

# **RHINO**

## **Husbandry Manual**



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**Editors**

**INTERNATIONAL RHINO FOUNDATION**

Association of Zoos and Aquariums Rhino Advisory Group



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# TABLE OF CONTENTS

<i>Acknowledgements</i> .....	6
<i>Preface</i> .....	7
<b>Part I.</b> .....	<b>8</b>
Crisis for Rhinos .....	8
Taxonomy and Conservation Status: Overview .....	8
<b>Part II. White Rhinoceros</b> .....	<b>15</b>
Taxonomy.....	15
Management.....	15
Reproduction.....	24
Enrichment.....	29
Training.....	33
Transportation.....	37
Enclosure Design.....	40
Health .....	49
Nutrition.....	65
Research .....	72
Behavior .....	80
<b>Part III. Black Rhinoceros</b> .....	<b>91</b>
Taxonomy.....	91
Management.....	92
Reproduction.....	100
Enrichment.....	104
Training.....	108
Transportation.....	112
Enclosure Design.....	115
Health .....	123
Nutrition.....	144
Research .....	152
Behavior .....	160
<b>Part IV. Greater One-Horned Rhinoceros</b> .....	<b>167</b>
Taxonomy.....	167
Management.....	167
Reproduction.....	176
Enrichment.....	180
Training.....	184
Transportation.....	188

Enclosure Design.....	191
Health .....	200
Nutrition.....	216
Research .....	224
Behavior .....	231
<b>Part V. Sumatran Rhinoceros .....</b>	<b>237</b>
Taxonomy.....	237
Management.....	238
Reproduction.....	243
Enrichment.....	245
Training.....	249
Transportation.....	252
Enclosure Design.....	255
Health .....	263
Nutrition.....	281
Research .....	288
Behavior .....	296
<b>References .....</b>	<b>300</b>
<b>Appendix A. Javan Rhinoceros .....</b>	<b>320</b>
<b>Appendix B. Trained Behaviors.....</b>	<b>321</b>
<b>Appendix C. Example Training Plans.....</b>	<b>322</b>
<b>Appendix D. Training Documentation Form.....</b>	<b>323</b>
<b>Appendix E. Instructions for Rhinoceros Estrus     Record-Keeping System .....</b>	<b>324</b>
<b>Appendix F. White Rhino Estrous Behavior Record Sheet .....</b>	<b>326</b>
<b>Appendix G. Greater One-Horned Rhino Estrous Behavior     Record Sheet.....</b>	<b>327</b>

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# PREFACE

A rhinoceros husbandry manual is a dynamic document, representing our current understanding of the rhinoceros in managed settings, and presenting a diverse set of parameters and recommendations for managing this unique, iconic mammalian taxon in zoo collections. In 1996, the Association of Zoos and Aquariums Rhino Advisory Group (AZA RAG) and the International Rhino Foundation (IRF) collaborated to produce the first Rhinoceros Husbandry Resource Manual, published by the Fort Worth Zoo and edited by Michael Fouraker and Tarren Wagener. This excellent reference has weathered the times, and with great relevance to rhino husbandry in 2013, the historic document has formed the basis for this new edition.

Recognizing that the depth of our rhino management experience has improved, that our skills, tools and techniques have been refined, and that targeted research projects have shed new light on our base of knowledge, the RAG and IRF have worked to gather crucial new information and input to update the Rhinoceros Husbandry Manual. At the 2009 bi-annual working meeting of the AZA RAG, held at Fossil Rim Wildlife Center, the Steering Committee formed a working committee and developed the outline for the 2014 Rhinoceros Husbandry Manual. A second planning meeting was held at the San Diego Zoo Safari Park in 2010 to review progress and refine the production process.

Management of four rhinoceros species, white rhino, black rhino, greater one-horned rhino and Sumatran rhino, all established in zoological facilities in North America, is the focus of this manual. The life history and distribution of the Javan rhino is discussed in Part I and in Appendix A. This manual presents recommended guidelines for the successful maintenance of rhinos in the zoo setting given the scientific data currently available. These guidelines represent current optimal recommendations for participation in the AZA's Species Survival Plans (SSPs) and in no way reflect U.S. Department of Agriculture (USDA) minimum standards. Although necessary for participation in rhinoceros SSPs, the guidelines do not supersede USDA mandates for the exhibition of rhino species. Additional information concerning these recommendations may be gathered by contacting the respective SSP coordinators. It should also be emphasized that these recommendations are guidelines, and in all cases common sense concerning enclosure design and routine zoo animal management should be used. Parameters exclusive to an individual institution also must be considered (e.g., climate, local and state laws, etc.).

As a living document, the AZA RAG and IRF intend this information to be revised and updated on a regular basis. The electronic format provides opportunities for revision as new information comes forward and/or as new husbandry and management techniques are developed. While much data was compiled for this manual, many holes in the knowledge-base still exist. It is our intention that this manual be the catalyst for scientific inquiry into the management of rhinos in zoos and conservation centers. To that end, there have been updates made to this document over the years, and this DVD is the most up-to-date document available at this time. The goal is to be able to update the manual quickly and to keep the most relevant issues available to rhino managers and facilities that may decide to work with rhino species in the future. The format has been developed in anticipation of the inclusion of reports and projects developed through international collaboration between SSPs, EEPs and TAGs in other zoo associations and regions. We hope you find this document to be an important resource for rhino management.



# PART I

## CRISIS FOR RHINOS

In the middle of the last century, all five rhino species were widely distributed and most abundant throughout Asia and Africa. As of mid-2013 only about 29,000 rhinos of all kinds survive in the wild. Another 865 exist in captivity (Table 1.1). However, more than 80% of these rhinos, both in the wild and in captivity, are of a single species, the white rhino (*Ceratotherium simum*). The other four species combined comprise fewer than 8,500 individuals. Populations have been growing for the past several years for all but the Sumatran rhino (*Dicerorhinus sumatrensis*), which has rapidly decreased, and the Javan rhino (*Rhinoceros sondaicus*), which we believe has remained relatively stable (Fig. 1.1). Although there has long been a debate among conservationists as to whether subspecies are different enough that they should be conserved as separate units, in some cases, such as the Sumatran and Javan rhinos, subspecies issues are superseded by the species' severely limited numbers. This provides only a small number of management options—the least of which may be genetic in nature.

Table 1.1. Conservation status of the rhinoceros.

Species or Subspecies	Wild Population	Global Managed Breeding Population
Southwestern black rhino	1,957	0
Northwestern black rhino	0	0
Eastern black rhino	799	145
Southern central black rhino	2,299	41
<i>Total black rhino</i>	5,055	186
Northern white rhino	4	3
Southern white rhino	20,405	550
<i>Total white rhino</i>	20,409	553
<b>TOTAL AFRICAN RHINO SPECIES</b>	25,464	739
Greater one-horned rhino	3,333	198
Javan rhino	35-44	0
Eastern Sumatran rhino (Borneo)	3-5	3
Western Sumatran rhino (Sumatra/Malaya)	100	7
<i>Total Sumatran rhino</i>	105	10
<b>TOTAL ASIAN RHINO SPECIES</b>	3,482	208
<b>TOTAL ALL RHINO SPECIES</b>	28,946	947

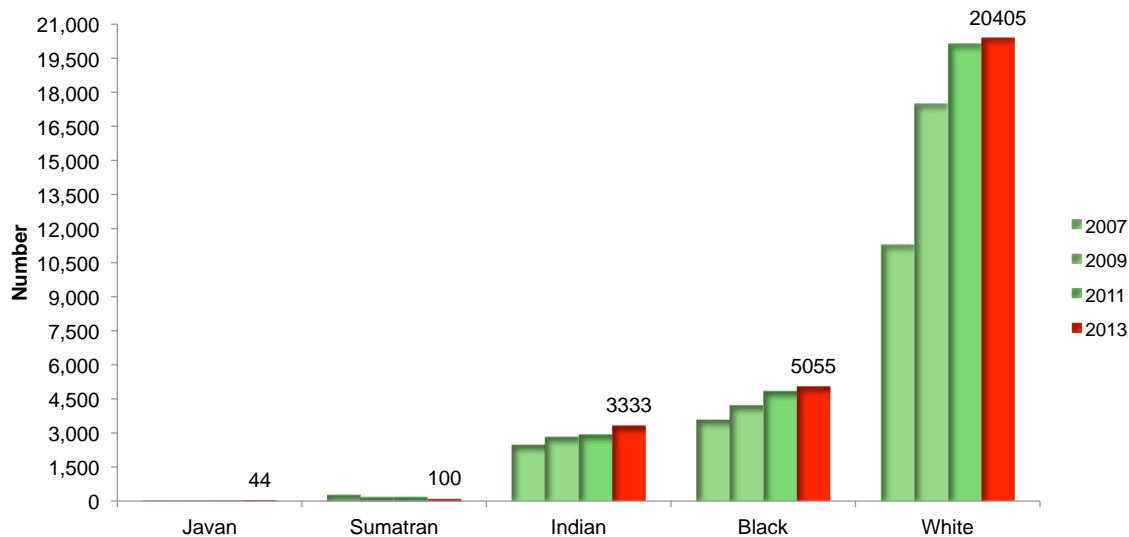


Fig. 1.1. Population numbers of the five rhino species 2007 through July 1, 2013.

The Javan rhino is the rarest of the species, with a total population estimated at between 35 and 44 in the wild and none in managed breeding situations. The last individual of the Javan rhino subspecies *Rhinoceros sondaicus annamiticus* was declared extinct in Vietnam in 2010 by World Wildlife Fund and the International Rhino Foundation; now only one population remains in Indonesia's Ujung Kulon National Park (Brook et al., 2012). However, while the Javan rhino is fewer in number, the Sumatran rhino's rapid decline (roughly 75% over the last 20 yr) makes it the most critically endangered of all the rhino species and perhaps of any large mammal. The IUCN officially declared the northwestern black rhino (*Diceros bicornis longipes*) extinct in the wild in 2013, and the northern white rhino (*Ceratotherium simum cottoni*) became extinct in the wild in 2008.

There are, however, success stories in rhino conservation. With concerted government commitment and efforts by dedicated conservationists, including protection and consolidation of populations, three of the five rhinoceros species have slowly recovered from the brink of extinction. Southern white (*Ceratotherium simum simum*) and greater one-horned or Indian rhinos (*Rhinoceros unicornis*) have both come back from fewer than 100 animals in the early 1900s, now numbering more than 20,000 and 3,000 respectively. Black rhinos (*Diceros bicornis*) faced a serious poaching crisis in the early 1990s, which led to the loss of 97% of the population. Management measures, including consolidation of populations through targeted translocations, strategic dehorning, and active protection have helped the species recover to more than 5,000 animals, most living in fragmented populations. Significant black rhino populations now survive in only nine countries (South Africa, Namibia, Kenya, Zimbabwe, Tanzania, Zambia, Malawi, Swaziland and Botswana, in descending order of population size). Unfortunately, rhino poaching is now rampant again, with poaching losses threatening to overshadow previous conservation progress in population growth.

### The Cause of the Crisis

Rhino species have faced many different challenges over the past few decades. As with many endangered species, fragmentation and loss of habitat is an important factor, but not the major cause of the rhino's decline. The greater global problem is overexploitation through poaching for rhino horn. Rhino horn has been used for centuries in traditional Chinese medicine as a fever reducer. China has been the primary consumer country until recently. Now, the burgeoning market

is in Vietnam, where the economy has rapidly grown and purchasing power is increasing. In addition to traditional use, rhino horn also has been touted as a cancer cure and a purported hangover preventative in Vietnam. It also is given as a high-value gift item. In the Middle East, horn has been used to make dagger handles that confer social status, but this threat has paled in comparison to the growing consumption in Asia.

### **Africa**

Rhino poaching is driven by crime syndicates that might have entered Africa as infrastructure (e.g., roads and building construction), and extractive industry (e.g., mining) contracts have been awarded to Asian companies operating in Africa. Poaching syndicates are highly systematic and strategic; poaching gangs are generally well-funded, well-equipped, and ruthless. From 2000–2007 there was a reduction in poaching in southern Africa, partly because of increased anti-poaching efforts. Since 2008, however, poaching has been on a steady increase. In South Africa alone, 333 rhinos were killed in known poaching events in 2010; by 2012, numbers had increased to 668 (about one rhino every 9 hr) and by the end of 2013, three rhinos were lost in South Africa to poaching each day (Fig. 1.2). The year 2013 may well prove to be the tipping point for African rhinos, with population growth unable to keep pace with poaching losses.

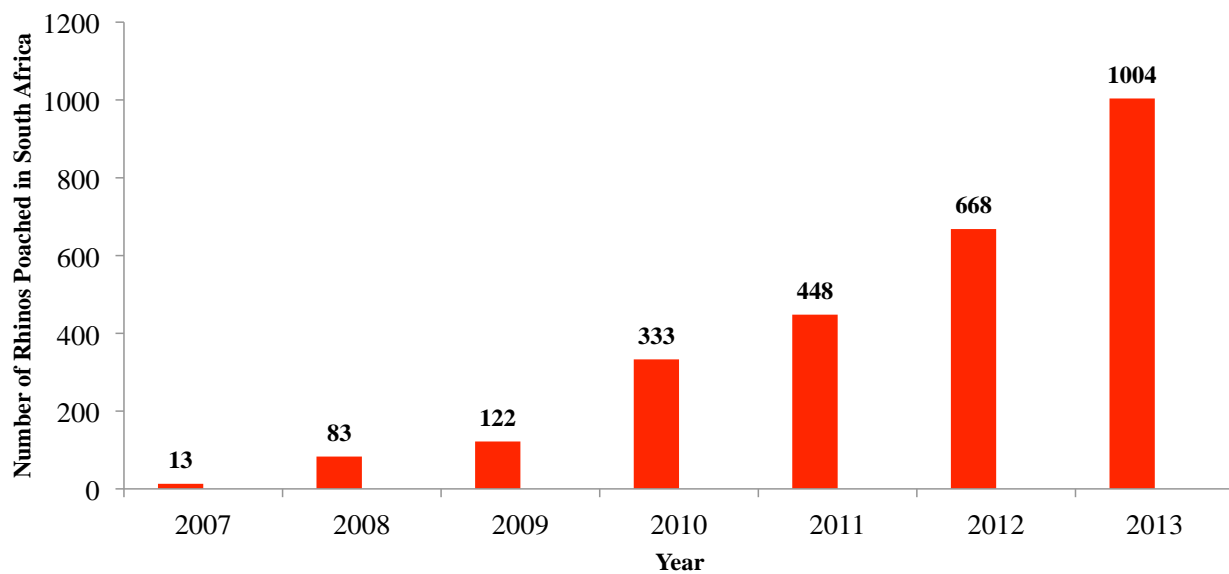


Fig. 1.2. Rhino poaching losses in South Africa alone 2007 to 31 December 2013.

### **Asia**

Nepal and India's greater one-horned or Indian rhino also is threatened by active and expanding poaching, but so far, to a lesser degree than African rhinos. In Malaysia, Sumatran rhinos have essentially been wiped out in the past ten years by poaching and habitat loss with recent information suggesting that only a handful of animals remain in one area in the state of Sabah.

Rapid decrease in numbers due primarily to poaching is the major, but not the only, problem encountered in the conservation of rhinos. Habitat loss and fragmentation is occurring in many areas, which leads to small isolated populations. Small and fragmented populations make it difficult for genetic exchange and adaptation to changing environmental conditions. This also may lead to long-term reproductive abnormalities as animals are less and less able to find each other to breed.

In Indonesia, rhinos are under greater threat from habitat fragmentation and loss to infrastructure development such as roads. Even with protection, with the exception of one national park (Way Kambas), Sumatran rhino numbers continue to slowly decline. Fortunately to-date, documented poaching has been rare in the last stronghold for Javan and Sumatran rhinos. If Indonesia's populations of rhino were to experience the poaching pressure seen in Africa, these two species would be wiped out within months.

## Conservation of Rhinos

If rhinos are to survive long-term in the wild, they must be intensively managed and protected, with a spectrum of options to maximize options for the future need. These options represent a continuum with respect to intensive management required and range from management within protected areas or semi-free-ranging reserves to conservation centers to zoos.

Zoos and conservation centers may increasingly provide key portions to the available options within the conservation spectrum. Animals in these facilities can play a number of important roles, serving as: (1) ambassadors for their wild counterparts; (2) instruments for education for local communities and the general public-at-large, (3) research populations that allow scientists to learn as much as possible about the basic biology of species (which may be difficult to study in nature); (4) 'flagship species' to protect and call attention to other threatened wildlife that share their habitat; (5) an 'insurance' population that can be used to re-establish or revitalize wild populations that have been severely reduced or extirpated (provided that adequate protection measures can be put in place in former ranges); and (6) a means to attract attention and support, financial and otherwise. However, it must be emphasized that *ex situ* populations and programs are not a be-all and end-all in themselves; the primary purpose should be to support applied learning that can be used to assist the survival or recovery of the species in the wild.

As the rhino crisis intensifies, expertise in small population management may become more and more important. For some species, such as Sumatran, Javan and black rhinos, implementation of these principles may hold the only hope for future survival. Zoos and conservation centers are well-positioned to provide inputs using examples from highly-organized and scientific programs such as Species Survival Plans (SSPs). SSPs have traditionally been managed in the United States and Canada by Species Coordinators and Management Committees in cooperation with Taxon Advisory Groups (TAGs), which provide strategic perspectives and technical advice for SSPs and participating rhino-holding institutions.

Managed breeding, however, is not all genetics and demography. Basic husbandry, how to maintain healthy individuals and induce them to breed, is fundamental. There are major challenges for rhino husbandry in zoos and conservation centers, which may present some of the most formidable captive management challenges of any species. As a consequence, all taxa of rhinos maintained in zoos and conservation centers are in some degree of demographic difficulty. These demographic problems are causing genetic difficulties because some lineages are at risk of being lost as their representatives are not reproducing. Clearly, husbandry, demography and genetics interact; and all three are crucial to the conservation of rhinos, especially in managed breeding situations. Husbandry problems can impede intensive management of rhino species (Table 1.2). Specific problems include poor survivorship and high mortality, poor reproductive success and a fundamental lack of knowledge regarding basic rhino biology.

Table 1.2. Status of managed rhino populations within the AZA (modified from Foose and Reece, 1994).

<b>Species</b>	<b>Status</b>
<b>Eastern black rhino</b>	This species reproduces rather reliably in captivity, but management has not maximized the reproductive potential, and health/husbandry problems continue to negate the breeding success that has occurred. As a result, the species is in a demographic crisis; thus, it is imperative to increase reproduction through improved management and expanded capacity to grow the population.
<b>Southern black rhino</b>	Reproduction in this species has been moderate, with many of the initial births in captivity actually conceived in the wild. Captive reproduction is on the decline and mortality has been high (although much is probably due to toxin exposure in Africa). This species is now managed as a non-AZA population by the International Rhino Foundation, which is working with AZA facilities, private owners, and the native range countries.
<b>Southern white rhino</b>	Reproduction in this subspecies has been very uneven with only a few facilities, particularly those able to maintain larger social groups, propagating well. The majority of rhinos in institutions are not breeding at all; thus, the population is in demographic and genetic crisis. The age structure of the population is senescing, and not enough of the original wild-caught founders have reproduced. There have been numerous imports over the years, but many of those new imports continue to not breed. The demographics of the southern white rhino population are still in need of work, and hopefully some of the new imports will breed.
<b>Northern white rhino</b>	The program for this subspecies has been a failure to date. Only seven individuals survive, and reproduction in captivity has been limited (none in North America and none anywhere since 1989).  Intense efforts are in progress to induce reproduction, but the prospects are limited at best. Four of the last northern white rhinos in Dvur Kralove went to Ol Pejeta in Kenya and have been “released” with the southern white rhinos in the hope that even with hybridization, the northern white rhino genes may be maintained through offspring. All the wild northern white rhinos that had previously been in Garamba National Park, Democratic Republic of the Congo, were poached. There are no longer any wild northern white rhinos left in their former range.
<b>Greater one-horned rhino</b>	This program has been relatively successful with the annual population growth rate about equal to what is occurring in the wild. However, much of the reproduction to date has been by a limited number of breeders; thus, the genetic diversity in the captive-born population is inadequate. Prospects do seem good for recruitment of more breeders from the existing captive population.

Table 1.2. Continued.

Species	Status
Sumatran rhino	This program has initially failed with numbers of individuals and founders low, no reproduction occurring and the death rate high (30% of those imported in the 1980's). However, in 2001, the Cincinnati Zoo was successful in breeding their pair of Sumatran rhinos and producing the first birth in captivity in 112 years. Since then, they have produced a total of three calves. One male was shipped back to Sumatra to join the managed breeding program at the Sumatran Rhino Sanctuary in Way Kambas National Park and has sired a calf there. Unfortunately, the adult female at Cincinnati has since died and the prospect of future zoo breeding in the U.S. is dependent on a sibling pairing at Cincinnati.

### **Poor Survivorship/High Mortality**

The browsing rhinos, black and Sumatran, in particular have problems with poor survivorship/high mortality under intensive management. The black rhino has been afflicted with many health problems (e.g., hemolytic anemia, severe ulcers on skin and mucous membranes, liver dysfunction). Both species are affected by iron storage issues, which may lead to other complications.

### **Poor Reproductive Success**

Reproduction in all four of the species that have been maintained in zoos and conservation centers is less than optimal. In general, greater one-horned, black and, to a lesser extent, white rhinos reproduce well in managed breeding situations if species-specific needs are met. Captive managers are still struggling to understand the spectrum of these needs. Although reproductive challenges have now been overcome for the Sumatran rhino, the learning curve was very steep before the species could be reliably maintained and bred. For no species of rhino in captivity is reproduction reliable or routine.

### **Poor Understanding of Basic Biology**

Compared with many other groups of organisms under intensive management, aspects of the basic biology (e.g., nutritional, reproductive, behavioral) of rhinos is poorly known. Nutritional problems are suspected to be of particular significance to the health and perhaps the reproductive difficulties of rhinos, particularly the browsing species. Behavioral issues also may interfere with successful husbandry. Various physiological and psychological challenges are believed by some researchers and managers to be underlying causal factors for many of the specific disease syndromes in rhinos. In recognition of husbandry challenges, a major goal of SSPs and the TAGs that facilitate them is the production of husbandry manuals, which can lead to successful management and propagation of species in captivity.

## **The Future**

In the coming decades, as rhino husbandry and small population management are refined in zoos and conservation centers, these techniques will undoubtedly have broader application to the intensive management of increasingly fragmented wild rhinoceros populations. *Ex situ* populations can contribute positively to the conservation of rhino species in nature, but only if we use these populations to gather as much information as possible that can be applied to proactive management *in situ*. Rhino-holding institutions also have the obligation to educate the public about the plight facing this magnificent taxonomic group, which grows more imperiled every day.



## TAXONOMY AND CONSERVATION STATUS: OVERVIEW

The following chapter outlines the taxonomy and conservation status of the five rhinoceros species. The *Rhinocerotidae* is a family of mammals in decline, with three of the five surviving species (black, Sumatran, and Javan) in crisis or on the verge of extinction. The other species (the white rhino and the greater one-horned rhino) have recovered to population levels less precarious than those of the other rhino species, but even these species suffer from limited distribution and ongoing, even escalating in some cases, poaching threats. The northern white rhino and western black rhino are now considered to be extinct in the wild.

### A Brief History and Overview of Rhinos

The family *Rhinocerotidae* has had a glorious history. Since the first rhinoceros appeared about 50 million years ago, the rhino family has enjoyed abundance and diversity, with many different species that assumed a wide variety of forms and occupied a broad range of niches often associated with other kinds of animals today. Some extinct rhinos had horns; others did not. Some rhinos appeared and acted like horses, others like hippos, some like tapirs, at least one like a small elephant, and several even like giraffes. Indeed, the largest land mammal that ever lived was a rhino.

Moreover, rhinos have not always been confined to Africa and Asia. During most of the last 50 million years, they also inhabited Europe and particularly North America. Rhinos were the most common of the large herbivores in North America for 40 million years and became extinct only because of drastic climatic changes about 5 million years ago. Today, five rhino species survive: three species in Asia and two in Africa.

In many ways, rhinos are typical ungulates. Ecologically, they function as primary consumers or herbivores, and like most ungulates, their weapons are primarily defensive rather than offensive. Rhinos generally inhabit savannahs, shrubby regions and forests in tropical and subtropical regions. Moreover, they are usually restricted to areas in which a frequent trip to water or mud holes is possible. Mud wallowing is especially effective in accelerating heat loss, as mud is slow to dry and absorbs body heat in the process. A thick coating of mud also helps protect against insects and parasites and keeps skin supple.

Generally speaking, rhinos require large individual areas because of their size and daily nutritional requirements. Most are fairly territorial, defending their home ranges to ensure adequate food and minimal reproductive competition. Territories are often marked with urine or dung, as rhino vision is poor but olfactory abilities are well-developed. Flakes of skin left on trees used as rubbing posts, as well as dried mud that falls from the skin, also carry individual scents and thus help establish territorial boundaries. Wide variation does exist, however, among the rhino species with regard to the size of individual ranges, the degree of territoriality, and social organization. General species descriptions are provided in (Owen-Smith, 1975; Kingdon, 1979; Laurie, 1982; Estes, 1991; Nowak, 1991; Dinerstein, 2003; Wilson and Mittermeier, 2011). The species descriptions included in the following chapters are summaries; specific ecological studies may cite data that differ slightly from these general descriptions. Word-origin information was obtained from Borror (1960).



# PART II

# WHITE RHINOCEROS

## TAXONOMY

### Scientific Name and Origin

*Ceratotherium simum*

*Ceratotherium*: Greek *cerato*, meaning “horn” and *thorium*, meaning “wild beast”

*simum*: Greek *simus*, meaning “flat-nosed”

### Common Names

African white rhinoceros: Afrikaans word describing its mouth, *weit*, meaning “wide”

Square-lipped rhinoceros: lacking a prehensile “hook”

### Distribution and Habitat

Southern and central Africa

Long- and short-grass savannas

### Size

1,800 to 2,200 kg (4,000 to 5,000 lb)

1.5 to 1.8 m (5 to 6 ft) tall at shoulder

Primary horn 94 to 201 cm (37 to 79 in) southern subspecies, 94 to 102 cm (37 to 40 in) northern subspecies

Largest land mammal, after elephants, along with the greater one-horned rhino

### Physical Description

Neutral gray, almost hairless

Two horns

### Life History Characteristics

Grazer

Semi-social and territorial: females and subadults rarely solitary; bulls typically solitary, though satellite males may reside within the territory of the dominant bull

Females sexually mature at five to six years in the wild; males at ten to 12 years

Gestation period approximately 16 months; interbirth interval of two to three years

## MANAGEMENT

### Group Composition in Captivity

White rhinos are considered semi-social, though adult males appear to be somewhat territorial and basically solitary. Adult females typically associate in pairs consisting of a female and her most recent offspring. A cow without her own calf may tolerate one or more juveniles, or two cows without offspring may associate together. Stable groups of as many as six rhinos may be formed in this way. Data on rhinoceros social organization, combined with preliminary analyses of parameters

affecting reproductive success in captivity, may be used to generate recommendations regarding possible social groupings in zoos and conservation centers (Tables 2.1 and 2.2). Many variables affect the probability of success for any managed social group, including the animals' dispositions and available holding space. Further, depending on space and animal and staffing availability, institutions may hold animals for breeding or for exhibit-only. Facilities that wish to hold rhinos for exhibit purposes only are advised to maintain two animals (male:female: 1:1 or 0.2 or, more recently, 2:0 because of the skew in male births, as well as the ability to house multiple females but only a single, possibly two, males at the breeding institutions). In white rhinos, bachelor male groups have been maintained in very large enclosures (e.g., game parks or ranches). The desire to hold exhibit animals should be expressed to the appropriate species coordinator so that pre- or post-reproductive or single-sex animals may be assigned.

Table 2.1. Possibilities for rhino social groupings within the same exhibit in captivity.

Rhino species	Multiple Animals of Same Sex		Multiple Animals of Opposite Sexes
	Adult males	Adult females	
White	rare (possible in exhibits >100 acres)	possible and recommended	possible and recommended for breeding (optimal: one male and two or more females); if possible, an additional male in proximity but not in same enclosure
Black	not recommended	possible in very large enclosures only; temporarily successful in some situations, but does not last	possible and recommended for breeding (optimal: 1:1)
Greater one-horned	not recommended	possible in enclosures $\geq$ 100 acres	pairings recommended only during peak estrus (with the exception of very large exhibits, which may hold a single male and female together consistently)

Table 2.2. Recommended numbers for institutional holding.

Rhino Species	Recommended Minimum Groupings for Breeding <sup>a</sup>	Preferred Optimal Holding for a Breeding Institution	Exhibit Only (per institution)
White	1.2	2.4 (1 herd/ 1 back-up male)	1.1 or 0.2 <sup>b</sup>
Black	1.1	2.2 (2 pairs)	1.1 or 0.2
Greater one-horned	1.1 <sup>c</sup>	2.2 (2 pairs)	1.1 <sup>c</sup> or 0.2

<sup>a</sup> See Enclosure Design chapter. Breeding institutions must have space for offspring to be held for up to three years following birth.

<sup>b</sup> Multi-male bachelor groups have been maintained in very large enclosures.

<sup>c</sup> In the case of greater one-horned rhinos, males and females should be introduced only during the female's estrous period. Institutions with very large enclosures may be able to hold opposite-sex animals together consistently.

For institutions that have the space and staff available for rhino breeding, it is recommended that managers commit one male and two or more females for this purpose (plus a back-up male). In addition, breeding institutions must also have space to hold offspring for up to three years after birth. Within a single exhibit or holding area, the recommended minimum numbers for breeding are one male and two females. In general, mature males should not be held together because of the increased likelihood of serious aggression. Furthermore, breeding success may be enhanced by separating males from females as little as possible.

Grouped or multiple-species exhibits are possible for white rhinos if ample exhibit space is available. Examples of institutions that have successfully maintained mixed-species exhibits include Fossil Rim Wildlife Center, San Diego Zoo Safari Park, Columbus Zoo, the Wilds, Lowry Park Zoo and Riverbanks Zoo. Species that have been successfully paired in an exhibit with rhinos include sarus cranes, herons, some antelope species (nilgai, blackbuck, gaur, Persian goitered gazelle), mouflon, zebra, and some deer species. In all cases, the dispositions of the individual animals, as well as adequate space and exhibit structure (i.e., visual barriers, refuge areas, etc.), are important to consider prior to attempting a mixed-species exhibit.

## Introductions

Changing social groupings of rhinos through the introduction of additional individuals to an established individual, pair or group is a process requiring care and planning. Rhino species vary widely in social structure, and rhinos periodically vary their grouping patterns in the wild according to factors such as reproduction and the rearing of young. Social groupings in captivity, therefore, should also vary according to species, as well as to the circumstances within each institution. Rhinos may be very protective of their individual boundaries, but proper introduction procedures can minimize injury from conflict and aggression. The following section outlines general considerations for any rhino introduction and provides systematic descriptions of aggression, procedural recommendations, and descriptions of potential species-specific introduction types.

Factors that must be considered in any introduction include individual animal personalities, staff experience and confidence level, and enclosure type (i.e., indoor/outdoor, public/off-exhibit,

relatively small/large). Barrier types and temperature should also be considered. Introductions often result in aggression, and it should be noted that rhinos of both sexes have been the aggressors. Territorial defense is often limited to ritualized confrontations, in which two rhinos advance toward each other but stop nose-to-nose and engage in a staring contest to gauge each other's size and strength. Also as part of this ritual, the two individuals may touch horns, back apart and wipe their horns on the ground (Nowak, 1991). More intensive conflicts involve head-on charges and the infliction of injuries by horning or ramming. In general, behaviors that have been noted during rhino introductions are listed in Table 2.3. It is important to note that what is often perceived as serious or dangerous aggression between rhinos is, in fact, normal behavior requiring no intervention of any kind. Along with increased size and thick skin comes decreased vulnerability compared with many other animals. Table 2.4 lists a descriptive hierarchy of aggression levels in rhinos.

Table 2.3. Behaviors noted during rhino introductions (Fouraker and Wagener, 1996).

Non-aggressive Behaviors	Ritualized Confrontations	Potential Stress-Related Behaviors	Aggressive Behaviors
Follow	Head sweep	Pacing	Charge/chase
Touch/rub/lick	Face-to-face stare	Running (excessive)	Open-mouth threat
Anal or genital investigation		Space-maintenance and threat vocalizations (excessive)	Sparring
		Diarrhea	Goring

In some cases, aggression may proceed to a point at which management should intervene to prevent serious injury. Rhino managers should allow some aggression during an introduction but be prepared to intervene in the event that aggression threatens the lives of one or more animals. Protocols for intervening may vary across institutions, but in general, careful consideration should be given to intervening in an introduction before aggression reaches Level 5 (Table 2.4). Stopping an introduction at a level prior to this will not lessen aggression during a subsequent introduction attempt. Animals that are allowed to “settle their differences” will establish some territorial boundaries and will usually not engage in serious aggression again, with the exception of a male attempting to approach an estrous female. In sum, moderate aggression is commonplace in any rhino introduction; sparring and fighting will occur and result in minor injuries (cutaneous wounds). However, in most cases, aggression levels prior to Level 5 may be allowed to continue using the discretion of management.

Table 2.4. Levels of aggression in rhinos (Fouraker and Wagener, 1996).

Level of Aggression	Definition
1	Rhinos are charging each other but do not make physical contact.
2	Rhinos are charging each other with physical contact resulting in some cuts and scrapes to the facial area.
3	Rhinos are charging each other with physical contact resulting in cuts and scrapes to the facial area and body.
4	Charging and/or pursuit <sup>a</sup> proceeds to the point that one or both rhinos are knocked down at least once. Scrapes and cuts are deeper and more numerous.
5	Aggression and pursuit proceed to the point that one or both rhinos have subcutaneous wounds or arterial blood flow.

<sup>a</sup> It should be noted that one animal might break away from the confrontation and attempt to escape. The aggressor often will pursue and begin horn-prodding the underbelly of the escapee as the two run around the enclosure. Often a rear leg is hooked and held aloft while pursuit continues. If the escapee does not stop and resume a defensive posture, the animals might continue until heat or exhaustion becomes a critical factor. Aggression at this point is more serious.

The introduction process requires much planning and cooperation among managers. Table 2.5 outlines recommended steps for rhino introductions. Familiarization through visual, olfactory and tactile contact should be permitted if at all possible prior to a full-scale introduction. If the facility permits, this may be accomplished by first placing individuals in the same barn or in nearby outdoor lots, or by providing olfactory cues such as urine, feces, or skin rubbings from the animal being introduced. As the animals acclimate, managers may move them to adjacent barred stalls or fenced outdoor yards. These barriers prevent confrontations leading to serious injury but allow acclimation and familiarization prior to introduction.

The actual introduction should be attempted in the largest available enclosure. Enclosures should be large enough to allow ample space for shading, mock-fighting, aggression and defense. The enclosure should contain visual barriers such as brush or earth piles or boulders (“run-arounds”), which give rhinos places to hide without becoming cornered or trapped. These features may lessen overt aggression if a rhino is able to escape the sight-line of another. An enclosure should not contain dead ends in which an individual may become trapped by an aggressor. The enclosure should allow for the use of high-pressure fire hoses, CO<sub>2</sub> fire extinguishers and/or vehicles to aid in separating individuals.

Animal personality and disposition should always be considered in introductions. A subordinate animal should be introduced to a more dominant animal in an enclosure familiar to the subordinate. In the case of multiple-animal introductions (such as introduction of a new female to an established male-female group, discussed below), the most subordinate animal should be introduced to the next most subordinate, and so on up the dominance hierarchy. Greater aggression may be noted in some individuals in the presence of an estrous female; therefore, any introduction attempt at this time should be especially well-monitored or possibly avoided if the attempt involves a male.

Table 2.5. Steps in the introduction process.

Step	Description
1	Animals in the same barn or multiple outdoor lots should have olfactory and auditory exposure to each other. If the animals are not housed near each other (i.e., enclosures on opposite sides of the zoo, etc.), they should be moved to the same exhibit area.
2	Animals should be given visual contact with each other in addition to the above sensory modalities. Animals may be shifted within a barn or in adjacent outdoor lots. If at any point during this process the animals display symptoms associated with stress (e.g., pacing, diarrhea, excessive vocalizations) for more than 2-3 hours, the introduction should return to the previous step.
3	If animals are not already positioned adjacent to each other, they should be moved closer together (e.g., to adjacent stalls or adjacent outdoor enclosures).
4	The actual introduction (full tactile exposure) should take place in the largest enclosure available and follow guidelines stated in this chapter. Preferably, the enclosure should be familiar to the least dominant animal and include ample “run-arounds”.
5	Within institutions in which rhinos can be left together 24 hours per day, they should be separated during the first several nights or until they show only minor aggression.

Appropriate personnel for first-time introductions include the primary animal manager, a vet with immobilization equipment, and the curator and keepers most familiar with rhinos. Other staff also may be needed at critical points around the enclosure’s perimeter so that the animals may be observed at all times in case separation becomes necessary. It should be noted that if a barn is opened and used to separate individuals, only one individual should be allowed inside the barn, and it must not be trapped inside by an aggressor.

White rhinos are preferably maintained in herd-like (male and multiple female) groups in captivity; therefore, many types of introductions may need to be attempted. Following are recommended protocols for potential white rhino introduction types.

#### ***Introduction of a New Male to a Female (or Vice Versa) to Form a Pair***

The introduction should occur in the largest lot available, following the general introduction protocols above. If a single large lot is not available, adjoining lots should be opened to form a large area for the introduction. If the latter strategy is used, care should be taken to modify any resulting dead ends in the exhibit where a rhino may become trapped during an aggressive interaction.

#### ***Introduction of a New Female to an Established Male-Female Group***

If given the opportunity, female white rhinos will establish social bonds with one another. New females should be introduced to a group one female at a time. As each subgroup of females becomes stable, additional females may be introduced one at a time. Finally, the stable female group (including the new female) should be introduced to the male. The time required for stable integration ranges from one to ten weeks.

### ***Introduction of a New Male to an Established Female Group***

A group of females to which a male is to be introduced should be compatible prior to the introduction of the male. Unlike the introduction of a female to a same-sex group, the male should be introduced to the group as a whole rather than to one individual at a time. The reported time required for stable integration has been estimated at five weeks. If the females are too aggressive to the new male, it may be necessary to reduce the numbers of females at introduction. If splitting the females into subgroups, it is useful to keep together those females that seem to be behaviorally bonded, particularly if there are four or more females. If only three females, that group should not be broken up to introduce the male.

### ***Reintroduction of a Female with New Calf to a Male-Female Group***

Following parturition, the reintroduction of a female and her new calf to a group should be treated as a first-time introduction of a female to an established group. The two should be allowed to acclimate to one female at a time, successively forming larger and larger female-female/calf subgroups. The final step is introducing the entire female group (including the new female and her calf) to the male. Some institutions have placed infants and their mothers back with the herd or single male as early as two weeks after birth.

### **Pregnancy and Parturition**

Table 2.6 lists behaviors associated with pregnancy and impending birth. In general, pregnant white rhinos will cease behaviors associated with estrus and exhibit a lack of breeding behavior. In all species, there may be a mucus discharge, noticeable weight gain or increase in girth size, as well as increased frequencies of defecation and urination throughout gestation. Pregnant white rhino females have been observed isolating themselves from other individuals. If pregnancy is confirmed (pregnancy fecal hormone tests are available, or through ultrasound), and/or the breeding date is known, the physical separation of the pregnant female from the bull/herd should take place as early as 30 days and as late as 24 hours prior to birth. Institutions with very large enclosures have had successful births in the yard with the male present; however, the female(s) and any males must be watched very closely.

The onset of labor often takes place at night or in the early morning and may last one to three hours. Parturition usually lasts ten to 12 hours from breaking of the water, though first-time mothers may take longer to calve. The presentation of a calf is generally head-first, although rear-foot presentations do occur and may take longer than head-first births, but usually deliver without assistance. Capabilities for monitoring births remotely through closed-circuit television or other means are advisable.



Table 2.6. Physiological and behavioral indicators of impending parturition.

30 Days Prior to Birth	2 Weeks Prior to Birth	24 to 48 Hours Prior to Birth
Increase in teat size	Nipples enlarge	Udders increase dramatically in size
Beginnings of milk production	Nipples develop wax plugs	Inappetance
Milk may be expended with pressure on the teats	Vulva swelling occurs	Becomes irritable and aggressive to stimuli, including staff
Female may prolapse vaginally when defecating		Mucus plug forms
		Increased vulva dilation
		Increased restlessness, lies down often

## Calf Development

A single calf is generally the rule. Few data are available on birth weights, but in general, calves weigh 54 to 70 kg (range=120-155 lb; n=5). Immediately following birth, the newborn calf is usually cleaned by its mother and stands for the first time within 30 minutes to five hours of birth. A newborn calf may require a substrate that allows traction to help steady it. Suitable materials may include sand, gravel, straw, hay or rubber matting. In all cases, both the dam and calf should be monitored closely to prevent ingestion of the substrate. A calf should begin nursing within one to two hours of standing (though in a single case, a calf removed from its dam for medical intervention nursed 16 hr post-birth). The dam will nurse her calf while standing or lying on her side.

Infants less than two months old may nurse hourly, while older calves nurse at intervals of about 2.5 hours. It has been reported that calves may gain up to 4.54 kg (10 lb) per day for the first ten days. The first defecation has been reported at two to ten days of age (n=2). Calves may nurse for up to two years, although they have been observed first sampling solid food at less than one week to one month of age. Calves may be offered supplemental feedings of milk if the dam is believed to be a poor milk producer or the calf is not gaining weight (see Nutrition chapter). Infant rhinos have been successfully pulled from their mothers because of rejection, medical issues related to the mother or infant, or from a failure to nurse. Otherwise, it should be noted that weaning for management purposes can be accomplished if necessary at six months, but one year is preferable. One attempt to use a surrogate mother was unsuccessful; however, hand-reared infants have been assimilated into existing groups and have shown reproductive success. Keeping the calf with the dam or the entire herd even longer helps to facilitate social learning. Male calves are usually weaned by the dam at an earlier age than female calves, but even male calves could stay with the white rhino herd until two to three years of age without problems. Depending on the facility, a white rhino dam and her newborn calf may be reintroduced to the male/herd after two weeks. White rhino calves

chase and mock-fight with any male, female, calf or juvenile in the herd. Non-aggressive sexual behaviors may be exhibited as early as 18 months of age in males.

In general, the long-term social effects of removing rhino calves from dams should be investigated. For all species, weaning or permanent separation of the calf except for medical reasons should not occur before one year of age. A calf can, however, be temporarily separated from its mother at as early as one month of age for short periods of time (e.g., re-breeding of dam). Generally, the procedure is to separate the calf for short periods of time (e.g., 15-20 min during cleaning) and gradually increase the separation time. If a dam is not going to be re-bred, her calf may remain with her until it reaches sexual maturity (at approximately 4.5-5 yr of age). It should be noted that available data indicate that nursing does not inhibit conception. In a herd-like situation, a female white rhino may temporarily abandon her calf during estrus and rejoin it immediately after breeding until the birth of her next calf. The first calf may be forced away before parturition of the second calf as the dam seeks to isolate herself. Following the birth of the second calf, the first calf, then a sub-adult, may rejoin its mother and her new calf in a social group for up to four years or until it reaches sexual maturity.

## **Identification**

Although physical characteristics such as horn size and shape make individual rhinos fairly easy to distinguish from one another, sound rhino management requires that animals be identified through permanent and reliable methods. Trovan<sup>®</sup> transponders (Electronic Identification Devices, Ltd., Santa Barbara, CA), implanted at the base of the left ear during post-natal examination or as soon after birth as possible, are one means of permanent primary identification for all individuals. Adults should be transpondered opportunistically. Transponder numbers need to be reported to the studbook keeper. In addition to a transponder, each individual should have a secondary visual means of identification, such as an ear tag or ear notch. Photographs or sketches in the animal's records may also serve this purpose.

## **Keeper Training and Interaction**

As with any position involving the management of large animals, rhinoceros keepers should have as much formal training and experience as possible and should be familiar with rhino behavior and husbandry. In order to ensure safety and to properly meet the requirements of management, it is recommended that more than one keeper be responsible for the care of these animals on a daily basis. Keeper interaction should be restricted to designated areas and should be conducted in accordance with institutional protocols. Finally, consistency of routine is vital.

There are no conclusive data to indicate the effects of different styles of keeper interaction on rhinoceros behavior or reproductive success in captivity. Interaction styles range from no contact at all to daily hands-on contact. In an effort to create an environment patterned after the wild, however, at no time should relationships with keepers substitute for natural interaction among individuals. It is important that rhinoceros personnel keep a daily log, noting any unusual behavioral or physical changes. It is the responsibility of management to supply all pertinent data to the studbook keeper.

## **Daily Regimen**

Fresh water should be available at all times and should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset,

spillage or leakage. White rhinos need access to mud baths to keep their skin healthy; mud wallows should be renovated periodically to prevent contamination (see Enclosure Design chapter for more information on wallows).

Natural substrates in confined areas (e.g., holding corrals) can be spot-cleaned daily, but daily removal of feces from larger enclosures is not necessary as defecating at dung piles is an important natural behavior (Owen-Smith, 1973). Hard-surfaced areas not exposed to the elements should be dry-cleaned or hosed daily and disinfected at least weekly. Indoor housing surfaces, as well as walls and rub areas, should be cleaned daily. For institutions holding free-ranging herds, these daily cleaning procedures are not practical, but periodic removal of dung heaps and the turning of soil and scattering of manure with proper equipment are necessary to reduce parasite loads. Additionally, the daily hosing or showering of rhinos in the barn with temperature-controlled water is recommended to promote healthy skin during the winter months.

In order to facilitate participation in many research programs involving rhinos, it is recommended that institutions implement training programs following the protocols recommended in the Training chapter. Additionally, it is recommended that some form of environmental/behavioral enrichment be integrated into daily rhino management.

## **REPRODUCTION**

### **White Rhino Reproductive Age**

According to the North American Regional Studbook for white rhinos that includes data on 254 dams, 155 sires and their 850 calves (Christman, 2011), the youngest white rhino to give birth was four years old which means she conceived at about two years eight months of age. By comparison, data from *in situ* populations indicate a somewhat later age of first reproduction, with average age of first reproduction occurring between 6.5 and 7.5 years of age in the wild (Owen-Smith, 1973) and small private game reserves (Swaigood et al., unpublished data). The youngest age of first birth reported *in situ* was 5.0 in a sample of 67 births (Swaigood et al., unpublished data). Field studies also indicate little reproductive behavior before age five, with the mean age of first copulation at 6.1 years (Swaigood et al., unpublished data). The oldest white rhino to give birth was 41.5 years at the time of conception. The youngest and oldest male white rhinos to sire a calf were three years two months and 48 years eight months, respectively, at the time of conception. Therefore, it appears that white rhinos have the longest period of reproductive potential among rhino species starting at approximately three years of age and extending into their late 30's and early 40's. Although the oldest female at first reproduction was 28 years at the time of the birth, only 3% of dams reproduced for the first time after the age of 20, indicating that first conception needs to occur before the females reach their late teens or fertility may be lost.

### **Seasonal Changes in Physiology Associated with Reproduction and Management Implications**

The African white rhinoceros is not seasonally restricted in its breeding activity. Based on year-long fecal hormone metabolite analyses (Schwarzenberger et al., 1998; Patton et al., 1999; Brown et al., 2001), it appears that reproductively active female white rhinos are capable of exhibiting reproductive cycles and breeding throughout the year. Although there is variability in reproductive cycle lengths with some being ~35 days and others ~70 days, season does not appear to be associated with the expression of different cycle lengths. Additionally, many white rhinos

are acyclic, but those that are, do not cycle during any season of the year. Finally, North American studbook data confirm that white rhinos conceive and give birth every month of the year with only a slight skew towards successful matings in April to September (see figures below), which is almost certainly due to increased mating opportunities that result from the management strategies employed during the warmer weather.

## **Reproductive Cycle**

Sound scientific data from both hormone metabolite monitoring and ultrasonography support the conclusion that white rhinoceroses can exhibit reproductive cycles of different lengths. Both 30-35 day (Hindle et al., 1992; Radcliffe et al., 1997; Patton et al., 1999; Brown et al., 2001) and 65-70 day (Schwarzenberger et al., 1998; Patton et al., 1999; Brown et al., 2001) cycles have been reported. The difference in the two cycle lengths cannot simply be attributed to individual animal differences because several females have been known to exhibit both cycle lengths over a period of months (Patton et al., 1999). The physiological mechanism differentiating these cycles is not yet understood, but based on incidence of pregnancy following matings, it is generally believed that the 30-35 day cycle can be fertile (Radcliffe et al., 1997; Schwarzenberger et al., 1998; Patton et al., 1999). Whereas, pregnancy has not yet been documented following a mating associated with a 65-70 day cycle (Schwarzenberger et al., 1998; Brown et al., 2001). In some cases, the longer cycles were associated with pregnancy and early embryonic death, indicating that elevated progesterone levels were the result of a lost pregnancy rather than an extended luteal phase (Radcliffe et al., 1997; Patton et al., 1999). An intensive field study documented 17 cycles circa 30 days in length but no longer cycle types, providing evidence supporting the shorter cycle as normal for the species (Swaisgood et al., unpublished data). The longer cycle length in white rhinoceroses is more prevalent in older females and may be a characteristic of aging animals that have not been reproducing consistently throughout their lives (Schwarzenberger et al., 1998; Patton et al., 1999; Brown et al., 2001). Another pattern that is cause for concern due to its prevalence within the captive white rhinoceros population is the acyclic or “flat liner” pattern, wherein progesterone remains at baseline. In studies conducted on relatively large sample sizes, close to 50% of the females exhibited this inactive pattern (Schwarzenberger et al., 1998; Patton et al., 1999; Brown et al., 2001). One hypothesis for cessation of reproductive activity is premature senescence resulting from oocyte depletion in animals that cycle continuously for years without becoming pregnant (Hermes et al., 2004). However, not all acyclic animals are aged, and some encouraging, anecdotal information suggests that cyclicity may resume in some acyclic females if they are introduced to a new male (Patton et al., 1999).

The white rhinoceros is a spontaneous ovulator with a follicular phase that lasts about nine days. The preovulatory follicle reaches a size of ~30 mm in diameter and changes from spherical to pear-shaped about 48 hours before ovulation (Radcliffe et al., 1997). Ovulation has been confirmed 24 hours after breeding but cases of anovulation, hemorrhagic follicle formation and early embryo loss have also been reported in this rhino species (Radcliffe et al., 1997). Fecal progesterone metabolite concentrations increase six to nine days after mating and ovulation (Patton et al., 1999; Radcliffe et al., 1997).

## **Peak Breeding Season for White Rhinos in North America**

The mature captive, female white rhinoceros will breed throughout the year, and calves have been produced in every month of the year with no particular bias for a specific season. Wild populations also give birth throughout the year but have higher birth rates in the wet season when there are more resources available to support lactation (Owen-Smith, 1973; White et al., 2007).

The distribution of births by month for African white rhinos is shown in Figure 2.1 for the 845 white rhino births reported in the 2011 North American Regional Studbook (Christman, 2011). Figure 2.2 shows the months of conception for white rhinos based on an approximate gestation of 16 months. These data demonstrate that white rhinos are fertile and conceive throughout the year. Although a slight seasonal effect on male rhino testosterone and mating activity has been reported for wild white rhinos (Kretzschmar et al., 2004), the trend towards more fertile matings from April to September in *ex situ* white rhinos could be management related since temperate zoos are more likely to pair their rhinos for mating during months with good weather.

## Reproductive Monitoring

White rhinos breed best when maintained in larger social groups, comprised of at least one male and greater than two females, in large enclosures (Swaisgood et al., 2006; Metrione, 2010). Most males are fairly compatible with females, so reproductive monitoring is not typically necessary for timing introductions. However, hormone monitoring can be a valuable tool for determining if a rhino has truly stopped cycling when overt signs of estrus are not observed. Monitoring is also useful for assessing reproductive cycle length since white rhinos with the longer (~70 day) cycles are less likely to produce calves. Hormonal analyses are most commonly employed to diagnose and monitor pregnancy. Several hormones and their metabolites have been employed for monitoring reproductive activity in the African white rhinoceros (Table 2.7), with fecal progesterone metabolites most commonly chosen.

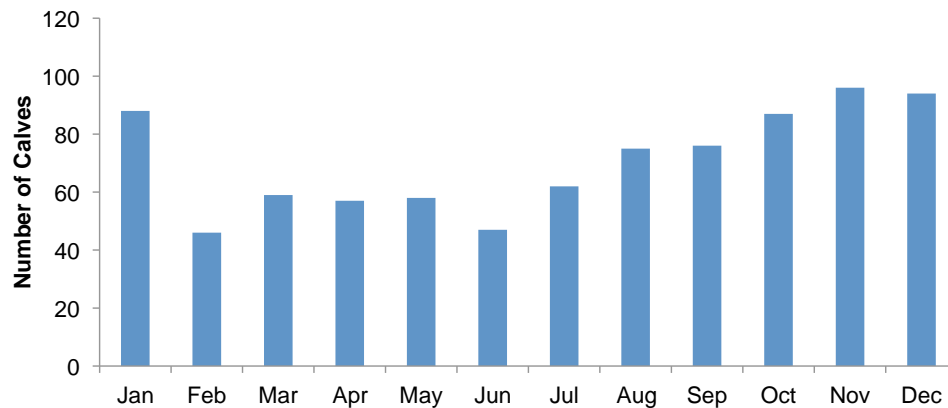


Fig. 2.1. Number of southern white rhino calves born per month (n=845).

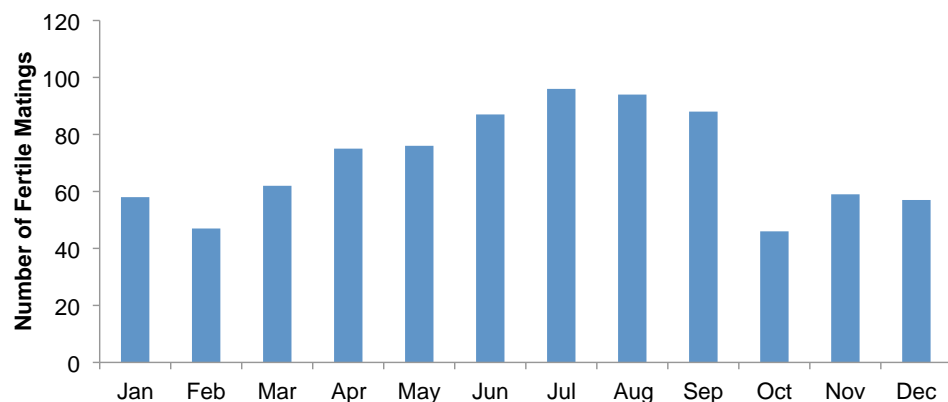


Fig. 2.2. Number of fertile southern white rhino matings by month (n=845).

Table 2.7. Reproductive hormones monitored in the African white rhinoceros.\*

Sample	Estrogen	Progesterone	Testosterone
Urine	♀ <sup>3</sup>	♀ <sup>3</sup>	
Feces		♀ <sup>1,4,6,7,8</sup>	♂ <sup>1,5</sup>
Serum			♂ <sup>2,5</sup>

\*Table adapted from Roth, 2006.

<sup>1</sup> Brown et al., 2001; <sup>2</sup>Christensen et al., 2009; <sup>3</sup>Hindle et al., 1992; <sup>4</sup>Hodges and Green, 1989;

<sup>5</sup> Kretzschmar et al., 2004; <sup>6</sup>Patton et al., 1999; <sup>7</sup>Radcliffe et al., 1997; <sup>8</sup>Schwarzenberger et al., 1998

## Pregnancy Detection and Loss

In the white rhino, pregnancy can be detected by rectal ultrasonography as early as 15 days after ovulation (Radcliffe et al., 1997) if the female is conditioned to stand for the procedure. However, many white rhinos are not conditioned for ultrasound exams. Therefore, fecal or serum progesterone concentrations often are evaluated to diagnose and monitor pregnancy in white rhinos. Following mating, sustained elevated progesterone is a good indicator of pregnancy, but pregnancy can only be confirmed approximately three months into gestation when progesterone concentrations increase above post-ovulatory luteal concentrations (Patton et al., 1999; Brown et al., 2001). Early pregnancy loss, abortions and stillbirths have all been reported in the white rhino (Radcliffe et al., 1997; Patton et al., 1999; Hildebrandt et al., 2007) and the incidence of early pregnancy loss could be quite high. It has been suggested that many of the longer (~70 day) reproductive cycles could be the result of early pregnancy followed by embryonic loss, which extends the luteal phase of the cycle. However, until more white rhinos are monitored regularly by ultrasound to confirm the presence of an embryo followed by its loss, we can only speculate about the prevalence of early embryonic death in white rhinos.

## Reproductive Technologies (Semen Collection, Artificial Insemination, etc.)

Viable white rhino semen samples can be collected by electroejaculation (Hermes et al., 2005; Roth et al., 2005) or harvested from the epididymis post-mortem (Roth, unpublished). White rhino sperm obtained by either method can survive processing and cryopreservation, but post-thaw motility is significantly reduced (Hermes et al., 2005; 2009b), indicating more research is needed to improve the cryopreservation protocols employed. Regardless, the successful production of a white rhino calf following AI with frozen-thawed semen proves that some white rhino sperm are fully functional post-thaw (Hermes et al., 2009b).

Artificial insemination (AI) techniques have been developed for the white rhino. To date, five calves have been produced (Hildebrandt et al., 2007; Hermes et al., 2009b), and four survived. Three of the five pregnancies were in the same female white rhino maintained at the Budapest Zoo. Numerous attempts to produce pregnancies in many other white rhinos at zoos across Europe, North America and Australia have failed to yield additional offspring. Therefore, although tremendous progress has been made in establishing that AI can be successful, more research is needed to improve the efficiency of AI for facilitating the management of this rhino species. There are no reports of in vitro fertilization attempts in the white rhino.

Sperm sorting to segregate X- versus Y-bearing sperm has been conducted and appears to be possible in white rhinos (Behr et al., 2009; O'Brien et al., 2011). However, maintaining adequate



sperm quality throughout the collection, transport, staining, sorting and cryopreservation process has proven challenging, and the sorting rate can be slow. Therefore, additional research and refinement of techniques will be necessary before samples of sufficient concentration and quality for AI can be produced.

## **Fertility Assessment**

Male fertility assessments should include: 1) an ultrasound examination of the testicles to evaluate tissue consistency and size, 2) electroejaculation to confirm sperm production, and 3) serum testosterone analysis. It is important to note that a positive result from electroejaculation is very meaningful, but a negative result should not be used alone to diagnose a male with infertility because electroejaculation is not always successful in producing good quality sperm samples, even when conducted on proven bulls.

Female rhinos should be examined by rectal ultrasound to determine if any masses or cysts exist in the vagina or uterus. These types of pathologies appear to be very prevalent in older and even mid-age white rhinos (Hermes et al., 2006). Ultrasonography can also be used to determine if the ovaries are active, however, any fertility assessment should include some form of longitudinal endocrine analysis which is more informative for assessing ovarian activity than a single ultrasound exam of the ovaries. These assessments can be conducted using serum or fecal samples (Table 2.7). Given that some white rhinos exhibit reproductive cycles that last 70 to 75 days in length, samples should be collected three times a week for at least three months to evaluate ovarian activity.

## **Genome Resource Banking**

Several white rhino sperm banks already exist at the Cincinnati Zoo's Center for Conservation and Research of Endangered Wildlife, San Diego Zoo's Institute for Conservation Research and at the SeaWorld/Busch Gardens Reproductive Department. The latter primarily contains samples that have been sorted for X- and Y-bearing sperm, whereas the former contain samples comprised of a natural mixture of X- and Y-bearing sperm collected either by electroejaculation or post-mortem from the epididymis. Fibroblast cell lines from many white rhinos are banked in San Diego Zoo's Frozen Zoo and efforts are underway to develop white rhino stem cells. Oocyte and embryo cryopreservation techniques in the white rhino are still in the experimental stages.

## **Challenges**

There are three significant challenges to white rhino reproduction in North American zoos: 1) a significant proportion of the females are acyclic; 2) the F1 generation of female rhinos appears to be reproductively active and mating but many are not producing calves; and 3) the prevalence of uterine pathology is quite high, even in females less than 20 years old. The cause(s) of acyclicity in female white rhinos remains unknown. There is speculation that it is the result of old age or premature reproductive aging, but to-date, there is no robust, scientific evidence to support this hypotheses. Reproductive suppression by dominant females is unlikely. The presence of other females appears to increase reproduction in both zoos (Swaigood et al., 2006; Metrione 2010) and small game reserves (Swaigood et al., unpublished data), and subordinate females were not more likely than dominant females to be nulliparous or acyclic (Metrione and Harder, 2011). Exogenous hormone treatments have been effective in stimulating ovarian activity in acyclic rhinos (Hildebrandt et al., 2007; Hermes et al., 2009a) but to date they have not been very effective in returning the female to a state of natural reproductive cyclicity. In contrast, the F1 female white rhinos



appear to be exhibiting ovarian activity and behavioral estrus, are mating with the bulls, but are not producing calves (Swaigood et al., 2006), suggesting that they are either failing to ovulate appropriately, failing to conceive or are conceiving and losing the pregnancies early in gestation. Reproductive tract pathologies have been reported in many species and it is pretty well accepted that the development of pathology can be avoided if the female is allowed to breed and does so successfully. However, some species are more prone to develop reproductive tract pathologies than others, and white rhinos appear to be more susceptible than black rhinos. How early in life and how often the females have to become pregnant to avoid developing reproductive tract cysts and masses are unanswered questions, but it is probably best to try and produce calves from females before they reach ten years of age.

## ***ENRICHMENT***

A planned and implemented enrichment program can contribute to better health by providing animals with opportunities to exert some form of control over their environment (Carlstead and Shepherdson, 1994; Baser, 1998). As it pertains to zoo-managed rhinos, we strive to encourage species-specific behaviors in rhinos while providing them response options to environmental change. This will ultimately result in their mental stimulation and the development of naturalistic behaviors (Swaigood and Shepherdson, 2005).

Each institution may have its own set of goals and criteria for its program, which fundamentally should start with an examination of the animal's natural history, activity patterns and behaviors seen in the wild. For white rhinos, simply providing a wallow permits both mud bathing as well as an opportunity for environmental manipulation. Since rhinos spend the majority of their day eating, keepers could look at modifying feeding schedules or providing enrichment throughout the day rather than just once a day. Keepers also could investigate the possibility of changing herd dynamics or composition.

Holding facilities, exhibit spaces and/or any constraints that may be unique to the facility should be examined since these areas will directly impact options for enrichment. The temperament and behavior of the specific individual(s) also needs to be taken into consideration. Once these criteria have been reviewed, a set of goals can be established. Generally speaking, the primary goal should be to “promote opportunities for the expression of species-appropriate behaviors” (Joseph and Sevenich, 1999). The Rhinoceros Husbandry Resource Manual (Fouraker and Wagener, 1996) clearly delineated the various functions enrichment can serve, such as (1) improving the well-being of the animal by increasing exercise, satisfying behavioral needs, and optimizing the level of stimulation that animals receive; (2) educating zoo visitors by increasing the levels of natural and interesting behaviors, visibility and activity levels; and (3) conserving endangered species by improving the success of breeding and reintroduction programs. The second goal of enrichment can be loosely defined as creating mental stimulation for the animal(s)(Fig. 2.3).

A successful enrichment program can be briefly summarized as one that:

- a) Establishes goals for the program
- b) Creates an enrichment approval form
  - States the purpose/goal of this enrichment
  - Provides a detailed description of enrichment items (construction material, thickness, dimensions, size of holes, etc.)
  - Identifies and addresses facility or exhibit constraints
  - Identifies and addresses safety concerns
  - Cost estimates
- c) Identifies approval protocols for enrichment submissions
- d) Creates an enrichment calendar (monthly or weekly) to ensure implementation schedule
- e) Determines how staff will document and/or track animal response to enrichment offered

To accomplish the goals mentioned above, an enrichment plan should be tailored with a set of criteria for either the individual or the species in general. The final proposal should then be submitted through the appropriate channels for approval. Ideally, an enrichment approval system should be set up to allow keepers, managers, and veterinarians the ability to assess the proposed enrichment and approve/reject it. Institutions can alter their enrichment scheduling as well as vary the type of enrichment offered (e.g., toy, food, sensory, environmental, behavioral and social) and keep track through their record-keeping or on a barn calendar (Connett, 2009). One of the most intimidating aspects of enrichment is allocating the time to document animal behavior and/or responses to the enrichment offered, however observations of responses can either be done in a direct or indirect manner as time allows. This data will enable evaluation as to whether the enrichment goals have been met. Since enrichment is dynamic in nature, adjustments can be made at any time so that the most effective enrichment is offered to the animal(s).



Fig. 2.3. Enrichment items, even an old log, can create a more stimulating environment (photo taken at Tulsa Zoo).

