Using this value as a threshold, each faecal sample was tested. A sample was considered to give a positive result when above the threshold value.

The evaluation of the test results followed the method described by Altman (1991). The positive predictive value is defined as the proportion of positives which are pregnant and the negative predictive value is defined as the proportion of negatives which are not pregnant. Specificity is defined as the proportion of negatives that are correctly identified by the test, while sensitivity is the proportion of positive results correctly identified by the test. The proportion of false positives was determined as (100 – specificity) and the proportion of false negatives as (100 – sensitivity).

3.2.2 Intergestation

3.2.2.1 Animals and data collection

Hormonal profiles during the intergestation period were derived from samples collected regularly from six individual wild females in the Conservancy. These

included the five animals which had been monitored during the gestation period, as well as the female Jete which gave birth to her first calf during the study. When the immediate post-partum coincided with the rainy season, faecal samples could not be collected as regularly and frequently (every two to three days) as during the dry season.

The intergestation period started on the day of parturition and lasted until a cyclic pattern of faecal 20α-progestagen excretion could no longer be detected and concentrations remained permanently above 2000 ng/g.

3.2.2.2 Statistical analysis

The initial observation of intergestation profiles showed that there were differences in background faecal progestagen concentrations on each profile. For this reason, each intergestation period was divided into distinct periods, I1, I2, etc. The definition of each period corresponded to the part of the profile that presented similar background concentrations, although the distinction between one period to the next was subjectively determined. Mean background concentrations for each period were subsequently calculated by removing progressively higher and lower values from the original data set until the coefficient of skewness was no longer significant as well as by calculating the mean of the remaining concentrations (Brinkley, 1981). Differences between mean background concentrations were tested by one-way analysis of variance.

Because an initial attempt to determine a significant rise in hormonal metabolite concentrations by an increase in concentrations 1.5-2 times above the mean nadir failed, another approach was undertaken. A cyclic pattern, on the basis of endocrine criteria, was identified as two consecutive values below mean background

levels followed by a sustained elevation in faecal progestagen concentrations above mean background concentrations, before decreasing again to a nadir of at least two consecutive values below mean background level. An elevation of concentrations above mean background was considered to be sustained if two of three consecutive values were above mean background concentration.

The cycle length was defined as the time interval between successive periods of sustained elevation of faecal progestagen concentrations as defined above. Cycle analysis only applied to parts of the hormonal profile which had regular sampling intervals, with no sampling interval longer than six days, since a longer sampling interval could result in the lack of identification of the period of low progestagen concentrations. This estimate is based on results from individual profiles in this study and from the observations that periods of oestrous were described to last six to seven days in wild black rhinoceros females (Hitchins & Anderson, 1983).

The period of sustained high concentration was designated as HC, while the period of low concentration was designated as LC and the total length of the cycle as TL. Because samples were not collected daily, the number of days between the end of HC and the subsequent period of LC was equally divided between HC and LC. The same applied between the end of a period of LC and a subsequent period of HC.

Cycles were subsequently categorised into different types. Cycles that were the most represented and whose TLs best fitted a Normal distribution were categorised as Type I cycles while the others were categorised as Type II cycles. In order to evaluate whether the cycle length was a reflection of lutcal activity, the correlation coefficient between the cycle TL and HC was assessed. The Pearson or Spearman rank correlation were used depending on whether the cycle length data was compatible with a Normal distribution, as evaluated by the Shapiro-Wilk test. Type

IIa cycles corresponded to cycles with a significant correlation between TL and HC and Type IIb cycles included cycles with no correlation between TL and HC. Comparison between cycle lengths used a Mann-Whitney test.

Profiles of hormonal levels are only shown for those periods during which cyclic patterns could be identified. The occurrence of reproductive behaviour (mating, fighting between males around the female and consortship with males) is indicated. Only interactions with males that were directly observed in the field are considered. Intervals between interactions with males were calculated by measuring the interval between first days of periods of observed interactions, considering only intervals that exceeded ten days, since it was found that periods of interactions with the same male could last as long as 12 days.

3.3 NON-INVASIVE MONITORING OF PREGNANCY

3.3.1 Examples of individual profiles

The pattern of faecal 20α-progestagen excretion throughout a complete pregnancy in a semi-wild female (DJ) is shown in Figure 3.1. This animal was presumed to have conceived on 25/11/96, when the last mating was observed. Mating was associated with a nadir in concentrations and within ten days following mating, levels increased to concentrations > 2000 ng/g. Concentrations decreased after the first month to levels slightly higher than background levels. Until around 100 days after mating, concentrations remained mainly below 2000 ng/g, showing only variations of small amplitude. After the third month of gestation, faecal concentrations of 20α-progestagen increased gradually and consistently to reach peak levels of around 12 000 ng/g by the fifth month of gestation. During the last ten months of gestation, levels remained between 4000 and 12 000 ng/g. Concentrations

started to decline during the last three weeks preceding parturition, which occurred in the presence of some 20α-progestagen levels. Concentrations declined to premating levels within four days following parturition. The female DJ gave birth on 22/2/98, or 454 days after the last observation of mating, suggesting a gestation period of 14.9 months.

Another profile of faecal 20α-progestagen concentrations established from the monitoring of the female Sara throughout pregnancy is shown in Figure 3.2.a. Signs of mating and chasing activity were observed on 28/2/97, when this female was accompanied by the male Guy for more than 10 days. Within ten days of presumed conception, levels increased and remained mainly between 1000 and 3000 ng/g for the first three months of gestation, except for a slight and transient decline around 45 days after oestrous behaviour. After another transient decrease around 90 days of gestation, levels increased dramatically and concentrations remained between 4000 and 16 000 ng/g afterwards. During the last 1.5 months preceding parturition, faecal samples were not collected from this female. The female Sara gave birth on 26/5/98, suggesting a gestation period of 450 days (14.8 months). During the first 6.5 months of pregnancy, she was observed to only interact occasionally with the male Guy but no further interactions were noted afterwards. She started to reject her previous calf around three months before parturition.

The pattern of faecal 20α-progestagen excretion in another wild female (Bulawayo) during gestation is presented in Figure 3.2.b. The animal is presumed to have conceived around 3-8/1/97, when she was observed to consort with male Buttom (see Figure 3.4). Concentrations increased within six days of the observed consortship and remained elevated for one month, although below 2000 ng/g. After a transient decline, concentrations gradually increased 80 days after presumed conception and

remained above 2000 ng/g. They remained above 5000 ng/g after the eighth month of gestation. A value below 5000 ng/g was recorded three weeks before parturition, which represented the first evidence of a possible decline in concentrations preceding parturition. Samples were not collected during the last three weeks of gestation. This female gave birth on 22/4/98, suggesting a gestation length of 15.3-15.4 months. She was not observed with any male during the entire gestation period and separated from her older calf one week prior to parturition.

3.3.2 Composite profile

Since progestagen concentrations increased markedly between 80 and 100 days of pregnancy on the individual profiles presented above, the gestation period was divided into early, mid, and late phases, which included months 1-3, months 4-12 and months 13-15 of gestation. Mean 20α-progestagen concentrations during each phase of pregnancy were not significantly different between the six wild study animals and their data was therefore combined in Table 3.1.a. Mean concentrations in each different phase of pregnancy were significantly higher ($P<0.001$) than in non-pregnant animals.

A composite profile of faecal 20α-progestagen concentrations during six pregnancies is illustrated in Figure 3.3. Mean 20α-progestagen concentrations during the first month (1359 ± 186 ng/g) and third month (2000 ± 265 ng/g) of gestation were significantly higher ($P=0.014$ and $P=0.002$ respectively) than non-pregnant concentrations (800 ± 58 ng/g). However, mean concentrations during the second month of pregnancy (1106 ± 23 ng/g) did not differ significantly ($P=0.08$) from non-pregnant concentrations. Between months 4 and 15 of gestation, mean concentrations remained between five and ten times greater than non-pregnant concentrations.

3.3.3 Diagnostic test

An important aspect of the non-invasive monitoring of gestation in wild females consisted in determining whether this technique could be used as a pregnancy test in black rhinoceros females living under free-ranging conditions. The 95% upper tolerance limit for 20α-progestagen concentrations in non-pregnant females was 2005 ng/g. Using 2000 ng/g as a threshold, all individual samples collected from the six wild females were tested retrospectively. This enabled the determination of the percentage of false negatives and false positives that would occur when diagnosing pregnancy from progestagen analysis in a single sample (Table 3.1.b).

In order to assess the accuracy of the test in a clinically useful way, the positive predicted value and negative predicted value were calculated and showed that a positive diagnosis of pregnancy or non-pregnancy could be made with 96.5% and 76.8% confidence respectively (Table 3.1.b). Because these predictive percentages depend on the prevalence of pregnant samples in the test, the specificity and sensitivity of the test were estimated as they represent a better indicator of reliability. The sensitivity of the test was 82.4%, while the specificity was 95.1%. The probability of a false positive or false negative occurring would therefore be 4.9 and 17.6% respectively. However, after the third month of gestation, the specificity of the test remained the same but the sensitivity increased to 97.6%, thus reducing the probability of a false negative to 2.4%.

3.3.4 Gestation length

During the whole study, the length of the gestation period was determined for 14 pregnancies in 12 females, including five wild, four semi-wild and four captive animals (see chapter 4). The mean gestation length was determined to be 469 ± 3.8

days (range, 447-489 days, n=14), or 15.4 months. Half of these gestation periods lasted 15.1-15.4 months.

3.4 NON-INVASIVE MONITORING OF THE POST-PARTUM PERIOD AND REPRODUCTIVE CYCLES

3.4.1 Individual profiles

The mean background concentrations calculated for each individual female are presented in Table 3.2.

3.4.1.1 Female Bulawayo

This female was radio-collared and the sampling frequency was therefore extremely consistent during the year following parturition (mean = 3.1 ± 0.1 days), with only two intervals exceeding six days (Figure 3.4).

3.4.1.1.1 Mean background concentrations

A first intergestation period I1 lasted 4 months until day 112 of the profile. It was characterised by a mean background concentration that was significantly higher than for I2, which lasted until day 240 (P<0.001) (Table 3.2). A third period lasting until day 311 had a mean background concentration which was significantly higher than for I2 (P<0.001). After day 311, faecal 20α-progestagen concentrations remained mainly above 2000 ng/g.

3.4.1.1.2 Cycles and interactions with males

Between days 1 and 120, levels varied widely between high and low concentrations and did not remain at low levels for long enough to enable the identification of a cyclic pattern of activity during this part of the profile. The female

Bulawayo was not observed to interact with any other black rhinoceros during that period.

During I2, an initial period of LC of 14 days was followed by cyclic variations lasting 72.5, 45 and 59 days (Table 3.3). The longer cycle was associated with an extended period of LC lasting 45.5 days, during which slight increases of short duration occurred. The exact duration of periods of HC and LC during cycle 2 were determined to last at least 18.5 and 14 days respectively, but their exact determination was hampered by a sampling interval of 11 days between these two periods. During that interval, the female could not be located, despite her radio-collar and intensive searching efforts. When she was found, she was observed twice with the male Buttom (days 233-238). Together with another observation ten days later with the same male, these were the only records of interactions with a male (Figure 3.10).

3.4.1.1.3 Period of cyclic activity, conception and gestation period

A cyclic pattern of faecal 20α-progestagen excretion was observed between four and 10 months post-partum, coinciding with the periods I2 and I3. The fact that a higher background concentration was detected after the only observed interaction of this female with a male and prior to a sustained increase in faecal 20α-progestagens to levels > 2000 ng/g, suggests that the female mated during cycle 2. She subsequently gave birth after a gestation period of ca. 15.3-15.4 months.

3.4.1.2 Female Pukwani

This female gave birth on 18/1/97.

3.4.1.2.1 Mean background concentration

The period I1 lasted 4.5 months (Table 3.2) and the sampling frequency was 4.7 ± 0.4 days during the first half of this period, with nine sampling intervals

exceeding six days before day 110 post-partum. The mean background concentration during I1 was significantly higher than during I2 (P=0.02). The mean background concentration during I3 further decreased compared to I2 (P<0.001). A fourth period I4 comprised between day 313 and 365 had a mean background concentration significantly higher than the previous one (P<0.001). During periods I2, I3 and I4, no sampling interval exceeded six days and the sampling frequency was high and regular (mean = 2.4 ± 0.1 days).

After day 350 of the profile, concentrations started to rise consistently to reach levels above 2000 ng/g after day 367 post-partum.

3.4.1.2.2 Cycles and interactions with males

A cyclic pattern of faecal 20α-progestagen excretion was observed from day 130 until around day 350 of the profile (Figure 3.5). Cycle lengths were calculated as being 26, 21, 48.5, 46, 28 and 40 days (Table 3.3). Two longer cycles (48.5 and 40 days) corresponded to longer periods of HC (34.5 and 32 days) while the cycle of 46 days corresponded to an extended period of LC of 36 days, bounded by two periods of consortship with males separated by a 26-day interval.

Four different black rhinoceros males were observed to interact with the female Pukwani between days 1 and 367 post-partum (Figure 3.10). They included three adults (Shava, Pumula, Nhamo) and one sub-adult (Mafupi). A male white rhinoceros (Mangosi) was also seen with her on one occasion on day 317.

Pumula was the male that was most often observed with the female Pukwani (52%) and which also had the most prolonged periods of interactions with her, lasting up to nine days. All periods of consortship with this male coincided with periods of LC and one started at the end of a period of HC (Table 3.5).

Interactions with the other two adult males occurred during periods of HC and those with Mafupi were detected either within periods of observations with Pumula or during the period of increment of faecal hormonal levels after the last cycle was observed.

Intervals between periods of consortship with the male Pumula were 42, 45, 23, 26, 17 and 61 days. When interactions with all adult males were considered during the first half of the profile until day 235 (excluding intervals less than 10 days and above 20 days), interactions occurred with a certain regularity, being separated by an average interval of 15 ± 0.7 days.

3.4.1.2.3 Periods of cyclic activity, conception and gestation period

The female Pukwani presented a cyclic pattern of faecal 20α-progestagen excretion for at least seven months, between 4.5 and 11.5 months post-partum, corresponding to the end of the period I1, and to I2, I3 and I4. The last cyclic variation identified in the profile was associated with a higher mean background concentration and occurred prior to the sustained elevation to faecal 20α-progestagen levels >2000 ng/g, similar to that of the previous profile (Figure 3.4), suggesting that the female became pregnant. The observation of a prolonged period of interactions with the male Pumula during the period of LC of cycle 5 suggests that the female Pukwani conceived at that time. This female gave birth in April 1999, indicating a gestation period of ca.16 months. She was not observed to interact with any adult males during the gestation period, except occasionally during the first two months.

3.4.1.3 Female Netsai

This female gave birth on 6/2/97. She had separated from her older calf approximately three weeks before parturition.

3.4.1.3.1 Mean background concentration

The first post-partum period I1 lasted until day 159 and had a mean background concentration of faecal 20α-progestagens that was not significantly different from that of Bulawayo and Pukwani during the same period (P=0.13) (Table 3.2). Faecal samples were collected infrequently during this period (mean = 4.6 ± 0.4 days), with six sampling intervals exceeding six days, all of which occurring before day 120. The female Netsai was not observed with any male during that period.

Period I2 lasted until day 283 and had a mean background concentration that was nearly a third of I1, while the last intergestation period I3 lasted until day 364 and had a significantly higher mean background concentration compared to I2 (P=0.0063). The sampling frequency was very high during I2 and I3 (mean = 2.8 ± 0.2 days).

After I3, levels consistently increased to reach concentrations that remained above 2000 ng/g after day 375.

3.4.1.3.2 Cycles and interactions with males

The first behavioural observation of this female with a male after she gave birth was made on day 124 post-partum with the male Buttom. The observation of spoor patterns indicated that the male had extensively chased the female and had mated her. Faecal 20α-progestagen concentrations increased above mean background concentration two days following that observation and a cyclic pattern of faecal 20α-progestagen excretion can subsequently be observed on the profile. Cycle lengths of 52.5, 24.5, 26.5, 24.5, 31 and 56 days were determined (Table 3.3). Periods of LC lasted between six and 21 days, but were less variable (range: 6-11 days) for cycles 2 to 5. Periods of HC lasted between 14.5 and 41 days.

The female Netsai was observed to interact with three different males between days 1 and 377 post-partum (Figure 3.10). These included one adult (Buttom) and two subadults (Jaggers and Bonus), one of which was known to be Netsai's previous calf, while the other was also identified as being one of her older calf through a genetic study (Garnier *et al.*, in press). These three males could be observed occasionally with the female Netsai and her young calf, thus representing a family group of five animals.

Interactions with Buttom were the most frequent (59%) and lasted up to 12 days. Intervals between periods of consortship with him were 35, 62, 23, 31 and 59 days. Periods of interactions with this male coincided with periods of LC and to the end of periods of HC (Table 3.5), except for the last observation on day 371 that occurred when levels had already started to increase consistently. On two occasions (days 159 and 166), the male Buttom had a fight with the subadult Jaggers.

The cycle lengths and intervals between interactions with males correlated best during the second half of the profile, after cycle 4.

3.4.1.3.3 Periods of cyclic activity, conception and gestation period

The period of cyclic activity identified in the female Netsai lasted for at least seven months, between four and 11 months post-partum, which coincided with the end of periods I1, I2 and I3. The last cycle (6) observed in this female also occurred during a period of higher mean background concentration and prior to the marked increase in faecal 20α-progestagens concentrations, suggesting that this female was in a phase of early pregnancy like the previous two females studied. She therefore most likely conceived during cycle 5 and gave birth in April 1999, suggesting a gestation period of ca. 16 months. During gestation, she was only observed to interact a few times with Buttom during the first three months.

3.4.1.4 Female Jete

This female gave birth to her first calf on 2/10/96. The mother left her calf hidden in the same area during the two weeks following birth.

3.4.1.4.1 Mean background concentration

The first intergestation period I1 lasted until d.282 (Table 3.2). The sampling frequency was regular during the first month post-partum, every three days, but became infrequent and irregular afterwards (mean = 4.3 ± 0.2 days), with eight sampling intervals exceeding six days. No clear pattern of variation of faecal 20α-progestagen concentrations could be seen before day 200 and the profile is therefore not shown before that time.

Between d. 287 and d. 520 of the profile, period I2 was characterised by a significantly lower mean background concentration compared to I1 ($P < 0.001$) and by a very regular sampling frequency (mean = 2.3 ± 0.14 days), with no sampling interval exceeding six days.

Between days 429 and 495 post-partum, no clear pattern of variations of faecal 20α-progestagens concentrations could be detected and levels became consistently above 2000 ng/g after day 523 post-partum.

3.4.1.4.2 Cycles and interaction with males

During the first month following parturition, faecal progestagen levels remained within HC range during the first 20 days post-partum, before decreasing sharply to HC for around 10 days. Between day 35 and day 215, concentrations increased progressively, both in background concentration and peak amplitude but no cyclic pattern of activity could be observed. Observations of consortship with males during that period were recorded on days 50, 172-278, and 211.

Between day 215 and 422 of the profile, cyclic variations of faecal 20α-progestagen concentrations were detected (Figure 3.7). Cycle lengths of 45, 36, 26.5, 28.5, 23 and 22 days were calculated. The variation in cycle lengths corresponded to a variation in lengths of the periods of HC between 37.5 and 10 days, while the period of LC remained between eight and 13 days (Table 3.3).

Two mating were observed on day 236 and 422 of the profile, coinciding with mid- or late periods of LC (Table 2.5). Levels increased to HC within two to six days following mating activity. A fight between males occurred during a period of HC, 11 days prior to the next sustained elevation of concentrations to HC (Table 2.5).

Between parturition and day 521, this female was observed to interact with five males, including three adult (Penga, No Name, Buttom) and two subadults (Sun, Handboy). Penga and Sun interacted the most with the female Jete, being identified in 38 and 31% of the consortship observed respectively (Figure 3.10). However only Penga was observed to mate the female Jete during this study, the first mating having been observed around eight months post partum.

Intervals between observations with the adult male Penga between days 200 and 422 post-partum were calculated as being 25, 29, 67, 37, 20 and 33 days.

3.4.1.4.3 Periods of cyclic activity, conception and gestation period.

The female Jete showed cyclic variations of faecal 20α-progestagens concentrations between seven and 14 months post-partum. It is possible that cyclic activity started earlier, around six months post-partum, when this female was first observed to interact with the male Penga during two successive observations. Unfortunately, sampling frequency was irregular during this period and no cyclic pattern of concentrations could be identified on the profile.

This female was last observed to be mated on day 422 by the male Penga, around 120 days before levels became consistently >2000 ng/g. She subsequently gave birth during the first week of March 1999, between 459 and 465 days (ca. 15-15.2 months) after the last observation of mating. Interactions with males were only observed during the first six months of gestation.

3.4.1.5 Female Sirica

This female gave birth around 1/12/96. The first signs of separation between the mother and her older calf were seen towards mid-September and she was alone in November.

3.4.1.5.1 Mean background concentration

Period I1 lasted for seven months until day 213 and was characterised by a mean background that was not significantly different from that of Jcte during I1 (P= 0.05). Sampling was infrequent during this period (mean = 4.9 ± 0.4 days), with eight intervals exceeding six days. No clear pattern of variation of faecal 20α-progestagen concentrations could be seen during this period and the profile is therefore not shown.

I2 lasted eight months until day 454 of the profile and had a mean background concentration that was not significantly different than I1 (P = 0.48). Faecal samples were collected very regularly and nearly every other day during this period (mean = 2.4 ± 0.1 days).

After I2, concentrations increased consistently to reach levels above 2000 ng/g after day 507, where they remained until around day 520. Between days 520 and 530, concentrations decreased dramatically and abruptly to below mean background concentration. Concentrations then started to show cyclic variations again.

3.4.1.5.2 Cycles and interactions with males

Cyclic variations of faecal 20α-progestagens concentrations were observed between approximately days 210 and 450 of the profile (Figure 3.8). Cycle lengths of 26.5, 36.5, 19, 43, 31, 30, 22.5 and 30 days were determined. Periods of HC varied between 13 and 30 days, while periods of LC varied between six and 11 days, except during the longer cycle of 43 days where it lasted for 19 days (Table 3.3).

The female Sirica was observed to interact with three different males (two adults and one sub-adult) between parturition and day 507 (Figure 3.10). The male which interacted predominantly (84%) with her and was observed to mate with her was No Name. Mating activity was recorded at the end or at the beginning of a period of HC (Table 3.5). A fight that was observed between No Name and another male occurred at the beginning of an extended period of LC (19 days).

Intervals between interactions with the adult male No Name was 44, 35, 19, 33, 49 and 18 days.

The female Sirica was not observed with any other black rhinoceros between days 450 and 530 post-partum, after which both Sun and No name were seen again with her.

3.4.1.5.3 Period of cyclic activity and conception

Cyclic variations in the female Sirica lasted for at least eight months, between seven and 15 months post-partum. After day 507, concentrations remained >2000 ng/g, suggesting that she became pregnant. Conception presumably occurred during the phase of LC of cycle 7, when two successive interactions with the male No Name were detected. A cyclic pattern of faecal 20α-progestagen excretion however resumed 110 days after presumed conception.

3.4.1.6 Female Sara

The female Sara gave birth to her second calf on 26/5/98, approximately two months after having separated from her older calf. After birth, the mother hid her calf until day 18 post-partum, when it was first observed to move with its mother. Unfortunately, the calf was killed by lions on day 22.

3.4.1.6.1 Mean background concentration

I1 lasted until day 35 of the profile (Figure 3.9) and had a mean background concentration of faecal progestagens that was significantly higher than that of I2 (P<0.001) (Table 3.2). Sampling frequency was extremely regular until day 118 of the profile (mean = 2 ± 0.06 days), after which date a gap of 11 days existed due to the impossibility of locating this female. She was then found again in her usual range on day 129.

After day 114, concentrations remained above 2000 ng/g for 25 days but decreased abruptly afterwards.

3.4.1.6.2 Cycles and interactions with males

After parturition, an initial period of HC lasted for 8 days and was followed by a period of LC of 15 days. Two subsequent cycles of 22 and 28.5 days were observed (Table 3.3). Oestrous behaviour occurred at the end of a period of HC and beginning of period of LC.

Within two days of the calf's death, the female Sara was observed to be re-united with her older calf, then three years old, and the adult male Muzamani. Both males were nearly constantly observed with this female until day 118. Reproductive behaviour was observed on days 33 and 37, when her spoor revealed that she had been squirting small amounts of urine very frequently, suggestive of oestrus. On day

97, the male Muzamani mated her. Between days 129 and 138, the female Sara was observed with her older calf and afterwards, the male Muzamani joined them again.

3.4.1.6.3 Periods of cyclic activity and conception

Cycle 1 was observed as soon as 24 days post-partum and two days after the calf had died. After the second cycle, levels started to increase consistently and although a transient decline was observed after 42 days, levels became >2000 ng/g after day 114, suggesting that she had become pregnant. Conception most likely occurred at the end of cycle 2 between days 35 and 44. The dramatic decrease in concentrations, associated with the resumption of interactions with an adult male, occurred ca. 100 days after presumed conception. In November 2000, the female Sara still had not produced another calf (C. Stockil, personal communication).

3.4.2 Cycle lengths and cycle types

The different cycle lengths that were established on the basis of hormonal concentrations of faecal 20α-progestagens are presented in Table 3.3. Total cycle lengths varied between 19 and 72.5 days, but cycles that were < 40 days best fitted a Normal distribution (W=0.97, n=21). These cycles were identified as Type I and represented 68% of all cycles. They were characterised by a mean TL of 26.8 ± 1 days (range, 19-36 days), a mean length of HC of 18 ± 1.1 days and a mean length of LC of 9 ± 0.5 days (Table 3.4). The correlation between TL and HC length was significant for those cycles (r=0.91, P<0.001, n=21).

In order to try to identify a common pattern and possible significance for cycles longer than 40 days, identified as Type II cycles, two groups were considered on the basis of the length of LC and subsequent correlation between TL and HC length. A cut off point of 15 days was use for LC since the best correlation between

TL and HC was obtained for cycles that had periods of LC ≤ 15 days (r=0.97, $P<0.001$, $n=26$).

The first group of cycles (Type IIa) represented 16% of all cycles and had a TL ≥ 40 days with a period of LC ≤ 15 days. This group presented a very good correlation between TL and HC (r=0.93, $P=0.01$, $n=5$). It was characterised by a mean total length of 48.7 ± 3 days, with means HC and LC of 37.8 ± 2.2 days and 11.9 ± 1.6 days respectively (Table 3.4). Both mean TL and length of HC were significantly longer than means calculated for the group I ($P<0.001$), while mean LC were not significantly different between the two groups ($P=0.06$).

Amongst Type IIa cycles, three of them occurred during periods described as I3 and I4 and were characterised by mean background concentration around 700-750 ng/g. They were identified in the females Bulawayo (cycle 3), Netsai (cycle 6) and Pukwani (cycle 6). They preceded a consistent increase in faecal 20α-progestagens concentrations to levels above 2000 ng/g and were detected between nine and 12 months post-partum.

Two other Type IIa cycles were preceded by the observation of mating activity (Jete: cycle 1, Pukwani: cycle 3). Another cycle identified as Type II (Bulawayo: cycle 2) could not be identified as belonging to Type IIa or IIb since a lag of 11 days occurred in the sample collection.

The second group (Type IIb) of cycles had a very poor correlation between TL and HC (r=0.36, $P=0.54$, $n=4$). Such cycles were identified once in four of the six females studied. Their mean TL (52.3 ± 4.5 days) was not significantly different from that of cycles IIa ($P=0.7$). However, both periods of HC (mean = 26.7 ± 5 days) and LC (mean = 27.3 ± 5.7) were significantly different from that of cycles IIa ($P=0.02$).

Of Type IIb cycles, two were associated with the observation of a fight between males during the extended period of LC (Netsai: cycle 1 and Sirica: cycle 4). One had a period of LC that was surrounded by two periods of prolonged consortship with males separated by 26 days (Pukwani: cycle 4). The last cycle type IIb was identified in the female Bulawayo (cycle 1) but was not associated with any interactions with males. The last two cycles were both identified to occur in September.

Excluding Type IIa cycles that corresponded to the early gestation period in three females, the cycles identified as Type I represented 75% of all cycles, while Type IIa cycles represented at least 7% and Type IIb at least 13% (one cycle could not be classified between IIa or IIb).

3.5 DISCUSSION

3.5.1 Locating and identifying wild black rhinoceros

Amongst the study animals, only one was radio-collared. However, despite the immense size of the main study area and the presence of a large number of black rhinoceros, the collection of faecal samples from known individual females was undertaken on a very regular basis.

The rapidity and success in finding a sample from a particular individual varied greatly with animals, the time of the year and the reproductive status. Some females were much easier to find than others, as they tended to move less within their home ranges. In general, females could also be located more rapidly during the dry season, when they tended to remain nearby known water points and became inactive as soon as the temperature became too hot. During this season, monitoring was undertaken before 9.00-10.00 a.m. This routine was established to follow patterns of

daily activity observed in other black rhinoceros populations (Goddard, 1967; Mukiniya, 1973; Owen-Smith, 1988). In the afternoon, animals became active again as the temperature declined, but were difficult to track since their spoor had often dried up in the sun.

The lower monitoring frequency of females during the immediate post-partum was mainly due to the increasing difficulty in finding females which had just given birth associated with the occurrence of the rainy season. The study animals were observed to hide in very dense thicket to give birth, where they kept the calf hidden for the following two or three weeks if undisturbed. This habit of hiding the newborn calf had been described as lasting three to four weeks in previous studies (Hall-Martin & Penzhorn, 1977; Joubert & Eloff, 1971). After this initial period, females were observed to move around with their calves until they could find another suitable place, still in dense bush with suitable browse and close to a water source, where they remained for another few days. Because most study animals gave birth during the rainy season, water was available everywhere in the bush and females did not have to search for it over long distances. They did not leave any spoor around the usual water sources and were therefore more difficult to locate. The presence of a radio-collar also affected the time and efforts required to find an individual, since the average time it took to monitor a non-collared animal was between two to six hours while it took less than two hours to find the collared female.

3.5.2 Use of faecal 20α-progestagen analysis for reproductive monitoring

The results of faecal hormonal analysis during the gestation and intergestation periods demonstrate that both pregnancy and oestrous cycles can be monitored by

measuring faecal 20α-progestagen immunoreactivity. The measurement of 20α-progestagen concentrations had previously been reported in captive black rhinoceros females. In both saliva and faecal samples, their measurement was found to be useful for monitoring pregnancy (Czekala & Callison, 1996; Schwarzenberger *et al.*, 1993). Patterns of cyclic ovarian activity could also be monitored through faecal and urinary 20α-progestagen analysis (Hindle *et al.*, 1992; Schwarzenberger *et al.*, 1993).

In this study, we used an EIA that had been previously validated for urinary steroid analysis in order to monitor cyclic ovarian activity in both African rhinoceros species (Hindle *et al.*, 1992). No radioactive-metabolism study has been undertaken in the black rhinoceros. However, the analysis of faecal samples collected from both captive and wild black rhinoceros, using high performance liquid chromatography analysis and gas chromatography-mass spectrophotometry, identified 5α- and 5β-reduced pregnanes as the main faecal progesterone metabolites (Patton *et al.*, 1996; Schwarzenberger *et al.*, 1996b).

The immunoreactivity measured in this study therefore most likely originates from pregnanes containing a 20α-hydroxyl group with which the antiserum utilised in the 20α-OHP assay cross-reacted. The antiserum also cross-reacted with pregnenes, even if present in small amounts as found by Schwarzenberger *et al.* (1993). This shows that although radio-metabolism studies are always valuable in determining the specific steroid catabolites in excreta, they are not absolutely necessary for the successful use and application of faecal steroid analysis (Schwarzenberger *et al.*, 1997). Nevertheless, it should be emphasised that faecal progesterone metabolites can be highly variable, even in closely related rhinoceros species (Heistermann *et al.*, 1998; Hindle & Hodges, 1990),

Similarly, the measurement of faecal 20α-progestagens has been useful in monitoring gestation and ovarian cycles in the Indian rhinoceros, in which 5β-pregnanes appear to be the dominant faecal metabolites (Schwarzenberger *et al.*, 2000a). It is therefore probable that 20α-progestagen evaluation in faeces could also be useful for monitoring reproductive activity in the Sumatran rhinoceros, which also predominantly excretes pregnanes from the 5β-series in faeces (Heistermann *et al.*, 1998).

3.5.3 Gestation period

Individual profiles obtained during gestation indicated that concentrations increased markedly to levels above 2000 ng/g within 80-100 days following conception. Later, concentrations continued to gradually increase until levels became above 4000-5000 ng/g within three to seven months of pregnancy. On the composite profile, mean faecal progestagen concentrations after the second month of gestation were significantly higher than concentrations in non-pregnant females, although they were already higher during the first month of gestation but showed a transient decline during the second month.

These results compare favourably with those from previous studies on captive black rhinoceros, which reported that the timing for the marked increase in progestagens was also around three months of gestation when using faecal 20α-progestagens or 20-oxo-pregnanes analysis (Schwarzenberger *et al.*, 1993, 1996b). Both Brown *et al.* (1997) and Berkeley *et al.* (1997), who used a progesterone assay, describe this pattern as occurring later, after only 4.5 to five months of gestation, and this difference is probably due to the different antiserum used. In the Indian and the white rhinoceros, patterns of faecal progestagen immunoreactivity also presented a

marked elevation after the third month of gestation (Patton *et al.*, 1999; Schwarzenberger *et al.*, 2000a).

By contrast, the excretion of urinary progestagen metabolites during the gestation period in captive rhinoceros showed less consistent patterns. Urinary pregnanediol was reported to increase after two and three months of gestation in captive black and white rhinoceros females respectively, but only after seven months in another study in black rhinoceros (Hindle, 1991; Ramsay *et al.*, 1987). Urinary pregnanediol also increased only after mid-gestation in the Indian rhinoceros (Hodges & Green, 1989; Kasman *et al.*, 1986), while urinary 20α-OHP did not become elevated until late gestation in the black rhinoceros (Hindle, 1991). It has been suggested that such differences between patterns of urinary progesterone metabolites excretion might be attributed to shifts in progesterone metabolism and/or excretion during pregnancy (Hindle, 1991). Whether or not this is the case, the consistency in patterns of faecal progestagen immunoreactivity between different species of rhinoceros suggests that this technique is more widely applicable than urinary progestagen analysis for detecting pregnancy in rhinoceroses, while also allowing an earlier diagnosis of pregnancy.

In the domestic horse, the use of faecal 20α-progestagen analysis also allowed the detection of a significant increase in concentration at a similar timing, around week 11 of gestation (Schwarzenberger *et al.*, 1991). In this species, the placenta becomes the sole source of progestagens after the corpora lutea have regressed, i.e. after four to five months of gestation. The progestagens secreted by the placenta include mainly 5α-pregnanes, which are also the main faecal progesterone metabolites in this species (Daels *et al.*, 1991). Because these metabolites are predominant in black rhinoceros faeces (Patton *et al.*, 1996; Schwarzenberger *et al.*,

1996b), the marked increase in faecal 20α-progestagen excretion observed after two to three months of gestation most likely reflects the onset of placental steroid production (Schwarzenberger *et al.*, 1993).

During mid- and late pregnancy, mean faecal 20α-progestagen concentrations remained approximately 8-9 times higher than concentrations in non-pregnant animals. A similar scale of differences between pregnant and non-pregnant animals was also found in previous studies on captive black rhinoceros (Berkeley *et al.*, 1997; Schwarzenberger *et al.*, 1993). The absolute values of faecal 20α-progestagen concentrations were also similar between this study and those of Schwarzenberger *et al.*, (1993) in captive animals. It is therefore unlikely that the difference in diets that exists between wild and captive rhinoceroses affects progesterone excretion, as could have been suggested by the findings of Wasser *et al.*, (1993), who reported that variations in dietary fiber content affected progesterone metabolites excretion in baboons.

By contrast, faecal progestagen values in mid- and late pregnancy were lower in the study of Berkeley *et al.* (1997), while concentrations were much higher in the study reported by Schwarzenberger *et al.* (1996b). Such differences are probably linked to the different assays used, since the antiserum used by Schwarzenberger *et al.* (1996b) was more specific of the progesterone metabolites that had been identified in black rhinoceros faeces (Patton *et al.*, 1996).

Parturition in the present study was found to occur in the presence of still elevated faecal 20α-progestagen concentrations, although levels started to decline between three to four weeks before parturition and returned to background levels around 2-4 days following parturition. Schwarzenberger *et al.* (1993) also observed a decline in faecal 20α-progestagen around two weeks before parturition, while faecal

20-oxo pregnanes were found to decline as early as the last quarter of gestation (Schwarzenberger *et al.*, 1996b). By contrast in the white rhinoceros, faecal pregnanes only decreased after parturition (Patton *et al.*, 1999). It can therefore be concluded that the measurement of faecal 20α-progestagen immunoreactivity represents the most useful monitor of the whole gestation period in the black rhinoceros, as well as the best predictor of impending parturition. This is particularly useful both in captivity, in order to prepare the most suitable environment for the parturient female, as well as in the wild, in order to detect the calf's birth and subsequently monitor the newborn calf's health.

The lag between parturition and return to background levels has also been observed in previous studies (Berkeley *et al.*, 1997; Schwarzenberger *et al.*, 1993). It is most likely attributable to the delay associated with the gastrointestinal time and enterohepatic circulation (Gower & Honour, 1984).

The mean gestation period determined in this study lasted 15.4 months, which is in close agreement with gestation length reported from behavioural observations both in the wild (Goddard, 1967b; Hall-Martin & Penzhorn, 1977; Hitchins & Anderson, 1983) and in captivity (Lindemann, 1982; Schwarzenberger et al., 1993, 1996b). Other gestation lengths in the present study were of ca. 16 months and previous reports also indicate that such longer gestation periods can occur, although less frequently than the 15-month gestation (Hindle, 1991; Hitchins & Anderson, 1983; Lindemann, 1982).

The evaluation of faecal 20α-progestagen measurement as a diagnostic test, when collecting only one faecal sample from a wild black rhinoceros female, showed that 97.6% of the pregnant animals were correctly identified by the test after the third month of gestation. Previous studies on free-ranging wildlife species, for example

feral horse, bison, elk, caribou and moose, established the potential use of faecal progestagen assessment to diagnose pregnancy (Berger *et al.*, 1999; Garrott *et al.*, 1998; Kirkpatrick *et al.*, 1991, 1992; Messier *et al.*, 1990; Stoops *et al.*, 1999; White *et al.*, 1995). These studies reported an ability to discriminate between pregnant and non-pregnant animals from a single sample or very few samples varying from 55 to 100 %, depending on the species, the extraction and assay procedure, the antiserum used and the timing of sample collection.

The specificity of the diagnostic procedure tested in this study was 95 %. This suggested that 5% of non-pregnant females can be misdiagnosed as pregnant, due to detection of some concentrations above 2000 ng/g in non-pregnant animals. Consequences associated with false positives are important for captive animals, which are usually separated once a female is confirmed pregnant, therefore preventing future conceptive opportunities. In the wild, the detection of a false positive could also lead to the focused monitoring of that animal and the incorrect suspicion of abortion in the absence of parturition. Furthermore, such a result could affect translocation decisions. Consequences of false positives would therefore mainly be associated with a loss in time as far as reproductive performances are concerned, by eventually restraining the animals from future conception. However, the false positives do not represent a risk for the animal itself since greater care is given to females that are diagnosed pregnant.

The sensitivity of the test increased from 82.4 % when considering the whole gestation period to 97.6 % when only considering the last 12 months of gestation. Such increased sensitivity is a reflection of the dramatic increase in faecal progestagen concentrations observed after the third month of pregnancy. The probability of false negative therefore decreases considerably after that time, since they only represent around 2.5 % after the first trimester of gestation. Although this

percentage is relatively small in absolute value, the consequences associated with false negatives could however be very important. They include the eventual conclusion that an animal has aborted if a positive diagnosis of pregnancy has previously been established. If the animal has not previously been tested before, the false diagnosis of non-pregnancy would lead managers to keep a male with that female for future conception. This might in turn lead to abortion, as has been observed after a male attacked violently a pregnant female in captivity (B. White, personal communication.). In the wild, management decisions for non-pregnant animals could include immobilisation for dehorning, earnotching and translocation. The impact of immobilisation on pregnancy in the black rhinoceros has not yet been studied and will be discussed in greater detail in chapter 4. However, until risks associated with such procedures are better known, managers try to prioritise non-pregnant females for immobilisation. The sensitivity of the pregnancy test therefore needs to be maximum.

The sensitivity of this test could be increased by selecting a lower value for pregnancy discrimination, but in this case the specificity would be diminished. Another possibility would be to use another antiserum, such as one raised against 20-oxo-pregnanes, as used by Schwarzenberger *et al.* (1996b). However, this assay does not allow the prediction of impending parturition, and its ability to monitor oestrous cycles has not been evaluated. One way to increase both specificity and sensitivity in this test would be to collect more than one sample for a pregnancy diagnosis. The collection of two samples at a three-month interval would overcome the problem of false negatives during the first three months of the gestation period. However, the collection of monthly samples during three months may be easier to organise by managers, since a routine pattern is more easily to follow.

3.5.4 Post-partum

In all females, the immediate post-partum period was characterised by a higher mean background concentration (700-1400 ng/g) compared to subsequent periods where it was ca. 500 ng/g. Such a higher background concentration could not be attributed to individual variations. They were observed in all study animals, being similar between three females in which this period lasted around four months, as well as between two females in which I1 lasted between seven and nine months.

Because the sampling frequency was lower during I1 in most females, the higher mean background concentration could have been associated with the failure to detect short nadirs in faecal 20α-progestagen immunoreactivity during this period, thus resulting in generally higher concentrations. However, a higher mean background concentration was also detected in the two females that could be sampled regularly during this period.

Since most I1 ended between late June and early July, coinciding with the onset of winter and the post-rainy season, the higher mean background concentration observed then might have been caused by the change of diet that occurred between the rainy and the dry season. This could have led to the appearance of certain plant compounds that could cross-react with the assay used, and/or to a more concentrated diet and a decrease in dietary fibre, that can have a positive effect on progesterone excretion (Wasser *et al.*, 1993). Alternatively, the duration of I1 could have been influenced by a change in photoperiodicity. However, because I1 in one female ended at a different time of the year, in September, the higher mean background concentration is probably not associated with the presence of an exogenous cross-reacting substance, and its duration is unlikely to be influenced by a change in season or photoperiod.

Another possibility to explain the higher mean background concentration observed during the post-partum period may be the development of luteinised follicles during this period. This hypothesis is reinforced by the failure to detect cycles in one female that could be monitored frequently during the immediate post-partum and by the presence of fluctuating levels of faecal progesterone metabolites concentrations. The resumption of follicular maturation after parturition was in fact confirmed in four captive black rhinoceros females through the measurement of oestrogens and progestagens in either faeces or urine, within two to four weeks following birth (Berkeley *et al.*, 1997; Hindle, 1991; Schwarzenberger *et al.*, 1993). In these studies, it is not clear whether these females also exhibited behavioural oestrus.

In the present study, four females exhibited either no or only one interaction with an adult male (around day 50 post-partum) during the first four to five months post-partum, while another female only resumed regular interactions with males after eight months post-partum. In the female who lost her calf at three weeks of age, the resumption of male interaction occurred within two days after the calf died, suggesting that lactation and/or suckling play a role in inhibiting behavioural oestrus during this time.

Other possibilities to explain the higher mean background concentrations during I1 are represented by the presence of progestagens of extra-ovarian origin, such as from the adrenal gland. The adrenal cortex has been recognised as a source of steroids in man and possibly other species (Hsu & Crump, 1989). Oestrogens, androgens and progesterone are present in the extracts of normal adrenal glands but overproduction of a sex steroid could occur if there was a modulation of metabolic pathways involved in the steroidogenic scheme.

3.5.5 Oestrous cycles

Individual hormonal profiles in six wild females showed that between two and eight cyclic variations of faecal 20α-progestagens concentrations were identified in each female (total of 31 cycles), occurring between four and 15 months post-partum. The majority of these cycles (75%) were described as Type I and characterised by a mean total length of 26.8 ± 1 days. This is similar to the cycle length reported in captive black rhinoceros by using non-invasive steroid analysis (Berkeley *et al.*, 1997; Godfrey *et al.*, 1991; Hindle *et al.*, 1992; Schwarzenberger *et al.*, 1993).

Type I cycles were characterised by a period of HC (mean = 18 ± 1.1 days) that lasted around twice as long as the period of LC (mean = 9 ± 0.5 days) and that was significantly correlated with the cycle TL. Most observations of reproductive activity (fight between males, period of prolonged interactions with the dominant male, mating) occurred during periods of LC. It can therefore be concluded that in Type I cycles, periods of LC can be associated with the follicular phase of an ovarian cycle, while the periods of HC are a reflection of the luteal phase.

Previous studies had detected an interval of seven to nine days between ovulation/mating and the luteal phase and subsequent increase in faecal progestagens in white rhinoceros (Radcliffe *et al.*, 1999; Schwarzenberger *et al.*, 1999b)., coinciding with the average LC length determined in this study. It can therefore be presumed that Type I cycles, which are characterised by an average phase of LC of nine days, are ovulatory.

Type IIa cycles were characterised by a longer TL that correlated significantly with the HC length, suggesting that these prolonged cycles corresponded to extended luteal activity. Although the HC length of Type IIa cycles corresponded to approximately twice the length of the luteal phase of Type I cycles, it is unlikely that

these longer cycles were caused by a failure to detect a decrease in faecal 20α-progestagens concentrations, as the sampling frequency was very high during the periods involved (around every two to three days). In addition, most of these longer cycles were bounded on either side by periods of prolonged interactions with an adult male.

Type II cycles were identified in all females except Sara, although a transient decrease occurred in this female 42 days after the observation of the last cycle. Three Type II cycles were associated with a period of elevated mean background concentration corresponding to I3 or I4. They also occurred just prior to the sustained elevation of faecal 20α-progestagens concentrations to levels above 2000 ng/g, indicating that these females were in a phase of early pregnancy. At the end of each of these cyclic variations, very limited or no interaction with males was observed. The occurrence of a cyclic pattern of faecal 20α-progestagen excretion during the early gestation period had also been noted on the profiles covering gestation periods in this study. The decline in concentrations that occurred between 40 and 60 days of gestation in the study animals explains why mean concentrations during the second month of gestation are lower than during the first and third month of gestation, as had been noticed by Schwarzenberger *et al.* (1993).

Some Type IIa cyclic variations are therefore associated with prolonged luteal phases in the early gestation period in the black rhinoceros. The resulting profile is very similar to what has been described in the horse, in which a decline in progesterone secretion occurs 40-50 days after conception (Daels *et al.*, 1991). The fact that intervals between conception and the time when levels remain above 2000 ng/g vary between two and four months in all study animals might suggest the possibility that accessory corpora lutea develop in the black rhinoceros, as has been

described in the horse (Daels *et al.*, 1991). In the horse, equine chorionic gonadotropin (eCG) secreted by the endometrial cups after ca. day 40 of gestation promotes the ovulation or luteinization of follicles that develop during the first four to five months of pregnancy (Daels *et al.*, 1991). However, attempts to detect the presence of eCG in serum collected from pregnant black rhinoceros have so far been unsuccessful (W.R. Allen, personal communication). Alternatively, it is possible that by about three months of gestation, placental progestagens support pregnancy.

The other longer cycles that corresponded to Type IIa or IIb were not followed by a sustained increase in faecal 20α-progestagens concentrations and subsequent birth. Some of these cycles were characterised by an extended luteal phase (Type IIa). Such extended luteal phases have also been identified in captive white rhinoceros females monitored over long periods of time through faecal progestagen analysis (Schwarzenberger *et al.*, 1998; Patton *et al.*, 1999).

The occurrence of extended luteal phases can be attributed to several causes. Uterine diseases such as pyometra are known to cause prolonged luteal phases in the domestic horse as a result of a retained corpus luteum, since the altered uterus is unable to release prostaglandins (Evans *et al.*, 1997). Although pyometra has been diagnosed in captive white rhinoceros by ultrasound examination (Radcliffe *et al.*, 1997; Patton *et al.*, 1999), this pathology is unlikely to be responsible for the prolonged luteal phases observed in the wild females studied. Indeed, these females either subsequently gave birth, or they exhibited Type I cycles after these Type II cycles.

The idiopathic persistence of the corpus luteum up to three months has also been described in the horse as a potential cause for prolonged luteal phases (Evans *et al.*, 1997). In captive white rhinoceros, persistent corpora lutea have been diagnosed

by ultrasound but the animals were described as "flatliners", with very low levels of faecal progestagen immunoreactivity (Hermes *et al.*, 2000; Schwarzenberger *et al.*, 1998). It therefore remains unclear whether the longer cycles observed in the study could be associated with persistent corpus luteum.

Another possible cause for a prolonged luteal phase is the occurrence of ovulation during dioestrus, as has been reported in the domestic horse. When it occurred during the late dioestrus, the resulting corpus luteum is too young to respond to prostaglandins and luteal activity therefore persists (Evans *et al.*, 1997).

Another potential explanation for these prolonged luteal phases is embryo loss. Pregnancy loss in mares between day 13 and day 34 of gestation results in the prolongation of the corpus luteum for 35 to 90 days following ovulation (Hinrichs, 1997). This pathology has been identified in captive white and Sumatran rhinoceros females and the corresponding luteal phases identified by faecal progesterone metabolites analysis lasted longer than the average ones (Patton *et al.*, 1999; Radcliffe *et al.*, 1999; Roth *et al.*, 2001). These reports, combined with the fact that a history of mating followed by anoestrus without pregnancy in mares is suggestive of early embryo loss (Hinrichs, 1997), indicate that such pathology cannot be excluded for some Type II cycles. This hypothesis is further reinforced by the fact that lengths of extended cyclic variations resulting from a prolonged corpus luteum during the early gestation period in this study are similar.

Some Type IIb cycles were characterised by a follicular phase that lasted around three times that of Type I cycles, with short surges of small amplitude of faecal progestagens occurring during these periods. This suggests that they might be associated with a pattern of follicle growth but that they were anovulatory. Follicles are anovulatory during the vernal transition in domestic mares before the first

ovulation of the season, suggesting that in this species they are seasonally linked (Sharp *et al.*, 1997). The fact that some of these Type IIb cycles were also the first cycles identified in two females might suggest that they correspond to a transitional phase into the breeding season.

Another possible cause of longer follicular phases is represented by heat stress, which is known to affect follicular growth in cows (Hansen, 1997). Some type IIb cycles occurred in September/October, during the hottest months of the year in Zimbabwe.

One Type IIb cycle was also characterised by a short luteal phase. A decrease in dioestrus length indicates premature luteolysis and is often associated with endometritis in the horse (Evans *et al.*, 1997). Such cycle was detected after a potential embryo loss in one study animal and it is possible that embryo loss caused a short-term infection responsible for the premature luteolysis.

Certain cyclic variations of faecal 20α-progestagen concentrations had a period of LC during which no interactions with males could be detected. The observed absence of males might have resulted from a failure to detect males with these females, but the sampling frequency was very frequent and regular during this period. These cycles represented 16% of all cycles observed. The absence of signs of oestrus, or "silent heat" and is known to occur in normally cyclic mares (Hinrichs, 1997). It has also been identified in a white rhinoceros female (Hindle, 1991).

3.5.6 Interactions with males

Each female was observed to interact with between one and three adult males, as well as with up to two subadults. One male always dominated the consortship for a female and will subsequently be called the dominant male for that female. Interactions

with dominant males represented between 38 and 100% of interactions with both adult and subadult males. In all cases, it is the dominant male that was observed to mate the female and to fight with other males. Most observations of consortship with the dominant male were coincidental with the detected periods of LC, while periods of consortship with other males, including sub-adults, could occur during periods of HC.

Significant increases in faecal 20α-progestagens were detected within six days after the observation of mating, although in two instances they were detected one day and 18 days before such increase. A time lag of up to 12 days had also been detected in a captive black rhinoceros female (see section 2.5.4.3). It most likely results from the delay necessary for the faecal excretion of steroid hormones through the entero-hepatic circulation, with probably another delay that exists between ovulation and an increase in progesterone, as has been observed in the white and Sumatran rhinoceros (Radcliffe *et al.*, 1997; Roth *et al.*, 2001).

Periods of interactions with the dominant male lasted up to 12 days, which is about twice as long as previous report on the duration of consortship (Hitchins & Anderson, 1983). Such difference may be attributed to the high frequency of observations that were undertaken during the present study. Fighting between the dominant male and another male was observed during such periods of consortship, occurring between 11 and 18 days before a significant increase in faecal progestagen concentrations was detected. This suggests that the dominant male may try to prevent other males from accessing the receptive female, including her previous calves after they have become sub-adult. It is however not known whether the dominant male exhibits this behaviour of mate guarding on his home range, similarly to what has been observed with white rhinoceros territorial males (Owen-Smith, 1988), or

whether he prevents other males from accessing the receptive female within her home range. The observations that two adult females with overlapping home ranges also had the same dominant male and also had overlapping home ranges with him suggest that a dominant male may become territorial over their common home range when she becomes receptive (Garnier, unpublished observation).

No mating was detected to occur during periods of consortship with other adult males. The finding that the dominant male for each female was also the breeding male for that female may suggest differences in fertility levels amongst males (Garnier *et al.*, in press).

There were also great individual differences in the number of male-interactions recorded for each female and some females kept interacting with their previous male calves. Such consortship represented up to 25% of all direct observations recorded. As a result, associations of up to five animals were seen, including the breeding male, the cow-calf unit and her two previous calves.

3.5.7 Abortion

In two females (Sirica and Sara), a cyclic pattern of faecal 20α-progestagen excretion was detected prior to a significant increase to levels above 2000 ng/g, suggesting that these females became pregnant. However, levels remained elevated only for a short time and decreased abruptly between 70 and 100 days after presumed conception. Such a dramatic and rapid decline in concentrations, associated with the resumption of regular interactions with males and with cyclic variations of faecal 20α-progestagen levels, indicate that there is a very strong probability that these two females aborted.

Abortion has been reported in some captive black rhinoceros females between months two and 14 of gestation, but most reports are isolated cases and no causal agent has been mentioned (Berkeley *et al.*, 1997; Hodges & Green, 1989; Schwarzenberger *et al.*, 1993, 1996b). Abortion was also diagnosed in Sumatran females between one and four months of gestation (Roth & Brown, 1999; Roth *et al.*, 2001). In the present study, it has been established that a marked increase in faecal progestagen concentrations occurring between two and four months of gestation probably reflected the onset of the steroid placental production. It is possible that this particular time in the gestation represents a vulnerable phase in the black rhinoceros.

Table 3.1.a. Faecal 20α-progestagen concentrations (ng/g dry faeces) in wild black rhinoceros (n=6) at various stages of pregnancy and non-pregnancy.

Reproductive status	Mean	SE	n	95% confidence limits[3]
Non-pregnant[1]	800	58	111	684-919
Early pregnancy[2]	1417[a]	131	57	1153-1680
Mid-pregnancy[2]	6410[a]	261	164	5895-6925
Late pregnancy[2]	7243[a]	472	47	6293-8192

[1] Non-pregnant samples were collected one to two times per week during three months preceding gestation, or between four to seven months after parturition during lactation

[2] Early pregnancy: month 1-3 of gestation; mid-pregnancy: month 4-12 of gestation; late pregnancy: month 13-15 of gestation.

[3] mean ± 1.96 x SE.

[a] P<0.05 when compared to non-pregnant concentrations.

Table 3.1.b. Characteristics of the pregnancy diagnostic test using faecal 20α-progestagen measurement in a single sample collected from wild black rhinoceros. Each unit tested represents one faecal sample.
All samples tested (n=432) were collected from the study animals.

	Known status		
Predicted status	Not pregnant	Pregnant	Total
Not pregnant[1]	156	47	203
Pregnant[2]	8	221	229
Total	164	268	

Positive predictive value: 221/229 = 96.5%
Negative predictive value: 156/203 = 76.8%
Sensitivity: 221/268=82.4% (for definition see text).
Specificity: 156/164=95.1% (for definition see text).

[1] Faecal 20α-progestagen value <2000 ng/g

[2] Faecal 20α-progestagen value ≥2000 ng/g

Table 3.2. Mean background concentrations of faecal 20α-progestagens from parturition until levels ≥ 2000 ng/g.
Different periods I1, I2, etc were considered on each hormonal profile corresponding to differences in background observed on the profile.