Enrichment can correlate aspects of ethology, psychology and animal husbandry to create a more stimulating environment for the animal (Mellen and Ellis, 1996). This has led to the inclusion of enrichment options in exhibit designs. Exhibit enrichment can be done by varying topography, landscaping, utilizing deadfall and trees, creating dirt mounds, planting vegetation, providing a wallow, and alternating the substrate (dirt, leaf litter, mulch, etc.). Other options (public view versus privacy, shaded area versus sun, etc.) can all provide the animal some control over their environment and the ability to make choices throughout the day. The aforementioned exhibit variables can contribute to effective enrichment, especially when used in conjunction with other approved enrichment activities. For example, novel scents/perfumes/extracts can be used to create a “trail” throughout the exhibit, while holes drilled in deadfall can serve as an anchoring point for browse, and either one can provide the potential for exploration and create options for the animal. The American Association of Zookeepers (AAZK) has created The Enrichment Notebook (Chan, 2004) that provides suggested guidelines and contains information on exhibit enrichment, dietary enrichment and a section on safety considerations. When using enrichment devices that are awkward and heavy, it is important to secure these items safely, not only for the animals, but for keeper staff as well. Fortunately, these somewhat bulky items can be secured safely by using pulley systems and/or other equipment can be used to hoist devices to enable hanging them higher. A synopsis of rhino enrichment options that are currently being used at zoological facilities can be found in Table 2.8. This table gives suggestions as to the primary area of use as well as its presentation. Note that food items, especially biscuits, treats, and produce, should be fed sparingly. Examples of enrichment type and some options that can be used are outlined below. These options must be used in compliance with a facility’s enrichment protocol, regulations and safety considerations.

Toy:

- Boomer Ball (Boomer Ball, Grayslake, Illinois)
- Weeble (Otto Environmental, LLC., Milwaukee, Wisconsin)
- Suspended log

Food:

- Scatter food around exhibit to stimulate grazing/foraging
- Place food items in enrichment devices to be randomly dispensed
- Fruits and vegetables frozen in bucket of water

Sensory:

- Use conspecific’s dung for smell (olfaction)
- Play different animal vocalizations or hang bamboo “chimes” for hearing (audition)
- Mount street sweeper brush for touch (tactition)

Environmental:

- Changing substrate (leaf litter, dirt, mulch, etc.)
- Altering daily routine
- Rotating to different enclosure (pen)

Behavioral:

- Training new behaviors
- Training for veterinary procedures

Social:

- Creating mixed species exhibit
- Rotating individuals (add or remove animals when possible)

Training for husbandry behaviors is not only stimulating for the animal, but it also can provide an invaluable opportunity for the veterinarian to perform diagnostic procedures with potentially less stress and more cooperation from the animal(s) (Dover, et al., 1994). The chapter for training will delve more deeply into this topic.

Table 2.8. Synopsis of rhinoceros enrichment ideas.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
<i>Amazing Graze</i> <sup>1</sup> (with biscuits/treats/produce)	X	X		X
Audio clips of vocalizations (rhino, other sp.)		X	X	
Beer keg		X	X	X
Biscuits (gorilla-sized leaf eater; horse treats)	X	X		X
Boat mooring buoy		X	X	
<i>Boomer Ball</i> <sup>2</sup>	X	X	X	X
Bowling ball		X		X
Branch/twig with produce skewered on it	X	X	X	X
Browse or approved plants	X	X	X	X
Brush	X	X	X	
Brushing by keeper		X		
Cardboard box (with or without produce)		X		X
Firehose “wall”		X	X	
Ice block (with or without flavor/produce)	X	X		X
Large cardboard tube		X		X
Log, stump, rootball	X	X	X	X
Melon (whole or chunks)	X	X		X
Mirror		X	X	
Mister	X	X	X	
Mud wallow	X	X		
Non-radial tire (with cuts in sidewall for safety)		X	X	
Oblong stone	X	X		X
Painting with non-toxic finger paint		X		
Paper grain bag filled with hay or produce		X	X	X
Paper mache ball	X	X	X	X
Peanut butter (on toys/walls/“furniture”)	X	X		
Planter bucket	X	X	X	X
Plastic soda concentrate container		X	X	X
Plastic drum (55 gal.)		X	X	X
Plastic ice block or iceberg toy		X		X
Plastic jug feeder		X	X	
Produce	X	X	X	X
PVC or bamboo chimes		X	X	
PVC tube with bells suspended inside		X	X	
Rolling treat bucket/feeder		X		X
Scent, flavoring, or extract	X	X		X
Snow-pile, -people (with or without fruit mix)		X		X

Table 2.8. Continued.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
Substrate pile or change (dirt/gravel/sand/leaf)	X	X		
Traffic cone or pylon		X	X	
Training with keeper	X	X		
Triangle rattle		X	X	
Urine, feces, or soiled bedding from others	X	X		X
Weeble <sup>3</sup>	X	X		X
Weeto <sup>3</sup>	X	X		X

<sup>a</sup> Area of use may be dictated by institutional guidelines and criteria

<sup>b</sup> Presentation format is based on that which is most commonly used

<sup>1</sup> Sanctuary Supplies, Rootstown, OH; sanctuariesupplies.com

<sup>2</sup> Boomer Ball, Grayslake, IL; boomerball.com

<sup>3</sup> Otto Environmental, LLC., Milwaukee, WI; ottoenvironmental.com

## TRAINING

A husbandry training program is considered an integral component of progressive animal husbandry programs in zoos today. Providing zoo-managed animals with choices and behavioral opportunities in their environments through a training program can result in healthier animals and better educational experiences for the zoo visitors. Training rhinos leads to a wide variety of animal management opportunities, from shifting to safer, less stressful medical procedures.

The purpose of this chapter is to give trainers an idea of the types of behaviors that can be trained with rhinos (Appendix B). This chapter will also give direction on starting a training program, ideas for shaping techniques, possible reinforcements, specific challenges to training rhinos, and resources for additional training information.

### Setting Up a Training Program

A well-planned, consistently-delivered training process is critical to the success of any program. To achieve this type of program many facilities utilize a framework that is taught in an Association of Zoos and Aquariums (AZA) course, Managing Animal Enrichment and Training Programs. This framework is called the “SPIDER” model. Steps in this framework include: Setting goals, Planning, Implementation, Documentation, Evaluation, and Re-adjusting. For more information on this process, please see [www.animaltraining.org](http://www.animaltraining.org). It is beneficial to start a training program by determining the overall behavioral goals (i.e., detailing the specific behaviors to be trained). During this goal development process it is important to include all parties involved with the management of the animals. This may include meeting with and seeking feedback from keepers, veterinary staff, nutritionists, behavioral husbandry staff, curators, and managers. Having everyone on the same page with clearly laid-out plans, assignments, and timelines helps to facilitate a smooth process. Defining roles and creating clear channels of communication and decision-making authority among all participants is also important. This can be accomplished through regularly scheduled team meetings, a consistent method of documentation, and continual communication among all staff involved in training. Facility design can have an effect on setting up a training



program. Discussions must also include how the rhino will be reinforced. The next two sections will discuss both of these topics further.

### ***Facility***

When beginning a training program, it is important to start training in an area that is safe for the animal care staff and the animal, and where the rhino is comfortable. This is usually the night quarters or holding area (Fig. 2.4). Training can also be done in barn stalls, outdoor barn paddocks, training chutes, or even open exhibit areas. Because all facility designs are different, training staff will have to be creative and utilize the space available. For examples of possible body positioning behaviors, see Appendix B. For many of these behaviors trainers will require areas with no or minimal obstructions, which allow safe access to the desired body parts. It is important to remember that a fancy, expensive facility is not necessary to accomplish a successful training program, just a creative mind.



Fig. 2.4. Indoor chute for training and procedures (courtesy of C. Nordin, St. Louis Zoo).

### ***Reinforcement***

A critical component in training is finding a positive reinforcement or a reward for which an animal is willing to work. In most cases, the rhino's regular diet can be utilized for training. Any manner of storage container for the reinforcement can be used. Reinforcement items need to be easily retrieved and delivered as soon as possible after the desired behavior occurs. Reinforcement can be delivered by tossing it through posts or by hand feeding if it can be safely accomplished. White rhinos tend to be less motivated by food-rewards, and tactile reinforcement is often the preferred method of reward.

### **Training Methods**

There are several steps to creating a husbandry training program for rhinos. After the behavioral goals are set, a safe facility to work the animals is identified, reinforcement type and how the reinforcement will be delivered has been determined, the next steps are learning about the animal

to be trained, building a relationship with that animal, and designing a training plan. An overview on learning and training can be found in Mellen and Ellis (1996).

### ***Natural History/Individual History***

An understanding of the natural history of rhinos, paired with information about an individual animal's specific background and level of experience, play an integral part in a husbandry training program. For example, wild-caught or hand-reared rhinos may react differently to trainers when compared to captive, parent-reared individuals. It is helpful to start training in a location where the animal is most comfortable. Some animals need to have more space around them to feel comfortable within the training environment. The space needs for a rhino often center around the size of the enclosure in which the animal is being trained. Other relevant issues regarding an animal's space requirement may include proximity to conspecifics, other species, and issues such as noise levels and extraneous activity. Keeping training sessions short and moving at a comfortable pace for the rhino may help keep their focus. Tactile interaction is important—most rhinos enjoy being scratched. Care must be taken to avoid startling the animal or putting yourself in the danger zones—areas where the rhino is able to horn, kick, or injure someone. When desensitized, rhinos will interact positively with their keepers and will seek out tactile interaction. Keepers need to always maintain awareness and caution as startled rhinos can react violently and quickly. Studying the animal's natural history helps to gain insight into the animal's behavior. For more information on rhino natural history, see the Behavior chapter of this manual. Insight also can be gathered from staff, the animal's ZIMS records, or other institutions about the animal's individual history. There is no substitute for simple observation—just watching the rhino's behavior in different situations, for example, what it looks like when the rhino is calm or aggressive. Watch the rhino on and off exhibit, at different times throughout the day, and when the rhino is unaware it is being observed. This greatly helps understanding and interpretation of the rhino's behavior during a training session.

### ***Relationship***

Developing a trusting relationship between the trainer and the animal being worked with can be very beneficial to training. If the animal is not comfortable being in close proximity to people, the rhino might back away from the trainer or try to horn the trainer. Developing a relationship can help address these issues. A relationship can be developed through normal daily animal care, such as feeding, observing, regular cleaning routines, and avoiding negative interactions. The more positive interactions the animal has with staff, the more comfortable the rhino will be. For example, instead of just putting the diet in the enclosure, the trainer can hand-feed the diet. If the animal is particularly skittish, the trainer may start by placing or tossing the food in the enclosure and sitting nearby while the rhino eats. As the animal becomes habituated to the trainer's presence, s/he can begin to hand-feed and work their positioning closer and closer to the animal.

### ***Training Plans***

Creating a training plan can be a good process to help trainers think through what steps they are going to take to train a behavior. One way to do this is to establish what the final behavior will look like, and then break-down the behavior into a series of small steps called "successive approximations". Examples of training plans can be found in Appendix C.

### ***Bridging Stimulus***

Utilizing a bridging stimulus can be a very useful tool in training rhinos but not a necessity. A bridge is a signal that pinpoints the exact moment in time the behavioral criterion was met. First a trainer must select a bridge. Common bridges used are clickers or whistles. Once selected, the



trainer needs to associate the sound with the delivery of reinforcement. For example, if the bridge selected was the clicker, the trainer would click and feed, click and feed, and continue this for several sessions with the rhino. Once the association has been established, the sound of the clicker with a food reward, the trainer can begin to use the bridge to shape behaviors. For more information on a bridging stimulus, see Pryor (1984), Mellen and Ellis (1996), and Ramirez (1999).

### ***Shaping Behavior***

For consistency, one trainer should shape new behaviors with an animal. Once the behaviors are trained and performed on cue consistently, other trainers can work with the animal. A shaping technique that works well with rhinos is using the sight of food as a lure. In most cases, rhinos focus on the food and follow it wherever it is placed. Trainers can also use their body positioning when shaping behaviors. The animal will, on many occasions, shift its body position when the trainer moves. For example, if a rhino is facing a trainer and the trainer takes a step to the left, many times the rhino will follow and shift its body to the left as well. Another useful tool in the shaping process is a target. A target is an object to which an animal is trained to touch a part of its body (Mellen and Ellis, 1996). Training a rhino to target different parts of its body is a good method of shaping many body-positioning behaviors such as standing, presenting a side of the body, or presenting a foot (Appendix C). When starting a program it is best to begin by training some basic body-positioning behaviors such as open mouth, target, and back before moving on to more complex behaviors like standing for blood collection or other veterinary procedures.

### ***Record Keeping***

It is important for trainers to keep records of all sessions. Trainers can go back and look for patterns in behavior, which helps keep consistency among trainers and leaves a historical record for others. For an example of a documentation form, please see Appendix D.

### ***Safety***

Establishing a safety protocol is another valuable component to a rhino training program. These protocols will allow a facility and training team to set clear guidelines to ensure the safety and well-being of the trainers and the animals. The disposition of the individual rhinos should be taken into account during any training session, and the ability to read the animal's body language and temperament will allow the trainer to know when the rhino is agitated and the session needs to end. In general, it is advisable to end a session on a positive note and not wait until an animal is agitated.

In any training session where the trainer is in close proximity to the rhino, as in blood-draws, it is important for keepers to work in groups of two or more. If there are multiple keepers involved in a session, then there are multiple people that are able to watch the keeper that is working close to the rhino, the rhino's behavior, and the location of its head and horn(s).

### **Summary and Resources**

The purpose of this chapter is to give trainers an idea of the type of behaviors that can be trained with rhinos and what these behaviors might look like. This chapter provides direction on starting a training program, ideas for shaping techniques and reinforcement, some specific challenges to training rhinos, and resources for additional training information. This chapter is meant as a reference for basic training information and contains just a small amount of the information that is available.

The following is a list of additional resources that can be helpful in developing a training program:

- *Animal Keeper's Forum*, a publication of the American Association of Zoo Keepers
- [www.animaltraining.org](http://www.animaltraining.org)
- Animal Training Organizations –
  - IMATA (International Marine Animal Trainers Association)
  - AAZK (American Association of Zoo Keepers)
  - IAATE (International Association of Avian Trainers and Educators)
  - ABMA (Animal Behavior Management Alliance)
  - PEM (Principles of Elephant Management)
- International Rhino Keeper Association (IRKA)
  - [www.rhinokeeperassociation.org](http://www.rhinokeeperassociation.org)

## **TRANSPORTATION**

Crating and shipping of rhinos is one of the most difficult transport procedures. While rhinos themselves are fairly hardy, the limitations of temperament, peculiarities of chemical immobilization, and rigorous shipping equipment necessitate a strict yet flexible protocol for optimizing successful crating and shipping.

### **Pre-shipment Medical Procedures**

Communication at the veterinary level between receiving and shipping institutions prior to rhino translocations is essential in order to discuss specific institutional and/or state requirements. Standard medical procedures for all moves should include the following: (1) a tuberculosis test within 30 days to six months of shipment (depending on where the rhino is being shipped) or as particular state, federal or international requirements dictate, (2) brucellosis serology if dictated by particular state or international requirements, (3) a physical examination, (4) three negative fecal screens 30 days prior to shipment, and (5) a review and update of vaccinations (see Health chapter). In addition, medical or research **protocols** defined by the SSP should be reviewed during the planning process.

### **Crating**

Crating is the recommended transport method, although transport in trailer stalls has also been successful. It is important in the latter case that the trailer is well-reinforced. In all situations, the animal's behavior and conditions should be constantly monitored. Typical problems that can occur during shipping include the following: (1) animals destroying and/or climbing out of the crate top; (2) animals becoming inverted in the crate and unable to right themselves; (3) animals destroying end panels or doors, resulting in eye, horn or facial injuries; and (4) prolonged, excessive exertion resulting in hyperthermia and/or myopathy.

### **Design**

The International Air Transport Association (IATA) crate design specifications are listed in Table 2.9. Crates are usually constructed of wood, metal, or wood with steel reinforcements. Crate dimensions should be determined by the animal's size (Table 2.9), but a general principle is that the crate should be 0.3 m (1 ft) longer and wider than the animal when it is lying on its side. Crates with vertical bars situated at the head end will decrease injuries to the head and face but must be spaced correctly with at least 10-15 cm (4-6 in) gaps. Horizontal bars at the head end should be avoided,

as they tend to cause horn breakage and/or damage. Crates with bars and doors at both ends are optimal.

Table 2.9. Approximate crate dimensions by species (modified from IATA, 1995).

Species	Length	Height
Black rhino	271 cm (107 in)	191 cm (75 in)
White rhino	475 cm (187 in)	221 cm (87 in)
Greater one-horned rhino	335 cm (132 in)	201 cm (79 in)

## Principles of Design

Frames should be constructed of strong metal with sides of solid hardwood. Vertical metal bars should be bolted in place at the front and back with sliding or hinged wooden doors to the exterior of the bars. The upper third of the wooden doors must have ventilation spaces or openings. There are several new requirements for lower ventilation as well. IATA specifies that the roof must be solid over the animal's head and slatted over the loin and hindquarters for ventilation. For ground transportation, however, removable panels or hinged doors over the animal's head can be useful for administering to medical needs and monitoring the animal. Hatches also allow for more ventilation when an animal is standing calmly.

The interior must be smooth with no projections. Wooden crates have often been modified to include solid metal sheets at the head so that the horn deflects and cannot damage the wood. Only nuts and bolts should be used in the container. Entry and exit doors must be closed and bolted in strategic places to be strong enough to resist the animal, and to withstand the rigors of the equipment necessary to move the crate and rhino. The floor must be at least 2.5 cm (1 in) thick and be a non-slip surface. Some crate floors are slatted so that urine and feces can flow through so that the animal is not standing in excreta. For international and air transport, the container must be constructed in such a way that the floor and lower sides are leak-proof. In view of the diversity in size, strength and temperament of rhinos, the size and strength of the container must be sufficient to restrict the movement of and restrain the animal. Dimensions must be large enough to prevent cramping without allowing unnecessary movement. In general, the crate should be 0.3 m (1 ft) longer and wider than the animal when it is lying on its side.

At the front of the container, there must be provisions for water and food access at the base of the door and between the bars, if present. For airline transport, this access point must be clearly marked FEEDING and be adequately secured when not in use. A water container must be provided and must be sufficiently large for the entry of the animal's muzzle. Some rhinos will not drink from a tub, and so offering a hose directly into the crate (either into the animal's mouth or just puddling in front of the rhino) is sufficient to provide water. Entrance and exit must be clearly indicated on crates used for airline transport. Many crates are designed to be used in either direction. The above recommendations are modified from IATA standards to include specifications for ground transport. Before shipping by air, consult the current IATA specifications and/or the airline.

## Acclimation Training

Crate acclimation can require two to six weeks, although several zoos have crate-trained rhinos in seven days or less. Training should be completed by a method of approximation (with

reinforcement given as rhinos demonstrate progress towards the desired behaviors). The first step is to introduce the crate as a non-interactive part of the animal's environment. Gradually, the food is moved toward and finally into the crate. If the animal acclimates to the point of completely entering the crate and will allow the door to be shut, the door should be left closed for short acclimation periods under close observation. It is not advisable to close the animal into the crate if the training period is short. No rhino likes to be closed into the crate, and even the best-trained rhinos will react negatively to being locked in. If there is not adequate time to train the rhino to acclimate to a closed crate, closing him/her in will only make the experience a negative one, and there will be a major setback to the ability to close the animal in for shipment. If the rhino does not completely acclimate to entering the crate, partial immobilization (standing restraint) may need to be utilized for shipping. Forced crating without training or immobilization is strongly discouraged.

## **Crating with Chemical Immobilization**

Immobilization offers a fairly simple way of crating a rhino. First, it should be noted that the usual pre-immobilization procedures (e.g., fasting, detainment in an adequate holding area, etc.) should be observed for any procedure requiring the use of chemical immobilization/tranquilization agents. For rhinos, etorphine (M-99) remains the drug of choice, although several alternatives are available. For specific drugs and dosages, refer to the Health chapter of this publication. The duration of immobilization without the administration of an antagonist may range from 30 minutes to two hours.

Following crating, all rhinos should be held for 24 hours at the loading location for observation or accompanied by a veterinarian during transport. This step is necessary because renarcotization is common in hoofed animals, especially rhinos, given opioids. This step, however, may not be necessary if the butorphanol/detomidine anesthetic protocol is used (P. Morris, pers. comm.). Trained personnel should be present to administer the correct reversal agent(s) in the event of a renarcotization. Any other complications of crating can be managed more easily and effectively before departure rather than en route.

## **Transport**

Numerous options for transporting rhinos are available. Each method has its advantages, and each should be scrutinized by evaluating the distance to be traveled, the personnel needed and the temperatures to which the animals will be subjected. A flat-bed truck and open trailer is temperature-restrictive. A crate within an open trailer should be protected from excessive wind, rain and sun. Enclosed trucks or trailers are other options that are necessary in extreme hot or cold temperatures. In any case, the transport vehicles must be climate-controlled if shipping in inclement weather (either hot or cold). If weather conditions and ventilation are appropriate, many rhinos have been moved in enclosed trailers without climate-control. Air transport, rather than ship transport, is the preferred option for any transoceanic translocation. Transport by ship is undesirable because of the excessive time at sea, variable conditions and more intensive personnel requirements. When transporting by air, it should be noted that some airlines may require the rhino crate to be placed in an aluminum air cargo box, which can restrict ventilation and subject the rhino to excessive heat buildup during both the airplane-loading process and transport.

During all rhino shipments, the shipper must be aware that any animal that has been immobilized (and to a lesser extent, some that have not) will be less capable of maintaining thermal homeostasis than a normal animal, and appropriate accommodations for this are necessary (e.g.,

ventilation, climate-control). If during the course of a transport procedure a situation arises in which the safety of the animal is jeopardized, a decision should be made through the appropriate channels to postpone or cancel the shipment. Leaving the decision of whether to transport an animal to the transporter or the recipient may be disadvantageous to the animal's welfare. The transporter is not familiar with pertinent medical and practical husbandry information, and recipients are at a disadvantage because they are often not present.

## ***ENCLOSURE DESIGN***

### **Design**

In designing facilities to maximize rhino health and reproductive success, it is important that the environment contain as many salient features as possible found in the natural environment (for more detailed information on the natural habitats of the various rhino species, see the Behavior chapter). The species to be exhibited will dictate the design of rhino facilities, as species differ in their group compositions and enclosure requirements. Additionally, whether an institution wishes to maintain rhinos only for exhibit or for breeding will determine the design of rhino enclosures. Whenever possible, institutions are encouraged to plan for breeding capabilities, but the various SSP coordinators and RAG chairs recognize the need for display-only exhibits, which can facilitate education and/or research. These exhibits serve rhino management programs by holding non-reproductive and single-sex specimens. The following section outlines design considerations for indoor and outdoor rhino facilities, as well as aspects of chute design for rhino restraint.

### **Enclosures**

The design of zoo enclosures for rhinos requires an understanding of rhino biology, behavior and social organization. As previously stated, black, white and greater one-horned rhinos vary in their levels of sociality and thus have different housing requirements. The design of rhino enclosures also depends on the type of rhino program (exhibit only or breeding) and the number of animals. In all cases, the larger and more varied the enclosure, the better. Tables 2.10 and 2.11 list the recommended animal numbers for institutions holding rhinos (see also Management and Behavior chapters) and the enclosure types recommended depending on institutional goals. It is important to note that for the most part, exhibit and holding-space availability will dictate an institution's designation as either a breeding or an exhibit facility. Design elements for a breeding facility should include an outdoor primary enclosure (with separation capabilities), indoor holding and an isolation area. Additionally, breeding institutions must have space for any offspring to be held for up to three years of age. Exhibit-only facilities should have an outdoor primary enclosure and indoor holding areas (both with separation capabilities). It will be recommended that exhibit-only facilities receive pre- or post-reproductive-age or single-sex groups of animals.

Table 2.10. Recommended numbers for institutional holding.

Rhino Species	Recommended Minimum Groupings for Breeding <sup>a</sup>	Preferred Optimal Holding for a Breeding Institution	Exhibit Only (per institution)
Black	1.1	2.2 (2 pairs)	1.1 or 0.2
White	1.2	2.4 (1 herd/ 1 back-up male)	1.1 or 0.2 <sup>b</sup>
Greater one-horned	1.1 <sup>c</sup>	2.2 (2 pairs)	1.1 <sup>c</sup> or 0.2

<sup>a</sup>Breeding institutions must have space for offspring to be held for up to 3 years following birth.

<sup>b</sup>Multi-male bachelor groups have been maintained in very large enclosures.

<sup>c</sup>In the case of greater one-horned rhinos, males and females should be introduced only during the female's estrous period. Institutions with very large enclosures may be able to hold opposite-sex animals together consistently.

In general, it is recommended that enclosures be designed such that animals may be kept outdoors as much as is possible within the following temperature constraints. Rhinos should not be locked outside when the temperature is below 4.4°C (40°F); sun, wind chill and rain should be considered in calculating temperature. During extremely cold weather, rhinos should not have access to mud wallows, and they should be filled with substrate. Animals should not be let out if enclosures are icy. Temporary exposure to temperatures below 4.4°C (40°F) for cleaning is left to the discretion of management. Localities that experience average daily temperatures below 10°C (50°F; average of high and low temperatures over a 24-hr period), should provide heated facilities capable of maintaining a minimum temperature of 13°C (55°F).

Table 2.11. Recommended enclosure types and sizes for captive rhinos by species [in sq m and (sq ft)].

	Individual Holding (per rhino)		Exhibit Only (per rhino)		Breeding/Communal	
	Indoor	Outdoor	Indoor (as primary exhibit area)	Outdoor	Indoor	Outdoor
Black	18 (200)	186 (2,000)	204 (2,200)	771 (8,300)	not recommended	2,322 (25,000)
White	30 (320)	186 (2,000)	215 (2,320)	929 (10,000)	not recommended	2,787 (30,000)
Greater one-horned	30 (320)	186 (2,000)	215 (2,320)	929 (10,000)	not recommended	2,787 (30,000)



## Outdoor Housing

Several general outdoor enclosure designs are recommended that incorporate the data available on the behavior and ecology of wild black, white and greater one-horned rhinos. As previously described, rhinos of all species may be less solitary than was originally thought. For the most part, however, rhinos are somewhat territorial; therefore, more than one outdoor yard is strongly recommended. In many respects, the critical enclosure characteristic is the availability of escape routes and visual barriers, which serve to hide or prevent access to an animal that is being pursued. Gates may be used as escape routes, provided that care is taken to prevent dead-end corners and to create “run-arounds” (brush piles, earth, or boulders) so that an animal can enter or leave the yard without an aggressor blocking or guarding the only exit.

Enclosure size depends on whether rhinos are kept for exhibit-only or breeding purposes (Table 2.11). It should be noted that a calf is considered an adult with respect to minimum space requirements after weaning; this should be considered in determining minimum enclosure size.

### *Primary Barriers*

The barrier between rhinos and the viewing public is a critical element in the design of the outdoor exhibit. This primary barrier should allow visitors a clear view of the animals from a safe location. Many types of primary barriers are available, the most common of which are walls, fencing, dry moats and water moats. One consideration in choosing fence type should be the size of the enclosure. For example, smaller exhibits should be constructed with barriers that provide as much visual exposure as possible. Moats, both dry and water, are less desirable for breeding groups because of the potential for accidents.

### *Fencing*

Because any of the rhino species may climb, a primary barrier should be a minimum of 1.5 m (5 ft) high and non-climbable. In small enclosures, particular attention should be given to the climbing ability of rhinos and to the need for separating aggressive animals. A secondary barrier or a taller primary barrier may serve to counter these problems. It is important to consider fence spacing and keeper access/exit in the event of an emergency as well.

Recommended materials for primary fencing include solid concrete or rock walls, horizontal pipe or cable spaced 25 to 30 cm (10-12 in) apart, and vertical pipe or posts spaced 25 to 30 cm (10-12 in) apart. Cable should be used only for horizontal fences. The size of the exhibit to be fenced will determine the strength and type of fencing material used, as each type has both advantages and disadvantages. Concrete surfaces and bare steel cable create surfaces that may encourage rhinos to horn-rub excessively, causing abnormal horn wear. If necessary, surfaces should be covered with a non-abrasive material; one solution is to insert steel cable through plastic pipe, or concrete surfaces can be covered with non-toxic wood.

If poles are used, each should be approximately 30 cm (12 in) in diameter and set in concrete with approximately 1.8 m (6 ft) underground. Poles should be spaced as closely together as possible to prevent rhinos from getting their horns through and uprooting the fence. ***Creosote-treated poles, which are dangerous to rhinos, should not be used.***

Rocks or a rock apron can be utilized to protect the poles or other objects in the exhibit from damage. A rock apron should extend 1.8 m (6 ft) from the leading edge of the object to offer adequate protection. If small rocks are used, they should be several layers thick; otherwise, a single layer of very large rocks is probably adequate.

## **Dry Moat**

The use of a dry moat requires one vertical wall, which should be a minimum of 1.5 m (5 ft) high, located on the public side. The second wall should be sloped at a maximum of 30° so that the animals can climb out. This gradual decline of the exhibit substrate down to a solid wall can be used to create a moat effect, but ***ditch moats with two vertical walls are considered dangerous to rhinos and are not recommended.*** The floor space in the moat should be a minimum of 1.5 m (5 ft) across to prevent rhinos from being trapped, and surface substrate for the moat should provide stable footing (recommended materials include dirt, gravel, sand, etc.).

## **Water Moat**

Water should not be utilized as a primary barrier as it carries the risk of drowning or injury.

## **Secondary Barriers**

Though not critical to the design of outdoor enclosures, secondary barriers may protect exhibit features or lessen stress on primary barriers. Recommended types are butt rails, vertical poles and electrically charged, or “hot,” wire. Plantings can also serve as a secondary barrier when used to create a visual screen. For example, plantings that extend above a low wall can give the appearance of a bigger wall [although the primary barrier height minimum of 1.5 m (5 ft) still applies]. Electric fencing can deter animals from destroying plantings, trees and other secondary barriers. Rock aprons may also be used around trees and fence lines as secondary barriers.

## **Gates**

Enclosure gates can be the weakest points of the exhibit; therefore, adequate hinge and lock strengths are very important. Interior doors are usually constructed of heavy-gauge steel or pipe that is hinged or sliding. Sliding gates are optimal, as they have the ability for partial opening, and should be a minimum of 1.8 m (6 ft) wide and 2 m (6 ft 8 in) high. If the gate uses a track, care should be taken in the construction of the track to avoid injuring the feet of the animals as they run through gates during introductions. Exterior building doors may be made of steel or wood reinforced with steel, with the lower part covered by a steel plate to minimize damage. Gates should be constructed to allow keepers to open and close them without entering rhino space. Also, where appropriate, vehicle access to an enclosure should be provided.

There are a variety of options available to operate gates. The simplest systems are manually operated, either push-pull or cable driven. Rhino-size doors can be heavy and difficult to move. Mechanical systems can make this easier, using electric motors or hydraulic or pneumatic pressure to move the doors. These systems require some form of back-up system in case of a mechanical or power failure. Mechanical systems can generate significant forces that can injure or even kill an animal. Safety measures have to be incorporated into the design of the gate operating system to prevent accidents.

## **Substrate**

The outdoor enclosure should have a well-drained surface that provides adequate footing for rhinos. Substrate options for white rhinos include grass, limestone, sand and other natural materials in combination. For example, one institution reported an outdoor enclosure substrate composition of 75% grass and 25% sand, rocky areas and decomposed granite. Rhinos should be carefully observed upon introduction to a new substrate, as excessive ingestion of the substrate from feeding on the ground has caused impaction in other hind-gut digesters.

## **Water**

Fresh, potable water should be available at all times. Water should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be substantially constructed to prevent injury, upset, spillage or leakage.

## **Mud Wallows**

White rhinos need access to mud wallows, not pools, for skin health, temperature regulation and behavioral enrichment (Fig. 2.5). The size of mud wallows should be gauged by the number of animals in the exhibit so that room is provided for each individual. It should be noted that given a start, rhinos may construct their own mud wallows. Construction of wallows varies by institution and exhibit. Some facilities use a concrete basin or pool filled with substrate, while others simply dig out a section of the enclosure. If a concrete pool is used, it should have the ability to be drained when needed, but care should also be taken to protect the drain line from being filled with substrate. If a wallow is dug on exhibit, it should be situated in an area that will allow adequate drainage away from the wallow. It is also important that a good source of water be nearby to keep the area wet. Wallows are much easier to manage if the enclosure allows access for a skid steer or tractor with front loading bucket.

Mud wallows must be actively managed. If the local soil does not create mud of a satisfying consistency, it can be adjusted with the addition of topsoil, clay or sand. Standing water in an animal area can be a potential USDA compliance issue. The wallow should be monitored for the growth of algae. If the algae cannot be removed by raking or scraping, it may be necessary to fill the area in to allow it to dry completely for several days, and then dug out again. Depending on the local environment, the substrate may need to be changed several times during a year to prevent contamination. Institutions in northern climates may need to fill in the wallow during winter months.



Fig. 2.5. Mud wallows are an essential component of white rhino enclosures (photo taken at Audubon Zoo).

## ***Visual Barriers***

When rhinos are maintained together in a more herd-like situation, naturalistic visual and physical barriers (refuges) in outdoor enclosures may help decrease aggression by permitting animals to separate themselves from others during introductions or in a group situation. Barriers should be large and high enough to provide “safe zones” that allow an animal to pass from another’s sight but should not hinder public viewing. Types of visual barriers include deadfall, logs and boulders, as well as trees and natural plantings. Trees and plantings may be protected from rhinos by pipe caging, rock aprons or barrier fencing. If permanent physical structures are not available as barriers, dirt mounds may be used to give individuals additional visual barrier points in the enclosure.

## ***Shade/Rain Shelter***

Access to shade is a necessity as well as a USDA requirement under the Animal Welfare Act. A variety of both natural and constructed options are possible. It is also important that a shade option be adequate as a rain shelter if barn shelter is not always accessible; therefore, trees may not be completely adequate. It is a good idea for wallows to be located in areas that are shaded at least part of the day.

## ***Additional Furnishings***

Additional furnishings for the outdoor exhibit should include scratching posts, which may be particularly effective if placed near mud wallows. Post materials must be non-toxic to rhinos (i.e., non-creosote). Several institutions have buried deadfall or logs upright in concrete sewer culverts, which are routed in place with 0.9 to 1.2 m (3-4 ft) of gravel. This enables managers to remove and replace posts as they deteriorate. Feed should be available at all times in the form of browse, feed stations and mineral salt licks.

## ***Indoor Housing***

Indoor housing is recommended for additional separation capabilities (beyond the primary enclosure) and is critical for those institutions in colder latitudes. At no time should rhinos be forced to endure temperatures below freezing for any length of time; animals may go out for short periods when temperatures are below freezing, but they should have access to radiant heat or heated enclosures during these times. An indoor facility in the winter should be heated to a minimum of 13°C (55°F) with the capability of maintaining some areas of the barn at 23.9°C (75°F). Supplemental heat may be needed when dealing with infants or with sick or older animals. Some acclimation may be necessary before moving animals from a warm barn to the outdoors during winter months. The humidity level should be maintained at 40 to 70%. Shower sprays or water baths should be offered in areas of relatively low humidity. Indoor facilities should be maintained with a negative air pressure, and ventilation should be provided to accommodate at least four air exchanges per hour (USDA recommendations for a cold-weather heated barn). Institutions are encouraged to check with their local authorities for air-exchange requirements when the public or personnel occupy the facility.

Within any indoor facility, areas must be provided for food and water. Fresh water should be available at all times and should be changed daily or be supplied from an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset, spillage or leakage.

For white rhinos, stalls should be available when needed. Stalls may not be necessary for females but are mandatory for bulls. The indoor enclosure should include a minimum of 30 sq



m (320 sq ft) per animal for white rhinos (Table 2.11). An additional 50% of adult space should be provided when a calf is present. This may be achieved by using more than one stall. Following weaning, a calf should be treated as an adult with respect to space requirements. If the institution has only indoor facilities in which to maintain and/or exhibit rhinos, the minimum requirement is 186 sq m (2,000 sq ft) per rhino [15.2 x 12.2 m (50 x 40 ft)] plus the recommended indoor holding [30 sq m (320 sq ft) per individual for white rhinos].

### ***Separation Capabilities***

The indoor facility should have the capacity to separate individuals for a variety of purposes. For white rhinos, although females may be housed together in a community barn following the guidelines of 30 sq m (320 sq ft) per animal, males should always be stalled individually. The facility should also have an extra space or large stall to make it possible to isolate mothers and calves or to quarantine sick animals.

Currently, no quantitative data are available on the visual, olfactory or auditory capabilities of rhinos in relation to breeding success. Based on species ecology and behavior, however, it is believed that rhinos rely heavily on both olfactory and auditory senses for social communication. It is therefore recommended that indoor facilities facilitate these types of communications at certain times among individuals. Options include partial walls or pipe fencing to allow for physical separation without visual, auditory or olfactory separation.

### ***Substrate and Special Features***

A brushed or broom-finished concrete floor that is well-drained and ensures adequate footing is recommended. Dirt flooring as the main substrate is not recommended. In addition, floor heat is recommended in colder climates. Bedding materials such as hay, wood shavings and hoofed-stock rubber matting are optional for white rhinos. Other situations in which bedding is required include barns with rough substrates (which may cause skin ulcerations) or for additional warmth for sick animals or young calves. When introducing rhinos to new substrates, careful observations should be made to avoid the animals' excessive ingestion of the novel substrate, which could potentially lead to health problems, such as impaction. The use of a power washing machine is recommended to disinfect barn areas. Additionally, rubber matting and bedding materials should be disinfected or changed regularly to prevent contamination. Permanent rubberized flooring (poured floors or encapsulated mats) is more expensive but reduces cleaning time and risk of contamination.

Normal light cycles seem to be adequate for rhinos. However, if an animal is to be held indoors for more than 12 hours (e.g., winter at cold-climate institutions), artificial or natural light sources to simulate natural cycles should be provided. Fluorescent lighting is an efficient light source that provides broad-spectrum illumination; skylights should also be included whenever possible.

Any new exhibit should include the capability for video systems. In addition, a scale for weighing animals is desirable and strongly recommended. Vehicle access to an indoor facility is also recommended. A restraint device or an area for restraint should be included in every facility design, as well as an area to set up crates for training, loading, and unloading.

### **Physical Restraint Designs**

Numerous institutions have constructed permanent physical devices to restrain their rhinos when necessary. Such "chutes" can be very valuable for physical exams as well as nutritional, reproductive or veterinary research. In addition to the following general information, please consult the Health chapter of this publication as well as Schaffer (1993) and Eyres et al. (1995). Institutions

in the United States that currently have chutes and may be able to provide additional information include Henry Vilas, Saint Louis, Sedgwick County, Oklahoma City, Henry Doorly, Cincinnati, Caldwell, and Milwaukee County Zoos, Fossil Rim Wildlife Center, and the Wilds. Companies that may assist in chute design and construction include Animar Systems, Inc. (Springfield, MO, USA), Cummings and Son, Inc. (Garden City, KS, USA), and Mark McNamara of Fauna Research (Tamer, Fauna Research, Inc., Red Hook, NY, USA). In general, institutions modifying rhino exhibits or constructing new ones should incorporate a physical restraint area or device into their design.

Several physical restraint designs are effective for rhinos. These range from a small restricted area in which to contain the animal to an area that contains one or more hydraulics that will “squeeze” together to restrict an animal’s movement. In general, major restraint chute design considerations include strength, durability, type and function. It should be noted, however, that available space and animal size and disposition vary across institutions and should be individually addressed.

In general, both zoological managers and researchers emphasize that the general restraint area should be an active component of daily rhino management. Methods to accomplish this vary. A restraint chute or restraint area can be designed so that the rhinos must pass through it to exit the barn into their yard. If rhinos are fed indoors, part of the feed (e.g., produce, grain) can be offered in the chute area. Finally, more extensive conditioning (see Training chapter) can be particularly effective in habituating rhinos to physical restraint. Such a program should be attempted prior to detaining a rhino in a chute for an exam.

Rhino chutes should be manufactured out of steel or a combination of steel and steel-reinforced wood. Some institutions have also used steel-strength aluminum (6061-T52 aluminum). Aluminum of this type is lighter and more maneuverable than steel, as well as potentially less stressful to rhinos because of “deader” sound properties than steel (i.e., when metal scrapes metal).

Permanent pass-through indoor restraint chutes (similar to those constructed for elephants) are especially effective for rhinos. With training, this type of chute may allow for detailed daily rhino observations. Further, inclement weather will not affect the use of an indoor restraint chute. The chute should allow restraint of the animal when it is passing through in either direction so that the shifting routine of the animal is not interrupted (Schaffer, 1993). The width of the chute should limit side-to-side movement while still allowing the animal to comfortably lie down. Animals can become wedged in tight-fitting chutes if the sides cannot be released. To alleviate excessive forward movement of the animal when it lowers its head, two vertical bars that push in from the sides of the chute to the shoulders of the rhino may be utilized. Quick release of these shoulder bars often relieves agitated animals without having to release them completely.

High-walled chutes or bars over the top of the chute keep the animal from climbing or rearing up. Horizontal bars in the chute’s entry gates and sides are hazardous for examiners when the animal lies down. Vertical bars on the sides can trap researchers’ arms if the animal can move forward. If the animal’s forward and side-to-side mobility can be limited, vertical bars or walls on all sides are recommended. The distance between these bars along the sides of the chute should be great enough to prevent the animal’s foot from becoming wedged if the animal rolls on its side in the chute. For personnel safety, this distance can be divided with removable vertical bars.

Rhinos may slam swinging doors; thus, sliding or guillotine gates are safer. A rectangular opening in these gates so that palpation can be performed should not pin the arm of an examiner when the animal is shifting. The distance between the vertical sides of this rectangular opening must be wide enough to provide for staff safety while still limiting the space through which a rhino could squeeze. Also, the horizontal bottom bar of this rectangle should be only a few inches from



the ground, as animals frequently lie down. Solid doors on the outside of these gates can be used to stop rhinos, as they may attempt to charge even small openings. Additionally, good lighting and accessible electrical sources are useful.

A closed chute (Fig. 2.6) is another option that has been used successfully for the treatment of a rhino with a urinary-tract infection and another with infected lesions on its foot (Eyres et al., 1995). As noted in Figure 2.4, a typical closed chute has both front and back gates. The back gate restricts the rhino's movement by sliding forward. Additionally, the hind end of the rhino is supported by a V-design that prevents it from lying down. This design also allows additional safety for staff while working with the animal. In many respects, a closed chute does not depend as strongly on conditioning of the rhinos as does a squeeze chute, although acclimation is recommended prior to attempting any treatments within the chute. The design of a closed chute might necessitate an outdoor location in most cases; therefore, the use of this type of chute may be limited by weather.

A free-stall chute can be used for animals more sensitive to a confined enclosure (Fig. 2.7). The design of this type of chute allows the rhino to enter or exit at its will and thus may help to keep rhinos calmer during procedures. Because there is free access, however, rhinos must be conditioned to target or stand still; thus, relatively non-invasive procedures also work best. Procedures that have been accomplished with a conditioned rhino in a free-stall include ultrasound and serial collection of blood and feces (Eyres et al., 1995).

A free-stall design can easily be incorporated into an existing pen or stall, indoors or outdoors. The open back of this type of chute allows the animal to enter and leave the structure at will. Protection of staff when working with the rhino is critical; a partial back wall constructed of vertical pipes allows staff to step out of the way (Fig. 2.7).

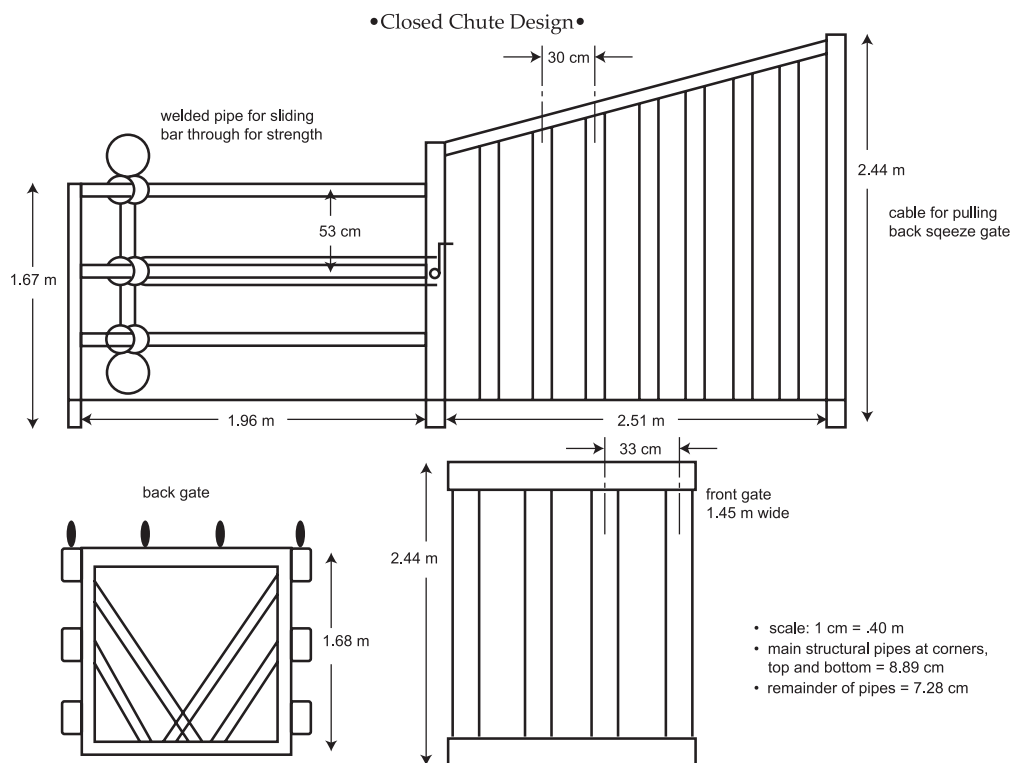


Fig. 2.6. Closed stall rhino restraint chute. Note that a typical closed chute has gates that restrict the rhino's movement and prevent it from lying down. The advantage of a closed chute is that it does not depend as strongly on conditioning as does a squeeze or free-stall chute (Eyres et al., 1995).

•Free-Stall Chute Design•

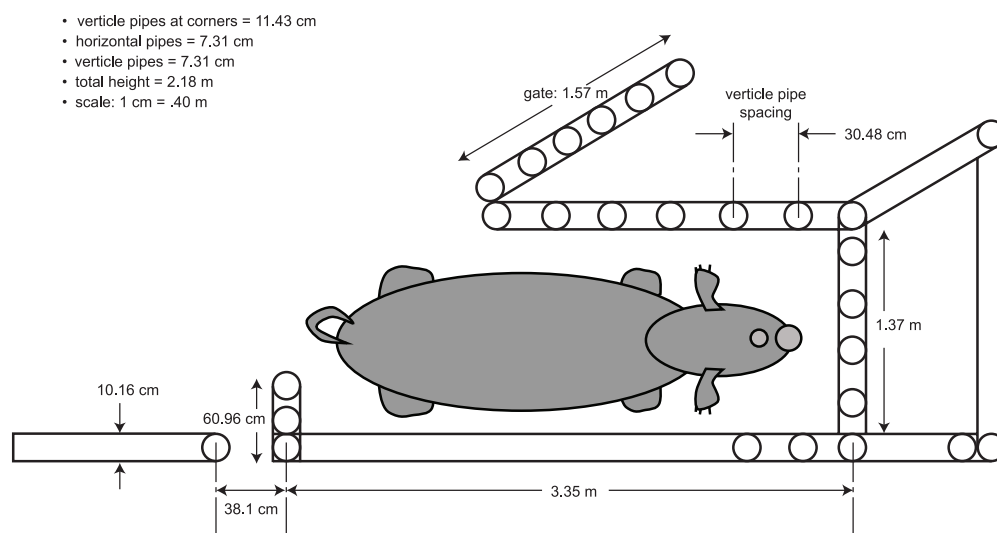


Fig. 2.7. Free-stall rhino restraint chute. The design of a free-stall chute allows the rhino to enter or exit at will and can be used for animals more sensitive to a confined enclosure. This type of restraint chute, however, is best used for relatively non-invasive procedures and with rhinos that have been conditioned to target or stand still (Eyres et al., 1995).

## HEALTH

### Introduction

This section provides a general overview of preventive medical and disease management, and chemical restraint of captive rhinoceroses. More detailed information for many of these topics is available in the references provided as well as through the Rhino Resource Center ([www.rhinoresourcecenter.com](http://www.rhinoresourcecenter.com)).

### Physiological Normals and Sample Collection

With the increased use of training for husbandry and medical procedures, resting values for heart rate, respiratory rate, temperature and other values have been obtained for non-restrained white rhinos. The various species appear to be similar with heart rates of 30 to 40 beats per minute and respiratory rates of six to 12 breaths per minute. Rectal temperatures are typically 34.5 to 37.5°C (94-99.5°F), although temperatures may be higher in anesthetized rhinos (37-39°C; 98.6-102°F) due to exertion or muscle tremors (Miller, 2003; Radcliffe and Morkel, 2007; Morkel et al., 2011). Values are comparable to domestic horse ranges. Limited information about electrocardiography (ECG) is available in rhinos (Jayasinge and Silva, 1972). Indirect blood pressure has been measured in unsedated white rhinoceros using a human blood pressure cuff around the base of the tail. Mean values reported for unanesthetized white rhino are 160 ± 2.9 mm Hg (systolic), 104 ± 2.3 mm Hg (diastolic), and 124 ± 2.2 mm Hg (mean blood pressure)(Citino and Bush, 2007). In anesthetized animals, etorphine can cause hypertension, although variable mean blood pressure measurements (107-280 mm Hg) have been observed, depending on drugs used and time values measured.

Hematologic, biochemical, mineral, protein electrophoresis, and blood gas values have been previously published (Tables 2.12-2.14; Flesness, 2002; Miller, 2003). Although most parameters can be generally interpreted similar to other perissodactyls, there are several differences that appear to be normal in rhinos. Total protein and globulins tend to be higher than in domestic horses. Other values that differ include lower sodium and chloride. Free-ranging rhinos tend to have higher creatine phosphokinase (CPK) than captive animals (this may be biased by immobilization technique)(Kock et al., 1990; Mathebula et al., 2012).

Venipuncture can be routinely performed on awake captive rhinoceroses using training and/or restraint devices (Fig. 2.8). The most commonly used sites for blood collection are the ear (auricular) vein, metacarpal vein (lower inside forelimb), and radial vein (inside of forelimb crossing the carpus)(Figs. 2.8-2.10). The tail (coccygeal) vein has also been used and is approached from the ventral aspect similar to the technique for blood collection in domestic cattle. Arterial access is available for blood gas sampling using the medial auricular artery (inside of the ear)(Fig. 2.11). Large volumes of blood can be collected from the radial or metacarpal veins for diagnostic testing, plasma/serum banking, or for therapeutic phlebotomy (Mylniczenko et al., 2012). Rhinoceros blood cells resemble those of domestic horses. Nucleated red blood cells (NRBC) and reticulocytes may be observed in anemic animals. Elevated total white blood cell counts (wbc) and eosinophil numbers have been observed in wild rhinoceroses, presumably due to the response to capture and parasite loads, respectively.

Table 2.12. Mean hematology values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
WBC x 10 <sup>3</sup> / $\mu$ l	8.42 (2.48)	9.30 (2.46)	7.20 (1.33)	8.27 (1.55)
RBC x 10 <sup>6</sup> / $\mu$ l	4.01 (0.88)	5.77 (1.28)	6.43 (0.86)	5.32 (1.09)
HBG g/dl	12.0 (2.0)	13.8 (3.8)	13.4 (1.5)	12.4 (1.6)
Hct %	33.4 (5.7)	36.9 (9.3)	37.0 (4.6)	36.9 (4.2)
MCV FL	85.7 (9.0)	63.8 (7.8)	57.8 (4.9)	71.5 (11.2)
MCH pg/cell	30.5 (3.3)	23.5 (1.9)	21.3 (3.0)	23.9 (3.8)
MCHC g/dl	35.7 (2.7)	37.9 (7.3)	36.3 (3.2)	33.5 (2.1)
Platelets x 10 <sup>3</sup> / $\mu$ l	284 (83)	378 (103)	178 (53)	198 (135)
Nucleated RBC/100 WBC	0	1 (1)	0	–
Reticulocytes %	1.6 (2.9)	–	–	–
Neutrophils x 10 <sup>3</sup> /ml	5.24 (2.18)	5.42 (2.05)	5.13 (1.24)	4.86 (1.16)
Lymphocytes x 10 <sup>3</sup> /ml	2.48 (1.1)	2.35 (1.15)	1.74 (0.67)	2.52 (0.90)
Monocytes x 10 <sup>3</sup> /ml	0.43 (0.32)	0.65 (0.55)	0.22 (0.15)	0.36 (0.22)
Eosinophils x 10 <sup>3</sup> /ml	0.25 (0.22)	0.54 (0.59)	0.32 (0.31)	0.37 (0.21)
Basophils x 10 <sup>3</sup> /ml	0.17 (0.09)	0.10 (0.05)	0.13 (0.05)	0.08 (0.01)
Neutrophilic bands x 10 <sup>3</sup> /ml	0.27 (0.35)	0.71 (1.18)	0.22 (0.20)	0.31 (0.24)

Table 2.13. Mean blood chemistry values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
BUN mg/dl	13 (3)	16 (3)	3 (2)	6 (2)
Creatinine mg/dl	1.1 (0.2)	1.8 (0.4)	1.3 (0.2)	0.9 (0.1)
Uric acid mg/dl	0.5 (0.2)	0.9 (0.8)	0.3 (0.2)	-
Bilirubin mg/dl	0.3 (0.1)	0.3 (0.3)	0.4 (0.3)	0.2 (0.1)
Glucose mg/dl	69 (21)	97 (39)	82 (25)	76 (13)
Cholesterol mg/dl	102 (37)	93 (26)	53 (21)	48 (21)
CPK IU/L	255 (248)	409 (722)	260 (203)	617 (398)
LDH IU/L	595 (427)	537 (320)	267 (149)	231 (38)
Alk Phos IU/L	80 (55)	92 (51)	80 (41)	17 (6)
ALT IU/L	16 (7)	16 (9)	7 (7)	6 (3)
AST IU/L	85 (27)	71 (25)	61 (27)	39 (9)
GGT IU/L	27 (18)	19 (14)	18 (16)	6 (2)
Total protein g/dl	7.6 (0.9)	8.5 (1.0)	7.5 (0.9)	7.5 (0.4)
Globulin g/dl (electrophoresis)	4.9 (0.9)	5.3 (0.8)	4.5 (0.7)	3.8 (0.7)
Albumin g/dl (electrophoresis)	2.6 (0.4)	3.2 (0.5)	2.9 (0.5)	3.6 (0.6)
Fibrinogen mg/dl	104 (195)	101 (241)	350 (84)	324 (85)

Table 2.14. Mean serum mineral values and blood gases in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
Ca mg/dl	12.7 (1.0)	11.8 (0.9)	11.4 (0.8)	13.3 (1.1)
P mg/dl	4.8 (1.1)	4.0 (0.9)	4.0 (0.9)	3.7 (0.7)
Na mEq/L	133 (3)	134 (5)	132 (3)	133 (4)
K mEq/L	4.7 (0.6)	4.7 (0.8)	4.1 (0.4)	4.6 (0.6)
Cl mEq/L	96 (0.3)	95 (4)	91 (3)	100 (3)
Bicarb mEq/L	23.3 (4.2)	18 (0)	27.0 (0)	-
CO <sub>2</sub> mEq/l	25.4 (9.9)	25.3 (8.8)	27.3 (3.7)	22.8 (2.4)
Iron $\mu$ g/dl	227 (66)	176 (67)	152 (70)	-
Mg mg/dl	3.34 (3.45)	118.2 (232.5)	7.95 (8.56)	-



Fig. 2.8. Voluntary blood collection from auricular vein in awake rhinoceros.



Fig. 2.9. Blood collection from the metacarpal vein in a standing rhinoceros.





Fig. 2.10. Blood collection from radial vein in immobilized rhinoceros.



Fig. 2.11. Arterial blood sample collection from medial auricular artery.



Urinalysis panels in captive rhinos are similar to horses with large numbers of calcium carbonate crystals, creating a normal milky yellow appearance to the urine. Calcium oxalate, phosphate, and ammonium crystals may also occur, depending on the diet. Occasionally dark discoloration of urine associated with pigmentation of certain browse species (e.g. ash, mulberry) can be mistaken for blood or myoglobin. However, analysis should be performed to rule-out any abnormalities. Normal values for the different rhino species have been published (Table 2.15; Haffey et al., 2008).

Table 2.15. Reported range of mean urinalysis results from three captive rhinoceros species.

	pH	Specific Gravity
Greater Asian one-horned rhinoceros	8.08-8.70	1.019-1.031
Sumatran rhinoceros	8.16-8.32	1.010-1.021
Black rhinoceros	8.10-8.26	1.010-1.012

Due to size, cerebral spinal fluid collection has not been successful except in a few rhino calves (S. Citino, pers. comm.). Therefore, extrapolation of normal values from domestic horses and other perissodactyls should be used for interpretation.

Incorporation of scales into rhino facilities has permitted monitoring of body weights and physical condition. Regular weighing is recommended to observe trends associated with growth, diet changes, and disease. Average range for adult body weight is 1800-2200 kg (3,968-4,850 lb; Miller, 2003).

## Preventive Medical Health Programs

Routine health monitoring should be performed on all rhinoceros on an ongoing basis. Animals should be trained to permit sampling and examination. The following **protocol** advises that specific laboratory tests be performed for the purpose of evaluating current health status. Additional tests are recommended to increase baseline information on other diseases to determine their significance to rhinoceros health. The final decision for specific procedures should be made by the animal care and veterinary staff based on individual circumstances.

- Physical exam by a veterinarian familiar with rhinoceros health problems. This should include a review of all systems (if performed in a restraint device, exam may be limited by training and temperament of the individual and design of facility).
- Body weight—Actual weight should be recorded whenever possible; body scores and/or digital photos can be used when scales are not available.
- Blood collection for complete blood count (CBC), serum chemistry panel, fibrinogen, serum protein electrophoresis and extra serum/plasma for banking (minimum of 10-20 ml). Blood smears should be carefully screened for the presence of hemoparasites, especially in recently captured or imported animals. The current Rhino SSP/TAG tissue/sample collection **protocol** should be consulted for additional samples that may be **requested** for research, disease screening, etc.
- Fecal samples should be collected semiannually or at least annually (depending on management system) for direct, flotation, and sedimentation to detect internal parasites.

- Annual enteric pathogen screening may be included, especially for animals in intensive management situations (e.g., breeding herd). Aerobic culture of feces for enteric pathogens should include special media for the detection of *Salmonella spp.* Because *Salmonella* organisms may be shed intermittently, at least three to five fecal cultures should be performed (may be done on consecutive days).
- Vaccinations—Vaccination for rabies, tetanus, and arboviruses (EEE/WEE/WNV) may be considered if the diseases are considered endemic in the area or increased risk factors are identified. There have been isolated cases of rabies and tetanus documented in rhinoceros (Jones, 1978; Mukherjee et al., 1984; Selvam et al., 2003). Check with the SSP veterinary advisor for the most current recommendations on species-specific vaccination protocols.

Additional preventive health recommendations have been included for consideration when performing examinations.

- Serological screening for leptospirosis (multiple serovars) and West Nile Virus (WNV). Although these tests are not species-specific and have not been validated for rhinoceros, they may detect cross-reactive antibodies in exposed animals. The presence of antibodies does not necessarily denote current infection or disease. Antibodies to leptospirosis have been detected in vaccinated rhinoceros and may be used to monitor response and possibly determine frequency of vaccination, although data is insufficient to determine protective titers. Insufficient data is available at this time to determine the significance of WNV antibodies in rhinoceroses; although it is important to note that a greater one-horned rhinoceros with clinical signs developed a WNV titer during a period of known exposure (P. Calle, pers. comm.). One study in greater Asian one-horned rhinoceroses did not find seroconversion in response to vaccination with a commercial equine WNV vaccine (Wolf et al., 2008).
- Serum/plasma vitamin E levels should be checked on a regular basis to assess adequacy of diet and supplementation protocols (see Nutrition chapter). See the most current Rhino TAG/SSP preventive health protocol for recommended laboratories.
- Reproductive tract examination—When feasible, a complete reproductive examination should be conducted to include transrectal ultrasound, semen collection and analysis, and serum or fecal collection for hormone analysis (Radcliffe et al., 1997). Uterine leiomyomas, cystic ovaries, and irregular cycling have been observed in captive animals (Hermes et al., 2004). Since these conditions can have potentially significant effects on reproduction, a careful evaluation is warranted if the animal is being considered for breeding. Standing laparoscopy enabled visual examination and uterine biopsy of a leiomyoma in a southern white rhinoceros (Radcliffe et al., 2000b). A number of publications describe the technique for ultrasonography in rhinoceroses and normal reproductive biology. The reader is referred to the Rhino Resource Center for further information ([www.rhinoresourcecenter.com](http://www.rhinoresourcecenter.com)).
- Urinalysis should include both fluid and sediment evaluation of a clean voided sample. Microbial culture should be considered if there is evidence of blood cells or bacteria.
- Radiographs of feet are strongly recommended if any signs of pododermatitis or nail cracks are observed (Atkinson et al., 2002). Regular foot trimming and care may require immobilization in certain individuals (i.e., those that have a history of chronic foot problems). See section on pododermatitis.

- Diagnostic tests for tuberculosis—Periodic testing for tuberculosis in rhinoceroses should be considered, especially if there has been a history in the institution or herd. Intradermal testing can be performed using bovine PPD (0.1 ml ID) in the eyelid, behind the ear, caudal tailfold, or axillary region. Both false positive and false negative results have been found when performing intradermal tuberculin testing in rhinos (Godfrey et al., 1990). Ancillary tests such as nasal swabs, tracheal washes, and gastric lavages for mycobacterial culture have also been used. Serological tests [ElephantTB STAT-PAK®, MAPIA, DPP (dual pathway platform); Chembio Diagnostic Systems, Inc.] are being investigated for use in rhinos. See section on tuberculosis.
- Other vaccination regimens will depend on regional requirements and exposure risks (consider multivalent vaccination for Clostridial diseases). Contact the SSP veterinary advisor for the most up-to-date information.

Animals being moved between institutions should receive a preshipment examination and testing. This is similar to the routine health procedures but should also include screening for tuberculosis and other requirements dictated by the receiving institution (see quarantine section below).

## Neonatal Examinations

Ideally, neonates should be examined within 24 to 72 hours of birth to detect any congenital defects. Often the dam can be separated while the calf is manually restrained for a brief, but thorough exam, including body weight, and if feasible, blood collection. Complete blood count, biochemical panel, tests for passive transfer of immunoglobulins [e.g. glutaraldehyde coagulation, zinc sulfate turbidity, radial immunodiffusion plates (equine plates work well in rhinos and can be standardized by testing healthy rhinos), and serum protein electrophoresis], vitamin E level, and banking should be performed. A microchip may be placed behind the left ear for future identification. Particular attention during the exam should be paid to the umbilical stalk for signs of infection, urine leakage, or hernia; passage of meconium, normal rectal/anal anatomy and tone; suckling reflex and neurological status. Regular weights and developmental progress should be documented. Lack of weight gain may be due to inadequate maternal milk production and care, or an indication of health or developmental problems in the calf. White rhinoceros calves have been successfully hand-reared (covered under Nutrition chapter). In one case, a white rhinoceros calf was orphaned when its dam died, and the calf was taught to drink from a bucket at the age of six weeks. Weakness or other problems resulting in prolonged recumbency can cause decubital ulcers. Treatment of two neonatal white rhinos has been described using serial sedation with butorphanol alone or in combination with detomidine for restraint (Gandolf et al., 2006). Rectal prolapse has been reported in black rhinoceros calves, with surgical intervention required in at least two cases (Pearson et al., 1967; Abou-Madi et al., 1996).

## Parasites

Internal parasites are more commonly found in free-ranging rhinos than in captivity. Stomach botfly larvae (*Gyrostigma pavesii*) have been reported in white rhinoceroses (Velleyan et al., 1983). Other parasites reported include nematodes (*Diceronema versterae*, *Parabronemia roundi*—associated with stomach nodules), *Draschia megastoma*, multiple species of *Kiluluma*, *Khalilia rhinocerotis* and pinworms (*Oxyuris karamoja*). Tapeworms (*Anoplocephala sp.*) can cause asymptomatic infestation in both captive and wild rhinos (Miller, 2003).

Diarrhea secondary to *Entamoeba sp.*, *Giardia sp.*, and unidentified flagellates have been observed in captive white rhinoceroses. One case of neosporosis led to acute fatal myocarditis in a neonatal white rhino calf and abortion in another case (Williams et al., 2002; Sangster et al., 2010).

Ticks are regularly found on free-ranging rhinoceroses, and imported animals should be carefully examined and treated. Multiple species have been identified on African rhinos (*Amblyomma hebraeum*, *Dermacentor rhinocerinus*, *Rhipicephalus maculates*, *R. muehlensi*, *R. simus*, *R. appendiculatus*, *R. zambeziensis*, *Haemaphysalis silacea*, *Hyalomma truncatum*)(Penzhorn et al., 2008).

Vector-borne parasitic infections occur in endemic areas. Trypanosomiasis can infect white rhinoceros and lead to anemia in tsetse fly-endemic areas. Antibodies to heartwater (*Cowdria ruminantium*) have been found in both African species where the *Amblyomma* ticks are present (Kock et al., 1992).

Capture and translocation or importation may exacerbate potential parasitic infestations and increase the risk of introduction of novel pathogens to a new environment. Therefore, careful screening for external and internal parasites, prophylactic treatment of wounds with fly repellent, pour-on tick treatments (coumaphos, flumethrin), and judicious use of antiparasitic medications (pyrantel pamoate, fenbendazole, ivermectin, praziquantel) should be considered. Check with the SSP veterinary advisor for current recommendations.

## Quarantine

Due to the size, strength, and temperament of a rhinoceros, it may be logistically difficult to maintain isolation from other animals during arrival and quarantine. The Rhinoceros TAG/SSP Recommended Preshipment Protocol for Rhinoceros lists a comprehensive battery of tests for health assessment prior to shipment. Since most zoological institutions will not have the facilities available to safely house and manage a newly arriving rhinoceros, it is important that the receiving institution work closely with the sending institution to ensure that all (or as many as possible) of the listed tests are conducted and results reviewed before shipment. Following the preshipment protocol may help compensate for some of the quarantine compromises that may be required. Regardless of preshipment test results, every attempt should be made to maintain some degree of physical separation of the incoming animal from the resident rhinos after arrival.

Current quarantine practices recommend a minimum 30 to 90 day quarantine period for most mammalian species in zoos and aquaria. Social concerns, physical facility design, and availability of trained rhino staff may dictate a modified quarantine protocol. Specific quarantine guidelines and protocols at each institution should be reviewed jointly and decisions made by the veterinary and animal management staff. Recommended **procedures** to consider as part of a comprehensive plan for rhinoceros quarantine include:

- Thorough physical examination including a review of all organ systems.
- Blood collection for CBC (including blood smear examination for hemic parasites), serum chemistry panel, fibrinogen, serum protein electrophoresis, and serum bank.
- Fecal collection for parasite screening (direct, flotation, sedimentation) conducted weekly for the first three weeks.
- Fecal cultures for *Salmonella* spp. conducted at least weekly for the first three weeks.
- Any procedures that were not completed prior to transport or that may be due, such as vaccination, serologic screening, or TB testing.

It should be emphasized that the quarantine test requirements should be strongly considered regardless of the results of preshipment testing. The stress of transport and quarantine may result in health changes (for example, Salmonella shedding) that were not detected during testing at the sending institution.

## **Hospitalization and Critical Care**

In most cases, hospitalization is impractical with adult rhinoceroses. Most animals will be treated in their holding areas. Rhino calves may be hospitalized in adequate large animal holding facilities if the severity of their medical condition warrants. Barns or holding areas that incorporate species-appropriate restraint devices facilitate medical treatment. Some of the following medical problems require active intervention, including sedation, immobilization, injectable drug therapy, and/or fluid therapy. Although it has been achieved, fluid therapy in rhinoceros presents logistical challenges. In addition to the intravenous route, it is possible to improve hydration using rectal enemas with warm physiologic solutions or even tap water. Animal and staff safety should be a priority in any planned intervention.

## **Diseases**

### ***Tuberculosis***

*Mycobacterium bovis* and *M. tuberculosis* have caused infections in captive rhinoceroses (Stetter et al., 1995; Miller, 2008). Initial infection may be asymptomatic or result in progressive weight loss and emaciation, with coughing and dyspnea occurring in the terminal stages. Nasal discharge may be present but is not a consistent sign. Most infections are pulmonary. Antemortem testing includes intradermal tuberculin test, tracheal and/or gastric lavage for mycobacterial culture, and serological tests (see preventive health section for details). Retrospective analyses of serum from *M. tuberculosis*-infected black rhinos showed positive results using the ElephantTB Stat-Pak® (Duncan et al., 2009). Treatment has been attempted using isoniazid, rifampin, ethambutol, and pyrazinamide (Barbiers, 1994; Duncan et al., 2009). However, assessment of successful response is limited. Concerns for other collection animals, staff and public health need to be considered prior to initiation of therapy. Since this is a reportable disease, notification of the appropriate state veterinary officials should occur promptly once a diagnosis is made. Consultation with the SSP veterinary advisor is also recommended.

### ***Salmonellosis***

Salmonella infection can cause enteritis and fatal septicemia in captive and newly captured wild rhinoceroses (Windsor and Ashford, 1972; Kenny et al., 1997). In a retrospective survey of captive black, white, and greater Asian one-horned rhinos in the U.S., 11% reported positive cultures, usually associated with clinical signs (Kenney, 1999). Clinical infection may result secondary to transport, changes in diet, immobilization, concurrent disease, or exposure to a large number of organisms. Lethargy, anorexia, signs of colic, diarrhea, and death may be observed. Successful treatment using trimethoprim-sulfamethoxazole and supportive care is possible if initiated early. However, treatment of asymptomatic animals is NOT recommended.

### ***Leptospirosis***

Leptospirosis usually presents with depression and anorexia. Other signs may include hemolytic anemia (not present in all cases), hemoglobinuria, signs of colic, and development of skin ulcers. Fatality rates are high in clinically affected black rhinos although successful treatment with



trimethoprim-sulfamethaxazole and ceftiofur has been reported (Neiffer et al., 2001). Diagnosis is based on high antibody titers (microagglutination test – MAT) and confirmed by detection of leptospiral organisms in urine or tissues (fluorescent antibody test). Preventive measures include rodent and wildlife control programs, and good husbandry to minimize contamination of feed and water.

### **Gastrointestinal Infections**

Fatal enteritis due to Clostridial infection has occurred in adult white rhinoceroses. Clostridial enterocolitis has been observed in white rhinoceros with signs of diarrhea, lethargy, and colic. Neosporosis has also been documented in a white rhino calf (Williams et al., 2002). See salmonellosis and GI torsion, impaction, and ulcers for other GI conditions.

### **Encephalomyocarditis Infection (EMC)**

EMC viral infection usually results in acute death due to myocarditis (Gaskin et al., 1980). The southeastern/Gulf Coast states in the U.S. are considered an endemic area. Diagnosis is usually based on virus isolation at necropsy from heart, spleen, or other tissues (<http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/53600.htm>). Prevention should target rodent control, although experimental vaccines have been proposed. A commercial vaccine is not currently available.

### **Fungal Pneumonia**

Fungal pneumonia is usually due to *Aspergillus spp.* and primarily observed in black rhinoceroses secondary to immunocompromise from concurrent disease, broad spectrum antibiotic therapy, or corticosteroid use (Weber and Miller, 1996). Clinical signs may include weakness, weight loss, epistaxis, or other signs consistent with pneumonia. Diagnosis is challenging, although serology and bronchoscopy, with cytology and fungal culture, may be useful. Long-term treatment with antifungal drugs (e.g., itraconazole) is expensive and has unknown efficacy. Definitive diagnosis is often made at necropsy.

### **Anthrax**

Death due to anthrax has been observed in wild rhinoceroses. Most cases result in sudden death. Foamy discharge from the mouth and nostrils can be seen and may appear similar to EMC infection. Diagnosis is based on identification of anthrax bacilli in blood or tissue smears. Vaccination of ranches rhinos has been used in some endemic areas of Africa. Sporadic outbreaks in domestic livestock occur in the U.S., although there have been no reports of rhino mortality in these areas (e.g., Texas).

### **Skin Conditions and Dermatopathy Syndromes**

There are several distinct syndromes reported in captivity, but these are primarily found in black rhinoceros (Munson and Miller, 1999).

- Eosinophilic granuloma syndrome—This syndrome usually presents with oral and nasal non-healing ulcers and granulomas in black rhinoceros (Pessier and Munson, 2004). Cytology shows a predominance of eosinophils associated with lysis of collagen and mineralization. Development of significant ulceration may lead to epistaxis (nose bleeds) or oral bleeding. A similar syndrome has been described in domestic cats. Although lesions may resolve spontaneously, usually over one to seven months, they may recur. Treatment has included corticosteroid therapy, which can result in fungal pneumonia, or local cryotherapy. Laser therapy has been used to treat ulcers but may exacerbate hemorrhage. Supportive care is recommended in severe cases. Eosinophilic granulomas in wild rhinos are typically associated with *Stephanofilaria dinniki*.

- Neoplasia—Squamous cell carcinoma (SCC) has been found on the sclera and skin of white rhino. SCC has been treated by surgical excision in a captive white rhino (Goodman et al., 2007). A greater one-horned rhino with SCC of the horn has also been successfully treated with surgery and radiation (Greer et al., 2010). Cutaneous melanomas have been reported in both black and greater Asian one-horned rhinoceroses (Wack et al., 2010).
- Environmental—Dermatitis may be due to inadequate access to wallows. Exfoliative dermatitis has been reported in a captive white rhino.

These cases illustrate the importance of biopsy and culture for identifying the etiology of and directing treatment in dermatologic cases in rhinos.

### ***Pododermatitis and Chronic Foot Problems***

All species of rhinoceros are susceptible to developing pododermatitis due to inappropriate substrate or other husbandry conditions in captivity. Abrasive substrate, long-term indoor housing in northern climates, and limited access to a wallow contribute to chronic foot trauma in captive rhinos (von Houwald, 2005). Management of the condition includes improvements in husbandry and medical/surgical interventions. Medical treatment may be in the form of oral antimicrobial medication and topical use of copper sulfate and oxytetracycline. Regular hoof trimming and surgical debridement of necrotic lesions, along with use of collagen products for granulation tissue stimulation, can lead to improvement in appearance and comfort of the animal. In addition to the factors mentioned above, nutritional imbalances (e.g., zinc) are also being investigated.

### ***Corneal Ulcers and Keratitis***

Corneal trauma and secondary infection can result in corneal ulceration and perforation. Surgical management of a melting corneal ulcer using a conjunctival graft has been described in a greater Asian one-horned rhino (Gandolf et al., 2000).

### ***Horn Problems***

Horn avulsion, cracks, or other trauma can occur as a result of acute or chronic rubbing, pressure, self-induced, or conspecific fighting. This may lead to myiasis, abscessation, osteomyelitis, or pain with behavioral changes. Radiographs, thermography, or fluoroscopy can be used to assess the extent of the damage. Treatment may involve debridement, antibiotics, wound treatment, and fly control (Suedmeyer, 2007). A squamous cell carcinoma of the horn in a greater Asian one-horned rhinoceros was diagnosed and managed with partial amputation and radiation therapy (Greer et al., 2010). The horn is attached to the basal dermal layer, not unlike the laminae of the horse's hoof wall, and therefore perturbations in blood flow can presumably lead to laminitis.

### ***Gastrointestinal Torsion, Impaction, and Ulcers and Other Conditions***

Torsion may result from abdominal trauma or severe GI disease. Severe torsion may lead to acute death; other signs are related to abdominal pain. Dietary changes, dehydration, ingestion of foreign material (i.e., sand), and inadequate fiber content may result in GI impaction. Gastric ulcers have been observed on gastroscopy and at necropsy in rhinos that have received long-term non-steroidal therapy or have concurrent disease. A 32-year old black rhinoceros was euthanized due to rapid onset of wasting secondary to perforation of a pyloric ulcer and peritonitis. Treatment depends on identifying the cause and site of the gastrointestinal condition. Surgical correction of torsion is usually recommended, although most cases are not diagnosed prior to necropsy. Impaction can be treated using rectal enemas, oral administration of psyllium, mineral oil, or

other products to increase GI water content. Equine anti-ulcer medications such as omeprazole, sucralfate, and H<sub>2</sub>-blockers have been used in rhinos by scaling the equine dose. Prevention includes adequate dietary fiber and fluid intake, and avoiding abdominal trauma and ingestion of foreign material. Esophageal dilation was observed in a black rhinoceros following ingestion of a foreign body; successful management involved diagnosis by endoscopy and a change in feeding practices to a low fiber diet (Radcliffe et al., 1998). Inflammatory bowel disease was confirmed at necropsy in a greater Asian one-horned rhinoceros that presented with weight loss and progressive diarrhea (Ferrell et al., 2010).

### ***Gingivitis and Dental Tartar***

All rhino species appear to accumulate dental tartar in captivity, especially if they are not given access to hard or course food items. Similar to horses, rhinoceros also develop dental points that may eventually create clinical mastication problems with age and require periodic dental flotation.

### ***Renal Disease***

Since 2001, chronic glomerulonephritis and/or renal failure have been recognized as a contributing cause of death in at least seven black rhinos. Mineralization of other tissues has been associated with chronic renal changes as well as three cases of accidental vitamin D toxicosis (Murnane et al., 1994; Fleming and Citino, 2003). Weight loss, decreased appetite, dermatitis, and signs of gastroenteritis may be present, including gastric ulcers. Significant changes in blood urea nitrogen, creatinine or urinalysis were not always evident in these cases, making diagnosis difficult until necropsy. However, two white rhinos (aged >40 yr) both developed progressive chronic renal failure characterized by uremia, isosthenuria and hypercalcemia. Nutritional management using a high energy, low protein feed (Equine Senior, Purina Mills, St. Louis, MO, USA) was instituted in both cases and appeared to stabilize the renal disease (S. Ferrell and R. Radcliffe, pers. comm.). Further investigation into causes of renal disease (other than toxicosis-related) is needed.

### ***Creosote Toxicosis***

Seven fatalities occurred among a group of 20 black rhinoceroses captured and housed in bomas constructed with creosote-treated wood in Zimbabwe (Kock et al., 1994). Although five cases died after transport to the U.S. and Australia, they exhibited the same clinical signs and lesions as the initial cases. Animals were lethargic, partially anorectic, developed swollen limbs, and passed brown urine. Abnormal blood results included anemia, and elevated liver enzymes and bilirubin. Post-mortem findings included widespread hemorrhages; swollen, pigmented liver; and ulcers of the upper gastrointestinal tract. The presumptive cause of death was liver dysfunction due to creosote toxicosis. Exposure to creosote-treated housing materials should be avoided for all rhino species.

## **Common Injuries and Treatment: Skin Lacerations, Punctures, Wounds**

Trauma due to conspecific fighting, mating injuries, collision with obstacles in enclosures or in crates, interspecific conflict or, in free-ranging situations, poaching is relatively common. Common sense application of wound treatment principles apply, although the thick skin does not lend itself to primary closure and has a tendency to undermine abscesses along the fascial planes. Therefore, wounds are usually treated by second intention using debridement, hydrotherapy, topical and/or systemic antibiotics, analgesics, and the array of wound healing products that accelerate granulation. A recent article describes the use of vacuum-assisted healing of a surgical wound in a black rhinoceros to improve healing time (Harrison et al., 2011).

## **Necropsy Protocol**

Any rhinoceros that dies or is euthanized should have a complete necropsy performed and tissues submitted for histopathology. The Rhinoceros TAG/SSP Veterinary/Nutrition Blood and Tissue Collection **Protocol** for Rhinoceros should be consulted for current information on the species pathology advisors as well as specific sample **requests** for research and banking purposes.

## **Capture, Restraint, and Immobilization Techniques**

Management of captive rhinoceroses often requires procedures that involve handling of these large and powerful animals. Husbandry training techniques have advanced the application of preventive medical, diagnostic, and minor therapeutic procedures. However, intensive medical and surgical interventions may require physical and/or chemical restraint for safety and comfort of the patient and staff. Since new developments are continuously advanced in this field, the following recommendations should be used as guidelines, and the veterinary advisors and literature should be consulted for specific situations.

### ***Physical Restraint***

Advances have been made in facilities that include a variety of stall and chute designs for physical restraint of rhinoceros. These will be addressed in the Training and Enclosure Design chapters.

### ***Chemical Restraint***

Significant changes in options for chemical restraint of captive rhinoceroses have occurred during the last two decades. The degree of sedation, analgesia, or immobilization required will vary with the procedure to be performed. Health status and temperament of the individual animal, as well as facility design and staff experience may dictate preference for one technique over another.

## **Chemical Restraint and Anesthesia of Rhinoceros**

### ***Introduction***

Anesthesia of rhinoceroses requires adequate preparation, equipment, and experience. It is highly recommended that rhinoceros anesthesia should be attempted only with experienced personnel or after consulting knowledgeable practitioners. Contingency plans for emergencies are a key component of preparations. Clearly defined roles for all personnel should be outlined and reviewed. Adequate equipment for moving a large animal should also be available.

The choice of chemical restraint techniques will be determined by a number of factors, including physical facilities where immobilization will be performed, health status of the individual, procedures to be achieved and level of analgesia required, length of intended immobilization, and experience of the veterinary and animal care staff. Only captive rhinoceros anesthesia techniques will be covered in this section. For more information on immobilization of free-ranging rhinoceros, the reader is referred to specific references on this subject ([www.rhinosourcecenter.com](http://www.rhinosourcecenter.com)) (Miller, 2003; Bush et al., 2004; Portas, 2004; Kock et al., 2006; Radcliffe and Morkel, 2007; Wenger et al., 2007).

### ***Equipment***

With training and availability of physical restraint devices (“chutes”), drug delivery for chemical restraint can be more easily and accurately achieved, create less stress for the individual, and provide greater control of the induction. Standing restraint is also feasible in these situations.

For rhinoceros in stalls, pens or exhibits, drug delivery often requires use of darting equipment. Depending on the situation, pole syringes with robust needles may be used on selected occasions. Hand-injection is also easily achieved in conditioned animals, although care must be taken when using potent opioids to ensure human safety. Most darting systems can be used in captive situations, as long as a robust needle (minimum 40-60 mm x 2 mm needle) is used to penetrate the thick skin and deliver the drug into the muscle. Nylon darts (Teleinject<sup>®</sup>, Telinject USA, Inc., Agua Dulce, CA, USA; Dan-Inject<sup>®</sup>, Danwild LLC, Austin, TX, USA) are preferred in these situations since they are less traumatic than metal darts. Ideal sites for drug injection are just caudal to the ear on the lateral cervical area, upper caudal hindlimb, or shoulder. However, any site can be used if the dart is placed perpendicular to the skin and is adequate to penetrate muscle.

Additional equipment that should be available include a blindfold, ear plugs (cotton cast padding, stuffed socks), heavy ropes, and padding for recumbency. Inflated truck inner tubes can be used to reduce pressure points. Otherwise, heavy mats or padding should be used if the procedure will take place on a concrete floor. Immobilizations carried out in sandy or grassy areas may provide enough padding for shorter procedures.

Contingency plans for moving a rhino that becomes recumbent in a difficult position should also be made. Lining stalls with plywood sheeting to prevent the head/horn from getting stuck should be considered in areas with open bollards or poles.

Additional equipment should include the availability of oxygen and other emergency equipment. If a field procedure is being planned, an axe or chain saw and bolt cutter may be needed if there are trees/vegetation/other obstacles in the area where the rhino can get caught.

### ***Preparations***

Depending on the health status, environment, and procedure planned, removal of food and water prior to the procedure should be considered at least overnight. Consult the veterinarian for specific instructions.

### ***Positioning***

Rhinoceros are prone to developing myopathy and neuropathy after recumbency. The optimal position remains controversial and may be dictated by the planned procedure. Lateral positioning is often preferred since it provides the optimal circulation to the limbs, although sternal recumbency may provide better ventilation (Morkel et al., 2010). During the procedure, limbs should be “pumped” about every 20 minutes to encourage circulation (Radcliffe and Morkel, 2007). If the animal needs to be in sternal recumbency for the procedure, it is ideal to roll the animal into lateral recumbency whenever possible and pump the legs.

### ***Anesthetic Monitoring***

With the advent of new tools for monitoring physiological parameters in veterinary patients, it is imperative that monitoring be performed on immobilized rhinoceros to minimize complications. Ideally, an accurate weight or weight estimate will facilitate optimal drug calculation and prevent drug over- or under-dosing and associated complications (Adams et al., 2005). Thorough physical examination should be performed, along with regular recording of vital signs (respiratory rate and depth, heart rate, temperature, mucous membrane color and capillary refill time). Ideally, this should be assigned to dedicated personnel and measured at five-minute intervals throughout the procedure.

Pulse oximetry is a useful tool for monitoring trends in hemoglobin oxygen saturation but can be prone to false readings due to placement and the thick skin of the patient. Therefore, it



should not be a substitute for basic clinical assessment. Sites for placement of the sensor include the ear pinnae (scraping can sometimes improve readings) and mucosal folds of the penis, vulva, or rectum. Side-by-side placement of sensor pads has been used in the conjunctival sac, gingival and nasal mucosa, and inside the rectum, vagina, or prepuce. Ideally, readings should be greater than 90%, but interpretation should be made in conjunction with the color of the mucous membranes, blood, and other clinical signs. Capnography may also enhance patient monitoring by enabling early detection of adverse respiratory events, including hypoxia. While pulse oximetry gives trends in patient oxygenation, capnography provides information about CO<sub>2</sub> production, pulmonary perfusion, alveolar ventilation, respiratory patterns, and elimination of CO<sub>2</sub> from the anesthesia circuit and ventilator.

### ***Standing Sedation***

Standing sedation should only be attempted under conditions that take into consideration animal and staff safety. The type of procedure, as well as the temperament of the animal, restraint device, and husbandry training of the individual, will determine the level of sedation and analgesia required. Excitement and environmental or painful stimuli can override drug effects. As a general rule, individuals that are acclimated to people and husbandry training and restraint practices tend to require lower doses of immobilizing agent. Opioids tend to have more potent effects than the tranquilizer/sedative classes of drugs. A combination of butorphanol and azaperone has proven effective for repeat procedures, inducing standing sedation and recumbency in the white rhinoceros (Radcliffe et al., 2000a). See Table 2.16 for suggested doses for standing sedation of captive rhinoceros.

Table 2.16. Standing chemical restraint doses for adult captive white rhinoceros.

<b>Chemical Restraint Drug(s)</b>	<b>Reversal Agent(s)</b>
0.8-1.5 mg etorphine IM	naltrexone 50 mg/mg etorphine
50-70 mg butorphanol + 100 mg azaperone IM	naltrexone 2.5 mg/mg butorphanol
1.1 mg etorphine + 5 mg acepromazine + 15 mg detomidine + 15 mg butorphanol IM	naltrexone 50 mg/mg etorphine; atipamezole 5 mg/mg detomidine
120-150 mg butorphanol + 5-7 mg medetomidine IM (give 1-2 mg nalorphine IV to keep standing)	naltrexone 2.5 mg/mg butorphanol; atipamezole at 5 mg/mg medetomidine

### ***Immobilization/General Anesthesia***

The primary class of drugs for general anesthesia in rhinoceros is the opioids. Etorphine is most commonly used, although carfentanil and more recently, combinations of etorphine and thiafentanil have also been administered to rhinos. Opioids are typically combined with azaperone, an alpha-2 agonist (e.g., xylazine, detomidine), or acepromazine to provide muscle relaxation and to counteract the hypertensive effect of the opioids (LeBlanc et al., 1987). Midazolam, diazepam, or guaifenesin infusion can also provide additional muscle relaxation. White rhinos appear to be more sensitive to the effects of opioids than black rhinos and exhibit muscle tremors, limb paddling, hypoxia, hypercapnia, and hypertension (Weber and Miller, 1996; Portas, 2004). Butorphanol has been administered to antagonize respiratory depressive effects in white rhino (10-20:1 butorphanol:etorphine in mgs); however, it may also lighten the plane of anesthesia (Miller et al., 2013).

Other partial opioid agonist-antagonists are routinely used in the field and can be adapted for captive rhinos when available (e.g., nalbuphine). Butorphanol-azaperone and butorphanol-medetomidine/detomidine combinations have successfully induced recumbency in captive white rhinos (Radcliffe et al., 2002; Portas, 2004; Waltzer et al., 2010). Supplemental ketamine, opioids, guifanesisin, propofol, or isoflurane can be used to deepen the level of anesthesia and lengthen immobilization as required (Ball et al., 2001). See Table 2.17 for recommended doses used for recumbent immobilization of rhinoceros.

Oxygen supplementation by intratracheal intubation or nasal insufflation (flow rates of 15-30 liters/minute) can increase oxygen saturation values (Morkel et al., 2010). Doxapram administration for apnea has also been used in rhinos but may only provide short-term relief. Partial or complete reversal should be considered in severe cases of hypoxia.

Table 2.17. Recumbent immobilizing doses for adult captive rhinoceros.

Chemical Restraint Drug(s)	Reversal Agent(s)
1.2 mg carfentanil IM	naltrexone 100 mg/mg carfentanil
2-3 mg etorphine + 20-40 mg azaperone IM	naltrexone 50 mg/mg etorphine
120-150 mg butorphanol + 5-7 mg medetomidine IM ± 5% guafenisin drip	naltrexone 2.5 mg/mg butorphanol; atipamezole 5 mg/mg medetomidine
70-120 mg butorphanol + 100-160 mg azaperone IM	naltrexone 2.5 mg/mg butorphanol

### ***Use of Tranquilizers/Sedatives for Transport and Other Uses***

There may be occasions other than medical procedures when rhinoceros need to be sedated for short or more extended periods of time, such as during crating and transport, or confinement for other reasons. With the advent of husbandry training, the need for drugs in captive rhinos has become more limited but should always be available as an option. Tranquilizer/sedative drugs may be used in rhinoceroses to relieve anxiety, reduce hostility, decrease motor activity, alleviate excitement, and to facilitate animal introductions. Drug choice is based on the desired duration of action and expected outcome. For short duration tranquilization/sedation, azaperone (20-60 mg) or detomidine (2-4 mg) can be used (Kock et al., 2006). Long-acting neuroleptics (LANs) are typically administered in free-ranging rhino after capture for transport and boma acclimation, although they have also been used in captive rhino for longer duration tranquilization (see Table 3.18 in black rhinoceros Health chapter).

## ***NUTRITION***

Many of the health problems identified in captive rhinos are believed by some to be linked to nutritional factors. Rhinos consume a large number of species of plants with a diverse array of physical characteristics and nutrients. They represent a range of feeding strategies and, consequently, diet, from browsers (or selective feeders) to unselective grazers. Diets in the zoological setting may have possible imbalances in dietary fats (particularly essential fatty acids) and soluble and insoluble carbohydrates, as well as minerals and vitamins for some species. This chapter outlines current dietary information for maintaining rhinos in captivity and includes a section on hand-rearing.

## Nutritional Requirements

Due to similarities in digestive tract morphology, the domestic horse still represents the best nutritional model for all rhinoceros species. Until further information is obtained, diets should be formulated using current National Research Council (NRC; 2007) recommendations for horses of various physiological stages. Minimum nutrient requirements are listed in Table 2.18.

Table 2.18. Nutrient concentrations in total diets for horses and ponies (dry matter basis; modified from NRC, 2007).

Nutrient	Growing	Mature/ Maintenance	Pregnant/ Lactating
Dig. Energy (Mcal/kg)	2.45 - 2.90	2	2.25 - 2.60
Crude Protein (%)	12-15	8	10-13
Ca (%)	0.6	0.3	0.4
P (%)	0.3	0.2	0.3
Mg (%)	0.1	0.1	0.1
K (%)	0.3	0.3	0.4
Se (mg/kg)	0.1	0.1	0.1
Vit A (IU/kg)	2000	2000	3000
Vit D (IU/kg)	800	300	600
Vit E (IU/kg)	80	50	80

Good quality forages should provide primary nutrients for all herbivores, with concentrate feeds used to balance energy, protein, mineral or vitamin needs. Hay storage is particularly important for ensuring proper dietary management. Moldy or dusty hay may cause colic and/or heaves. Large amounts of poor-quality hay should not be fed to rhinos, as it may be so poorly digested that impaction and/or colic will result. Very high-quality legume or small-grain hay may be so readily digested that when fed with concentrates, loose feces or colic may result. The larger, grazing rhino species should have *ad libitum* access to grass hay and water. Given the propensity of captive grazing rhinos to become over-conditioned, use of hays or low-glycemic index concentrates are highly recommended for white rhinos (Berkeley et al., 2011). The concentrate portion of the ration should be given in at least two feedings daily for better utilization. When practical, a small feeding of hay should be encouraged prior to each concentrate feeding.

In studies of intake, digestion and passage in zoo herbivores, Foose (1982) measured dry matter intakes of approximately 1% of body mass when white rhinos (n=5) were fed grass hays and slightly higher levels (1.2-1.6% of body mass) when fed alfalfa hay. Diets were 67% digestible (white rhinos eating alfalfa). Thus, a guideline for as-fed diet quantity would be approximately 1.5% of body mass, but the exact quantity of high-fiber pellets will depend on their formulation. Offering sweet feeds in excess of 33% of the total calories in the diet is not advised. Large (>1.0 cm diameter), high-fiber pellets work well with grazing species.

Animals can sometimes be encouraged to consume less palatable forages if hays are soaked in water or sprinkled with molasses. Applesauce has proved to be helpful in administering unpalatable medications and/or supplements.

Produce generally is not offered to white rhinos in their daily diets (indeed, 15/28 institutions do not offer any at all), but some institutions do feed up to 2.3 kg of produce daily. Produce items that are fed for enrichment, training, or as part of a daily diet are listed below (Table 2.19). Similarly, browse generally is not offered to white rhinos in their daily diets (16/28 institutions do not offer any). Where it is offered, a few kg of browse might be offered a few times per year up to free choice when the particular forage is growing (Table 2.19). The most common hays and concentrated pellets fed among white rhinos are noted below (Table 2.19). Hay is often fed in variable amounts, from 0 kg to ad libitum, depending on the available grazing. Two of the surveyed institutions do not feed any pellets. It is important to remember that all diets should be based on forages, not concentrates.

### **Feeding Location**

As with all zoo species, feed should be offered on a concrete pad or in livestock troughs or bins. Sand impaction has previously been documented in rhinos (Nouvel and Pasquier, 1946); therefore, feeding directly on the ground is not recommended. To reduce competition for food, individual feeding stations or adequate space at communal feeders is recommended.

### **Supplements**

Dietary supplements should be unnecessary in properly formulated rations. A possible vitamin-E deficiency has been suggested but not confirmed in zoo rhinos; current recommendations based on natural browse composition suggest that diets should contain 150 to 200 IU vitamin E/kg dry matter. Salt blocks and water should be available at all times. If grown in an area prone to soil selenium (Se) deficiency, forage should be tested routinely for determination of Se content in order to provide data needed for balancing rations (Table 2.18).

### **Problematic Diets**

High-quality alfalfa as an exclusive forage is unnecessary and may lead to mineral imbalances, colic and diarrhea. There is some evidence that white rhinos are more sensitive to phytoestrogens, such as those found in alfalfa hay and some pellets, than greater one-horned rhinos (Tubbs et al., 2012), but the implications of this sensitivity for white rhino reproductive success are still being evaluated. The consumption of fresh red maple browse has been associated with hemolytic anemia in horses and should therefore be avoided.

Table 2.19. Food items fed to white rhinoceros. Amounts (kg) reflect daily rations. Some food items are not offered daily but are offered during enrichment and training; other food items are offered free choice (f.c.).

Produce	Amount	Browse/Grasses <sup>1</sup>	Amount	Hay	Amount	Pellets and Mixes	Amount
Apple	0-0.5	<i>Acacia</i>	0-2/wk	Alfalfa with coastal bermudagrass	20-25	Bran	4.6/wk
Banana	0-2.2	<i>Acer</i> <sup>1</sup>	0-f.c.	Brome w/prairie	Up to 22	Equine Senior	2.9
Beets	0-0.2	<i>Albizia</i>	0-f.c.	Coastal bermudagrass	2.5-f.c.	HMS ADF 16	1.8-9.1
Carrot	0-1.5	<i>Andropogon</i>		Mixed grass	9.3-18	Mazuri ADF 16	2.2-13.6
Celery	0-0.23	<i>Arundo domax</i>		Orchardgrass w/ timothy	0-35	Mazuri ADF 25	5.4-15
Green beans		<i>Bamboo</i>		Peanut		Mazuri Browser Rhino Cube	5.5
Kale		<i>Celtis</i>	0-f.c.	Prairie	Up to 18	Mazuri Wild Herbivore	2.3-20
Lettuce	0-1 head	<i>Cymbopogon citratus</i>		Sudangrass		Mazuri Wild Herbivore Plus	6.5
Parsnips	0-0.2	<i>Ficus</i>		Timothy	0-f.c.	NuZu Low Isoflavinoid	7-10
Pear		<i>Hibiscus</i>		Timothy w/ alfalfa	79.2-f.c.	Open formula herbivore grain	
Potato	0-0.75	<i>Ipomoea</i>	0-f.c.			SDZ Global High Fiber	10.8
Squash	0-0.23	<i>Liquidambar</i>	0-f.c.			ADF-25 Herbivore	
Sweet potato	0-0.75	<i>Lonicera japonica</i> <sup>1</sup>	0-f.c.			Sweetfeed	7.2/wk
		<i>Miscanthus sinensis</i>	0-5/wk			TMR Pennfield	0.5/wk
		<i>Morus</i>	0-f.c.			Toronto Zoo Formula	6.25
		<i>Myrica</i>				ADF 22%	
		<i>Panicum</i>				Wheat bran	0.5
		<i>Pennisetum alopecuroides</i>	0-5/wk				
		<i>Phyllostachys</i>	0-f.c.				
		<i>Plantanus</i>	0-2.72				
		<i>Populus</i>					
		<i>Quercus</i> <sup>1</sup>					
		<i>Saccharum</i>					
		<i>Salix</i>	0-f.c.				
		<i>Sassafras</i>	0-2.72				
		<i>Schinus terebinthifolius</i> <sup>1</sup>					
		<i>Schizachrium</i>					
		<i>Spondias mombin</i>					
		<i>Ulmus crassifolia</i>					

<sup>1</sup> Some browse species are potentially toxic when offered in large quantities. Variety and moderation are key. Animal managers should consult a nutritionist before feeding substantial quantities of any one browse item.



## Hand-Rearing

A limited number of rhino calves have been and are currently being raised using various formulas. Reports and published information must be carefully scrutinized for measures of success and methodology in milk-sample analysis. The following information uses the ungulate hand-rearing chapter in the AZA Infant Diet Notebook as a base for general feeding guidelines and formula selection (Reiter et al., 1994). This recommendation is to be used as a guideline for standardization of a hand-rearing diet.

### *Milk Composition and Formula Selection*

Based on available data, rhinoceros milk is more dilute than milks of other ungulate species. It is low in solids, low in protein, very low in fat and high in sugar compared with milk of equids, bovids and cervids (Ofstedal, 1984). Formula selected should mimic mother's milk in composition as much as possible (Table 2.20). In Table 2.21, Formula One has been used to raise a calf to one year of age; Formula Two more closely mimics mother's milk. In Europe, Mazuri® makes a rhino milk replacer for white rhinos ([www.mazuri.eu](http://www.mazuri.eu)). Land O'Lakes® Mare's Match® (Table 2.22) has been used to supplement a greater-one horned rhino calf at San Diego Zoo Safari Park and possibly could be used for hand-raising white rhino calves (powder:H<sub>2</sub>O = 1:6 but formula may need to be mixed at a more dilute ratio (1:8) for the first few days to avoid problems with constipation). The San Diego Zoo Safari Park has used a low fat cow's milk: nonfat cow's milk: lactose powder: water (27:9:1:1 by weight)(Blakeslee and Zuba, 2002).

Table 2.20. Nutrient compositions of rhino milk (Gregory et al., 1965) and recommended formulas (% as-fed basis).

Formula	Solids	Protein	Fat	Sugar
Rhino milk	8.8	1.4	0.2	6.6
Formula 1	10.3	3.3	0.3	5.9
Formula 2	8.3	1.7	0.2	6.6

Table 2.21. Compositions of rhino hand-rearing formulas.

Ingredients	Parts by Volume	
	Formula 1	Formula 2
Water	32	9
Skim milk	32	9
Karo Syrup	1	1

Table 2.22. Products used in hand-rearing diets.

Product	Manufacturer/Distributor
Mare's Match Milk Replacer Colostrum Replacement	Land O' Lakes Animal Milk Products Shoreview, MN 55126
Colostrx	Protein Technology, Inc. Minneapolis, MN 95403
Seramune Oral	Sera, Inc. Shawnee Mission, KS 66285
Replenish	Fermenta Animal Health Co. Distributor Kansas City, MO 64153
Fer-In-Sol Poly-Vi-Sol with Iron	Mead Johnson Nutritionals Bristol-Meyer Co. Evansville, IN 47721
Major Multi-Vita Drops	Major Pharmaceutical Corp. Distributor Chicago, IL 60612
Lixotinic	Revival Animal Health Distributor Orange City, Iowa 51041
Probios	Vet Plus, Inc. Menomonie, WI 54751
Lactaid	Lactaid, Inc. Pleasantville, NJ 08232

Though rhinoceros' milk is different from cow's milk, the latter may still be appropriate for hand-rearing rhinos if used in combination with other ingredients. Cow's milk is low in iron; consequently, an iron source such as Fer-In-Sol<sup>®</sup> (Table 2.22) should be added to the formula at two drops per 100 g of formula. In addition, infant vitamins, such as Major<sup>®</sup> Multi-Vita Drops<sup>®</sup> (Table 2.22), should also be added to the formula at two drops per 100 g of formula. Some infant vitamins, such as Mead Johnson<sup>®</sup> Poly-Vi-Sol with Iron<sup>®</sup> (Table 2.22), contain added iron. San Diego Zoo Safari Park uses Probios<sup>®</sup> (2 tbsp) and Lixotinic<sup>®</sup> (0.44 ml /kg body weight)(Table 2.22) as daily supplements added in the first bottle. The animal may also benefit from the addition of Lactaid<sup>®</sup> at one drop per 100 g of formula. Lactaid<sup>®</sup> (Table 2.22) aids in carbohydrate digestion and helps prevent possible gastrointestinal tract distress. If the neonate is less than 24 hours old, colostrum diluted 50% with water or an electrolyte solution for ungulates, such as Replenish<sup>®</sup> (Table 2.22), should be administered for the first 24 hours. Though species-specific colostrum is preferred, cow colostrum may be used. San Diego Zoo Safari Park uses Land O'Lakes<sup>®</sup> Colostrum

Replacement<sup>®</sup> (Table 2.22) in the first 24 hours after nursing followed by a mix of 50% colostrum and 50% formula during the next 24 hours, and then 100% formula until weaning. Products such as Colostrx<sup>®</sup> and Seramune<sup>®</sup> Oral may also be used (Table 2.22). To avoid gastrointestinal distress, a diluted formula should be offered beginning on day two. The formula can be gradually increased to full concentration depending on the animal's health, including weight gain and stool condition. Prior to feeding, the formula should be warmed to approximately 37°C (99°F). Rhinoceros calves prefer their milk cooler than many other ungulates.

### **Feeding Regimen**

The calf should be stabilized and hydrated before any feeding. Quantity fed should range from 10 to 13% of body weight (BW). Animals should be fed every two hours. Because infants suckle during daylight hours, feeding should be equally spaced in a 12-hour period not to exceed 3 to 4% of body weight at any one feeding. It is recommended that feeding begin with 10% of body weight split equally into 12 feeds one hour apart during daylight hours. The quantity of formula fed should be adjusted daily based on the animal's weight. Animals should be weighed at the same time each day. Fresh water should be available at all times.

Table 2.23. Example feeding regimen, provided by San Diego Zoo Safari Park.

Week 1 & 2	6am, 8, 10, 12, 2, 4, 6, 8pm	(18-20% BW) 8 feedings
Week 3 & 4	6am, 8, 10, 12, 2, 4, 6pm	(17-19% BW) 7 feedings
Week 5 & 6	6am, 8, 10:30, 1, 3:30, 6pm	(16-18% BW) 6 feedings
Week 7 & 8	6am, 9, 12, 3, 6pm	(14-16% BW) 5 feedings
Week 9 – 14	6am, 10, 2, 6pm	(12-14% BW) 4 feedings
Week 15	No more increases to daily volume	
Week 15 – 30	6am, 12, 6pm	(8-12% BW) 3 feedings
Week 30 – 40		(5-7% BW)
Week 41		(3-4% BW)
Week 52	Start dropping amount on all bottles	
Week 60	Weaned	

If diarrhea occurs, the quantity of formula fed should be decreased or the formula diluted until stool condition returns to normal. If diarrhea is persistent, an electrolyte solution can be used to dilute the formula, replacing some or all of the water. In addition, the number of feedings can be increased to lessen the quantity fed at any one time.

Formula can be prepared ahead of time and warmed as needed. Water should be boiled to decrease possible contamination due to pathogens and refrigerated before being added to the formula. The formula should be refrigerated and used within 72 hours. Prior to feeding, the formula should be warmed to the animal's body temperature. Calf nipples work well with large species. Bottles should be boiled before use. Diluted bleach may be used as a disinfectant. Formula left over from each feed should be discarded.

### **Weaning**

Weaning may begin as early as six months if necessary and should be completed in one year. Weaning is a slow process involving carefully monitoring of body weight and solid food consumption. Animals should have access to solid food at all times. A nutritionally complete pellet diet such as Calf Manna (Manna Pro Products, LLC., Chesterfield, MO, USA), horse feeds or high fiber

ungulate pellets, in addition to high-quality grass hay, is appropriate. Formula may be decreased by gradually eliminating the number of feeds or decreasing the amount offered per feed and gradually decreasing the number of feeds.

## RESEARCH

### Introduction and Top Research Priorities

Largely due to the rhino poaching crisis that far out-weighs any other challenge facing rhinos today, the top five priorities of the Rhino Research Council, listed below, are skewed towards *in situ* issues. It is important to note that these research priorities are not ranked in order of importance, so the first and only priority focused on the *ex situ* population is not considered more important than the others. The Rhino Research Council realizes that conservation of genetic diversity via cell/gamete rescue from remnant populations is really not a research issue and is more of an implementation challenge, but it was identified as an important action to emphasize at this time of crisis. Following this list of the highest research priorities, the chapter continues with important areas of *ex situ* research, including reproduction, health, nutrition, behavior/ecology, and genetics. For more detailed discussions of *in situ* research needs, the reader is referred to the Rhino Research Council's 2014 Rhino Research Masterplan.

#### *Investigate Major Factors Affecting Health and Reproduction Ex Situ*

- Epidemiology of browser rhino health issues—Health concerns and unusual syndromes continue to impact black rhinos in captivity, but the incidence of various conditions shifts over time, and patterns require constant monitoring and re-evaluation to track current trends and conditions. Otherwise, outdated problems and historic records continue to be referenced at the cost of realizing emerging trends and realities. One example is the significant increase in the number of black rhino deaths due to renal disease compared to five years ago and a decreased incidence of mortality due to hemolytic anemia compared to 20 years ago. It would be extremely useful to secure ongoing support for a point person whose priority is to evaluate the historical data and current situations to keep abreast of the problems and factors associated with those health issues.
- Iron overload syndrome (significance, detection, treatment, prevention)—Evidence that hemosiderosis is occurring in browsing rhinos is solid, but there are still many questions surrounding the impact of this condition on rhino health, whether it is primary or secondary, why it is occurring, and what can be done about it. In the past decade, relatively little progress has been made. Although there has been some progress in developing oral iron chelators for humans, the efficacy and safety of such medications are unknown in rhinos, and they are cost prohibitive. Research on dietary tannins as iron chelators has produced inconclusive results. Recent studies on the impact of large volume, regular phlebotomies for reducing iron marker values has shown promise but is unlikely to be adopted as a long-term management strategy at many institutions. Research in any of the four areas—cause, detection, prevention and treatment—could be valuable in producing insight on how to manage/prevent this condition.
- Obesity/body condition scoring—Overconditioning in captive rhinos can lead to a multitude of health problems, including musculoskeletal, foot, and reproductive problems. One example is the almost certain exacerbation of pododermatitis associated with captive husbandry conditions in the greater one-horned rhino. Research on standardizing body

condition scores for all rhino species, along with improved nutritional management, should be a priority for captive health.

- Sub-optimal reproduction (stillbirths, embryo loss, phytoestrogens, pathology)—Although reproductive success has improved in white and greater one-horned rhinos by changing management strategies, providing larger spaces and more complex social groups that include experienced and inexperienced individuals, and allowing mate choice options, there are a few reproductive challenges that remain unresolved and challenge our ability to develop self-sustaining populations. For example, < 50% of all captive white rhinos are reproductively successful, almost 50% of greater one-horned rhino calves are stillborn, and as our ability to evaluate larger numbers of female rhinos has improved, incidence of known embryo loss and reproductive pathology continue to increase.

### ***Improve Rhino Identification and Monitoring***

- Optimize/standardize survey methodologies—Given the current poaching crisis, research that can improve our ability to monitor and survey wild populations is extremely important. Post-release monitoring is our primary method of determining the success of translocation, dehorning and many other conservation efforts. Data compiled from tracking individuals after translocation can be used to develop specific criteria that individual rhinos should meet (sex, age, reproductive status) prior to translocation in order to improve the success rate. Better methods for signal transduction and reception would be helpful for forest-dwelling animals. Creative methods of transmitter attachment are needed since horn transmitters are difficult to insert in de-horned animals, and radiocollars are a challenge given the neck morphology of rhinos. Also, lesions can occur with collars. Given the abundance of research on this topic in the military field, adaptation and application of this newer technology may be useful for rhinos.
- Genetic census—Advances in genetic analyses of fecal samples, hair, environmental-DNA and microbiomes could be used to determine the genetic diversity among wild populations, including sex ratios, number of individuals and extent of inbreeding.

### ***Identify Most Important Factors in Translocation Success***

Translocations have proven extremely valuable in saving rhinos from poaching, re-populating parks/ranches, bolstering dwindling populations and establishing new populations. However, there is room for improvement, and given the importance of this action for long-term rhino survival, research is needed to enhance survival and reproductive success of every rhino moved. Studies might focus on the following example topics: white rhino boma maladaptation, mortality rates in different habitats, impacts of dehorning, impact on source populations, and identifying the type of individuals most likely to succeed.

### ***Determine Ecological Factors Impacting Rhino Populations***

To choose the best locations for rhinos and provide guidance for those who want to improve their land in support of rhino populations, more information is required regarding specific ecological traits that impact rhino population survival and ability to thrive. Spatial ecology is especially important and is integrated with habitat quality, usage and preference.



## ***Conserve Genetic Diversity via Cell/Gamete Rescue from Remnant Populations (small/sub-populations and highly endangered)***

Given the poaching crisis in Africa and India and the rapid decline of Sumatran rhinos throughout their range, it seems prudent that every effort should be made to preserve cell lines and gametes from all genetically valuable rhinos/populations. The methodologies already exist but may need to be modified for the field conditions encountered in the African savannah and the tropical forests of Asia.

### **Areas in Need of Research *Ex Situ***

#### ***Reproduction***

It is still apparent based on studbook data that < 50% of the captive white rhino population has successfully reproduced. There are some young first-generation-captive-born (F1) animals that are not reproducing, but the problem is not solely with F1s. Results from several studies have demonstrated that many of the female F1s that fail to reproduce are exhibiting reproductive cycles, are behaving normally and are being mated by males. Therefore, interest has shifted towards examining post-copulatory events that may be causing infertility, such as inflammation, early embryo loss and/or developmental abnormalities potentially due to dietary phytoestrogens. However, there are still many adult white rhinos with irregular reproductive cycles (long ~70 day cycles versus normal ~35 day cycles), and with the increased use of ultrasonography for monitoring white rhino ovarian activity, it has been determined that some acyclic white rhinos are developing only small follicles that do not grow and mature into pre-ovulatory follicles. Fortunately, hormonal therapy has shown promise for these animals, although resumption of natural, regular cyclicity has not been restored following hormone treatment. High reproductive success at several facilities with large spaces and high numbers of white rhinos continue to support the long-held theory that management is the most significant factor impacting captive white rhino propagation. Interestingly, data from “wild” populations have also indicated that white rhinos at higher densities are more successful, and mating success is not fairly distributed. Some males are far more successful than others.

The following is a summary of the primary issues with white rhino reproduction:

- F1 females are mating but not producing calves.
- White rhino estrogen receptors are more sensitive to phytoestrogens than those of greater one-horned rhinos. Is there a negative impact of dietary phytoestrogens on reproductive success?
- Irregular reproductive cycles (long versus short) are still occurring.
- Acyclic females appear to be producing small follicles that do not mature. Hormonal therapy shows some promise but, so far, has not restored regular, normal cyclicity, and more work is needed.
- Mate choice appears to be important in white rhinos since introducing new males often solves reproductive problems. How can we determine suitable mates before moving animals?

Other important areas of reproduction research are as follows:

- Early embryonic death (EED)—Repeated documentation of EED in all captive rhino species is puzzling. In the past 15 to 20 years, there have been at least six confirmed cases of EED in white rhinos at four different institutions, five confirmed cases of EED in greater one-horned rhinos at three different locations, and seven EEDs confirmed in two Suma-

tran rhinos at two locations. Few rhinos are monitored closely enough to diagnose EED, so considering the number of confirmed cases, it appears to be a common occurrence in the captive population. It has also been reported in at least one field study in wild black rhinos. However, in most field studies females breed only once or twice before conceiving and carrying pregnancies to term, so the high incidence of EED in captivity seems aberrant. In addition to EED, abortions later in gestation have also been reported. There are many potential causes of EED and abortion in rhinos, including uterine infection, uterine scarring from a previous dystocia, endometrial hyperplasia, reproductive pathology, hormonal insufficiency, dietary insufficiency or toxicity, social/behavioral influence of enclosure co-inhabitants or other health-related conditions of the female rhinos. It is quite possible EED is occurring in many females that are breeding repeatedly without producing calves. Further investigation into the prevalence and potential causes of EED and abortion is warranted.

- Reproductive pathology—Reproductive tract pathology in female rhinos has been reported in all captive rhino species and prevalence can be as high as 50% in females over 15 years of age (white rhinos). It can range from small, inconsequential cysts that do not affect fertility to large, invasive tumors that jeopardize the life of the rhino. There is general agreement that these pathologies typically develop in rhinos that spend the majority of their lives in a non-pregnant state and could be related to repeated exposure to hormone fluctuations, a condition not natural in wild rhinos that spend much of their time pregnant. However, there are some younger rhinos that also develop severe pathology, and more recent research has revealed that a fairly substantial proportion of white and greater one-horned rhinos develop pathologies in their early teens. Understanding the etiology of this pathology and identifying hormone therapies that might prevent it would be very useful. Furthermore, criteria for determining when the degree and types of pathology observed during an exam can be tolerated without loss of fertility versus those that render a female infertile would be very useful to establish so that appropriate management recommendations could be made.
- Silent estrus—Behaviorally silent estrous cycles have now been documented in a few white rhinos. This phenomenon challenges efforts to breed this species in zoos because introductions often are made based on behavior. In white rhinos, silent estrus seems to be associated with the development of small follicles that do not reach pre-ovulatory size. When these white rhinos are treated with hormonal therapy, they do develop pre-ovulatory sized follicles and ovulate but still do not exhibit appropriate receptivity towards the male.
- Measuring estrogen—Quantifying changes in estrogen levels that correlate with meaningful reproductive events has been difficult in several of the rhino species and continues to be challenging. A more accurate and meaningful way to assess estrogen concentrations could be useful for monitoring cyclicity, timing introductions and assessing other reproductive characteristics.
- Early pregnancy marker—Pregnancy can be diagnosed in rhinos through progesterone monitoring and ultrasound. It would be useful for management and for learning more about EED if a pregnancy-specific marker could be identified in rhinos not trained for ultrasound, but this is not a high priority.
- Low libido/lack of reproductive activity—For unknown reasons, some male white rhinos fail to exhibit appropriate mating behaviors when in the presence of an estrous female.

The use of assisted reproduction has the potential of becoming a very useful tool for regional and metapopulation management of rhinos. Additionally, assisted reproduction may provide a means of overcoming physical and/or behavioral problems that currently prevent reproduction in specific individuals. Progress towards these goals has been impressive, and it is now time to look at priorities considered feasible to overcome during the next five years.

- Semen collection—Semen collection by electroejaculation (EEJ) has become more reliable and has been successful in all four captive rhino species. However, there are differences in the quality of samples collected with greater one-horned rhinos being the most reliable in producing a high quality, concentrated sample. In black, white and Sumatran rhinos, samples often are more dilute and more likely to be contaminated with urine or red blood cells. Samples are often adequate for cryopreservation, artificial insemination (AI) or in vitro fertilization but often are not able to withstand more complex processing like sperm sorting. More research into the effects of different anesthetic protocols and attempts at penile catheterization may prove valuable. Chemical induction of ejaculation has been attempted but has not proven very effective yet.
- In vitro fertilization (IVF)—To date, IVF success has been minimal (one IVF attempt produced one embryo in a black rhino and one IVM/IVF attempt produced one embryo in a black rhino) and more research is needed. Success has been minimal, in part, because of limited opportunities.
- Sperm sorting—Since 2005, 25 EEJ attempts across three species (n=10 black, n=9 white, n=6 greater one-horned) have been conducted for sperm sorting. Inconsistent ejaculate quality is the greatest challenge to application of sorting technology and integration into AI programs for each species. Only 36% of collections were good enough for sorting. Greater one-horned rhino sperm has a unique problem: an interaction between the seminal plasma and egg yolk that prevents staining of X and Y populations. Most progress has been with white rhino where sorted, cryopreserved samples are now available for use in AI procedures.
- Artificial insemination—Successful AI protocols using cryopreserved semen now exist for white rhinos (five term pregnancies) and greater one-horned rhinos (three term pregnancies). AI has not yet been successful in Sumatran or black rhinos. Additional research is needed in developing successful procedures for black rhinos and Sumatran rhinos, and research to improve efficiency/success rates in all rhinos is also needed to ensure the technology is integrated into the captive management plan. Some challenges include: breaking down intact hymens in females that have never mated, determining ovulatory versus anovulatory cycles in greater one-horned rhinos, and controlling the cycle for planned, timed AI to improve feasibility of use.
- Estrous cycle manipulation—In Europe, significant progress has been made with white rhinos using synthetic progestin and human chorionic gonadotropin or gonadotropin releasing hormone. Similar methodologies are being tested in the U.S. An ovulation induction protocol has been established for Sumatran rhinos. Research is still needed on effective methods for controlling the cycle in greater one-horned rhinos so that specifically-timed AI procedures can be planned and implemented.
- Gamete rescue—Protocols for rescuing sperm post-mortem are well established but protocols for oocyte rescue are not. A few attempts have been made with oocyte rescue, and one two-cell embryo was produced following maturation and IVF with frozen-thawed sperm. However, all protocols still need substantial research before they will be applicable for management purposes.

## **Health**

- Obesity/body condition index – Overconditioning in captive rhinos leads to a multitude of health problems, including musculoskeletal, foot, and reproductive problems. Research examining methods of standardizing body condition scores for all rhino species, along with improved nutritional management, should be a priority for captive health. Methods applied to domestic livestock, and more recently elephants, should be considered for use in rhinos. Also, is the inclusion of cereal-based concentrates in rhino diets detrimental?
- Idiopathic neurologic disease—Several white rhinos have exhibited episodic tremors/ataxia in Australia. Survey of neurologic disease in white rhino would be worthwhile to determine if this is an underreported condition.
- Neosporosis—This infection has resulted in abortion and acute death in white rhino. An experimental ELISA is available. Research to determine seroprevalence may reveal if this is a widespread issue for reproductive or potentially systemic health problems.
- Gastrointestinal health issues—Investigation into potential factors such as diet/nutrition, management, infectious diseases, and stress are warranted to determine if morbidity/mortality can be prevented.
- Pharmacokinetics/dynamics of commonly used antibiotics and analgesics—Little scientific work has been done in rhinoceros species on therapeutic drugs. Non-empirical use may lead to inadequate or potentially adverse effects. With the advent of husbandry training and restraint devices, sample collection is now possible for these types of studies.
- Quantifying stress (especially chronic stress)—Develop laboratory and behavioral markers of stress. What is the health impact, for instance, on the development of gastrointestinal ulcers, increased iron stores, etc.? New ideas include evaluation of neutrophil function using a portable luminometer and the analyses of five markers in fecal samples including thyroid hormones.

## **Nutrition**

There is concern that phytoestrogens may be impacting white rhino reproductive success. Work is ongoing to evaluate the estrogen activity of rhino feeds: Alfalfa and soy have high activity, but sudangrass also has high activity. West coast Bermuda is low in activity, but East coast Bermuda is high in activity. Therefore, it is clear that both type of hay and location where hay is grown can affect phytoestrogen content. Pending outcomes of ongoing research by San Diego Zoo Global, it might be valuable to develop a low phytoestrogen diet.

Body condition scoring systems have been established for the African rhino species, however preliminary trials have proven that these scoring systems are very subjective if utilized by staff at each institution. Furthermore, efforts to control for bias by sending images of animals from different institutions to one person for evaluation were equally as difficult because of the variation in animal appearance based on photo angle/quality. Although similar scoring systems should be developed for Asian rhino species, less subjective methods for all species are needed. Perhaps physiological values for leptin, glucose, insulin, etc., could provide a more solid method of evaluating body condition in addition to computerized assessments, body measurements matched with weights and/or characterizing body types. This is an important issue since body condition has now been suggested as a factor involved in iron storage problems, disease, skewed sex ratio of calves and

reproductive failure of captive-born white rhinos. Other important topics for nutritional research are as follows:

- Research needs related to reproduction include determining if there is an impact of diet on calf sex ratio, evaluation of new hand-rearing formulas for supplementation and complete rearing, determining milk production, and estimating energy requirements of lactation.
- Minimum dietary nutrient concentrations for rhinos need to be established for maintenance, gestation and breeding to determine if utilizing the horse guidelines are adequate.
- Science-based recommendations need to be developed regarding the use of alfalfa for rhinos.
- The use of fecal DNA analyses as a means of defining wild rhino diets should be investigated.
- The cause of tooth overgrowth could be related to diet (nutritional and/or mechanical), and further investigation could be helpful. There have been several cases of rhinos requiring repeated teeth floating procedures. Additional browse could be helpful since silica is known to reduce tooth growth.
- Recent investigations of trace minerals are ongoing, especially with copper, which is an important anti-oxidant that could combat iron. One study has changed the diet to enhance copper. Appropriate dietary concentrations of zinc and copper need to be determined as both are being supplemented for both hoof health and to bind with iron.

### ***Behavior and Ecology***

Large-scale manipulative experiments to test hypotheses relating to social environment, stress, foraging/nutrition, movement patterns, etc. on reproduction, health and survival are encouraged. To achieve this goal, multiple large, naturalistic enclosures will need to be established and experimental groups assigned to different treatment conditions in a way that establishes multiple experimental replicates to address each question. Such a systematic approach, on a more limited number of variables of interest, will advance understanding of the factors governing whether captive (and wild) populations thrive or fail. Areas of *ex situ* research might include:

- Role of communication (olfactory, acoustic) in reproduction, maintaining social relationships, endocrinology (stress and reproduction), and overall social organization (consider experimental manipulations, such as with chemical signals and acoustic playbacks)
- Evaluate social processes (courtship, aggression) leading to successful copulation
- How social mechanisms lead to establishment of social outcomes, such as territoriality, dominance, group composition, etc., and how these change through time as new animals move in and out of the group
- How do changes in ecological parameters affect social behavior, endocrinology (stress and reproduction) and organization?
- Role of climatic variables (temperature/rainfall/photoperiod) on social organization and behavior
- Development of methods to reduce bodily injuries in male-male conflict and inter-sexual conflict that inhibits reproduction
- Behavioral, hormonal, and health perspectives regarding social stress
- Manipulate social variables such as density, group size, and age-sex ratios to determine outcomes for social behavior, organization, stress, health, reproduction, and survival
- Avoidance and attraction patterns
- Social density and composition effects



## Genetics

The potential benefits of employing genetic tools for studying rhino conservation and population genetics are tremendous, but considerable research and development is still needed in many areas before direct, efficient, reliable application will be possible. Areas where conservation genetics could be most valuable include studies on genetic structure /connectivity of surviving populations, dispersal, paternity, and census. The potential methodologies would include analyses of mitochondrial DNA, microsatellites, genetic sexing, single nucleotide polymorphisms, immunogenetics and the microbiome. Over the next five years, there needs to be an emphasis on developing appropriate methodologies, including:

- Microsatellite analysis—Microsatellites are powerful if they can be amplified from DNA from feces and should be a priority.
- Next generation sequencing (NGS)—Since fecal DNA typing is difficult and genetic diversity in some rhinoceros species is low, NGS may be of great value in providing the initial screening for polymorphisms needed to develop markers to examine rhino population genetics.
- Standardization—Different labs using various loci are reporting different results. There needs to be some consistency, or the techniques may wrongly come to be considered unreliable for answering conservation/population questions.
- Shorter amplicons—Amplicons of 75-150 base pairs need to be targeted by genetic markers and made available for fecal DNA studies since degradation can occur rapidly in fecal material.
- Fecal microbiome analysis—Fecal microbiome analysis may be useful in some cases as an alternative to analysis of an individual's own genetic material. Microbial DNA is more plentiful and in better condition when excreted in the feces, and each individual has their own microbial profile. This was the methodology employed to determine that the fecal samples from the Viet Nam Javan rhino were all from the same individual.
- Immunogenetic variability—Immunogenetic variability and its relationship to mate choice would be interesting to investigate. If pre-testing for mate compatibility or interest could occur prior to animal transport, it would be very valuable to animal managers in zoos and managed reserves/ranches.
- Environmental DNA—eDNA is an emerging tool in genetic studies but may be more challenging under tropical conditions.

Ongoing priority genetic studies that might include *ex situ* components include:

- Major Histocompatibility Complex (MHC) variability—MHC analyses are being planned and, once established, will help significantly when analyzing populations for variation in genes that are under selection. This is particularly important information for species that appear to have low neutral genetic variation, for example the greater one-horned and white rhinoceros.
- Fecal DNA analyses—Some work has been done, but the method needs to be optimized so that individual genotypes from fecals can be used for census work and assessing and studying breeding strategies, dispersal, etc. A project to optimize non-invasive genotyping from fecals as a tool for estimating numbers and sex of white and black rhinos from the Kruger National Park has been initiated.

- Study of disease history and parasite load—A critical emerging arena to which genetics can contribute is disease risk assessment of different rhino populations. This is particularly relevant in light of the tragic loss of five captive Sumatran rhino in Malaysia. A particular avenue worth pursuing is polymerase chain reaction techniques that can determine presence and load of different pathogens from tissue collections and fecals. A major study investigating these possibilities is proposed for the Kruger National Park for their *C.s.simum* and *D.b.minor* populations.

## **BEHAVIOR**

### **Ranging Behavior and Sociality**

#### ***Females and Subadults***

The behavioral ecology of the social white rhinoceros differs from that of the other rhino species, which tend to be more solitary (Owen-Smith, 1988; Hutchins and Kreger, 2006). White rhinos have a rich social repertoire, displaying a variety of behaviors and vocalizations (Table 2.24) regularly during interactions. Pairs or small groups of white rhino females and adolescents are the norm (Owen-Smith, 1973, 1975; Pienaar, 1994; Shrader and Owen-Smith, 2002), although most of these associations are transient, lasting only a few months (Owen-Smith, 1973, 1975; Shrader and Owen-Smith, 2002). Close companions are typically two animals, such as mother and calf, two adolescents, or an adolescent and an adult female; only rarely are two adult females found in long-lasting associations in the wild (Owen-Smith, 1973; Hillman-Smith, 1987; Shrader and Owen-Smith, 2002). In contrast, in zoos, companion subgroups can be composed of only adult females (Kuneš and Bičík, 2002; Swaisgood et al., 2006; Metrione et al., 2007). The space between companions is usually less than 5 m (16.4 ft) and rarely more than 25 m (82 ft)(Owen-Smith, 1973; Metrione 2005), and aggressive interactions between close companions are exceedingly rare.

Female white rhinos and any social partners accompanying them occupy large home ranges in the wild (6.5-66.8 km<sup>2</sup>/1,606-16,507 acres; Pienaar, 1994; White et al., 2007a), which overlap extensively with those of other female groups, but the majority of activity may be in smaller core areas about a quarter of the size of the home range (Owen-Smith, 1973, 1975; Pienaar, 1994; White et al., 2007a). Core area use may also shift seasonally, presumably to track resources. Home range area is also influenced by social density, with females ranging farther at lower densities (Owen-Smith, 1975; Rachlow et al., 1999; White et al., 2007a), suggesting that even the relatively gregarious white rhino female limits its movements to avoid social encounters with less familiar rhinos. Female home ranges are substantially larger than male territories, which are socially constrained, with the average female range overlapping with seven male territories (range: 4-15; White et al., 2007a). While extensive space might be available at some zoological institutions for rhinos, an entire group, subdivided into companion subgroups, often can be found in the same general area of a large pasture (Metrione et al., 2007), consistent with the temporary social aggregations seen in wild rhino. Some analyses indicate that larger enclosures are associated with improved reproduction (Swaisgood et al., 2006; Metrione, 2010).

As noted above, associations between free-ranging individuals, particularly those that involve adolescents, frequently last less than a month, but some may persist for five or more months (Owen-Smith, 1973, 1975; Shrader and Owen-Smith, 2002). Relationships in zoological institutions can be long-lasting (years) and might change only when a calf is born to one of the companions

(Metrione et al., 2007). In fact, female white rhinos housed with a female companion known from adolescence tended to have a lower average concentration of corticosterone, a hormone associated with the stress response, than females housed with recently-introduced female companions or no female companion (Metrione and Harder, 2011). In addition, in managed breeding settings, better reproduction is associated with housing females in groups of three or more females/adolescents (Metrione, 2010), including housing captive-born females with wild-caught females (Swaigood et al., 2006).