	I1		I2		I3		I4	
Female ID	Length Days on profile *Dates*	Mean F20α-progestagens Mean ± SE	Length Days on profile *Dates*	Mean F20α-progestagens Mean ± SE	Length Days on profile *Dates*	Mean F20α-progestagens Mean ± SE	Length Days on profile *Dates*	Mean F20α-progestagens Mean ± SE
Bulawayo	1-112 *15/5/96-4/9/96*	987 ± 49	115-241 *7/9/96-11/1/97*	487 ± 26	244-311 *14/1/97-22/3/97*	698 ± 33		
Pukwani	1-164 *18/1/97-2/7/97*	915 ± 64	170-266 *8/7/97-12/10/97*	682 ± 59	268-309 *14/10/97-4/11/97*	259 ± 24	313-365 *28/11/97-22/1/98*	749 ± 50
Netsai	1-159 *6/2/97-9/7/97*	1189 ± 82	163-283 *13/7/97- 10/11/97*	458 ± 12	285-364 *12/11/97-30/1/98*	734 ± 24		
Jete	1-282 *2/10/96-10/7/97*	820 ± 31	287-521 *15/7/97-6/3/99*	470 ± 22				
Sirica	1-213 *1/12/96-2/7/97*	720 ± 37	215-454 *2/12/97-28/2/98*	494 ± 14				
Sara	1-35 *26/5/98-30/6/98*	1375 ± 117	37-81 *2/7/98-15/8/98*	575 ± 37				

Table 3.3. Individual cycle lengths in wild black rhinoceros females

Animal ID	Cycle Length[a]	HC Length	LC Length	Cycle Number	Cycle Type[1]
Bulawayo	72.5	27	45.5	1	IIb
	45	N/A	N/A	2	II
	59	44.5	14.5	3	IIa
Pukwani	26	14.5	11.5	1	I
	21	12.5	8.5	2	I
	48.5	34.5	14	3	IIa
	46	10	36	4	IIb
	28	19	9	5	I
	40	32	8	6	IIa
Netsai	52.5	31.5	21	1	IIb
	24.5	14.5	10	2	I
	26.5	18	8.5	3	I
	24.5	18.5	6	4	I
	31	20	11	5	I
	56	41	15	6	IIa
Jete	45	37	8	1	IIa
	36	28	8	2	I
	26.5	16	10.5	3	I
	28.5	20.5	8	4	I
	23	10	13	5	I
	22	14	8	6	I
Sirica	26.5	20.5	6	1	I
	36.5	30	6.5	2	I
	19	13	6	3	I
	43	24	19	4	IIb
	31	20	11	5	I
	30	24	6	6	I
	22.5	15	7.5	7	I
	30	21.5	8.5	8	I
Sara	22	11	11	1	I
	28.5	17	11.5	2	I

[a] Cycle total length (TL) was calculated as the intervals between sustained elevation of faecal 20α-progestagen concentrations above mean background concentrations (HC), after at least two successive points below mean background concentration (LC). Cycle length calculations only applied when samples were collected every 2-3 days.

[1] Type I: TL<40 days
Type IIa: TL≥40 days and LC≤15 days
Type IIb: TL≥40 days and LC>15 days

Table 3.4. Characteristics of different cycle types.
Type I: TL < 40 days
Type IIa: TL ≥ 40 days and LC ≤ 15 days
Type IIb: TL ≥ 40 days and LC > 15 days

Cycle type	**Type I** n= 21	**Type IIa** n=5	**Type IIb** n=4
TL[1] mean ± SEM (days) range (days)	26.8 ± 1 19 - 36	48.7 ± 3 40 - 59	53 ± 6.6 43 - 72.5
HC[2] mean ± SEM (days) range (days)	18 ± 1.1 10 - 30	37.8 ± 2.2 32 - 44	23.1 ± 4.6 10 - 31
LC[3] mean ± SEM (days) range	9 ± 0.5 6 - 13	11.9 ± 1.6 8 - 15	30.3 ± 6.3 19 - 45

[1] Total Length
[2] High concentrations
[3] Low concentrations

Table 3.5. Relationship between cycle parameters and behavioural observations of consortship with the dominant adult male in wild black rhinoceros females. The dominant male was defined as the male that dominated the consortship for a female.

Animal	Cycle parameters					Behavioural observations
	N°	Type°	TL[1]	HC[1] Days profile	LC[1] Days profile	Behaviour[2] Days profile
Pukwani	1	I	26	147.5-162	162-173.5	148,160
	2	I	21	173.5-186	186-194.5	190,193,198
	3	IIa	48.5	194.5-229	229-243	235,237
	4	IIb	46	243-253	253-289	258,260,262 /284,286,288
	5	I	28	289-308	308-317	*301,305*,309
	6	IIa	40	317-349	349-357	
Netsai						124**
	1	IIb	52.5	125-156.5	156.5-177.5	159*,163,166*,170,171
	2	I	24.5	177.5-192	192-202	
	3	I	26.5	202-220	220-228.5	221,223,227
	4	I	24.5	228.5-247	247-253	*244,246*,248,250,252
	5	I	31	253-273	273-284	275,277,279
	6	IIa	56	284-325	325-340	334
Jete						236**
	1	IIa	45	242-279	279-287	*265*
	2	I	36	287-315	315-323	
	3	I	26.5	323-339	339-349.5	*332,338**
	4	I	28.5	349.5-370	370-378	*369*
	5	I	23	378-388	388-401	389
	6	I	22	401-415	415-423	422**
Sirica	1	I	26.5	219-239.5	239.5-245.5	*239***
	2	I	36.5	245.5-275.5	275.5-282	
	3	I	19	282-295	295-301	*283***
	4	IIb	43	301-325	325-344	*318*,326*,337
	5	I	31	344-364	364-375	370
	6	I	30	375-399	399-405	
	7	I	22.5	405-420	420-427.5	*419*,424
	8	I	30	427.5-449	449-457.5	*437*

° Type I: TL<40 days; Type IIa: TL≥40 days and LC≤15 days; Type IIb: TL≥40 days and LC>15 days

[1] Cycle total length (TL) was calculated as the intervals between sustained elevation of faecal 20α-progestagen concentrations above mean background concentrations (HC), after at least two successive points below mean background concentration (LC). Cycle length calculations only applied when samples were collected every 2-3 days.

[2] Consortship with dominant male. * indicate that fighting between males was also observed and ** indicate that mating was observed. Days in italic indicate that behavioural observations were made during periods of HC.

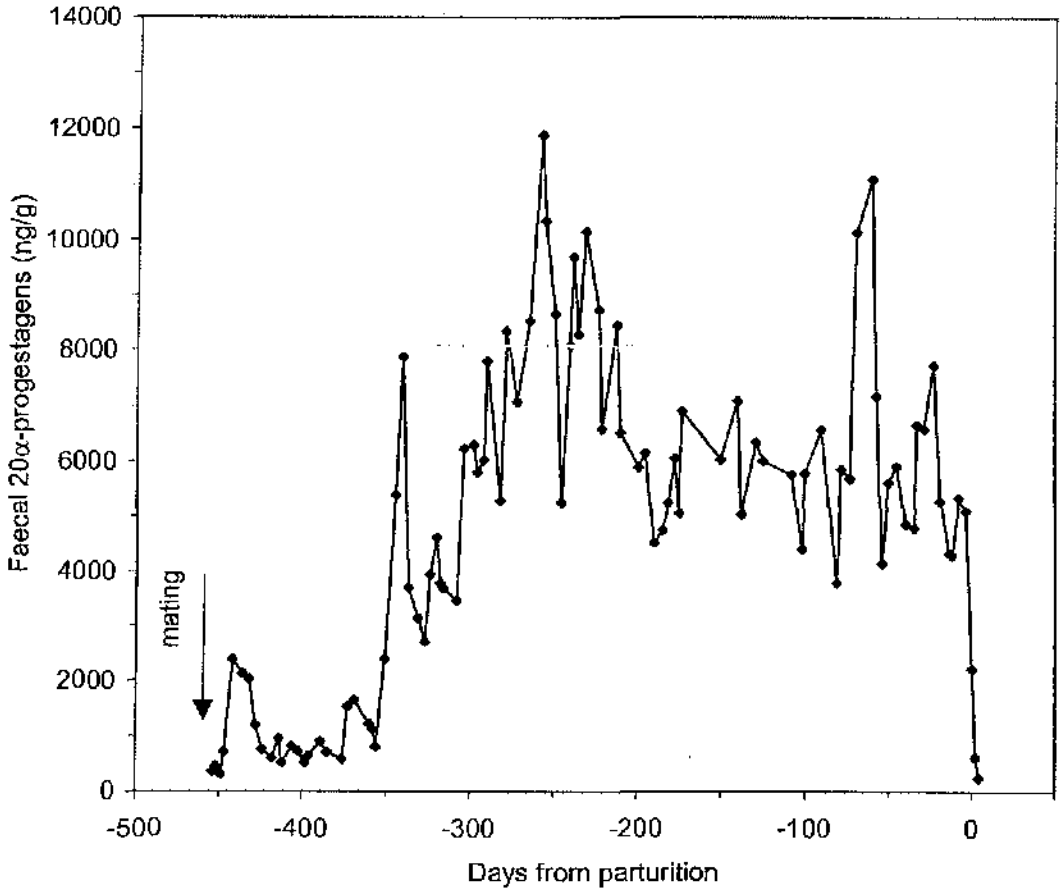


Figure 3.1. Concentrations of immunoreactive 20α-progestagens in faecal samples of a wild black rhinoceros (DJ) during pregnancy

Figure 3.2.a. Female Sara

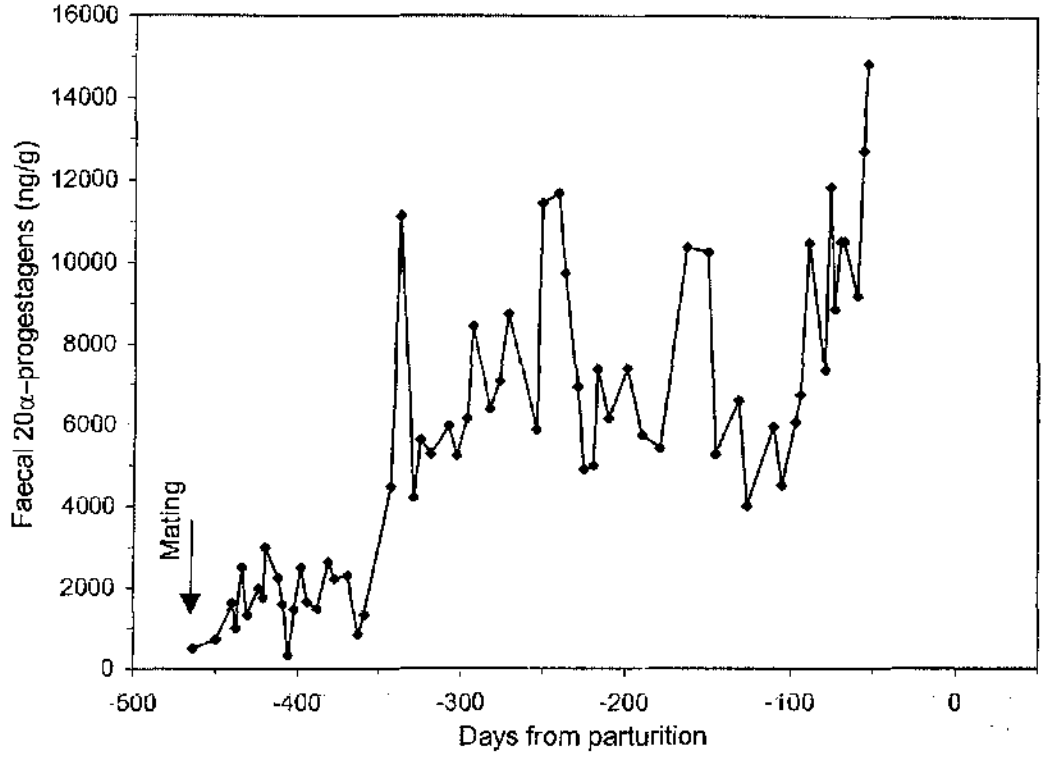


Figure 3.2.b. Female Bulawayo

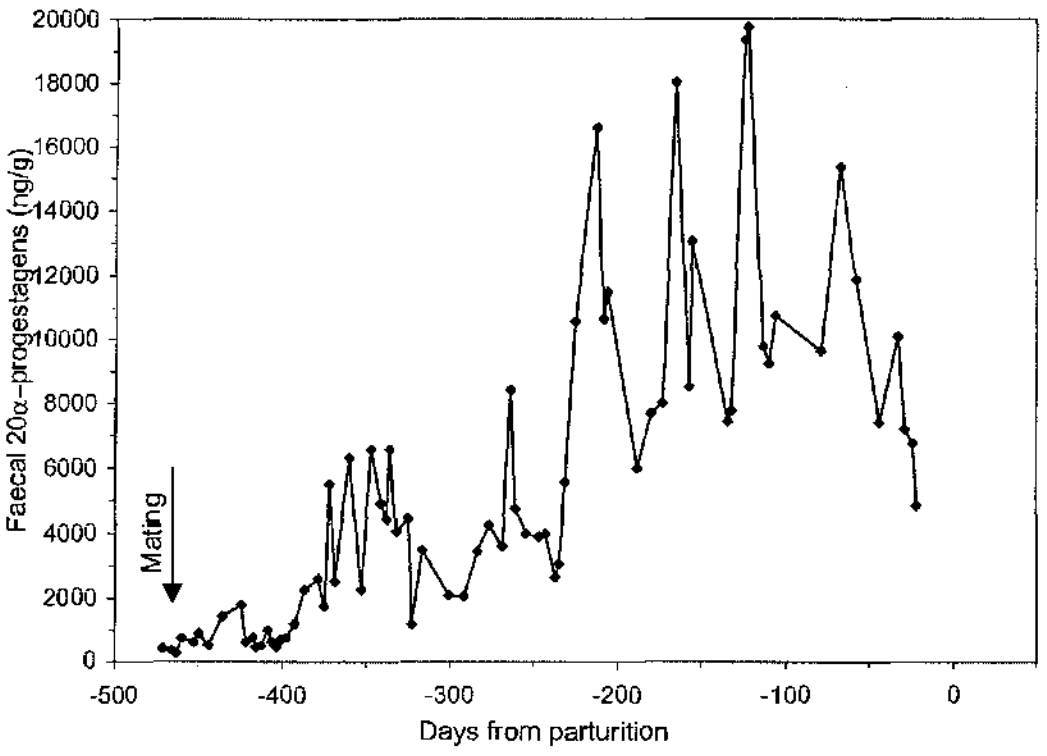


Figure 3.2. Concentrations of immunoreactive 20α-progestagens in faecal samples of two wild black rhinoceros females during pregnancy

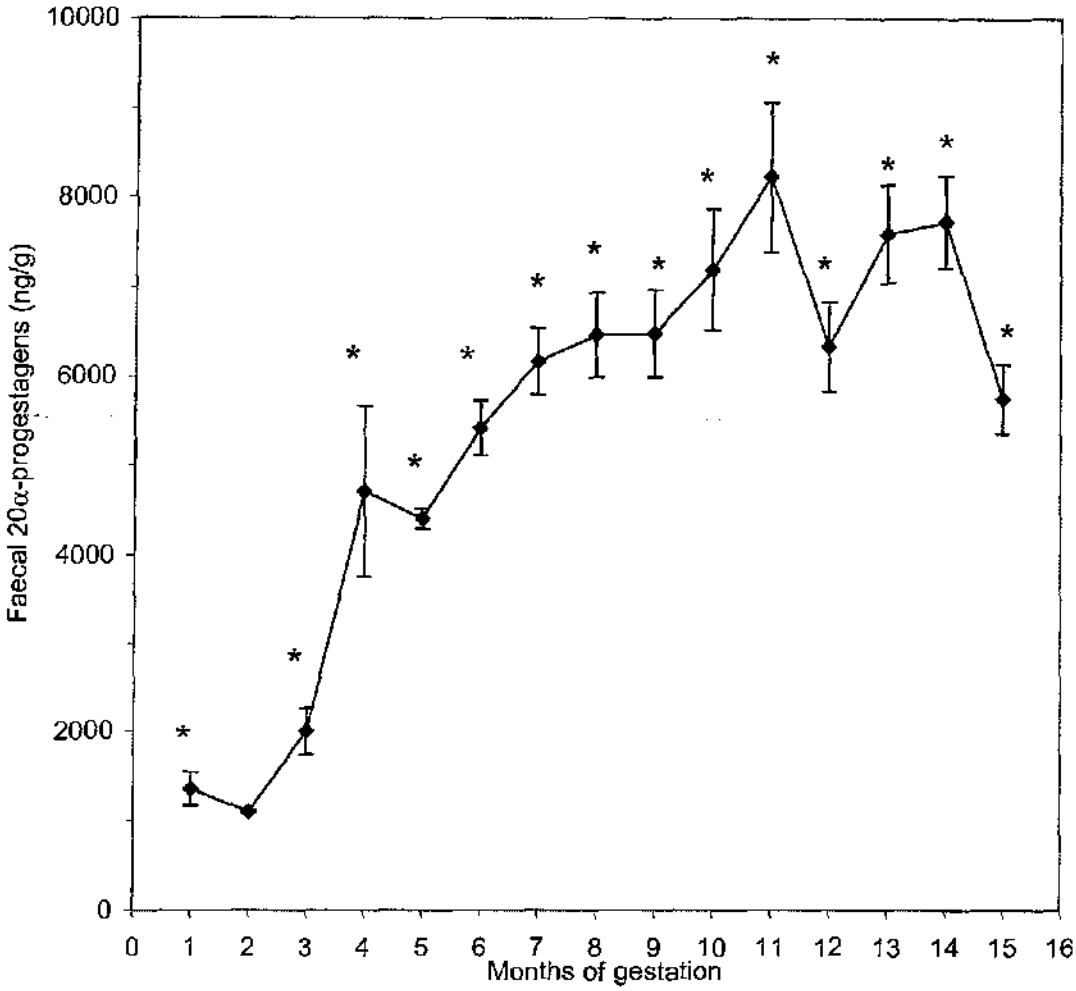
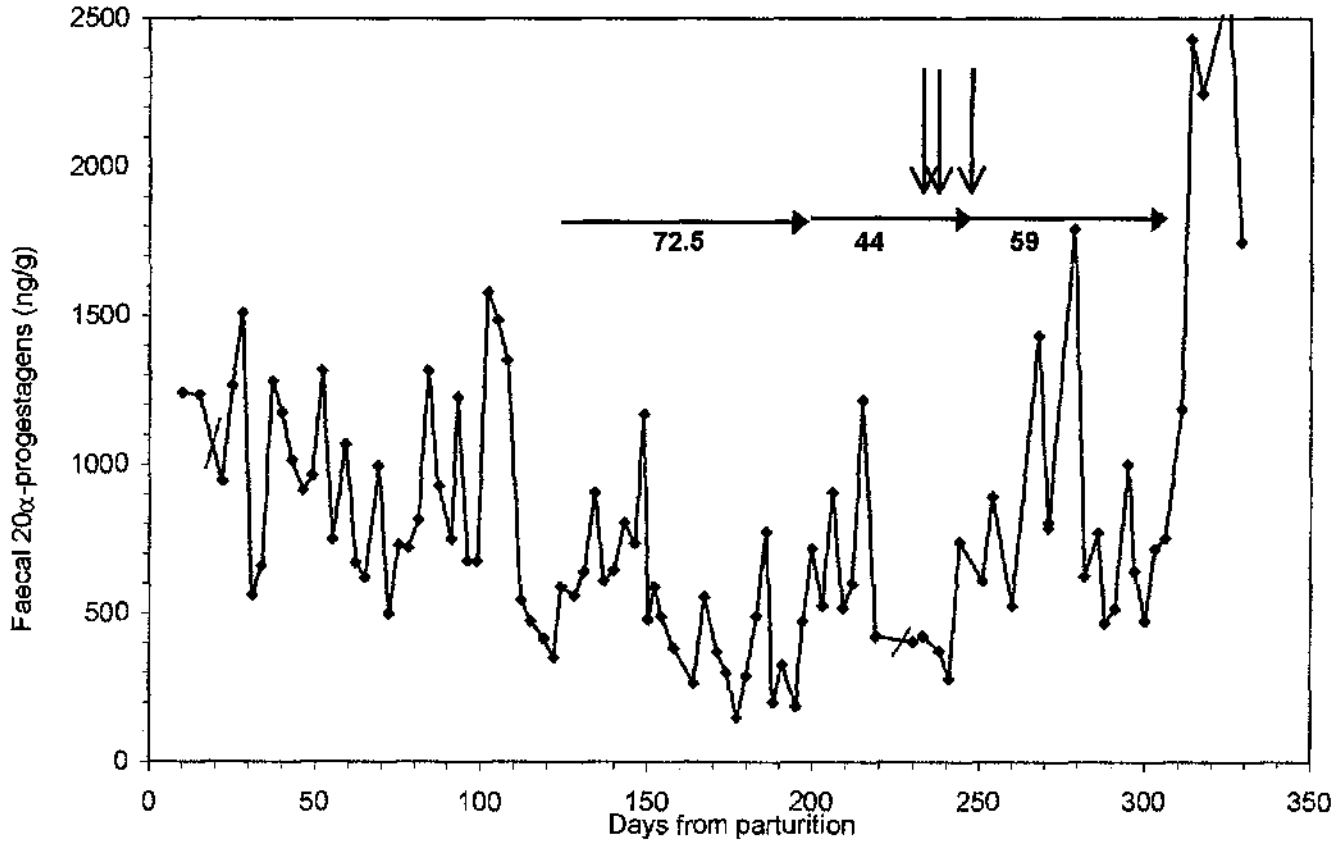


Figure 3.3. Mean concentrations of faecal 20α-progestagens (+/- SE, n =10-25 per point) during six pregnancies in wild black rhinoceros.
Asterisks indicate $P < 0.05$ when compared to non-pregnant concentrations.



Male:

↓ Buttom

Figure 3.4. Concentrations of faecal 20α-progestagens in a wild black rhinoceros (Bulawayo) during reproductive cycles and early pregnancy.
Vertical arrows indicate interactions with a male and horizontal arrows indicate cycle length.
/ indicate a sampling interval > 6 days

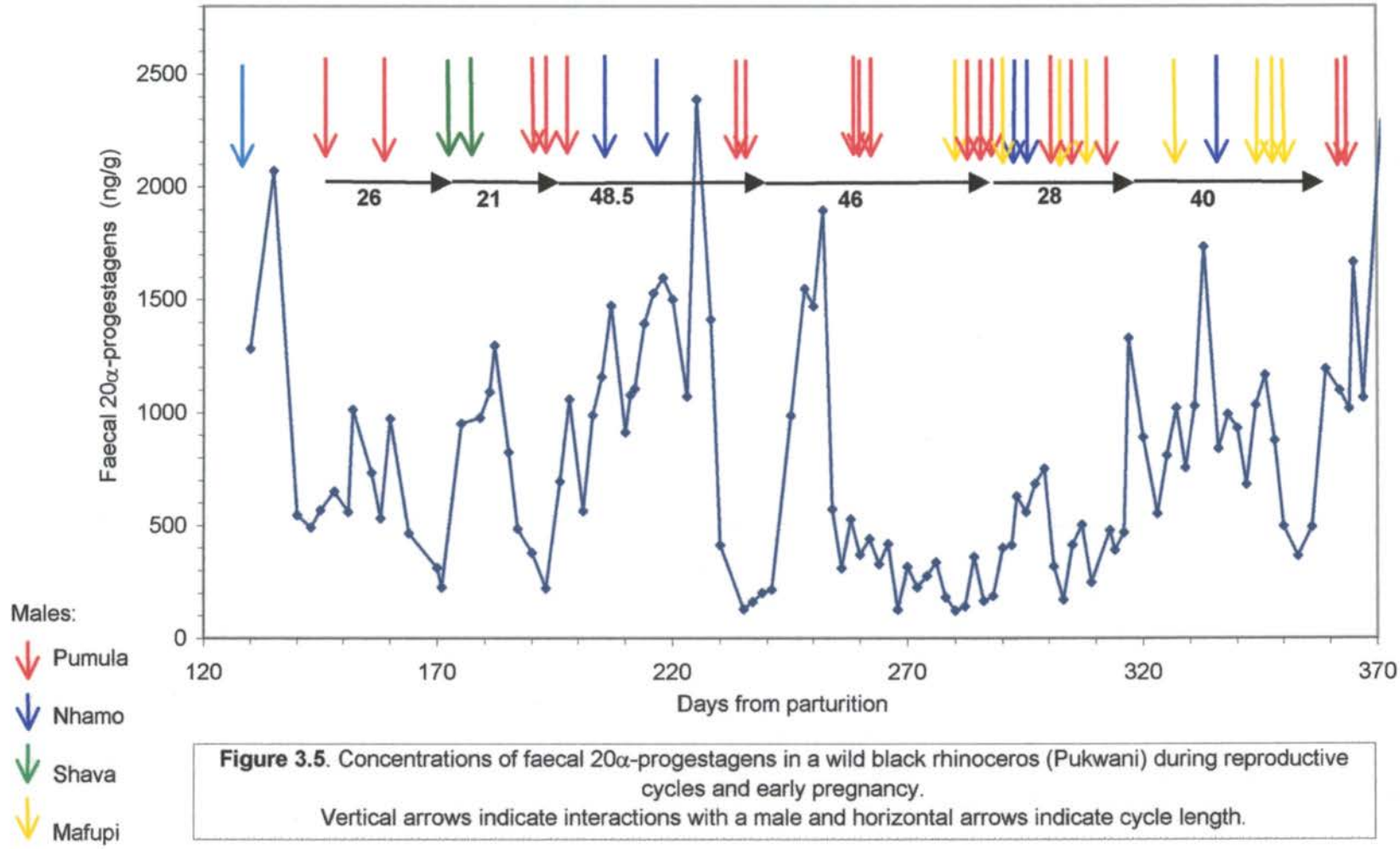


Figure 3.5. Concentrations of faecal 20α-progestagens in a wild black rhinoceros (Pukwani) during reproductive cycles and early pregnancy.
Vertical arrows indicate interactions with a male and horizontal arrows indicate cycle length.