Dominance hierarchies probably do not occur in the wild (Owen-Smith, 1973), but in the zoo setting, dominant females can gain access to food sooner and feed for longer durations than subordinate females when the group is fed in specific and spatially restricted areas (Metrione et al., 2007). Aggression and the frequency of space-maintenance vocalizations, such as the snort and snarl (Owen-Smith, 1975; Table 2.24), usually are higher during these feeding times than at other times during the day (Metrione et al., 2007). Positive contact behaviors, such as play, might help to mitigate any distress caused by aggressive interactions, especially for subordinate females (Metrione and Harder, 2011). Excessive horn rubbing (Hutchins and Kreger, 2006; Table 2.24), on the other hand, is a behavior that might indicate boredom or distress. Higher rates of stereotypic behavior were reported for non-cycling female white rhinos compared with cycling females (Carlstead and Brown, 2005).

## **Males**

Reproductive white rhino males, which are only 33 to 67% of all wild adult males, establish and defend non-overlapping territories approximately 5 km<sup>2</sup> (1,235 acres) in size, while non-reproductive, subordinate males live within a dominant male's territory (Owen-Smith, 1971, 1973, 1975; Rachlow, 1997; Rachlow et al., 1999; White et al., 2007a). Territory boundaries are delineated by scent marks left only by territorial males during spray-urination, dung-kicking, scraping with the fore- and/or hindlegs, and horn-scraping (Table 2.24; Owen-Smith, 1973, 1975). These behaviors also are expressed by zoo-managed white rhino males throughout their enclosures. When wild territorial male rhinos temporarily move off of their territory in search of water during drought, they adopt the behavior of a subordinate male (e.g., urinating in a stream as opposed to spraying urine), even in the presence of a female (Owen-Smith, 1973). Subordinate males rarely associate with estrous females for any significant length of time, while territorial males associate with a significantly larger number of such females (Rachlow, 1997; Rachlow et al., 1998).

Confrontations between territorial males are highly ritualized, involving advancements, retreats, horn-scraping, horn-clashes directed mainly at the opponent's horn, and checked jabbing gestures as opposed to injury-causing movements (Table 2.24; Owen-Smith, 1973, 1975). Some confrontations, however, particularly during a territory take-over, might be prolonged and bloody (Owen-Smith, 1973, 1975), corresponding with Level 5 aggression in Table 2.25. Most wild males, therefore, will not become territory holders until they are 12 years of age or older (Owen-Smith, 1973). The average duration of occupation of the same territory by a male was 5.4 years, after which he might continue to be a territorial male in a different territory (Owen-Smith, 1973).

Table 2.24. Ethogram of southern white rhinoceros behaviors (adapted from Owen-Smith, 1973 and Fouraker and Wagener, 1996).

<b>Vocalization</b>	<b>Purpose</b>	<b>Description</b>
Snort (vocalization)	Mild “keep-away” warning	Nasal exhalation or inhalation
Snarl (vocalization)	More powerful distance-increasing signal	A gruff roar, brief or rumbling, made with the mouth open, head thrust back, and ears laid back
Pant (vocalization)	Contact seeking or maintaining call	A chesty exhalation or inhalation
Hic (vocalization)	Signifies bull’s intent to court with a throb produced at the	Repetitive wheezy exhalations beginning of each inhalation
Squeal (vocalization)	Emitted by the bull towards a cow to prevent her from leaving his territory	High pitched then falling off; may become a singing wail
Shriek (vocalization)	Attack inhibiting	Intense/shrill; ears thrust back, head thrust forward
Whine (vocalization)	Calf seeking udder or adolescents approaching companions	A thin, mewling tone that rises and falls in pitch
Squeak (vocalization)	Distress signal used by calves	Abrupt and high pitched
Gruff squeal (vocalization)	Emphasizes bull’s presence	Throaty, rumbling squeal
Gasp-puff (vocalization)	Response to sudden fright	Sudden inhalation or exhalation
<b>General Behaviors</b>	<b>Purpose</b>	<b>Description</b>
Locomote	Relocate position	Movement from one location to another
Resting	Energy conservation	Recumbent on the ground
Wallow	Cooling, skin protection	Roll, lay down, or move about in an area that is wet or muddy
Forage	Energy intake	Search for and consume food
Nurse	Energy intake	Calf suckles from udder
Urinate	Excretion	Discharge urine in a stream
Urine spray/squirt	Scent marking	Project urine in a strong spray (male) or in distinct squirts (female); may be directed on a substrate
Defecate	Excretion	Discharge fecal material, usually at communal latrine
Dung-kick	Scent marking	Rapid alternation of hind feet against the ground and dung while remaining stationary; often associated with defecation by the male

Table 2.24. Continued.

<b>General Behaviors Cont.</b>	<b>Purpose</b>	<b>Description</b>
Scraping	Scent marking, territorial demarcation	Hindlegs or forelegs dragged with nail pressed against the ground
Urine/Dung Smelling or Tasting	Olfactory investigation	Smelling or consuming urine or dung; may be followed by flehmen
Flehmen	Olfactory investigation	Raise head and curl underside of upper lip upward
Mouthing	Tactile and olfactory investigation	Repeated chewing or gummin motion with mouth open; not associated with eating
Tail curled	Associated with situations of general autonomic stimulation	Curling of tail; may be held or repeated
Horn rub	Uncertain	Rubbing horn against hard object; often occurs if horn becomes wet; considered stereotypical when excessive
Object toss	Exploration and play	Tilt or lift inanimate object off the ground
<b>Social Behaviors</b>	<b>Purpose</b>	<b>Description</b>
Follow	Social interaction	Locomote to remain in close proximity of another rhino
Nasonasal meeting	Potentially for individual identification	Movements slow and relaxed eventually allowing noses to meet
Anal and genital investigation	Identification	Sniff anogenital region of another animal
Acceptance of tactile contact	To strengthen bonds	Expression of a close bond through non-aggressive physical contact (touch, rub, lick)
Horn wrestling	Play	Horn-against-horn movements that are relatively gentle and more slow than horn clashes
Head flings	Play invitation and indication of excitement	Head swung up and down rapidly
Horn scraping	Assertion of presence/status	Sideways, twisting movements of the horn on the ground
Head/horn sweep	Distance increasing display	Head swings laterally relative to the ground, rooting air with the horn but not contacting another rhino



Table 2.24. Continued.

<b>Social Behaviors Cont.</b>	<b>Purpose</b>	<b>Description</b>
Pinning ears back	Distance increasing display	Ears laid back, usually couple with head thrust and snort or snarl
Advancing steps	More powerful distance-increasing effect than a snarl or snort alone	Actor steps quickly toward the recipient and simultaneously gives a snarl, snort, or shriek
Horn against horn stare	Intimidation display	Horns of two rhinos pressed together with heads raised and ears forward
Horn prod	Ritualized attack movement	Head lowered followed by upward jabbing movement that makes contact with another rhino but does not pierce skin
Horn clash	Gesture to repel encroachment	Horn lowered parallel to the ground then hit sideways against horn of the recipient
Charge	Intimidation display	Rapid advance
Attack	To drive recipient away	Repeated horn jabbing movements directed toward body of recipient
Fight	Opponents attempting to drive each other away	Attack gestures made by both opponents using horns and body
Chase	To drive recipient away	Locomote rapidly in pursuit or another
Gore	Injury-causing	Pierce or wound recipient with horn
Presenting the side	Act of appeasement	Turning head away from other rhino
<b>Reproductive Behaviors</b>	<b>Purpose</b>	<b>Description</b>
Anogenital investigation	Estrus identification, courtship	Bull smells cow's vaginal area; often followed by flehmen
Penis unsheathed	Excretion or sexual stimulation	Penis is dropped from the sheath, possibly in a partial erection
Erection	Sexual stimulation	Penis in erect position
Vulva wink	Sexual stimulation	Rapid contractions of vulva
Vulva swelling	Sexual stimulation	Swelling and/or dilation of vulva
Stand	Mating	Female remains stationary during a chin-rest or mount
Chin Resting	Mating	Bull rests his head on the rump or back of the cow

Table 2.24. Continued.

Reproductive Behaviors Cont.	Purpose	Description
Mounting	Mating	Bull straddles cow's back with forelegs while standing on hind legs; might be preceded by erection
Mis-mount	Mating	Male mounts female but not in proper orientation for copulation
Copulation	Mating	Penile penetration of vagina with successful ejaculation

Table 2.25. Levels of aggression in rhinos (Fouraker and Wagener, 1996).

Level of Aggression	Definition
1	Rhinos are charging each other but do not make physical contact
2	Rhinos are charging each other with physical contact resulting in some cuts and scrapes to the facial area
3	Rhinos are charging each other with physical contact resulting in cuts and scrapes to the facial area and body
4	Charging and/or pursuuta proceeds to the point that one or both rhinos are knocked down at least once. Scrapes and cuts are deeper and more numerous.
5	Aggression and pursuit proceed to the point that one or both rhinos have subcutaneous wounds or arterial blood flow

<sup>a</sup>It should be noted that one animal might break away from the confrontation and attempt to escape. The aggressor often will pursue and begin horn-prodding the underbelly of the escapee as the two run around the enclosure. Often a rear leg is hooked and held aloft while pursuit continues. If the escapee does not stop and resume a defensive posture, the animals might continue until heat or exhaustion becomes a critical factor. Aggression at this point is more serious.

## Reproductive Behavior

Both in the wild and in managed settings, male and female rhinos defecate almost exclusively at communal dung piles and often spend time investigating excrement at the dung piles (Table 2.24; Owen-Smith, 1973; Mettrione, 2005). Female rhinos often urinate at dung piles as well. It has been suggested that the dung piles serve as a “bulletin board” (Owen-Smith, 1973). Both urine and dung serve as chemical signals, and experiments have shown that rhinos can discriminate sex, reproductive condition and individual identity, and they gain other information from these odor sources (Swaisgood, unpublished data). Probably by identifying cues of upcoming sexual receptivity in female excreta, males have been observed intensively following a female as much as a week prior to the onset of estrus (Owen-Smith, 1973).

Anestrous female white rhinos are intolerant of a male's presence and will show aggression to males within 10 m (30 ft) in the wild (Owen-Smith, 1973, 1975). In zoos, the acceptable male-female inter-individual distance seems to vary from rhino to rhino. Male aggression towards females is rare, occurring less than 1/100th as commonly as affiliative behavior toward the female (Swais-

good et al., 2006). The female shows substantially less affiliative behavior toward the male than vice-versa and more aggression, although rates are still low. The pre-estrus consort period lasts up to 20 days in the wild, during which the territorial male is generally obedient to the space-maintenance threats of the female, unless she approaches the border of his territory, upon which the male will approach, vocalize, and charge the female to keep her from leaving (Owen-Smith, 1973). Prior to full estrus, females will use mock charges and defensive threats to drive a bull back (Fouraker and Wagener, 1996). In the case of zoo-managed rhinos, the bull may attempt to split female groupings apart during courtship and breeding attempts. In some cases, these bonds may interfere with breeding success if the females are able to continually drive-off an approaching male (Fouraker and Wagener, 1996).

Mating is a long, slow process. Estrus typically lasts for 24 hours (Owen-Smith, 1973, 1975; Metrione, 2005), during which the male makes regular advances and “hiccing” vocalizations (Table 2.24; Owen-Smith, 1973) and unfailingly makes olfactory investigations of the squirts of urine released by the female (Tables 2.24 and 2.26; Fig. 2.12; Owen-Smith, 1973, 1975; Kuneš and Bičík, 2002; Metrione, 2005). Eventually, the female tolerates chin-resting by the male, usually on her hindquarters or back (Fig. 2.13), mounting attempts (Fig. 2.14), and finally copulation (Tables 2.24 and 2.26; Owen-Smith, 1973, 1975; Swaisgood et al., 2006). White rhino males often mount before an erection occurs (Fouraker and Wagener, 1996). Copulation lasts 15-30 minutes and might include multiple ejaculations (Owen-Smith, 1973, 1975; Swaisgood et al., 2006). It is a good idea to keep a record of all observations of sexual behavior, and suggested instructions for doing so, as well as a data sheet, are provided in Appendices E and F, respectively.

Table 2.26. General reproductive behaviors observed during estrus and courtship (Fouraker and Wagener, 1996).

Female Behaviors	Male Behaviors
Vocalizations <sup>a</sup>	Vocalizations
Urine squirt/spray <sup>a</sup>	Frequent urination; urine spray
Urogenital changes (e.g., vulva swelling)	Erection
Vulva “winking”	Genital inspection of female
Vaginal discharge <sup>a</sup>	Flehmen response
Aggression toward male	Charge/chase female
Maintains proximity to male	Maintains proximity to female
Nuzzles male’s belly and/or genitals	Follows female
Stands for male	Chin-rest
Inappetance	Mounts female

<sup>a</sup>Estrous behaviors in the absence of a male are often difficult to distinguish. In general, increased activity, agitation, vocalizations, spray-squirting urine, and vaginal discharge have been cited. As the female approaches peak estrus, these behaviors usually increase in frequency. Some females have been reported to successfully breed without exhibiting any overt behavioral signs of estrus.



Fig. 2.12. Many, but not all, female white rhinos squirt urine and have vaginal discharge during estrus (photo taken at Wildlife Safari).

There is no evidence for the suppression of sexual behavior in captive-born females by wild-caught females, including the mother, and copulatory behavior is not compromised in captive-born females (Swaigood et al., 2006). There also is no evidence that reproduction is influenced by social dominance (Swaigood et al., 2006; Metrione and Harder, 2011). White rhino males are polygynous (Owen-Smith, 1973), and males were equally likely to direct courtship behaviors toward wild-caught and captive-born females during peak estrus (Swaigood et al., 2006). Among females that have copulated, captive-born females are significantly less likely to give birth, even when years of opportunity is held constant (Swaigood et al., 2006). These results indicate that the captive-born reproduction problem is post-copulatory, and there must be problems with conception or pregnancy maintenance (Swaigood et al., 2006; Metrione, 2010).

While companions may accompany adult females with older calves, females do not tolerate companions when they are accompanied by a young calf (Owen-Smith, 1973; Shrader and Owen-Smith, 2002; Metrione et al., 2007). In fact, females seek dense brush in areas not frequented by other rhinoceros to give birth in the wild (Owen-Smith, 1973) and sometimes will separate from the rest of the group approximately 20 hours prior to parturition in the zoo setting (Metrione et al., 2007). Parturition usually lasts ten to 12 hours from water-break, though first-time mothers may take longer to calve (Fouraker and Wagener, 1996).





Fig. 2.13. Chin-resting by the male (photo taken at Wildlife Safari).

Maternal care is also highly developed in white rhinos, and the mother-calf bond is undoubtedly the strongest social association in the species (Owen-Smith, 1975; White et al., 2007b). Mothers respond to solicitation vocalizations by calves by cessation of activity and repositioning to allow nursing. Young calves in the wild suckle almost once an hour for about three minutes, spending about 4% of their time suckling (White et al., 2007b). These values decrease as the calf ages, and weaning occurs at about 27 months for males and 19 months for females. Female mothers in good condition invest more resources in male than in female calves (White et al., 2007b). In the managed setting, if the dam and calf were separated from the group, a dam and her newborn calf may be reintroduced to the male/herd after two weeks (Fouraker and Wagener, 1996).

### **Adolescent White Rhinos**

Adolescent dispersal in the wild is instigated by the mother's aggression at the birth of her next calf (Owen-Smith, 1973). In zoos and conservation centers, adolescent females might remain close companions with their mothers if a new calf is not born to either one. Owen-Smith (1973) observed that male adolescents were sometimes chased by territorial males. Regardless of sex, excursions from home ranges usually occur in pairs or small groups, either with another adolescent(s) or an unrelated adult female (Owen-Smith, 1973, 1975; Shrader and Owen-Smith, 2002).





Fig. 2.14. The male usually mounts the female multiple times before successful copulation finally occurs (photo taken at Wildlife Safari).

### **Behavior During Introductions (from Fouraker and Wagener, 1996)**

As noted above, white rhinos in stable groups are highly gregarious and aggression is rare, although there is some variability across individuals and facilities. By contrast, introductions of unfamiliar animals to the group often result in aggression, and it should be noted that rhinos of both sexes have been the aggressors. Behaviors that have been noted during rhino introductions are listed in Table 2.27. Territorial defense is often limited to ritualized confrontations, in which two rhinos advance toward each other but stop and engage in a horn-to-horn stare (Tables 2.24 and 2.27). Also as part of this ritual, the two individuals may touch horns, back apart, and wipe their horns on the ground (Table 2.24; Nowak, 1991). More intensive conflicts (Table 2.25) involve charges and inflicting injuries by horning or charging. It is important to note that what is often perceived as serious or dangerous aggression between rhinos is, in fact, normal behavior requiring no intervention of any kind. Along with increased size and thick skin comes decreased vulnerability compared with many other animals.

In some cases, however, aggression may proceed to a point at which management should intervene to prevent serious injury. Managers should allow some aggression during an introduction but be prepared to intervene in the event that aggression threatens the lives of one or more rhinos. Protocols for intervening may vary across institutions, but in general, careful consideration should be given to intervening in an introduction before aggression reaches Level 5 (Table 2.25). Stopping

an introduction at a level prior to this will not lessen aggression during a subsequent introduction attempt. Animals that are allowed to “settle their differences” will establish some territorial boundaries and will usually not engage in serious aggression again, with the exception of a male attempting to approach an estrous female. In sum, moderate aggression is commonplace in any rhino introduction; sparring and fighting will occur and result in minor injuries (e.g., cutaneous wounds). However, in most cases, aggression levels prior to Level 5 may be allowed to continue using the discretion of management.

Table 2.27. Behaviors noted during rhino introductions (Fouraker and Wagener, 1996).

<b>Non-aggressive Behaviors</b>	<b>Ritualized Confrontations</b>	<b>Potential Stress-Related Behaviors</b>	<b>Aggressive Behaviors</b>
Follow	Head sweep	Pacing	Charge/chase
Touch/rub/lick	Face-to-face stare	Running (excessive)	Open-mouth threat
Anal or genital investigation		Space-maintenance and threat vocalizations (excessive)	Sparring
		Diarrhea	Goring

Animal personality and disposition should always be considered in introductions. A subordinate animal should be introduced to a more dominant animal in an enclosure familiar to the subordinate. In the case of multiple-animal introductions (e.g., introduction of a new female to an established male-female group), the most subordinate animal should be introduced to the next most subordinate, and so on up the dominance hierarchy. Greater aggression may be noted in some individuals in the presence of an estrous female; therefore, any introduction attempt at this time should be especially well-monitored or possibly avoided if the attempt involves a male. Aggression may be meaningfully reduced by allowing individuals to become familiar with each other through protected contact (e.g., through enclosure bars) or, possibly, by exposing them to one another’s odors for a period prior to introduction.

# PART III

## ***BLACK RHINOCEROS***

### ***TAXONOMY***

#### **Scientific Name and Origin**

*Diceros bicornis*

*Diceros*: Greek *di*, meaning “two” and *ceros*, meaning “horn”

*bicornis*: Latin *bi*, meaning “two” and *cornis*, meaning “horn”

#### **Common Names**

African black rhinoceros: Not black at all. The black rhino probably derives its name from the dark-colored local soil covering its skin from wallowing, and possibly to distinguish it from the other African species of rhino, the white rhino.

Prehensile-lipped rhinoceros: The upper lip of the black rhino is adapted for feeding from trees and shrubs and is the best distinguishing characteristic.

Hook-lipped rhinoceros: also referring to the prehensile lip

#### **Distribution and Habitat**

Sub-Saharan Africa

Tropical bush lands and savannahs

#### **Size**

800 to 1,350 kg (1,750 to 3,000 lb)

1.4 to 1.7 m (4.5 to 5.5 ft) tall at shoulder

Primary horn 0.5 to 1.3 m (1 ft 8 in to 4 ft 4 in)

#### **Physical Description**

Gray to brownish-gray, hairless

Two horns

#### **Life History Characteristics**

Browser

Not as solitary as is commonly portrayed: adult females share overlapping home ranges; males usually solitary and possibly territorial

Females sexually mature at four to seven years of age; males at seven to ten years

Gestation period approximately 15 to 16 months; interbirth interval of 2.5 to four years

# MANAGEMENT

## Group Composition in Captivity

Black rhinos may not be as solitary as is commonly portrayed. Black rhinos often associate briefly at water holes or salt licks. Although females are rarely observed alone, adult males are usually solitary and possibly territorial (Estes, 1991). Cows with calves tend to stay alone; however, the twosome sometimes allow an unrelated immature male or female to join them until that individual reaches maturity (Goddard, 1967). Data on rhinoceros social organization, combined with preliminary analyses of parameters affecting reproductive success in captivity, may be used to generate recommendations regarding possible social groupings in zoos and conservation centers (Tables 3.1 and 3.2). Many variables affect the probability of success for any managed social group, including the animals' dispositions and available holding space. Further, depending on space and animal and staffing availability, institutions may hold animals for breeding or for exhibit-only. Facilities that wish to hold rhinos for exhibit purposes only are advised to maintain two animals (male: female: 1.1 or 0.2 or, more recently 2.0 because of the skew in male births, as well as the ability to house multiple females, but only a single, or possibly two, males at the breeding institutions).

Table 3.1. Possibilities for rhino social groupings within the same exhibit in captivity.

Rhino species	Multiple Animals of Same Sex		Multiple Animals of Opposite Sexes
	Adult males	Adult females	
White	rare (possible in exhibits >100 acres)	possible and recommended	possible and recommended for breeding (optimal: one male and two or more females); if possible, an additional male in proximity but not in same enclosure
Black	not recommended	possible in very large enclosures only; temporarily successful in some situations, but does not last	possible and recommended for breeding (optimal: 1.1)
Greater one-horned	not recommended	possible in enclosures $\geq 100$ acres	pairings recommended only during peak estrus (with the exception of very large exhibits, which may hold a single male and female together consistently)

For institutions that have the space and staff available for rhino breeding, it is recommended that managers commit to two pairs of black rhinos. In addition, breeding institutions must also have space to hold offspring for up to three years after birth. Within a single exhibit or holding area, the recommended minimum numbers for breeding are one male and one female. In general, mature males should not be held together because of the increased likelihood of serious aggression.

Table 3.2. Recommended numbers for institutional holding.

Rhino Species	Recommended Minimum Groupings for Breeding <sup>a</sup>	Preferred Optimal Holding for a Breeding Institution	Exhibit Only (per institution)
White	1.2	2.4 (1 herd/ 1 back-up male)	1.1 or 0.2 <sup>b</sup>
Black	1.1	2.2 (2 pairs)	1.1 or 0.2
Greater one-horned	1.1 <sup>c</sup>	2.2 (2 pairs)	1.1 <sup>c</sup> or 0.2

<sup>a</sup> See Enclosure Design chapter. Breeding institutions must have space for offspring to be held for up to three years following birth.

<sup>b</sup> Multi-male bachelor groups have been maintained in very large enclosures.

<sup>c</sup> In the case of greater one-horned rhinos, males and females should be introduced only during the female's estrous period. Institutions with very large enclosures may be able to hold opposite-sex animals together consistently.

Grouped or multiple-species exhibits are possible for black rhinos if ample exhibit space is available. Examples of institutions that have successfully maintained mixed-species exhibits include Fossil Rim Wildlife Center, San Diego Zoo Safari Park, Columbus Zoo, the Wilds, Lowry Park Zoo and Riverbanks Zoo. Species that have been successfully paired in an exhibit with rhinos include sarus cranes, herons, some antelope species (nilgai, blackbuck, gaur, Persian goitered gazelle), mouflon, zebra, and some deer species. In all cases, the dispositions of the individual animals, as well as adequate space and exhibit structure (i.e., visual barriers, refuge areas, etc.), are important to consider prior to attempting a mixed-species exhibit.

## Introductions

Changing social groupings of rhinos through the introduction of additional individuals to an established individual, pair or group is a process requiring care and planning. Rhino species vary widely in social structure, and rhinos periodically vary their grouping patterns in the wild according to factors such as reproduction and the rearing of young. Social groupings in captivity, therefore, should also vary according to species, as well as to the circumstances within each institution. Rhinos may be very protective of their individual boundaries, but proper introduction procedures can minimize injury from conflict and aggression. The following section outlines general considerations for any rhino introduction and provides systematic descriptions of aggression, procedural recommendations, and descriptions of potential species-specific introduction types.

Factors that must be considered in any introduction include individual animal personalities, staff experience and confidence level, and enclosure type (i.e., indoor/outdoor, public/off-exhibit, relatively small/large). Barrier types and temperature should also be considered. Introductions



often result in aggression, and it should be noted that rhinos of both sexes have been the aggressors. Territorial defense is often limited to ritualized confrontations, in which two rhinos advance toward each other but stop nose-to-nose and engage in a staring contest to gauge each other's size and strength. Also as part of this ritual, the two individuals may touch horns, back apart and wipe their horns on the ground (Nowak, 1991). More intensive conflicts involve head-on charges and inflicting injuries by horning or ramming. In general, behaviors that have been noted during rhino introductions are listed in Table 3.3. It is important to note that what is often perceived as serious or dangerous aggression between rhinos is, in fact, normal behavior requiring no intervention of any kind. Along with increased size and thick skin comes decreased vulnerability compared with many other animals. Table 3.4 lists a descriptive hierarchy of aggression levels in rhinos.

Table 3.3. Behaviors noted during rhino introductions (Fouraker and Wagener, 1996).

Non-aggressive Behaviors	Ritualized Confrontations	Potential Stress-Related Behaviors	Aggressive Behaviors
Follow	Head sweep	Pacing	Charge/chase
Touch/rub/lick	Face-to-face stare	Running (excessive)	Open-mouth threat
Anal or genital investigation		Space-maintenance and threat vocalizations (excessive)	Sparring
		Diarrhea	Goring

In some cases, aggression may proceed to a point at which management should intervene to prevent serious injury. Rhino managers should allow some aggression during an introduction but be prepared to intervene in the event that aggression threatens the lives of one or more animals. Protocols for intervening may vary across institutions, but in general, careful consideration should be given to intervening in an introduction before aggression reaches Level 5 (Table 3.4). Stopping an introduction at a level prior to this will not lessen aggression during a subsequent introduction attempt. Animals that are allowed to “settle their differences” will establish some territorial boundaries and will usually not engage in serious aggression again, with the exception of a male attempting to approach an estrous female. In sum, moderate aggression is commonplace in any rhino introduction; sparring and fighting will occur and result in minor injuries (cutaneous wounds). However, in most cases, aggression levels prior to Level 5 may be allowed to continue using the discretion of management.

Table 3.4. Levels of aggression in rhinos (Fouraker and Wagener, 1996).

Level of Aggression	Definition
1	Rhinos are charging each other but do not make physical contact.
2	Rhinos are charging each other with physical contact resulting in some cuts and scrapes to the facial area.
3	Rhinos are charging each other with physical contact resulting in cuts and scrapes to the facial area and body.
4	Charging and/or pursuit <sup>a</sup> proceeds to the point that one or both rhinos are knocked down at least once. Scrapes and cuts are deeper and more numerous.
5	Aggression and pursuit proceed to the point that one or both rhinos have subcutaneous wounds or arterial blood flow.

<sup>a</sup> It should be noted that one animal might break away from the confrontation and attempt to escape. The aggressor often will pursue and begin horn-prodding the underbelly of the escapee as the two run around the enclosure. Often a rear leg is hooked and held aloft while pursuit continues. If the escapee does not stop and resume a defensive posture, the animals might continue until heat or exhaustion becomes a critical factor. Aggression at this point is more serious.

The introduction process requires much planning and cooperation among managers. Table 3.5 outlines recommended steps for rhino introductions. Familiarization through visual, olfactory and tactile contact should be permitted if at all possible prior to a full-scale introduction. If the facility permits, this may be accomplished by first placing individuals in the same barn or in nearby outdoor lots, or by providing olfactory cues such as urine, feces or skin rubbings from the animal being introduced. As the animals acclimate, managers may move them to adjacent barred stalls or fenced outdoor yards. These barriers prevent confrontations leading to serious injury but allow acclimation and familiarization prior to introduction.

The actual introduction should be attempted in the largest available enclosure. Enclosures should be large enough to allow ample space for shading, mock-fighting, aggression and defense. The enclosure should contain visual barriers such as brush, earth piles, or boulders (“run-arounds”), which give rhinos places to hide without becoming cornered or trapped. These features may lessen overt aggression if a rhino is able to escape the sight-line of another. An enclosure should not contain dead ends in which an individual may become trapped by an aggressor. The enclosure should allow for the use of high-pressure fire hoses, CO<sub>2</sub> fire extinguishers and/or vehicles to aid in separating individuals.

Animal personality and disposition should always be considered in introductions. A subordinate animal should be introduced to a more dominant animal in an enclosure familiar to the subordinate. Greater aggression may be noted in some individuals in the presence of an estrous female; therefore, any introduction attempt at this time should be especially well-monitored or possibly avoided if the attempt involves a male.

Table 3.5. Steps in the introduction process.

Step	Description
1	Animals in the same barn or multiple outdoor lots should have olfactory and auditory exposure to each other. If the animals are not housed near each other (i.e., enclosures on opposite sides of the zoo, etc.), they should be moved to the same exhibit area.
2	Animals should be given visual contact with each other in addition to the above sensory modalities. Animals may be shifted within a barn or in adjacent outdoor lots. If at any point during this process the animals display symptoms associated with stress (e.g., pacing, diarrhea, excessive vocalizations) for more than 2-3 hours, the introduction should return to the previous step.
3	If animals are not already positioned adjacent to each other, they should be moved closer together (e.g., to adjacent stalls or adjacent outdoor enclosures).
4	The actual introduction (full tactile exposure) should take place in the largest enclosure available and follow guidelines stated in this chapter. Preferably, the enclosure should be familiar to the least dominant animal and include ample “run-arounds”.
5	Within institutions in which rhinos can be left together 24 hours per day, they should be separated during the first several nights or until they show only minor aggression.

Appropriate personnel for first-time introductions include the primary animal manager, a vet with immobilization equipment, and the curator and keepers most familiar with rhinos. Other staff also may be needed at critical points around the enclosure’s perimeter so that the animals may be observed at all times in case separation becomes necessary. It should be noted that if a barn is opened and used to separate individuals, only one individual should be allowed inside the barn, and it must not be trapped inside by an aggressor.

The social nature of the black rhinoceros is intermediate between that of the white and greater one-horned species. In general, a 1.1 pair is the recommended group size. Additionally, in comparison with the other rhino species, black rhinos have a much longer average birth interval. In an effort to decrease this interval and re-breed females earlier, a female black rhino should be temporarily reintroduced to the male for breeding following her first post-partum estrus (see calf development below for separation periods). Following are recommended protocols for potential black rhinoceros introduction types.

***Introduction of a New Male to a Female (or Vice Versa) to Form a Pair***

The introduction should occur in the largest lot available, following the general introduction protocols above. If a single large lot is not available, adjoining lots should be opened to form a large area for the introduction. If the latter strategy is used, care should be taken to modify any resulting dead ends in the exhibit where a rhino may become trapped during an aggressive interaction. If all yards are the same size, it is best to introduce in the subordinate’s yard, usually, but not necessarily, the female’s yard.

***Introduction of a New Female to an Established Male-Female Group (if adequate space is available)***

Unlike white rhinos, female black rhinos generally do not tend to form strong pair bonds. Therefore, a new female should be introduced to an established male-female group one individual at a time, but it is not necessary that she be introduced to all females before being introduced to the

male. For breeding introductions, a calf should be trained to be temporarily separated from its dam so that she can be introduced to a male (see calf development, this chapter).

***Introduction of a New Male to an Established Female Group (if adequate space is available)***

As stated above, female black rhinos do not generally tend to form strong pair bonds. However, if a multiple-female group is established and managers perceive that the females have formed strong bonds, the new male should be introduced to the females as a group rather than to one female at a time. If the females are not as compatible as managers would like but an introduction is necessary (SSP recommendation, breeding, etc.), the new male should be introduced to each female individually. Following all successful male-female introductions, the male should be introduced to all the females at the same time.

***Reintroduction of a Post-partum Female (Without Calf) to a Male***

The reintroduction of a post-partum female to a male is usually recommended for the dam to be re-bred. Therefore, this type of introduction is usually temporary, and following breeding, the female should be placed back with the newborn calf. In order to attempt this introduction, the calf must be trained to be separated from the female (see calf development, this chapter). The introduction of the post-partum female to a male should be attempted following the first post-partum heat.

**Pregnancy and Parturition**

Table 3.6 lists behaviors associated with pregnancy and impending birth. Black rhinos may breed continually throughout gestation. In all species, there may be a mucus discharge, noticeable weight gain or increase in girth size, as well as increased frequencies of defecation and urination throughout gestation. Pregnant black rhino females have been observed isolating themselves from other individuals. If pregnancy is confirmed (pregnancy fecal hormone tests are available, or through ultrasound), and/or the breeding date is known, the physical separation of the pregnant female from the bull/herd should take place as early as 30 days and as late as 24 hours prior to birth. Institutions with very large enclosures have had successful births in the yard with the male present; however, the female(s) and any males must be watched very closely.

Table 3.6. Physiological and behavioral indicators of impending parturition.

<b>30 Days Prior to Birth</b>	<b>2 Weeks Prior to Birth</b>	<b>24 to 48 Hours Prior to Birth</b>
Increase in teat size	Nipples enlarge	Udders increase dramatically in size
Beginnings of milk production	Nipples develop wax plugs	Inappetance
Milk may be expended with pressure on the teats	Vulva swelling occurs	Becomes irritable and aggressive to stimuli, including staff
Female may prolapse vaginally when defecating		Mucus plug forms Increased vulva dilation Increased restlessness, lies down often

The onset of labor often takes place at night or in the early morning and may last one to three hours. Parturition usually lasts ten to 12 hours from breaking of the water, though first-time mothers may take longer to calve. The presentation of a calf is generally head-first, although rear-foot presentations do occur and may take longer than head-first births, but usually deliver without assistance. Capabilities for monitoring births remotely through closed-circuit television or other means are advisable.

## **Calf Development**

A single calf is generally the rule. Few data are available on birth weights, but in general, calves weigh 27 to 41 kg (range=60-90 lb, n=4). Immediately following birth, the newborn calf is usually cleaned by its mother and stands for the first time within 30 minutes to five hours of birth. A newborn calf may require a substrate that allows traction to help steady it. Suitable materials may include sand, gravel, straw, hay or rubber matting. In all cases, both the dam and calf should be monitored closely to prevent ingestion of the substrate. A calf should begin nursing within one to two hours of standing (though in a single case, a calf removed from its dam for medical intervention nursed 16 hr post-birth). The dam will nurse her calf while standing or lying on her side.

Infants less than two months old may nurse hourly, while older calves nurse at intervals of about 2.5 hours. Few data are available on nursing durations and frequencies, but it has been reported that as the calf ages and grows stronger, nursing will usually increase in duration but decrease in frequency. It has been reported that calves may gain up to 4.54 kg (10 lb) per day for the first ten days. The first defecation has been reported at two to ten days of age (n=2). Calves may nurse for up to two years, although they have been observed first sampling solid food at less than one week to one month of age. Calves may be offered supplemental feedings of milk if the dam is believed to be a poor milk producer or the calf is not gaining weight (see Nutrition chapter). Infant rhinos have been successfully pulled from their mothers because of rejection, medical issues related to the mother or infant, or from a failure to nurse. Otherwise, it should be noted that weaning for management purposes can be accomplished if necessary at six months, but one year is preferable. One attempt to use a surrogate mother was unsuccessful; however, hand-reared infants have been assimilated into existing groups and have shown reproductive success. Keeping the calf with the dam even longer helps to facilitate social learning. Male calves are usually weaned by the dam at an earlier age than female calves. Depending on the facility, a cow and her newborn calf may be reintroduced to the male/herd following the cow's first heat. Calves generally do not have peers to play with, though they are generally very curious and often chase and mock-fight with their dams or occasionally keepers. Non-aggressive sexual behaviors may be exhibited at as early as 18 months of age in males.

In general, the long-term social effects of removing rhino calves from dams should be investigated. For all species, weaning or permanent separation of the calf except for medical reasons should not occur before one year of age. A calf can, however, be temporarily separated from its mother at as early as one month of age for short periods of time (e.g., re-breeding of dam). Generally, the procedure is to separate the calf for short periods of time (e.g., 15-20 minutes during cleaning) and gradually increase the separation time. If a dam is not going to be re-bred, her calf may remain with her until it reaches sexual maturity (at approximately 4.5-5 yr of age). It should be noted that available data indicate that nursing does not inhibit conception. The first calf may be forced away before parturition of the second calf as the dam seeks to isolate herself.



## Identification

Although physical characteristics such as horn size and shape make individual rhinos fairly easy to distinguish from one another, sound rhino management requires that animals be identified through permanent and reliable methods. Trovan<sup>®</sup> transponders (Electronic Identification Devices, Ltd., Santa Barbara, CA), implanted at the base of the left ear during post-natal examination or as soon after birth as possible, are one means of permanent primary identification for all individuals. Adults should be transpondered opportunistically. Transponder numbers need to be reported to the studbook keeper. In addition to a transponder, each individual should have a secondary visual means of identification, such as an ear tag or ear notch. Photographs or sketches in the animal's records may also serve this purpose.

## Keeper Training and Interaction

As with any position involving the management of large animals, rhinoceros keepers should have as much formal training and experience as possible and should be familiar with rhino behavior and husbandry. In order to ensure safety and to properly meet the requirements of management, it is recommended that more than one keeper be responsible for the care of these animals on a daily basis. Keeper interaction should be restricted to designated areas and should be conducted in accordance with institutional protocols. Finally, consistency of routine is vital.

There are no conclusive data to indicate the effects of different styles of keeper interaction on rhinoceros behavior or reproductive success in captivity. Interaction styles range from no contact at all to daily hands-on contact. In an effort to create an environment patterned after the wild, however, at no time should relationships with keepers substitute for natural interaction among individuals. It is important that rhinoceros personnel keep a daily log, noting any unusual behavioral or physical changes. It is the responsibility of management to supply all pertinent data to the studbook keeper.

## Daily Regimen

Fresh water should be available at all times and should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset, spillage or leakage. Rhinos need access to mud baths to keep their skin healthy; mud wallows should be renovated periodically to prevent contamination (see Enclosure Design chapter for more information on wallows).

Natural substrates in confined areas (e.g., holding corrals) can be spot-cleaned daily, but daily removal of feces from larger enclosures is not necessary as defecating at dung piles is an important natural behavior. Hard-surfaced areas not exposed to the elements should be dry-cleaned or hosed daily and disinfected at least weekly. Indoor housing surfaces, as well as walls and rub areas, should be cleaned daily. Additionally, the daily hosing or showering of rhinos in the barn with temperature-controlled water is recommended to promote healthy skin during the winter months.

In order to facilitate participation in many research programs involving rhinos, it is recommended that institutions implement training programs following the protocols recommended in the Training chapter. Additionally, it is recommended that some form of environmental/behavioral enrichment be integrated into daily rhino management.

# **REPRODUCTION**

## **Black Rhino Reproductive Age**

The North American Regional Studbooks for black rhinos contain data on 67.63 eastern black rhino sires and dams and their 200 calves, as well as 13.23 southern black rhino sires and dams and their 60 calves (Christman, 2011). The youngest southern black rhino to give birth was five years eight months old, which means she conceived at four years two months of age. The youngest eastern black rhino to give birth was five years one month, which means she conceived at three years nine months. By comparison, in a 22-year old re-introduced black rhino population in South Africa, age at first reproduction was between six and nine years of age, with the age of first calving tending to increase with rhino density (Hrabar and du Toit, 2005). The oldest southern black rhino to give birth was 26 years eight months and the oldest eastern black rhino to give birth was 35 years 11 months. The youngest male eastern black rhino to sire a calf was four years three months (at the time of conception) and the youngest southern black rhino was three years two months. The oldest to sire a calf was 36 years three months (eastern) and 30 years eight months (southern). In general, captive black rhinos can potentially reproduce between the ages of four and 30 if they are maintained in good health. Although fertility tends to decline in nulliparous females as they age, the oldest dam at first reproduction was 24 years and the oldest sire at first reproduction was 25, suggesting that a few black rhinos can breed for the first time towards the end of their lifespan.

## **Seasonal Changes in Physiology Associated with Reproduction and Management Implications**

The African black rhinoceros is not seasonally restricted in its breeding activity. Based on year-long fecal hormone metabolite analyses (Brown et al., 2001), it appears the captive female black rhino is consistently exhibiting reproductive cycles throughout the year, and hormone concentrations associated with the cycles do not differ by season. Although some of these cycles are anovulatory (Radcliffe et al., 2001), data are insufficient to determine if anovulation is seasonally influenced, and studbook data indicating that black rhinos produce calves every month of the year would argue against a strong seasonal effect on ovulation and conception. In contrast, results from fecal hormone metabolite research on wild southern black rhinos suggest that the females are more fertile during late spring and early summer, which corresponds to the early rainy season, and results in more females giving birth during the late rainy season when food is plentiful for lactating dams (Garnier et al., 2002). However, this apparent nutrient related seasonal skew in fertility is likely to diminish in captivity where high-quality food is available year-round.

## **Reproductive Cycle**

The female black rhinoceros is a spontaneous ovulator with an average reproductive cycle length of ~26 days (Schwarzenberger et al., 1993; Berkeley et al., 1997; Brown et al., 2001; Radcliffe et al., 2001; Garnier et al., 2002). Although the black rhino appears to be more consistent in exhibiting regular reproductive cycles compared to Indian rhinos and white rhinos, there is some variation in cycle length with about 20% of the cycles being shorter than 20 days and 20% being longer than 32 days in length (Brown et al., 2001). The studies cited above have reported other variations in reproductive function, including anovulation, brief acyclic intervals and early embryo loss. The follicular phase of the cycle, when progesterone is at baseline concentrations, is relatively short, lasting three to five days (Brown et al., 2001). The black rhino preovulatory follicle is large, reaching ~5 cm in diameter and changing from spherical to pear-shaped just prior to ovulation (Radcliffe

et al., 2001). Females ovulate 24 to 48 hours after estrus, and fecal progesterone increases about six days after ovulation (Schwarzenberger et al., 1993; Radcliffe et al., 2001).

### Peak Breeding Season for Black Rhinos in North America

The mature captive, female black rhinoceros will breed throughout the year and calves have been produced in every month of the year with no particular bias for a specific season. The distribution of births by month for eastern black rhinos is shown in Figure 3.1 for the 199 births reported in the 2011 North American Regional Studbook (Christman, 2011). Figure 3.2 shows the months of conception based on an approximate gestation of 16 months. Similarly, Figure 3.3 shows the distribution of births by month for the 59 recorded southern black rhino births (Christman, 2011), and Figure 3.4 shows the associated months of fertile matings. These data demonstrate that eastern and southern black rhinos are fertile and conceive throughout the year. The trend towards more fertile matings in eastern black rhinos during the interval from March to September is almost certainly management related since temperate zoos are more likely to pair their rhinos for mating during months with good weather. Such a trend is not noted for the southern black rhinos, most of which are maintained in southern states like Texas and Florida. In the wild, an increase in the number of conceptions was associated with increased monthly rainfall in a re-introduced population in South Africa (Hrabar and du Toit, 2005).

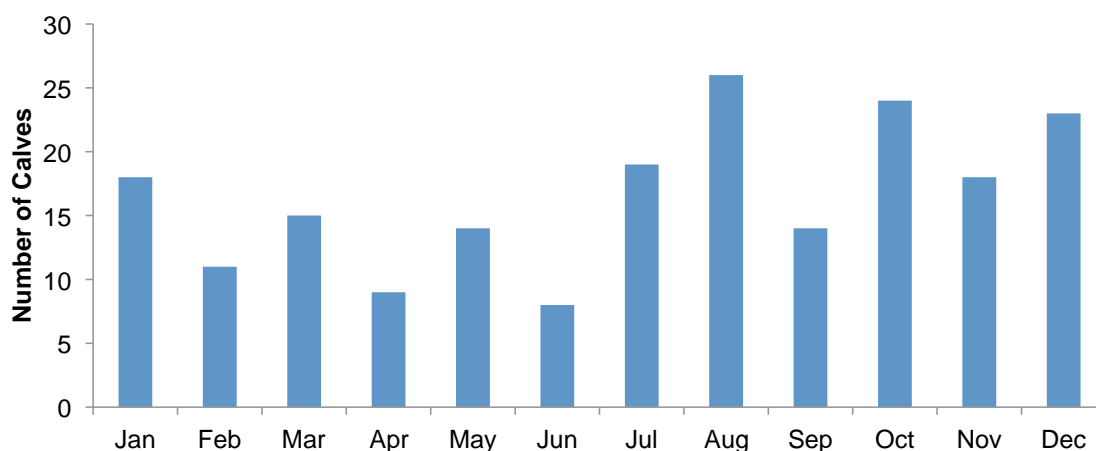


Fig. 3.1. Number of eastern African black rhino calves born per month (n=199).

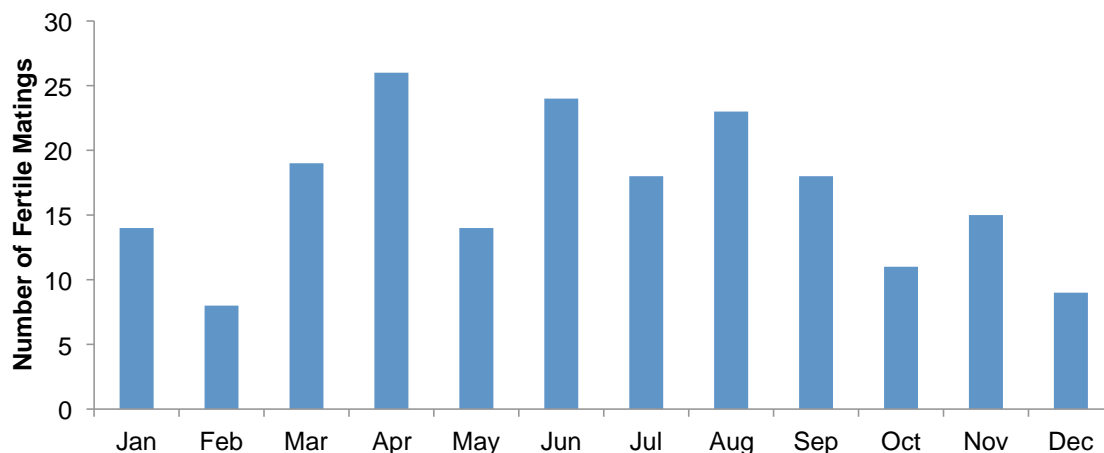


Fig. 3.2. Number of fertile eastern African black rhino matings by month (n=199).

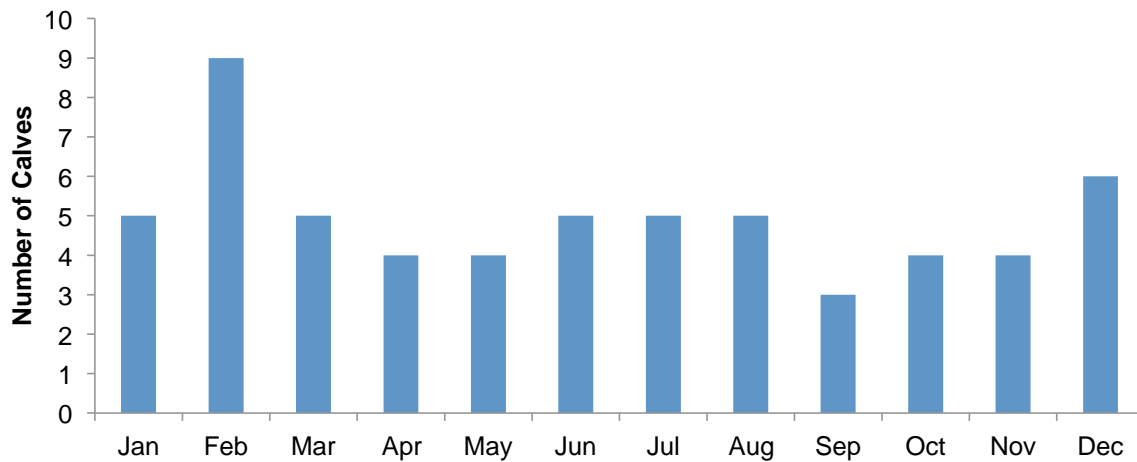


Fig. 3.3. Number of southern African black rhino calves born per month (n=59).

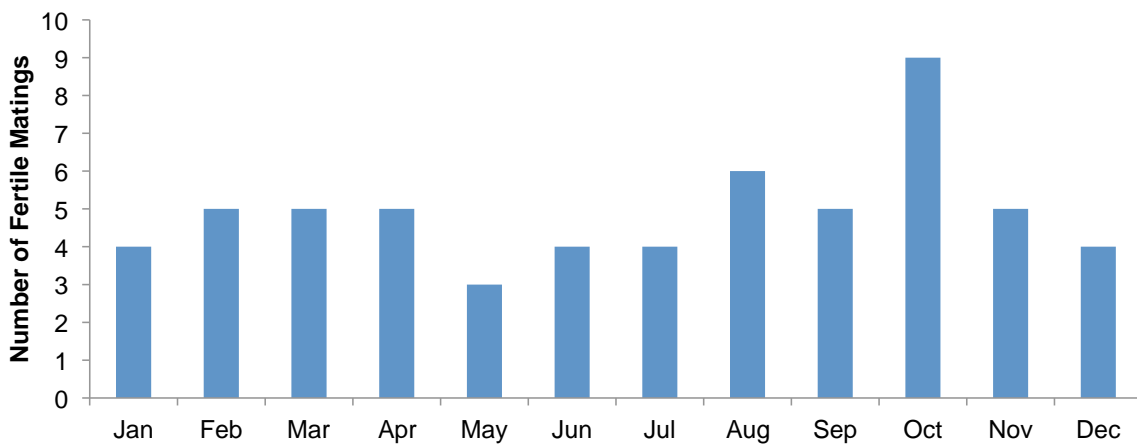


Fig. 3.4. Number of fertile southern African black rhino matings by month (n=59).

### Reproductive Monitoring

In general, black rhinos breed fairly well in zoos. Unlike the Asian species, the African rhino species typically are not dangerously aggressive when introduced for mating. Therefore, most animal care staff can simply introduce males and females when they observe estrous behaviors without a significant risk of animal injuries. However, reproductive monitoring can be very useful for pregnancy diagnosis and for determining if a female is still exhibiting reproductive activity when overt signs of estrus are not observed.

Several hormones and their metabolites have been employed for monitoring reproductive activity in the African black rhinoceros (Table 3.7). Several studies have reported attempts to analyze urinary and fecal estrogen metabolites in black rhinos, but in most cases, results have been negative. Estrogen metabolites excreted by the Indian rhino accurately reflect reproductive status, whereas in the other rhinos species, only progesterone metabolite monitoring has proven useful for tracking ovarian activity.

Table 3.7. Reproductive hormones monitored in the African black rhinoceros.\*

Sample	Estrogen	Progesterone	Testosterone
Urine	♀ 7 ♂ 2	♀ 2,7,8,10	♂ 2
Feces	♀ 1,3	♀ 1,3,6,9,11,12	♂ 3
Serum	♀ 1	♀ 1	♂ 4
Saliva	♀ 5	♀ 5	

\* Table adapted from Roth, 2006.

<sup>1</sup> Berkeley et al., 1997; <sup>2</sup>Brett et al., 1989; <sup>3</sup>Brown et al., 2001 ; <sup>4</sup>Christensen et al., 2009; <sup>5</sup>Czekala and Callison, 1996;

<sup>6</sup> Garnier et al., 2002; <sup>7</sup>Hindle et al., 1992; <sup>8</sup>Hodges and Green, 1989; <sup>9</sup>Radcliffe et al., 2001; <sup>10</sup>Ramsay et al., 1987;

<sup>11</sup>Schwarzenberger et al., 1993; <sup>12</sup>Schwarzenberger et al., 1996

## Pregnancy Detection and Loss

In the black rhino, pregnancy can be detected early and monitored throughout gestation via rectal ultrasonography (Radcliffe et al., 2001). However, many black rhinos are not conditioned for ultrasound exams. Therefore, hormone concentrations in serum or hormone metabolite concentrations in feces are typically monitored to determine pregnancy status in the black rhino. Sustained elevations in progesterone concentrations four to eight weeks after mating is a good indicator of pregnancy (Schwarzenberger et al., 1993), but pregnancy can only be confirmed about three months after conception when progesterone concentrations increase above post-ovulatory luteal concentrations (Brown et al., 2001). Pregnancy loss, abortions and stillbirths have been reported in the black rhino, but the prevalence of stillbirths is much less than that for Indian rhinos. The first reported case of prescribing a progesterone supplement to help a rhino carry a pregnancy to term was in a black rhino (Berkeley et al., 1997). Since then, the synthetic progesterone, altrenogest (Regu-Mate®, Intervet Inc., Millsboro, DE), has been prescribed to additional black rhinos, Indian rhinos and Sumatran rhinos with confirmed histories of early pregnancy loss, and the anecdotal evidence suggests it is effective in helping to sustain pregnancies.

## Reproductive Technologies (Semen Collection, Artificial Insemination, etc.)

Viable black rhino semen samples can be collected by electroejaculation (Roth et al., 2005) or harvested from the epididymis post-mortem (O'Brien and Roth, 2000). Black rhino sperm obtained by either method withstand processing and cryopreservation procedures fairly well. Post-thaw functionality of frozen-thawed black rhino sperm has looked promising during in vitro fertilization studies (Stoops et al., 2011), but to-date, no calves have been produced with frozen-thawed black rhino sperm.

Artificial insemination (AI) techniques have also been developed for the black rhino, and multiple AI attempts on multiple black rhinos have been conducted. However, to-date, no offspring have resulted from AI in the black rhino.

In vitro fertilization has been attempted in black rhinos, but with limited success to-date. It has been possible to obtain oocytes from hormone-treated cows via transvaginal aspiration, and one four-cell embryo was produced following insemination of the oocytes with non-frozen semen (Hermes et al., 2009a). It has also been possible to rescue viable oocytes from the ovaries of cows post-mortem and several of these oocytes successfully matured in vitro with one two-cell embryo forming after insemination with frozen-thawed sperm (Stoops et al., 2011). However, neither embryo produced by IVF continued to develop. This progress in rhino IVF is exciting, but also demonstrates that it is still in the very early experimental stages.



Sperm sorting to segregate X- versus Y-bearing sperm has also been conducted and appears to be possible in black rhinos (Behr et al., 2009; O'Brien et al., 2011). However, maintaining adequate sperm quality throughout the collection, transport, staining, sorting and cryopreservation process has proven challenging, and samples robust enough for AI have not yet been produced.

## **Fertility Assessment**

Male fertility assessments should include: 1) an ultrasound examination of the testicles to evaluate tissue consistency and size, 2) electroejaculation to confirm sperm production, and 3) serum testosterone analysis. It is important to note that a positive result from electroejaculation is very meaningful, but a negative result should not be used alone to diagnose a male with infertility because electroejaculation is not always successful in producing good quality sperm samples, even when conducted on proven bulls.

Female rhinos should be examined by rectal ultrasound to determine if any masses or cysts exist in the vagina or uterus. These types of pathologies appear to be very prevalent in older Indian, white and Sumatran rhinos and much less prevalent in African black rhinos, but there have been a couple of reports of uterine or ovarian pathology in black rhinos (Godfrey et al., 1991; Hermes et al., 2009a). Ultrasonography can also be used to determine if the ovaries are active, however, any fertility assessment should include some form of longitudinal endocrine analysis which is more informative for assessing ovarian activity than a single ultrasound exam of the ovaries. These assessments can be conducted using serum or fecal samples (Table 3.7). Given the length of the black rhino reproductive cycle, samples should be collected and evaluated for at least 6-8 weeks.

## **Genome Resource Banking**

A black rhino sperm bank already exists at the Cincinnati Zoo's Center for Conservation and Research of Endangered Wildlife. Samples primarily include sperm rescued from the epididymis of testicles recovered post-mortem. A second black rhino sperm bank has been established at SeaWorld/Busch Gardens in San Diego and contains primarily electroejaculated samples, several of which have been sorted for X- and Y-bearing sperm. Fibroblast cell lines from many black rhinos are banked in San Diego Zoo's Frozen Zoo, which also contains black rhino semen samples. Oocytes and embryos have not yet been banked and additional research is necessary before such efforts will be fruitful.

## **Challenges**

Many zoos have been successful in breeding black rhinos, however there are some pairs that are behaviorally incompatible, or compatible and mating regularly without producing a pregnancy. These isolated cases can be a challenge. It appears that reproductive success in black rhinos is best if established, compatible, fertile pairs are allowed to remain at the same zoo and mate at fairly regular intervals (~3-4 yr).

# **ENRICHMENT**

A planned and implemented enrichment program can contribute to better health by providing animals with opportunities to exert some form of control over their environment (Carlstead and Shepherdson, 1994; Baser, 1998). As it pertains to zoo-managed rhinos, we strive to encourage species-specific behaviors in rhinos while providing them response options to environmental change. This will ultimately result in their mental stimulation and the development of naturalistic behaviors (Swaigood and Shepherdson, 2005).

Each institution may have its own set of goals and criteria for its program, which fundamentally should start with an examination of the animal's natural history, activity patterns and behaviors seen in the wild. For example, the use of the black rhino prehensile lip can be demonstrated by providing manipulative devices on exhibit for enrichment. Since rhinos spend the majority of their day eating, keepers could look at modifying feeding schedules or providing enrichment throughout the day rather than just once a day. Keepers also could investigate the possibility of changing group composition at some facilities.

Holding facilities, exhibit spaces and/or any constraints that may be unique to the facility should be examined since these areas will directly impact options for enrichment. The temperament and behavior of the specific individual(s) also needs to be taken into consideration. Once these criteria have been reviewed, a set of goals can be established. Generally speaking, the primary goal should be to “promote opportunities for the expression of species-appropriate behaviors” (Joseph and Sevenich, 1999). The Rhinoceros Husbandry Resource Manual (Fouraker and Wagener, 1996) clearly delineated the various functions enrichment can serve, such as (1) improving the well-being of the animal by increasing exercise, satisfying behavioral needs, and optimizing the level of stimulation that animals receive; (2) educating zoo visitors by increasing the levels of natural and interesting behaviors, visibility and activity levels; and (3) conserving endangered species by improving the success of breeding and reintroduction programs. The second goal of enrichment can be loosely defined as creating mental stimulation for the animal(s).

A successful enrichment program can be briefly summarized as one that:

- a) Establishes goals for the program
- b) Creates an enrichment approval form
  - States the purpose/goal of this enrichment
  - Provides a detailed description of enrichment items (construction material, thickness, dimensions, size of holes, etc.)
  - Identifies and addresses a facility or exhibit constraints
  - Identifies and addresses safety concerns
  - Cost estimates
- c) Identifies approval protocols for enrichment submissions
- d) Creates an enrichment calendar (monthly or weekly) to ensure implementation schedule
- e) Determines how staff will document and/or track animal response to enrichment offered.

To accomplish the goals mentioned above, an enrichment plan should be tailored with a set of criteria for either the individual or the species in general. The final proposal should then be submitted through the appropriate channels for approval. Ideally, an enrichment approval system should be set up to allow keepers, managers, and veterinarians the ability to assess the proposed enrichment and approve/reject it. Institutions can alter their enrichment scheduling as well as vary the type of enrichment offered (e.g., toy, food, sensory, environmental, behavioral and social) and keep track through their record-keeping or on a barn calendar (Connett, 2009). One of the most intimidating aspects of enrichment is allocating the time to document animal behavior and/or responses to the enrichment offered, however observations of responses can either be done in a direct or indirect manner as time allows. This data will enable evaluation as to whether the enrichment goals have been met. Since enrichment is dynamic in nature, adjustments can be made at any time so that the most effective enrichment is offered to the animal(s).

Enrichment can correlate aspects of ethology, psychology and animal husbandry to create a more stimulating environment for the animal (Mellen and Ellis, 1996). This has led to the inclusion of enrichment options in exhibit designs. Exhibit enrichment can be done by varying topography, landscaping, utilizing deadfall and trees, creating dirt mounds, planting vegetation, providing a wallow, and alternating the substrate (dirt, leaf litter, mulch, etc.). Other options (public view versus privacy, shaded area versus sun, etc.) can all provide the animal some control over their environment and the ability to make choices throughout the day. The aforementioned exhibit variables can contribute to effective enrichment, especially when used in conjunction with other approved enrichment activities. For example, novel scents/perfumes/extracts can be used to create a “trail” throughout the exhibit, while holes drilled in deadfall can serve as an anchoring point for browse, and either one can provide the potential for exploration and create options for the animal. The American Association of Zookeepers (AAZK) has created *The Enrichment Notebook* (Chan, 2004) that provides suggested guidelines and contains information on exhibit enrichment, dietary enrichment and a section on safety considerations. When using enrichment devices that are awkward and heavy, it is important to secure these items safely, not only for the animals, but for keeper staff as well. Fortunately, these somewhat bulky items can be secured safely by using pulley systems and/or other equipment can be used to hoist devices to enable hanging them higher. A synopsis of rhino enrichment options that are currently being used at zoological facilities can be found in Table 3.8. This table gives suggestions as to the primary area of use as well as its presentation. Note that food items, especially biscuits, treats, and produce, should be fed sparingly. Examples of enrichment type and some options that can be used are outlined below. These options must be used in compliance with a facility’s enrichment protocol, regulations and safety considerations.

Toy:

- Boomer Ball (Boomer Ball, Grayslake, Illinois)
- Weeble (Otto Environmental, LLC., Milwaukee, Wisconsin)
- Suspended log

Food:

- Scatter food around exhibit to stimulate grazing/foraging
- Place food items in enrichment devices to be randomly dispensed
- Fruits and vegetables frozen in bucket of water

Sensory:

- Use conspecific’s dung for smell (olfaction)
- Play different animal vocalizations or hang bamboo “chimes” for hearing (audition)
- Mount street sweeper brush for touch (tactition)

Environmental:

- Changing substrate (leaf litter, dirt, mulch, etc.)
- Altering daily routine
- Rotating to different enclosure (pen)

Behavioral:

- Training new behaviors
- Training for veterinary procedures

Social:

- Creating mixed species exhibit
- Rotating individuals (add or remove animals when possible)

Table 3.8. Synopsis of rhinoceros enrichment ideas.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
<i>Amazing Graze</i> <sup>1</sup> (with biscuits/treats/produce)	X	X		X
Audio clips of vocalizations (rhino, other sp.)		X	X	
Beer keg		X	X	X
Biscuits (gorilla-sized leaf eater; horse treats)	X	X		X
Boat mooring buoy		X	X	
<i>Boomer Ball</i> <sup>2</sup>	X	X	X	X
Bowling ball		X		X
Branch/twig with produce skewered on it	X	X	X	X
Browse or approved plants	X	X	X	X
Brush	X	X	X	
Brushing by keeper		X		
Cardboard box (with or without produce)		X		X
Firehose “wall”		X	X	
Ice block (with or without flavor/produce)	X	X		X
Large cardboard tube		X		X
Log, stump, rootball	X	X	X	X
Melon (whole or chunks)	X	X		X
Mirror		X	X	
Mister	X	X	X	
Mud wallow	X	X		
Non-radial tire (with cuts in sidewall for safety)		X	X	
Oblong stone	X	X		X
Painting with non-toxic finger paint		X		
Paper grain bag filled with hay or produce		X	X	X
Paper mache ball	X	X	X	X
Peanut butter (on toys/walls/“furniture”)	X	X		
Planter bucket	X	X	X	X
Plastic soda concentrate container		X	X	X
Plastic drum (55 gal.)		X	X	X
Plastic ice block or iceberg toy		X		X
Plastic jug feeder		X	X	
Produce	X	X	X	X
PVC or bamboo chimes		X	X	
PVC tube with bells suspended inside		X	X	
Rolling treat bucket/feeder		X		X
Scent, flavoring, or extract	X	X		X
Snow-pile, -people (with or without fruit mix)		X		X
Substrate pile or change (dirt/gravel/sand/leaf)	X	X		
Traffic cone or pylon		X	X	
Training with keeper	X	X		
Triangle rattle		X	X	

Table 3.8. Continued.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
Urine, feces, or soiled bedding from others	X	X		X
<i>Weeble</i> <sup>3</sup>	X	X		X
<i>Weeto</i> <sup>3</sup>	X	X		X

<sup>a</sup> Area of use may be dictated by institutional guidelines and criteria

<sup>b</sup> Presentation format is based on that which is most commonly used

<sup>1</sup> Sanctuary Supplies, Rootstown, OH; [sanctuarysupplies.com](http://sanctuarysupplies.com)

<sup>2</sup> Boomer Ball, Grayslake, IL; [boomerball.com](http://boomerball.com)

<sup>3</sup> Otto Environmental, LLC., Milwaukee, WI; [ottoenvironmental.com](http://ottoenvironmental.com)

Training for husbandry behaviors is not only stimulating for the animal, but it also can provide an invaluable opportunity for the veterinarian to perform diagnostic procedures with potentially less stress and more cooperation from the animal(s) (Dover, et al., 1994). The chapter for training will delve more deeply into this topic.

## TRAINING

A husbandry training program is considered an integral component of progressive animal husbandry programs in zoos today. Providing zoo-managed animals with choices and behavioral opportunities in their environments through a training program can result in healthier animals and better educational experiences for the zoo visitors. Training rhinos leads to a wide variety of animal management opportunities, from shifting to safer, less stressful medical procedures.

The purpose of this chapter is to give trainers an idea of the types of behaviors that can be trained with rhinos (Appendix B). This chapter will also give direction on starting a training program, ideas for shaping techniques, possible reinforcements, specific challenges to training rhinos, and resources for additional training information.

### Setting Up a Training Program

A well-planned, consistently-delivered training process is critical to the success of any program. To achieve this type of program many facilities utilize a framework that is taught in an Association of Zoos and Aquariums (AZA) course, Managing Animal Enrichment and Training Programs. This framework is called the “SPIDER” model. Steps in this framework include: Setting goals, Planning, Implementation, Documentation, Evaluation, and Re-adjusting. For more information on this process, please see [www.animaltraining.org](http://www.animaltraining.org). It is beneficial to start a training program by determining the overall behavioral goals (i.e., detailing the specific behaviors to be trained). During this goal development process it is important to include all parties involved with the management of the animals. This may include meeting with and seeking feedback from keepers, veterinary staff, nutritionists, behavioral husbandry staff, curators, and managers. Having everyone on the same page with clearly laid-out plans, assignments, and timelines helps to facilitate a smooth process. Defining roles and creating clear channels of communication and decision-making authority among all participants is also important. This can be accomplished through regularly scheduled team meetings, a consistent method of documentation, and continual communication among all staff involved in training. Facility design can have an effect on setting up a training program. Discussions must also include how the rhino will be reinforced. The next two sections will discuss both of these topics further.



## ***Facility***

When beginning a training program, it is important to start training in an area that is safe for the animal care staff and the animal, and where the rhino is comfortable. This is usually the night quarters or holding area (Fig. 3.5). Training can also be done in barn stalls, outdoor barn paddocks, training chutes, or even open exhibit areas. Because all facility designs are different, training staff will have to be creative and utilize the space available. For examples of possible body positioning behaviors, see Appendix B. For many of these behaviors trainers will require areas with no or minimal obstructions, which allow safe access to the desired body parts. It is important to remember that a fancy, expensive facility is not necessary to accomplish a successful training program, just a creative mind.



Fig. 3.5. Indoor chute for training and procedures (courtesy of C. Nordin, St. Louis Zoo).

## ***Reinforcement***

A critical component in training is finding a positive reinforcement or a reward for which an animal is willing to work. In most cases, the rhino's regular diet can be utilized for training. Any manner of storage container for the reinforcement can be used. Reinforcement items need to be easily retrieved and delivered as soon as possible after the desired behavior occurs. Reinforcement can be delivered by tossing it through posts or by hand feeding if it can be safely accomplished. Black rhinos tend to be food-reward motivated.

## ***Training Methods***

There are several steps to creating a husbandry training program for rhinos. After the behavioral goals are set, a safe facility to work the animals is identified, reinforcement type and how the reinforcement will be delivered has been determined, the next steps are learning about the animal to be trained, building a relationship with that animal, and designing a training plan. An overview on learning and training can be found in Mellen and Ellis (1996).

## ***Natural History/Individual History***

An understanding of the natural history of rhinos, paired with information about an individual animal's specific background and level of experience, play an integral part in a husbandry training program. For example, wild-caught or hand-reared rhinos may react differently to trainers when compared to captive, parent-reared individuals. It is helpful to start training in a location where the animal is most comfortable. Some animals need to have more space around them to feel comfortable within the training environment. The space needs for a rhino often center around the size of the enclosure in which the animal is being trained. Other relevant issues regarding an animal's space requirement may include proximity to conspecifics, other species, and issues such as noise levels and extraneous activity. Keeping training sessions short and moving at a comfortable pace for the rhino may help keep their focus. Tactile interaction is important—most rhinos enjoy being scratched. Care must be taken to avoid startling the animal or putting yourself in the danger zones—areas where the rhino is able to horn, kick, or injure someone. When desensitized, rhinos will interact positively with their keepers and will seek out tactile interaction. Keepers need to always maintain awareness and caution as startled rhinos can react violently and quickly. Studying the animal's natural history helps to gain insight into the animal's behavior. For more information on rhino natural history, see the Behavior chapter of this manual. Insight also can be gathered from staff, the animal's ZIMS records, or other institutions about the animal's individual history. There is no substitute for simple observation—just watching the rhino's behavior in different situations, for example, what it looks like when the rhino is calm or aggressive. Watch the rhino on and off exhibit, at different times throughout the day, and when the rhino is unaware it is being observed. This will greatly help understanding and interpretation of the rhino's behavior during a training session.

## ***Relationship***

Developing a trusting relationship between the trainer and the animal being worked with can be very beneficial to training. If the animal is not comfortable being in close proximity to people, the rhino might back away from the trainer or try to horn the trainer. Developing a relationship can help address these issues. A relationship can be developed through normal daily animal care, such as feeding, observing, regular cleaning routines, and avoiding negative interactions. The more positive interactions the animal has with staff, the more comfortable the rhino will be. For example, instead of just putting the diet in the enclosure, the trainer can hand-feed the diet. If the animal is particularly skittish, the trainer may start by placing or tossing the food in the enclosure and sitting nearby while the rhino eats. As the animal becomes habituated to the trainer's presence, s/he can begin to hand-feed and work their positioning closer and closer to the animal.

## ***Training Plans***

Creating a training plan can be a good process to help trainers think through what steps they are going to take to train a behavior. One way to do this is to establish what the final behavior will look like, and then break-down the behavior into a series of small steps called "successive approximations". Examples of training plans can be found in Appendix C.

## ***Bridging Stimulus***

Utilizing a bridging stimulus can be a very useful tool in training rhinos but not a necessity. A bridge is a signal that pinpoints the exact moment in time the behavioral criterion was met. First a trainer must select a bridge. Common bridges used are clickers or whistles. Once selected, the trainer needs to associate the sound with the delivery of reinforcement. For example, if the bridge selected was the clicker, the trainer would click and feed, click and feed, and continue this for

several sessions with the rhino. Once the association has been established, the sound of the clicker with a food reward, the trainer can begin to use the bridge to shape behaviors. For more information on a bridging stimulus, see Pryor (1984), Mellen and Ellis (1996), and Ramirez (1999).

### ***Shaping Behavior***

For consistency, one trainer should shape new behaviors with an animal. Once the behaviors are trained and performed on cue consistently, other trainers can work with the animal. A shaping technique that works well with rhinos is using the sight of food as a lure. In most cases, rhinos focus on the food and follow it wherever it is placed. Trainers can also use their body positioning when shaping behaviors. The animal will, on many occasions, shift its body position when the trainer moves. For example, if a rhino is facing a trainer and the trainer takes a step to the left, many times the rhino will follow and shift its body to the left as well. Another useful tool in the shaping process is a target. A target is an object to which an animal is trained to touch a part of its body (Mellen and Ellis, 1996). Training a rhino to target different parts of its body is a good method of shaping many body-positioning behaviors such as standing, presenting a side of the body, or presenting a foot (Appendix C). When starting a program it is best to begin by training some basic body-positioning behaviors such as open mouth, target, and back before moving on to more complex behaviors like standing for blood collection or other veterinary procedures.

### ***Record Keeping***

It is important for trainers to keep records of all sessions. Trainers can go back and look for patterns in behavior, which helps keep consistency among trainers and leaves a historical record for others. For an example of a documentation form, please see Appendix D.

### ***Safety***

Establishing a safety protocol is another valuable component to a rhino training program. These protocols will allow a facility and training team to set clear guidelines to ensure the safety and well-being of the trainers and the animals. The disposition of the individual rhinos should be taken into account during any training session, and the ability to read the animal's body language and temperament will allow the trainer to know when the rhino is agitated and the session needs to end. In general, it is advisable to end a session on a positive note and not wait until an animal is agitated.

In any training session where the trainer is in close proximity to the rhino, as in blood-draws, it is important for keepers to work in groups of two or more. If there are multiple keepers involved in a session, then there are multiple people that are able to watch the keeper that is working close to the rhino, the rhino's behavior, and the location of its head and horn(s).

## **Summary and Resources**

The purpose of this chapter is to give trainers an idea of the type of behaviors that can be trained with rhinos and what these behaviors might look like. This chapter provides direction on starting a training program, ideas for shaping techniques and reinforcement, some specific challenges to training rhinos, and resources for additional training information. This chapter is meant as a reference for basic training information and contains just a small amount of the information that is available.

The following is a list of additional resources that can be helpful in developing a training program:

- *Animal Keeper's Forum*, a publication of the American Association of Zoo Keepers
- [www.animaltraining.org](http://www.animaltraining.org)

- Animal Training Organizations –
  - IMATA (International Marine Animal Trainers Association)
  - AAZK (American Association of Zoo Keepers)
  - IAATE (International Association of Avian Trainers and Educators)
  - ABMA (Animal Behavior Management Alliance)
  - PEM (Principles of Elephant Management)
- International Rhino Keeper Association (IRKA)
  - [www.rhinokeeperassociation.org](http://www.rhinokeeperassociation.org)

## **TRANSPORTATION**

Crating and shipping of rhinos is one of the most difficult transport procedures. While rhinos themselves are fairly hardy, the limitations of temperament, peculiarities of chemical immobilization, and rigorous shipping equipment necessitate a strict yet flexible protocol for optimizing successful crating and shipping.

### **Pre-shipment Medical Procedures**

Communication at the veterinary level between receiving and shipping institutions prior to rhino translocations is essential in order to discuss specific institutional and/or state requirements. Standard medical procedures for all moves should include the following: (1) a tuberculosis test within 30 days to six months of shipment (depending on where the rhino is being shipped) or as particular state, federal or international requirements dictate, (2) brucellosis serology if dictated by particular state or international requirements, (3) a physical examination, (4) three negative fecal screens 30 days prior to shipment, and (5) a review and update of vaccinations (see Health chapter). In addition, medical or research **protocols** defined by the SSP should be reviewed during the planning process.

### **Crating**

Crating is the recommended transport method, although transport in trailer stalls has also been successful. It is important in the latter case that the trailer is well-reinforced. In all situations, the animal's behavior and conditions should be constantly monitored. Typical problems that can occur during shipping include the following: (1) animals destroying and/or climbing out of the crate top; (2) animals becoming inverted in the crate and unable to right themselves; (3) animals destroying end panels or doors, resulting in eye, horn or facial injuries; and (4) prolonged, excessive exertion resulting in hyperthermia and/or myopathy.

### **Design**

The International Air Transport Association (IATA) crate design specifications are listed in Table 3.9. Crates are usually constructed of wood, metal, or wood with steel reinforcements. Crate dimensions should be determined by the animal's size (Table 3.9), but a general principle is that the crate should be 0.3 m (1 ft) longer and wider than the animal when it is lying on its side. Crates with vertical bars situated at the head end will decrease injuries to the head and face but must be spaced correctly with at least 10-15 cm (4-6 in) gaps. Horizontal bars at the head end should be avoided, as they tend to cause horn breakage and/or damage. Crates with bars and doors at both ends are optimal.

Table 3.9. Approximate crate dimensions by species (modified from IATA, 1995).

Species	Length	Height
Black rhino	271 cm (107 in)	191 cm (75 in)
White rhino	475 cm (187 in)	221 cm (87 in)
Greater one-horned rhino	335 cm (132 in)	201 cm (79 in)

## Principles of Design

Frames should be constructed of strong metal with sides of solid hardwood. Vertical metal bars should be bolted in place at the front and back with sliding or hinged wooden doors to the exterior of the bars. The upper third of the wooden doors must have ventilation spaces or openings. There are several new requirements for lower ventilation as well. IATA specifies that the roof must be solid over the animal's head and slatted over the loin and hindquarters for ventilation. For ground transportation, however, removable panels or hinged doors over the animal's head can be useful for administering to medical needs and monitoring the animal. Hatches also allow for more ventilation when an animal is standing calmly.

The interior must be smooth with no projections. Wooden crates have often been modified to include solid metal sheets at the head so that the horn deflects and cannot damage the wood. Only nuts and bolts should be used in the container. Entry and exit doors must be closed and bolted in strategic places to be strong enough to resist the animal, and to withstand the rigors of the equipment necessary to move the crate and rhino. The floor must be at least 2.5 cm (1 in) thick and be a non-slip surface. Some crate floors are slatted so that urine and feces can flow through so that the animal is not standing in excreta. For international and air transport, the container must be constructed in such a way that the floor and lower sides are leak-proof. In view of the diversity in size, strength and temperament of rhinos, the size and strength of the container must be sufficient to restrict the movement of and restrain the animal. Dimensions must be large enough to prevent cramping without allowing unnecessary movement. In general, the crate should be 0.3 m (1 ft) longer and wider than the animal when it is lying on its side.

At the front of the container, there must be provisions for water and food access at the base of the door and between the bars, if present. For airline transport, this access point must be clearly marked FEEDING and be adequately secured when not in use. A water container must be provided and must be sufficiently large for the entry of the animal's muzzle. Some rhinos will not drink from a tub, and so offering a hose directly into the crate (either into the animal's mouth or just puddling in front of the rhino) is sufficient to provide water. Entrance and exit must be clearly indicated on crates used for airline transport. Many crates are designed to be used in either direction. The above recommendations are modified from IATA standards to include specifications for ground transport. Before shipping by air, consult the current IATA specifications and/or the airline.

## Acclimation Training

Crate acclimation can require two to six weeks, although several zoos have crate-trained rhinos in seven days or less. Training should be completed by a method of approximation (with reinforcement given as rhinos demonstrate progress towards the desired behaviors). The first step is to introduce the crate as a non-interactive part of the animal's environment. Gradually, the food is moved toward and finally into the crate. If the animal acclimates to the point of completely entering the crate and will allow the door to be shut, the door should be left closed for short acclimation



periods under close observation. It is not advisable to close the animal into the crate if the training period is short. No rhino likes to be closed into the crate, and even the best-trained rhinos will react negatively to being locked in. If there is not adequate time to train the rhino to acclimate to a closed crate, closing him/her in will only make the experience a negative one, and there will be a major setback to the ability to close the animal in for shipment. If the rhino does not completely acclimate to entering the crate, partial immobilization (standing restraint) may need to be utilized for shipping. Forced crating without training or immobilization is strongly discouraged.

### **Crating with Chemical Immobilization**

Immobilization offers a fairly simple way of crating a rhino. First, it should be noted that the usual pre-immobilization procedures (e.g., fasting, detainment in an adequate holding area, etc.) should be observed for any procedure requiring the use of chemical immobilization/tranquilization agents. For rhinos, etorphine (M-99) remains the drug of choice, although several alternatives are available. For specific drugs and dosages, refer to the Health chapter of this publication. The duration of immobilization without the administration of an antagonist may range from 30 minutes to two hours.

Following crating, all rhinos should be held for 24 hours at the loading location for observation or accompanied by a veterinarian during transport. This step is necessary because renarcotization is common in hoofed animals, especially rhinos, given opioids. This step, however, may not be necessary if the butorphanol/detomidine anesthetic protocol is used (P. Morris, pers. comm.). Trained personnel should be present to administer the correct reversal agent(s) in the event of a renarcotization. Any other complications of crating can be managed more easily and effectively before departure rather than en route.

### **Transport**

Numerous options for transporting rhinos are available. Each method has its advantages, and each should be scrutinized by evaluating the distance to be traveled, the personnel needed and the temperatures to which the animals will be subjected. A flat-bed truck and open trailer is temperature-restrictive. A crate within an open trailer should be protected from excessive wind, rain and sun. Enclosed trucks or trailers are other options that are necessary in extreme hot or cold temperatures. In any case, the transport vehicles must be climate-controlled if shipping in inclement weather (either hot or cold). If weather conditions and ventilation are appropriate, many rhinos have been moved in enclosed trailers without climate-control. Air transport, rather than ship transport, is the preferred option for any transoceanic translocation. Transport by ship is undesirable because of the excessive time at sea, variable conditions and more intensive personnel requirements. When transporting by air, it should be noted that some airlines may require the rhino crate to be placed in an aluminum air cargo box, which can restrict ventilation and subject the rhino to excessive heat buildup during both the airplane-loading process and transport.

During all rhino shipments, the shipper must be aware that any animal that has been immobilized (and to a lesser extent, some that have not) will be less capable of maintaining thermal homeostasis than a normal animal, and appropriate accommodations for this are necessary (e.g., ventilation, climate-control). If during the course of a transport procedure a situation arises in which the safety of the animal is jeopardized, a decision should be made through the appropriate channels to postpone or cancel the shipment. Leaving the decision of whether to transport an animal to the transporter or the recipient may be disadvantageous to the animal's welfare. The transporter is not familiar with pertinent medical and practical husbandry information, and recipients are at a disadvantage because they are often not present.

# ENCLOSURE DESIGN

## Design

In designing facilities to maximize rhino health and reproductive success, it is important that the environment contain as many salient features as possible found in the natural environment (for more detailed information on the natural habitats of the various rhino species, see the Behavior chapter). The species to be exhibited will dictate the design of rhino facilities, as species differ in their group compositions and enclosure requirements. Additionally, whether an institution wishes to maintain rhinos only for exhibit or for breeding will determine the design of rhino enclosures. Whenever possible, institutions are encouraged to plan for breeding capabilities, but the various SSP coordinators and RAG chairs recognize the need for display-only exhibits, which can facilitate education and/or research. These exhibits serve rhino management programs by holding non-reproductive and single-sex specimens. The following section outlines design considerations for indoor and outdoor rhino facilities, as well as aspects of chute design for rhino restraint.

## Enclosures

The design of zoo enclosures for rhinos requires an understanding of rhino biology, behavior and social organization. As previously stated, black, white and greater one-horned rhinos vary in their levels of sociality and thus have different housing requirements. The design of rhino enclosures also depends on the type of rhino program (exhibit only or breeding) and the number of animals. In all cases, the larger and more varied the enclosure, the better. Tables 3.10 and 3.11 list the recommended animal numbers for institutions holding rhinos (see also the Management and Behavior chapters) and the enclosure types recommended depending on institutional goals. It is important to note that for the most part, exhibit and holding-space availability will dictate an institution's designation as either a breeding or an exhibit facility. Design elements for a breeding facility should include an outdoor primary enclosure (with separation capabilities), indoor holding and an isolation area. Additionally, breeding institutions must have space for any offspring to be held for up to three years of age. Exhibit-only facilities should have an outdoor primary enclosure and indoor holding areas (both with separation capabilities). It will be recommended that exhibit-only facilities receive pre- or post-reproductive-age or single-sex groups of animals.

Table 3.10. Recommended numbers for institutional holding.

Rhino Species	Recommended Minimum Groupings for Breeding <sup>a</sup>	Preferred Optimal Holding for a Breeding Institution	Exhibit Only (per institution)
Black	1.1	2.2 (2 pairs)	1.1 or 0.2
White	1.2	2.4 (1 herd/ 1 back-up male)	1.1 or 0.2 <sup>b</sup>
Greater one-horned	1.1 <sup>c</sup>	2.2 (2 pairs)	1.1 <sup>c</sup> or 0.2

<sup>a</sup>Breeding institutions must have space for offspring to be held for up to 3 years following birth.

<sup>b</sup>Multi-male bachelor groups have been maintained in very large enclosures.

<sup>c</sup>In the case of greater one-horned rhinos, males and females should be introduced only during the female's estrous period. Institutions with very large enclosures may be able to hold opposite-sex animals together consistently.

In general, it is recommended that enclosures be designed such that animals may be kept outdoors as much as is possible within the following temperature constraints. Rhinos should not be locked outside when the temperature is below 4.4°C (40°F); sun, wind chill and rain should be considered in calculating temperature. During extremely cold weather, rhinos should not have access to mud wallows, and they should be filled with substrate. Animals should not be let out if enclosures are icy. Temporary exposure to temperatures below 4.4°C (40°F) for cleaning is left to the discretion of management. Localities that experience average daily temperatures below 10°C (50°F; average of high and low temperatures over a 24-hr period), should provide heated facilities capable of maintaining a minimum temperature of 13°C (55°F).

Table 3.11. Recommended enclosure types and sizes for captive rhinos by species [in sq m and (sq ft)].

	Individual Holding (per rhino)		Exhibit Only (per rhino)		Breeding/Communal	
	Indoor	Outdoor	Indoor (as primary exhibit area)	Outdoor	Indoor	Outdoor
Black	18 (200)	186 (2,000)	204 (2,200)	771 (8,300)	not recommended	2,322 (25,000)
White	30 (320)	186 (2,000)	215 (2,320)	929 (10,000)	not recommended	2,787 (30,000)
Greater one-horned	30 (320)	186 (2,000)	215 (2,320)	929 (10,000)	not recommended	2,787 (30,000)

## Outdoor Housing

Several general outdoor enclosure designs are recommended that incorporate the data available on the behavior and ecology of wild black, white and greater one-horned rhinos. As previously described, rhinos of all species may be less solitary than was originally thought. For the most part, however, rhinos are somewhat territorial; therefore, more than one outdoor yard is strongly recommended. To provide a large area for introductions of black rhinos, a communal yard adjacent to individual yards should be available. If the space is not available, two adjacent yards may be opened for male/female introductions. In many respects, the critical enclosure characteristic is the availability of escape routes and visual barriers, which serve to hide or prevent access to an animal that is being pursued. Gates may be used as escape routes, provided that care is taken to prevent dead-end corners and to create “run-arounds” (brush piles, earth, or boulders) so that an animal can enter or leave the yard without an aggressor blocking or guarding the only exit.

Enclosure size depends on whether rhinos are kept for exhibit-only or breeding purposes (Table 3.11). It should be noted that a calf is considered an adult with respect to minimum space requirements after weaning; this should be considered in determining minimum enclosure size.

### Primary Barriers

The barrier between rhinos and the viewing public is a critical element in the design of the outdoor exhibit. This primary barrier should allow visitors a clear view of the animals from a safe location. Many types of primary barriers are available, the most common of which are walls,

fencing, dry moats and water moats. One consideration in choosing fence type should be the size of the enclosure. For example, smaller exhibits should be constructed with barriers that provide as much visual exposure as possible. Moats, both dry and water, are less desirable for breeding groups because of the potential for accidents.

### **Fencing**

Because any of the rhino species may climb, a primary barrier should be a minimum of 1.5 m (5 ft) high and non-climbable. In small enclosures, particular attention should be given to the climbing ability of rhinos and to the need for separating aggressive animals. A secondary barrier or a taller primary barrier may serve to counter these problems. It is important to consider fence spacing and keeper access/exit in the event of an emergency as well.

Recommended materials for primary fencing include solid concrete or rock walls, horizontal pipe or cable spaced 25 to 30 cm (10-12 in) apart, and vertical pipe or posts spaced 25 to 30 cm (10-12 in) apart. Cable should be used only for horizontal fences. The size of the exhibit to be fenced will determine the strength and type of fencing material used, as each type has both advantages and disadvantages. Concrete surfaces and bare steel cable create surfaces that may encourage rhinos to horn-rub excessively, causing abnormal horn wear. If necessary, surfaces should be covered with a non-abrasive material; one solution is to insert steel cable through plastic pipe, or concrete surfaces can be covered with non-toxic wood.

If poles are used, each should be approximately 30 cm (12 in) in diameter and set in concrete with approximately 1.8 m (6 ft) underground. Poles should be spaced as closely together as possible to prevent rhinos from getting their horns through and uprooting the fence. ***Creosote-treated poles, which are dangerous to rhinos, should not be used.***

Rocks or a rock apron can be utilized to protect the poles or other objects in the exhibit from damage. A rock apron should extend 1.8 m (6 ft) from the leading edge of the object to offer adequate protection. If small rocks are used, they should be several layers thick; otherwise, a single layer of very large rocks is probably adequate.

### **Dry Moat**

The use of a dry moat requires one vertical wall, which should be a minimum of 1.5 m (5 ft) high, located on the public side. The second wall should be sloped at a maximum of 30° so that the animals can climb out. This gradual decline of the exhibit substrate down to a solid wall can be used to create a moat effect, but ***ditch moats with two vertical walls are considered dangerous to rhinos and are not recommended.*** The floor space in the moat should be a minimum of 1.5 m (5 ft) across to prevent rhinos from being trapped, and surface substrate for the moat should provide stable footing (recommended materials include dirt, gravel, sand, etc.).

### **Water Moat**

Water should not be utilized as a primary barrier as it carries the risk of drowning or injury, especially for calves.

### **Secondary Barriers**

Though not critical to the design of outdoor enclosures, secondary barriers may protect exhibit features or lessen stress on primary barriers. Recommended types are butt rails, vertical poles and electrically charged, or “hot” wire. Plantings can also serve as a secondary barrier when used to create a visual screen. For example, plantings that extend above a low wall can give the appearance of a bigger wall [although the primary barrier height minimum of 1.5 m (5 ft) still

applies]. Electric fencing can deter animals from destroying plantings, trees and other secondary barriers. Rock aprons may also be used around trees and fence lines as secondary barriers.

### **Gates**

Enclosure gates can be the weakest points of the exhibit, therefore, adequate hinge and lock strengths are very important. Interior doors are usually constructed of heavy-gauge steel or pipe that is hinged or sliding. Sliding gates are optimal, as they have the ability for partial opening, and should be a minimum of 1.8 m (6 ft) wide and 2 m (6 ft 8 in) high. If the gate uses a track, care should be taken in the construction of the track to avoid injuring the feet of the animals as they run through gates during introductions. Exterior building doors may be made of steel or wood reinforced with steel, with the lower part covered by a steel plate to minimize damage. Gates should be constructed to allow keepers to open and close them without entering rhino space. Also, where appropriate, vehicle access to an enclosure should be provided.

There are a variety of options available to operate gates. The simplest systems are manually operated, either push-pull or cable driven. Rhino-size doors can be heavy and difficult to move. Mechanical systems can make this easier, using electric motors or hydraulic or pneumatic pressure to move the doors. These systems require some form of back-up system in case of a mechanical or power failure. Mechanical systems can generate significant forces that can injure or even kill an animal. Safety measures have to be incorporated into the design of the gate operating system to prevent accidents.

### **Substrate**

The outdoor enclosure should have a well-drained surface that provides adequate footing for rhinos. Black rhinos should be maintained on grass if the space is available, but limestone is adequate. Rhinos should be carefully observed upon introduction to a new substrate, as excessive ingestion of the substrate from feeding on the ground has caused impaction in other hind-gut digesters.

### **Water**

Fresh, potable water should be available at all times. Water should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be substantially constructed to prevent injury, upset, spillage or leakage.

### **Mud Wallows**

Black rhinos need access to mud wallows for skin health, temperature regulation and behavioral enrichment. The size of mud wallows should be gauged by the number of animals in the exhibit so that ample room is provided for each individual. It should be noted that given a start, rhinos may construct their own mud wallows. Construction of wallows varies by institution and exhibit. Some facilities use a concrete basin or pool filled with substrate, while others simply dig out a section of the enclosure. If a concrete pool is used, it should have the ability to be drained when needed, but care should also be taken to protect the drain line from being filled with substrate. If a wallow is dug on exhibit, it should be situated in an area that will allow adequate drainage away from the wallow. It is also important that a good source of water be nearby to keep the area wet. Wallows are much easier to manage if the enclosure allows access for a skid steer or tractor with front loading bucket.



Mud wallows must be actively managed. If the local soil does not create mud of a satisfying consistency, it can be adjusted with the addition of topsoil, clay or sand. Standing water in an animal area can be a potential USDA compliance issue. The wallow should be monitored for the growth of algae. If the algae cannot be removed by raking or scraping, it may be necessary to fill the area in to allow it to dry completely for several days, and then dug out again. Depending on the local environment, the substrate may need to be changed several times during a year to prevent contamination. Institutions in northern climates may need to fill in the wallow during winter months.

### ***Visual Barriers***

Naturalistic visual and physical barriers (refuges) in outdoor enclosures may help decrease aggression by permitting animals to separate themselves from others during introductions or in a group situation. Barriers should be large and high enough to provide “safe zones” that allow an animal to pass from another’s sight but should not hinder public viewing. Types of visual barriers include deadfall, logs and boulders, as well as trees and natural plantings. Trees and plantings may be protected from rhinos by pipe caging, rock aprons or barrier fencing. If permanent physical structures are not available as barriers, dirt mounds may be used to give individuals additional visual barrier points in the enclosure.

### ***Shade/Rain Shelter***

Access to shade is a necessity as well as a USDA requirement under the Animal Welfare Act. A variety of both natural and constructed options are possible. It is also important that a shade option be adequate as a rain shelter if barn shelter is not always accessible; therefore, trees may not be completely adequate. It is a good idea for wallows to be located in areas that are shaded at least part of the day.

### ***Additional Furnishings***

Additional furnishings for the outdoor exhibit should include scratching posts, which may be particularly effective if placed near mud wallows. Post materials must be non-toxic to rhinos (i.e., non-creosote). Several institutions have buried deadfall or logs upright in concrete sewer culverts, which are routed in place with 0.9 to 1.2 m (3-4 ft) of gravel. This enables managers to remove and replace posts as they deteriorate. Feed should be available at all times in the form of browse, feed stations and mineral salt licks.

### **Indoor Housing**

Indoor housing is recommended for additional separation capabilities (beyond the primary enclosure) and is critical for those institutions in colder latitudes. At no time should rhinos be forced to endure temperatures below freezing for any length of time; animals may go out for short periods when temperatures are below freezing, but they should have access to radiant heat or heated enclosures during these times. An indoor facility in the winter should be heated to a minimum of 13°C (55°F) with the capability of maintaining some areas of the barn at 23.9°C (75°F). Supplemental heat may be needed when dealing with infants or with sick or older animals. Some acclimation may be necessary before moving animals from a warm barn to the outdoors during winter months. The humidity level should be maintained at 40 to 70%. Shower sprays or water baths should be offered in areas of relatively low humidity. Indoor facilities should be maintained with a negative air pressure, and ventilation should be provided to accommodate at least four air exchanges per hour (USDA recommendations for a cold-weather heated barn). Institutions are

encouraged to check with their local authorities for air-exchange requirements when the public or personnel occupy the facility.

Within any indoor facility, areas must be provided for food and water. Fresh water should be available at all times and should be changed daily or be supplied from an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset, spillage or leakage.

For black rhinos, isolated stalls are essential. The indoor enclosure should include a minimum of 18 sq m (200 sq ft) for black rhinos (Table 3.11). An additional 50% of adult space should be provided when a calf is present. This may be achieved by using more than one stall. Following weaning, a calf should be treated as an adult with respect to space requirements. If the institution has only indoor facilities in which to maintain and/or exhibit rhinos, the minimum requirement is 186 sq m (2,000 sq ft) per rhino [15.2 x 12.2 m (50 x 40 ft)] plus the recommended indoor holding [18 sq m (200 sq ft) per individual for black rhinos].

### ***Separation Capabilities***

The indoor facility should have the capacity to separate individuals for a variety of purposes. As indicated above, black rhinos should be kept in individual stalls. The facility should also have an extra space or large stall to make it possible to isolate mothers and calves or to quarantine sick animals.

Currently, no quantitative data are available on the visual, olfactory or auditory capabilities of rhinos in relation to breeding success. Based on species ecology and behavior, however, it is believed that rhinos rely heavily on both olfactory and auditory senses for social communication. It is therefore recommended that indoor facilities facilitate these types of communications at certain times among individuals. Options include partial walls or pipe fencing to allow for physical separation without visual, auditory or olfactory separation.

### ***Substrate and Special Features***

A brushed or broom-finished concrete floor that is well-drained and ensures adequate footing is recommended. Dirt flooring as the main substrate is not recommended. In addition, floor heat is recommended in colder climates. Bedding materials such as hay, wood shavings and hoofed-stock rubber matting are optional for black rhinos. Other situations in which bedding is required include barns with rough substrates (which may cause skin ulcerations) or for additional warmth for sick animals or young calves. When introducing rhinos to new substrates, careful observations should be made to avoid the animals' excessive ingestion of the novel substrate, which could potentially lead to health problems, such as impaction. The use of a power washing machine is recommended to disinfect barn areas. Additionally, rubber matting and bedding materials should be disinfected or changed regularly to prevent contamination. Permanent rubberized flooring (poured floors or encapsulated mats) is more expensive but reduces cleaning time and risk of contamination.

Normal light cycles seem to be adequate for rhinos. However, if an animal is to be held indoors for more than 12 hours (e.g., winter at cold-climate institutions), artificial or natural light sources to simulate natural cycles should be provided. Fluorescent lighting is an efficient light source that provides broad-spectrum illumination; skylights should also be included whenever possible.

Any new exhibit should include the capability for video systems. In addition, a scale for weighing animals is desirable and strongly recommended. Vehicle access to an indoor facility is also recommended. A restraint device or an area for restraint should be included in every facility design, as well as an area to set up crates for training, loading, and unloading.

## Physical Restraint Designs

Numerous institutions have constructed permanent physical devices to restrain their rhinos when necessary. Such “chutes” can be very valuable for physical exams as well as nutritional, reproductive or veterinary research. In addition to the following general information, please consult the Health chapter of this publication as well as Schaffer (1993) and Eyres et al. (1995). Institutions in the United States that currently have chutes and may be able to provide additional information include Henry Vilas, Saint Louis, Sedgwick County, Oklahoma City, Henry Doorly, Cincinnati, Caldwell, and Milwaukee County Zoos, Fossil Rim Wildlife Center, and the Wilds. Companies that may assist in chute design and construction include Animar Systems, Inc. (Springfield, MO, USA), Cummings and Son, Inc. (Garden City, KS, USA), and Mark McNamara of Fauna Research (Tamer, Fauna Resources, Inc., Red Hook, NY, USA). In general, institutions modifying rhino exhibits or constructing new ones should incorporate a physical restraint area or device into their design.

Several physical restraint designs are effective for rhinos. These range from a small restricted area in which to contain the animal to an area that contains one or more hydraulics that will “squeeze” together to restrict an animal’s movement. In general, major restraint chute design considerations include strength, durability, type and function. It should be noted, however, that available space and animal size and disposition vary across institutions and should be individually addressed.

In general, both zoological managers and researchers emphasize that the general restraint area should be an active component of daily rhino management. Methods to accomplish this vary. A restraint chute or restraint area can be designed so that the rhinos must pass through it to exit the barn into their yard. If rhinos are fed indoors, part of the feed (e.g., produce, grain) can be offered in the chute area. Finally, more extensive conditioning (see Training chapter) can be particularly effective in habituating rhinos to physical restraint. Such a program should be attempted prior to detaining a rhino in a chute for an exam.

Rhino chutes should be manufactured out of steel or a combination of steel and steel-reinforced wood. Some institutions have also used steel-strength aluminum (6061-T52 aluminum). Aluminum of this type is lighter and more maneuverable than steel, as well as potentially less stressful to rhinos because of “deader” sound properties than steel (i.e., when metal scrapes metal).

Permanent pass-through indoor restraint chutes (similar to those constructed for elephants) are especially effective for rhinos. With training, this type of chute may allow for detailed daily rhino observations. Further, inclement weather will not affect the use of an indoor restraint chute. The chute should allow restraint of the animal when it is passing through in either direction so that the shifting routine of the animal is not interrupted (Schaffer, 1993). The width of the chute should limit side-to-side movement while still allowing the animal to comfortably lie down. Animals can become wedged in tight-fitting chutes if the sides cannot be released. To alleviate excessive forward movement of the animal when it lowers its head, two vertical bars that push in from the sides of the chute to the shoulders of the rhino may be utilized. Quick release of these shoulder bars often relieves agitated animals without having to release them completely.

High-walled chutes or bars over the top of the chute keep the animal from climbing or rearing up. Horizontal bars in the chute’s entry gates and sides are hazardous for examiners when the animal lies down. Vertical bars on the sides can trap researchers’ arms if the animal can move forward. If the animal’s forward and side-to-side mobility can be limited, vertical bars or walls on all sides are recommended. The distance between these bars along the sides of the chute should be great enough to prevent the animal’s foot from becoming wedged if the animal rolls on its side in the chute. For personnel safety, this distance can be divided with removable vertical bars.

Rhinos may slam swinging doors; thus, sliding or guillotine gates are safer. A rectangular opening in these gates so that palpation can be performed should not pin the arm of an examiner when the animal is shifting. The distance between the vertical sides of this rectangular opening must be wide enough to provide for staff safety while still limiting the space through which a rhino could squeeze. Also, the horizontal bottom bar of this rectangle should be only a few inches from the ground, as animals frequently lie down. Solid doors on the outside of these gates can be used to stop rhinos, as they may attempt to charge even small openings. Additionally, good lighting and accessible electrical sources are useful.

A closed chute (Fig. 3.6) is another option that has been used successfully for the treatment of a rhino with a urinary-tract infection and another with infected lesions on its foot (Eyres et al., 1995). As noted in Figure 3.6, a typical closed chute has both front and back gates. The back gate restricts the rhino's movement by sliding forward. Additionally, the hind end of the rhino is supported by a V-design that prevents it from lying down. This design also allows additional safety for the staff while working with the animal. In many respects, a closed chute does not depend as strongly on conditioning of the rhinos as does a squeeze chute, although acclimation is recommended prior to attempting any treatments within the chute. The design of a closed chute might necessitate an outdoor location in most cases; therefore, the use of this type of chute may be limited by weather.

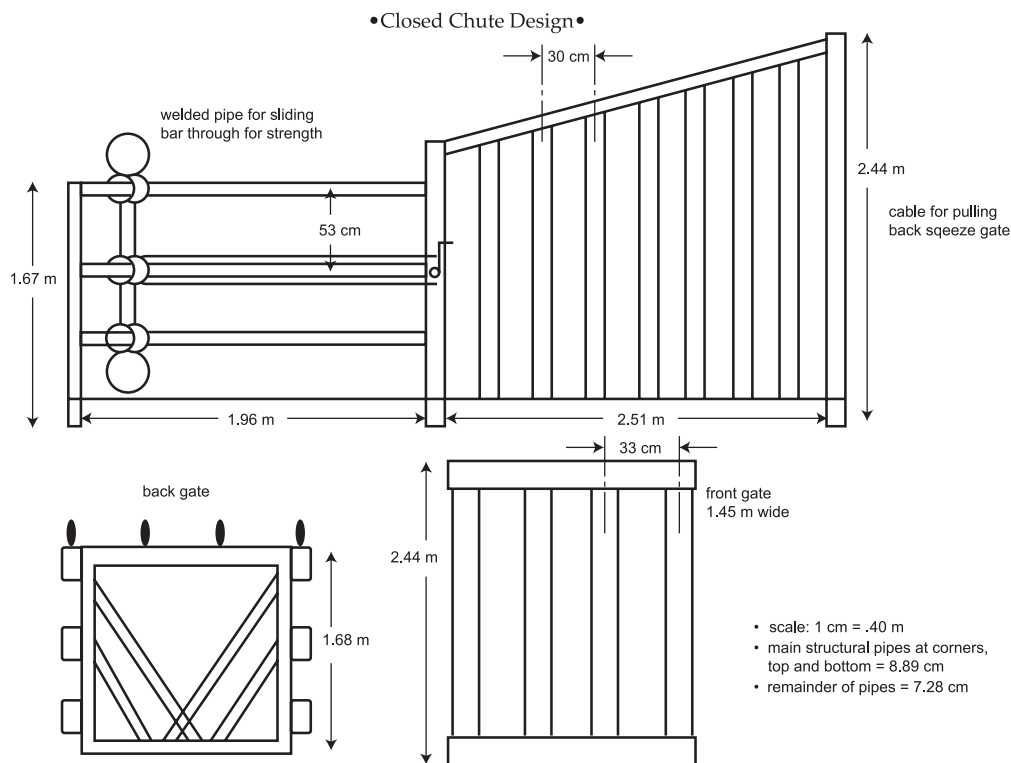


Fig. 3.6. Closed stall rhino restraint chute. Note that a typical closed chute has gates that restrict the rhino's movement and prevent it from lying down. The advantage of a closed chute is that it does not depend as strongly on conditioning as does a squeeze or free-stall chute (Eyres et al., 1995).

A free-stall chute can be used for animals more sensitive to a confined enclosure (Fig. 3.7). The design of this type of chute allows the rhino to enter or exit at its will and thus may help to keep rhinos calmer during procedures. Because there is free access, however, rhinos must be conditioned to target or stand still; thus, relatively non-invasive procedures also work best. Procedures that have been accomplished with a conditioned rhino in a free-stall include ultrasound and serial collection of blood and feces (Eyres et al., 1995).

A free-stall design can easily be incorporated into an existing pen or stall, indoors or outdoors. The open back of this type of chute allows the animal to enter and leave the structure at will. Protection of staff when working with the rhino is critical; a partial back wall constructed of vertical pipes allows staff to step out of the way (Fig. 3.7).

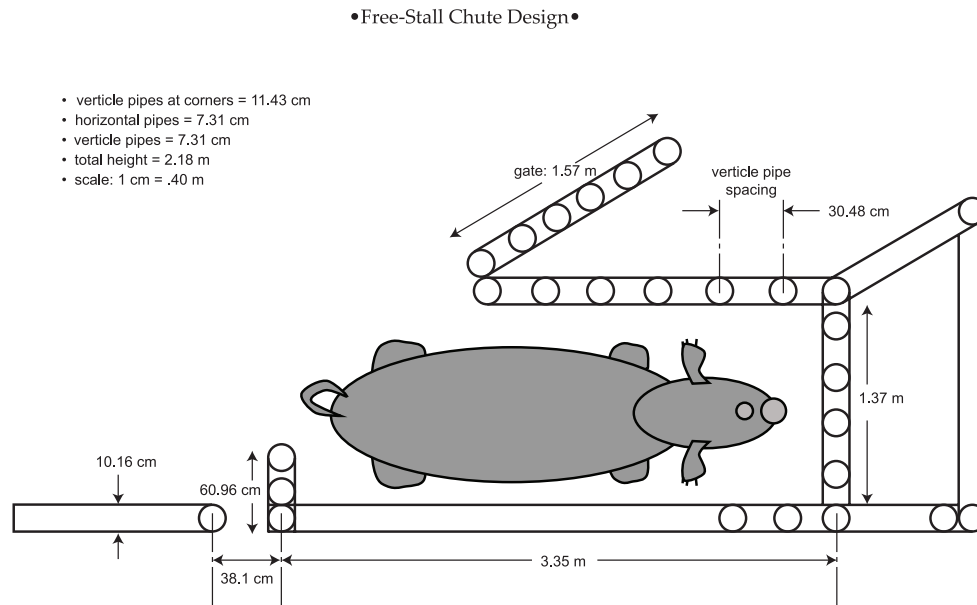


Fig. 3.7. Free-stall rhino restraint chute. The design of a free-stall chute allows the rhino to enter or exit at will and can be used for animals more sensitive to a confined enclosure. This type of restraint chute, however, is best used for relatively non-invasive procedures and with rhinos that have been conditioned to target or stand still (Eyres et al., 1995).

## **HEALTH**

### **Introduction**

This section provides a general overview of preventive medical and disease management, and chemical restraint of captive rhinoceroses. More detailed information for many of these topics is available in the references provided as well as through the Rhino Resource Center ([www.rhinoresourcecenter.com](http://www.rhinoresourcecenter.com)).

### **Physiological Normals and Sample Collection**

With the increased use of training for husbandry and medical procedures, resting values for heart rate, respiratory rate, temperature and other values have been obtained for non-restrained black rhinos. The various species appear to be similar with heart rates of 30 to 40 beats per minute and respiratory rates of six to 12 breaths per minute. Rectal temperatures are typically 34.5-37.5°C (94-99.5°F), although temperatures may be higher in anesthetized rhinos (37-39°C; 98.6-102°F)



due to exertion or muscle tremors (Miller, 2003; Radcliffe and Morkel, 2007; Morkel et al., 2011). Values are comparable to domestic horse ranges. Limited information about electrocardiography (ECG) is available in rhinos (Jayasinge and Silva, 1972). Indirect blood pressure has been measured in unsedated black rhinoceros using a human blood pressure cuff around the base of the tail. Mean values reported for unanesthetized white rhino are  $160 \pm 2.9$  mm Hg (systolic),  $104 \pm 2.3$  mm Hg (diastolic), and  $124 \pm 2.2$  mm Hg (mean blood pressure)(Citino and Bush, 2007). In anesthetized animals, etorphine can cause hypertension, although variable mean blood pressure measurements (107-280 mm Hg) have been observed, depending on drugs used and time values measured.

Hematologic, biochemical, mineral, protein electrophoresis, and blood gas values have been previously published (Tables 3.12-3.14; Flesness, 2002; Miller, 2003). Although most parameters can be generally interpreted similar to other perissodactyls, there are several differences that appear to be normal in rhinos. Total protein and globulins tend to be higher than in domestic horses. Other values that differ include lower sodium and chloride. Free-ranging rhinos tend to have higher creatine phosphokinase (CPK) than captive animals (this may be biased by immobilization technique)(Kock et al., 1990; Mathebula et al., 2012). Hypophosphatemia (low blood phosphorus) is a recognized problem in captive black rhinoceros, with levels dropping below 1 mg/dl. Low serum phosphorus (P) has been linked with hemolytic anemia and other blood disorder syndromes (Miller, 1993; Dennis et al., 2007). Oral and intravenous supplementation may be instituted in these cases. Anecdotal reports of doses used in black rhinos are 10 to 24 g elemental phosphorus (preferably chelated) orally once per day until normal serum levels are reestablished (E. Valdes, pers. comm.; Gillespie et al., 1990). In critical cases, intravenous sodium or potassium phosphate can be administered at 14.5 mmol P/hour but serum calcium should be carefully monitored.

Table 3.12. Mean hematology values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
WBC x $10^3/\mu\text{l}$	8.42 (2.48)	9.30 (2.46)	7.20 (1.33)	8.27 (1.55)
RBC x $10^6/\mu\text{l}$	4.01 (0.88)	5.77 (1.28)	6.43 (0.86)	5.32 (1.09)
HBG g/dl	12.0 (2.0)	13.8 (3.8)	13.4 (1.5)	12.4 (1.6)
Hct %	33.4 (5.7)	36.9 (9.3)	37.0 (4.6)	36.9 (4.2)
MCV FL	85.7 (9.0)	63.8 (7.8)	57.8 (4.9)	71.5 (11.2)
MCH pg/cell	30.5 (3.3)	23.5 (1.9)	21.3 (3.0)	23.9 (3.8)
MCHC g/dl	35.7 (2.7)	37.9 (7.3)	36.3 (3.2)	33.5 (2.1)
Platelets x $10^3/\mu\text{l}$	284 (83)	378 (103)	178 (53)	198 (135)
Nucleated RBC/100 WBC	0	1 (1)	0	–
Reticulocytes %	1.6 (2.9)	–	–	–
Neutrophils x $10^3/\text{ml}$	5.24 (2.18)	5.42 (2.05)	5.13 (1.24)	4.86 (1.16)
Lymphocytes x $10^3/\text{ml}$	2.48 (1.1)	2.35 (1.15)	1.74 (0.67)	2.52 (0.90)
Monocytes x $10^3/\text{ml}$	0.43 (0.32)	0.65 (0.55)	0.22 (0.15)	0.36 (0.22)
Eosinophils x $10^3/\text{ml}$	0.25 (0.22)	0.54 (0.59)	0.32 (0.31)	0.37 (0.21)
Basophils x $10^3/\text{ml}$	0.17 (0.09)	0.10 (0.05)	0.13 (0.05)	0.08 (0.01)
Neutrophilic bands x $10^3/\text{ml}$	0.27 (0.35)	0.71 (1.18)	0.22 (0.20)	0.31 (0.24)

Table 3.13. Mean blood chemistry values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
BUN mg/dl	13 (3)	16 (3)	3 (2)	6 (2)
Creatinine mg/dl	1.1 (0.2)	1.8 (0.4)	1.3 (0.2)	0.9 (0.1)
Uric acid mg/dl	0.5 (0.2)	0.9 (0.8)	0.3 (0.2)	-
Bilirubin mg/dl	0.3 (0.1)	0.3 (0.3)	0.4 (0.3)	0.2 (0.1)
Glucose mg/dl	69 (21)	97 (39)	82 (25)	76 (13)
Cholesterol mg/dl	102 (37)	93 (26)	53 (21)	48 (21)
CPK IU/L	255 (248)	409 (722)	260 (203)	617 (398)
LDH IU/L	595 (427)	537 (320)	267 (149)	231 (38)
Alk Phos IU/L	80 (55)	92 (51)	80 (41)	17 (6)
ALT IU/L	16 (7)	16 (9)	7 (7)	6 (3)
AST IU/L	85 (27)	71 (25)	61 (27)	39 (9)
GGT IU/L	27 (18)	19 (14)	18 (16)	6 (2)
Total protein g/dl	7.6 (0.9)	8.5 (1.0)	7.5 (0.9)	7.5 (0.4)
Globulin g/dl (electrophoresis)	4.9 (0.9)	5.3 (0.8)	4.5 (0.7)	3.8 (0.7)
Albumin g/dl (electrophoresis)	2.6 (0.4)	3.2 (0.5)	2.9 (0.5)	3.6 (0.6)
Fibrinogen mg/dl	104 (195)	101 (241)	350 (84)	324 (85)

Table 3.14. Mean serum mineral values and blood gases in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
Ca mg/dl	12.7 (1.0)	11.8 (0.9)	11.4 (0.8)	13.3 (1.1)
P mg/dl	4.8 (1.1)	4.0 (0.9)	4.0 (0.9)	3.7 (0.7)
Na mEq/L	133 (3)	134 (5)	132 (3)	133 (4)
K mEq/L	4.7 (0.6)	4.7 (0.8)	4.1 (0.4)	4.6 (0.6)
Cl mEq/L	96 (0.3)	95 (4)	91 (3)	100 (3)
Bicarb mEq/L	23.3 (4.2)	18 (0)	27.0 (0)	-
CO <sub>2</sub> mEq/l	25.4 (9.9)	25.3 (8.8)	27.3 (3.7)	22.8 (2.4)
Iron $\mu$ g/dl	227 (66)	176 (67)	152 (70)	-
Mg mg/dl	3.34 (3.45)	118.2 (232.5)	7.95 (8.56)	-

Venipuncture can be routinely performed on awake captive rhinoceroses using training and/or restraint devices (Fig. 3.8). The most commonly used sites for blood collection are the ear (auricular) vein, metacarpal vein (lower inside forelimb), and radial vein (inside of forelimb crossing the carpus)(Fig. 3.8-3.10). The tail (coccygeal) vein has also been used and is approached from the ventral aspect similar to the technique for blood collection in domestic cattle. Arterial access is available for blood gas sampling using the medial auricular artery (inside of the ear)(Fig. 3.11). Large volumes of blood can be collected from the radial or metacarpal veins for diagnostic testing,

plasma/serum banking, or for therapeutic phlebotomy (Mylniczenko et al., 2012). One to eight liters have been obtained from trained standing rhinoceros (Fig. 3.12; V. Clyde, pers. comm.). Rhinoceros blood cells resemble those of domestic horses. Nucleated red blood cells (NRBC) and reticulocytes may be observed in anemic animals. Elevated total white blood cell counts (wbc) and eosinophil numbers have been observed in wild rhinoceroses, presumably due to the response to capture and parasite loads, respectively.



Fig. 3.8. Voluntary blood collection from auricular vein in awake rhinoceros.



Fig. 3.9. Blood collection from the metacarpal vein in a standing rhinoceros.





Fig. 3.10. Blood collection from radial vein in immobilized rhinoceros.



Fig. 3.11. Arterial blood sample collection from medial auricular artery.



Fig. 3.12. Therapeutic phlebotomy in a trained rhinoceros (courtesy of V. Clyde).

Urinalysis panels in captive rhinos are similar to horses with large numbers of calcium carbonate crystals, creating a normal milky yellow appearance to the urine. Calcium oxalate, phosphate, and ammonium crystals may also occur, depending on the diet. Occasionally dark discoloration of urine associated with pigmentation of certain browse species (e.g. ash, mulberry) can be mistaken for blood or myoglobin. However, analysis should be performed to rule-out any abnormalities. Normal values for the different rhino species have been published (Table 3.15; Haffey et al., 2008).

Table 3.15. Reported range of mean urinalysis results from three captive rhinoceros species.

	pH	Specific Gravity
Greater Asian one-horned rhinoceros	8.08-8.70	1.019-1.031
Sumatran rhinoceros	8.16-8.32	1.010-1.021
Black rhinoceros	8.10-8.26	1.010-1.012

Due to size, cerebral spinal fluid collection has not been successful except in a few rhino calves (S. Citino, pers. comm.). Therefore, extrapolation of normal values from domestic horses and other perissodactyls should be used for interpretation.

Incorporation of scales into rhino facilities has permitted monitoring of body weights and physical condition. Regular weighing is recommended to observe trends associated with growth, diet changes, and disease. Average range for adult body weight is 800-1350 kg (1,764-2,976 lb; Miller, 2003).

### Preventive Medical Health Programs

Routine health monitoring should be performed on all rhinoceros on an ongoing basis. Animals should be trained to permit sampling and examination. The following **protocol** advises that specific laboratory tests be performed for the purpose of evaluating current health status.



Additional tests are recommended to increase baseline information on other diseases to determine their significance to rhinoceros health. The final decision for specific procedures should be made by the animal care and veterinary staff based on individual circumstances.

- Physical exam by a veterinarian familiar with rhinoceros health problems. This should include a review of all systems (if performed in a restraint device, exam may be limited by training and temperament of the individual and design of facility). Special attention for certain species include:
  - Black rhinos—Oral exam due to prevalence of dental problems/gingivitis; nasal exam due to epistaxis
- Body weight—Actual weight should be recorded whenever possible; body scores and/or digital photos can be used when scales are not available.
- Blood collection for complete blood count (CBC), serum chemistry panel, fibrinogen, serum protein electrophoresis and extra serum/plasma for banking (minimum of 10-20 ml). Blood smears should be carefully screened for the presence of hemoparasites, especially in recently captured or imported animals. The current Rhino SSP/TAG tissue/sample collection **protocol** should be consulted for additional samples that may be **requested** for research, disease screening, etc.
- Fecal samples should be collected semiannually or at least annually (depending on management system) for direct, flotation, and sedimentation to detect internal parasites.
- Annual enteric pathogen screening may be included, especially for animals in intensive management situations (e.g., breeding herd). Aerobic culture of feces for enteric pathogens should include special media for the detection of *Salmonella spp.* Because *Salmonella* organisms may be shed intermittently, at least three to five fecal cultures should be performed (may be done on consecutive days).
- Vaccinations—Annual vaccination for leptospirosis is recommended for black rhinoceroses. Vaccination for rabies, tetanus, and arboviruses (EEE/WEE/WNV) may be considered if the diseases are considered endemic in the area or increased risk factors are identified. There have been isolated cases of rabies and tetanus documented in rhinoceros (Jones, 1978; Mukherjee et al., 1984; Selvam et al., 2003). Check with the SSP veterinary advisor for the most current recommendations on species-specific vaccination protocols.

Additional preventive health recommendations have been included for consideration when performing examinations.

- Serological screening for leptospirosis (multiple serovars) and West Nile Virus (WNV). Although these tests are not species-specific and have not been validated for rhinoceros, they may detect cross-reactive antibodies in exposed animals. The presence of antibodies does not necessarily denote current infection or disease. Antibodies to leptospirosis have been detected in vaccinated rhinoceros and may be used to monitor response and possibly determine frequency of vaccination, although data is insufficient to determine protective titers. Insufficient data is available at this time to determine the significance of WNV antibodies in rhinoceroses; although it is important to note that a greater one-horned rhinoceros with clinical signs developed a WNV titer during a period of known exposure (P. Calle, pers. comm.). One study in greater Asian one-horned rhinoceroses did not find seroconversion in response to vaccination with a commercial equine WNV vaccine (Wolf et al., 2008).

- Serum/plasma vitamin E levels should be checked on a regular basis to assess adequacy of diet and supplementation protocols (see Nutrition chapter). Levels should be checked in any black rhinoceros with signs of illness. See the most current Rhino TAG/SSP preventive health protocol for recommended laboratories.
- Reproductive tract examination—When feasible, a complete reproductive examination should be conducted to include transrectal ultrasound, semen collection and analysis, and serum or fecal collection for hormone analysis (Radcliffe et al., 1997). Uterine leiomyomas, cystic ovaries, and irregular cycling have been observed in captive animals (Hermes et al., 2004). Since these conditions can have potentially significant effects on reproduction, a careful evaluation is warranted if the animal is being considered for breeding. Standing laparoscopy enabled visual examination and uterine biopsy of a leiomyoma in a southern white rhinoceros (Radcliffe et al., 2000b). A number of publications describe the technique for ultrasonography in rhinoceroses and normal reproductive biology. The reader is referred to the Rhino Resource Center for further information ([www.rhinosourcecenter.com](http://www.rhinosourcecenter.com)).
- Urinalysis should include both fluid and sediment evaluation of a clean voided sample. Microbial culture should be considered if there is evidence of blood cells or bacteria.
- Radiographs of feet are strongly recommended if any signs of pododermatitis or nail cracks are observed (Atkinson et al., 2002). Regular foot trimming and care may require immobilization in certain individuals (i.e., those that have a history of chronic foot problems). See section on pododermatitis.
- Diagnostic tests for tuberculosis—Periodic testing for tuberculosis in rhinoceroses should be considered, especially if there has been a history in the institution or herd. Intradermal testing can be performed using bovine PPD (0.1 ml ID) in the eyelid, behind the ear, caudal tailfold, or axillary region. Both false positive and false negative results have been found when performing intradermal tuberculin testing in rhinos (Godfrey et al., 1990). Ancillary tests such as nasal swabs, tracheal washes, and gastric lavages for mycobacterial culture have also been used. Serological tests [ElephantTB STAT-PAK®, MAPIA, DPP (dual pathway platform); Chembio Diagnostic Systems, Inc.] are being investigated for use in rhinos. See section on tuberculosis.
- Other vaccination regimens will depend on regional requirements and exposure risks (consider multivalent vaccination for Clostridial diseases). Contact the SSP veterinary advisor for the most up-to-date information.

Animals being moved between institutions should receive a preshipment examination and testing. This is similar to the routine health procedures but should also include screening for tuberculosis and other requirements dictated by the receiving institution (see quarantine section below).

## Neonatal Examinations

Ideally, neonates should be examined within 24 to 72 hours of birth to detect any congenital defects. Often the dam can be separated while the calf is manually restrained for a brief, but thorough exam, including body weight, and if feasible, blood collection. Complete blood count, biochemical panel, tests for passive transfer of immunoglobulins [e.g. glutaraldehyde coagulation, zinc sulfate turbidity, radial immunodiffusion plates (equine plates work well in rhinos and can be standardized by testing healthy rhinos), and serum protein electrophoresis], vitamin E level, and banking should be performed. A microchip may be placed behind the left ear for future identification. Particular attention during the exam should be paid to the umbilical stalk for signs

of infection, urine leakage, or hernia; passage of meconium, normal rectal/anal anatomy and tone; suckling reflex and neurological status. Regular weights and developmental progress should be documented. Lack of weight gain may be due to inadequate maternal milk production and care, or an indication of health or developmental problems in the calf. Black rhinoceros calves have been successfully hand-reared (covered under Nutrition chapter). In one case, a white rhinoceros calf was orphaned when its dam died, and the calf was taught to drink from a bucket at the age of six weeks. Weakness or other problems resulting in prolonged recumbency can cause decubital ulcers. Treatment of two neonatal white rhinos has been described using serial sedation with butorphanol alone or in combination with detomidine for restraint (Gandolf et al., 2006). Rectal prolapse has been reported in black rhinoceros calves, with surgical intervention required in at least two cases (Pearson et al., 1967; Abou-Madi et al., 1996). Leukoencephalomalacia has also been described in black rhinoceros calves (see disease section for more details)(Miller et al., 1987).

## Parasites

Internal parasites are more commonly found in free-ranging rhinos than in captivity. Stomach botfly larvae (*Gyrostigma pavesii*) have been reported in black rhinoceroses (Velleyan et al., 1983). Other parasites reported include nematodes (*Diceronema versterae*, *Parabronemia round*—associated with stomach nodules), *Draschia megastoma*, multiple species of *Kilulumma*, *Khalilia rhinocerotis* and pinworms (*Oxyuris karamoja*). Tapeworms (*Anoplocephala sp.*) can cause asymptomatic infestation in both captive and wild rhinos (Miller, 2003). One case of neosporosis led to acute fatal myocarditis in a neonatal white rhino calf and abortion in another case (Williams et al., 2002; Sangster et al., 2010).

Ticks are regularly found on free-ranging rhinoceroses, and imported animals should be carefully examined and treated. Multiple species have been identified on African rhinos (*Amblyomma hebraeum*, *Dermacentor rhinocerinus*, *Rhipicephalus maculates*, *R. muehlensi*, *R. simus*, *R. appendiculatus*, *R. zambeziensis*, *Haemaphysalis silacea*, *Hyalomma truncatum*)(Penzhorn et al., 2008). Filarid (*Stephanofilaria dinniki*) and screwworms (*Chrysomia bezziana*) can lead to skin lesions in wild black rhinos, particularly prevalent in the axial region (Round, 1964; Penzhorn et al., 1994).

Vector-borne parasitic infections occur in endemic areas. Trypanosomiasis can infect black rhinoceros and lead to anemia in tsetse fly-endemic areas. Evidence of tick-borne disease has been documented in black rhinoceros due to *Babesia bicornis sp. nov.* and *Theileria bicornis sp. nov.* (Nijhof et al., 2003). In addition, antibodies to heartwater (*Cowdria ruminantium*) have been found in both African species where the *Amblyomma* ticks are present (Kock et al., 1992).

Capture and translocation or importation may exacerbate potential parasitic infestations and increase the risk of introduction of novel pathogens to a new environment. Therefore, careful screening for external and internal parasites, prophylactic treatment of wounds with fly repellent, pour-on tick treatments (coumaphos, flumethrin), and judicious use of antiparasitic medications (pyrantel pamoate, fenbendazole, ivermectin, praziquantel) should be considered. Check with the SSP veterinary advisor for current recommendations.

## Quarantine

Due to the size, strength, and temperament of a rhinoceros, it may be logistically difficult to maintain isolation from other animals during arrival and quarantine. The Rhinoceros TAG/SSP Recommended Preshipment Protocol for Rhinoceros lists a comprehensive battery of tests for health assessment prior to shipment. Since most zoological institutions will not have the facilities

available to safely house and manage a newly arriving rhinoceros, it is important that the receiving institution work closely with the sending institution to ensure that all (or as many as possible) of the listed tests are conducted and results reviewed before shipment. Following the preshipment protocol may help compensate for some of the quarantine compromises that may be required. Regardless of preshipment test results, every attempt should be made to maintain some degree of physical separation of the incoming animal from the resident rhinos after arrival.

Current quarantine practices recommend a minimum 30 to 90 day quarantine period for most mammalian species in zoos and aquaria. Social concerns, physical facility design, and availability of trained rhino staff may dictate a modified quarantine protocol. Specific quarantine guidelines and protocols at each institution should be reviewed jointly and decisions made by the veterinary and animal management staff. Recommended **procedures** to consider as part of a comprehensive plan for rhinoceros quarantine include:

- Thorough physical examination including a review of all organ systems.
- Blood collection for CBC (including blood smear examination for hemic parasites), serum chemistry panel, fibrinogen, serum protein electrophoresis, and serum bank.
- Fecal collection for parasite screening (direct, flotation, sedimentation) conducted weekly for the first three weeks.
- Fecal cultures for *Salmonella spp.* conducted at least weekly for the first three weeks.
- Any procedures that were not completed prior to transport or that may be due, such as vaccination, serologic screening, or TB testing.

It should be emphasized that the quarantine test requirements should be strongly considered regardless of the results of preshipment testing. The stress of transport and quarantine may result in health changes (for example, *Salmonella* shedding) that were not detected during testing at the sending institution.

## Hospitalization and Critical Care

In most cases, hospitalization is impractical with adult rhinoceroses. Most animals will be treated in their holding areas. Rhino calves may be hospitalized in adequate large animal holding facilities if the severity of their medical condition warrants. Barns or holding areas that incorporate species-appropriate restraint devices facilitate medical treatment. Some of the following medical problems require active intervention, including sedation, immobilization, injectable drug therapy, and/or fluid therapy. Although it has been achieved, fluid therapy in rhinoceros presents logistical challenges. In addition to the intravenous route, it is possible to improve hydration using rectal enemas with warm physiologic solutions or even tap water. Animal and staff safety should be a priority in any planned intervention.

## Diseases

### *Tuberculosis*

*Mycobacterium bovis* and *M. tuberculosis* have caused infections in captive rhinoceroses (Stetter et al., 1995; Miller, 2008). Although not currently reported in free-ranging rhinoceroses, there was a recent report of *M. bovis* infection in a black rhinoceros brought into captivity in South Africa (Espie et al., 2009). Initial infection may be asymptomatic or result in progressive weight loss and emaciation, with coughing and dyspnea occurring in the terminal stages. Nasal discharge may be present but is not a consistent sign. Most infections are pulmonary. Antemortem testing includes intradermal tuberculin test, tracheal and/or gastric lavage for mycobacterial culture, and serological tests (see preventive health section for details). Retrospective analyses of serum from

*M. tuberculosis*-infected black rhinos showed positive results using the ElephantTB Stat-Pak® (Duncan et al., 2009). Treatment has been attempted using isoniazid, rifampin, ethambutol, and pyrazidamide (Barbiers, 1994; Duncan et al., 2009). However, assessment of successful response is limited. Concerns for other collection animals, staff and public health need to be considered prior to initiation of therapy. Since this is a reportable disease, notification of the appropriate state veterinary officials should occur promptly once a diagnosis is made. Consultation with the SSP veterinary advisor is also recommended.