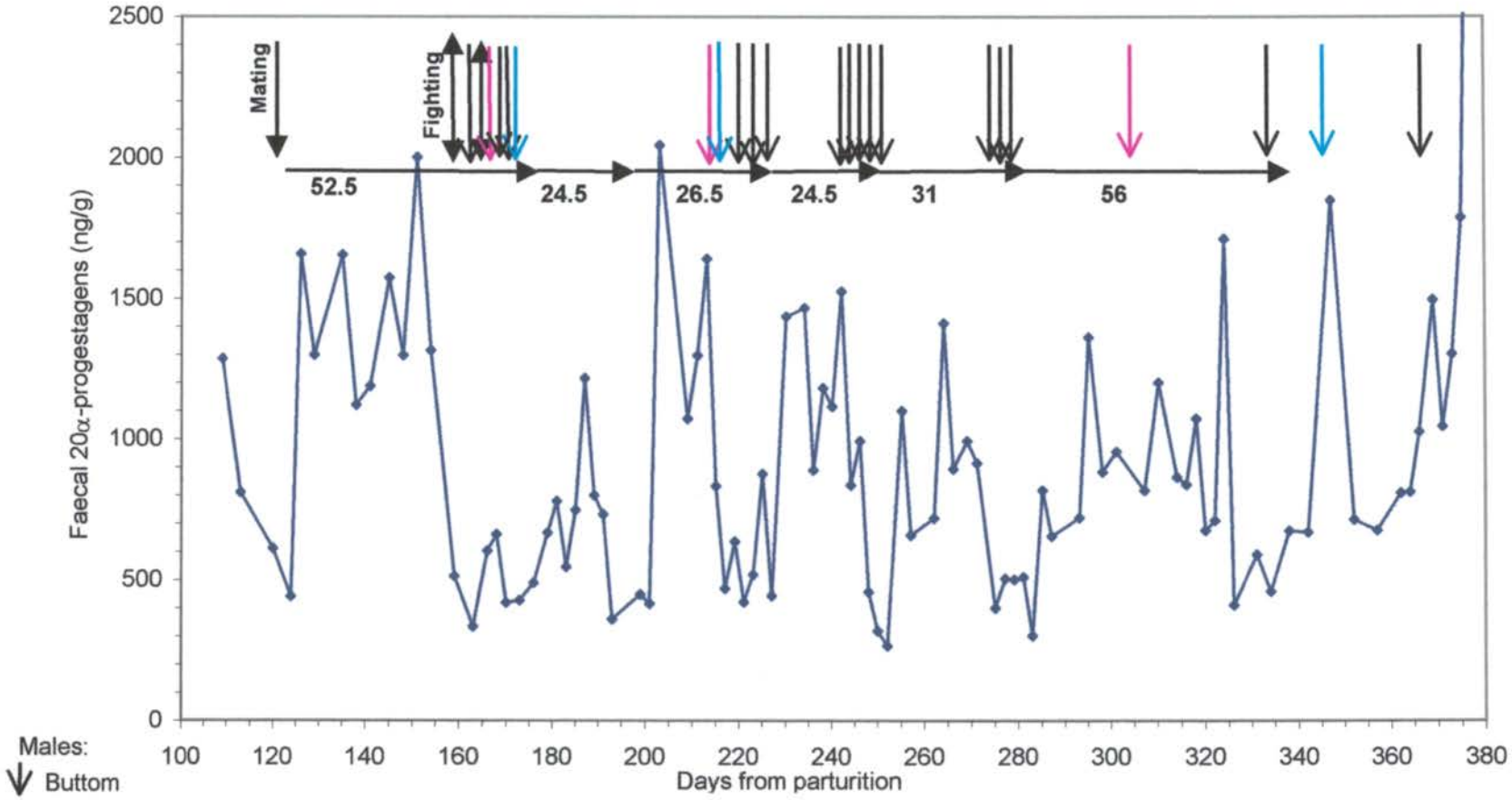


Figure 3.6. Concentrations of faecal 20α-progestagens in a wild black rhinoceros (Netsai) during reproductive cycles and early pregnancy. Vertical arrows indicate interactions with a male and horizontal arrows indicate cycle length. When more than one male interacted with the female, only one arrow (Buttom) is shown. Full arrowheads indicate mating and double arrowheads indicate fighting between males

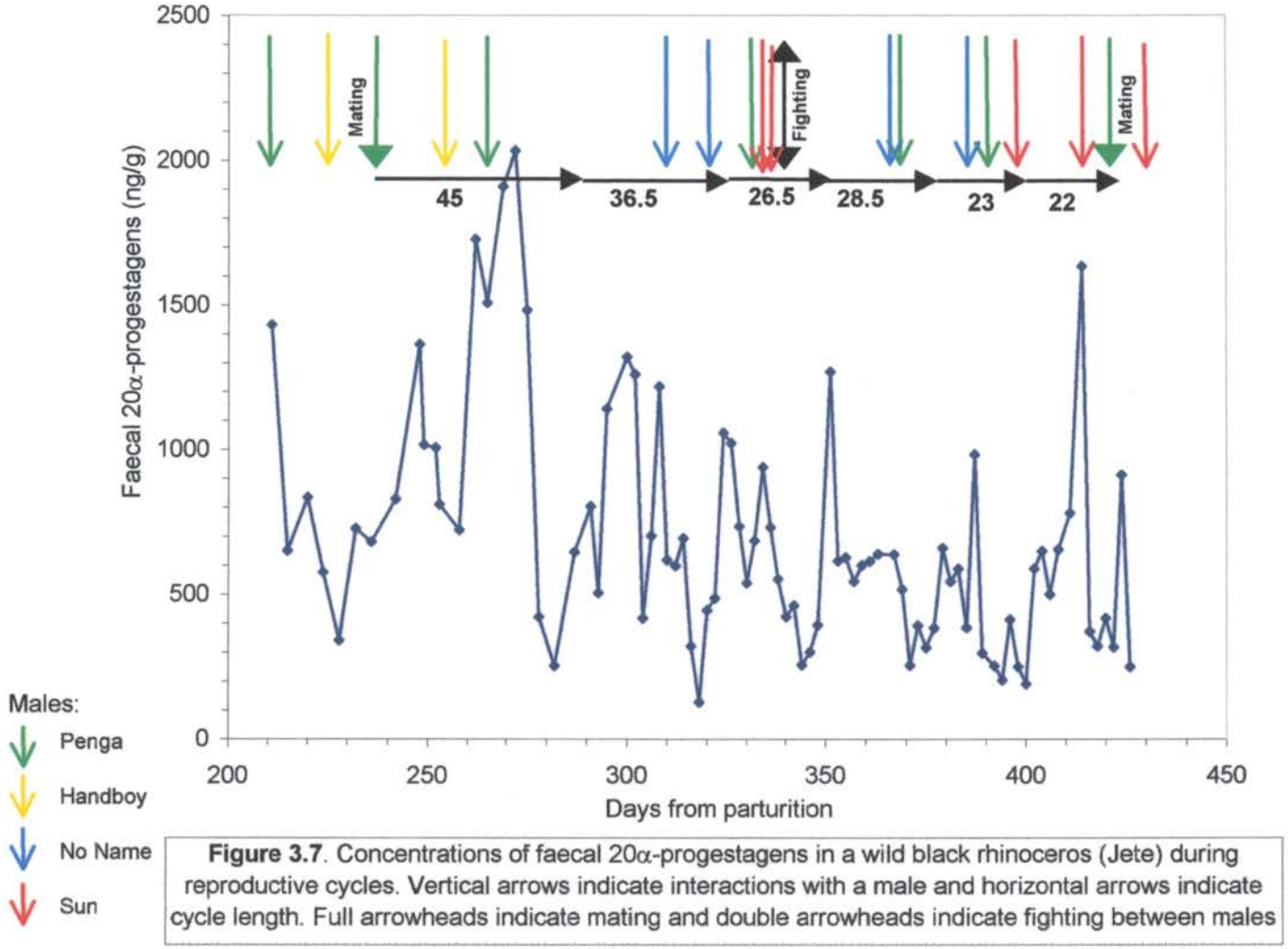


Figure 3.7. Concentrations of faecal 20α-progestagens in a wild black rhinoceros (Jete) during reproductive cycles. Vertical arrows indicate interactions with a male and horizontal arrows indicate cycle length. Full arrowheads indicate mating and double arrowheads indicate fighting between males

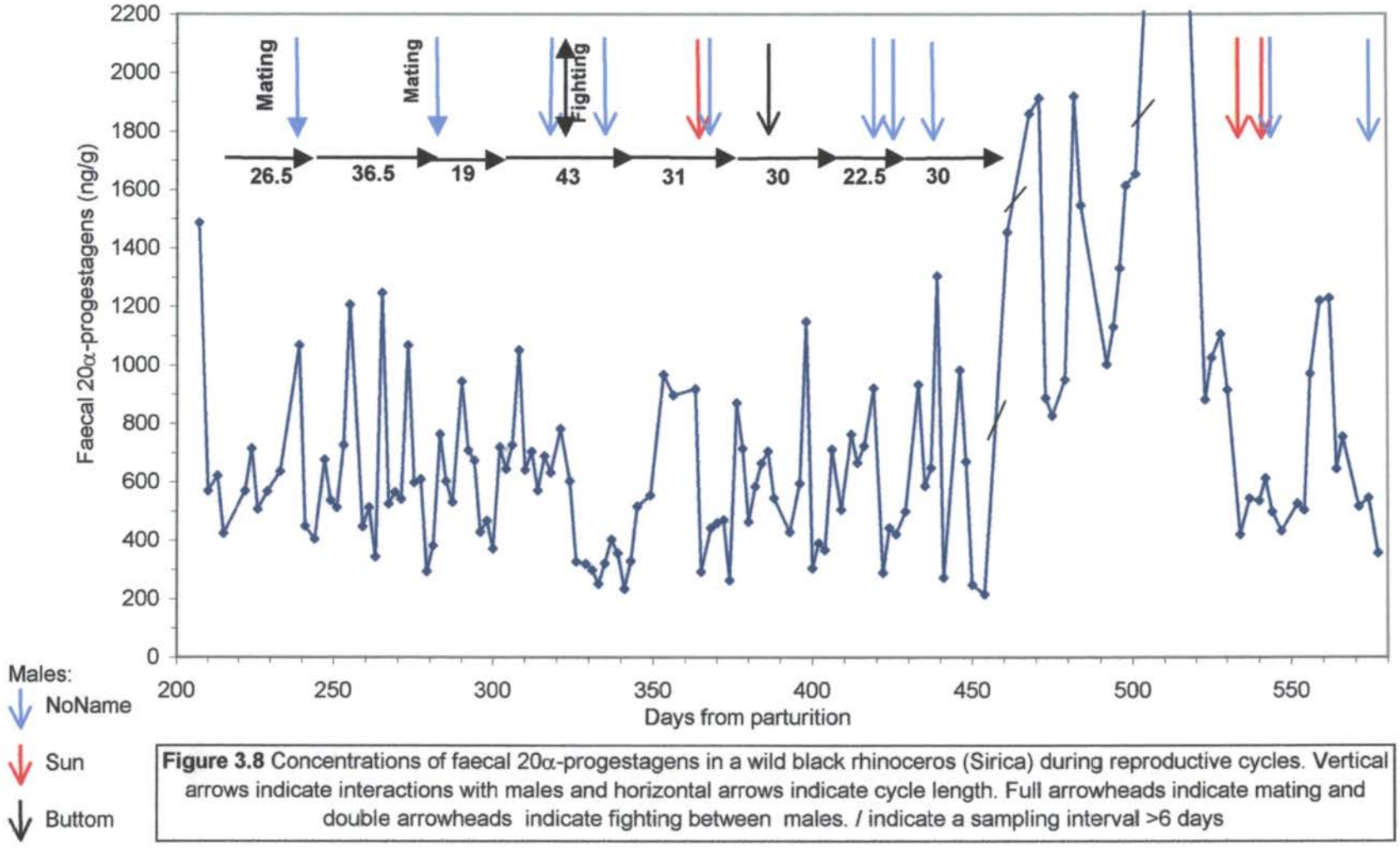


Figure 3.8 Concentrations of faecal 20α-progestagens in a wild black rhinoceros (Sirica) during reproductive cycles. Vertical arrows indicate interactions with males and horizontal arrows indicate cycle length. Full arrowheads indicate mating and double arrowheads indicate fighting between males. / indicate a sampling interval >6 days

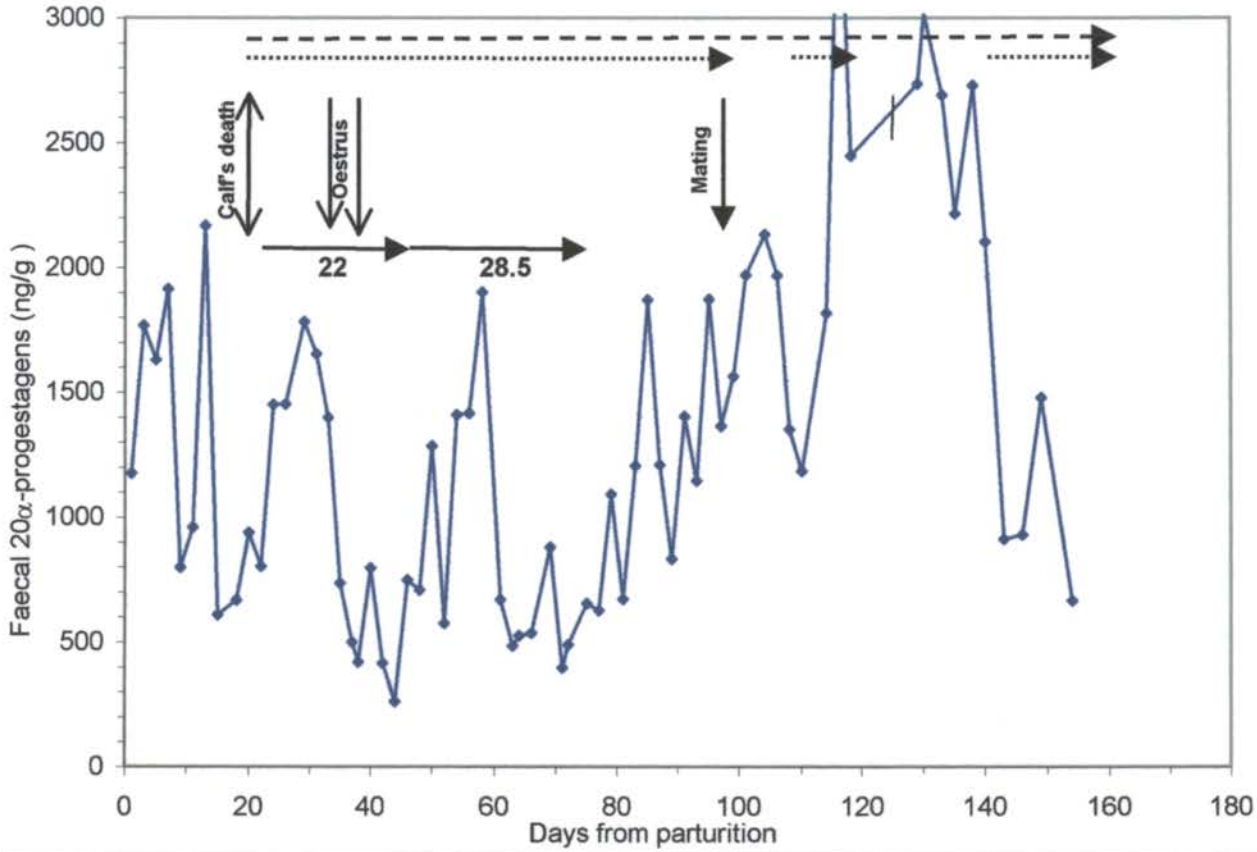


Figure 3.9. Concentrations of faecal 20α-progestagens in a wild black rhinoceros (Sara) during reproductive cycles and suspected abortion. Vertical arrows indicate oestrous behaviour and horizontal arrows indicate cycle length. Full arrowheads indicate mating and double arrowhead indicate the calf's death. Hatched horizontal arrows indicate the presence of her older calf (Monarch). Dotted horizontal arrows indicate the presence of an adult male (Muzamani) .
/ indicate a sampling interval > 6 days.

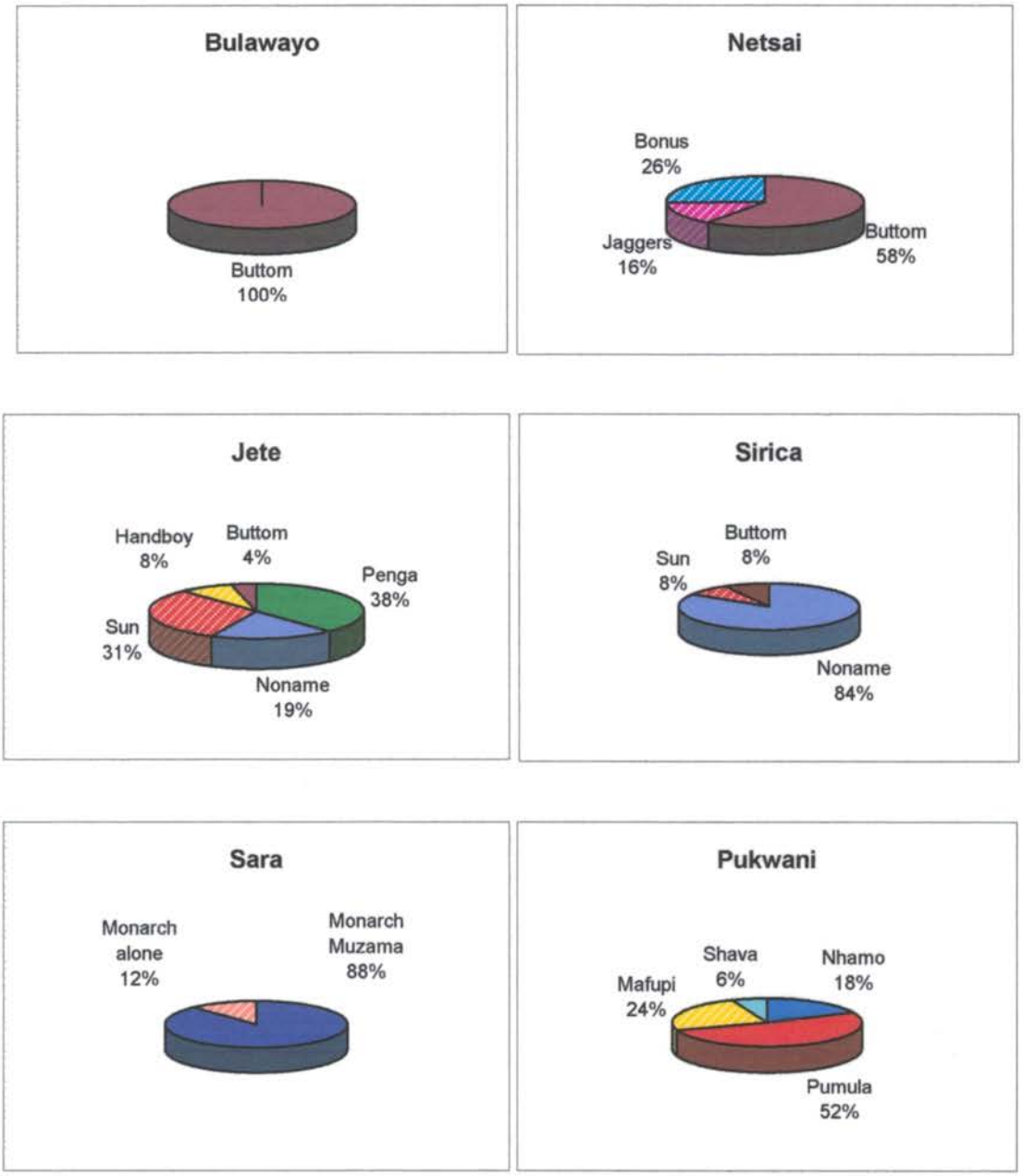


Figure 3.10. Consortship of black rhinoceros females studied in SVC with different males. Proportions used number of interactions with each male between parturition until faecal 20α-progestagen concentrations increased to levels > 2000 ng/g .
Only direct visual observations were taken into account.
Hatched areas indicate that males were subadults.

4 INFLUENCE OF ENVIRONMENTAL FACTORS ON FERTILITY IN BLACK RHINOCEROS

4.1 INTRODUCTION

We have demonstrated in the preceding chapter that wild females exhibited a period of cyclic ovarian activity that lasted between four and seven months and the onset of which occurred between four and seven months post-partum. The gestation period was determined to be around 15 months in most animals, with some lasting up to 16 months. Because of such a long gestation period, it has been difficult to evaluate whether the black rhinoceros presented a seasonal pattern of reproduction in previous studies (Goddard, 1967; Hall-Martin & Penzhorn, 1977; Hitchins & Anderson, 1983; Joubert & Eloff, 1971; Shenkel & Shenkel-Hulliger, 1969a). Since such knowledge is essential for captive breeding, we investigated the incidence of seasonal breeding in wild and semi-wild black rhinoceros, as well as in captive animals.

In addition, fertility parameters were evaluated for the wild black rhinoceros population in the southern section of the SVC, so that the population reproductive output could be related to individual performance determined in chapter 3. This population was presumed to be able to present optimum or near-to-optimum reproductive performances for this species, since it occupied at low density a very large area providing a high availability of suitable habitat (Caughley & Sinclair, 1994). Fertility parameters were also evaluated for the translocated black rhinoceros population in the northern section of the SVC. They were subsequently compared with those of the wild population, so that the effect of translocation on fertility parameters could be evaluated in this species. This is particularly necessary as translocations have

become a major component of black rhinoceros conservation strategies (Emslie & Brooks, 1999). Fertility parameters were also assessed for the captive population maintained in British institutions. They were then compared to those of the wild population, in order to determine the impact of captive management on breeding success of this species.

4.2 MATERIALS AND METHODS

4.2.1 Seasonality

Seasonality was assessed by: 1. Aligning the periods of cyclic ovarian activity and gestation with time of the year, 2. Assessing the relative proportion of reproductive cycles of Type I and II for each month and 3. Assessing the monthly distribution of births. The study included the same six wild females that had been monitored with faecal progestagen analysis in the preceding chapter. In addition, four semi-wild females (Amber, Mvu, DJ, Cuckoo) kept at Imire game ranch in Zimbabwe and six captive females (Rosie, Vuyu, Arusha, Naivasha, Rukwa and Esther) maintained in three British zoological institutions were also studied. The beginning of periods of cyclic activity was determined through results of faecal steroid analysis and behavioural observations for the six wild females, and through observations of behavioural oestrus in the four semi-wild and six captive females studied. On the basis of the findings of the preceding chapter, cycle lengths were categorised into two types: Type I had a cycle length ≤ 40 days and Type II > 40 days. The assigning of a cycle to each month used dates related to the onset of the luteal phase for cycles determined by faecal steroid analysis and dates related to the first signs of oestrous behaviour for cycles determined through behavioural observations.

The monthly distribution of births in the wild used all births recorded in the southern part of the SVC between March 1994 and March 1999 and which resulted from conception in the SVC, as well as the first births recorded from semi-wild nulliparous females at Imire. Birth dates recorded in the International Studbook for African rhinoceroses (Göltenboth, 1999) were used for the distribution of births in captivity. Only captive-born specimens of the eastern ecotype *Diceros bicornis michaeli* were considered, since the other ecotype (*Diceros bicornis minor)* was poorly represented in captivity. Cases of abortions were excluded, but not those of stillbirth or perinatal mortality. The proportions of births between different times of the year were compared using a Chi-Squared test with Yates' continuity correction.

4.2.2 Fertility parameters

Animals in each population were classified into different age classes that included adults, sub-adults and calves. Animals were considered to be adult if they were potentially or actually reproductively active. This age class included males older than 8 years of age and females older than 5 years of age, since the mean age at first conception was found to be 5 years for females born in the SVC (see section 4.4.1.3). However, if a female had given birth at a younger age, she was considered to have become adult at the time of conception, based on a 15 months gestation period. Subadults were those which had either separated permanently from their mother or were still with their mother but above 3.5 years of age. Calves were those under 3.5 years of age still living permanently with their mother.

The following fertility parameters were evaluated for a five-year period in each population:

- The **calving interval** represented the interval between two successive births. The observed calving interval (CIo) was measured after recording successive dates of birth, while the calculated calving interval (CIc) was measured from the calving rate by 1: Calf/Year/Female.
- The **conception rate** represented the proportion of oestrous cycles resulting in conception. The number of oestrous cycles was determined on the basis of behavioural data in captive females and on the basis of endocrine data in wild females.
- The **calf index** represented the proportion of adult females with a calf (Calf/Female).
- The **calving rate** represented the proportion of adult females giving birth each year (Calf/Year/Female).
- The **fecundity rate** was the number of calves born each year expressed as a proportion of the total population (Calf/Year/Population).
- The **recruitment rate** expressed the rate of increase of the population. It was determined according to the equation: $Nt=Noe^{rt}$ in which Nt is the population size at time t, No is the population size at time to, e is the base of natural logs and r is the exponential rate of increase (Caughley & Sinclair, 1994). In order to compare recruitment rates between populations with different sex ratios, the recruitment rate was adjusted to a hypothetical sex ratio. The actual sex ratio was determined for the middle of the period of analysis. If that sex ratio was biased towards males or females, either the number of surplus males was deduced from Nt and No or the number of surplus females was added to Nt and No respectively (R. Du Toit, personal communication).

- The **mortality rate** was the proportion of animals dying each year in the population.

Differences in sex ratios and fertility parameters (conception rate, calf index, calving rate and fecundity rate) between populations were tested for significance by using a Chi-Squared test with Yates' continuity correction. Differences between mean calving intervals and age at first parturition were tested with a Mann-Whitney test.

4.3 ASSESSMENT OF REPRODUCTIVE SEASONALITY

4.3.1 Wild and semi-wild females (Diceros bicornis minor)

4.3.1.1 Reproductive cycles in wild females

The periods of post-partum anoestrus, oestrous cycles and pregnancy described in six wild females in chapter 3 were aligned with months in Figure 4.1. In five females, oestrous cycling started between May and June, except for one female for which it started in September. A female which lost her three-week old calf in June resumed oestrous cycles a few days afterwards. Detected periods of oestrous cycles therefore lasted between four and seven months, except for the female whose calf died for which it only lasted one month.

In wild females which produced calves that remained alive, all conceptions (n=6) determined through faecal steroid analysis occurred between November and February, corresponding to the early rainy season and late spring-early summer. Three of these conceptions (50%) coincided with the month of November 1998 only. This synchrony in conception resulted in all females (except the two females which aborted) giving birth between March and May in the years 1998/99, i.e. during the late and post-rainy season. However, the observation of calving dates for the same females

at the beginning of the profiles of Figure 4.1 indicates that birth dates were more spread out in 1996/97.

4.3.1.2 Reproductive cycles in semi-wild females

Oestrous behaviour was recorded for the four females kept in Imire Game Ranch between August 1994 and March 1998 and periods of reproductive cycles are presented in Figure 4.2.

From September 1994 until the third week of March 1995, three females presented periods of more or less regular oestrous cycles. The range of oestrous cycle lengths was (18-83 days, n=5) for the female DJ, (23-76 days, n=4) for the female Mvu and (14-58 days, n=6) for the female Amber. The longer inter-oestrous intervals detected in each of these females during this period occurred in September/October.

From the third week of March until the end of June/early July, these three females did not show any signs of oestrous behaviour. These longer inter-oestrous intervals lasted between three and 3.5 months and were extremely synchronised between females. They began between 19/3 and 24/3 and ended between 25/6 and 12/7. Afterwards, these females resumed periods of regular cycles that lasted between five to eight months. All cycle lengths were of Type I and mean cycle lengths were 24 ± 2.5 days (range=14-32, n=6) for the female Amber, 31.9 ± 2.1 days (range=25-40, n=8) for the female DJ, and 27.4 ± 1.4 days (range, 23-33, n=7) for the female Mvu.

The females Mvu and Amber were last mated on 5/1/96 and 22/11/95 respectively. In 1997, they gave birth when they were 10.2 and 9.9 years, after a gestation period of 489 and 447 days respectively. They stayed away from the other animals until around seven-month post-partum. After this time, their calves were

removed from them in order to be hand-reared. Subsequently, they successfully bred nine days and two months after the calf's removal.

The female DJ presented another extended inter-oestrous interval of 96 days between 24/3/96 and 28/6/96. Afterwards, Type II cycles were observed between July and October, followed by two Type I cycles in October/November. In February 1998, she was estimated to be 10.9 years and gave birth 454 days after the last observation of mating.

For these three females, the time of conception occurred between the end of November and January, during the early rainy season, while parturition dates coincided with the late rainy season.

Another female (Cuckoo) gave birth to her first calf in July 1994 when she was 7.3 years. The calf was accidentally drowned in a water pan at three months of age. Six days after the calf's death, the mother was mated and she subsequently gave birth after a gestation period of 466 days. She stayed away from the other black rhinoceroses during 5.5 months post-partum. The calf was then separated from her and she returned to oestrus five days later, when she was successfully mated. She subsequently gave birth 487 days after presumed conception.

The mean age at first parturition for these four semi-wild females was 9.5 years.

4.3.1.3 Distribution of cycle lengths

The monthly distribution of cycle lengths determined by faecal progestagen analysis in wild females in chapter 3 is presented in Figure 4.3.a). It can be seen that Type I cycles were recorded between May and February. Among Type II cycles, those distributed in November-January corresponded to the early pregnancy phase. Other

Type II cycles occurred either in May/June (Cycle 1 in Jete and Netsai) or in September/October (Cycle 1 in Bulawayo, cycle 4 in Pukwani and Sirica).

The monthly distribution of cycle lengths in semi-wild nulliparous females (Figure 4.3.b.) showed that Type I cycles occurred from June to February, but predominated in August and in November-February. Inter-oestrous intervals of around 100 days occurred in March while others of approximately 80 days were detected in each female in October.

4.3.1.4 Distribution of births

The monthly distribution of births (n=21) recorded in Zimbabwe from wild and semi-wild females is presented in Figure 4.3.c. The proportion of births recorded in December-May (71%) was significantly higher that in June-Nov (χ^2=3.85, P=0.05). More births (62%) in fact occurred between February and May, coinciding with the late rainy season. Considering a gestation period of 15 to 16 months, this reflects a conception peak between November and February, i.e. in the early rainy season. Some births were also recorded in mid-winter in July and August, half of which were observed in nulliparous females.

4.3.2 Captive females (Diceros bicornis michaeli)

4.3.2.1 Reproductive cycles

The different periods of reproductive activity in six captive females are presented in Figure 4.4.

4.3.2.1.1 Nulliparous females

In three nulliparous females, periods of oestrous cycling lasted between 12 and at least 36 months. The longer period of 36 months was observed in the female Rosie,

but it might have lasted nearly four years since periods during which no oestrus was recorded coincided with times during which the male did not have access to the female. Between February 1994 and December 1997, the mean cycle length was 29.2 ± 2.3 days (range, 12-82 days, *n*=37). Faecal progestagen analysis undertaken between May 1996 and March 1997 showed that the female had regular cyclic activity during that period (see Figure 2.3). However, the analysis of faecal samples after November 1997 showed very low levels of faecal progestagens, indicating that she had stopped cycling.

Another nulliparous female (Arusha) exhibited an extended period of oestrous cycles lasting 18 months after September 1996. Detailed records of oestrous dates were only undertaken at the beginning of this period and indicated an average cycle length of 28.2 ± 4.7 days (range, 23-47 days, *n*=5). Regular oestrous cycling resumed after the female was introduced to a different male and successful breeding occurred while she was also managed with another cycling female that was her mother.

The other captive nulliparous female (Vuyu) exhibited regular oestrous cycles for 12 months before she successfully conceived. The first signs of regular behavioural oestrous were recorded four months after she was introduced to a young pair. Between August 1996 and August 1997, the mean cycle length was 33.5 ± 5.6 days (range, 19-78, *n*=11). The last observation of mating activity was noted in August 1997, i.e. 468 days before the delivery of a newborn calf.

4.3.2.1.2 Multiparous females

Periods of oestrous cycling lasted between five and 14 months in captive multiparous black rhinoceros (Figure 4.4). The shortest period of five months was observed in a female (Naivasha) after she had a stillborn calf in 1997, following a

gestation length of 467 days. One month after the stillbirth, the female was introduced to another pair with a cycling female. Prior to the gestation period, the female had exhibited a period of oestrous cycles between May 1995 and February 1996, during which the mean cycle length was 23.4 ± 3.4 days (range, 10-58 days, n=12). After six months of regular mating by a male that was a proven breeder, the female was introduced to another male and successfully bred with him.