### ***Salmonellosis***

Salmonella infection can cause enteritis and fatal septicemia in captive and newly captured wild rhinoceroses (Windsor and Ashford, 1972; Kenny et al., 1997). In a retrospective survey of captive black, white, and greater Asian one-horned rhinos in the U.S., 11% reported positive cultures, usually associated with clinical signs (Kenney, 1999). Research has shown that asymptomatic black rhinoceroses can carry and intermittently shed Salmonella in their feces (Miller et al., 2008). Clinical infection may result secondary to transport, changes in diet, immobilization, concurrent disease, or exposure to a large number of organisms. Lethargy, anorexia, signs of colic, diarrhea, and death may be observed. Successful treatment using trimethoprim-sulfamethoxazole and supportive care is possible if initiated early. However, treatment of asymptomatic animals is NOT recommended.

### ***Leptospirosis***

Leptospirosis usually presents with depression and anorexia. Other signs may include hemolytic anemia (not present in all cases), hemoglobinuria, signs of colic, and development of skin ulcers. Fatality rates are high in clinically affected black rhinos although successful treatment with trimethoprim-sulfamethoxazole and ceftiofur has been reported (Neiffer et al., 2001). Diagnosis is based on high antibody titers (microagglutination test – MAT) and confirmed by detection of leptospiral organisms in urine or tissues (fluorescent antibody test). Low levels of antibodies have been observed in free-ranging and non-vaccinated black rhinos without evidence of disease (Jessup et al., 1992). Preventive measures include annual vaccination of black rhinos with a multivalent large animal product, rodent and wildlife control programs, and good husbandry to minimize contamination of feed and water. Abscesses and systemic anaphylactic reactions, including hives and skin sloughing, have occurred following leptospirosis vaccination in the black rhinoceros. Those individuals with a history of systemic adverse reactions should forego regular vaccination.

### ***Gastrointestinal Infections***

Colitis of unknown etiology and secondary endotoxic shock led to the death of an adult black rhinoceros. Clostridial enterocolitis has been observed in black rhinoceros with signs of diarrhea, lethargy, and colic. Fatal cases of enterotoxemia due to Clostridial enteritis have occurred in black rhinoceros (Ndeereh et al., 2012). See salmonellosis and GI torsion, impaction, and ulcers for other GI conditions.

### ***Encephalomyocarditis Infection (EMC)***

EMC viral infection usually results in acute death due to myocarditis (Gaskin et al., 1980). The southeastern/Gulf Coast states in the U.S. are considered an endemic area. Diagnosis is usually based on virus isolation at necropsy from heart, spleen, or other tissues (<http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/53600.htm>). Prevention should target rodent control, although experimental vaccines have been proposed. A commercial vaccine is not currently available.



## ***Fungal Pneumonia***

Fungal pneumonia is usually due to *Aspergillus spp.* and primarily observed in black rhinoceroses secondary to immunocompromise from concurrent disease, broad spectrum antibiotic therapy, or corticosteroid use (Weber and Miller, 1996). Clinical signs may include weakness, weight loss, epistaxis, or other signs consistent with pneumonia. Diagnosis is challenging, although serology and bronchoscopy, with cytology and fungal culture, may be useful. Fungal pneumonia in the black rhinoceros is often associated with elevations in serum globulin, above levels generally observed in other chronic diseases (S. Citino, pers. comm.). Long-term treatment with antifungal drugs (e.g., itraconazole) is expensive and has unknown efficacy. Definitive diagnosis is often made at necropsy.

## ***Anthrax***

Death due to anthrax has been observed in wild rhinoceroses. Most cases result in sudden death. Foamy discharge from the mouth and nostrils can be seen and may appear similar to EMC infection. Diagnosis is based on identification of anthrax bacilli in blood or tissue smears. Vaccination of ranches rhinos has been used in some endemic areas of Africa. Sporadic outbreaks in domestic livestock occur in the U.S., although there have been no reports of rhino mortality in these areas (e.g., Texas).

## ***Skin Conditions and Dermatopathy Syndromes***

Black rhinoceros have a disproportionate number of skin conditions among the captive rhino species. Skin disease may be the most common health problem of black rhinos with over 50% having at least one episode during their lifetime (Munson and Miller, 1999). There are several distinct syndromes reported in captivity.

- Superficial necrolytic dermatopathy (SND)—This has also been called ulcerative skin disease, vesicular and ulcerative dermatopathy, and mucosal/cutaneous ulcerative syndrome (Munson and Miller, 1999; Dennis et al., 2007). More than 40 black rhinos have been affected. Initial signs are epidermal plaques or vesicles that progress to ulcers, often over pressure points, ear margins, coronary bands and tail tip. Oral or nasal ulcers may develop concurrently. Clinical signs are intermittent and, during episodes, rhinos may also be anorexic, depressed, lame, have oral or nasal bleeding, and lose weight. Affected animals may have decreased albumin and hematocrits. In most cases, the skin lesions are associated with other concurrent health issues, including GI and respiratory disease. Research suggests that the underlying cause may be an imbalance of dietary essential micronutrients with related metabolic changes. Management includes symptomatic treatment such as topical or systemic antibiotics, topical ointments, and hydrotherapy. If lesions become extensive, it can be fatal. Treatments with cryotherapy and steroids have been successful, but rhinos treated with systemic steroids can develop fatal fungal infections. Occasionally, lesions may resolve spontaneously.
- Eosinophilic granuloma syndrome—This syndrome usually presents with oral and nasal non-healing ulcers and granulomas (Pessier and Munson, 2004). Cytology shows a predominance of eosinophils associated with lysis of collagen and mineralization. Development of significant ulceration may lead to epistaxis (nose bleeds) or oral bleeding. A similar syndrome has been described in domestic cats. Although lesions may resolve spontaneously, usually over one to seven months, they may recur. Treatment has included corticosteroid therapy, which can result in fungal pneumonia, or local cryotherapy. Laser therapy has been used to treat ulcers but may exacerbate hemorrhage. Supportive care

is recommended in severe cases. Eosinophilic granulomas in wild rhinos are typically associated with *Stephanofilaria dinniki*.

- Neoplasia—Squamous cell carcinoma (SCC) has been treated by surgical excision in a captive white rhino (Goodman et al., 2007). A greater Asian one-horned rhinoceros with SCC of the horn has also been successfully treated with surgery and radiation (Greer et al., 2010). Cutaneous melanomas have been reported in both black and greater Asian one-horned rhinoceroses (Wack et al., 2010).
- Fungal—*Malassezia pachydermatis* and *Candida parapsilosis* were identified in black rhino and successfully treated using natamycin solution.
- Environmental—Dermatitis may be due to inadequate access to wallows.
- Other—There has been a rare single case of primary vitiligo reported in a black rhino that began around the nares at two years of age and progressed to include multiple areas without evidence of the other syndromes (Takle et al., 2010).

These cases illustrate the importance of biopsy and culture for identifying the etiology of and directing treatment in dermatologic cases in rhinos.

### ***Pododermatitis and Chronic Foot Problems***

All species of rhinoceros are susceptible to developing pododermatitis due to inappropriate substrate or other husbandry conditions in captivity. Abrasive substrate, long-term indoor housing in northern climates, and limited access to a wallow contribute to chronic foot trauma in captive rhinos (von Houwald, 2005). Management of the condition includes improvements in husbandry and medical/surgical interventions. Medical treatment may be in the form of oral antimicrobial medication and topical use of copper sulfate and oxytetracycline. Regular hoof trimming and surgical debridement of necrotic lesions, along with use of collagen products for granulation tissue stimulation, can lead to improvement in appearance and comfort of the animal. In addition to the factors mentioned above, nutritional imbalances (e.g., zinc) are also being investigated.

Black rhinos have also been reported to develop laminitis that may or may not be related to idiopathic hemorrhagic vasculopathy syndrome (Fig. 3.13; Nance, 1998). Frequent foot trimmings, analgesics, and antibiotics are needed to manage this condition, similar to laminitis in domestic horses.



Fig. 3.13. Evidence of laminitis in rhinoceros.

### ***Corneal Ulcers and Keratitis***

Corneal trauma and secondary infection can result in corneal ulceration and perforation. Surgical management of a melting corneal ulcer using a conjunctival graft has been described in a greater Asian one-horned rhino (Gandolf et al., 2000).

### ***Horn Problems***

Horn avulsion, cracks, or other trauma can occur as a result of acute or chronic rubbing, pressure, self-induced, or conspecific fighting. This may lead to myiasis, abscessation, osteomyelitis, or pain with behavioral changes. Radiographs, thermography, or fluoroscopy can be used to assess the extent of the damage. Treatment may involve debridement, antibiotics, wound treatment, and fly control (Suedmeyer, 2007). A squamous cell carcinoma of the horn in a greater Asian one-horned rhinoceros was diagnosed and managed with partial amputation and radiation therapy (Greer et al., 2010). The horn is attached to the basal dermal layer not unlike the laminae of the horse's hoof wall and therefore perturbations in blood flow can presumably lead to laminitis.

### ***Gastrointestinal Torsion, Impaction, and Ulcers and Other Conditions***

Torsion may result from abdominal trauma or severe GI disease. Severe torsion may lead to acute death; other signs are related to abdominal pain. Dietary changes, dehydration, ingestion of foreign material (i.e., sand), and inadequate fiber content may result in GI impaction. Gastric ulcers have been observed on gastroscopy and at necropsy in rhinos that have received long-term non-steroidal therapy or have concurrent disease. A 32-year old black rhinoceros was euthanized due to rapid onset of wasting secondary to perforation of a pyloric ulcer and peritonitis. Treatment depends on identifying the cause and site of the gastrointestinal condition. Surgical correction of torsion is usually recommended, although most cases are not diagnosed prior to necropsy. Impaction can be treated using rectal enemas, oral administration of psyllium, mineral oil, or other products to increase GI water content. Equine anti-ulcer medications such as omeprazole, sucralfate, and H<sub>2</sub>-blockers have been used in rhinos by scaling the equine dose. Prevention includes adequate dietary fiber and fluid intake, and avoiding abdominal trauma and ingestion of foreign material. Esophageal dilation was observed in a black rhinoceros following ingestion of a foreign body; successful management involved diagnosis by endoscopy and a change in feeding practices to a low fiber diet (Radcliffe et al., 1998). Inflammatory bowel disease was confirmed at necropsy in a greater Asian one-horned rhinoceros that presented with weight loss and progressive diarrhea (Ferrell et al., 2010).

### ***Gingivitis and Dental Tartar***

All rhino species appear to accumulate dental tartar in captivity, especially if they are not given access to hard or course food items. However, black rhinoceros appear to develop severe proliferative gingivitis, not always directly associated with the degree of accumulation of calculus (Beagley et al., 2010). Lesions are vascular and periodically hemorrhage, causing blood-tinged saliva. The gross appearance is similar to raw ground meat. The cause of this condition is unknown but may be related to lack of browse in the diet. Similar to horses, rhinoceros also develop dental points that may eventually create clinical mastication problems with age and require periodic dental flotation.

### ***Renal Disease***

Since 2001, chronic glomerulonephritis and/or renal failure have been recognized as a contributing cause of death in at least seven black rhinos. Mineralization of other tissues has been

associated with chronic renal changes as well as three cases of accidental vitamin D toxicosis (Murnane et al., 1994; Fleming and Citino, 2003). Weight loss, decreased appetite, dermatitis, and signs of gastroenteritis may be present, including gastric ulcers. Significant changes in blood urea nitrogen, creatinine or urinalysis were not always evident in these cases, making diagnosis difficult until necropsy. However, two white rhinos (aged >40 yr) both developed progressive chronic renal failure characterized by uremia, isosthenuria and hypercalcemia. Nutritional management using a high energy, low protein feed (Equine Senior, Purina Mills, St. Louis, MO, USA) was instituted in both cases and appeared to stabilize the renal disease (S. Ferrell and R. Radcliffe, pers. comm.). Further investigation into causes of renal disease (other than toxicosis-related) is needed.

### ***Hemolytic Anemia***

Cases of hemolytic anemia in the captive black rhinoceros population appear to be decreasing since peak occurrence during the 1990's. A high mortality rate (75%) was observed in the 39 animals that have been affected, with 47 known occurrences (Miller, 1993; Dennis et al., 2007). Despite intensive research, the definitive cause has not been determined. Possible etiologies include a hereditary deficiency of glucose-6-phosphate dehydrogenase leading to decreased red blood cell ATP levels, hypophosphatemia, and hypovitaminosis E. Leptospirosis is a known cause of hemolytic anemia in this species but was not associated with all cases. Management of this syndrome includes supplementation of phosphorus (intravenous or oral), vitamin E, prophylactic antibiotics for secondary infections, and whole blood transfusion in severe cases.

### ***Acute Lymphoblastic Leukemia***

The only case of leukemia ever described in a rhinoceros was reported in a two-year old southern black rhino (Radcliffe et al., 2000c). The disease was characterized by a marked elevation in white blood cell numbers with immature lymphoblastic forms predominating in blood smears. Treatment was attempted following established equine acute lymphoblastic leukemia chemotherapy protocols and included a combination drug program. Although the disease was moved to remission by treatment, the rhinoceros died from complications of doxorubicin therapy that may have been exacerbated by hemochromatosis (Paglia and Radcliffe, 2000).

### ***Idiopathic Hemorrhagic Vasculopathy Syndrome (IHVS)***

This disorder was first recognized in 1995 in North American black rhinoceroses with characteristic signs of severe limb, facial, and neck swelling associated with a nonhemolytic anemia (Murray et al., 2000). The syndrome presents acutely without any known cause, and recurrent episodes are likely. The case fatality rate is high, but a number of animals have recovered with antibiotic and non-steroidal therapy. As of 2007, there have been at least 13 documented cases, the majority occurring during cooler months of the year (October-March), with most of the black rhinos having lived in Texas or the southern U.S. It has been proposed that the syndrome is an immune-mediated vasculitis, similar to equine purpura hemorrhagica. Additional signs include lethargy, respiratory stridor, laminitis and nail sloughing, aural hematomas (swelling of the ears), and oral or skin ulcers. A possible association with *Streptococcus* spp. infection has been observed. Treatment is usually supportive, including antibiotics, non-steroidal anti-inflammatories, fatty acid and phosphorus supplementation, and topical therapy of ulcers or foot lesions. Steroids have also been proposed, but the risks of fungal pneumonia as a sequelae make it a controversial treatment. See the current Black Rhinoceros SSP Tissue/Sample Research protocol for sample collection recommendations for these cases.

### ***Hemosiderosis/Hemochromatosis (Iron Overload Syndrome)***

Serum iron and ferritin levels in captive black rhinoceros are significantly higher than those measured in wild or recently captured animals (Miller et al., 2012). Levels appear to increase with time in captivity. In contrast, values for ferritin and tissue iron do not appear to be elevated in captive white or greater Asian one-horned rhinos (Smith et al., 1995; Paglia et al., 2001). Although hemolytic anemia, vitamin E deficiency, and hereditary disorders have all been proposed as potential causes, it is now believed to be related to dietary factors in the captive browser species. Hemosiderosis (tissue iron accumulation) is a common finding in multiple organs in black rhinoceros, although inflammation and lesions associated with these changes may also be observed (hemochromatosis). It has been suggested that high iron load may play a role in some of the black rhino disease syndromes, although evidence is limited. A fatality in a captive Sumatran rhino was associated with multi-organ hemochromatosis. Recommendations to minimize accumulation and reduce iron load include low iron diets, provision of browse, therapeutic phlebotomy (regular large-volume blood collection) and treatment with iron chelating agents in those individuals with suspected clinical disease. Recent success with large-volume phlebotomy and low iron diets have shown promising results in lowering elevated ferritin levels in captive black rhinos (Mylniczzenko et al., 2012). Captive browsing rhinoceroses may be best managed in native habitat that can offer appropriate nutrition; for instance, Sumatran rhinos appear to have fewer problems with iron overload syndrome when managed in captive situations in range countries (D. Candra, pers. comm.).

### ***Leukoencephalomalacia***

Severe neurological disease due to necrosis of the cerebrum has been diagnosed in four female black rhino calves, with three cases becoming comatose within one to seven days of signs, and eventually proving fatal (Miller et al., 1987). The fourth case developed its first neurologic episode at three weeks of age and subsequently experienced 14 separate episodes before being euthanized at 16 months. Clinical signs include depression, hyperexcitability, or aggression before progressing to severe neurologic disease. No obvious changes were noted in hematologic, biochemical, or tests for viral, bacterial, and vitamin E levels. Some investigators hypothesize that the cause may be related to dam age (mean 17.3 yr), or excessive maternal iron (Paglia et al., 2001; Dennis et al., 2007). Other research has focused on possible congenital or hereditary causes of encephalomalacia. There is no known curative treatment.

### ***Creosote Toxicosis***

Seven fatalities occurred among a group of 20 black rhinoceroses captured and housed in bomas constructed with creosote-treated wood in Zimbabwe (Kock et al., 1994). Although five cases died after transport to the U.S. and Australia, they exhibited the same clinical signs and lesions as the initial cases. Animals were lethargic, partially anorectic, developed swollen limbs, and passed brown urine. Abnormal blood results included anemia, and elevated liver enzymes and bilirubin. Post-mortem findings included widespread hemorrhages; swollen, pigmented liver; and ulcers of the upper gastrointestinal tract. The presumptive cause of death was liver dysfunction due to creosote toxicosis. Exposure to creosote-treated housing materials should be avoided for all rhino species.

### **Common Injuries and Treatment: Skin Lacerations, Punctures, Wounds**

Trauma due to conspecific fighting, mating injuries, collision with obstacles in enclosures or in crates, interspecific conflict or, in free-ranging situations, poaching is relatively common. Common sense application of wound treatment principles apply, although the thick skin does not



lend itself to primary closure and has a tendency to undermine abscesses along the fascial planes. Therefore, wounds are usually treated by second intention using debridement, hydrotherapy, topical and/or systemic antibiotics, analgesics, and the array of wound healing products that accelerate granulation. A recent article describes the use of vacuum-assisted healing of a surgical wound in a black rhinoceros to improve healing time (Harrison et al., 2011).

## **Necropsy Protocol**

Any rhinoceros that dies or is euthanized should have a complete necropsy performed and tissues submitted for histopathology. The Rhinoceros TAG/SSP Veterinary/Nutrition Blood and Tissue Collection **Protocol** for Rhinoceros should be consulted for current information on the species pathology advisors as well as specific sample **requests** for research and banking purposes.

## **Capture, Restraint, and Immobilization Techniques**

Management of captive rhinoceroses often requires procedures that involve handling of these large and powerful animals. Husbandry training techniques have advanced the application of preventive medical, diagnostic, and minor therapeutic procedures. However, intensive medical and surgical interventions may require physical and/or chemical restraint for safety and comfort of the patient and staff. Since new developments are continuously advanced in this field, the following recommendations should be used as guidelines, and the veterinary advisors and literature should be consulted for specific situations.

### ***Physical Restraint***

Advances have been made in facilities that include a variety of stall and chute designs for physical restraint of rhinoceros. These will be addressed in the Training and Enclosure Design chapters.

### ***Chemical Restraint***

Significant changes in options for chemical restraint of captive rhinoceroses have occurred during the last two decades. The degree of sedation, analgesia, or immobilization required will vary with the procedure to be performed. Health status and temperament of the individual animal, as well as facility design and staff experience may dictate preference for one technique over another.

## **Chemical Restraint and Anesthesia of Rhinoceros**

### ***Introduction***

Anesthesia of rhinoceroses requires adequate preparation, equipment, and experience. It is highly recommended that rhinoceros anesthesia should be attempted only with experienced personnel or after consulting knowledgeable practitioners. Contingency plans for emergencies are a key component of preparations. Clearly defined roles for all personnel should be outlined and reviewed. Adequate equipment for moving a large animal should also be available.

The choice of chemical restraint techniques will be determined by a number of factors, including physical facilities where immobilization will be performed, health status of the individual, procedures to be achieved and level of analgesia required, length of intended immobilization, and experience of the veterinary and animal care staff. Only captive rhinoceros anesthesia techniques will be covered in this section. For more information on immobilization of free-ranging rhinoceros, the reader is referred to specific references on this subject ([www.rhinosourcecenter.com](http://www.rhinosourcecenter.com)) (Miller, 2003; Bush et al., 2004; Portas, 2004; Kock et al., 2006; Radcliffe and Morkel, 2007; Wenger et al., 2007).

## ***Equipment***

With training and availability of physical restraint devices (“chutes”), drug delivery for chemical restraint can be more easily and accurately achieved, create less stress for the individual, and provide greater control of the induction. Standing restraint is also feasible in these situations.

For rhinoceros in stalls, pens or exhibits, drug delivery often requires use of darting equipment. Depending on the situation, pole syringes with robust needles may be used on selected occasions. Hand-injection is also easily achieved in conditioned animals, although care must be taken when using potent opioids to ensure human safety. Most darting systems can be used in captive situations, as long as a robust needle (minimum 40-60 mm x 2 mm needle) is used to penetrate the thick skin and deliver the drug into the muscle. Nylon darts (Teleinject<sup>®</sup>, Telinject USA, Inc., Agua Dulce, CA, USA; Dan-Inject<sup>®</sup>, Danwild LLC, Austin, TX, USA) are preferred in these situations since they are less traumatic than metal darts. Ideal sites for drug injection are just caudal to the ear on the lateral cervical area, upper caudal hindlimb, or shoulder. However, any site can be used if the dart is placed perpendicular to the skin and is adequate to penetrate muscle.

Additional equipment that should be available include a blindfold, ear plugs (cotton cast padding, stuffed socks), heavy ropes, and padding for recumbency. Inflated truck inner tubes can be used to reduce pressure points. Otherwise, heavy mats or padding should be used if the procedure will take place on a concrete floor. Immobilizations carried out in sandy or grassy areas may provide enough padding for shorter procedures.

Contingency plans for moving a rhino that becomes recumbent in a difficult position should also be made. Lining stalls with plywood sheeting to prevent the head/horn from getting stuck should be considered in areas with open bollards or poles.

Additional equipment should include the availability of oxygen and other emergency equipment. If a field procedure is being planned, an axe or chain saw and bolt cutter may be needed if there are trees/vegetation/other obstacles in the area where the rhino can get caught.

## ***Preparations***

Depending on the health status, environment, and procedure planned, removal of food and water prior to the procedure should be considered at least overnight. Consult the veterinarian for specific instructions.

## ***Positioning***

Rhinoceros are prone to developing myopathy and neuropathy after recumbency. The optimal position remains controversial and may be dictated by the planned procedure. Lateral positioning is often preferred since it provides the optimal circulation to the limbs, although sternal recumbency may provide better ventilation (Morkel et al., 2010). During the procedure, limbs should be “pumped” about every 20 minutes to encourage circulation (Radcliffe and Morkel, 2007). If the animal needs to be in sternal recumbency for the procedure, it is ideal to roll the animal into lateral recumbency whenever possible and pump the legs.

## ***Anesthetic Monitoring***

With the advent of new tools for monitoring physiological parameters in veterinary patients, it is imperative that monitoring be performed on immobilized rhinoceros to minimize complications. Ideally, an accurate weight or weight estimate will facilitate optimal drug calculation and prevent drug over- or under-dosing and associated complications (Adams et al., 2005). Thorough physical examination should be performed, along with regular recording of vital signs (respiratory rate

and depth, heart rate, temperature, mucous membrane color and capillary refill time). Ideally, this should be assigned to dedicated personnel and measured at five-minute intervals throughout the procedure.

Pulse oximetry is a useful tool for monitoring trends in hemoglobin oxygen saturation but can be prone to false readings due to placement and the thick skin of the patient. Therefore, it should not be a substitute for basic clinical assessment. Sites for placement of the sensor include the ear pinnae (scraping can sometimes improve readings) and mucosal folds of the penis, vulva, or rectum. Side-by-side placement of sensor pads has been used in the conjunctival sac, gingival and nasal mucosa, and inside the rectum, vagina, or prepuce. Ideally, readings should be greater than 90%, but interpretation should be made in conjunction with the color of the mucous membranes, blood, and other clinical signs. Capnography may also enhance patient monitoring by enabling early detection of adverse respiratory events, including hypoxia. While pulse oximetry gives trends in patient oxygenation, capnography provides information about CO<sub>2</sub> production, pulmonary perfusion, alveolar ventilation, respiratory patterns, and elimination of CO<sub>2</sub> from the anesthesia circuit and ventilator.

### **Standing Sedation**

Standing sedation should only be attempted under conditions that take into consideration animal and staff safety. The type of procedure, as well as the temperament of the animal, restraint device, and husbandry training of the individual, will determine the level of sedation and analgesia required. Excitement and environmental or painful stimuli can override drug effects. As a general rule, individuals that are acclimated to people and husbandry training and restraint practices tend to require lower doses of immobilizing agent. Opioids tend to have more potent effects than the tranquilizer/sedative classes of drugs. A combination of butorphanol and azaperone has proven effective for repeat procedures, inducing standing sedation and recumbency in the white rhinoceros (Radcliffe et al., 2000a). See Table 3.16 for suggested doses for standing sedation of captive rhinoceros.

Table 3.16. Standing chemical restraint doses for captive black rhinoceros.

<b>Chemical Restraint Drug(s)</b>	<b>Reversal Agent(s)</b>
<b>Adults (800-1200 kg)</b>	
0.1-0.25 mg etorphine IV	naltrexone at 40-50 mg/mg etorphine
0.2-0.6 mg etorphine IM female	naltrexone at 40-50 mg/mg etorphine
0.3-0.8 mg etorphine IM male	naltrexone at 40-50 mg/mg etorphine
0.8-1.5 mg etorphine IM by dart	naltrexone at 40-50 mg/mg etorphine
1.25-1.5 mg etorphine + 5-6 mg acepromazine IM	naltrexone at 40-50 mg/mg etorphine
0.25-0.4 mg etorphine + 10 mg detomidine IM	naltrexone 40-50 mg/mg etorphine; atipamezole 5 mg/mg detomidine
20-30 mg butorphanol + 20-50 mg detomidine IM	naltrexone 1 mg/mg butorphanol; atipamezole 5 mg/mg detomidine
0.15 mg/kg butorphanol + 0.03-0.055 mg/kg detomidine IM	naltrexone/atipamezole as above
70 mg butorphanol + 100 mg azaperone IM	naltrexone 1 mg/mg butorphanol

Table 3.16. Continued.

Chemical Restraint Drug(s)	Reversal Agent(s)
0.5-0.85 mg etorphine IM (as low as 0.25 mg used to walk rhino into crate)	naltrexone 40-50 mg/mg etorphine
25-50 mg butorphanol IM/IV	naltrexone 1 mg/mg butorphanol
<b>Subadults</b>	
30-50 mg butorphanol + 30-40 azaperone IM	naltrexone 1 mg/mg butorphanol
0.5-1.25 mg etorphine + 2-5 mg acepromazine IM	naltrexone 40-50 mg/mg etorphine
<b>Juvenile (≤2 year old)</b>	
30 mg butorphanol IM	naltrexone 1 mg/mg butorphanol
40 mg butorphanol + 40 mg azaperone IM	naltrexone 1 mg/mg butorphanol

### ***Immobilization/General Anesthesia***

The primary class of drugs for general anesthesia in rhinoceros is the opioids. Etorphine is most commonly used, although carfentanil and more recently, combinations of etorphine and thiafentanil have also been administered to rhinos. Opioids are typically combined with azaperone, an alpha-2 agonist (e.g., xylazine, detomidine), or acepromazine to provide muscle relaxation and to counteract the hypertensive effect of the opioids (LeBlanc et al., 1987). Midazolam, diazepam, or guaifenesin infusion can also provide additional muscle relaxation. Butorphanol has been administered to antagonize respiratory depressive effects in white rhino (10-20:1 butorphanol:etorphine in mgs); however, it may also lighten the plane of anesthesia (Miller et al., 2013). It should be used with caution in black rhinos since they appear to be more sensitive and can suddenly get to their feet (Portas, 2004). Other partial opioid agonist-antagonists are routinely used in the field and can be adapted for captive rhinos when available (e.g., nalbuphine). Butorphanol-azaperone and butorphanol-medetomidine/detomidine combinations have successfully induced recumbency in captive Sumatran, Indian, and white rhinos (Radcliffe et al., 2002; Portas, 2004; Waltzer et al., 2010). Supplemental ketamine, opioids, guaifenesin, propofol, or isoflurane can be used to deepen the level of anesthesia and lengthen immobilization as required (Ball et al., 2001). See Table 3.17 for recommended doses used for recumbent immobilization of rhinoceros.

Oxygen supplementation by intratracheal intubation or nasal insufflation (flow rates of 15-30 liters/minute) can increase oxygen saturation values (Morkel et al., 2010). Doxapram administration for apnea has also been used in rhinos but may only provide short-term relief. Partial or complete reversal should be considered in severe cases of hypoxia.

### ***Use of Tranquilizers/Sedatives for Transport and Other Uses***

There may be occasions other than medical procedures when rhinoceros need to be sedated for short or more extended periods of time, such as during crating and transport, or confinement for other reasons. With the advent of husbandry training, the need for drugs in captive rhinos has become more limited but should always be available as an option. Tranquilizer/sedative drugs may be used in rhinoceroses to relieve anxiety, reduce hostility, decrease motor activity, alleviate excitement, and to facilitate animal introductions. Drug choice is based on the desired duration of action and expected outcome. For short duration tranquilization/sedation, benzodiazepines (2-6 hr; adult doses: midazolam 25-50 mg IM; diazepam 20-30 mg IM) and azaperone (2-4 hr; 80-200 mg IM) are useful choices in black rhinoceros. Long-acting neuroleptics (LANs) are typically

administered in free-ranging rhino after capture for transport and boma acclimation, although they have also been used in captive rhino for longer duration tranquilization (see Table 3.18 for recommended doses in black rhinoceros).

Table 3.17. Recumbent immobilizing doses for captive black rhinoceros.

Chemical Restraint Drug(s) Adults (800-1200 kg)	Reversal Agent(s)
2.5-3 mg etorphine + 60 mg azaperone IM dart	naltrexone 40-50 mg/mg etorphine
1.5-2 mg etorphine + 100 mg azaperone IM hand-inj	naltrexone 40-50 mg/mg etorphine
2-3 mg etorphine + 20-40 mg azaperone IM	naltrexone 40-50 mg/mg etorphine
1 mg etorphine + 60 mg azaperone IM hand-inj	naltrexone 40-50 mg/mg etorphine
1 mg etorphine + 10 mg detomidine IM (female)	naltrexone 40-50 mg/mg etorphine; atipam 5 mg/mg detomidine
2-3.5 mg etorphine +10-15 mg detomidine (or 75 mg xylazine) IM	naltrexone 40-50 mg/mg etorphine; atipam 5 mg/mg detomidine
3.5-3.8 mg etorphine +14 mg detomidine + 400 mg ketamine IM	naltrexone 40-50 mg/mg etorphine; atipam 5 mg/mg detomidine
1.5-2 mg etorphine + 2-3 mg medetomidine IM	naltrexone 40-50 mg/mg etorphine; atipam 5 mg/mg medetom
1.5-2 mg etorphine + 2-3 mg medetomidine + 1 g/L ketamine in 5% guaifenesin drip	naltrexone 40-50 mg/mg etorphine; atipam 5 mg/mg medetom
0.9-1.2 mg carfentanil IM	naltrexone 100 mg/mg carfentanil
0.7-1 mg carfentanil + 50-60 mg xylazine IM	naltrexone 100 mg/mg carfentanil; yohimbine 0.125 mg/kg IV
1 mg carfentanil + 10 mg midazolam IM	naltrexone 100 mg/mg carfentanil
0.7-1.2 mg carfentanil + 10 mg midazolam	naltrexone 100 mg/mg carfentanil
1-1.5 mg carfentanil + 0.3 mg/kg butorphanol + 0.06 mg/kg detomidine IM	naltrexone 100 mg/mg carfentanil; atipam 5 mg/mg detomidine
70-120 mg butorphanol + 100-160 mg azaperone IM (hand inj)	naltrexone 2.5 mg/mg butorphanol ½ IV, ½ IM
30-50 mg butorphanol + 50-60 mg azaperone IM hand-inj	naltrexone 2.5 mg/mg butorphanol ½ IV, ½ IM
120-150 butrophanol +5-7 mg medetomidine IM dart	naltrexone 1 mg/mg butorphanol; atipam 5 mg/mg medetom



Table 3.18. Suggested doses for tranquilizers and sedatives in black rhinoceros.

	Onset of action (IM)	Duration of effect	Antagonist
Haloperidol	20 min	3-8 hours	Congentin
decanoate form	6 days	3 weeks	Congentin
Azaperone	10-15 min	2-4 hours	None
Diazepam	immediate (IV)	2-21 hours	Flumazinil
Midazolam	5-10 min	2-6 hours	Flumazinil
Xylazine	10-15 min	2-4 hours	Yohimbine
Detomidine/Medetomidine	10-15 min	1-2 hours	Atipamezole
Zuclopenthixol acetate	1 hour	72 hours	None
(Clopixol-Acuphase)	*Not available in the US		
Perphenazine enathate	12-18 hours	7-10 days	None
(Trilafon-LA)	*Not available in the US		
<b>Doses</b>			
Haloperidol	0.05-0.1 mg/kg PO/IM		
Azaperone	0.05-0.25 mg/kg IM; 40-100 mg IM; adults 100-200 mg IM		
Diazepam	0.1-0.2 mg/kg PO/IM/IV; 5-10 mg IV		
Midazolam	0.10.2 mg/kg IM/IV; 5-10 mg IV/IM		
Detomidine/Medetomidine	0.004-0.05 mg/kg IM		
Zuclopenthixol acetate	50-100 mg IM juveniles/subadults; 100-200 mg adults; 0.6 mg/kg IM		
Perphenazine enathate	50-150 mg juveniles/subadults; 100-400 mg IM adults; 0.05-0.25 mg/kg IM		

## **NUTRITION**

Many of the health problems identified in captive rhinos are believe by some to be linked to nutritional factors. Rhinos consume a large number of species of plants with a diverse array of physical characteristics and nutrients. They represent a range of feeding strategies and, consequently, diet, from browsers (or selective feeders) to unselective grazers. Diets in the zoological setting may have possible imbalances in dietary fats (particularly essential fatty acids) and soluble and insoluble carbohydrates, as well as minerals and vitamins for some species. This chapter outlines current dietary information for maintaining rhinos in captivity and includes a section on hand-rearing.

### **Nutritional Requirements**

Due to similarities in digestive tract morphology, the domestic horse still represents the best nutritional model for all rhinoceros species (Clauss et al., 2006a,b). Until further information is obtained, diets should be formulated using current National Research Council (NRC; 2007) recommendations for horses of various physiological stages. Minimum nutrient requirements are listed in Table 3.19.

Table 3.19. Nutrient concentrations in total diets for horses and ponies (dry matter basis; modified from NRC, 2007).

Nutrient	Growing	Mature/ Maintenance	Pregnant/ Lactating
Dig. Energy (Mcal/kg)	2.45–2.90	2	2.25 –2.60
Crude Protein (%)	12-15	8	10–13
Ca (%)	0.6	0.3	0.4
P (%)	0.3	0.2	0.3
Mg (%)	0.1	0.1	0.1
K (%)	0.3	0.3	0.4
Se (mg/kg)	0.1	0.1	0.1
Vit A (IU/kg)	2000	2000	3000
Vit D (IU/kg)	800	300	600
Vit E (IU/kg) <sup>1</sup>	80	50	80

<sup>1</sup>Black rhino diets should contain 150-200 IU vitamin E /kg dry matter (Dierenfeld et al., 1995).

Good quality forages should provide primary nutrients for all herbivores, with concentrate feeds used to balance energy, protein, mineral or vitamin needs. Hay storage is particularly important for ensuring proper dietary management. Moldy or dusty hay may cause colic and/or heaves. Large amounts of poor-quality hay should not be fed to rhinos, as it may be so poorly digested that impaction and/or colic will result. Very high-quality legume or small-grain hay may be so readily digested that when fed with concentrates, loose feces or colic may result. Browsing species should be fed mixed grass-legume hays and/or a mixture of legume hay and less digestible browse. Alfalfa hay contains more iron than grass hays and should not be fed as the sole forage to black rhinos. Animals should have *ad libitum* access to hay, water, and salt (Clauss et al., 2006b). The concentrate portion of the ration should be given in at least two feedings daily for better utilization. When practical, a small feeding of hay should be encouraged prior to each concentrate feeding.

In studies of intake, digestion and passage in zoo herbivores, Foose (1982) measured dry matter intakes of approximately 1% of body mass when black rhinos (n=3) were fed grass hays and slightly higher levels (1.2-1.6% of body mass) when fed alfalfa hay. Diets were 43% digestible (black rhinos eating grass). Thus, a guideline for as-fed diet quantity would be approximately 1.5% of body mass. Later studies confirm these earlier data (Clauss et al., 2006a). Smaller pellets can be readily manipulated by browsing species.

Animals can sometimes be encouraged to consume less palatable forages if hays are soaked in water or sprinkled with molasses. Applesauce has proved to be helpful in administering unpalatable medications and/or supplements.

Produce items and browse often fed to black rhinos are listed below (Table 3.20). The most common hays and concentrated pellets fed among black rhinos also are noted below (Table 3.20). Hay and browse often is fed in variable amounts, depending on the seasonal browse available. It is important to remember that all diets should be based on forages, not concentrates.

## **Feeding Location**

As with all zoo species, feed should be offered on a concrete pad or in livestock troughs or bins. Sand impaction has previously been documented in rhinos (Nouvel and Pasquier, 1946); therefore, feeding directly on the ground is not recommended. To reduce competition for food, individual feeding stations or adequate space at communal feeders is recommended.

## **Supplements**

Dietary supplements should be unnecessary in properly formulated rations. A vitamin-E deficiency has been documented in zoo-housed black rhinos (Clauss et al., 2002); current recommendations based on natural browse composition (Dierenfeld et al., 1995) suggest that diets should contain 150 to 200 IU vitamin E/kg dry matter. Salt blocks and water should be available at all times. If grown in an area prone to soil selenium (Se) deficiency, forage should be tested routinely for determination of Se content in order to provide data needed for balancing rations (Table 3.19).

## **Problematic Diets**

Alfalfa hay as an exclusive forage may lead to iron storage issues and mineral imbalances, and high-quality alfalfa may lead to colic and diarrhea. The consumption of fresh red maple browse has been associated with hemolytic anemia in horses and should therefore be avoided.

## **Browse**

Particularly for the browsing rhino species, the addition of fresh and/or frozen browse may be essential to dietary health. Browse may contribute required nutrients that have not yet been quantified and may also be of benefit to dilute a captive diet that is too digestible. Table 3.20 lists North American browse species currently fed to black rhinos.

Table 3.20. Food items fed to white rhinoceros. Amounts (kg) reflect daily rations. Some food items are not offered daily but are offered during enrichment and training; other food items are offered free choice (f.c.).

Produce	Amount	Browse/Grasses <sup>1</sup>	Amount	Hay	Amount	Pellets and Mixes	Amount
Apple	0.05-1.2	<i>Abies fraseri</i>	8-14	Alfalfa	2-20	Custom formula herbivore diet	4.6
Banana	0.1-0.4	<i>Acacia</i>	0-36	Brome	3.3-3.8	Elephant supplement	0.5-2.7
Beets	0.28	<i>Acer</i> <sup>1</sup>	0-10	Coastal bermudagrass	2.8-15	HMS ADF 16	4.5-9
Bell pepper	0.28	<i>Alnus</i>	1-10	Coastal bermudagrass w/ sudangrass	10.4	Mazuri ADF 16	4-5.5
Bok choy	0.2-0.28	<i>Alpinia zerumbet</i>	8-14	Oat hay w/ timothy	9-12	Mazuri ADF 16, Purina Stocker Grower, Purina Equine Senior	10
Cantaloupe	0.13-0.28	<i>Arundo</i>	0-3	Orchardgrass w/alfalfa	5.6-22.7	Mazuri ADF 25 and Wild Herbivore	4.3-7.2
Carrot	0.13-0.59	<i>Bauhinia</i>	8-14	Peanut	1	Mazuri Browser Rhino Cube	6.1
Cauliflower	0.2	<i>Bismarkia nobilis</i>	8-14	Prairie	3.3-22	Mazuri Wild Herbivore	2-9
Celery	0.2-0.3	<i>Broussonetia papyrifera</i>	8-14	Timothy	3.3-22	Moose Maintenance	33
Cucumber	0.28	<i>Carpinus</i>	1-5	Timothy w/ alfalfa (3:1)	14.6-21.1	NuZu Low Iron Herbivore	6-7
Grapes	0.4	<i>Casuarina equisetifolia</i>	8-14	Triple Crown Safe Starch	3-9	SDZ Global High Fiber ADF-25 Herbivore	8.6
Green beans	0.2-0.28	<i>Celtis</i>	0-10			University of Florida Zoo Blend milled at Pennfield	10-13.6
Honeydew	0.13-0.28	<i>Cenchrus pupureus</i>	8-14			Zoo Nutrition Network Herbivore	6.8
Kale	0.2-0.25	<i>Cersis</i>	0-5				
Lettuce	0.2-0.4	<i>Coccoloba uvifera</i>	8-14				
Orange	0.3	<i>Cocos nucifera</i>	8-14				
Pear	0.17-0.3	<i>Coprosma repens</i>	27-36				
Pineapple	0.22-0.3	<i>Cornus</i> <sup>1</sup>	0-3				
Potato	0.22-0.4	<i>Elaeagnus</i>	0-7				
Pumpkin	0.28	<i>Elaeocarpus</i>	1				
Squash	0.2-0.23	<i>Escallonia</i>	27-36				
Sugar cane	0.28	<i>Ficus</i>	0-10				
Sweet potato	0.13-1.5	<i>Fraxinus</i>	1-10				
Watermelon	0.13-0.28	<i>Gleditsia</i>	0-6.8				
		<i>Gordonia lasianthus</i>	7				
		<i>Hibiscus</i>	0-14				
		<i>Lagerstroemia indica</i>	8-14				
		<i>Latania loddigesii</i>	8-14				
		<i>Liquidambar</i>	0-14				
		<i>Lirodendron</i>	0-3				

Table 3.20. Continued.

Produce	Amount	Browse/Grasses <sup>1</sup>	Amount	Hay	Amount	Pellets and Mixes	Amount
<i>Lonicera</i>			0-10				
<i>Malus</i> <sup>1</sup>			0-10				
<i>Morus</i>			0-14				
<i>Musa</i>			0-14				
<i>Musaceae</i>			27-36				
<i>Myrica</i>			7				
<i>Nyssa</i> <sup>1</sup>			7				
<i>Pennisetum</i>			1-2				
<i>Persea borbonia</i>			7				
<i>Pinus</i> <sup>1</sup>			0-3				
<i>Phragmites australis</i>			8-14				
<i>Phyllostachys</i>			0-3				
<i>Phoenix canariensis</i>			8-14				
<i>Phoenix dactylifera</i>			8-14				
<i>Plantanus</i>			0-7				
<i>Poaceae</i>			8-14				
<i>Populus</i>			0-10				
<i>Pyrus</i>			2.2-7				
<i>Quercus</i> <sup>1</sup>			0-14				
<i>Rhus</i>			0-5				
<i>Robinia</i> <sup>1</sup>			0-10				
<i>Rubus</i>			1-5				
<i>Sabal palmetto</i>			8-14				
<i>Saccharum</i>			0-3				
<i>Salix</i>			0-36				
<i>Schefflera</i>			4-8				
<i>Syagrus romanzoffiana</i>			8-14				
<i>Symphoricarpos</i> <sup>1</sup>			1-5				
<i>Tilia</i>			7				
<i>Tillandsia usneodes</i>			8-14				
<i>Tipuana karoo</i>			8-14				
<i>Ulmus</i>			0-14				
<i>Vitis</i>			1-5				
<i>Wodyetia bifurcata</i> <sup>1</sup>			8-14				

<sup>1</sup> Some browse species are potentially toxic when offered in large quantities. Variety and moderation are key. Animal managers should consult a nutritionist before feeding substantial quantities of any one browse item.



## Hand-Rearing

A limited number of rhino calves have been and are currently being raised using various formulas. Reports and published information must be carefully scrutinized for measures of success and methodology in milk-sample analysis. The following information uses the ungulate hand-rearing chapter in the AZA Infant Diet Notebook as a base for general feeding guidelines and formula selection (Reiter et al., 1994). This recommendation is to be used as a guideline for standardization of a hand-rearing diet.

### *Milk Composition and Formula Selection*

Based on available data, rhinoceros milk is more dilute than milks of other ungulate species. It is low in solids, low in protein, very low in fat and high in sugar compared with milk of equids, bovids and cervids (Oftedal, 1984). Formula selected should mimic mother's milk in composition as much as possible (Table 3.21). In Table 3.22, Formula One has been used to raise a calf to one year of age; Formula Two more closely mimics mother's milk. In Europe, Mazuri® makes a rhino milk replacer for black rhinos ([www.mazuri.eu](http://www.mazuri.eu)). Land O'Lakes® Mare's Match® (Table 3.23) has been used to supplement a greater one-horned rhino calf at San Diego Zoo Safari Park and possibly could be used for hand-raising black rhino calves (powder:H<sub>2</sub>O = 1:6 but formula may need to be mixed at a more dilute ratio (1:8) for the first few days to avoid problems with constipation). The San Diego Zoo Safari Park has used a low fat cow's milk: nonfat cow's milk: lactose powder: water (27:9:1:1 by weight)(Blakeslee and Zuba, 2002).

Table 3.21. Nutrient compositions of rhino milk (Gregory et al., 1965) and recommended formulas (% as-fed basis).

Formula	Solids	Protein	Fat	Sugar
Rhino milk	8.8	1.4	0.2	6.6
Formula 1	10.3	3.3	0.3	5.9
Formula 2	8.3	1.7	0.2	6.6

Table 3.22. Compositions of rhino hand-rearing formulas.

Ingredients	Parts by Volume	
	Formula 1	Formula 2
Water	32	9
Skim milk	32	9
Karo Syrup	1	1

Table 3.23. Products used in hand-rearing diets.

<b>Product</b>	<b>Manufacturer/Distributor</b>
Mare's Match Milk Replacer Colostrum Replacement	Land O' Lakes Animal Milk Products Shoreview, MN 55126
Colostrx	Protein Technology, Inc. Minneapolis, MN 95403
Seramune Oral	Sera, Inc. Shawnee Mission, KS 66285
Replinish	Fermenta Animal Health Co. Distributor Kansas City, MO 64153
Fer-In-Sol Poly-Vi-Sol with Iron	Mead Johnson Nutritionals Bristol-Meyer Co. Evansville, IN 47721
Major Multi-Vita Drops	Major Pharmaceutical Corp. Distributor Chicago, IL 60612
Lixotinic	Revival Animal Health Distributor Orange City, Iowa 51041
Probios	Vet Plus, Inc. Menomonie, WI 54751
Lactaid	Lactaid, Inc. Pleasantville, NJ 08232

Though rhinoceros' milk is different from cow's milk, the latter may still be appropriate for hand-rearing rhinos if used in combination with other ingredients. Cow's milk is low in iron; consequently, an iron source such as Fer-In-Sol<sup>®</sup> (Table 3.23) should be added to the formula at two drops per 100 g of formula. In addition, infant vitamins, such as Major<sup>®</sup> Multi-Vita Drops<sup>®</sup> (Table 3.23), should also be added to the formula at two drops per 100g of formula. Some infant vitamins, such as Mead Johnson<sup>®</sup> Poly-Vi-Sol with Iron<sup>®</sup> (Table 3.23), contain added iron. San Diego Zoo Safari Park uses Probios<sup>®</sup> (2 tbsp) and Lixotinic<sup>®</sup> (0.44 ml /kg body weight)(Table 3.23) as daily supplements added in the first bottle. The animal may also benefit from the addition of Lactaid<sup>®</sup> at one drop per 100 g of formula. Lactaid<sup>®</sup> (Table 3.23) aids in carbohydrate digestion and helps prevent possible gastrointestinal tract distress. If the neonate is less than 24 hours old, colostrum diluted 50% with water or an electrolyte solution for ungulates, such as Replenish<sup>®</sup> (Table 3.23), should be administered for the first 24 hours. Though species-specific colostrum is preferred, cow

colostrum may be used. San Diego Zoo Safari Park uses Land O'Lakes® Colostrum Replacement® (Table 3.23) in the first 24 hours after nursing followed by a mix of 50% colostrum and 50% formula during the next 24 hours, and then 100% formula until weaning. Products such as Colostrx® and Seramune® Oral may also be used (Table 3.23). To avoid gastrointestinal distress, a diluted formula should be offered beginning on day two. The formula can be gradually increased to full concentration depending on the animal's health, including weight gain and stool condition. Prior to feeding, the formula should be warmed to approximately 37°C (99°F). Rhinoceros calves prefer their milk cooler than many other ungulates.

### **Feeding Regimen**

The calf should be stabilized and hydrated before any feeding. Quantity fed should range from 10 to 13% of body weight (BW). Animals should be fed every two hours. Because infants suckle during daylight hours, feeding should be equally spaced in a 12-hour period not to exceed 3 to 4% of body weight at any one feeding. It is recommended that feeding begin with 10% of body weight split equally into 12 feeds one hour apart during daylight hours. The quantity of formula fed should be adjusted daily based on the animal's weight. Animals should be weighed at the same time each day. Fresh water should be available at all times.

Table 3.24. Example feeding regimen, provided by San Diego Zoo Safari Park.

Week 1 & 2	6am, 8, 10, 12, 2, 4, 6, 8pm	(18-20% BW) 8 feedings
Week 3 & 4	6am, 8, 10, 12, 2, 4, 6pm	(17-19% BW) 7 feedings
Week 5 & 6	6am, 8, 10:30, 1, 3:30, 6pm	(16-18% BW) 6 feedings
Week 7 & 8	6am, 9, 12, 3, 6pm	(14-16% BW) 5 feedings
Week 9 – 14	6am, 10, 2, 6pm	(12-14% BW) 4 feedings
Week 15	No more increases to daily volume	
Week 15 – 30	6am, 12, 6pm	(8-12% BW) 3 feedings
Week 30 – 40		(5-7% BW)
Week 41		(3-4% BW)
Week 52	Start dropping amount on all bottles	
Week 60	Weaned	

If diarrhea occurs, the quantity of formula fed should be decreased or the formula diluted until stool condition returns to normal. If diarrhea is persistent, an electrolyte solution can be used to dilute the formula, replacing some or all of the water. In addition, the number of feedings can be increased to lessen the quantity fed at any one time.

Formula can be prepared ahead of time and warmed as needed. Water should be boiled to decrease possible contamination due to pathogens and refrigerated before being added to the formula. The formula should be refrigerated and used within 72 hours. Prior to feeding, the formula should be warmed to the animal's body temperature. Calf nipples work well with large species. Bottles should be boiled before use. Diluted bleach may be used as a disinfectant. Formula left over from each feed should be discarded.

### **Weaning**

Weaning may begin as early as six months if necessary and should be completed in one year. Weaning is a slow process involving carefully monitoring of body weight and solid food consumption. Animals should have access to solid food at all times. A nutritionally complete pellet diet such

as Calf Manna (Manna Pro Products, LLC., Chesterfield, MO, USA), horse feeds or high fiber ungulate pellets, in addition to high-quality grass hay, is appropriate. Formula may be decreased by gradually eliminating the number of feeds or decreasing the amount offered per feed and gradually decreasing the number of feeds.

## RESEARCH

### Introduction and Top Research Priorities

Largely due to the rhino poaching crisis that far out-weighs any other challenge facing rhinos today, the top five priorities of the Rhino Research Council, listed below, are skewed towards *in situ* issues. It is important to note that these research priorities are not ranked in order of importance, so the first and only priority focused on the *ex situ* population is not considered more important than the others. The Rhino Research Council realizes that conservation of genetic diversity via cell/gamete rescue from remnant populations is really not a research issue and is more of an implementation challenge, but it was identified as an important action to emphasize at this time of crisis. Following this list of the highest research priorities, the chapter continues with important areas of *ex situ* research, including reproduction, health, nutrition, behavior/ecology, and genetics. For more detailed discussions of *in situ* research needs, the reader is referred to the Rhino Research Council's 2014 Rhino Research Masterplan.

#### *Investigate Major Factors Affecting Health and Reproduction Ex Situ*

- Epidemiology of browser rhino health issues—Health concerns and unusual syndromes continue to impact black rhinos in captivity, but the incidence of various conditions shifts over time, and patterns require constant monitoring and re-evaluation to track current trends and conditions. Otherwise, outdated problems and historic records continue to be referenced at the cost of realizing emerging trends and realities. One example is the significant increase in the number of black rhino deaths due to renal disease compared to five years ago and a decreased incidence of mortality due to hemolytic anemia compared to 20 years ago. It would be extremely useful to secure ongoing support for a point person whose priority is to evaluate the historical data and current situations to keep abreast of the problems and factors associated with those health issues.
- Iron overload syndrome (significance, detection, treatment, prevention)—Evidence that hemosiderosis is occurring in browsing rhinos is solid, but there are still many questions surrounding the impact of this condition on rhino health, whether it is primary or secondary, why it is occurring, and what can be done about it. In the past decade, relatively little progress has been made. Although there has been some progress in developing oral iron chelators for humans, the efficacy and safety of such medications are unknown in rhinos, and they are cost prohibitive. Research on dietary tannins as iron chelators has produced inconclusive results. Recent studies on the impact of large volume, regular phlebotomies for reducing iron marker values has shown promise but is unlikely to be adopted as a long-term management strategy at many institutions. Research in any of the four areas—cause, detection, prevention and treatment—could be valuable in producing insight on how to manage/prevent this condition.
- Obesity/body condition scoring—Overconditioning in captive rhinos can lead to a multitude of health problems, including musculoskeletal, foot, and reproductive problems. One example is the almost certain exacerbation of pododermatitis associated with captive

husbandry conditions in the greater one-horned rhino. Research on standardizing body condition scores for all rhino species, along with improved nutritional management, should be a priority for captive health.

- Sub-optimal reproduction (stillbirths, embryo loss, phytoestrogens, pathology)—Although reproductive success has improved in white and greater one-horned rhinos by changing management strategies, providing larger spaces and more complex social groups that include experienced and inexperienced individuals, and allowing mate choice options, there are a few reproductive challenges that remain unresolved and challenge our ability to develop self-sustaining populations. For example, < 50% of all captive white rhinos are reproductively successful, almost 50% of greater one-horned rhino calves are stillborn, and as our ability to evaluate larger numbers of female rhinos has improved, incidence of known embryo loss and reproductive pathology continue to increase.

### ***Improve Rhino Identification and Monitoring***

- Optimize/standardize survey methodologies—Given the current poaching crisis, research that can improve our ability to monitor and survey wild populations is extremely important. Post-release monitoring is our primary method of determining the success of translocation, dehorning and many other conservation efforts. Data compiled from tracking individuals after translocation can be used to develop specific criteria that individual rhinos should meet (sex, age, reproductive status) prior to translocation in order to improve the success rate. Better methods for signal transduction and reception would be helpful for forest-dwelling animals. Creative methods of transmitter attachment are needed since horn transmitters are difficult to insert in de-horned animals, and radiocollars are a challenge given the neck morphology of rhinos. Also, lesions can occur with collars. Given the abundance of research on this topic in the military field, adaptation and application of this newer technology may be useful for rhinos.
- Genetic census—Advances in genetic analyses of fecal samples, hair, environmental-DNA and microbiomes could be used to determine the genetic diversity among wild populations, including sex ratios, number of individuals and extent of inbreeding.

### ***Identify Most Important Factors in Translocation Success***

Translocations have proven extremely valuable in saving rhinos from poaching, re-populating parks/ranches, bolstering dwindling populations and establishing new populations. However, there is room for improvement, and given the importance of this action for long-term rhino survival, research is needed to enhance survival and reproductive success of every rhino moved. Studies might focus on the following example topics: white rhino boma maladaptation, mortality rates in different habitats, impacts of dehorning, impact on source populations, and identifying the type of individuals most likely to succeed.

### ***Determine Ecological Factors Impacting Rhino Populations***

To choose the best locations for rhinos and provide guidance for those who want to improve their land in support of rhino populations, more information is required regarding specific ecological traits that impact rhino population survival and ability to thrive. Spatial ecology is especially important and is integrated with habitat quality, usage and preference.



## ***Conserve Genetic Diversity via Cell/Gamete Rescue from Remnant Populations (small/sub-populations and highly endangered)***

Given the poaching crisis in Africa and India and the rapid decline of Sumatran rhinos throughout their range, it seems prudent that every effort should be made to preserve cell lines and gametes from all genetically valuable rhinos/populations. The methodologies already exist but may need to be modified for the field conditions encountered in the African savannah and the tropical forests of Asia.

### **Areas in Need of Research *Ex Situ***

#### ***Reproduction***

- Early embryonic death (EED)—Repeated documentation of EED in all captive rhino species is puzzling. In the past 15 to 20 years, there have been at least six confirmed cases of EED in white rhinos at four different institutions, five confirmed cases of EED in greater one-horned rhinos at three different locations, and seven EEDs confirmed in two Sumatran rhinos at two locations. Few rhinos are monitored closely enough to diagnose EED, so considering the number of confirmed cases, it appears to be a common occurrence in the captive population. It has also been reported in at least one field study in wild black rhinos. However, in most field studies females breed only once or twice before conceiving and carrying pregnancies to term, so the high incidence of EED in captivity seems aberrant. In addition to EED, abortions later in gestation have also been reported. There are many potential causes of EED and abortion in rhinos, including uterine infection, uterine scarring from a previous dystocia, endometrial hyperplasia, reproductive pathology, hormonal insufficiency, dietary insufficiency or toxicity, social/behavioral influence of enclosure co-inhabitants or other health-related conditions of the female rhinos. It is quite possible EED is occurring in many females that are breeding repeatedly without producing calves. Further investigation into the prevalence and potential causes of EED and abortion is warranted.
- Reproductive pathology—Reproductive tract pathology in female rhinos has been reported in all captive rhino species and prevalence can be as high as 50% in females over 15 years of age (white rhinos). It can range from small, inconsequential cysts that do not affect fertility to large, invasive tumors that jeopardize the life of the rhino. There is general agreement that these pathologies typically develop in rhinos that spend the majority of their lives in a non-pregnant state and could be related to repeated exposure to hormone fluctuations, a condition not natural in wild rhinos that spend much of their time pregnant. However, there are some younger rhinos that also develop severe pathology, and more recent research has revealed that a fairly substantial proportion of white and greater one-horned rhinos develop pathologies in their early teens. Understanding the etiology of this pathology and identifying hormone therapies that might prevent it would be very useful. Furthermore, criteria for determining when the degree and types of pathology observed during an exam can be tolerated without loss of fertility versus those that render a female infertile would be very useful to establish so that appropriate management recommendations could be made.
- Measuring estrogen—Quantifying changes in estrogen levels that correlate with meaningful reproductive events has been difficult in several of the rhino species and continues to be challenging. A more accurate and meaningful way to assess estrogen concentrations could be useful for monitoring cyclicity, timing introductions and assessing other reproductive characteristics.

- Early pregnancy marker—Pregnancy can be diagnosed in rhinos through progesterone monitoring and ultrasound. It would be useful for management and for learning more about EED if a pregnancy-specific marker could be identified in rhinos not trained for ultrasound, but this is not a high priority.

The use of assisted reproduction has the potential of becoming a very useful tool for regional and metapopulation management of rhinos. Additionally, assisted reproduction may provide a means of overcoming physical and/or behavioral problems that currently prevent reproduction in specific individuals. Progress towards these goals has been impressive, and it is now time to look at priorities considered feasible to overcome during the next five years.

- Semen collection—Semen collection by electroejaculation (EEJ) has become more reliable and has been successful in all four captive rhino species. However, there are differences in the quality of samples collected with greater one-horned rhinos being the most reliable in producing a high quality, concentrated sample. In black, white and Sumatran rhinos, samples often are more dilute and more likely to be contaminated with urine or red blood cells. Samples are often adequate for cryopreservation, artificial insemination (AI) or in vitro fertilization but often are not able to withstand more complex processing like sperm sorting. More research into the effects of different anesthetic protocols and attempts at penile catheterization may prove valuable. Chemical induction of ejaculation has been attempted but has not proven very effective yet.
- In vitro fertilization (IVF)—To date, IVF success has been minimal (one IVF attempt produced one embryo in a black rhino and one IVM/IVF attempt produced one embryo in a black rhino) and more research is needed. Success has been minimal, in part, because of limited opportunities.
- Sperm sorting—Since 2005, 25 EEJ attempts across three species (n=10 black, n=9 white, n=6 greater one-horned) have been conducted for sperm sorting. Inconsistent ejaculate quality is the greatest challenge to application of sorting technology and integration into AI programs for each species. Only 36% of collections were good enough for sorting. Greater one-horned rhino sperm has a unique problem: an interaction between the seminal plasma and egg yolk that prevents staining of X and Y populations. Most progress has been with white rhino where sorted, cryopreserved samples are now available for use in AI procedures.
- Artificial insemination—Successful AI protocols using cryopreserved semen now exist for white rhinos (five term pregnancies) and greater one-horned rhinos (three term pregnancies). AI has not yet been successful in Sumatran or black rhinos. Additional research is needed in developing successful procedures for black rhinos and Sumatran rhinos, and research to improve efficiency/success rates in all rhinos is also needed to ensure the technology is integrated into the captive management plan. Some challenges include: breaking down intact hymens in females that have never mated, determining ovulatory versus anovulatory cycles in greater one-horned rhinos, and controlling the cycle for planned, timed AI to improve feasibility of use.
- Estrous cycle manipulation—In Europe, significant progress has been made with white rhinos using synthetic progestin and human chorionic gonadotropin or gonadotropin releasing hormone. Similar methodologies are being tested in the U.S. An ovulation induction protocol has been established for Sumatran rhinos. Research is still needed on effective methods for controlling the cycle in greater one-horned rhinos so that specifically-timed AI procedures can be planned and implemented.

- Gamete rescue—Protocols for rescuing sperm post-mortem are well established but protocols for oocyte rescue are not. A few attempts have been made with oocyte rescue, and one two-cell embryo was produced following maturation and IVF with frozen-thawed sperm. However, all protocols still need substantial research before they will be applicable for management purposes.

## **Health**

- Obesity/body condition index – Overconditioning in captive rhinos leads to a multitude of health problems, including musculoskeletal, foot, and reproductive problems. Research examining methods of standardizing body condition scores for all rhino species, along with improved nutritional management, should be a priority for captive health. Methods applied to domestic livestock, and more recently elephants, should be considered for use in rhinos. Also, is the inclusion of cereal-based concentrates in rhino diets detrimental?
- Quantifying stress (especially chronic stress)—Develop laboratory and behavioral markers of stress. What is the health impact, for instance, on the development of gastrointestinal ulcers, increased iron stores, etc.? New ideas include evaluation of neutrophil function using a portable luminometer and the analyses of five markers in fecal samples including thyroid hormones.
- Iron overload syndrome—Does iron overload cause disease in captive browser rhinos (i.e., what is the clinical significance)? Does it increase susceptibility to other diseases, such as infections? Determine what is the most useful marker (or suite of markers) for iron overload, e.g., is rhino-specific ferritin an accurate marker? Research is needed to determine whether dietary and therapeutic interventions are effective or even necessary, and if other methods such as stress or management play a role. What are the clinical/laboratory indications for therapy? Is there a link between excessive body condition and/or chronic stress and the presence of chronic inflammatory mediators in captive rhino? Is it possible that there is a ‘rhino metabolic syndrome’ characterized by insulin resistance and the chronic elaboration of inflammatory mediators by adipocytes that contributes to excessive iron uptake and other black rhino diseases?
- Oro-dental health—Factors in the development of dental disease in black rhino and methods for treating and preventing routine dental issues need further research.
- In addition to the causes of black rhino mortality, a superficial necrolytic dermatitis (SND)-like disease continues to plague captive black rhinos, affecting both their appearance and welfare, though not necessarily causing mortality. In the past, data suggested that SND affected as many as 50% of captive black rhinos. In one study, the hypothesis that SND was caused by hypoaminoacidemia was refuted. Furthermore, there was no positive correlation between corticoid levels and animals with lesions compared to those without lesions. Animals without lesions exhibited higher mean corticoid levels over time than animals with lesions. Disease could be immune-mediated.
- Renal disease—Necropsy reports on captive black rhinos in the U.S. have shown an increased number of cases of renal disease as a cause of death with secondary tissue mineralization. Does renal disease predispose individuals to hypertension with secondary arterio/atherosclerosis and possibly other health issues such as intermittent epistaxis? Ante-mortem blood-work and urinalysis has not typically been useful diagnostically. Research to investigate predisposing factors and improved methods for early diagnosis and intervention may benefit the captive population. In addition, the relationship of renal disease to other health syndromes should be re-examined.

- Idiopathic hemorrhagic vasculopathy syndrome, idiopathic epistaxis—Cases continue to occur with no obvious etiology or consistently-successful treatment. Idiopathic syndromes in black rhinos should continue to receive research attention, including the role of stress, nutrition, management, and infectious diseases.
- Pharmacokinetics/dynamics of commonly used antibiotics and analgesics—Little scientific work has been done in rhinoceros species on therapeutic drugs. Non-empirical use may lead to inadequate or potentially adverse effects. With the advent of husbandry training and restraint devices, sample collection is now possible for these types of studies.

### **Nutrition**

With respect to iron overload disorder, it could be useful to re-evaluate the population for iron markers since it has been many years since the original data were generated, and many dietary/management changes have been implemented. Currently at Disney's Animal Kingdom, horses are being used as a model to evaluate iron chelators and develop a specific ferritin test for black rhinos. Other research needs include development of a low iron diet or dietary supplement and determining the upper limit for dietary iron concentrations.

It is also necessary to investigate the use of phytase, in conjunction with phosphorus (P) supplement, to combat hypophosphatemia. Black rhinos have demonstrated serum hypophosphatemia and hypercalcemia. Serum fluctuations of both minerals seem related to dietary P levels. Currently, high levels of NaPO<sub>4</sub> are required to provide low normal serum P values. Additional research needs related to dietary supplementation include tyrosine supplementation, continued monitoring of serum vitamin E, determining the appropriate concentration and form of vitamin E in pellets, evaluating the effectiveness of Emcelle<sup>®</sup> in increasing serum vitamin E, and establishing normal serum vitamin E ranges.

Fatty acid ratios differ significantly between captive and wild populations of black rhinos and could be associated with changes in immune function and or diseases such as superficial necrolytic dermatitis. The appropriate dietary ratios of fatty acids need to be determined, and the minimum amount of supplement that needs to be offered to increase linolenic acid to provide benefits also needs to be determined. Seasonal fatty acid concentrations of four acacia species are being evaluated by San Diego Zoo Global.

Body condition scoring systems have been established for the African rhino species, however preliminary trials have proven that these scoring systems are very subjective if utilized by staff at each institution. Furthermore, efforts to control for bias by sending images of animals from different institutions to one person for evaluation were equally as difficult because of the variation in animal appearance based on photo angle/quality. Although similar scoring systems should be developed for Asian rhino species, less subjective methods for all species are needed. Perhaps physiological values for leptin, glucose, insulin, etc., could provide a more solid method of evaluating body condition in addition to computerized assessments, body measurements matched with weights and/or characterizing body types. This is an important issue since body condition has now been suggested as a factor involved in iron storage problems, disease, skewed sex ratio of calves and reproductive failure of captive-born white rhinos.

There are often requests for browse lists. Many papers exist on the browse chosen by wild rhinos as well as the nutritional components of the browse. However, the fact is that most zoos will feed what browse they can get locally and with the least amount of cost/effort. Regional browse studies on nutritional value and palatability of local species are helpful as general guides. Results of such studies have been reported for East coast species and a similar study on West coast species (in

particular acacia) is ongoing. In addition, a study comparing sumac and willow was also recently reported. San Diego Zoo Global is studying the nutrient composition of elephant grass (*Pennisetum purpureum*) following one to six months of regrowth. Institutions with black or Sumatran rhino are encouraged to develop browse farms to help provide better diets for their animals, and these regional studies are a great reference when initiating a browse farm. Other important topics for nutritional research are as follows:

- Research needs related to reproduction include determining if there is an impact of diet on calf sex ratio, evaluation of new hand-rearing formulas for supplementation and complete rearing, determining milk production, and estimating energy requirements of lactation.
- Minimum dietary nutrient concentrations for rhinos need to be established for maintenance, gestation and breeding to determine if utilizing the horse guidelines are adequate.
- Science-based recommendations need to be developed regarding the use of alfalfa for rhinos.
- The use of fecal DNA analyses as a means of defining wild rhino diets should be investigated.
- The cause of tooth overgrowth could be related to diet (nutritional and/or mechanical), and further investigation could be helpful. There have been several cases of rhinos requiring repeated teeth floating procedures. Additional browse could be helpful since silica is known to reduce tooth growth.
- Recent investigations of trace minerals are ongoing, especially with copper, which is an important anti-oxidant that could combat iron. One study has changed the diet to enhance copper. Appropriate dietary concentrations of zinc and copper need to be determined as both are being supplemented for both hoof health and to bind with iron.

### ***Behavior and Ecology***

Large-scale manipulative experiments to test hypotheses relating to social environment, stress, foraging/nutrition, movement patterns, etc. on reproduction, health and survival are encouraged. To achieve this goal, multiple large, naturalistic enclosures will need to be established and experimental groups assigned to different treatment conditions in a way that establishes multiple experimental replicates to address each question. Such a systematic approach, on a more limited number of variables of interest, will advance understanding of the factors governing whether captive (and wild) populations thrive or fail. Areas of *ex situ* research might include:

- Role of communication (olfactory, acoustic) in reproduction, maintaining social relationships, endocrinology (stress and reproduction), and overall social organization (consider experimental manipulations, such as with chemical signals and acoustic playbacks)
- Evaluate social processes (courtship, aggression) leading to successful copulation
- How social mechanisms lead to establishment of social outcomes, such as territoriality, dominance, group composition, etc., and how these change through time as new animals move in and out of the group
- How do changes in ecological parameters affect social behavior, endocrinology (stress and reproduction) and organization?
- Role of climatic variables (temperature/rainfall/photoperiod) on social organization and behavior
- Development of methods to reduce bodily injuries in male-male conflict and inter-sexual conflict that inhibits reproduction



- Behavioral, hormonal, and health perspectives regarding social stress
- Manipulate social variables such as density, group size, and age-sex ratios to determine outcomes for social behavior, organization, stress, health, reproduction, and survival
- Avoidance and attraction patterns
- Social density and composition effects

### **Genetics**

The potential benefits of employing genetic tools for studying rhino conservation and population genetics are tremendous, but considerable research and development is still needed in many areas before direct, efficient, reliable application will be possible. Areas where conservation genetics could be most valuable include studies on genetic structure /connectivity of surviving populations, dispersal, paternity, and census. The potential methodologies would include analyses of mitochondrial DNA, microsatellites, genetic sexing, single nucleotide polymorphisms, immunogenetics and the microbiome. Over the next five years, there needs to be an emphasis on developing appropriate methodologies, including:

- Microsatellite analysis—Microsatellites are powerful if they can be amplified from DNA from feces and should be a priority.
- Next generation sequencing (NGS)—Since fecal DNA typing is difficult and genetic diversity in some rhinoceros species is low, NGS may be of great value in providing the initial screening for polymorphisms needed to develop markers to examine rhino population genetics.
- Standardization—Different labs using various loci are reporting different results. There needs to be some consistency, or the techniques may wrongly come to be considered unreliable for answering conservation/population questions.
- Shorter amplicons—Amplicons of 75-150 base pairs need to be targeted by genetic markers and made available for fecal DNA studies since degradation can occur rapidly in fecal material.
- Fecal microbiome analysis—Fecal microbiome analysis may be useful in some cases as an alternative to analysis of an individual's own genetic material. Microbial DNA is more plentiful and in better condition when excreted in the feces, and each individual has their own microbial profile. This was the methodology employed to determine that the fecal samples from the Viet Nam Javan rhino were all from the same individual.
- Immunogenetic variability—Immunogenetic variability and its relationship to mate choice would be interesting to investigate. If pre-testing for mate compatibility or interest could occur prior to animal transport, it would be very valuable to animal managers in zoos and managed reserves/ranches.
- Environmental DNA—eDNA is an emerging tool in genetic studies but may be more challenging under tropical conditions.

Ongoing priority genetic studies that might include *ex situ* components include:

- Major Histocompatibility Complex (MHC) variability—MHC analyses are being planned and, once established, will help significantly when analyzing populations for variation in genes that are under selection.
- Fecal DNA analyses—Some work has been done, but the method needs to be optimized so that individual genotypes from fecals can be used for census work and assessing and studying breeding strategies, dispersal, etc. A project to optimize non-invasive genotyping

from fecals as a tool for estimating numbers and sex of white and black rhinos from the Kruger National Park has been initiated. A project optimizing fecal genotyping of *D. b. bicornis* for census of the Etosha population is being launched.

- Study of disease history and parasite load—A critical emerging arena to which genetics can contribute is disease risk assessment of different rhino populations. This is particularly relevant in light of the tragic loss of five captive Sumatran rhino in Malaysia. A particular avenue worth pursuing is polymerase chain reaction techniques that can determine presence and load of different pathogens from tissue collections and fecals. A major study investigating these possibilities is proposed for the Kruger National Park for their *C.s.simum* and *D.b.minor* populations.

## BEHAVIOR

### Ranging Behavior and Sociality

Black rhinos primarily are browsers, so they may roam long distances (>500 km<sup>2</sup>; >123,553 acres) in search of food and water in arid habitats (Hutchins and Kreger, 2006). Home range sizes (6.8-235 km<sup>2</sup>; 1,680-58,070 acres) and core areas (0.6-74 km<sup>2</sup>; 148-18,286 acres) can be highly variable depending on habitat type and duration of occupancy (Tatman et al., 2000; Lent and Fike, 2003; Göttert et al., 2010), and they might also shift in size and position over time (Lent and Fike, 2003; Göttert et al., 2010). Female home ranges do overlap, especially in areas with preferred forage (Tatman et al., 2000; Lent and Fike, 2003; Göttert et al., 2010). Wild female and subadult black rhinos might be observed in small groups on occasion, but these are usually transient (Owen-Smith, 1988; Hutchins and Kreger, 2006; Göttert et al., 2010). Cows with calves tend to stay alone; however, the twosome sometimes allow an unrelated immature male or female to join them until that individual reaches maturity (Goddard, 1967).

Black rhino males often occupy and defend mutually exclusive territories in southern Africa (Owen-Smith, 1988), but they might occupy undefended, overlapping home ranges (Owen-Smith, 1988) or non-overlapping home ranges (Tatman et al., 2000) in East Africa. Males (n=2) in South Africa did not establish their territories until approximately age nine, and “satellite males” up to seven or eight years of age roamed within the territory of an older male (Lent and Fike, 2003). It is likely that variability in male spatial distribution across populations and over time is affected by vegetation type and structure, climate, and female spatial distribution. Territory and home range boundaries are delineated by scent marks left by males during spray-urination, dung-kicking, scraping with the fore- and/or hindlegs, and horn-scraping (Table 3.25; Owen-Smith, 1988). Both in the wild and in managed settings, male and female rhinos defecate almost exclusively at communal dung piles, and these dung piles are distributed over the entire home range, but especially near paths and water sources (Tatman et al., 2000).

Injuries from fighting among unfamiliar rhinos were more likely when male and female black rhinos were translocated into reserves ≤11,500 ha with densities of ≤9 km<sup>2</sup> (2,224 acres) per rhino compared to those ≥18,000 ha (Linklater and Swaisgood, 2008). Given larger space, encounter rates were lower and the rhinos could become familiar with one another more gradually and with less injurious aggression. A large-scale analysis of more than 600 translocations demonstrated that younger rhino, especially dependent calves, were most vulnerable and had lower survival rates, especially when introduced into an existing population (Linklater et al. 2011, 2012). These studies have implications for introductions in zoo facilities. Aggression is much rarer when animals are familiar with one another, and unfamiliar animals should be introduced gradually and in the largest space possible. Extra precaution should be taken when young animals are involved.

Table 3.25. Ethogram to assist with general rhino management (adapted from Fouraker and Wagener, 1996).

<b>General Behaviors</b>	<b>Definition</b>
Locomote	Move about covering ground
Resting	Recumbent on the ground
Wallow	Roll, lay down, or move about in an area that is wet or muddy
Forage	Search for and consume food
Object toss	Tilt or lift inanimate object off the ground
Horn rub	Rub horn against an object; often occurs if horn becomes wet but also occurs when dry
Head sweep	Head swings laterally relative to the ground, rooting the air with the horn
Mouthing	Repeated chewing or gumming motion with mouth open; not associated with eating
Nurse	Calf becomes still and suckles; tail may wag
<b>Elimination Behavior</b>	<b>Definition</b>
Urinate	Discharge or pass urine in a stream
Defecate	Discharge fecal material
Hind foot kicking	Rapid alternation of hind feet against ground while remaining stationary; often associated with elimination by the male
Hind leg drag	Walk with hind legs stiff and straight, producing a scrape-mark on the ground (usually performed by males during scent marking before or after urine spraying)
Urine spray/squirt	Project urine in a strong spray (usually male) or in distinct squirts (usually female); may be directed on a substrate
Urine/feces investigation	Smell or taste urine pool or feces; flehmen often exhibited
Defecation on pile	Defecate on an area that has been used repeatedly as a dung pile
<b>Social Behaviors</b>	<b>Definition</b>
Proximity	Rhinos are within one body length of each other
Face-to-face stare	Rhinos stand less than one body length from and facing each other
Affiliative physical contact	Touch, rub, or lick other animal
Follow	Locomote to remain within close proximity of another animal
Charge	Locomote rapidly toward another animal with the head lowered
Chase	Locomote rapidly in pursuit of another animal
Open-mouth threat	Display in which one rhino faces another with open mouth and bellowing; also associated with charge/chase behavior

Table 3.25. Continued.

<b>Social Behaviors Cont.</b>	<b>Definition</b>
Spar	Rhinos use horns in offensive or defensive manner; may contact heads/horns and use body weight against each other
Horn strike	Strike another rhino with horn
Gore	Pierce or wound another rhino with horn
<b>Reproductive Behaviors</b>	<b>Definition</b>
Flehmen	Raise head and curl underside of upper lip upward; often seen in males in response to female urine
Anal and genital investigation	Sniff anogenital region of another animal
Erection	Penis in erect position
Penis unsheathed	Penis is dropped from the sheath, possibly in a partial erection
Stand	Female remains stationary during a chin-rest or mount
Chin-rest	Male rests chin on female's back or hindquarters in preparation for copulation
Mount	Male's weight is on hind legs, head over female's withers; copulation posture
Mis-mount	Male mounts female but not in proper orientation for copulation
Copulation	Penile penetration of vagina with successful ejaculation
Vulva wink	Rapid contractions of vulva, exposing clitoris
Vulva swelling	Swelling, color change, and/or dilation of the vulva
<b>Vocalizations</b>	<b>Definition</b>
Call	Vocalization resembling a cry (may be emitted in anticipation of food or upon physical separation from another animal)
Snort	Vocalization in which air is gruffly forced through the nasal passages, usually aggressive or defensive in context
Bellow	Vocalization during a charge or other high-intensity confrontation, sometimes accompanied by open-mouth threats

## **Reproductive Behavior**

Black rhino males are polygynous, and only a few males in a wild population might sire most of the offspring (Garnier et al., 2001). Subadult males and less preferred mates do not associate with female black rhinos during their fertile period (Garnier et al., 2002). Reproductive behavior in wild black rhinos lasts up to four days (Garnier et al., 2002), during which the female might mate with several bulls (Estes, 1991). Multiple mountings by the male will occur before copulation takes place (Tables 3.25 and 3.26; Hutchins and Kreger, 2006). Black rhino males might mount with or without



an erection (Fig. 3.14; Fouraker and Wagener, 1996). At full copulation, rhinos may remain coupled for 30 minutes to one hour, with ejaculations every few minutes (Fouraker and Wagener, 1996). Interestingly, black rhinos may breed continually throughout gestation (Fouraker and Wagener, 1996).



Fig. 3.14. The male usually mounts the female multiple times with and without an erection before successful copulation finally occurs (courtesy of K. Meeks, White Oak Conservation Holdings).

Parturition usually lasts ten to 12 hours from water-break, though first-time mothers may take longer to calve (Fouraker and Wagener, 1996). Black rhino females do not tolerate the presence of other rhinos during, and for a few months after, the birth of a new calf (Hall-Martin and Penzhorn, 1977). Infants may nurse hourly while older calves nurse every couple of hours (Fouraker and Wagener, 1996). It has been reported that as the calf ages nursing will usually decrease in frequency (Fouraker and Wagener, 1996). Wild, newborn black rhinos may remain in hiding during their first week to month of life (Hall-Martin and Penzhorn, 1977; Hutchins and Kreger, 2006), and free-ranging mothers will move as far as 19 km (11.8 mi) from their young calves (< 6 months of age) when traveling to water (Berger, 1993). In zoos, calves might be expected to remain close to their mother 75% of the time during the first six weeks of life (Greene et al., 2006). Calves (n=5) might be expected to spend about ~55% of their time resting, ~15% of their time playing, ~10% of their time standing, ~10% of their time locomoting, and ~5% of their time nursing (Greene et al., 2006). Mothers spend most of their time resting (~40%) or standing (~30%) and less time feeding (~20%) or locomoting (~10%)(Greene et al., 2006).

Possibly owing to their more solitary tendencies and reduced space availability in zoos, captive females will calve for the first time at a younger age when they are the only female at a given zoo, and reproductive rate is higher when there are fewer females at a zoo (Carlstead et al., 1999a).



Greater amounts of aggressiveness and assertiveness by a female were found to contribute positively to zoo-housed female black rhinos' chances of breeding, and higher reproductive success also was found in black rhino females that were more dominant than the male (Carlstead et al., 1999a,b). Male black rhinos express their dominance less when housed in a larger enclosure, thus large enclosures that reduce dominant, aggressive behaviors in the male might promote greater breeding success (Carlstead et al., 1999a). Black rhino females that scored lower on stereotypical behaviors also tended to have greater breeding success (Carlstead et al., 1999b), and monitoring changes in these behavioral frequencies (chasing, mouthing, and stereotypical behaviors) as an indicator for reproductive performance was recommended (Carlstead et al., 1999b).

Table 3.26. General reproductive behaviors observed during estrus and courtship (Fouraker and Wagener, 1996).

Female Behaviors	Male Behaviors
Vocalizations <sup>a</sup>	Vocalizations
Urine squirt/spray <sup>a</sup>	Frequent urination; urine spray
Urogenital changes (e.g., vulva swelling)	Erection
Vulva “winking”	Genital inspection of female
Vaginal discharge <sup>a</sup>	Flehmen response
Aggression toward male	Charge/chase female
Maintains proximity to male	Maintains proximity to female
Nuzzles male’s belly and/or genitals	Follows female
Stands for male	Chin-rest
Inappetance	Mounts female

<sup>a</sup> Estrous behaviors in the absence of a male are often difficult to distinguish. In general, increased activity, agitation, vocalizations, spray-squirting urine, and vaginal discharge have been cited. As the female approaches peak estrus, these behaviors usually increase in frequency. Some females have been reported to successfully breed without exhibiting any overt behavioral signs of estrus.

## Adolescent Black Rhinos

In the wild, dispersal of two male calves occurred at three to four years of age. In contrast, females continued to maintain a home range overlapping that of their mother into adulthood, and disassociations were usually temporary following the birth of a new calf (Lent and Fike, 2003). If a cow and calf are separated from other rhinos at zoological institutions, they may be reintroduced following the cow’s first estrus (Fouraker and Wagener, 1996).

## Behaviors Specific to Zoo-Managed Rhinos

A common behavioral abnormality observed in zoo-managed rhinos is excessive horn rubbing (Hutchins and Kreger, 2006), which might indicate boredom or distress, e.g., when rhinos are housed indoors without activity or forage (Dieckhoefer et al., 2006). Stereotypical behaviors among female black rhinos were associated with a greater percentage of wall space over which the rhino could not see, whereas males’ stereotypical behavior was positively correlated with chlorine use (Carlstead et al., 1999a), possibly because it masks or removes their own scent marks. Males that scored higher for “fear” were housed at facilities where there was a greater percentage of

outdoor enclosure perimeter that allowed zoo visitors to have unobstructed view into the enclosure at a distance of less than 10 m (32.8 ft)(Carlstead et al., 1999a). This finding is not unexpected given the observation that wild black rhinos choose bedding sites enclosed by tall vegetation and dense thickets (Tatman et al., 2000), suggesting this species prefers the security offered by cover. Fighting among zoo-housed black rhinos was associated with variability in glucocorticoid metabolites, which are secreted during the adrenal stress response (Carlstead and Brown, 2005).

### **Behavior During Introductions (from Fouraker and Wagener, 1996)**

Aggression between newly introduced black rhinos can be severe, especially if a new rhino(s) is being introduced to an established rhino(s)(Hall-Martin and Penzhorn, 1977). Thus, a “howdy” period and plenty of space for escape upon the first physical introduction is highly advisable. Rhinos of both sexes have been the aggressors. Behaviors that have been noted during rhino introductions are listed in Table 3.27. Territorial defense is often limited to ritualized confrontations, in which two rhinos advance toward each other but stop and engage in a horn-to-horn stare (Tables 3.25 and 3.27). Also as part of this ritual, the two individuals may touch horns, back apart, and wipe their horns on the ground (Nowak, 1991). More intensive conflicts (Table 3.28) involve charges and inflicting injuries by horning or ramming. It is important to note that what is often perceived as serious or dangerous aggression between rhinos is, in fact, normal behavior requiring no intervention of any kind. Along with increased size and thick skin comes decreased vulnerability compared with many other animals.

Table 3.27. Behaviors noted during rhino introductions (Fouraker and Wagener, 1996).

<b>Non-aggressive Behaviors</b>	<b>Ritualized Confrontations</b>	<b>Potential Stress-Related Behaviors</b>	<b>Aggressive Behaviors</b>
Follow	Head sweep	Pacing	Charge/chase
Touch/rub/lick	Face-to-face stare	Running (excessive)	Open-mouth threat
Anal or genital investigation		Space-maintenance and threat vocalizations (excessive)	Sparring
		Diarrhea	Goring

Table 3.28. Levels of aggression in rhinos (Fouraker and Wagener, 1996).

Level of Aggression	Definition
1	Rhinos are charging each other but do not make physical contact
2	Rhinos are charging each other with physical contact resulting in some cuts and scrapes to the facial area
3	Rhinos are charging each other with physical contact resulting in cuts and scrapes to the facial area and body
4	Charging and/or pursuit <sup>a</sup> proceeds to the point that one or both rhinos are knocked down at least once. Scrapes and cuts are deeper and more numerous.
5	Aggression and pursuit proceed to the point that one or both rhinos have subcutaneous wounds or arterial blood flow

<sup>a</sup> It should be noted that one animal might break away from the confrontation and attempt to escape. The aggressor often will pursue and begin horn-prodding the underbelly of the escapee as the two run around the enclosure. Often a rear leg is hooked and held aloft while pursuit continues. If the escapee does not stop and resume a defensive posture, the animals might continue until heat or exhaustion becomes a critical factor. Aggression at this point is more serious.

In some cases, aggression may proceed to a point at which management should intervene to prevent serious injury. Rhino managers should allow some aggression during an introduction but be prepared to intervene in the event that aggression threatens the lives of one or more animals. Protocols for intervening may vary across institutions, but in general, careful consideration should be given to intervening in an introduction before aggression reaches Level 5 (Table 3.28). Stopping an introduction at a level prior to this will not lessen aggression during a subsequent introduction attempt. Animals that are allowed to “settle their differences” will establish some territorial boundaries and usually will not engage in serious aggression again, with the exception of a male attempting to approach an estrous female. In sum, moderate aggression is commonplace in any rhino introduction; sparring and fighting will occur and result in minor injuries (cutaneous wounds). However, in most cases, aggression levels prior to Level 5 may be allowed to continue using the discretion of management.

Animal personality and disposition should always be considered in introductions. A subordinate animal should be introduced to a more dominant animal in an enclosure familiar to the subordinate. In the case of multiple-animal introductions, the most subordinate animal should be introduced to the next most subordinate, and so on up the dominance hierarchy. Greater aggression may be noted in some individuals in the presence of an estrous female; therefore, any introduction attempt at this time should be especially well-monitored or possibly avoided if the attempt involves a male. Aggression may be meaningfully reduced by allowing individuals to become familiar with each other through protected contact (e.g., through enclosure bars) or, possibly, by exposing them to one another’s odors for a period prior to introduction.

# PART IV

## ***GREATER ONE-HORNED RHINOCEROS***

### ***TAXONOMY***

#### **Scientific Name and Origin**

*Rhinoceros unicornis*

*Rhinoceros*: Greek *rhino*, meaning “nose” and *ceros*, meaning “horn”

*unicornis*: Latin *uni*, meaning “one” and *cornus*, meaning “horn”

#### **Common Names**

Asian greater one-horned rhinoceros: referring to the single, large horn

Indian/Nepalese rhinoceros: referring to the species’ endemic range

#### **Distribution and Habitat**

Northern India, southern Nepal

Floodplains, riverine grasslands

#### **Size**

1,800 to 2,200 kg (4,000 to 5,000 lb)

1.75 to 2.0 m (5.75 to 6.5 ft.) tall at shoulder

Single horn 20 to 61 cm (8 to 24 in)

Largest land mammal, after elephants, along with the African white rhino

#### **Physical Description**

Brownish-gray, hairless, with rivet-plated (armor-plated), knobby skin

One horn

Upper lip semi-prehensile

#### **Life History Characteristics**

Grazer (primarily; will consume some browse)

Mostly solitary: temporary groups of females and young or of subadults possible

Females sexually mature at five to seven years of age; males at ten years

Gestation period approximately 15 to 16 months; interbirth interval of three years

### ***MANAGEMENT***

#### **Group Composition in Captivity**

The greater one-horned rhino is generally considered more solitary than black and white rhinos. Apart from cow-calf pairs, groups are rare (Laurie, 1982). Data on rhinoceros social organization, combined with preliminary analyses of parameters affecting reproductive success in captivity, may be used to generate recommendations regarding possible social groupings in the zoo setting (Tables 4.1 and 4.2). Many variables affect the probability of success for any zoo-managed

social group, including the animals' dispositions and available holding space. Further, depending on space and animal and staffing availability, institutions may hold animals for breeding or for exhibit only. Facilities that wish to hold rhinos for exhibit purposes only are advised to maintain two animals (male:female: 1.1 or 0.2 or, more recently, 2.0 because of the skew in male births, as well as the inability to house more than one, or possibly two, males at the breeding institutions).

Table 4.1. Possibilities for rhino social groupings within the same exhibit in captivity.

Rhino species	Multiple Animals of Same Sex		Multiple Animals of Opposite Sexes
	Adult males	Adult females	
White	rare (possible in exhibits >100 acres)	possible and recommended	possible and recommended for breeding (optimal: one male and two or more females); if possible, an additional male in proximity but not in same enclosure
Black	not recommended	possible in very large enclosures only; temporarily successful in some situations, but does not last	possible and recommended for breeding (optimal: 1.1)
Greater one-horned	not recommended	possible in enclosures $\geq 100$ acres	pairings recommended only during peak estrus (with the exception of very large exhibits, which may hold a single male and female together consistently)

For institutions that have the space and staff available for rhino breeding, it is recommended that managers commit to two pairs of greater one-horned rhinos. In addition, breeding institutions must also have space for offspring to be held for up to three years after birth. Within a single exhibit or holding area, the recommended minimum numbers for breeding are 1.1. In general, it is recommended that mature males not be held together because of the increased likelihood of serious aggression. Furthermore, males and females of this species should be kept separately and introduced only for breeding purposes.



Table 4.2. Recommended numbers for institutional holding.

Rhino Species	Recommended Minimum Groupings for Breeding <sup>a</sup>	Preferred Optimal Holding for a Breeding Institution	Exhibit Only (per institution)
White	1.2	2.4 (1 herd/ 1 back-up male)	1.1 or 0.2 <sup>b</sup>
Black	1.1	2.2 (2 pairs)	1.1 or 0.2
Greater one-horned	1.1 <sup>c</sup>	2.2 (2 pairs)	1.1 <sup>c</sup> or 0.2

<sup>a</sup> See Enclosure Design chapter. Breeding institutions must have space for offspring to be held for up to three years following birth.

<sup>b</sup> Multi-male bachelor groups have been maintained in very large enclosures.

<sup>c</sup> In the case of greater one-horned rhinos, males and females should be introduced only during the female's estrous period. Institutions with very large enclosures may be able to hold opposite-sex animals together consistently.

Grouped or multiple-species exhibits are possible for greater one-horned rhinos if ample exhibit space is available. Examples of institutions that have successfully maintained mixed-species exhibits include, San Diego Zoo Safari Park, Columbus Zoo, the Wilds, Lowry Park Zoo and Riverbanks Zoo. Species that have been successfully paired in an exhibit with rhinos include sarus cranes, herons, some antelope species (nilgai, blackbuck, gaur, Persian goitered gazelle), mouflon, zebra, and some deer species. In all cases, the dispositions of the individual animals, as well as adequate space and exhibit structure (i.e., visual barriers, refuge areas, etc.), are important to consider prior to attempting a mixed-species exhibit.

## Introductions

Changing social groupings of rhinos through the introduction of additional individuals to an established individual, pair or group is a process requiring care and planning. Rhino species vary widely in social structure, and rhinos periodically vary their grouping patterns in the wild according to factors such as reproduction and the rearing of young. Social groupings in captivity, therefore, should also vary according to species, as well as to the circumstances within each institution. Rhinos may be very protective of their individual boundaries, but proper introduction procedures can minimize injury from conflict and aggression. The following section outlines general considerations for any rhino introduction and provides systematic descriptions of aggression, procedural recommendations, and descriptions of potential species-specific introduction types.

Factors that must be considered in any introduction include individual animal personalities, staff experience and confidence level, and enclosure type (i.e., indoor/outdoor, public/off-exhibit, relatively small/large). Barrier types and temperature should also be considered. Introductions often result in aggression, and it should be noted that rhinos of both sexes have been the aggressors. Territorial defense is often limited to ritualized confrontations, in which two rhinos advance toward each other but stop nose-to-nose and engage in a staring contest to gauge each other's size and strength. Also as part of this ritual, the two individuals may touch horns, back apart and wipe their horns on the ground (Nowak, 1991). More intensive conflicts involve head-on charges and

inflicting injuries by horning or ramming. In general, behaviors that have been noted during rhino introductions are listed in Table 4.3. It is important to note that what is often perceived as serious or dangerous aggression between rhinos is, in fact, normal behavior requiring no intervention of any kind. Along with increased size and thick skin comes decreased vulnerability compared with many other animals. Table 4.4 lists a descriptive hierarchy of aggression levels in rhinos.

Table 4.3. Behaviors noted during rhino introductions (Fouraker and Wagener, 1996).

Non-aggressive Behaviors	Ritualized Confrontations	Potential Stress-Related Behaviors	Aggressive Behaviors
Follow	Head sweep	Pacing	Charge/chase
Touch/rub/lick	Face-to-face stare	Running (excessive)	Open-mouth threat
Anal or genital investigation		Space-maintenance and threat vocalizations (excessive)	Sparring
		Diarrhea	Goring

In some cases, aggression may proceed to a point at which management should intervene to prevent serious injury. Rhino managers should allow some aggression during an introduction but be prepared to intervene in the event that aggression threatens the lives of one or more rhinos. Protocols for intervening may vary across institutions, but in general, careful consideration should be given to intervening in an introduction before aggression reaches Level 5 (Table 4.4). Stopping an introduction at a level prior to this will not lessen aggression during a subsequent introduction attempt. Animals that are allowed to “settle their differences” will establish some territorial boundaries and will usually not engage in serious aggression again, with the exception of a male attempting to approach an estrous female. In sum, moderate aggression is commonplace in any rhino introduction; sparring and fighting will occur and result in minor injuries (cutaneous wounds). However, in most cases, aggression levels prior to Level 5 may be allowed to continue using the discretion of management.

The introduction process requires much planning and cooperation among rhino managers. Table 4.5 outlines recommended steps for rhino introductions. Familiarization through visual, olfactory and tactile contact should be permitted if at all possible prior to a full-scale introduction. If the facility permits, this may be accomplished by first placing individuals in the same barn or in nearby outdoor lots, or by providing olfactory clues such as urine, feces or skin rubbings from the animal being introduced. As the animals acclimate, managers may move them to adjacent barred stalls or fenced outdoor yards. These barriers prevent confrontations leading to serious injury but allow acclimation and familiarization prior to introduction.

Table 4.4. Levels of aggression in rhinos (Fouraker and Wagener, 1996).

Level of Aggression	Definition
1	Rhinos are charging each other but do not make physical contact.
2	Rhinos are charging each other with physical contact resulting in some cuts and scrapes to the facial area.
3	Rhinos are charging each other with physical contact resulting in cuts and scrapes to the facial area and body.
4	Charging and/or pursuit <sup>a</sup> proceeds to the point that one or both rhinos are knocked down at least once. Scrapes and cuts are deeper and more numerous.
5	Aggression and pursuit proceed to the point that one or both rhinos have subcutaneous wounds or arterial blood flow.

<sup>a</sup> It should be noted that one animal might break away from the confrontation and attempt to escape. The aggressor often will pursue and begin horn-prodding the underbelly of the escapee as the two run around the enclosure. Often a rear leg is hooked and held aloft while pursuit continues. If the escapee does not stop and resume a defensive posture, the animals might continue until heat or exhaustion becomes a critical factor. Aggression at this point is more serious.

The actual introduction should be attempted in the largest available enclosure. Enclosures should be large enough to allow ample space for shading, mock-fighting, aggression and defense. Prior to an attempted introduction, water pools in an outdoor enclosure should be filled with substrate to prevent injury in the event of serious aggression. The enclosure should contain visual barriers such as brush or earth piles or boulders (“run-arounds”), which give rhinos places to hide without becoming cornered or trapped. These features lessen overt aggression if a rhino is able to escape the sight-line of another. An enclosure should not contain dead ends in which an individual may become trapped by an aggressor. The enclosure should allow for the use of high-pressure fire hoses, CO<sub>2</sub> fire extinguishers and/or vehicles to aid in separating individuals.

Animal personality and disposition should always be considered in introductions. A subordinate animal should be introduced to a more dominant animal in an enclosure familiar to the subordinate. Male greater one-horned rhinos should be introduced only during the female’s estrous period.

Appropriate personnel for first-time introductions include the primary animal manager, a vet with immobilization equipment, and the curator and keepers most familiar with rhinos. Other staff may be needed at critical points around the enclosure’s perimeter so that the animals may be observed at all times in case separation becomes necessary. It should be noted that if a barn is opened and used to separate individuals, only one individual should be allowed inside the barn, and it must not be trapped inside by an aggressor.

Because of the relatively more aggressive, territorial nature of greater one-horned rhinos, introductions should be attempted only for breeding purposes (one male to one female). With the exception of very large facilities, individual rhinos should be held separately in all other situations.

Table 4.5. Steps in the introduction process.

Step	Description
1	Animals in the same barn or multiple outdoor lots should have olfactory and auditory exposure to each other. If the animals are not housed near each other (i.e., enclosures on opposite sides of the zoo, etc.), they should be moved to the same exhibit area.
2	Animals should be given visual contact with each other in addition to the above sensory modalities. Animals may be shifted within a barn or in adjacent outdoor lots. If at any point during this process the animals display symptoms associated with stress (e.g., pacing, diarrhea, excessive vocalizations) for more than 2-3 hours, the introduction should return to the previous step.
3	If animals are not already positioned adjacent to each other, they should be moved closer together (e.g., to adjacent stalls or adjacent outdoor enclosures).
4	The actual introduction (full tactile exposure) should take place in the largest enclosure available and follow guidelines stated in this chapter. Preferably, the enclosure should be familiar to the least dominant animal and include ample “run-arounds”.
5	Within institutions in which rhinos can be left together 24 hours per day, they should be separated during the first several nights or until they show only minor aggression.

### ***Introduction of a Male to a Female for Breeding Purposes***

Introductions should occur only for breeding purposes and when the female is in estrus (see Table 4.6 for behaviors associated with estrus). The introduction should take place in the largest area available. If two adjoining yards are opened to create a larger introduction area, the female should be placed in the yard she is most familiar with first and allowed to acclimate. After she is acclimated to the yard, the male should be introduced to her. As stated in the general introduction protocols, if the facility allows, preliminary visual and tactile contact may increase the likelihood of mating success. If intervention is required because of aggression between the rhinos, the introduction should cease and be attempted at a later date. If possible, the female should be kept from entering a barn if the individuals cannot be separated in the outdoor enclosure. In all likelihood, the male will follow her into the barn, and the chances of serious injury will increase. Because the introduction should occur during estrus, and introduction may be required at any time of the day or night. The rhinos should be monitored and separated following breeding.

Table 4.6. General reproductive behaviors observed during estrus and courtship (Fouraker and Wagener, 1996).

Female Behaviors	Male Behaviors
Vocalizations <sup>a</sup>	Vocalizations
Urine squirt/spray <sup>a</sup>	Frequent urination; urine spray
Urogenital changes (e.g., vulva swelling)	Erection
Vulva “winking”	Genital inspection of female
Vaginal discharge <sup>a</sup>	Flehmen response
Aggression toward male	Charge/chase female
Maintains proximity to male	Maintains proximity to female
Nuzzles male’s belly and/or genitals	Follows female
Stands for male	Chin-rest
Inappetance	Mounts female

<sup>a</sup> Estrous behaviors in the absence of a male are often difficult to distinguish. In general, increased activity, agitation, vocalizations, spray-squirting urine, and vaginal discharge have been cited. As the female approaches peak estrus, these behaviors usually increase in frequency. Some females have been reported to successfully breed without exhibiting any overt behavioral signs of estrus.

### ***Reintroduction of a Post-partum Female (Without Calf) to a Male***

An introduction of a post-partum female to a male for breeding should occur only during the female’s estrous period. It may be advisable to wait until after the first post-partum estrus cycle. The calf should not be introduced with the female when she is introduced to the male; therefore, the calf should be trained to be separated from the female to allow for the introduction of a male (see calf development, this chapter).

### **Pregnancy and Parturition**

Table 4.7 lists behaviors associated with pregnancy and impending birth. In all species, there may be a mucus discharge, noticeable weight gain or increase in girth size, as well as increased frequencies of defecation and urination throughout gestation.

The onset of birth often takes place at night or in the early morning and may last one to three hours. Parturition usually lasts ten to 12 hours from breaking of the water, though first-time mothers may take longer to calve. The presentation of a calf is generally head-first, although rear-foot presentations do occur and may take longer than head-first births, but usually deliver without assistance. Capabilities for monitoring births remotely through closed-circuit television or other means are advisable.



Table 4.7. Physiological and behavioral indicators of impending parturition.

30 Days Prior to Birth	2 Weeks Prior to Birth	24 to 48 Hours Prior to Birth
Increase in teat size	Nipples enlarge	Udders increase dramatically in size
Beginnings of milk production	Nipples develop wax plugs	Inappetance
Milk may be expended with pressure on the teats	Vulva swelling occurs	Becomes irritable and aggressive to stimuli, including staff
Female may prolapse vaginally when defecating		Mucus plug forms
		Increased vulva dilation
		Increased restlessness, lies down often

## Calf Development

A single calf is generally the rule. Few data are available on birth weights, but in general, calves weigh 65 to 81 kg at birth (144-178 lb; n=9). Immediately following birth, the newborn calf is usually cleaned by its mother and stands for the first time within 30 minutes to five hours of birth. A newborn calf may require a substrate that allows traction to help steady itself. Suitable materials may include sand, gravel, straw, hay or rubber matting. In all cases, both the dam and calf should be monitored closely to prevent ingestion of the substrate. A calf should begin nursing within one to two hours of standing (though in a single case, a calf removed from its dam for medical intervention nursed 16 hr post-birth). The dam will nurse her calf while standing or lying on her side.

Infant less than two months old may nurse hourly, while older calves nurse at intervals of about 2.5 hours. Few data are available on nursing durations and frequencies, but it has been reported that as the calf ages and grows stronger, nursing will usually increase in duration but decrease in frequency. It has been reported that calves may gain up to 4.54 kg (10 lb) per day for the first ten days. The first defecation has been reported at two to ten days of age (n=2). Calves may nurse for up to two years, although they have been observed first sampling solid food at less than one week to one month of age. Calves may be offered supplemental feedings of milk if the dam is believed to be a poor milk producer or the calf is not gaining weight (see Nutrition chapter). Infant rhinos have been successfully pulled from their mothers because of rejection, medical issues related to the mother or infant, or from a failure to nurse. Otherwise, it should be noted that weaning for management purposes can be accomplished if necessary at six months, but one year is preferable. One attempt to use a surrogate mother was unsuccessful; however, hand-reared infants have been assimilated into existing groups and have shown reproductive success. Keeping the calf with the dam even longer helps to facilitate social learning. Male calves are usually weaned by the dam at an earlier age than female calves. If the facility is able to run multiple female greater one-horned rhinos together, the cow and calf should not be introduced to the others for approximately four months

following birth. Calves generally do not have peers to play with, though they are generally very curious and often chase and mock-fight with their dams or occasionally keepers. Non-aggressive sexual behaviors may be exhibited at as early as 18 months of age in males.

In general, the long-term social effects of removing rhino calves from dams should be investigated. For all species, weaning or permanent separation of the calf except for medical reasons should not occur before one year of age. A calf can, however, be temporarily separated from its mother at as early as one month of age for short periods of time (e.g., re-breeding of dam). Generally, the procedure is to separate the calf for short periods of time (e.g., 15-20 minutes during cleaning) and gradually increase the separation time. If a dam is not going to be re-bred, her calf may remain with her until it reaches sexual maturity (at approximately 4.5-5 yr of age). It should be noted that available data indicate that nursing does not inhibit conception. The first calf may be forced away before parturition of the second calf as the dam seeks to isolate herself.

## **Identification**

Although physical characteristics such as horn size and shape make individual rhinos fairly easy to distinguish from one another, sound rhino management requires that animals be identified through permanent and reliable methods. Trovan<sup>®</sup> transponders (Electronic Identification Devices, Ltd., Santa Barbara, CA), implanted at the base of the left ear during post-natal examination or as soon after birth as possible, provide a means of permanent primary identification for all individuals. Adults should be transpondered opportunistically. Transponder numbers need to be reported to the studbook keeper. In addition to a transponder, each individual should have a secondary visual means of identification, such as an ear tag or ear notch. Photographs or sketches in the animal's records may also serve this purpose.

## **Keeper Training and Interaction**

As with any position involving the management of large animals, rhinoceros keepers should have as much formal training and experience as possible and should be familiar with rhino behavior and husbandry. In order to ensure safety and to properly meet the requirements of management, it is recommended that more than one keeper be responsible for the care of these animals on a daily basis. Keeper interaction should be restricted to designated areas and should be conducted in accordance with institutional protocols. Finally, consistency of routine is vital.

There are no conclusive data to indicate the effects of different styles of keeper interaction on rhinoceros behavior or reproductive success in captivity. Interaction styles range from no contact at all to daily hands-on contact. In an effort to create an environment patterned after the wild, however, at no time should relationships with keepers substitute for natural interaction among individuals. It is important that rhinoceros personnel keep a daily log, noting any unusual behavioral or physical changes. It is the responsibility of management to supply all pertinent data to the studbook keeper.

## **Daily Regimen**

Fresh water should be available at all times and should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset, spillage or leakage. Rhinos need access to water pools and/or mud baths to keep their skin healthy; mud wallows should be renovated periodically to prevent contamination (see Enclosure Design chapter for more information on pools and wallows).

Natural substrates in confined areas (e.g., holding corrals) can be spot-cleaned daily, but daily removal of feces from larger enclosures is not necessary as defecating at dung piles is an important natural behavior (Laurie, 1982). Hard-surfaced areas not exposed to the elements should be dry-cleaned or hosed daily and disinfected at least weekly. Indoor housing surfaces, as well as walls and rub areas, should be cleaned daily. Additionally, the daily hosing or showering of rhinos in the barn with temperature-controlled water is recommended to promote healthy skin during the winter months.

In order to facilitate participation in many research programs involving rhinos, it is recommended that institutions implement training programs following the protocols recommended in the Training chapter. Additionally, it is recommended that some form of environmental/behavioral enrichment be integrated into daily rhino management.

## ***REPRODUCTION***

### **Greater-One Horned Rhino Reproductive Age**

According to the North American Regional Studbook for greater one-horned rhinos (Christman, 2011) that contains data on 127 births, the youngest greater one-horned rhino to give birth was just four years old, which means she conceived at two years eight months. The oldest greater one-horned rhino to give birth was 31 years 4.5 months. The youngest male greater one-horned rhino to sire a calf was four years eight months (at time of conception) and the oldest to sire a calf was 38 years five months (at time of conception). In general, greater one-horned rhinos can potentially reproduce between the ages of three and 30 (females) and five and 40 (males) if they are maintained in good health. Although fertility tends to decline in nulliparous females as they age, the oldest dam at first reproduction was 24 years and the oldest sire at first reproduction was 30, suggesting that a few greater one-horned rhinos can breed for the first time fairly late in life. However, only two female greater one-horned rhinos successfully reproduced for the first time after they exceeded the age of 18, suggesting that most females need to conceive before they reach the age of 16.5 or they are unlikely to ever reproduce.

### **Seasonal Changes in Physiology or Behavior Associated with Reproduction and Management Implications**

The mature female greater one-horned rhinoceros will breed throughout the year. Based on year-long fecal hormone metabolite analyses (Schwarzenberger et al., 2000) and urine hormone metabolite analyses paired with serial ultrasound exams (Stoops et al., 2004), it appears the female greater one-horned rhino is consistently exhibiting reproductive cycles throughout the year, and hormone concentrations associated with the cycles do not differ by season. Although some of these cycles are anovulatory, data are insufficient to determine if anovulation is seasonally influenced (Stoops et al., 2004) and documentation of anovulatory cycles during all seasons of the year would argue against a seasonal association (Stoops, unpublished data). Similarly, male greater one-horned rhinos will mate with females throughout the year. Furthermore, ejaculate sperm concentration is high and semen quality good when collected by electroejaculation throughout the year, and no seasonal influences on either of these characteristics or serum testosterone concentrations have been reported (Stoops et al., 2010).

## Reproductive Cycle

The female greater one-horned rhinoceros is a spontaneous ovulator with a somewhat variable cycle length. Although most reports are in agreement that the average cycle length for a greater one-horned rhino is approximately 43 days, the range (36-61 days) is greater than that typically observed in other rhino species making the greater one-horned rhino's estrus somewhat more difficult to predict (Kassam and Lasley, 1981; Kasman et al., 1986; Hodges and Green, 1989; Schwarzenberger et al., 2000; Stoops et al., 2004). Most of the variation appears to be in the length of the luteal phase since the follicular phase is fairly consistent at about 14 days, which is a much longer follicular phase than those observed in other rhino species (Stoops et al., 2004). The greater one-horned rhino also differs from other rhinos by the size of follicle produced prior to ovulation. The greater one-horned rhino's preovulatory follicle is the largest reported for any mammalian species and measures about 12 cm in diameter. Its pattern of growth also is unusual in that it reaches maximum size many days before ovulation instead of just prior to ovulation (Stoops et al., 2004).

## Peak Breeding Season for Greater One-Horned Rhinos in North America

The distribution of births by month for greater-one horned rhinos is shown in Figure 4.1 for the 142 greater one-horned rhino births reported in the 2009 International studbook (Guldenschuh and von Houwald, 2009). Figure 4.2 shows the months of conception for greater-one horned rhinos based on an approximate gestation of 480 days. Based on these data, it is clear that greater one-horned rhinos are fertile and conceive throughout the year. The trend towards more fertile matings during the interval from March to September is almost certainly management related since temperate zoos are more likely to pair their rhinos for mating during months with good weather.

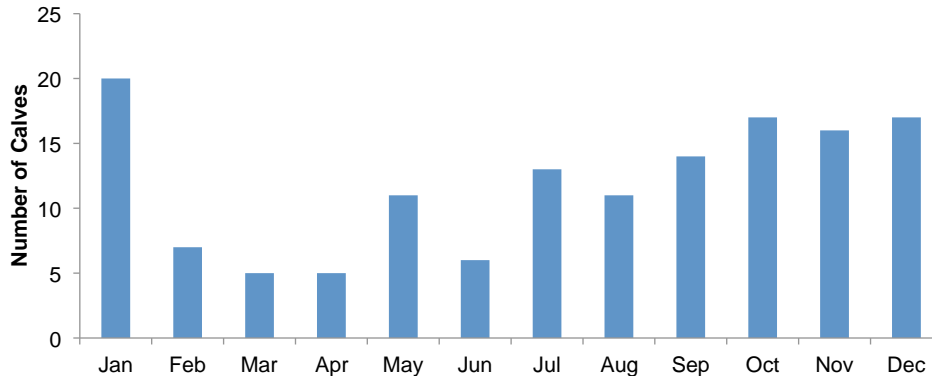


Fig. 4.1. Number of greater one-horned rhino calves born per month (n=142).

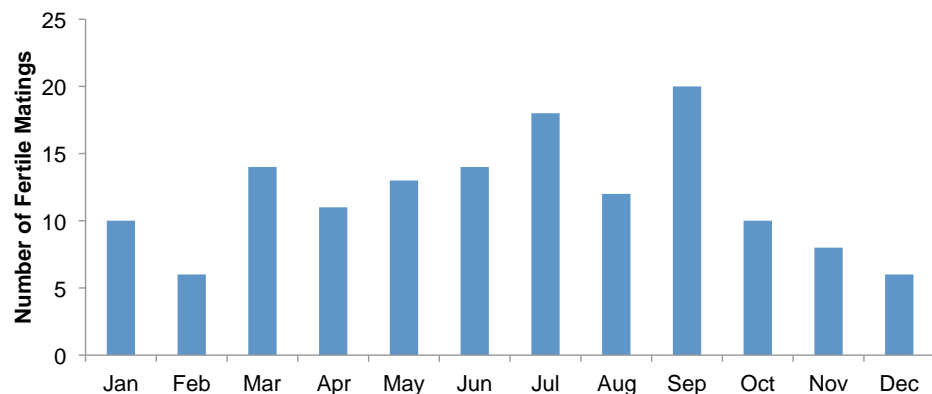


Fig. 4.2. Number of fertile greater one-horned rhino matings by month (n=142).

## Reproductive Monitoring and its Use in Timing Introductions for Mating

Although institutions with very large greater one-horned rhino enclosures have been very successful in breeding the species by simply allowing the bull to run with the cows much of the year and minimizing any intervention by animal care staff, most zoos do not have this luxury. For smaller facilities and more aggressive pairs, reproductive monitoring is extremely useful in timing introductions. This is especially true for the females that fail to exhibit behavioral estrus. Although estrous behaviors (including urine spraying, pacing, anorexia, vocalizations) can be very flamboyant in this species, some females, during some or all cycles, fail to exhibit any signs of estrus. Fortunately, greater one-horned rhinos produce very high concentrations of estrogen metabolites in their urine during their follicular phase, thereby providing an ideal non-invasive reproductive monitoring tool. Once a female's baseline urinary estrogen concentration is determined, the start of the follicular phase can be identified accurately as the first day urinary estrogen rises above baseline, and estrus can be predicted to occur approximately 12 days later (Stoops et al., 2004). Although ultrasonography can be useful in confirming that a dominant or preovulatory follicle is present, it cannot be used alone to accurately determine the day of estrus. In contrast, ultrasonography is essential to confirm ovulation after mating since anovulatory cycles often are associated with hormone profiles that appear identical to those of ovulatory cycles (Stoops et al., 2004).

Several hormones and their metabolites have been employed for monitoring reproductive activity in the greater one-horned rhinoceros (Table 4.8). Estrogen metabolites excreted by the greater one-horned rhino accurately reflect reproductive status, whereas in the other rhino species, only progesterone metabolite monitoring has proven useful for tracking ovarian activity.

Table 4.8. Reproductive hormones monitored in the greater one-horned rhinoceros.\*

Sample	Estrogen	Progesterone	Testosterone	LH	Other
Urine	♀ <sup>2,1,5</sup>	♀ <sup>3,2,5</sup>		♀ <sup>5</sup>	
Feces	♀ <sup>4</sup>	♀ <sup>4</sup>	♀ <sup>4</sup>		
Serum			♂ <sup>6</sup>		
Saliva		♀ <sup>7</sup>	♀ <sup>7</sup>		

\* Table adapted from Roth, 2006.

<sup>1</sup> Kassam and Lasley, 1981; <sup>2</sup> Kasman et al., 1986; <sup>3</sup> Hodges and Green, 1989; <sup>4</sup> Schwarzenberber et al., 2000;

<sup>5</sup> Stoops et al., 2004; <sup>6</sup> Stoops et al., 2010; <sup>7</sup> Gomez et al., 2004.

## Pregnancy Detection and Loss

Early pregnancy in the greater one-horned rhino has been diagnosed 18 days after ovulation using ultrasonography to identify the embryonic vesicle within the uterine lumen (Stoops et al., 2007). However, early diagnosis by ultrasound can be challenging in this species because portions of the uterine horns drop out of view to a depth that the ultrasound is incapable of penetrating. Therefore, early diagnosis by ultrasound examination is not always possible. However, urinary and fecal progesterin metabolites can be used to confirm pregnancy after three months of gestation because concentrations exceed those measured during the luteal phase. Throughout gestation, images of the fetus, fluid or membranes may be obtained via trans-abdominal ultrasound examinations, but due to the size and thickness of the greater one-horned rhino's skin, not every



exam yields good quality images (Stoops, pers. comm.). Early embryonic loss has now been documented in the greater one-horned rhino (Stoops et al., 2007), just as it has been documented in all other captive rhino species. Unfortunately, this species appears to be especially prone to miscarrying or producing stillborn offspring. Although such occurrences are more common in primiparous cows, many proven dams also fail to produce viable calves in subsequent pregnancies (Kock and Garnier, 1993; Pluhacek et al., 2007).

## **Reproductive Technologies (Semen Collection, Artificial Insemination, etc.)**

Semen collection by electroejaculation has proven very successful for collecting good quality greater one-horned rhino ejaculates (Roth et al., 2005; Stoops et al., 2010) and effective cryopreservation protocols have been established (Stoops et al., 2010). Post-thaw functionality of these samples has been proven by the production of multiple pregnancies following their use in artificial insemination procedures (Stoops et al., 2007). In the past, semen was collected by manual stimulation (Schaffer et al., 1990), but subsequent attempts to collect good ejaculates by manual stimulation in any rhino species have largely failed.

Artificial insemination (AI) techniques have also been established for this species. AI can be conducted either manually with customized insemination tubing that is guided by hand into and through the tortuous cervix or by using a standard flexible colonoscope that is inserted through the cervix with tubing in the instrument port for the insemination (Stoops et al., 2007). Method of choice depends upon the individual rhino's tolerance of vaginal manipulations.

Neither *in vitro* fertilization nor embryo transfer have been attempted in this species and would be considered highly experimental. However, ovaries obtained post-mortem could provide a source of oocytes for initiating some preliminary research on these higher-tech applications.

## **Fertility Assessment**

Male fertility assessments should include: 1) an ultrasound examination of the testicles to evaluate tissue consistency and size, 2) electroejaculation to confirm sperm production, and 3) serum testosterone analysis. It is important to note that a positive result from electroejaculation is very meaningful, but a negative result should not be used alone to diagnose a male with infertility because electroejaculation is not always successful in producing good quality sperm samples, even when conducted on proven bulls.

Female rhinos should be examined by rectal ultrasound to determine if any masses or cysts exist in the vagina or uterus. These types of pathologies appear to be very prevalent in older rhinos of this species and some masses grow to be life-threatening (Montali et al., 1982; Kock and Garnier, 1993). Ultrasonography can also be used to determine if the ovaries are active, however, any fertility assessment should include non-invasive urinary estrogen metabolite analysis which is more informative for assessing ovarian activity than a single ultrasound exam of the ovaries. Given the relatively long and sometimes variable reproductive cycle of this species, samples should be collected and evaluated for at least three months.

## **Genome Resource Banking**

A greater one-horned rhino sperm bank already exists at the Cincinnati Zoo's Center for Conservation and Research of Endangered Wildlife (Stoops et al., 2010). Samples include both electroejaculated semen and sperm rescued from the epididymis of testicles recovered post-mortem. Many zoos have participated in this collaborative effort to collect and bank semen from

male greater one-horned rhinos. An effort was made to target the most genetically valuable males in the North American population. Some of this semen is currently being used for AI procedures, but some will be stored long term. Samples include at least one male that is deceased without ever siring offspring. Fibroblast cell lines from many greater one-horned rhinos are banked in San Diego Zoo's Frozen Zoo. Oocytes and embryos have not yet been banked and additional research is necessary before such efforts will be fruitful.

## **Challenges**

Breeding greater one-horned rhinos in captivity can be quite successful if the enclosure space is large or the pair is behaviorally compatible. However, there continue to be problems at many zoos with incompatible pairs. In addition, females often experience an estrus without exhibiting any behavioral changes so animal care staff have a difficult time determining when the rhinos should be paired for breeding. Urinary estrogen metabolite monitoring offers a means of overcoming these challenges, but sample collection requires diligent commitment and is easier to achieve in some facilities than in others. Artificial insemination is another option that has proven successful for overcoming the challenge of trying to breed behaviorally incompatible pairs. Perhaps one of the greatest mysteries of greater one-horned rhino reproduction is the high percentage of stillborn calves produced, even from proven females. Research to investigate the causes of this phenomenon is needed.

## ***ENRICHMENT***

A planned and implemented enrichment program can contribute to better health by providing animals with opportunities to exert some form of control over their environment (Carlstead and Shepherdson, 1994; Baser, 1998). As it pertains to zoo-managed rhinos, we strive to encourage species-specific behaviors in rhinos while providing them response options to environmental change. This will ultimately result in their mental stimulation and the development of naturalistic behaviors (Swaigood and Shepherdson, 2005).

Each institution may have its own set of goals and criteria for its program, which fundamentally, should start with an examination of the animal's natural history, activity patterns and behaviors seen in the wild. For Indian rhinos, pools provide an important form of environmental enrichment. Since rhinos spend the majority of their day eating, keepers could look at modifying feeding schedules or providing enrichment throughout the day rather than just once a day. Keepers also could investigate the possibility of changing group composition at some facilities.

Holding facilities, exhibit spaces and/or any constraints that may be unique to the facility should be examined since these areas will directly impact options for enrichment. The temperament and behavior of the specific individual(s) also needs to be taken into consideration. Once these criteria have been reviewed, a set of goals can be established. Generally speaking the primary goal should be to "promote opportunities for the expression of species-appropriate behaviors" (Joseph and Sevenich, 1999). The Rhinoceros Husbandry Resource Manual (Fouraker and Wagener, 1996) clearly delineated the various functions enrichment can serve, such as (1) improving the well-being of the animal by increasing exercise, satisfying behavioral needs, and optimizing the level of stimulation that animals receive; (2) educating zoo visitors by increasing the levels of natural and interesting behaviors, visibility and activity levels; and (3) conserving endangered species by improving the success of breeding and reintroduction programs. The second goal of enrichment can be loosely defined as creating mental stimulation for the animal(s)(Fig. 4.3).

A successful enrichment program can be briefly summarized as one that:

- a) Establishes goals for the program
- b) Creates an enrichment approval form
  - States the purpose/goal of this enrichment
  - Provides a detailed description of enrichment items (construction material, thickness, dimensions, size of holes, etc.)
  - Identifies and addresses facility or exhibit constraints
  - Identifies and addresses safety concerns
  - Cost estimates
- c) Identifies approval protocols for enrichment submissions
- d) Creates an enrichment calendar (monthly or weekly) to ensure implementation schedule
- e) Determines how staff will document and/or track animal response to enrichment offered

To accomplish the goals mentioned above, an enrichment plan should be tailored with a set of criteria for either the individual or the species in general. The final proposal should then be submitted through the appropriate channels for approval. Ideally, an enrichment approval system should be set up to allow keepers, managers, and veterinarians the ability to assess the proposed enrichment and approve/reject it. Institutions can alter their enrichment scheduling as well as vary the type of enrichment offered (e.g., toy, food, sensory, environmental, behavioral and social) and keep track through their record-keeping or on a barn calendar (Connett, 2009). One of the most intimidating aspects of enrichment is allocating the time to document animal behavior and/or responses to the enrichment offered, however observations of responses can either be done in a direct or indirect manner as time allows. This data will enable evaluation as to whether the enrichment goals have been met. Since enrichment is dynamic in nature, adjustments can be made at any time so that the most effective enrichment is offered to the animal(s).



Fig. 4.3. Enrichment items, such as a plastic drum, can create a more stimulating environment (photo taken at Cincinnati Zoo and Botanical Garden).

Enrichment can correlate aspects of ethology, psychology and animal husbandry to create a more stimulating environment for the animal (Mellen and Ellis, 1996). This has led to the inclusion of enrichment options in exhibit designs. Exhibit enrichment can be done by varying topography, landscaping, utilizing deadfall and trees, creating dirt mounds, planting vegetation, providing a wallow, and alternating the substrate (dirt, leaf litter, mulch, etc.). Other options (public view versus privacy, shaded area versus sun, etc.) can all provide the animal some control over their environment and the ability to make choices throughout the day. The aforementioned exhibit variables can contribute to effective enrichment, especially when used in conjunction with other approved enrichment activities. For example, novel scents/perfumes/extracts can be used to create a “trail” throughout the exhibit, while holes drilled in deadfall can serve as an anchoring point for browse, and either one can provide the potential for exploration and create options for the animal. The American Association of Zookeepers (AAZK) has created The Enrichment Notebook (Chan, 2004) that provides suggested guidelines and contains information on exhibit enrichment, dietary enrichment and a section on safety considerations. When using enrichment devices that are awkward and heavy, it is important to secure these items safely, not only for the animals, but keeper staff as well. Fortunately, these somewhat bulky items can be secured safely by using pulley systems and/or other equipment can be used to hoist devices to enable hanging them higher. A synopsis of rhino enrichment options that are currently being used at zoological facilities can be found in Table 4.9. This table gives suggestions as to the primary area of use as well as its presentation. Note that food items, especially biscuits, treats, and produce, should be fed sparingly. Examples of enrichment type and some options that can be used are outlined below. These options must be used in compliance with a facility’s enrichment protocol, regulation and safety considerations.

Toy:

- Boomer Ball (Boomer Ball, Grayslake, Illinois)
- Weeble (Otto Environmental, LLC., Milwaukee, Wisconsin)
- Suspended log

Food:

- Scatter food around exhibit to stimulate grazing/foraging
- Place food items in enrichment devices to be randomly dispensed
- Fruits and vegetables frozen in bucket of water

Sensory:

- Use conspecific’s dung for smell (olfaction)
- Play different animal vocalizations or hang bamboo “chimes” for hearing (audition)
- Mount street sweeper brush for touch (tactition)

Environmental:

- Changing substrate (leaf litter, dirt, mulch, etc.)
- Altering daily routine
- Rotating to different enclosure (pen)

Behavioral:

- Training new behaviors
- Training for veterinary procedures

Social:

- Creating mixed species exhibit
- Rotating individuals (add or remove animals when possible)

Training for husbandry behaviors is not only stimulating for the animal, but it also can provide an invaluable opportunity for the veterinarian to perform diagnostic procedures with potentially less stress and more cooperation from the animal(s) (Dover, et al., 1994). The chapter for training will delve more deeply into this topic.

Table 4.9. Synopsis of rhinoceros enrichment ideas.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
<i>Amazing Graze</i> <sup>1</sup> (with biscuits/treats/produce)	X	X		X
Audio clips of vocalizations (rhino, other sp.)		X	X	
Beer keg		X	X	X
Biscuits (gorilla-sized leaf eater; horse treats)	X	X		X
Boat mooring buoy		X	X	
<i>Boomer Ball</i> <sup>2</sup>	X	X	X	X
Bowling ball		X		X
Branch/twig with produce skewered on it	X	X	X	X
Browse or approved plants	X	X	X	X
Brush	X	X	X	
Brushing by keeper		X		
Cardboard box (with or without produce)		X		X
Firehose “wall”		X	X	
Ice block (with or without flavor/produce)	X	X		X
Large cardboard tube		X		X
Log, stump, rootball	X	X	X	X
Melon (whole or chunks)	X	X		X
Mirror		X	X	
Mister	X	X	X	
Mud wallow	X	X		
Non-radial tire (with cuts in sidewall for safety)		X	X	
Oblong stone	X	X		X
Painting with non-toxic finger paint		X		
Paper grain bag filled with hay or produce		X	X	X
Paper mache ball	X	X	X	X
Peanut butter (on toys/walls/“furniture”)	X	X		
Planter bucket	X	X	X	X
Plastic soda concentrate container		X	X	X
Plastic drum (55 gal.)		X	X	X
Plastic ice block or iceberg toy		X		X
Plastic jug feeder		X	X	
Produce	X	X	X	X
PVC or bamboo chimes		X	X	
PVC tube with bells suspended inside		X	X	
Rolling treat bucket/feeder		X		X
Scent, flavoring, or extract	X	X		X
Snow-pile, -people (with or without fruit mix)		X		X
Substrate pile or change (dirt/gravel/sand/leaf)	X	X		
Traffic cone or pylon		X	X	
Training with keeper	X	X		



Table 4.9. Continued.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
Triangle rattle		X	X	
Urine, feces, or soiled bedding from others	X	X		X
<i>Weeble</i> <sup>3</sup>	X	X		X
<i>Weeto</i> <sup>3</sup>	X	X		X

<sup>a</sup> Area of use may be dictated by institutional guidelines and criteria

<sup>b</sup> Presentation format is based on that which is most commonly used

<sup>1</sup> Sanctuary Supplies, Rootstown, OH; [sanctuarysupplies.com](http://sanctuarysupplies.com)

<sup>2</sup> Boomer Ball, Grayslake, IL; [boomerball.com](http://boomerball.com)

<sup>3</sup> Otto Environmental, LLC., Milwaukee, WI; [ottoenvironmental.com](http://ottoenvironmental.com)

## TRAINING

A husbandry training program is considered an integral component of progressive animal husbandry programs in zoos today. Providing zoo-managed animals with choices and behavioral opportunities in their environments through a training program can result in healthier animals and better educational experiences for the zoo visitors. Training rhinos leads to a wide variety of animal management opportunities, from shifting to safer, less stressful medical procedures.