The other multiparous female (Rukwa) exhibited oestrous cycles for at least six months between March 1995, when the recording of behavioural activity started, and September 1995. The mean oestrous cycle length was 33.8 ± 7.8 days (range, 14-62, n=7). She gave birth 462 days after the last observation mating activity and became aggressive towards the male four months after conception.

A period of 14 months of very regular oestrous cycles was observed in another female (Esther), during which the mean cycle length was 28 ± 0.8 days (range, 23-34, n=14). She did not always have access to a male during the whole period but bred with a male from a younger pair, while she also fought with the older male that had traditionally been managed with her.

4.3.2.2 Distribution of cycle lengths

In nulliparous females (Figure 4.5.a.), inter-oestrous intervals of Type I were detected in all months of the year and represented 78 % of all cycles. Each nulliparous female exhibited an extended inter-oestrous interval of 80-100 days between late October/November and late January (In the female Arusha, such interval does not appear on Figure 4.4 as it was detected prior to the period shown on this figure). However, these intervals were found to be shorter in each female the following years, while they could also be preceded and followed by other extended inter-oestrous

intervals of 40-50 days. Other prolonged inter-oestrous intervals were detected in each female between March and July.

Multiparous females also exhibited Type I inter-oestrous intervals in all months of the year (Figure 4.5.b). The proportion of Type II cycles was only 6%, which was significantly less than in nulliparous females (χ^2=4.36, P=0.03). One female (Esther) exhibited no prolonged inter-oestrus intervals while the other multiparous females presented a Type II cycle of ca. 60 days only in May and July.

4.3.2.3 Distribution of births

The monthly distribution of 290 births recorded in captive institutions is presented in Figure 4.5.c. Parturition dates were distributed every month of the year, reflecting the distribution of Type I cycles all year round. Few parturition dates were recorded during the months of April and September, indicating that few successful conceptions occurred in January and in June. Excluding the months of April and September (which appeared anomalous), the proportion of births recorded during the first half of the year (39%) was significantly lower than during the second half of the year (χ^2=1.5, P=0.0004).

4.4 FERTILITY PARAMETERS

4.4.1 Wild population

4.4.1.1 Population number and structure

The population located in the southern section of the Conservancy consisted of 28 animals in 1994, which increased to 43 animals in 1999. During the five-year period, 16 calves were born in this population, including one from the female translocated in 1993 which had been conceived prior to translocation. Two males were introduced in 1994 and three animals died.

Between 1994 and 1999, the proportion of adults averaged 56% and the mean adult sex ratio was 1.2:1 (Table 4.1), which did not differ from parity (χ^2=1.37, P=0.28). It increased from 1.1:1 in 1994 to 1.6:1 in 1999, due to the preponderance of males being born until 1992. Between 1986 and 1992, the sex ratio of 11 calves born was 2.7:1 while between 1993 and 1998, the sex ratio at birth was of 0.8:1. Although there was no statistical difference between the sex ratio of calves born between these two periods (χ^2=1.2, P=0.27), the discrepancy between the number of males and females born until 1992 increased the proportion of males in this population. In 1999, there were approximately three adult males for two adult females.

4.4.1.2 Sexual maturity

4.4.1.2.1 Male sexual maturity

It could not be determined in the study when males first successfully bred. However, a change in ranging pattern was observed in two males during the study. One male left his original area, where he had been since birth and where his mother still was when he was seven years old in 1997. He joined another area with animals with which he had never been seen to interact before. Similarly, another male also moved from his maternal area when eight years old.

4.4.1.2.2 Age at first parturition

Three heifers gave birth during the study in the southern part of the Conservancy. One female which had been translocated with her mother in 1993 to the SVC was successfully fertilised at 41 months (3.4 years). At that time, she still interacted considerably with her mother and shared most of her home range. Excluding her, the mean age at first parturition for females born in SVC was of 6.2 years, giving a mean age at first conception of 5 years (Table 4.1).

4.4.1.3 Calving interval

The mean calving interval for eight females was 28 months (n=10) (Table 4.1). This excluded the calving interval of 47 months observed in the translocated female that became resident in the south of SVC. The shorter interval of 23 months was observed in two females which had overlapping home ranges. A young female exhibited the longer interval of 38 months between the birth of her first calf and second calf, while a 35-year old female also showed a longer interval between successive births of 33 months.

4.4.1.4 Calf index (Calf/Female)

The proportion of adult females accompanied by a calf averaged 76% between 1994 and 1999 (Table 4.1). In 1996, adult females without calves included one female that was still with her seven-year-old sub-adult calf and two heifers that were above five years of age but only gave birth the following year. In 1999, females without calves included the same animal as above that had finally separated from her eight-year old sub-adult calf, a young female which had lost her newborn calf, as well as a nulliparous female. The maximum calf to cow ratio was observed in 1996/97, after seven females gave birth the previous year (Figure 4.6.a).

4.4.1.5 Calving rate (Calf/Year/Female)

The mean calving rate was 35.4% (Table 4.1), suggesting that each female gave birth at an average of once every 34 months. The calving rate presented great annual variations, due to the fact that up to 90% of adult females could give birth the same year (Figure 4.6.a).

4.4.1.6 Conception rate

The mean proportion of cycles that resulted in conception, detected through faecal progestagen analysis, was 20% in five females whose calves survived after birth (Table 4.1). The conception rate ranged from 14.3% in an old female who subsequently aborted, to 50% in a female which only had a 23 months calving interval. Out of six conceptions determined by faecal analysis, two resulted in abortion.

4.4.1.7 Fecundity rate (Calf/Year/Population)

The mean fecundity rate was 9.5% (Table 4.1). It also showed great annual variations that reflected the fluctuations in calving rates (Figure 4.6.a).

4.4.1.8 Recruitment rate

The recruitment rate for the five-year period, excluding the two animals that had been introduced during the study, was 7.2% (Table 4.1). It remained the same when the population structure was corrected to a hypothetical equal sex ratio.

The mean annual mortality rate was 1.6%. Animals that died included a 37-year old male, for which the post-mortem examination revealed that he died of malnutrition and old age. A 25-year old female died in 1997 and the sudden character of her death, associated with the presence of necrotic lesion in the lower abdomen, led to suspect that she died of a snake bite. A three-week old calf was killed by lions.

4.4.2 Translocated population

4.4.2.1 Population structure

The population originated from 12 animals translocated in 1993 and it increased to 20 animals in 1999. The mean proportion of adults (56.2%) was very

similar to that in the wild population (Table 4.1). However, the adult sex ratio was strongly biased towards females (χ^2=7.84, P=0.005), with approximately one male for two females in the adult age class.

4.4.2.2 Sexual maturity

The mean age at first parturition for translocated females was 6.2 years of age, similar to that of wild females (Table 4.1). Great individual variations were observed. Three females that had been translocated with their mothers, including one in the south of SVC, produced their first calves before 5.5 years and therefore conceived at an average of just below 4 years. Another female produced her first calf at only 9.3 years of age, nearly four-years after translocation. She was translocated without her mother, was in a poor condition and was released after the main group of animals.

4.4.2.3 Calving interval

The mean calving interval for translocated females was 47 months, which was significantly longer than for non-translocated wild females (*Mann-WhitneyU*=1.5, P=0.009) (Table 4.1). Amongst these females, two were three and six months pregnant at the time of translocation and one conceived four months after translocation. Translocated females (n=5) had a mean lag of 42 months (3.5 years) between translocation and parturition.

4.4.2.4 Calf index (Calf/Female)

The mean calf/cow ratio was 62.7% (Table 4.1). It did not differ significantly (χ^2=1.1, P=0.29) from that observed in the wild population. Only two adult females out of five were accompanied by small calves one year after

translocation (Figure 4.6.b). Females without calves included two multiparous females that were both accompanied by sub-adults of ca. 3.7 years, as well as one nulliparous female. In 1999, this heifer still had not produced a calf while another female which had given birth in 1995 stopped breeding afterwards. Faecal progestagen analysis on one sample collected from this female 20 months after parturition suggested that she was pregnant then (Faecal 20α-progestagens concentration=8720 ng/g).

4.4.2.5 Calving rate (Calf/Year/Female)

The mean calving rate was 30.1% (Table 4.1), which did not differ significantly from the wild population (χ^2=0.03, P=0.85). This rate suggested that each female gave birth approximately every 40 months. The calving rate showed a progressive decrease from 40% two years after translocation to 17%, where it remained until five years following translocation (Figure 4.6.b).

4.4.2.6 Fecundity rate (Calf/Year/Population)

The mean fecundity rate was 12.6%, which compared favourably with that of the wild population (χ^2=0.33, P=0.57) (Table 4.1). Great annual variations were observed, with a maximum being observed five years after translocation (Figure 4.6.b).

4.4.2.7 Recruitment rate

During the period 1994/99, the translocated population showed an annual growth rate of 8.6%. The recruitment decreased to 6.9% when the population structure was corrected to an equal sex ratio. The carcass of an adult male was found in May 1994 and the cause of mortality was attributed to fighting (R. du Toit, personal

communication). The annual mortality rate during this period was of 1.5%, while post-release mortality was determined to be 8.3%.

4.4.3 Captive population

4.4.3.1 Population structure

The captive black rhinoceros population held in the UK nearly doubled in numbers in five years, increasing from 16 animals in 1995 to 29 animals in 2000. During this period, 11 births were recorded, one of which (9%) was stillborn. Four females were introduced either from European institutions or from South Africa. Some adult males were moved between institutions and one adult male was also transferred outside the UK in 1998 (see chapter 2).

The population structure was characterised by a higher proportion of adults (69.2%) compared to the wild population (χ^2=4.85, P=0.02) (Table 4.1).

The mean adult sex ratio was of one male to two females, being very similar to the sex ratio of the translocated population studied in the northern section of the SVC. It also differed from parity (χ^2=7.84, P=0.005). Amongst ten calves born (excluding the stillbirth) in 1995/2000, the sex ratio at birth was 0.43:1.

4.4.3.2 Sexual maturity

4.4.3.2.1 Male sexual maturity

A captive male held in Chester Zoo successfully fertilised a female when he was 65 months old (5.4 years). Another male in London Zoo was observed to start mating regularly the female when he was nearly 5 years of age, but they subsequently failed to breed.

4.4.3.2.2 Age at first parturition

The mean age at first parturition in captivity was determined to be 103 months (8.6 years) (Table 4.1). However, when the female that produced her first offspring at only 16 years of age was excluded, the mean age at first parturition decreased to 82 months (6.8 years). This suggests that captive females first conceived at an average of 67 months (5.6 years), which is very close to the age at first conception observed in the wild population.

The four females that reached sexual maturity between six and eight years included three females in Port Lympne and one in Chester Zoo. Both institutions managed more than a single pair of black rhinoceros. Two females (Etna and Vuyu) exhibited the first signs of reproductive activity a few months after they had been managed together with a male. They subsequently bred successfully one year after the onset of cyclic reproductive activity. Another female (Nakuru) which also first conceived at an early age (5.8 years) had been managed with another adult female on the two instances that she was successfully mated. The other female (Pangani) first conceived at 6.5 years with a young male that had also access intermittently to an older female.

The female (Arusha) which first gave birth at 16 years in 1999 had started to cycle at 8 years of age but subsequently developed irregular oestrous cycles and increased aggressiveness. The resumption of regular cyclic activity was observed after she was managed with a different male and successful breeding occurred when another adult female (her mother) was also managed with them.

4.4.3.3 Calving interval

The calving intervals recorded in captivity averaged 42 months, which was not significantly higher than for the wild population (Mann-Whitney U=16, P=0.09)

(Table 4.1). The shortest interval of 20 months occurred after one female had a stillbirth. The next shorter interval was of 37 months, while the longest calving interval of nearly five years occurred in one female that presented on-going feet problems necessitating her being isolated from males.

4.4.3.4 Calf index (Calf/Female)

An average calf index of 28.5% was determined in captivity, being significantly lower than for the wild population (χ^2=20.5, P<0.001) (Table 4.1). Great annual variations were observed since no females gave birth before 1997 (Figure 4.6.c). On 1.1.2000, four adult females were still without calves. They included three nulliparous females of between 7 and 11 years, two of which had been hand-reared and managed with a single male since they were very young, as well as a 27-year old female which had been brought from Rome in 1998.

4.4.3.5 Calving rate (Calf/Year/Female)

The mean calving rate was 21.7% and did not differ significantly from that calculated in the wild population (χ^2=1.22, P=0.27) (Table 4.1). This rate suggests that each female gave birth approximately every 54 months (4.5 years), which is one year longer than the observed calving interval for that population. The calving rate increased from nil the first year to 36% the fifth year, when four of the 11 adult females gave birth (Figure 4.6.c).

4.4.3.6 Conception rate

The conception rate was calculated for three captive females (Esther, Naivasha, Vuyu) for which inter-oestrous intervals could be recorded during whole

periods of management with males. The mean conception rate of 8.1% did not differ significantly from that of the wild population (χ^2=0.97, P=0.32).

4.4.3.7 Fecundity rate (Calf/Year/Population)

The captive population presented a mean fecundity rate of 10.4% (Table 4.1), with great annual variations reflecting those of the calving rate (Figure 4.6.c). It did not differ significantly from the mean fecundity rate measured in the wild population (χ^2=0.05, P=0.82).

4.4.3.8 Recruitment rate

Between 1995/2000, the captive population held in British institutions presented an annual growth rate of 5.2% (Table 4.1). It decreased to 4% when the population structure was corrected for an equal sex ratio. The annual mortality rate was of only 1% during that period, consisting in neonatal mortality (stillbirth). The loss of one calf amongst 11 that were born during the study indicates a neonatal mortality of 9%.

4.5 DISCUSSION

4.5.1 Breeding season in wild black rhinoceros

The analysis of the timing of reproductive cycles and gestation periods in females in Zimbabwe indicated that nearly three quarters of births occurred between December and May, coinciding with the rainy and post-rainy season in this area. Birth dates were more concentrated between February and May (62%), suggesting that more successful fertilisations took place between November and February. This was further confirmed by the finding that three out of six conceptions determined through faecal

progestagen measurement in wild females were found to have taken place in November.

Although the number of births was limited in this study, the results are partly in agreement with those of Hitchins & Anderson (1983), who studied the monthly distribution of 128 births of the same subspecies (*Diceros bicornis minor*) in South Africa. The authors recorded one calving peak during the rainy season and 65% of matings between October and December. Another study of a black rhinoceros population of the eastern ecotype (*Diceros bicornis michaeli*) in Addo National Park in South Africa also identified a parturition peak in the wettest months (December/March), during which 66% of births were recorded (Hall-Martin, 1986; Hall-Martin & Penzhorn, 1977). Similarly, a calving peak was detected during the rainy and post-rainy season in a population of black rhinoceros of the south-western ecotype (*Diceros bicornis bicornis*) that occurs in Namibia (Joubert & Eloff, 1971).

It therefore appears that black rhinoceros females in southern Africa, under tropical latitudes, tend to drop most of their calves around the time of the rainy season. This coincides with the most favourable time of the year for the nutrition of the late pregnant and early lactating female, when vegetation growth that follows rainfall provides the most suitable nutritional environment to these demanding periods.

It has also been established in the study that in multiparous females whose calves survived, periods of oestrous cycles lasted between four to six months. These started around May/June and ended between November and February, depending on when conception took place. Furthermore, three nulliparous semi-wild females presented a similar timing for periods of behavioural oestrous cycles, which occurred between the end of June until the third week of March. It can thus be presumed that

this species presents a breeding season between May/June and March under this latitude.

During the period of reproductive activity identified in wild females, the monthly distribution of cycle lengths showed that Type II cycles (apart from those identified during the early gestation period) occurred between May and October. Those detected in May/June corresponded to the first cycles identified during the breeding season in two females. Whether they were associated with early embryo loss, a persistent corpus luteum, or ovulation during dioestrus, as seen in chapter 3 is not known. However, their occurrence is suggestive of lowered reproductive success during this period. This is in turn reflected by the low parturition incidence observed in August/September. Other extended cycle lengths identified in wild females were observed to take place in September/October and are probably associated with the high temperatures during these months, as also discussed in the preceding chapter. This observation is corroborated by the fact that long inter-oestrous intervals of ca.70-80 days were also noted in each semi-wild female in October 1994 and by the low number of births detected in December/January.

4.5.2 Anoestrus in wild and semi-wild black rhinoceros

In Zimbabwe, three nulliparous females showed a three-month inter-oestrous interval whose onset was extremely synchronised, since it started between 19-24/3 and ended between 25/6 and 12/7. These patterns were established from a small number of animals over only three years. However, their precise timing from one year to another, combined with their synchronisation between animals, suggests the occurrence of a seasonal anoestrus between April and June in nulliparous females. It could not be determined whether the multiparous wild females also exhibited this seasonal

anoestrus, since at that time the females were either pregnant or during a phase of post-partum anoestrus.

The onset of this anoestrus period coincided with the southern hemisphere autumnal equinox (21/3) and its termination corresponded to the winter solstice (21/6), indicating that it occurred during a period of decreasing day lengths. It can therefore be suggested that photoperiodicity contributes to the occurrence of a seasonal anoestrus in nulliparous females.

Although previous studies of the distribution of births in wild black rhinoceros identified a certain synchrony in the timing of parturition, as has been discussed above, this is the first time that a seasonal pattern of activity, as well an anoestrous period, have been identified in this species.

The seasonal pattern of reproductive activity identified in black rhinoceros females in Zimbabwe is similar to that occurring in equids, which exhibit a period of reproductive quiescence during periods of decreasing day lengths and a breeding season during spring and summer (Sharp *et al.*, 1997). In the southern hemisphere, fertility levels in mares were found to be minimal between April and June (Hafez, 1993). This period coincides precisely with the period of anoestrus detected in nulliparous black rhinoceros females. In multiparous females, no behavioural oestrus was recorded during this time but the pattern of faecal progestagen excretion suggested that some ovarian activity occurred during this time of the year. Faecal progestagen levels showed some important fluctuations and presented a higher mean background concentration during this period compared to the breeding season. This contrasts with the seasonal anoestrus of equids, which reflects a time of complete reproductive incompetence and which is marked by the absence of ovarian steroids in the circulation (Sharp *et al.*, 1997).

It was also established that conception was possible after the post-partum anoestrus had been shortened by the death or removal of the calf. Oestrous cycles could resume within two days of the loss of the calf and breeding could be successful during the first oestrus. This may contribute to explain why some conceptions took place between March and May, as indicated by the detection of births between June and August in wild females. Interestingly, Hitchins & Anderson (1983) identified a marked parturition peak between June and August, reflecting an important conception peak between March and May. This conception peak might have been associated with the resumption of cyclic ovarian activity following neonate mortality, which was suspected to be very high (69%) in the study area.

Furthermore, one wild female was observed to interact with males during the months of March/April and it is likely that individual differences exist between animals with respect to the length of the anoestrous period, as observed in equids. Some mares have been found to continue to have cyclic activity throughout the year, while others might exhibit a six months anoestrous period (Daels *et al.*, 1991). However, the low incidence of parturition in wild black rhinoceros detected between March and May indicated that reproductive activity was much lower during this period compared to the breeding season.

4.5.3 Seasonality in captivity

It has been demonstrated that captive black rhinoceros females exhibited oestrous cycles each month of the year and also gave birth throughout the year. More animals were found to produce offspring during the months of August, and from October to December, compared to first half of the year. This is similar to the results of Rookmaker (1998), who used the same database but did not mention which species was considered.

The distribution of births in captive black rhinoceros could have been affected by management techniques, since most captive institutions usually restrict the periods of contact between males and females to periods of detected oestrus. In addition, they also preferably let out the animals together in outside paddocks during the spring and summer months. This could have explained the higher incidence of births during the second half of the year. However, the nadir in births recorded in September is unlikely to have been caused by management techniques, since animals usually start to go out and increase contacts during the spring. The lower incidence of births detected during September is therefore most likely linked to higher reproductive failure during the month of June. It is not known whether this can be attributed to a failure to conceive, from either the female or/and the male, or to a failure to carry the gestation to term.

Prolonged inter-oestrous intervals were observed between May and July in both multiparous and nulliparous females, while a change in mean background faecal progestagen concentrations was detected in June in a captive heifer. This period may therefore reflect a seasonal transition associated with a change in photoperiod.

Three captive heifers presented extended inter-oestrous intervals of around three months between November and February. Some multiparous females equally exhibited prolonged inter-oestrous intervals during this period, although they were shorter than in heifers. Lindemann (1982) had previously reported that a captive black rhinoceros female which had been cycling regularly between May and December suspended cycling activity from December to March. It is thus likely that these extended inter-oestrous intervals corresponded to periods of anoestrus, as was observed in the semi-wild nulliparous females. Although the starting dates and finishing dates of these anoestrous periods were less synchronous and timed in captivity than in Zimbabwe, they nevertheless corresponded to a period of reduced

day lengths. They started between late October and November and ended between late January and early March. This resembles the winter anoestrus observed in domestic equids in the northern hemisphere, which lasts between one and four months (Sharp *et al.*, 1997). The seasonal character of the anoestrus period identified in the present study is reinforced by the observation of irregular inter-oestrous intervals as soon as October in some nulliparous females. Interestingly, these anoestrous periods became shorter with years in nulliparous and could disappear in multiparous females.

4.5.4 Population structure and composition

The composition and sex ratio of the wild population studied in the southern section of the Save Valley Conservancy compares favourably with that of healthy natural black rhinoceros populations that previously occurred in East Africa (Goddard, 1967, 1970a; Mukinyia, 1973).

By contrast, the translocated and the captive populations examined in the study presented a biased adult sex ratio towards females, with a proportion of around two females per male. This resulted from a deliberate choice to establish breeding nuclei with a majority of females, as had previously been done in some sanctuaries in Kenya (Brett, 1998). The translocated population had an age composition that was similar to that of the wild population, reflecting comparable breeding performances of females since the proportion of calves was even greater in the translocated population. However, the captive population had a greater proportion of adults and a smaller calf age class that were also reflected in the reduced growth rate.

It must also be noticed that in the three populations studied, the sub-adult age class had more males than females, especially in the wild population. This was largely due to male births outnumbering female births during the first ten years following the establishment of this population in 1986/88. A comparable phenomenon

was noted after the establishment of new populations in South Africa (Bride *et al.*, 1996) but Brett (1998) noted that in Kenya, the sex ratio of calves born from translocated females was biased towards males in certain places, but also towards females in other areas.

4.5.5 Sexual maturity

Wild females were found to be first able to conceive at a very young age, since one heifer was first successfully fertilised when only 3.4 years old. This represents the earliest age recorded for sexual maturity in wild black rhinoceros, since the youngest age previously reported was 3.8 years (Goddard, 1970b). The mean age at first conception in wild females in the study was found to be 5 years, coinciding with anecdotal reports in wild and natural black rhinoceros populations in East Africa (Goddard, 1970a, Shenkel & Shenkel-Hulliger, 1969a).

By contrast, other studies in southern Africa indicated that black rhinoceros females produced their first offspring after eight or ten years of age (Hall-Martin, 1986; Hitchins & Anderson, 1983). The authors suggested that delayed puberty in these females was associated with the unusually high-density populations of these two areas. It can thus be supposed that the unusually low density of black rhinoceros in the study area could have contributed to the attaining of puberty at a very early age in most females. This is reinforced by the fact that the mean age at first calving in white rhinoceros was found to differ by around three years between high and low density populations (Rachlow & Berger, 1998).

Its was also established in the present study that females translocated to an area could produce their first calves at a similar age as the females resident and born in the same area. In fact, the three females that were translocated with their mothers to the Conservancy first conceived just before they were four years old. This result

contrasts markedly with other studies on translocated populations, which report a mean age at first calving of 7.5 years and 8.7 years (Adcock, 1996; Brett, 1998). Brett (1998) suggested that stress and lowered nutritional status of the animals, together with a disruption of social bonds between males and females, could have had some effects on delaying puberty. The disparity between Brett's results and ours may also be linked to the low density of black rhinoceros in the Conservancy. In addition, the fact that most females were translocated with their mothers may also have contributed to their attaining of sexual maturity at a young age (see below).

Semi-wild females produced their first offspring later than in the wild, at a mean age of 9.5 years, but with great individual differences. One semi-wild female first conceived at around six years while the three other females that were the same age were only successfully fertilised between 2.5 and 3.5 years later. These three females had however started to develop regular oestrous cycles up to two years before successful conception took place, showing that the process of sexual maturity is a progressive one). It is therefore probable that some extended and irregular cycle lengths observed in heifers are associated with the acquiring of reproductive competence, since the process of puberty represents a gradual maturation within the hypothalamic-hypophyseal-ovarian axis to produce specific hormones and to respond to hormonal signals to each other (Evans *et al.*, 1997). It is however also possible that some extended inter-oestrous intervals detected in January in two semi-wild females were associated with early embryo loss, since the most favourable time for conception had been identified to be the early rainy season for black rhinoceros in Zimbabwe.

Captive black rhinoceros females were found to produce their first offspring later than in the wild, at an average age of 8.6 years. This result compares with reports from other studies in captive animals (Lindemann, 1982; Smith & Read, 1992).

However, most captive females in this study first gave birth between 6 and 8 years old, which is only just slightly later than in wild females. By contrast, three captive females (25% of adult females) showed an important delay in producing their first calves, despite the fact that behavioural observations in these animals suggested that some cyclic ovarian activity had been initiated much earlier.

The environment in which a calf is reared is known to affect age at puberty in domestic animals and may possibly explain the differences in age at first conception observed in the study. Photoperiod is unlikely to have played a role since both wild and semi-wild females were exposed to the same photoperiod. Similarly, photoperiodic variations were also identical for captive females that were all maintained in the UK. The nutritional status of females is also known to affect the process of puberty in mares, ewes and cows (Ferrell, 1991). A common point between all semi-wild females and two captive females that had delayed puberty was that they had been hand-reared. Although body size was not measured during the course of this study, it is possible that hand-rearing might have affected normal body growth in these animals. This in turn could have delayed the attainment of a minimum threshold body weight that is necessary in some domestic and wild species for the attaining of puberty (Ferrell, 1991; see Owen-Smith, 1988).

However, the hand-rearing of these females has also provided them with an unusual social environment compared to a wild situation, depriving them of most maternal contact. The presence of the mother, or another female, could be a potentially important factor for a female calf in the process of sexual maturity. This hypothesis is reinforced by the fact that the three wild translocated animals that reached sexual maturity at an early age had also been translocated with their mothers. In addition, two young captive females started to develop regular cyclic activity after

they were managed together with a male. Furthermore, another captive female that had stopped cycling resumed her cyclic activity and subsequently conceived after she was put together with a cycling female, that was also her mother.

Non-environmental factors such as diseases may also have played a role in delaying first conception in some animals, either directly or indirectly. Recurrent feet pathologies in some captive animals have reduced the duration of periods of contact between males and females, thus decreasing conception opportunities. In addition, some captive females might have developed reproductive disorders. Two nulliparous females which had started to exhibit regular oestrous cycles, progressively developed an unusual aggressiveness and anxiety. This, coupled with the disappearance of behavioural oestrus, may suggest that they were affected by some reproductive pathologies. It has recently been found that a captive nulliparous black rhinoceros female with a history of long-term aggressive behaviour and suspected silent heat had some ovarian and endometrial cysts, as well as a necrotic vestibulo-vaginitis (Göltenboth *et al.*, 2000). It is thus possible that a similar pathology developed in the two nulliparous females that exhibited similar symptoms in this study. However, one of these two females (Arusha) resumed normal cycling activity after she was managed again with her mother, which was then cycling. This tends to indicate that her condition was reversible without medical treatment.

Age at sexual maturity in black rhinoceros males could only be evaluated in captivity during this study. A captive-born male first fertilised a female at 5.4 years, which coincides with results from a previous study indicating that captive males first initiate a pregnancy at an average of 6.2 years of age (Lindemann, 1982). The observation that two wild males moved from the maternal area might be linked to the process of sexual maturation. This is in agreement with results from a recent genetic

study that confirmed that a wild male of 7-8 years of age bred successfully (Garnier *et al.*, in press). However, males have been described to become territorial only later, after at least 9 years (Hitchins & Anderson, 1983) and the prime breeding period may only be reached at a later stage, when males are 17-25 years old (Adcock, 1994).

4.5.6 Calving interval

The mean calving interval in wild females in the study was determined to be 2.3 years, representing a better breeding output than in other black rhinoceros populations in Southern Africa where calving intervals averaged 3 years (Bride *et al.*, 1996; Adcock *et al.*, 1998).

Two wild study animals that exhibited the shortest calving interval of 23 months were also monitored through faecal progestagen analysis. Individual hormonal profiles indicated that their short calving to conception interval combined a short post-partum anoestrus of four months with an equally brief period of oestrous cycles of four to five months. Interestingly, these two females had overlapping home ranges, also overlapping with that of the dominant bull of the area, which had fathered nearly all calves born to these two females and which was the most genetically represented in the population (Garnier *et al.*, in press). It is therefore plausible that this prime breeding bull may associate with some of the most productive females of the population. Alternatively, the reduced calving intervals observed in the two females may be linked to the greater fertility levels of the dominant bull compared with other males in the population.

The longer calving intervals (33 and 38 months) were observed in two females that were 10-years old and a 35-years old, respectively. Reproductive monitoring in the younger female during most of this calving interval showed that she exhibited an extended period of oestrous cyclicity for at least nine months. In the older

female, hormonal monitoring undertaken during the following calving interval revealed that she had aborted after 3.5 months of gestation. The longer calving intervals exhibited by some animals may therefore be associated with age-related variations in fertility. This is corroborated by the fact that the older female shifted her home range to the periphery of the main rhinoceros area during the study, as had been previously observed in an elderly female which had not produced a calf for ten years.

The calving interval in translocated females was evaluated to average 3.8 years, which was significantly longer than in wild females. It was also determined that an average lag of 3.5 years occurred between translocation and the production of a calf, coinciding exactly with results from Brett (1998) on translocated populations in Kenya. The origin of such a lag could not be evaluated during the study. However, the fact that two females were three and six months pregnant at the time of translocation indicated that the translocation process did not disrupt pregnancy during the first six months of gestation. These results are encouraging since it was suggested in the preceding chapter that the early gestation period in the black rhinoceros might present some vulnerable phases during the first three to four months.

Nevertheless, there is also a probability that two other translocated females in this study were pregnant at the time of translocation but either aborted or lost the calf after birth. Pregnancy may be suspected in these two animals since both were with three-year old calves when translocation occurred, while another two females originating from the same place were pregnant at translocation when their calves were 1.5 and 2 years old. In addition, these two females conceived four to six months following translocation but still presented a long calving interval (>3.5 years) between their first and second birth in the Conservancy. Abortion or neonatal mortality may therefore be suspected in these females which conceived shortly after translocation.

Brett (1998) reported that 50% of the females pregnant at translocation lost their calves, probably more through neonatal mortality than through abortion.

In UK based zoological institutions, black rhinoceros females exhibited a longer calving interval (42 months) than wild females. This interval, however, compared favourably to results from previous surveys in captivity (Lindemann, 1982; Smith & Read, 1992).

The short calving interval of 20 months was associated with stillbirth, corresponding to the mean calving interval of 25 months determined in captive females which had lost their calves after birth or had stillborn calves (Lindemann, 1988). The longer calving intervals corresponded to prolonged periods of oestrous cycles, lasting for up to 14 months in some multiparous females. Some of these longer periods of oestrous cyclicity had inevitably been prolonged by the temporary unavailability of males or health problems. Nevertheless, they were much longer than in wild females, in which they lasted for a maximum of seven months. Such a difference was reflected in the disparity between conception rates, which were more than twice higher in wild females compared to captive animals.

Captive multiparous females nevertheless exhibited very regular oestrous cycles during these periods, except in June when extended inter-oestrous intervals were noted in some animals. The prolonged periods of cyclic activity might therefore have originated from a failure to successfully conceive. It is however improbable that these females were affected by reproductive pathologies since they would have not subsequently conceived without medical treatment. In addition, they would also have probably exhibited an abnormal reproductive behaviour.

Because a common point with three multiparous females is that a change in partners occurred before successful conception took place, it can thus be presumed

that the social environment in which captive females are kept plays a leading role in successful breeding for this species.

4.5.7 Calf index

In the wild population, an average proportion of three quarters of adult females were accompanied by calves, similar to that observed in natural and increasing populations (Goddard, 1967). The individual monitoring of wild females during the study enabled us to determine that the apparently unproductive females included an older female, a female which had lost her calf through predation and a heifer.

The older wild female had been estimated to be born ca. 1962, after examination of her tooth wear pattern at capture (Du Toit, 1986). She ceased reproducing in 1989, while another female estimated to be born the same year was still breeding in 1999. Another wild female, also estimated to be born the same year, started to present longer calving intervals, lower conception rates and a case of abortion during the study, indicating reduced fertility. Although these females exhibited sub-fertility or infertility conditions with a ten-year gap, it is probable that the ageing process contributed to their reduced fertility. This is further corroborated by the fact that two of the females exhibited a similar dispersal pattern during the study, moving their main home ranges from the main rhinoceros area to its periphery.

In the translocated population, the average proportion of females with calves (62.7%) was similar to the wild population but showed a marked increase between the fifth and sixth year following translocation. Unproductive females at the end of the study included a young nulliparous female as well as a multiparous female which had given birth 21 months after translocation but stopped reproducing afterwards. The fact that this female did not produce other calves within five years of

translocation contrasts with other females. Old age is unlikely to have caused infertility in this female, which was estimated to be only 16 years when she produced her last calf. Furthermore, she was diagnosed pregnant when tested 20 months after the birth of her last calf and this leads us to suspect that she failed to carry the gestation to term or that her calf did not survive. This emphasises the importance of individual reproductive monitoring in order to identify and subsequently treat potential fertility problems.

In captivity, the calf index was significantly lower than in the wild. The proportion of unproductive females was mainly represented by nulliparous females, for which delayed sexual maturity has already been discussed above. In a previous survey in captivity, Lindemann (1982) reported that 38% of adult females were not breeding and suspected incompatibility rather than infertility as the main causal factor. The age class of these animals was not mentioned in this study and such conclusion has to be taken with precaution considering that there were no means of diagnosing sub-fertility at that time. Similarly, Rookmaker (1998) established that only 43% of all females recorded in captivity had reproduced, despite having been kept in a breeding situation.

4.5.8 Calving rate

Around a third of adult females gave birth each year in the wild and translocated populations studied, which represents a better performance compared to most other free-ranging and translocated populations (Brett, 1998; Goddard, 1967; Hitchins & Anderson, 1983; Western & Sindyio, 1972).

Such a good calving rate in the wild population was linked to the fact that most females were breeding and exhibited short calving intervals. The apparently good calving rate of translocated females needs, by contrast, to be considered with

caution because two animals were pregnant at translocation and two others which conceived within six months of translocation might have aborted or lost their calves. The progressive decrease of the calving rate during the two years following translocation illustrates the pre- and post- translocation breeding. In addition, the stable but very low calving rate (≤20%) observed between three and five years after translocation reflects the calving interval of nearly four years in translocated females. Such annual variations emphasise the necessity to only study fertility parameters over at least a five-year period.

In captivity, an average of 21.7% of adult females gave birth every year, suggesting that each female should give birth approximately every 4.5 years. The discrepancy between the calculated calving interval and the one observed (3.5 years) was due to the fact that the observed calving interval only included females that were actually breeding. The calving rate was lower than in the wild population, due to the important proportion of captive adult females that are nulliparous as well as to the decreased conception rates in captive multiparous females compared to free-ranging ones.

4.5.9 Fecundity and recruitment rates

The fecundity rate (9.5%) in the wild black rhinoceros population in the Save Valley Conservancy reflected its high calving rate. It also represented an improved reproductive performance compared to those of remaining populations of the same ecotype in Southern Africa or those of natural populations that occurred in east Africa in the 1960s, which only presented fecundity rates of around 7% (Bride *et al.*, 1996; Goddard, 1967, 1970a).

The fecundity rate measured in both the translocated (12.6%) and captive populations (10.4%) were found to be higher than for the wild population. However, these rates decreased after correction to an equal sex ratio, emphasizing the influence of sex ratio on breeding performances of populations. Such influence also appeared in Kenyan sanctuaries, which presented the highest recruitment when they had been stocked with founder animals comprising twice as many females as males (Brett, 1998).

Because previous studies are unlikely to have performed such correction on recruitment rates, the non-corrected rates measured in the study are used for comparison. The annual recruitment measured in the translocated population in the northern section of the Conservancy (7.2%) was much higher compared to other translocated populations, where it averaged 5.7% and even 2.9% in Kenya (Adcock *et al.*, 1998; Brett, 1998).

Low mortality rates were determined in both the wild and translocated populations, averaging 1.5% per year. Mortality was thus reduced compared to other free-ranging populations in which adult mortality rates could reach 10% (Goddard, 1970a) and neonatal mortality 69% (Hitchins & Anderson, 1983). Causes of death amongst wild animals included old age. A male was estimated to be around 37 years when he died and this corresponds to reports that the life-span of black rhinoceros is around 40 years in the wild (Owen-Smith, 1988). In captivity, only 4% of captive animals have attained 30 years of age, but it is not known how much disease affected this survival since two thirds of the black rhinoceros in captivity died before they reached their tenth year (Rookmaker, 1998). One older black rhinoceros female however reached 48 years in captivity (Smith & Read, 1992).

Another black rhinoceros is suspected to have died from a snake bite. Very large mambas (*Dendroaspis poluepis*) were frequently seen in the study area and they have also caused the death of very large cattle bulls (*Bos sp.*). Although the skin of the black rhinoceros is very thick, its thin epidermis and very thick and vascularised dermis makes it particularly sensitive to wounds and infectious processes (Jones, 1979).

The death of a three-week-old calf through predation by lions represented a very improbable accident, since the lion density in the Conservancy is extremely low (less than ten animals occurring in the whole area). In addition, cases of predation by lions are rare and females usually defend their calves efficiently against these animals by goring them (Elliott, 1987; Goddard, 1967). It is thus probable that the lion pride came across the young calf that had been hidden by its mother while she went to drink. By contrast, cases of predation by hyaena (*Crocutta crocutta*) on calves less than three months old have been reported more frequently (Berger & Cunningham, 1995; Goddard, 1967; Hitchins & Anderson, 1983). Such attacks are not always successful and may only result in the calf partially or completely losing its tail and/or its ears (Hitchins & Anderson, 1983).

Amongst the translocated animals, the post-release mortality rate (8.3%) was similar to that observed in other translocated populations in Southern Africa (Adcock *et al.*, 1998) but lower than in Kenya (Brett, 1998). The fact that most of the translocated population was initially released into an empty area that offered a large carrying capacity probably contributed to the reduced mortality observed in the Conservancy.

In captivity, the only death that was recorded during the present study was a case of stillbirth of unknown origin (B.White, personal communication.). Although

this only represented an annual mortality rate of 1%, this nevertheless affected one eleventh of the progeny born during the study. A stillbirth rate of 11% has been reported for all the progeny born in captivity (Rookmaker, 1998) and efforts should attempt to reduce such rates as much as possible. Two other stillbirths that had occurred in the captive UK population before the study started were associated with a low birth weight of around 26 kg (Garnier, unpublished information), emphasizing the importance of diagnosing pregnancy to feed females according to their reproductive status.

Table 4.1. Comparison of mean sex ratios, composition and fertility parameters of three black rhinoceros populations during a five-year period.

	Wild	Translocated	Captive
Adult age class (%)	56	56.2	69.2*
Adult sex ratio (M:F)	1.2:1	0.4:1*	0.5:1*
Age first parturition (years) (range)	6.2[1] (6.1-6.3, n=2)	6.7[2] (4.6-9.3, n=4)	8.6 (5.8-15.6, n=5)
Age first conception (years) (range)	5[1] (4.9-5, n=2)	5[2] (3.4-8, n=4)	7.3 (4.5-14.3, n=5)
Calving Interval (months) (range)	28[1] (23-38, n=10)	47[2] * (42-50; n=4)	42 (20-67, n=6)
Calf index (Calf/Femalex100) (range)	76 (70-90)	62.7 (40-85.7)	28.5* (0-66.7)
Calving rate (Calf/Year/Femalex100) (range)	35.4 (0-70)	30.1 (16.7-57.1)	21.7 (0-36.4)
Conception rate (Conception/Cyclesx100) (range)	20 (14.3-50, n=5)		8.1 (7.1-9, n=3)
Fecundity rate (Calf/Year/Populationx100) (range)	9.5 (0-20.6)	12.6 (6.7-25)	10.4 (0-16.7)
Recruitment rate (%) (corrected to an equal sex ratio)	7.2 (7.2)	8.6 (6.9)	5.2 (4)

* $P<0.05$ compared to the same fertility parameter in the wild population, or to a sex ratio of 1:1

[1] only include females born in the SVC

[2] includes females translocated to the north of SVC and a mother/daughter unit translocated to the south of SVC

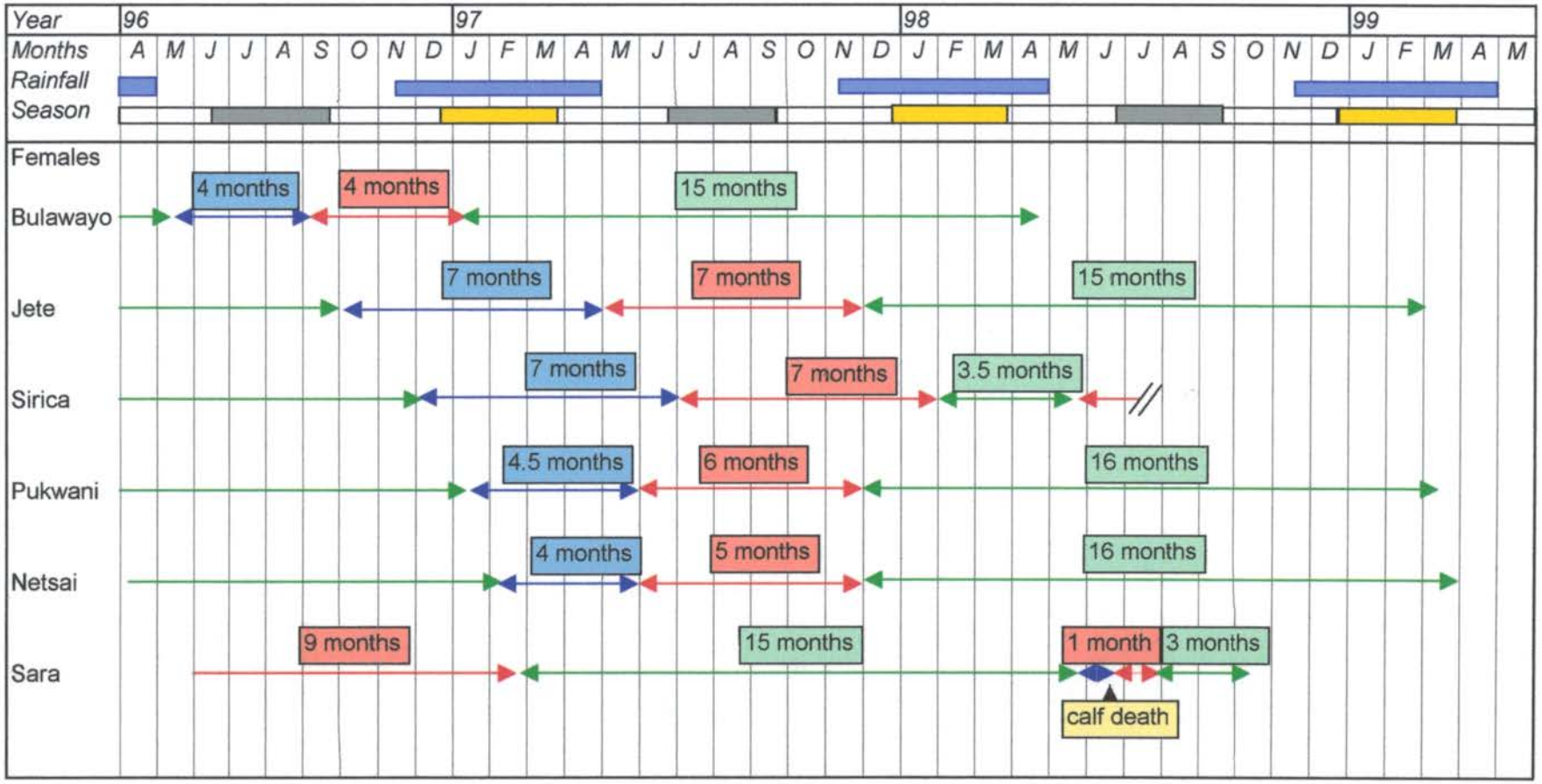


Figure 4.1. Annual reproductive cycles in wild black rhinoceros females (*D.b.minor*) in Zimbabwe.

Post-partum period without oestrous behaviour

Oestrous cyclicity detected through faecal steroid analysis.

Gestation or periods during which faecal 20a-progestagens levels increased consistently to reach levels above 2000 ng/g.

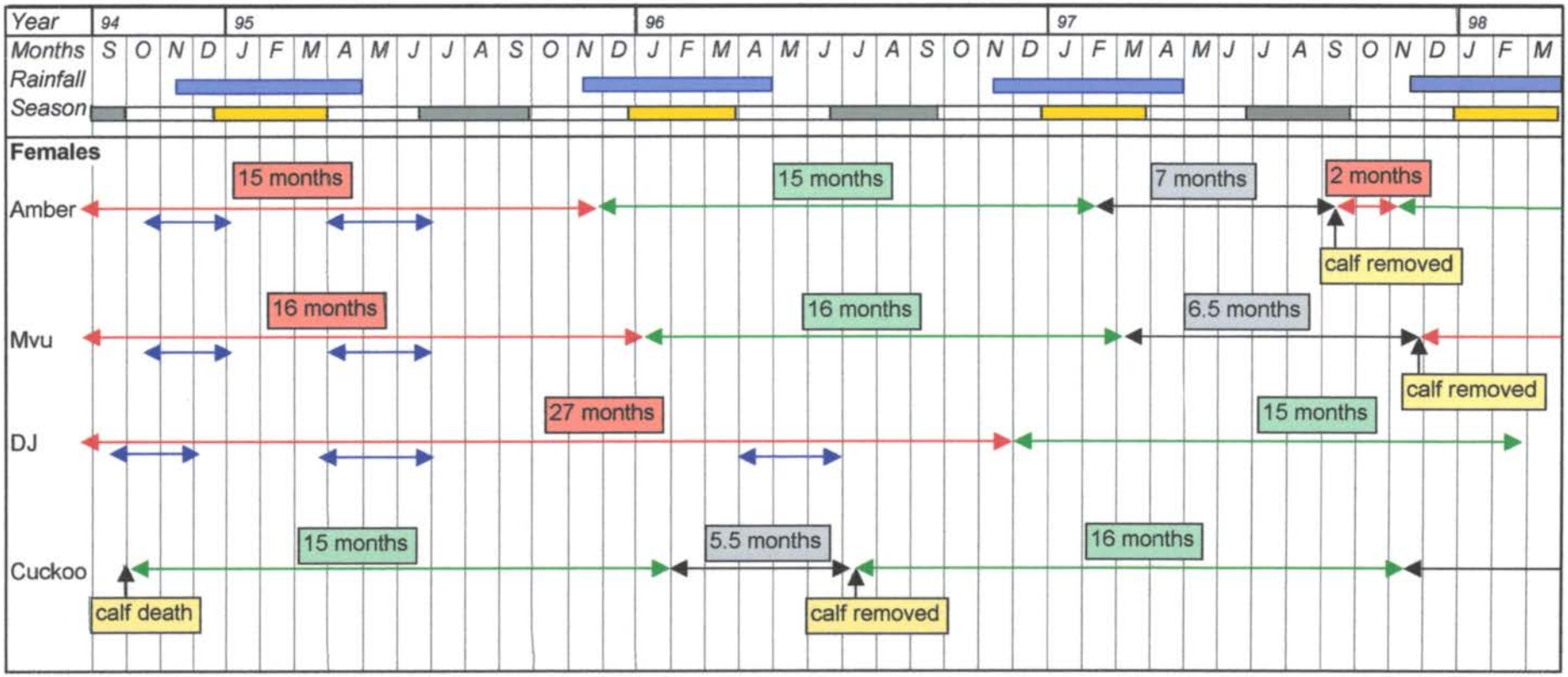
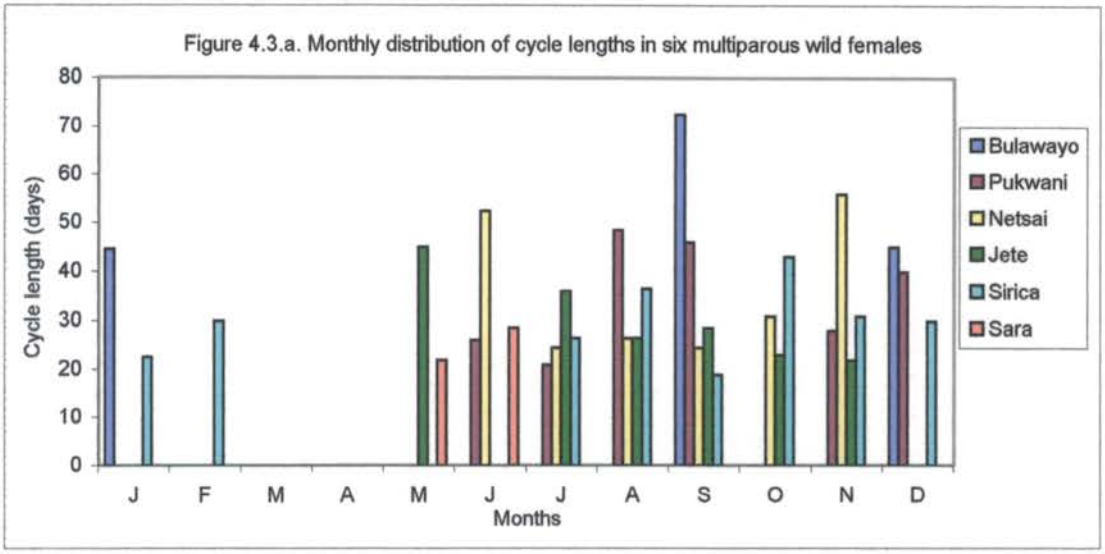
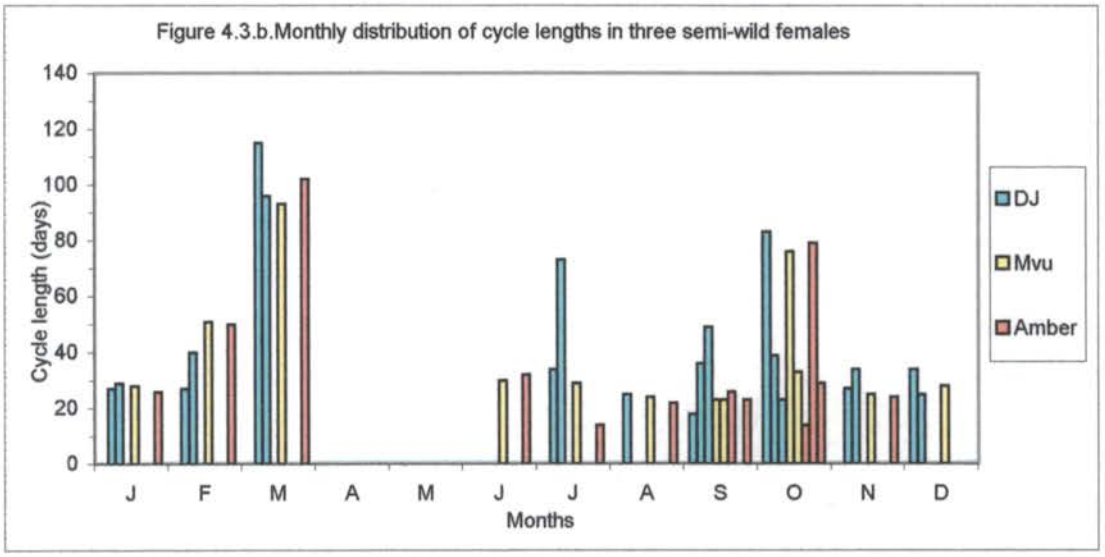


Figure 4.2. Annual reproductive cycles in semi-wild black rhinoceros females (*D.b.minor*) in Zimbabwe

- Inter-oestrous interval > 60 days
- Oestrous cyclicity detected through behavioural observations.
- Gestation
- Female and calf staying away from other animals





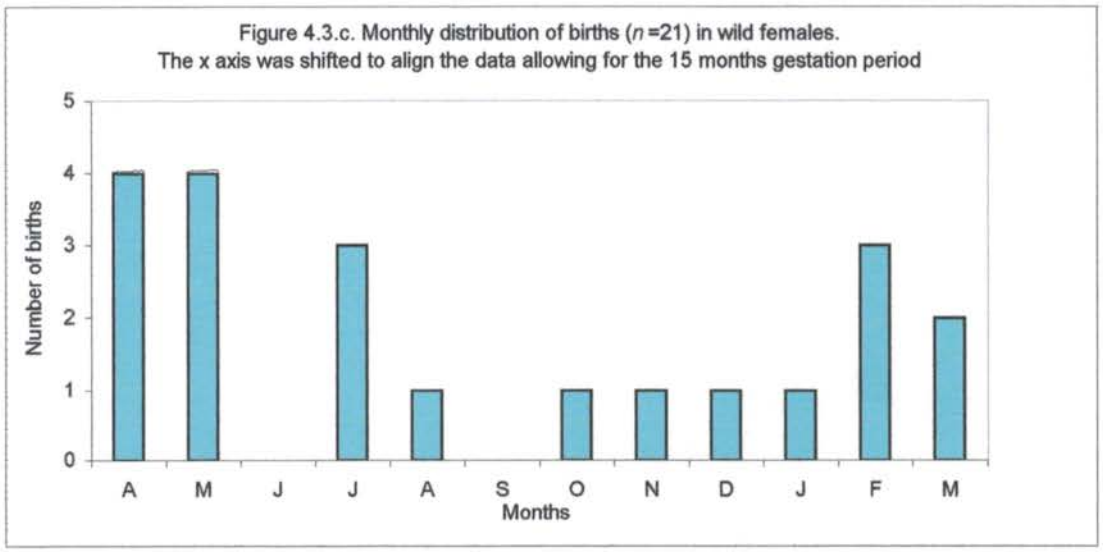


Figure 4.3. Monthly distribution of cycles and births in wild and semi-wild females *Diceros bicornis minor* in Zimbabwe.
Cycles lengths were determined through faecal progestagen analysis in wild females and through observations of behavioural oestrus in semi-wild females.

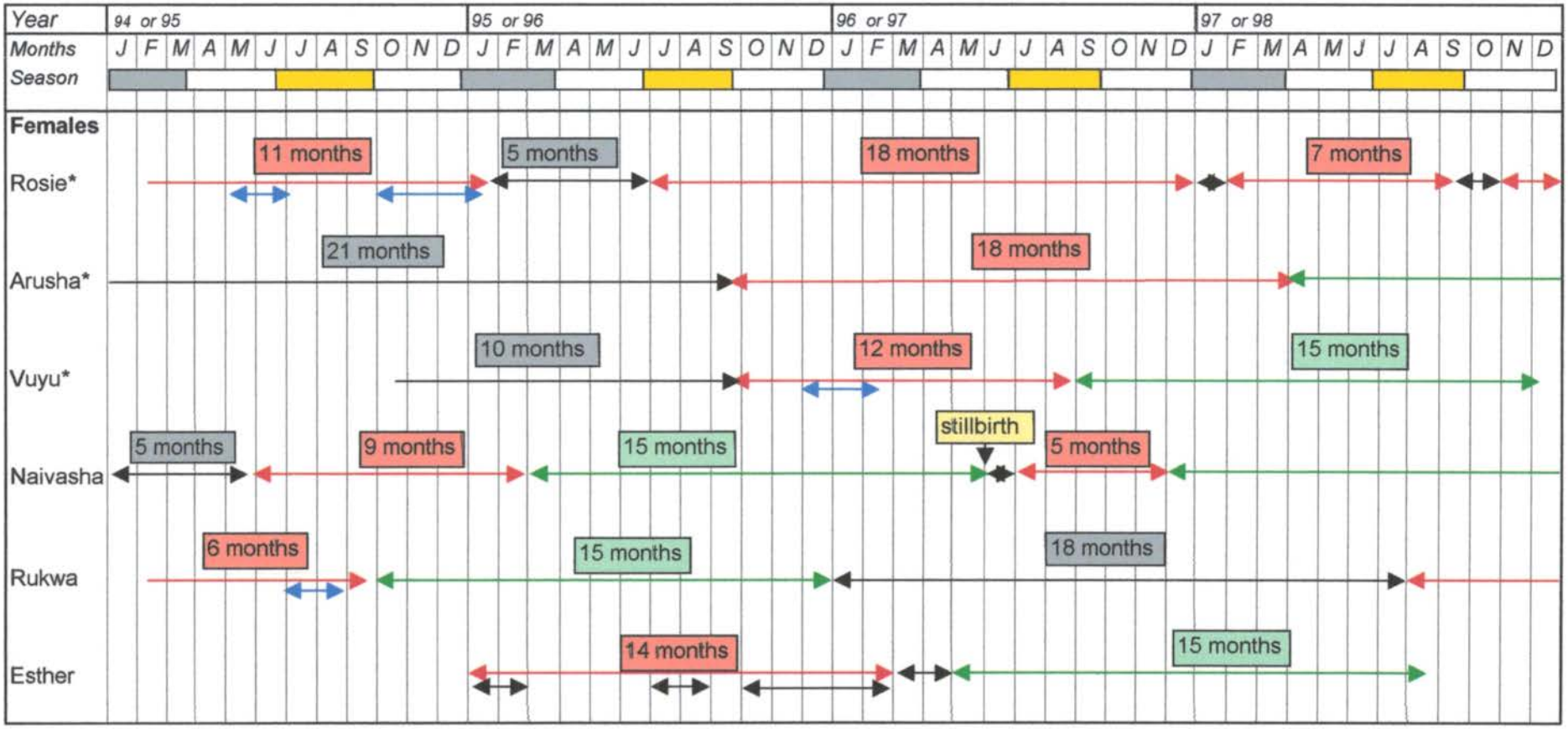


Figure 4.4. Annual reproductive cycles in captive black rhinoceros females (*D.b.michaeli*) in the UK

- Oestrous cyclicity detected through behavioural observations.
- Inter-oestrous intervals > 60 days
- Gestation
- Females kept separated from male and/or no record of oestrous behaviour

Periods of study covered 1994/97 for the female Rosie and 1995/96 for the other females. (*) indicate a nulliparous female

Figure 4.5.a. Monthly distribution of cycle lengths in three nulliparous captive females

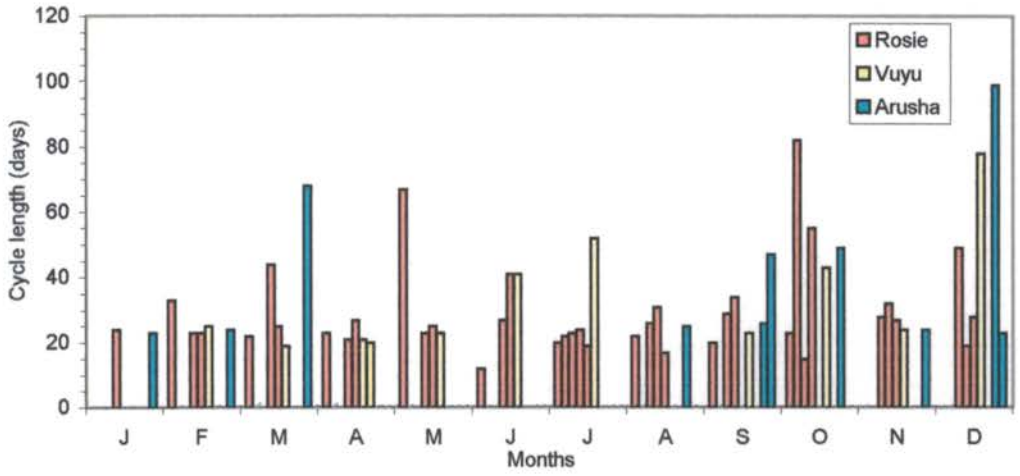


Figure 4.5.b. Monthly distribution of cycle lengths in three multiparous captive females

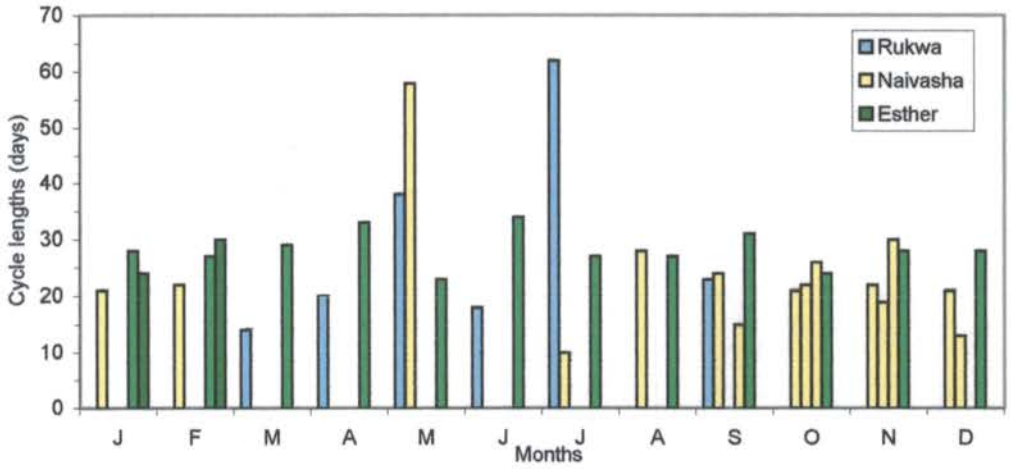


Figure 4.5.c. Frequency distribution of births (n =290) in captivity
The x axis was shifted to align the data allowing for the 15 months gestation period

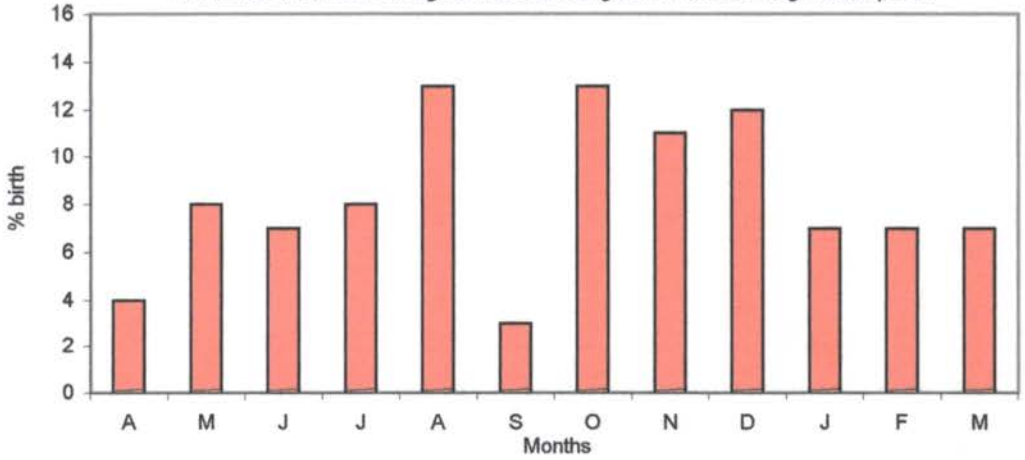
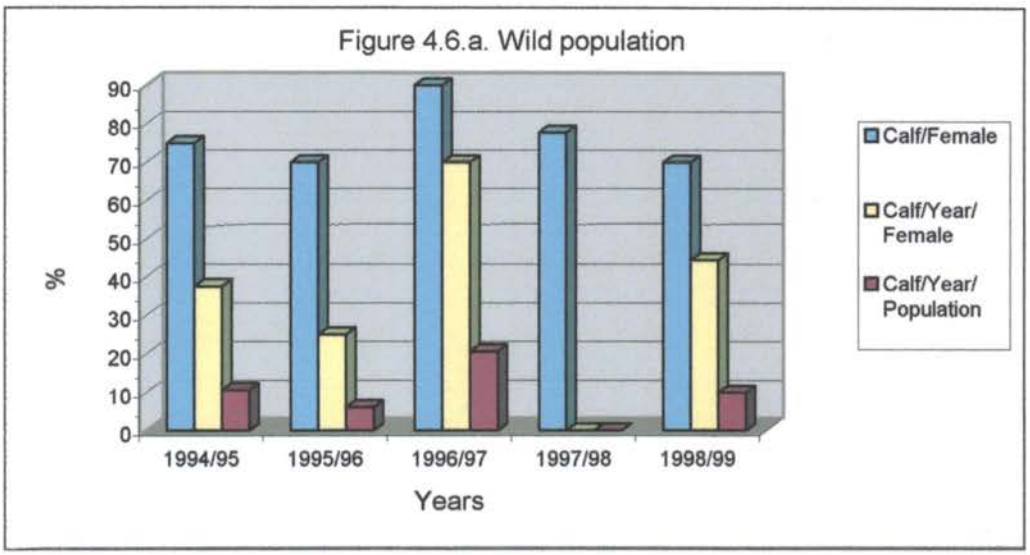
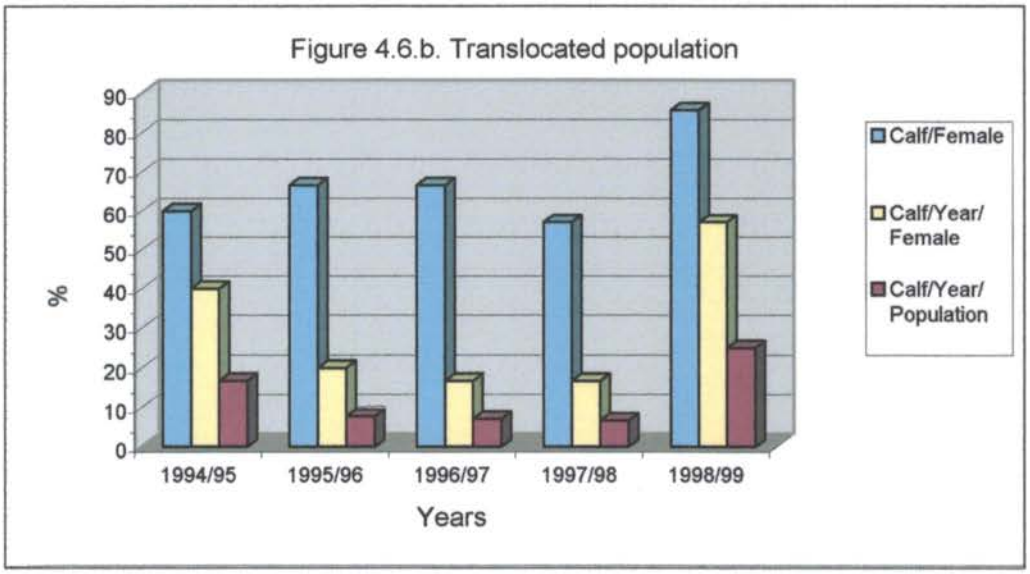


Figure 4.5. Monthly distribution of cycles and births in captive females *Diceros bicornis michaeli* in the UK.
Cycle lengths were determined through observations of behavioural oestrus





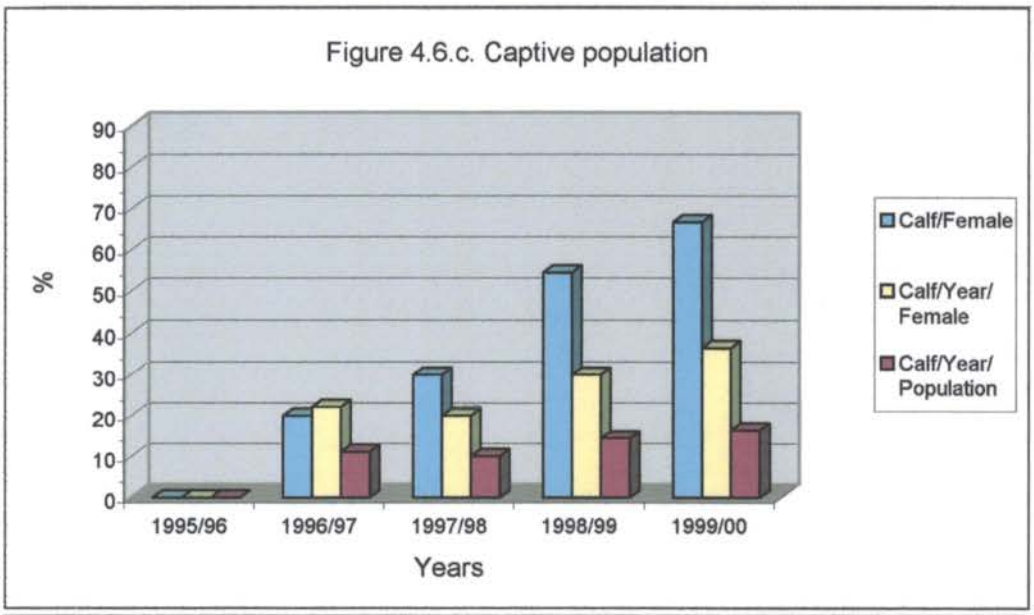


Figure 4.6. Fertility parameters in a wild population, a translocated population and a captive population of black rhinoceros during five years

5 GENERAL DISCUSSION

5.1 FERTILITY MONITORING IN THE WILD

This study represents the first long-term study of reproductive activity using faecal steroid analysis in wild black rhinoceros and more generally in a large African mammal. Urinary steroid analysis has previously been validated for monitoring reproductive status in the black rhinoceros in captivity (Hindle *et al.*, 1992), but an attempt to use this technique in the wild showed that the regular collection of urine samples proved to be too difficult because urine dried too quickly in the heat (Brett *et al.*, 1989). In other free-ranging African species, non-invasive hormonal studies of reproductive activity were possible in some species that were gregarious and easily observable, such as the mountain gorilla, baboon, mongoose, African wild dog (Creel *et al.*, 1992, 1997, 1998; Robbins & Czekala, 1997; Wasser, 1996). In North American species, for example bison, elk, caribou and feral horse, the collection of both urinary and faecal samples also proved to be feasible in the wild after direct observation of the animals from afar (see Lasley & Kirkpatrick, 1991; Messier *et al.*, 1990; Stoops *et al.*, 1999).

In the present study, the direct observation of wild black rhinoceros became possible only after the trackers gained sufficient experience to approach these animals at close range without disturbing them. Tracking these animals without being detected by them necessitated the greatest attention since they were very secretive and wary of humans, favouring the thickest and densest habitats. Black rhinoceros rely on their extremely developed sense of smell and hearing to detect any danger, as they have very poor eyesight, as well as on the alarm chirping of the oxpeckers which feed on external parasites on large ungulates (Ritchie, 1963; Shenkel & Shenkel-Hulliger,

1969a). The repeated disturbance of these animals had to be avoided as it would have put the observers at risk and might also have resulted in a change of the normal activity patterns of the rhinoceros. Repeated human interference with black rhinoceros in the wild has resulted in the disruption of mating bonds, a certain degree of local site abandonment and a shift to more nocturnal habits (Berger & Cunningham, 1995; Shenkel & Shenkel-Hulliger, 1969a). As a result, most direct observations lasted no longer than a few minutes.

Another difficulty of studying black rhinoceros in the wild was associated with the unpredictable character of these animals. Their truculence towards other living creatures has been reported and it is considered that their reactions to men were conditioned by the nature of their previous contacts with humans (Eltringham, 1979). Ritchie (1963) suggested that in areas where rhinoceros have been hunted by traditional hunting methods such as bows and arrows, there has been an evolutionary selection for aggressiveness as animals which flee are more likely to be killed. On the contrary, rhinoceros living in areas where they have been poached with modern weapons are extremely shy and wary of humans, since a charging rhinoceros will invariably be shot. This most likely explains the extreme wariness of the black rhinoceros in the Save Valley Conservancy, which originated from the Zambezi Valley where poachers used automatic rifles.

5.2 GESTATION PERIOD

The gestation length in captive and wild black rhinoceros was determined to average 15.4 months, although some lasted up to 16 months. Variability in pregnancy length is not unusual in domestic and wild Equids, in which it can also vary by as much as three weeks (Evans *et al.*, 1997). It was also observed that multiple mating

and interactions with males occurred in wild females before successful conception took place, while post-conception mating was recorded in captive animals not only four months after conception but also two weeks prior to parturition (B.White, personal communication). In the wild, a male showed signs of mate-guarding females around 50 days after she conceived. These observations of post-conception reproductive activity are likely to explain previous records indicating a gestation length of less than 14 months (Greed, 1967) and increase the difficulty of diagnosing gestation from behavioural observations. Such behaviour is probably associated with a temporary surge in oestrogen, which was noted by Berkeley *et al.* (1997). It also closely resembles that of the domestic mare, which can typically exhibit oestrus around three weeks after conception (Evans *et al.*, 1997).

The individual and composite profiles of faecal progestagen concentrations that were measured during the gestation period in black rhinoceros females showed two distinct phases. An early gestation period lasting 60-100 days was characterised by faecal progestagen concentrations remaining mainly below 2000 ng/g. During this phase, the luteal phase of the primary corpus luteum was extended up to 40-60 days. Concentrations then showed a transient decline before increasing markedly and consistently to reach levels >2000 ng/g. During mid- and late pregnancy, mean concentrations remained 8-9 times higher than non-pregnant concentrations and returned to non-pregnant levels within a few days following parturition.

The fact that this hormonal profile is so similar to that of the horse during gestation (Schwarzenberger *et al.*, 1991) emphasises the closeness of the Equid and Rhinocerotid families as members of the Order Perissodactylae. Although the underlying physiological mechanisms associated with the recognition and maintenance of pregnancy in the black rhinoceros are still unknown, they might

present some similarities with those in Equids. The formation of accessory corpora lutea is nevertheless doubtful in black rhinoceros, in which attempts to detect eCG have not been successful (W.R.Allen, personal communication, Ramsay *et al.*, 1987).

After the early gestation period, the marked increase in faecal progestagen excretion observed in black rhinoceros is likely to be a reflection of the onset of the placental secretion of progestagens. This is also corroborated by the similarity between the progestogens secreted by the placenta in the horse and the faecal metabolites identified during gestation in the black rhinoceros (Daels *et al.*, 1991; Patton *et al.*, 1996). The fact that faecal progestagen concentrations remained 8-9 times higher than non-pregnant concentrations after the third month of gestation enabled us to validate this technique as a reliable method for pregnancy diagnosis. After three months of gestation, the diagnosis of pregnancy based on faecal progestagen measurement in only one faecal sample had a probability of being falsely positive or negative of 4.9 or 2.4% respectively. During the early pregnancy phase, the probability of a false negative was 17.6%, emphasising the need to collect three samples at monthly intervals to avoid a potential misdiagnosis during this phase.

The first advantage gained by diagnosing gestation in the black rhinoceros is to provide pregnant females in captivity with the most suitable environment, both nutritionally and socially. The nutritional needs of the pregnant female will be discussed in the section on captive management, but require particular attention since low birth weights can be associated with neonatal mortality and stillbirths (Hutchins *et al.*, 1996). This has also been observed in captive black rhinoceroses (Garnier, unpublished observation). One of the most important social requirements for pregnant females is to be separated from the male during pregnancy. This is supported by observations during the study that wild females had very rare contacts with males

during the first six months of gestation and none afterwards, while captive females became aggressive also after six months of gestation. Furthermore, a pregnant black rhinoceros female aborted after she was violently attacked by a male in captivity (B.White, personal communication).

The other advantage of diagnosing pregnancy in this endangered species is that foetal viability can be monitored and abortion detected. This is particularly important in the wild, where abortion cannot otherwise be detected. This also applies to captivity, where early foetal loss can easily be missed, especially when animals are managed in outdoor paddocks. It was established in the study that two wild females aborted after 2.5 and 3.5 months of gestation. Considering that these females belonged to a population which exhibited some of the best fertility levels recorded in the species, it can be presumed that abortion in the wild is a more frequent occurrence than previously thought. Abortion may result from a variety of factors, including genetic abnormalities, developmental malformations, hormonal aberrations, infectious diseases, stress, trauma, drugs and inadequate nutrition (Hutchins *et al.*, 1996). In free-ranging wildlife, abortion could not be assessed before because of the lack of appropriate diagnosis procedure. Only recently could abortion be evaluated in feral horse, bison and elk by using faecal steroid analysis (Kirkpatrick *et al.*, 1996; Lasley & Kirkpatrick, 1991; Stoops *et al.*, 1999). In captivity, reports of abortion in black rhinoceros have only been anecdotal and unexplained (Berkeley *et al.*, 1997; Hodges & Green, 1989; Schwarzenberger *et al.*, 1993, 1996b).

Another consequence of diagnosing pregnancy in wild females is that the birth of a newborn calf can be predicted and detected, thus facilitating the monitoring of neonatal mortality in the wild. It was observed during the study that females became increasingly difficult to find around the time of birth as they hid in thick vegetation to

give birth and remained in hiding for the first few weeks after birth. Such habits most likely contributed to the scarcity of reports of neonatal mortality in the wild, which are mainly due to predation (Berger & Cunningham, 1995; Elliott, 1987; Goddard, 1967; Hitchins & Anderson, 1983). It is nevertheless possible that other causes of neonatal mortality occur in the wild, such as stillbirth or prematurity, which have been detected in captivity.

5.3 POST-PARTUM PERIOD

The individual monitoring of wild females established that during a post-partum period lasting between four months and seven months, females were never or very rarely seen with males. The reduced expression or absence of oestrous behaviour over at least four months, combined with the fact that this period was shortened by the death or removal of the calf, indicates that the expression of reproductive behaviour may be inhibited by the suckling stimulus during this period. The post-partum period in the black rhinoceros therefore bears some resemblance with that of domestic cattle, in which ovulation and oestrus are delayed by the suckling stimulus which suppresses the pulsatile release of LH (Robinson & Shelton, 1991). However, the final stages of follicular development in cattle occur around 50 days post-partum, whereas it appears that follicular activity may resume within a month after birth in the black rhinoceros, being similar to the horse. This was suggested by the post-partum hormonal profiles obtained in this study as well as in those of Berkeley *et al.* (1997), Hindle (1991), Schwarzenberger *et al.* (1993). It could not be determined in the study by which mechanisms the suckling stimulus prevented the exhibition of behavioural oestrus. However, the higher background of faecal progestagen concentrations and fluctuations of wide amplitude during this period might be suggestive of ovulation and subsequent

prolongation of the corpus luteum or luteinisation of the follicles that probably develop during this phase. In cattle, dominant follicles during this period become cystic until the first ovulation post-partum occurs, which requires an adequate LH pulse frequency to stimulate maximum oestradiol production and the ovulatory surge of LH (Stevenson, 1997).

The duration of lactation could not be determined during this study since the observation of suckling activity was impossible under field conditions. Lee *et al.* (1991) established that the duration of lactation was strongly related to maternal and neonatal weight in large bodied mammals. By using a mean body weight of 900 kg for a female *Diceros bicornis minor* and of 40 kg for a newborn calf (Skinner & Smithers, 1990), the use of their equation suggests a lactation period of around 12 months in the black rhinoceros. This result corroborates previous reports that suspected lactation to last around one year, although calves were observed to be still suckling at 1.5 to two years old (Goddard, 1967b; Hall-Martin & Penzhorn, 1977; Hitchins & Anderson, 1983; Joubert & Eloff, 1971).

The finding that the length of this post-partum period in wild females varied between four and seven months suggests that its duration is influenced by a variety of factors. Since the study has shown that in winter black rhinoceros presented reduced reproductive activity, are long day breeders, it is possible that photoperiodicity may contribute to influence the duration of this period, as has been observed in other seasonal breeders (Daels *et al.*, 1991; Lindsay, 1991).

The nutritional status of the animals might also play a role in determining the length of the post-partum interval, as is known to occur in sheep, cattle and red deer (Loudon, 1987). The author suggested that on high planes of nutrition the lactational anoestrus is reduced in length since suckling frequency declines with copious milk

production, thus permitting the pulsatile release of LH. Interestingly, the two black rhinoceros females that had the longest post-partum intervals produced their calves before or just at the onset of the rainy season, when food availability was reduced. By contrast, the three females which produced their calves during the mid-rainy season and just after the rainy season, when food availability was maximum, exhibited a short post-partum period of only four months.

5.4 OESTROUS CYCLES

Wild black rhinoceros females in the study exhibited periods of oestrous cyclicity lasting between four and seven months. These periods occurred between May and February and were characterised by a great variability in cycle lengths. The majority of cycles (75%) was classified as Type I and characterised by a mean total length of 26.8 ± 1 days, which can be considered to represent the normal ovulatory cycle in this species. These cycles coincided with the few other reports of cycle lengths in this species, determined through non-invasive hormonal analysis. (Berkeley *et al.*, 1997; Godfrey *et al.*, 1991; Hindle *et al.*, 1992; Schwarzenberger *et al.*, 1993) or behavioural observations (Hitchins & Anderson, 1983; Lindemann, 1982).

One of the unexpected finding of the study was the great variability of cycle lengths in the black rhinoceros and the natural occurrence of longer cycles classified as Type II: these were approximately twice as long as Type I cycles. They were detected between May and October and were characterised by either a prolonged luteal phase that lasted around twice as long as the normal one, or by an extended follicular phase.

It has already been discussed that cycles associated with an extended luteal phase may be linked to a persistent corpus luteum, to embryo loss or to ovulation during dioestrus. The incidence of these conditions was most likely underestimated in most wildlife prior to the development of non-invasive reproductive monitoring methods and these longer cycles have recently been identified in captive white rhinoceroses (Patton *et al.*, 1999; Schwarzenberger *et al.*, 1998). In domestic horses, it appears that they occur frequently, since it was reported that up to 25% of oestrous cycles were associated with persistent luteal activity and that between 5 and 30% of the gestation periods were affected by early embryo loss (Hinrichs, 1997). The fact that these longer cycles were detected before the period of optimum fertility in wild black rhinoceros females suggest that they may be associated with a transitional period during which full reproductive competence is acquired, as is observed in the horse at the onset of the breeding season (Sharp *et al.*, 1997).

Other extended ovarian cycles corresponded to a prolongation of the follicular phase lasting up to 4-5 times the length of the normal ones. They were mainly observed in September/October, during the hottest time of the year, suggesting that they might be associated with heat stress. Heat stress is known to affect follicular growth, to decrease the duration of oestrous behaviour and also to disrupt early pregnancy in domestic cattle in which conception rates markedly decrease during the warmest months of the year (Hansen, 1997).

Finally, another factor of variation affecting the oestrous cycles of wild black rhinoceros females was the occurrence of silent heats, identified in 16% of the cycles. These silent heats may be linked to individual characteristics of the animals, as in equids in which they are a common occurrence in young mares, timid mares or mares with foals by their side (Hinrichs, 1997). They may also be associated to the transition

into the breeding season, similar to what occurs in ewes in which the first ovulation of the breeding season is frequently associated with a silent heat as the exhibition of behavioural oestrous needs a priming by progesterone (Lindsay, 1991). This hypothesis is reinforced by the fact that some silent heats that were identified in wild black rhinoceros occurred after the first cycle of their breeding season, as well as by the observation that some extended inter-oestrous intervals were detected after the winter anoestrus in nulliparous females. This phenomenon has also been observed in the first cycle of the wildebeest, which has only two cycles per breeding season (Watson, 1969).

The variability in the ovarian cycles exhibited by wild black rhinoceros females is therefore very similar to that observed in the horse (Daels *et al.*, 1997). It also emphasizes the limitations of behavioural observations for monitoring reproductive activity in females. In the wild, the main signs associated with oestrus that could be recorded were the presence of an adult male with a female during successive days, as well as mating and fighting between males, as had been recorded (Brett *et al.*, 1989; Goddard, 1966; Hitchins & Anderson, 1983; Shenkel & Shenkel-Hulliger, 1969a). The opportunities to record mating activity were limited, due to short time that could be spend with these animals.

Oestrogens could not be measured during the study and previous reports on captive black rhinoceros females indicate very little correlation between reproductive behaviour and the hormonal profiles of oestrogens measured in faeces and serum (Berkeley *et al.*, 1997; Schwarzenberger *et al.*, 1996). Berkeley *et al.* (1997) described two serum oestrogen peaks during one cycle, one with a subsequent elevation in progesterone and another during the mid-luteal phase around 10 days before the second one. Hindle *et al.* (1992), who established a composite profile of

urinary 20α-OHP and oestrone, found that the mean oestrone peak lasted an average of 10 days, starting to increase five days before mating and returning to background concentrations five days after mating. However, the close examination of some of the individual profiles used for the composite profile (Hindle, 1991) reveals that these oestrogen peaks presented great variations. Some peaks of large amplitude lasted around 16 days, while others of smaller amplitude lasted around 8 days and some even smaller ones only lasted 3-4 days. The pattern of follicular growth has not been described in the black rhinoceros, nor in most wildlife species. However, the above oestrogen patterns might be suggestive of more than one follicular wave in this species.

Furthermore, it is not known whether black rhinoceros are spontaneous or induced ovulators. Most of wild and captive black rhinoceros that exhibited oestrus behaviour in this study were within visual or olfactory contact with a male and it has never been proved that black rhinoceros females kept in isolation ovulated spontaneously. It was observed in the study that the introduction of a new black rhinoceros male was beneficial to successful breeding in multiparous females when they had previously been kept with the same male. It was also recorded that when a new pair was introduced, mating occurred very soon afterwards (B.White, personal communication). Although most artiodactyls are spontaneous ovulators, the beneficial role of the male in the exhibition of oestrus has been demonstrated in some domestic and wild species such as the ewe, which is considered to be a semi-induced ovulator, as well as in the Arabian oryx (Blanvillain *et al.*, 1997; Lindsay, 1991; Sempere *et al.*, 1998). The induction or semi-induction of oestrus and/or ovulation by the male may be a concept that requires to be explored in the black rhinoceros, especially with the

recent finding that the Sumatran rhinoceros was an induced ovulator (Roth *et al.*, 2001).

5.5 SEASONALITY

The black rhinoceros females that were studied in Zimbabwe presented a seasonal pattern of reproductive activity. A higher proportion of births was detected during the late rainy season and more conceptions occurred between November and February, during the early rainy season. This timing of births ensures that temperature and food resources are most suitable for the optimum growth and survival of the young since the rainy season has always been considered to be the limiting factor in tropical zones (Flowerdew, 1987). Conception peaks during the rainy season have also been identified in a variety of African mammals, including elephant, giraffe, plains zebra and Grevy's zebra (Estes, 1992; Owen-Smith, 1988).

The individual reproductive monitoring of black rhinoceros females also tends to indicate that this species may be a long-day breeder. Periods of optimum fertility occurred during the late spring and summer in the northern and the southern hemispheres, closely resembling what occurs in domestic equids (Sharp *et al.*, 1997). In autumn, nulliparous females exhibited an anoestrous period which was very marked and synchronised in the southern hemisphere (April/June) but which seemed more flexible in the northern hemisphere (October/January). During this period of short day lengths, it is not known whether the ovaries were completely quiescent, as in the horse or whether they just exhibited silent heats as it has sometimes been observed in *Bos indicus* in winter. Alternatively, lutcal activity may have been prolonged as observed in some horses throughout the year (Daels *et al.*, 1991). In multiparous females, this anoestrus was not detected in captivity while in wild females, it

coincided to the post-partum period during which the expression of oestrus appeared to be reduced but ovaries were not quiescent.

In late June, a marked decline in mean faecal progestagen concentrations was detected on all the hormonal profiles, whether in the wild or in captivity. The significance of such pattern remains unclear since it was similar in both hemispheres. Nevertheless, its occurrence resulted in decreased fertility, as reflected by the low incidence of births in September.

The seasonal pattern of reproduction exhibited by black rhinoceros females therefore appeared to be influenced by rainfall and possibly temperatures and photoperiod in Zimbabwe, with a more visible influence of photoperiod on nulliparous females. The complexity of the interactions between various environmental factors has been demonstrated in zebu cattle in the Mexican gulf coast region (Robinson & Shelton, 1991). In this species, it was shown that pubertal heifers had their breeding season determined by photoperiodic constraints, while by the fifth calving, rainfall had become the factor of overriding influence.

In captivity, the seasonal pattern of reproduction was less marked than in the wild. However, the higher fertility observed in summer and the longer inter-oestrous intervals exhibited in winter by the anoestrus in nulliparous females suggest that photoperiodicity contributes to the regulation of reproductive patterns exhibited by this subspecies (*Diceros bicornis michaeli*). It has never been determined whether this ecotype exhibited a seasonal pattern of reproduction in its natural environment but the influence of photoperiod, which fluctuates only very slightly under equatorial latitudes, is unlikely to be determinant near the equator (Loudon & Brinklow, 1992). The fact that reproductive activity of this black rhinoceros ecotype under temperate climates appears to be influenced by photoperiod may suggest that the neuro-

endocrine pathways associated with seasonality can be developed when necessary, since they were more obvious in young animals that were captive-born. This would contrast with what has been described in deer species of equatorial origin; when transferred to temperate climates, they remained aseasonal or adopted a calving pattern that was the reverse of temperate species (Loudon & Brinklow, 1992).

5.6 FERTILITY PARAMETERS IN THE WILD POPULATION

Fertility parameters of the study wild black rhinoceros population indicated that it presented very good reproductive performances, reflected by an annual recruitment rate of 7.2%. Its age structure and fecundity rate (9.5%), together with a short calving interval (28 months), also compared favourably with those of some of the most successful black rhinoceros populations (Goddard, 1967, 1970a; Hitchins & Anderson, 1983). It can thus be concluded that the breeding output of this wild population represented near to optimum reproductive performances for female black rhinoceros.

Wild black rhinoceros females born or translocated to the Conservancy with their mother first conceived at an early age (5 years). It has been suggested that the minimum age at first conception in large herbivores increases in relation to their body weight (Owen-Smith, 1988). Considering a mean body mass of 900 kg for females of *Diceros bicornis minor* (Skinner & Smithers, 1990), the relationship of Owen-Smith (1988) determines a minimum age at first conception of 3.6 years for the black rhinoceros, which corroborates the findings of this study. Likewise, if body weight and sexual maturity are linked through this relation, other species of rhinoceros such as the Indian rhinoceros, which is much larger than the black, should then reach sexual maturity slightly later than the black species. Using a mean body mass of 1600

kg for females of this species (Owen-Smith, 1988), the same relationship suggests a minimum age at first conception of 4.3 years, which agrees with data available from wild Indian rhinoceros (Dinerstein & Jnawali, 1992).

Body weight is known to be a primary factor affecting age at puberty in most domestic species (Ferrell, 1991). More particularly, it has been found that retarded growth resulted in the suppression of LH pulse amplitude and frequency in domestic mares (Evans *et al.*, 1997). The concept that a threshold body weight needs to be attained for reaching puberty has also been demonstrated in cattle and sheep and various species of primates and deer (Ferrell, 1991; Asa, 1996). However, it is the nutritional plane, rather than an absolute body weight, which has been found to affect the timing of puberty. The nutritional status of the study animals was assessed by using a body condition index that had been established specifically for black rhinoceros. They exhibited a body index of 4 or 5 on a scale of 1 to 5, indicating their good nutritional status. This confirmed that habitats in the Save Valley Conservancy were most suitable for black rhinoceros (Price Waterhouse, 1994). It can thus be presumed that the early age at sexual maturity that was shown by wild females in the Conservancy was linked to their excellent nutritional status.

The low density of the wild black rhinoceros population studied (around 0.02/km^2) may also have contributed to the attaining of puberty at a young age in these females. This was corroborated by the mean calving interval which was determined to be much shorter than that observed in high-density areas of black rhinoceros (Hall-Martin, 1986; Hitchins & Anderson, 1983). The action of the density-dependent effects on reproductive output is poorly known but it has been identified in a number of species that included, among others, African elephant, white rhinoceros and red deer (Albon *et al.*, 1983; Rachlow & Berger, 1998; Fowler, 1987). It is possible that

they exert some of their influence through poor body condition, since white rhinoceros females in a high density area were found to be in significantly poorer condition compared to females in a low density area (Rachlow & Berger, 1998).

The lactational effect on fertility also plays an important role in the birth-spacing mechanism. It was determined in the study that no wild female conceived before eight months post-partum, but the duration of the post-partum period of reproductive inhibition ranged between four and seven months. It is likely that the timing of births also affects calving intervals, since the length of this post-partum period may be related to the nutritional status of females, as also discussed earlier.

Great individual differences were observed between fertility levels of wild females and infertility was probably related to the ageing process in some females. One female stopped breeding at 27 years of age while two others of around 35 years were still breeding during the study, although one of them aborted. In the wild, it has been suggested that the reproductive life of black rhinoceros females lasts until they are 30-35 years old, but these reports were not supported by precise examples of animals of known age (Goddard, 1970a; Shenkel & Shenkel-Hulliger, 1969a). In captivity, a study animal was still producing at 30 years, although a previous survey established that no captive female older than 24 years of age had reproduced (Smith & Read, 1992). It can thus be presumed that fertility might start to decrease in some females after 25 years, but that breeding can also continue in other females until 30-35 years. Longer calving intervals will be exhibited before old females becomes infertile, as suggested by observation that wild Indian rhinoceros females which were older than 20 years exhibited calving intervals of more than four years (Dinerstein & Raj Jnawali, 1991). It must be noted that reproductive pathologies such as endometrial

hyperplasia, leiomyomas and fibromas might also be associated with age-related infertility (Godfrey *et al.*, 1991; Schaffer *et al.*, 1994).

The proportion of wild black rhinoceros cows without calves, which was very similar to that observed in previous studies of free-ranging populations, was therefore mainly associated with age-related differences in fertility levels. It must be noted that neonatal mortality resulted, on the contrary, in the shortening of calving intervals to 20 months. The extreme shortening of the calving interval to 17 months has been described in a captive black rhinoceros after stillbirth and in two wild Indian rhinoceros which lost their young calves (Dinerstein & Raj Jnawali, 1991; Laurie, 1982; Smith & Read, 1992). It can thus be concluded that if neonatal mortality goes undetected, females will appear to exhibit calving intervals longer than 40 months. This interval corresponds to the sum of the shorter normal calving intervals observed in this study (23 months) and of the shortest interval reported after neonatal mortality (17 months). However, not all calving intervals longer than 40 months should be attributed to calf loss, since they can be observed in older females, translocated animals and possibly animals with reproductive disorders.

5.7 FERTILITY PARAMETERS IN THE TRANSLOCATED POPULATION

The analysis of reproductive performances in the study translocated black rhinoceros population between one and five years after translocation indicated a good recruitment rate during this period (6.9%). However, the high fecundity rate (12.6%) was linked to the biased sex ratio towards females and to the fact that at least 40% of the adult females were pregnant at translocation. Annual fecundity rates were in fact very low between three and five years post-translocation, reflecting an average lag of 3.5 years that occurs between translocation and the resumption of calf production.

A possible explanation for this lag is that translocated females need a certain time to establish links with breeding bulls, as suggested by Brett (1998). This theory is supported by the fact that a study animal translocated to the resident population in the south of the Conservancy first established her home range in the periphery of the main area where most resident rhinoceros were already established. She stayed in that area for 3.5 years, during which time she was only seen to interact with sub-adult animals, including her daughter. After 3.5 years, she shifted her home range to the main area where the prime breeding bull and breeding females were established and subsequently bred with the dominant male (Garnier *et al.*, in press). Adcock *et al.* (1998) had also observed that translocated black rhinoceros did not establish a proper home range before at least three years after release.

Such a delay in establishing breeding bonds with males probably also applies when animals are released into an empty area, as observed in females translocated to the north of the Conservancy. It is not known whether females that had young calves at translocation (and could therefore be presumed not to be pregnant) exhibited regular oestrous cycles afterwards, since they were not monitored regularly through faecal progestagen analysis. However, the observation that the female mentioned above did not interact with any breeding bull during 3.5 years following translocation may suggest that she did not exhibit oestrus during this period and/or was not within his ranging area.

The translocation process in black rhinoceros first involves the capture of the animals, which utilised an anaesthetic combination of opioid (etorphine) and α2-agonists (detomidine or xylazine) (Kock, 2000). The effects of the opioid are then reversed by an antagonist. In horses, endogenous opioids participate in the regulation of the reproductive function, by inhibiting LH release in mares during the luteal phase

as well as in seasonally anovulatory mares and stallions (Aurich *et al.*, 1996). The interference of exogenous opioids with the normal pattern of cyclic ovarian activity in translocated black rhinoceros females is however unlikely as reversal agents have been reported to reverse the morphine blockade of ovulation (Packman & Rothchild, 1976).

Another potential effect of translocation on breeding success in black rhinoceros is that some pregnant females might lose their foetus or calves, as discussed in chapter 4. Translocation is associated with some stress, as indicated by the blood parameters measured during such operations (Kock *et al.*, 1999). In domestic animals, stress associated with transport and transfer to a new location has resulted in higher embryo loss in ewes while transport has resulted in a high incidence of silent heats in cows (Hansen, 1997). Alternatively, reproductive failure following translocation might originate from the male. In llama, stress-induced testicular degenerations occurred after translocation, returning to normal within two years (Neely & Bravo, 1997). The reversible character of this condition bears some resemblance with what occurs in translocated black rhinoceros, as a translocated adult male of prime breeding age has been observed to be very unsettled and not associated with any breeding success during at least five years following translocation (Garnier *et al.*, in press). It is therefore possible that a combination of stress-induced reproductive inhibition/failure originates from both males and females in this species, which underlines the importance of conducting immobilisation and translocation procedures with minimum stress.

Reproductive success of translocated breeding nuclei to new areas is also dependent upon respecting of the social structure and mating systems of the species involved (see next section). This has recently been observed when young African

elephant developed a very aggressive following translocation, having been deprived of their normal social and learning environment (Roque de Pinho, 1998).

5.8 FERTILITY PARAMETERS IN THE CAPTIVE POPULATION

The captive population studied in the UK showed some of the best growth rates (5.2%) ever observed in captivity, although this was still lower than that of the wild population. The high fecundity rate (10.4%) was largely due to the predominance of females in the adult population and the relatively low calf index (28.5%) showed in fact that only a small proportion of adult females in this population had calves. Among females without calves, 75% were nulliparous and aged between seven and 11 years old. The mean age at first calving in the captive population was reached nearly 2.5 years later than in the wild and had still not improved since previous surveys were undertaken in captivity (Lindemann, 1982; Smith & Read, 1992). Great individual variations were however observed and some females did actually reach sexual maturity nearly as early as in the wild. In addition, adult females that had started to produce also exhibited long calving intervals (3.5 years).

Should captive females have similar performances to those determined in the wild population (considering that they last conceive between 25-30 years), each female would be able to produce around 8-10 calves during her reproductive life. Rookmaker (1998) established that 41% of captive black rhinoceros females had produced only one calf and 42% had produced 2-4 calves, showing that there is a clear need to improve reproductive output in captivity. It has been already been mentioned that fertility parameters may have been influenced by environmental factors (nutrition, social environment, photoperiod) as well as non-environmental factors (diseases).

These factors will therefore be considered separately in the following discussion, with the view to improve fertility levels of this species in captivity.

5.8.1 *Influence of nutrition*

The relation between body weight and age at sexual maturity has already been discussed concerning wild black rhinoceros females. The first two to three years of a black rhinoceros' life are key in the body growth process, since wild calves attain around half of the adult size between one and two years and three-quarters of the adult size between two and three years (Brooks, 1989; Shenkel & Shenkel-Hulliger, 1969a). The importance of nutrition during these first years is also corroborated by the facts that the semi-wild females that first gave birth at an average age of 9.5 years had been hand-raised, while two out of three captive nulliparous that had not bred successfully at 9 and 11 years had also been hand-reared. The providing of adequate milk composition to the calf is difficult since there is limited knowledge on black rhinoceros milk composition, which also changes during a lactation cycle (Fowler, 1986; Robbins, 1993). Furthermore, adequate feeding of young calves with solid food, as observed when the calf is a few weeks in the wild, may also be difficult to sustain in captivity. Black rhinoceros are selective browsers which can feed on more than 200 plant species in the wild (Estes, 1992; Skinner & Smithers, 1990). By contrast, white rhinoceros are pure grazers which are much easier to feed in captivity. The probable contribution of inadequate feeding to delayed sexual maturity in captive black rhinoceros is reinforced by results from a recent survey. It was determined that in captivity, 50% of primiparous black rhinoceros females were older than 10 years, while 56% of primiparous white rhinoceros were 4-6 years old when they first gave birth (Rookmaker, 1998).

The nutritional status of multiparous females at the time of breeding, as well as during gestation and lactation, is also important for successful conception, foetal and calf growth. In cervids, there is evidence that the physical condition at the time of breeding is a primary determinant of their productivity (Albon *et al.*, 1986). In the wild black rhinoceros population studied, most conceptions were determined to occur during the early rainy season. This time of the year probably corresponds to maximum in protein intake, as was observed in a study of the monthly changes in nutrient intakes of the greater kudu (*Tragelaphus strepsiceros*), which is also a browser (Owen-Smith & Cooper, 1989). The protein level is known to be an important factor for successful conception in ewes, in which there is also an inverse relationship between nutritional levels during early pregnancy and plasma progesterone levels (Clarke & Tilbrooke, 1992). In some cases, a simple increase in protein or caloric intake may be responsible for the breeding activity associated with the availability of green vegetation resulting from rainfall. In other cases such as in horses, it has been found that specific compounds, such as 6-methoxy-2-benzoxalolinone, stimulated reproduction (Asa, 1996). Vitamins and minerals also have an important role in metabolism and a deficiency in vitamin A or E, in phosphorus or magnesium can result in ovarian dysfunction, and even anoestrus, in domestic ungulates (Ferrell, 1991). The level of phosphorus might need to be particularly monitored in captive black rhinoceros, for which cases of hypophosphatemia have been recorded and associated with hemolysis in several captive black rhinoceros (Paglia *et al.*, 1996). The fact that results from the study of Owen-Smith & Cooper (1989) indicate that the phosphorus intake for kudu was maximum in November, when a conception peak was also observed in black rhinoceros, tends to corroborate the importance of this mineral in reproductive success.

During gestation, the nutritional needs of females also increase to cover those of foetal development although most foetal growth occurs after 50-60% of the gestation period has elapsed, i.e. after 8-9 months in the black rhinoceros (Robbins, 1993). The metabolic costs of female mammals have been estimated to amount to twice the average daily metabolic rate during the last third of pregnancy and to three times this metabolic rate during lactation (Caughley & Sinclair, 1994). The supplementation of black rhinoceros females during the last five months of gestation and during lactation is therefore essential in ensuring the normal growth of the foetus and the calf. The production of calves with low birth weight is not only potentially associated with stillbirths, but it also seems to be a predisposing factor for certain diseases such as leukoencephalomalacia in black rhinoceros (Kenny *et al.*, 1996).

5.8.2 Influence of social environment

The social environment in which captive black rhinoceros females are brought up and subsequently maintained was also found to be an important factor in successful breeding. The importance of respecting the social structure of wild species that are kept in captive exhibits has recently been demonstrated in cheetahs by using faecal steroid analysis (Brown *et al.*, 1996). In this species, it was found that reproductive suppression might occur in captive females housed together, since wild females are principally solitary.

Black rhinoceros have traditionally been considered to be solitary animals, the only stable bond being between the mother and calf (Goddard, 1967; Ritchie, 1963; Shenkel & Shenkel-Hulliger, 1969a). However, Joubert & Eloff (1971) had already noted that these animals might not be as solitary as previously thought, since black rhinoceros that had overlapping home ranges and shared the same water holes tolerated each other, thus forming a "community" or a "clan".

Recently, a non-invasive genetic analysis of paternity and relatedness in the wild black rhinoceros population monitored in the present study indicated that these animals might in fact have a loose family structure (Gamier *et al.*, in press). This was suggested by various facts: 1. A group of five animals that were occasionally seen together consisted of a mother and her young calf, her two previous calves that had become subadults and a breeding bull; 2. Subadult females remained in their mother's area until they attained sexual maturity and subsequently went back to their mother or to another adult female and calf until they produced their first calf; 3. Subadult females often paired with subadult males which were identified as being sometimes their brothers; 4. Subadult males left their maternal area only at 6-8 years old, when they probably reached sexual maturity.

Both Joubert & Eloff (1971) and Owen-Smith (1988) had also observed that black and white rhinoceros young females kept interacting with their mothers until they produced their first calves. The fact that in the present study, sexual maturity was observed to be reached at an early age when wild females were translocated with their mothers, while hand-reared females exhibited delayed sexual maturity compared to wild females, emphasises the importance of the mother/daughter relationship. This leads to the recommendation to translocate young females either with their mothers, or only once they have started to reproduce.

The importance of the role of the mother in the normal process of sexual maturity cannot be dissociated from the fact that young females spend at least the first 23 months of their lives permanently accompanied by their mothers. In addition, it was observed in two instances that the mother progressively shifted her home range away, thus leaving her daughter to occupy some of the area where she had been brought up. This concept would explain why immature females have been recently

found to sustain more mortality than older age classes after translocation (Adcock, 1996; Brett, 1998).

While a nutritional dependence obviously links the mother and daughter during the first year of life, it is likely that the mother keeps playing an important role afterwards, including both protective and educational ones. Young elephants have a nutritional dependence of 2-4 years but remain very close to their mothers during the first five years of their lives, during which they acquire foraging skills and gain experience in the social organisation that characterises this species (Moss, 1988).

Another potential role played by the mother, or another mature female, might be in favouring the onset of reproductive activity in the young female, possibly indirectly through contacts with adult males. In domestic animals, exposure to stallions is known to play a role in puberty in young mares, while the contact with a mature bull also affects age at sexual maturity in heifers (Evans *et al.*, 1997; Stevenson, 1997). The presence of a male has also been found to stimulate puberty in captive females oryx (Blanvillain *et al.*, 1997).

Traditionally, black rhinoceros have been kept in pairs in captivity, if possible from an early age, and females are sequentially penned with males during oestrus. However, two pairs that were monitored during the study have not been successful in reproducing and the females were still nulliparous at 9 and 11 years old, although they had started to show signs of reproductive activity as early as five years earlier. It has already been reported that sexual interest will decline in a pair that is continuously run together, both in the black and white rhinoceros (Lindemann, 1982; Schwarzenberger *et al.*, 1999b).

The lack of breeding success may be attributed to sub-fertility of the male. Although both males were of breeding age when reproductive activity was observed,

it is possible that a decline in male sexual activity occurs when a male is continuously run with the same female. This has also been observed in llamas and white rhinoceroses and could be reversed when the male was introduced to new females (Brown, 2000; Göltenboth *et al.*, 2000). This is further supported by observations during the study that multiparous females successfully bred after a change of male. The "Coolidge effect", or enhanced mating performance of males with novel females (Nelson, 1995), may also exist in the wild. A polygynous mating system was found in the wild, with one male clearly dominating the breeding during the study, suggesting potential differences in male fertility levels (Garnier *et al.*, in press). Most females bred with the same male for the period covered, i.e. for the production of two to three calves, but this apparent monogamy may be the result of the highly skewed reproductive success in males. It is probable that a change of the dominant male would have been observed if the study had involved more animals and had lasted longer, as it has been observed in the white rhinoceros (Owen-Smith, 1988).

It is also possible that the failure to breed in captive animals run as pairs originated from the females. It has already been seen that hand-rearing may have contributed to them being late in showing the first signs of reproductive activity compared to wild females. In addition, one female might also have developed a reproductive pathology, probably ovarian cysts.

Furthermore, the pairing of animals from a young age might lead them to consider each other as siblings and thus to prevent successful breeding from occurring. This has been suggested to occur in white rhinoceros (Schwarzenberger *et al.*, 1999b) and also in black rhinoceros (N. Lindsay, personal communication). This is also corroborated by observations made during the study, when young wild females paired temporarily with sub-adult males, which could in fact be their brothers. These

pairs of sub-adult are unlikely to subsequently become breeding pairs, since closely related individuals usually do not mate with each other in a wide variety of species (Berger & Stevens, 1996). In addition, it was also observed that sub-adult males shifted their home ranges from their maternal area when they were 6-8 years old, corroborating the observations of Adcock *et al.* (1998). This correspond to the time when they become sexually mature in the wild, although prime breeding age is only reached later (Adcock, 1994; Garnier *et al.*, in press).

Interestingly, another young pair which had been managed together since they were very young bred successfully. However, the female had not been hand-reared and the young male also reproduced with an older multiparous female. The pairing of a young male with an older female has also been evaluated as being a successful combination by Carlstead *et al.* (1999b), on the basis that breeding success in captive black rhinoceros was positively correlated with a higher dominance of the female over her male partner. Although their finding on the pairing of animals correlates with ours, their evaluation of dominance is only defined as a temperamental trait and cannot be considered as an accurate reflection of the dominance relationships that may occur in the wild.

The management of two female black rhinoceros together also appeared to be beneficial to successful breeding in captivity. In two instances, a captive nulliparous female successfully conceived after having been managed with another cycling female. In the wild, it was observed that a dominant black rhinoceros mated with two to three females, which also had overlapping home ranges (Garnier, unpublished observation). The management of one male with two females has equally been found to be a successful technique for breeding black rhinoceros in captivity (B.White, personal communication). These observations are however in contradiction with

results of Carlstead et al. (1999a), who established that reproductive success in captive black rhinoceroses was negatively correlated with the density of females in an institution. However, the authors did not consider the influence of reproductive disorders nor of nutrition in their study. The positive influence of managing two females together may exert itself by a certain induction of ovarian synchrony and changes in cycle length by stimuli from the same sex, as has been demonstrated in primates, cattle, sheep and goat (Asa, 1996; Nelson, 1995).

The social environment of captive black rhinoceros therefore appears to play a key role in reproductive success. Although no definite conclusion can be drawn, it nevertheless appears that black rhinoceros are not as solitary as previously thought and have a loose family structure. The mother-daughter bond seems important until the young female reaches sexual maturity and the mother, or another mature female, might play a certain role in the normal puberty process of young females. The pairing of young animals from a young age is also a pattern that occurs in the wild but which might not persist when they become sexually mature.

The association of two or three adult females with one male might also be beneficial for successful breeding, providing that a change of male does occur from time to time. For these reasons, it can be hypothesised that a good strategy when establishing a breeding nucleus of black rhinoceros might be to introduce two adult bulls, with three to four adult females and a pair of sub-adults, male and female. This also coincides with the recommendations made for establishing a breeding nucleus of white rhinoceros (Pienaar, 1994) and underlines the importance of managing captive animals in extensive systems of large paddocks.

5.8.3 *Influence of diseases*

Diseases can interfere with successful breeding either directly, through reproductive pathologies, or indirectly, by reducing the lengths of periods during which the male could be kept with the female. Feet problems were found to be a common source of illness, as had already been noticed in previous reports (Kock & Garnier, 1993). The deterioration of the general condition linked to serious diseases can contribute to decrease the breeding performances of the animals, as noted in most domestic species (Bondurant, 1991).

Reproductive pathologies were not diagnosed during the study, although it was suspected in two nulliparous females with symptoms of silent heat and increased aggressiveness. These symptoms coincided with reports in other nulliparous black rhinoceros diagnosed with ovarian and endometrial cysts (Göltenboth *et al.*, 2000). In domestic animals, cystic ovaries have been associated with adrenal hyperfunction, post-partum uterine infection and disease (Peter, 1997). It has been suggested that they develop more frequently in high producing cows but not every work supports this concept. However, there is a strong relationship between cystic ovarian disease and an impairment of the pre-ovulatory surge of LH, which might also be involved in black rhinoceros females suffering from that disorder. The incidence of reproductive disorders in black rhinoceros requires further evaluation.

5.9 CONCLUSION

This study has represented the first long-term and longitudinal non-invasive evaluation of fertility on a large African mammal in its natural environment. Individual reproductive monitoring of black rhinoceros females could be achieved by combining one of the oldest traditional local skills, tracking, with faecal steroid analysis, one of the latest techniques available for wildlife reproductive studies.

Non-invasive reproductive assessment in wild black rhinoceros females enabled the diagnosis of pregnancy, the monitoring of foetal viability and of oestrous cycles in this species. Such evaluations are essential for management decisions, which need to be based on an accurate knowledge of reproductive potential and performances, both at a population and individual level. It was also established in the study that black rhinoceros presented a seasonal pattern of reproductive activity that was influenced by rainfall pattern and possibly photoperiodicity and temperatures. A great variability in oestrous cycle lengths and characteristics was also found to occur in wild females exhibiting good fertility levels. Both findings emphasise the need to characterise naturally occurring reproductive patterns in wildlife for the development of appropriate captive breeding programmes.

The evaluation of fertility parameters for the wild black rhinoceros population studied in the Save Valley Conservancy indicated that its breeding output was one of the most successful recorded for this species. This was most likely linked to the very low density of animals and suitability of black rhinoceros habitats in the area. Great individual variations were nevertheless recorded, corroborating the importance of monitoring reproductive performances at an individual level.

It was also established in this study that translocation did not impact on sexual maturity in young females providing that were translocated with their mother, underlining the importance of respecting the mother/daughter bond. By contrast, this study confirmed that the main impact of translocation on reproductive output was to delay the resumption of calf production by 3.5 years. This may be linked to the social disruption associated with translocation, but also to the potential effects of stress on the reproductive physiology of males and/or females. The continuous non-invasive monitoring of black rhinoceros before and after translocation would enable the identification of a more precise origin.

Finally, the evaluation of reproductive output in captive females showed that it had improved compared to previous surveys, but that it was still lower compared to the wild. Some young females were still nulliparous although they had started to cycle, while multiparous females had lower conception rates than in the wild. Subtle social requirements appear to be fundamental in the successful breeding of this species. These do not seem to be fulfilled when only managing a single pair of black rhinoceros, but appear to be satisfied when animals are managed in an extensive system of large paddocks. These management systems obviously allow for a better stimulation of a free-living environment and permit a regular change of breeding males, as well as the running of more than one female with a male. The existence of relations of male dominance in the wild, as well as of polygyny, may lead to great variations in male fertility levels, which require further evaluations.

Male fertility was not evaluated during the study but the same non-invasive method could be developed and applied to black rhinoceros bulls. Together with the recent development of a non-invasive technique for assessing paternity and relatedness in wild black rhinoceros (Garnier *et al.*, in press), this underlines the

importance of developing new management tools for optimising the success of conservation strategies. Nevertheless, recent demographic trends for this species underline how precarious the short-term future of wild populations can be and how important it is to concentrate *in situ* research efforts in multi-facetted studies.

There is still much to learn from this extraordinary species, which has just started to reveal some of its unique characteristics. These have most likely contributed to its survival and adaptation throughout millions of years and need to be elucidated before it is too late.

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