The purpose of this chapter is to give trainers an idea of the types of behaviors that can be trained with rhinos (Appendix B). This chapter will also give direction on starting a training program, ideas for shaping techniques, possible reinforcements, specific challenges to training rhinos, and resources for additional training information.

### Setting Up a Training Program

A well-planned, consistently-delivered training process is critical to the success of any program. To achieve this type of program many facilities utilize a framework that is taught in an Association of Zoos and Aquariums (AZA) course, Managing Animal Enrichment and Training Programs. This framework is called the “SPIDER” model. Steps in this framework include: Setting Goals, Planning, Implementation, Documentation, Evaluation, and Re-adjusting. For more information on this process, please see [www.animaltraining.org](http://www.animaltraining.org). It is beneficial to start a training program by determining the overall behavioral goals (i.e., detailing the specific behaviors to be trained). During this goal development process it is important to include all parties involved with the management of the animals. This may include meeting with and seeking feedback from keepers, veterinary staff, nutritionists, behavioral husbandry staff, curators, and managers. Having everyone on the same page with clearly laid-out plans, assignments, and timelines helps to facilitate a smooth process. Defining roles and creating clear channels of communication and decision making authority among all participants is also important. This can be accomplished through regularly scheduled team meetings, a consistent method of documentation, and continual communication among all staff involved in training. Facility design can have an effect on setting up a training program. Discussions must also include how the rhino will be reinforced. The next two sections will discuss both of these topics further.

### Facility

When beginning a training program, it is important to start training in an area that is safe for the animal care staff and the animal, and where the rhino is comfortable. This is usually the

night quarters or holding area (Fig. 4.4). Training can also be done in barn stalls, outdoor barn paddocks, training chutes, or even open exhibit areas. Because all facility designs are different, training staff will have to be creative and utilize the space available. For examples of possible body positioning behaviors, see Appendix B. For many of these behaviors trainers will require areas with no or minimal obstructions, which allow safe access to the desired body parts. It is important to remember that a fancy, expensive facility is not necessary to accomplish a successful training program, just a creative mind.



Fig. 4.4. Indoor chute for training and procedures (courtesy of C. Nordin, St. Louis Zoo).

### ***Reinforcement***

A critical component in training is finding a positive reinforcement or a reward for which an animal is willing to work. In most cases, the rhino's regular diet can be utilized for training. Any manner of storage container for the reinforcement can be used. Reinforcement items need to be easily retrieved and delivered as soon as possible after the desired behavior occurs. Reinforcement can be delivered by tossing it through posts or by hand feeding if it can be safely accomplished.

### **Training Methods**

There are several steps to creating a husbandry training program for rhinos. After the behavioral goals are set, a safe facility to work the animals is identified, reinforcement type and how the reinforcement will be delivered has been determined, the next steps are learning about the animal to be trained, building a relationship with that animal, and designing a training plan. An overview on learning and training can be found in Mellen and Ellis (1996).

### ***Natural History/Individual History***

An understanding of the natural history of rhinos, paired with information about an individual animal's specific background and level of experience, play an integral part in a husbandry training program. For example, wild-caught or hand-reared rhinos may react differently to trainers when compared to captive, parent-reared individuals. It is helpful to start training in a location where the animal is most comfortable. Some animals need to have more space available for them to feel comfortable within the training environment. The space needs for a rhino often center around

the size of the enclosure in which the animal is being trained. Other relevant issues regarding an animal's space requirement may include proximity to conspecifics, other species, and issues such as noise levels and extraneous activity. Keeping training sessions short and moving at a comfortable pace for the rhino may help keep their focus. Tactile interaction is important—most rhinos enjoy being scratched. Care must be taken to avoid startling the animal or putting yourself in the danger zones—areas where the rhino is able to horn, kick, or injure someone. When desensitized, rhinos will interact positively with their keepers and will seek out tactile interaction. Keepers need to always maintain awareness and caution as startled rhinos can react violently and quickly. Studying the animal's natural history helps to gain insight into the animal's behavior. For more information on rhino natural history, see the Behavior chapter of this manual. Insight can also be gathered from staff, the animal's ZIMS records, or other institutions about the animal's individual history. There is no substitute for simple observation—just watching the rhino's behavior in different situations, for example, what it looks like when the rhino is calm or aggressive. Watch the rhino on and off exhibit, at different times throughout the day, and when the rhino is unaware it is being observed. This greatly helps understanding and interpretation of the rhino's behavior during a training session.

### ***Relationship***

Developing a trusting relationship between the trainer and the animal being worked with can be very beneficial to training. If the animal is not comfortable being in close proximity to people, the rhino might back away from the trainer or try to horn the trainer. Developing a relationship can help address these issues. A relationship can be developed through normal daily animal care, such as feeding, observing, regular cleaning routines, and avoiding negative interactions. The more positive interactions the animal has with staff, the more comfortable the rhino will be. For example, instead of just putting the diet in the enclosure, the trainer can hand-feed the diet. If the animal is particularly skittish, the trainer may start by placing or tossing the food in the enclosure and sitting nearby while the rhino eats. As the animal becomes habituated to the trainer's presence, s/he can begin to hand-feed and work their positioning closer and closer to the animal.

### ***Training Plans***

Creating a training plan can be a good process to help trainers think through what steps they are going to take to train a behavior. One way to do this is to establish what the final behavior will look like, and then break-down the behavior into a series of small steps called "successive approximations". Examples of training plans can be found in Appendix C.

### ***Bridging Stimulus***

Utilizing a bridging stimulus can be a very useful tool in training rhinos but not a necessity. A bridge is a signal that pinpoints the exact moment in time the behavioral criterion was met. First a trainer must select a bridge. Common bridges used are clickers or whistles. Once selected, the trainer needs to associate the sound with the delivery of reinforcement. For example, if the bridge selected was the clicker, the trainer would click and feed, click and feed, and continue this for several sessions with the rhino. Once the association has been established, the sound of the clicker with a food reward, the trainer can begin to use the bridge to shape behaviors. For more information on a bridging stimulus, see Pryor (1984), Mellen and Ellis (1996), and Ramirez (1999).

### ***Shaping Behavior***

For consistency, one trainer should shape new behaviors with an animal. Once the behaviors are trained and performed on cue consistently, other trainers can work with the animal. A shaping technique that works well with rhinos is using the sight of food as a lure. In most cases, rhinos focus

on the food and follow it wherever it is placed. Trainers can also use their body positioning when shaping behaviors. The animal will, on many occasions, shift its body position when the trainer moves. For example, if a rhino is facing a trainer and the trainer takes a step to the left, many times the rhino will follow and shift its body to the left as well. Another useful tool in the shaping process is a target. A target is an object to which an animal is trained to touch a part of its body (Mellen and Ellis, 1996). Training a rhino to target different parts of its body is a good method of shaping many body-positioning behaviors such as standing, presenting a side of the body, or presenting a foot (see Appendix C). When starting a program it is best to begin by training some basic body-positioning behaviors such as open mouth, target, and back before moving on to more complex behaviors like standing for blood collection or other veterinary procedures.

### **Record Keeping**

It is important for trainers to keep records of all sessions. Trainers can go back and look for patterns in behavior, which helps keep consistency among trainers and leaves a historical record for others. For an example of a documentation form, please see Appendix D.

### **Safety**

Establishing a safety protocol is another valuable component to a rhino training program. These protocols will allow a facility and training team to set clear guidelines to ensure the safety and well-being of the trainers and the animals. The disposition of the individual rhinos should be taken into account during any training session, and the ability to read the animal's body language and temperament will allow the trainer to know when the rhino is agitated and the session needs to end. In general, it is advisable to end a session on a positive note and not wait until an animal is agitated.

In any training session where the trainer is in close proximity to the rhino, as in blood-draws, it is important for keepers to work in groups of two or more. If there are multiple keepers involved in a session, then there are multiple people that are able to watch the keeper that is working close to the rhino, the rhino's behavior, and the location of its head and horn(s).

### **Summary and Resources**

The purpose of this chapter is to give trainers an idea of the type of behaviors that can be trained with rhinos and what these behaviors might look like. This chapter provides direction on starting a training program, ideas for shaping techniques and reinforcement, some specific challenges to training rhinos, and resources for additional training information. This chapter is meant as a reference for basic training information and contains just a small amount of the information that is available.

The following is a list of additional resources that can be helpful in developing a training program:

- *Animal Keeper's Forum*, a publication of the American Association of Zoo Keepers
- [www.animaltraining.org](http://www.animaltraining.org)
- Animal Training Organizations –
  - IMATA (International Marine Animal Trainers Association)
  - AAZK (American Association of Zoo Keepers)
  - IAATE (International Association of Avian Trainers and Educators)
  - ABMA (Animal Behavior Management Alliance)
  - PEM (Principles of Elephant Management)
- International Rhino Keeper Association (IRKA)
  - [www.rhinokeeperassociation.org](http://www.rhinokeeperassociation.org)

# TRANSPORTATION

Crating and shipping of rhinos is one of the most difficult transport procedures. While rhinos themselves are fairly hardy, the limitations of temperament, peculiarities of chemical immobilization, and rigorous shipping equipment necessitate a strict yet flexible protocol for optimizing successful crating and shipping.

## Pre-shipment Medical Procedures

Communication at the veterinary level between receiving and shipping institutions prior to rhino translocations is essential in order to discuss specific institutional and/or state requirements. Standard medical procedures for all moves should include the following: (1) a tuberculosis test within 30 days to six months of shipment (depending on where the rhino is being shipped) or as particular state, federal or international requirements dictate, (2) brucellosis serology if dictated by particular state or international requirements, (3) a physical examination, (4) three negative fecal screens 30 days prior to shipment, and (5) a review and update of vaccinations (see Health chapter). In addition, medical or research **protocols** defined by the SSP should be reviewed during the planning process.

## Crating

Crating is the recommended transport method, although transport in trailer stalls has also been successful. It is important in the latter case that the trailer is well-reinforced. In all situations, the animal's behavior and conditions should be constantly monitored. Typical problems that can occur in shipping include the following: (1) animals destroying and/or climbing out of the crate top; (2) animals becoming inverted in the crate and unable to right themselves; (3) animals destroying end panels or doors, resulting in eye, horn or facial injuries; and (4) prolonged, excessive exertion resulting in hyperthermia and/or myopathy.

## Design

The International Air Transport Association (IATA) crate design specifications are listed in Table 4.10. Crates are usually constructed of wood, metal, or wood with steel reinforcements. Crate dimensions should be determined by the animal's size (Table 4.10), but a general principle is that the crate should be 0.3 m (1 ft) longer and wider than the animal when it is lying on its side. Crates with vertical bars situated at the head end will decrease injuries to the head and face but must be spaced correctly with at least 10-15 cm (4-6 in) gaps. Horizontal bars at the head end should be avoided, as they tend to cause horn breakage and/or damage. Crates with bars and doors at both ends are optimal.

Table 4.10. Approximate crate dimensions by species (modified from IATA, 1995).

Species	Length	Height
Black rhino	271 cm (107 in)	191 cm (75 in)
White rhino	475 cm (187 in)	221 cm (87 in)
Greater one-horned rhino	335 cm (132 in)	201 cm (79 in)



## Principles of Design

Frames should be constructed of strong metal with sides of solid hardwood. Vertical metal bars should be bolted in place at the front and back with sliding or hinged wooden doors to the exterior of the bars. The upper third of the wooden doors must have ventilation spaces or openings. There are several new requirements for lower ventilation as well. IATA specifies that the roof must be solid over the animal's head and slatted over the loin and hindquarters for ventilation. For ground transportation, however, removable panels or hinged doors over the animal's head can be useful for administering to medical needs and monitoring the animal. Hatches also allow for more ventilation when an animal is standing calmly.

The interior must be smooth with no projections. Wooden crates have often been modified to include solid metal sheets at the head so that the horn deflects and cannot damage the wood. Only nuts and bolts should be used in the container. Entry and exit doors must be closed and bolted in strategic places to be strong enough to resist the animal, and to withstand the rigors of the equipment necessary to move the crate and rhino. The floor must be at least 2.5 cm (1 in) thick and be a non-slip surface. Some crate floors are slatted so that urine and feces can flow through so that the animal is not standing in excreta. For international and air transport, the container must be constructed in such a way that the floor and lower sides are leak-proof. In view of the diversity in size, strength and temperament of rhinos, the size and strength of the container must be sufficient to restrict the movement of and restrain the animal. Dimensions must be large enough to prevent cramping without allowing unnecessary movement. In general, the crate should be 0.3 m (1 ft) longer and wider than the animal when it is lying on its side.

At the front of the container, there must be provisions for water and food access at the base of the door and between the bars, if present. For air transport, this access point must be clearly marked FEEDING and be adequately secured when not in use. A water container must be provided and must be sufficiently large for the entry of the animal's muzzle. Some rhinos will not drink from a tub, and so offering a hose directly into the crate (either into the animal's mouth or just puddling in front of the rhino) is sufficient to provide water. Entrance and exit must be clearly indicated on crates used for air transport. Many crates are designed to be used in either direction. The above recommendations are modified from IATA standards to include specifications for ground transport. Before shipping by air, consult the current IATA specifications and/or the airline.

## Acclimation Training

Crate acclimation can require two to six weeks, although several zoos have crate-trained rhinos in seven days or less. Training should be completed by a method of approximation (with reinforcement given as rhinos demonstrate progress towards the desired behaviors). The first step is to introduce the crate as a non-interactive part of the animal's environment. Gradually, the food is moved toward and finally into the crate. If the animal acclimates to the point of completely entering the crate and will allow the door to be shut, the door should be left closed for short acclimation periods under close observation. It is not advisable to close the animal into the crate if the training period is short. No rhino likes to be closed into the crate, and even the best-trained rhinos will react negatively to being locked in. If there is not adequate time to train the rhino to acclimate to a closed crate, closing him/her in will only make the experience a negative one, and there will be a major setback to the ability to close the animal in for shipment. If the rhino does not completely acclimate to entering the crate, partial immobilization (standing restraint) may need to be utilized for shipping. Forced crating without training or immobilization is strongly discouraged.

## Crating with Chemical Immobilization

Immobilization offers a fairly simple way of crating a rhino. First, it should be noted that the usual pre-immobilization procedures (e.g., fasting, detainment in an adequate holding area, etc.) should be observed for any procedure requiring the use of chemical immobilization/tranquilization agents. For rhinos, etorphine (M-99) remains the drug of choice, although several alternatives are available. For specific drugs and dosages, refer to the Health chapter of this publication. The duration of immobilization without the administration of an antagonist may range from 30 minutes to two hours.

Following crating, all rhinos should be held for 24 hours at the loading location for observation or accompanied by a veterinarian during transport. This step is necessary because renarcotization is common in hoofed animals, especially rhinos, given opioids. This step, however, may not be necessary if the butorphanol/detomidine anesthetic protocol is used (P. Morris, pers. comm.). Trained personnel should be present to administer the correct reversal agent(s) in the event of a renarcotization. Any other complications of crating can be managed more easily and effectively before departure rather than en route.

## Transport

Numerous options for transporting rhinos are available. Each method has its advantages, and each should be scrutinized by evaluating the distance to be traveled, the personnel needed and the temperatures to which the animals will be subjected. A flat-bed truck and open trailer is temperature-restrictive. A crate within an open trailer should be protected from excessive wind, rain and sun. Enclosed trucks or trailers are other options that are necessary in extreme hot or cold temperatures. In any case, the transport vehicles must be climate-controlled if shipping in inclement weather (either hot or cold). If weather conditions and ventilation are appropriate, many rhinos have been moved in enclosed trailers without climate-control. Air transport, rather than ship transport, is the preferred option for any transoceanic translocation. Transport by ship is undesirable because of the excessive time at sea, variable conditions and more intensive personnel requirements. When transporting by air, it should be noted that some airlines may require the rhino crate to be placed in an aluminum air cargo box, which can restrict ventilation and subject the rhino to excessive heat buildup during both the airplane-loading process and transport.

During all rhino shipments, the shipper must be aware that any animal that has been immobilized (and to a lesser extent, some that have not) will be less capable of maintaining thermal homeostasis than a normal animal, and appropriate accommodations for this are necessary (e.g., ventilation, climate-control). If during the course of a transport procedure a situation arises in which the safety of the animal is jeopardized, a decision should be made through the appropriate channels to postpone or cancel the shipment. Leaving the decision of whether to transport an animal to the transporter or the recipient may be disadvantageous to the animal's welfare. In many cases, the transporter is not familiar with pertinent medical and practical husbandry information, and recipients are at a disadvantage because they are often not present.

# ENCLOSURE DESIGN

## Design

In designing facilities to maximize rhino health and reproductive success, it is important that the environment contain as many salient features as possible found in the natural environment (for more detailed information on the natural habitats of the various rhino species, see the Behavior chapter). The species to be exhibited will dictate the design of rhino facilities, as species differ in their group compositions and enclosure requirements. Additionally, whether an institution wishes to maintain rhinos only for exhibit or for breeding will determine the design of rhino enclosures. Whenever possible, institutions are encouraged to plan for breeding capabilities, but the various SSP coordinators and RAG chairs recognize the need for display-only exhibits which can facilitate education and/or research. These exhibits serve rhino management programs by holding non-reproductive and single-sex specimens. The following section outlines design considerations for indoor and outdoor rhino facilities, as well as aspects of chute design for rhino restraint.

## Enclosures

The design of zoo enclosures for rhinos requires an understanding of rhino biology, behavior and social organization. As previously stated, black, white and greater one-horned rhinos vary in their levels of sociality and thus have different housing requirements. The design of rhino enclosures also depends on the type of rhino program (exhibit only or breeding) and the number of animals. In all cases, the larger and more varied the enclosure, the better. Tables 4.11 and 4.12 list the recommended animal numbers for institutions holding rhinos (see Management chapter) and the enclosure types recommended depending on institutional goals. It is important to note that for the most part, exhibit and holding-space availability will dictate an institution's designation as either a breeding or an exhibit facility. Design elements for a breeding facility should include an outdoor primary enclosure (with separation capabilities), indoor holding and an isolation area. Additionally, breeding institutions must have space for any offspring to be held for up to three years of age. Exhibit-only facilities should have an outdoor primary enclosure and indoor holding areas (both with separation capabilities). It will be recommended that exhibit-only facilities receive pre- or post-reproductive-age or single-sex groups of animals.

Table 4.11. Recommended numbers for institutional holding.

Rhino Species	Recommended Minimum Groupings for Breeding <sup>a</sup>	Preferred Optimal Holding for a Breeding Institution	Exhibit Only (per institution)
Black	1.1	2.2 (2 pairs)	1.1 or 0.2
White	1.2	2.4 (1 herd/ 1 back-up male)	1.1 or 0.2 <sup>b</sup>
Greater one-horned	1.1 <sup>c</sup>	2.2 (2 pairs)	1.1 <sup>c</sup> or 0.2

<sup>a</sup>Breeding institutions must have space for offspring to be held for up to 3 years following birth.

<sup>b</sup>Multi-male bachelor groups have been maintained in very large enclosures.

<sup>c</sup>In the case of greater one-horned rhinos, males and females should be introduced only during the female's estrous period. Institutions with very large enclosures may be able to hold opposite-sex animals together consistently.

In general, it is recommended that enclosures be designed such that animals may be kept outdoors as much as is possible within the following temperature constraints. Rhinos should not be locked outside when the temperature is below 4.4°C (40°F); sun, wind chill and rain should be considered in calculating temperature. During extremely cold weather, rhinos should not have access to pools or mud wallows; pools should be drained and mud wallows filled with substrate. Animals should not be let out if enclosures are icy. Temporary exposure to temperatures below 4.4°C (40°F) for cleaning is left to the discretion of management. Localities that experience average daily temperatures below 10°C (50°F; average of high and low temperatures over a 24-hr period), should provide heated facilities capable of maintaining a minimum temperature of 13°C (55°F).

Table 4.12. Recommended enclosure types and sizes for zoo-housed rhinos by species [in sq m and (sq ft)].

	Individual Holding (per rhino)		Exhibit Only (per rhino)		Breeding/Communal	
	Indoor	Outdoor	Indoor (as primary exhibit area)	Outdoor	Indoor	Outdoor
Black	18 (200)	186 (2,000)	204 (2,200)	771 (8,300)	not recommended	2,322 (25,000)
White	30 (320)	186 (2,000)	215 (2,320)	929 (10,000)	not recommended	2,787 (30,000)
Greater one-horned	30 (320)	186 (2,000)	215 (2,320)	929 (10,000)	not recommended	2,787 (30,000)

## Outdoor Housing

Several general outdoor enclosure designs are recommended that incorporate the data available on the behavior and ecology of wild black, white and greater one-horned rhinos. As previously described, rhinos of all species may be less solitary than was originally thought. Given very large yards or ranch situations, even opposite-sex greater one-horned rhinos (considered the least social of the commonly held rhino species) may be housed together. For the most part, however, rhinos are somewhat territorial; therefore, more than one outdoor yard is strongly recommended. To provide a large area for introductions of greater one-horned rhinos, a communal yard adjacent to individual yards should be available. If the space is not available, two adjacent yards may be opened for male/female introductions. In many respects, the critical enclosure characteristic is the availability of escape routes and visual barriers, which serve to hide or prevent access to a pursued animal. Gates may be used as escape routes, provided that care is taken to prevent dead-end corners and to create “run-arounds” (brush piles, earth, or boulders) so that an animal can enter or leave the yard without an aggressor blocking or guarding the only exit.

Enclosure size depends on whether rhinos are kept for exhibit-only or breeding purposes (Table 4.12). It should be noted that a calf is considered an adult with respect to minimum space requirements after weaning; this should be considered in determining minimum enclosure size.

## **Primary Barriers**

The barrier between rhinos and the viewing public is a critical element in the design of the outdoor exhibit. This primary barrier should allow visitors a clear view of the animals from a safe location. Many types of primary barriers are available, the most common of which are walls, fencing, dry moats and water moats. One consideration in choosing fence type should be the size of the enclosure. For example, smaller exhibits should be constructed with barriers that provide as much visual exposure as possible. Moats, both dry and water, are less desirable for breeding groups because of the potential for accidents.

## **Fencing**

Because any of the rhino species may climb, a primary barrier should be a minimum of 1.5 m (5 ft) high and non-climbable. In small enclosures, particular attention should be given to the climbing ability of rhinos and to the need for separating aggressive animals. A secondary barrier or a taller primary barrier may serve to counter these problems. It is important to consider fence spacing and keeper access/exit in the event of an emergency as well.

Recommended materials for primary fencing include solid concrete or rock walls, horizontal pipe or cable spaced 25 to 30 cm (10-12 in) apart and vertical pipe or posts spaced 25 to 30 cm (10-12 in) apart. Cable should be used only for horizontal fences. The size of the exhibit to be fenced will determine the strength and type of fencing material used, as each type has both advantages and disadvantages. Concrete surfaces and bare steel cable create surfaces that may encourage rhinos to horn-rub excessively, causing abnormal horn wear. If necessary, surfaces should be covered with a non-abrasive material; one solution is to insert steel cable through plastic pipe, or concrete surfaces can be covered with non-toxic wood.

If poles are used, each should be approximately 30 cm (12 in) in diameter and set in concrete with approximately 1.8 m (6 ft) underground. Poles should be spaced as closely together as possible to prevent rhinos from getting their horns through and uprooting the fence. ***Creosote-treated poles, which are dangerous to rhinos, should not be used.***

Rocks or a rock apron can be utilized to protect the poles or other objects in the exhibit from damage. A rock apron should extend 1.8 m (6 ft) from the leading edge of the object to offer adequate protection. If small rocks are used, they should be several layers thick; otherwise, a single layer of very large rocks is probably adequate.

## **Dry Moat**

The use of a dry moat requires one vertical wall, which should be a minimum of 1.5 m (5 ft) high, located on the public side. The second wall should be sloped at a maximum of 30° so that the animals can climb out. This gradual decline of the exhibit substrate down to a solid wall can be used to create a moat effect, but ***ditch moats with two vertical walls are considered dangerous to rhinos and are not recommended.*** The floor space in the moat should be a minimum of 1.5 m (5 ft) across to prevent rhinos from being trapped, and surface substrate for the moat should provide stable footing (recommended materials include dirt, gravel, sand, etc.).

## **Water Moat**

Water can be utilized as a primary barrier, although it carries the risk of drowning or injury, especially for calves. Dimensions and specifications for a water moat should be the same as those listed above for a dry moat. Pools may also serve as primary barriers, provided the walls are high enough [at least 1.5 m (5 ft) high] to prevent animals from climbing out. Once a rhino has its chin over the top of a wall, it can get its front feet over as well.



## ***Secondary Barriers***

Though not critical to the design of outdoor enclosures, secondary barriers may protect exhibit features or lessen stress on primary barriers. Recommended types are butt rails, vertical poles and electrically charged, or “hot,” wire. Plantings can also serve as a secondary barrier when used to create a visual screen. For example, plantings that extend above a low wall can give the appearance of a bigger wall [although the primary barrier height minimum of 1.5 m (5 ft) still applies]. Electric fencing can be used to deter animals from destroying plantings, trees and other secondary barriers. Rock aprons may also be used around trees and fence lines as secondary barriers.

## ***Gates***

Enclosure gates can be the weakest points of the exhibit; therefore, adequate hinge and lock strengths are very important. Interior doors are usually constructed of heavy-gauge steel or pipe that is hinged or sliding. Sliding gates are optimal, as they have the ability for partial opening, and should be a minimum of 1.8 m (6 ft) wide and 2 m (6 ft 8 in) high. If the gate uses a track, care should be taken in the construction of the track to avoid injuring the feet of the animals as they run through gates during introductions. Exterior building doors may be made of steel or wood reinforced with steel, with the lower part covered by a steel plate to minimize damage. Gates should be constructed to allow keepers to open and close them without entering rhino space. Also, where appropriate, vehicle access to an enclosure should be provided.

There are a variety of options available to operate gates. The simplest systems are manually operated, either push-pull or cable driven. Rhino-size doors can be heavy and difficult to move. Mechanical systems can make this easier, using electric motors or hydraulic or pneumatic pressure to move the doors. These systems require some form of back-up system in case of a mechanical or power failure. Mechanical systems can generate significant forces that can injure or even kill an animal. Safety measures have to be incorporated into the design of the gate operating system to prevent accidents.

## ***Substrate***

The outdoor enclosure should have a well-drained surface that provides adequate footing for rhinos. Greater one-horned rhinos require substrates that “give,” including mulch (such as hardwood), grass (irrigation should be planned for all natural areas if needed) and bedding because of the incidence of foot problems. Rhinos should be carefully observed upon introduction to a new substrate, as excessive ingestion of the substrate from feeding on the ground has caused impaction in other hind-gut digesters.

## ***Water***

Fresh, potable water should be available at all times. Water should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be substantially constructed to prevent injury, upset, spillage or leakage.

## ***Mud Wallows***

All rhinos need access to pools and/or mud wallows for skin health, temperature regulation and behavioral enrichment. The size of mud wallows should be gauged by the number of animals in the exhibit so that ample room is provided for each individual. It should be noted that given a start, rhinos may construct their own mud wallows. Construction of wallows varies by institution and exhibit. Some facilities use a concrete basin or pool filled with substrate, while others simply dig out

a section of the enclosure. If a concrete pool is used, it should have the ability to be drained when needed, but care should also be taken to protect the drain line from being filled with substrate. If a wallow is dug on exhibit, it should be situated in an area that will allow adequate drainage away from the wallow. It is also important that a good source of water be nearby to keep the area wet. Wallows are much easier to manage if the enclosure allows access for a skid steer or tractor with front loading bucket.

Mud wallows must be actively managed. If the local soil does not create mud of a satisfying consistency, it can be adjusted with the addition of topsoil, clay or sand. Standing water in an animal area can be a potential USDA compliance issue. The wallow should be monitored for the growth of algae. If the algae cannot be removed by raking or scraping, it may be necessary to fill the area in to allow it to dry completely for several days, and then dug out again. Depending on the local environment, the substrate may need to be changed several times during a year to prevent contamination. Institutions in northern climates may need to fill in the wallow during winter months.

### **Pools**

When a calf is in the enclosure, pools should be modified or drained to a depth of 0.45 m (1.5 ft) or less. Ramps are preferable to steps for entry into the pool. They should be placed in at least two or three locations around a pool, if not the entire perimeter, to ensure safe access in and out of the pool. Ramp slopes should be no greater than 15 to 20°. If steps are used rather than ramps, they should have a 20- to 25-cm (8-10-in) rise with a 41- to 61-cm (16-24-in) step. Note that multiple entries to a pool prevent it from being a “dead end” in the enclosure. In the design of slopes or steps, keeper access for cleaning should be considered. The pool substrate should be broom-swept concrete. Table 4.13 lists pool specifications by species.

Table 4.13. Specifications for exhibit pools.

	Overall Size	Depth	Comments
Greater one-horned	37.2 sq m (400 sq ft)	At least 0.9 m (3 ft); optimally 1.5 m (5 ft)	Recommended

### **Visual Barriers**

Naturalistic visual and physical barriers (refuges) in outdoor enclosures may help decrease aggression by permitting animals to separate themselves from others if necessary during introductions or in a group situation. Barriers should be large and high enough to provide “safe zones” that allow an animal to pass from another’s sight but should not hinder public viewing. Types of visual barriers include deadfall, logs and boulders, as well as trees and natural plantings. Trees and plantings may be protected from rhinos by pipe caging, rock aprons or barrier fencing. If permanent physical structures are not available as barriers, dirt mounds may be used to give individuals additional visual barrier points in the enclosure.

### **Shade/Rain Shelter**

Access to shade is a necessity as well as a USDA requirement under the Animal Welfare Act. A variety of both natural and constructed options are possible. It is also important that a shade option be adequate as a rain shelter if barn shelter is not always accessible; therefore, trees may not be completely adequate. Pools should be located in areas that are shaded at least part of the day.

### ***Additional Furnishings***

Additional furnishings for the outdoor exhibit should include scratching posts, which may be particularly effective if placed near mud wallows or pools. Post materials must be non-toxic to rhinos (i.e., non-creosote). Several institutions have buried deadfall or logs upright in concrete sewer culverts, which are routed in place with 0.9 to 1.2 m (3-4 ft) of gravel. This enables managers to remove and replace posts as they deteriorate. Feed should be available at all times in the form of browse, feed stations and mineral salt licks.

### **Indoor Housing**

Indoor housing is recommended for additional separation capabilities (beyond the primary enclosure) and is critical for those institutions in colder latitudes. At no time should rhinos be forced to endure temperatures below freezing for any length of time; animals may go out for short periods when temperatures are below freezing, but they should have access to radiant heat or heated enclosures during these times. An indoor facility in the winter should be heated to a minimum of 13°C (55°F) with the capability of maintaining some areas of the barn at 23.9°C (75°F). Supplemental heat may be needed when dealing with infants or with sick or older animals. Some acclimation may be necessary before moving animals from a warm barn to the outdoors during winter months. The humidity level should be maintained at 40 to 70%. Shower sprays or water baths should be offered in areas of relatively low humidity. Indoor facilities should be maintained with a negative air pressure, and ventilation should be provided to accommodate at least four air exchanges per hour (USDA recommendations for a cold-weather heated barn). Institutions are encouraged to check with their local authorities for air-exchange requirements when the public or personnel occupy the facility.

Within any indoor facility, areas must be provided for food and water. Fresh water should be available at all times and should be changed daily or be supplied from an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset, spillage or leakage.

For greater one-horned rhinos, isolated stalls are essential. The indoor enclosure should include a minimum of 30 sq m (320 sq ft) per animal for greater one-horned rhinos (Table 4.12). An additional 50% of adult space should be provided when a calf is present. This may be achieved by using more than one stall. Following weaning, a calf should be treated as an adult individual with respect to space requirements. If the institution has only indoor facilities in which to maintain and/or exhibit rhinos, the minimum requirement is 186 sq m (2,000 sq ft) per rhino [15.2 x 12.2 m (50 x 40 ft)] plus the recommended indoor holding [30 sq m (320 sq ft) per individual for greater one-horned rhinos].

### ***Separation Capabilities***

The indoor facility should have the capacity to separate individuals for a variety of purposes. As indicated above, greater one-horned rhinos should be kept in individual stalls. The facility should also have an extra space or large stall to make it possible to isolate mothers and calves or quarantine sick animals.

Currently, no quantitative data are available on the visual, olfactory or auditory capabilities of rhinos in relation to breeding success. Based on species ecology and behavior, however, it is believed that rhinos rely heavily on both olfactory and auditory senses for social communication.

It is therefore recommended that indoor facilities allow these types of communications at certain times among individuals. Options include partial walls or pipe fencing to allow for physical separation without visual, auditory or olfactory separation.

### ***Substrate and Special Features***

A brushed or broom-finished concrete floor that is well-drained and insures adequate footing is recommended. Dirt flooring as the main substrate is not recommended. In addition, floor heat is recommended in colder climates. Bedding materials such as hay, wood shavings and hoofed-stock rubber matting are recommended for greater one-horned rhinos. Other situations in which bedding is required include barns with rough substrates (which may cause skin ulcerations) or for additional warmth for sick animals or young calves. When introducing rhinos to new substrates, careful observations should be made to avoid the animals' excessive ingestion of the novel substrate, which could potentially lead to health problems, such as impaction. The use of a power washing machine is recommended to disinfect barn areas. Additionally, rubber matting and bedding materials should be disinfected or changed regularly to prevent contamination. Permanent rubberized flooring (poured floors or encapsulated mats) is more expensive but reduces cleaning time and risk of contamination.

Normal light cycles seem to be adequate for rhinos. However, if an animal is to be held indoors for more than 12 hours (e.g., winter at cold-climate institutions), artificial or natural light sources to simulate natural cycles should be provided. Fluorescent lighting is an efficient light source that provides broad-spectrum illumination; skylights should also be included whenever possible. Additionally, because greater one-horned rhinos are introduced for breeding purposes for a limited amount of time and closely observed, lighting is also necessary in outdoor enclosures for the observation of breeding at night.

Any new exhibit should include the capability for video systems. In addition, a scale for weighing animals is desirable and strongly recommended. Vehicle access to an indoor facility is also recommended. A restraint device or an area for restraint should be included in the design of every facility, as well as an area to set up crates for training, loading, and unloading.

### **Physical Restraint Designs**

Numerous institutions have constructed permanent physical devices to restrain their rhinos when necessary. Such "chutes" can be very valuable for physical exams as well as nutritional, reproductive or veterinary research. In addition to the following general information, please consult the Health chapter of this publication as well as Schaffer (1993) and Eyres et al. (1995). Institutions in the United States that currently have chutes and may be able to provide additional information include Henry Vilas, Saint Louis, Sedgwick County, Oklahoma City, Henry Doorly, Cincinnati, Caldwell, and Milwaukee County Zoos, Fossil Rim Wildlife Center, and the Wilds. Companies that may assist in chute design and construction include Animar Systems, Inc. (Springfield, MO, USA), Cummings and Son, Inc. (Garden City, KS, USA), Mark McNamara of Fauna Research (Tamer, Fauna Research, Inc., Red Hook, NY, USA). In general, institutions modifying rhino exhibits or constructing new ones should incorporate a physical restraint area or device into their design.

Several physical restraint designs are effective for rhinos. These range from a small restricted area in which to contain the animal to an area that contains one or more hydraulics that will "squeeze" together to restrict an animal's movement. In general, major restraint chute design considerations include strength, durability, type and function. It should be noted, however, that

available space and animal size and disposition vary across institutions and should be individually addressed.

In general, both zoo managers and researchers emphasize that the general restraint area should be an active component of daily rhino management. Methods to accomplish this vary. A restraint chute or restraint area can be designed so that the rhinos must pass through it to exit the barn into their yard. If rhinos are fed indoors, part of the feed (e.g., produce, grain) can be offered in the chute area. Finally, more extensive conditioning (see Training chapter) can be particularly effective in habituating rhinos to physical restraint. Such a program should be attempted prior to detaining a rhino in a chute for an exam.

Rhino chutes should be manufactured out of steel or a combination of steel and steel-reinforced wood. Some institutions have also used steel-strength aluminum (6061-T52 aluminum). Aluminum of this type is lighter and more maneuverable than steel, as well as potentially less stressful to rhinos because of “deader” sound properties than steel (i.e., when metal scrapes metal).

Permanent pass-through indoor restraint chutes (similar to those constructed for elephants) are especially effective for rhinos. With training, this type of chute may allow for detailed daily rhino observations. Further, inclement weather will not affect the use of an indoor restraint chute. The chute should allow restraint of the animal when it is passing through in either direction so that the shifting routine of the animal is not interrupted (Schaffer, 1993). The width of the chute should limit side-to-side movement while still allowing the animal to comfortably lie down. Animals can become wedged in tight-fitting chutes if the sides cannot be released. To alleviate excessive forward movement of the animal when it lowers its head, two vertical bars that push in from the sides of the chute to the shoulders of the rhino may be utilized. Quick release of these shoulder bars often relieves agitated animals without having to release them completely.

High-walled chutes or bars over the top of the chute keep the animal from climbing or rearing up. Horizontal bars in the chute’s entry gates and sides are hazardous for examiners when the animal lies down. Vertical bars on the sides can trap researchers’ arms if the animal can move forward. If the animal’s forward and side-to-side mobility can be limited, vertical bars or walls on all sides are recommended. The distance between these bars along the sides of the chute should be great enough to prevent the animal’s foot from becoming wedged if the animal rolls on its side in the chute. For personnel safety, this distance can be divided with removable vertical bars.

Rhinos may slam swinging doors; thus, sliding or guillotine gates are safer. A rectangular opening in these gates for performing palpation should not pin the arm of an examiner when the animal is shifting. The distance between the vertical sides of this rectangular opening must be wide enough to provide for staff safety while still limiting the space through which a rhino could squeeze. Also, the horizontal bottom bar of this rectangle should be only a few inches from the ground, as animals frequently lie down. Solid doors on the outside of these gates can be used to stop rhinos, as they may attempt to charge even small openings. Additionally, good lighting and accessible electrical sources are useful.

A closed chute (Fig. 4.5) is another option that has been used successfully for the treatment of a rhino with a urinary-tract infection and another with infected lesions on its foot (Eyres et al., 1995). As noted in Figure 4.5, a typical closed chute has both front and back gates. The back gate restricts the rhino’s movement by sliding forward. Additionally, the hind end of the rhino is supported by a V-design that prevents it from lying down. This design also allows additional safety for staff while working with the animal. In many respects, a closed chute does not depend as strongly on conditioning of the rhinos as does a squeeze chute, although acclimation is recom-



mended prior to attempting any treatments within the chute. The design of a closed chute might necessitate an outdoor location in most cases; therefore, the use of this type of chute may be limited by weather.

A free-stall chute can be used for animals more sensitive to a confined enclosure (Fig. 4.6). The design of this type of chute allows the rhino to enter or exit at its will and thus may help to keep rhinos calmer during procedures. Because there is free access, however, rhinos must be conditioned to target or stand still; thus, relatively non-invasive procedures also work best. Procedures that have been accomplished with a conditioned rhino in a free-stall include ultrasound and serial collection of blood and feces (Eyres et al., 1995).

A free-stall design can easily be incorporated into an existing pen or stall, indoors or outdoors. The open back of this type of chute allows the animal to enter and leave the structure at will. Protection of staff when working with the rhino is critical; a partial back wall constructed of vertical pipes allows staff to step out of the way (Fig. 4.6).

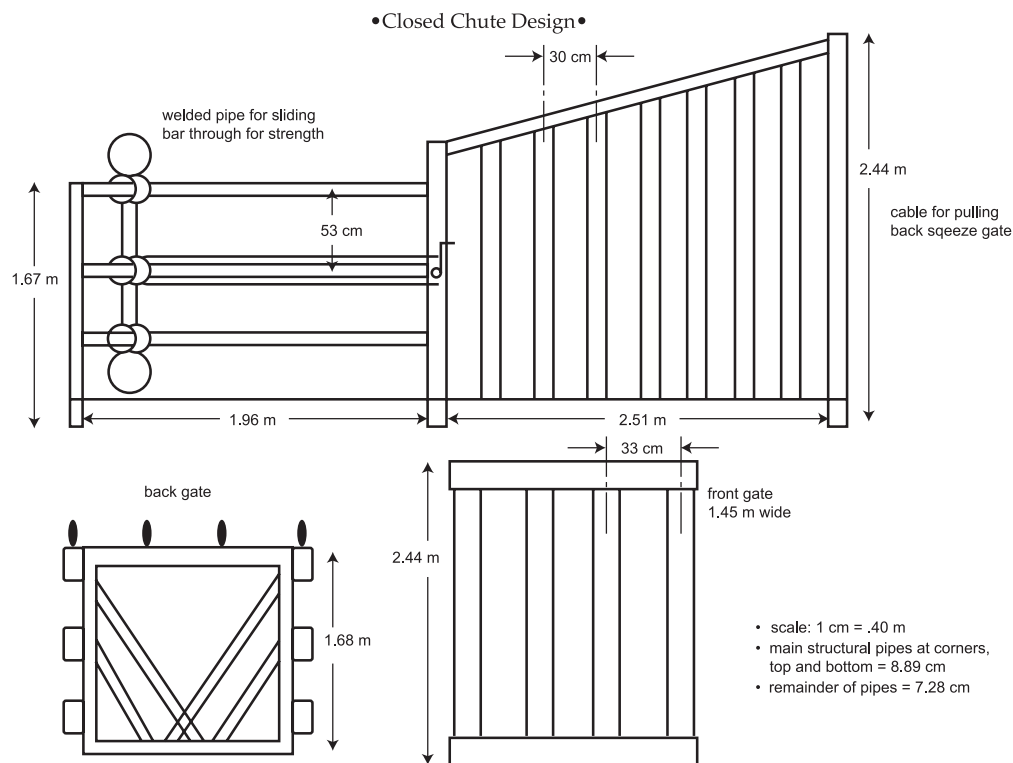


Fig. 4.5. Closed stall rhino restraint chute. Note that a typical closed chute has gates that restrict the rhino's movement and prevent it from lying down. The advantage of a closed chute is that it does not depend as strongly on conditioning as does a squeeze or free-stall chute (Eyres et al., 1995).

### •Free-Stall Chute Design•

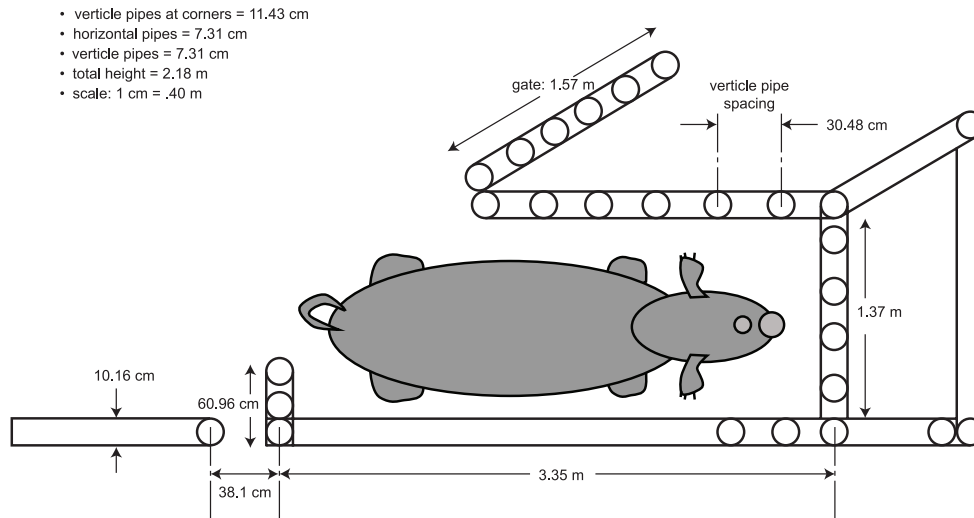


Fig. 4.6. Free-stall rhino restraint chute. The design of a free-stall chute allows the rhino to enter or it at will and can be used for animals more sensitive to a confined enclosure. This type of restraint chute, however, is best used for relatively non-invasive procedures and with rhinos that have been conditioned to target or stand still (Eyres et al., 1995).

## HEALTH

### Introduction

This section provides a general overview of preventive medical and disease management, and chemical restraint of captive rhinoceroses. More detailed information for many of these topics is available in the references provided as well as through the Rhino Resource Center ([www.rhinoresourcecenter.com](http://www.rhinoresourcecenter.com)).

### Physiological Normals and Sample Collection

With the increased use of training for husbandry and medical procedures, resting values for heart rate, respiratory rate, temperature and other values have been obtained for non-restrained black and white rhinos. The various species appear to be similar with heart rates of 30 to 40 beats per minute and respiratory rates of six to 12 breaths per minute. Rectal temperatures are typically 34.5-37.5°C (94-99.5°F), although temperatures may be higher in anesthetized rhinos (37-39°C; 98.6-102°F) due to exertion or muscle tremors (Miller, 2003; Radcliffe and Morkel, 2007; Morkel et al., 2011). Values are comparable to domestic horse ranges. Limited information about electrocardiography (ECG) is available in rhinos (Jayasinge and Silva, 1972). Indirect blood pressure has been measured in unsedated black and white rhinoceros using a human blood pressure cuff around the base of the tail. Mean values reported for unanesthetized white rhino are 160 ± 2.9 mm Hg (systolic), 104 ± 2.3 mm Hg (diastolic), and 124 ± 2.2 mm Hg (mean blood pressure) (Citino and Bush, 2007). In anesthetized animals, etorphine can cause hypertension, although variable mean blood pressure measurements (107-280 mm Hg) have been observed, depending on drugs used and time values measured.

Hematologic, biochemical, mineral, protein electrophoresis, and blood gas values have been previously published (Tables 4.14-4.16; Flesness, 2002; Miller, 2003). Although most parameters can be generally interpreted similar to other perissodactyls, there are several differences that appear to be normal in rhinos. Total protein and globulins tend to be higher than in domestic horses. Other values that differ include lower sodium and chloride. Free-ranging rhinos tend to have higher creatine phosphokinase (CPK) than captive animals (this may be biased by immobilization technique)(Kock et al., 1990; Mathebula et al., 2012).

Venipuncture can be routinely performed on awake captive rhinoceroses using training and/or restraint devices (Fig. 4.7). The most commonly used sites for blood collection are the ear (auricular) vein, metacarpal vein (lower inside forelimb), and radial vein (inside of forelimb crossing the carpus)(Fig. 4.7-4.9). The tail (coccygeal) vein has also been used and is approached from the ventral aspect similar to the technique for blood collection in domestic cattle. Arterial access is available for blood gas sampling using the medial auricular artery (inside of the ear)(Fig. 4.10). Large volumes of blood can be collected from the radial or metacarpal veins for diagnostic testing, plasma/serum banking, or for therapeutic phlebotomy (Mylniczenko et al., 2012). Rhinoceros blood cells resemble those of domestic horses. Nucleated red blood cells (NRBC) and reticulocytes may be observed in anemic animals. Elevated total white blood cell counts (wbc) and eosinophil numbers have been observed in wild rhinoceroses, presumably due to the response to capture and parasite loads, respectively.

Table 4.14. Mean hematology values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
WBC x 10 <sup>3</sup> / $\mu$ l	8.42 (2.48)	9.30 (2.46)	7.20 (1.33)	8.27 (1.55)
RBC x 10 <sup>6</sup> / $\mu$ l	4.01 (0.88)	5.77 (1.28)	6.43 (0.86)	5.32 (1.09)
HBG g/dl	12.0 (2.0)	13.8 (3.8)	13.4 (1.5)	12.4 (1.6)
Hct %	33.4 (5.7)	36.9 (9.3)	37.0 (4.6)	36.9 (4.2)
MCV FL	85.7 (9.0)	63.8 (7.8)	57.8 (4.9)	71.5 (11.2)
MCH pg/cell	30.5 (3.3)	23.5 (1.9)	21.3 (3.0)	23.9 (3.8)
MCHC g/dl	35.7 (2.7)	37.9 (7.3)	36.3 (3.2)	33.5 (2.1)
Platelets x 10 <sup>3</sup> / $\mu$ l	284 (83)	378 (103)	178 (53)	198 (135)
Nucleated RBC/100 WBC	0	1 (1)	0	–
Reticulocytes %	1.6 (2.9)	–	–	–
Neutrophils x 10 <sup>3</sup> /ml	5.24 (2.18)	5.42 (2.05)	5.13 (1.24)	4.86 (1.16)
Lymphocytes x 10 <sup>3</sup> /ml	2.48 (1.1)	2.35 (1.15)	1.74 (0.67)	2.52 (0.90)
Monocytes x 10 <sup>3</sup> /ml	0.43 (0.32)	0.65 (0.55)	0.22 (0.15)	0.36 (0.22)
Eosinophils x 10 <sup>3</sup> /ml	0.25 (0.22)	0.54 (0.59)	0.32 (0.31)	0.37 (0.21)
Basophils x 10 <sup>3</sup> /ml	0.17 (0.09)	0.10 (0.05)	0.13 (0.05)	0.08 (0.01)
Neutrophilic bands x 10 <sup>3</sup> /ml	0.27 (0.35)	0.71 (1.18)	0.22 (0.20)	0.31 (0.24)

Table 4.15. Mean blood chemistry values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
BUN mg/dl	13 (3)	16 (3)	3 (2)	6 (2)
Creatinine mg/dl	1.1 (0.2)	1.8 (0.4)	1.3 (0.2)	0.9 (0.1)
Uric acid mg/dl	0.5 (0.2)	0.9 (0.8)	0.3 (0.2)	-
Bilirubin mg/dl	0.3 (0.1)	0.3 (0.3)	0.4 (0.3)	0.2 (0.1)
Glucose mg/dl	69 (21)	97 (39)	82 (25)	76 (13)
Cholesterol mg/dl	102 (37)	93 (26)	53 (21)	48 (21)
CPK IU/L	255 (248)	409 (722)	260 (203)	617 (398)
LDH IU/L	595 (427)	537 (320)	267 (149)	231 (38)
Alk Phos IU/L	80 (55)	92 (51)	80 (41)	17 (6)
ALT IU/L	16 (7)	16 (9)	7 (7)	6 (3)
AST IU/L	85 (27)	71 (25)	61 (27)	39 (9)
GGT IU/L	27 (18)	19 (14)	18 (16)	6 (2)
Total protein g/dl	7.6 (0.9)	8.5 (1.0)	7.5 (0.9)	7.5 (0.4)
Globulin g/dl (electrophoresis)	4.9 (0.9)	5.3 (0.8)	4.5 (0.7)	3.8 (0.7)
Albumin g/dl (electrophoresis)	2.6 (0.4)	3.2 (0.5)	2.9 (0.5)	3.6 (0.6)
Fibrinogen mg/dl	104 (195)	101 (241)	350 (84)	324 (85)

Table 4.16. Mean serum mineral values and blood gases in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
Ca mg/dl	12.7 (1.0)	11.8 (0.9)	11.4 (0.8)	13.3 (1.1)
P mg/dl	4.8 (1.1)	4.0 (0.9)	4.0 (0.9)	3.7 (0.7)
Na mEq/L	133 (3)	134 (5)	132 (3)	133 (4)
K mEq/L	4.7 (0.6)	4.7 (0.8)	4.1 (0.4)	4.6 (0.6)
Cl mEq/L	96 (0.3)	95 (4)	91 (3)	100 (3)
Bicarb mEq/L	23.3 (4.2)	18 (0)	27.0 (0)	-
CO <sub>2</sub> mEq/l	25.4 (9.9)	25.3 (8.8)	27.3 (3.7)	22.8 (2.4)
Iron $\mu$ g/dl	227 (66)	176 (67)	152 (70)	-
Mg mg/dl	3.34 (3.45)	118.2 (232.5)	7.95 (8.56)	-



Fig. 4.7. Voluntary blood collection from auricular vein in awake rhinoceros.



Fig. 4.8. Blood collection from the metacarpal vein in a standing rhinoceros.





Fig. 4.9. Blood collection from radial vein in immobilized rhinoceros.



Fig. 4.10. Arterial blood sample collection from medial auricular artery.

Urinalysis panels in captive rhinos are similar to horses with large numbers of calcium carbonate crystals, creating a normal milky yellow appearance to the urine. Calcium oxalate, phosphate, and ammonium crystals may also occur, depending on the diet. Occasionally dark discoloration of urine associated with pigmentation of certain browse species (e.g. ash, mulberry) can be mistaken for blood or myoglobin. However, analysis should be performed to rule-out any abnormalities. Normal values for the different rhino species have been published (Table 4.17; Haffey et al., 2008).

Table 4.17. Reported range of mean urinalysis results from three captive rhinoceros species.

	pH	Specific Gravity
Greater Asian one-horned rhinoceros	8.08-8.70	1.019-1.031
Sumatran rhinoceros	8.16-8.32	1.010-1.021
Black rhinoceros	8.10-8.26	1.010-1.012

Due to size, cerebral spinal fluid collection has not been successful except in a few rhino calves (S. Citino, pers. comm.). Therefore, extrapolation of normal values from domestic horses and other perissodactyls should be used for interpretation.

Incorporation of scales into rhino facilities has permitted monitoring of body weights and physical condition. Regular weighing is recommended to observe trends associated with growth, diet changes, and disease. Average range for adult body weight is 1800-2200 kg (3,968-4,850 lb; Miller, 2003).

## Preventive Medical Health Programs

Routine health monitoring should be performed on all rhinoceros on an ongoing basis. Animals should be trained to permit sampling and examination. The following **protocol** advises that specific laboratory tests be performed for the purpose of evaluating current health status. Additional tests are recommended to increase baseline information on other diseases to determine their significance to rhinoceros health. The final decision for specific procedures should be made by the animal care and veterinary staff based on individual circumstances.

- Physical exam by a veterinarian familiar with rhinoceros health problems. This should include a review of all systems (if performed in a restraint device, exam may be limited by training and temperament of the individual and design of facility). Special attention for certain species include:
  - Greater Asian one-horned rhinos—Foot exam due to prevalence of pododermatitis; ophthalmic exam for keratitis
- Body weight—Actual weight should be recorded whenever possible; body scores and/or digital photos can be used when scales are not available.
- Blood collection for complete blood count (CBC), serum chemistry panel, fibrinogen, serum protein electrophoresis and extra serum/plasma for banking (minimum of 10-20 ml). Blood smears should be carefully screened for the presence of hemoparasites, especially in recently captured or imported animals. The current Rhino SSP/TAG tissue/sample collection **protocol** should be consulted for additional samples that may be **requested** for research, disease screening, etc.

- Fecal samples should be collected semiannually or at least annually (depending on management system) for direct, flotation, and sedimentation to detect internal parasites.
- Annual enteric pathogen screening may be included, especially for animals in intensive management situations (e.g., breeding herd). Aerobic culture of feces for enteric pathogens should include special media for the detection of *Salmonella*. Because *Salmonella* organisms may be shed intermittently, at least three to five fecal cultures should be performed (may be done on consecutive days).
- Vaccinations—Annual vaccination for leptospirosis is possible for greater Asian one-horned rhinoceroses. Vaccination for rabies, tetanus, and arboviruses (EEE/WEE/WNV) may be considered if the diseases are considered endemic in the area or increased risk factors are identified. There have been isolated cases of rabies and tetanus documented in rhinoceros (Jones, 1978; Mukherjee et al., 1984; Selvam et al., 2003). Check with the SSP veterinary advisor for the most current recommendations on species-specific vaccination protocols.

Additional preventive health recommendations have been included for consideration when performing examinations.

- Serological screening for leptospirosis (multiple serovars) and West Nile Virus (WNV). Although these tests are not species-specific and have not been validated for rhinoceros, they may detect cross-reactive antibodies in exposed animals. The presence of antibodies does not necessarily denote current infection or disease. Antibodies to leptospirosis have been detected in vaccinated rhinoceros and may be used to monitor response and possibly determine frequency of vaccination, although data is insufficient to determine protective titers. Insufficient data is available at this time to determine the significance of WNV antibodies in rhinoceroses; although it is important to note that a greater one-horned rhinoceros with clinical signs developed a WNV titer during a period of known exposure (P. Calle, pers. comm.). One study in greater Asian one-horned rhinoceroses did not find seroconversion in response to vaccination with a commercial equine WNV vaccine (Wolf et al., 2008).
- Serum/plasma vitamin E levels should be checked on a regular basis to assess adequacy of diet and supplementation protocols (see Nutrition chapter). See the most current Rhino TAG/SSP preventive health protocol for recommended laboratories.
- Reproductive tract examination—When feasible, a complete reproductive examination should be conducted to include transrectal ultrasound, semen collection and analysis, and serum or fecal collection for hormone analysis (Radcliffe et al., 1997). Uterine leiomyomas, cystic ovaries, and irregular cycling have been observed in captive animals (Hermes et al., 2004). Since these conditions can have potentially significant effects on reproduction, a careful evaluation is warranted if the animal is being considered for breeding. Standing laparoscopy enabled visual examination and uterine biopsy of a leiomyoma in a southern white rhinoceros (Radcliffe et al., 2000b). A number of publications describe the technique for ultrasonography in rhinoceroses and normal reproductive biology. The reader is referred to the Rhino Resource Center for further information ([www.rhinoresourcecenter.com](http://www.rhinoresourcecenter.com)).
- Urinalysis should include both fluid and sediment evaluation of a clean voided sample. Microbial culture should be considered if there is evidence of blood cells or bacteria.
- Radiographs of feet are strongly recommended if any signs of pododermatitis or nail cracks are observed, especially in greater Asian one-horned rhinoceroses (Atkinson et al., 2002). Regular foot trimming and care may require immobilization in certain individuals (i.e., those that have a history of chronic foot problems). See section on pododermatitis.

- Diagnostic tests for tuberculosis—Periodic testing for tuberculosis in rhinoceroses should be considered, especially if there has been a history in the institution or herd. Intradermal testing can be performed using bovine PPD (0.1 ml ID) in the eyelid, behind the ear, caudal tailfold, or axillary region. Both false positive and false negative results have been found when performing intradermal tuberculin testing in rhinos (Godfrey et al., 1990). Ancillary tests such as nasal swabs, tracheal washes, and gastric lavages for mycobacterial culture have also been used. Serological tests [ElephantTB STAT-PAK®, MAPIA, DPP (dual pathway platform); Chembio Diagnostic Systems, Inc.] are being investigated for use in rhinos. See section on tuberculosis.
- Other vaccination regimens will depend on regional requirements and exposure risks (consider multivalent vaccination for Clostridial diseases). Contact the SSP veterinary advisor for the most up-to-date information.

Animals being moved between institutions should receive a preshipment examination and testing. This is similar to the routine health procedures but should also include screening for tuberculosis and other requirements dictated by the receiving institution (see quarantine section below).

## Neonatal Examinations

Ideally, neonates should be examined within 24 to 72 hours of birth to detect any congenital defects. Often the dam can be separated while the calf is manually restrained for a brief, but thorough exam, including body weight, and if feasible, blood collection. Complete blood count, biochemical panel, tests for passive transfer of immunoglobulins [e.g. glutaraldehyde coagulation, zinc sulfate turbidity, radial immunodiffusion plates (equine plates work well in rhinos and can be standardized by testing healthy rhinos), and serum protein electrophoresis], vitamin E level, and banking should be performed. A microchip may be placed behind the left ear for future identification. Particular attention during the exam should be paid to the umbilical stalk for signs of infection, urine leakage, or hernia; passage of meconium, normal rectal/anal anatomy and tone; suckling reflex and neurological status. Regular weights and developmental progress should be documented. Lack of weight gain may be due to inadequate maternal milk production and care, or an indication of health or developmental problems in the calf. Greater Asian one-horned rhinoceros calves have been successfully hand-reared (covered under Nutrition chapter). In one case, a white rhinoceros calf was orphaned when its dam died, and the calf was taught to drink from a bucket at the age of six weeks. Weakness or other problems resulting in prolonged recumbency can cause decubital ulcers. Treatment of two neonatal white rhinos has been described using serial sedation with butorphanol alone or in combination with detomidine for restraint (Gandolf et al., 2006). Rectal prolapse has been reported in black rhinoceros calves, with surgical intervention required in at least two cases (Pearson et al., 1967; Abou-Madi et al., 1996).

## Parasites

Internal parasites are more commonly found in free-ranging rhinos than in captivity. Parasites reported include nematodes (*Diceronema versterae*, *Parabronemia roundi*—associated with stomach nodules), *Draschia megastoma*, multiple species of *Kiluluma*, *Khalilia rhinocerotis* and pinworms (*Oxyuris karamoja*). Tapeworms (*Anoplocephala* sp.) can cause asymptomatic infestation in both captive and wild rhinos (Miller, 2003). Liver flukes (*Fasciola gigantica*) have been reported in greater Asian one-horned rhinoceros. Diarrhea secondary to *Entamoeba* sp., *Giardia* sp., and unidentified



flagellates have been observed in captive Asian one-horned rhinoceroses. One case of neosporosis led to acute fatal myocarditis in a neonatal white rhino calf and abortion in another case (Williams et al., 2002; Sangster et al., 2010).

Ticks are regularly found on free-ranging rhinoceroses and, imported animals should be carefully examined and treated. Vector-borne parasitic infections occur in endemic areas.

Capture and translocation or importation may exacerbate potential parasitic infestations and increase the risk of introduction of novel pathogens to a new environment. Therefore, careful screening for external and internal parasites, prophylactic treatment of wounds with fly repellent, pour-on tick treatments (coumaphos, flumethrin), and judicious use of antiparasitic medications (pyrantel pamoate, fenbendazole, ivermectin, praziquantel) should be considered. Check with the SSP veterinary advisor for current recommendations.

## Quarantine

Due to the size, strength, and temperament of a rhinoceros, it may be logistically difficult to maintain isolation from other animals during arrival and quarantine. The Rhinoceros TAG/SSP Recommended Preshipment Protocol for Rhinoceros lists a comprehensive battery of tests for health assessment prior to shipment. Since most zoological institutions will not have the facilities available to safely house and manage a newly arriving rhinoceros, it is important that the receiving institution work closely with the sending institution to ensure that all (or as many as possible) of the listed tests are conducted and results reviewed before shipment. Following the preshipment protocol may help compensate for some of the quarantine compromises that may be required. Regardless of preshipment test results, every attempt should be made to maintain some degree of physical separation of the incoming animal from the resident rhinos after arrival.

Current quarantine practices recommend a minimum 30 to 90 day quarantine period for most mammalian species in zoos and aquaria. Social concerns, physical facility design, and availability of trained rhino staff may dictate a modified quarantine protocol. Specific quarantine guidelines and protocols at each institution should be reviewed jointly and decisions made by the veterinary and animal management staff. Recommended **procedures** to consider as part of a comprehensive plan for rhinoceros quarantine include:

- Thorough physical examination including a review of all organ systems.
- Blood collection for CBC (including blood smear examination for hemic parasites), serum chemistry panel, fibrinogen, serum protein electrophoresis, and serum bank.
- Fecal collection for parasite screening (direct, flotation, sedimentation) conducted weekly for the first three weeks.
- Fecal cultures for *Salmonella spp.* conducted at least weekly for the first three weeks.
- Any procedures that were not completed prior to transport or that may be due, such as vaccination, serologic screening, or TB testing.

It should be emphasized that the quarantine test requirements should be strongly considered regardless of the results of preshipment testing. The stress of transport and quarantine may result in health changes (for example, *Salmonella* shedding) that were not detected during testing at the sending institution.



## Hospitalization and Critical Care

In most cases, hospitalization is impractical with adult rhinoceroses. Most animals will be treated in their holding areas. Rhino calves may be hospitalized in adequate large animal holding facilities if the severity of their medical condition warrants. Barns or holding areas that incorporate species-appropriate restraint devices facilitate medical treatment. Some of the following medical problems require active intervention, including sedation, immobilization, injectable drug therapy, and/or fluid therapy. Although it has been achieved, fluid therapy in rhinoceros presents logistical challenges. In addition to the intravenous route, it is possible to improve hydration using rectal enemas with warm physiologic solutions or even tap water. Animal and staff safety should be a priority in any planned intervention.

## Diseases

### *Tuberculosis*

*Mycobacterium bovis* and *M. tuberculosis* have caused infections in captive rhinoceroses (Stetter et al., 1995; Miller, 2008). Initial infection may be asymptomatic or result in progressive weight loss and emaciation, with coughing and dyspnea occurring in the terminal stages. Nasal discharge may be present but is not a consistent sign. Most infections are pulmonary. Antemortem testing includes intradermal tuberculin test, tracheal and/or gastric lavage for mycobacterial culture, and serological tests (see preventive health section for details). Retrospective analyses of serum from *M. tuberculosis*-infected black rhinos showed positive results using the ElephantTB Stat-Pak® (Duncan et al., 2009). Treatment has been attempted using isoniazid, rifampin, ethambutol, and pyrazinamide (Barbiers, 1994; Duncan et al., 2009). However, assessment of successful response is limited. Concerns for other collection animals, staff and public health need to be considered prior to initiation of therapy. Since this is a reportable disease, notification of the appropriate state veterinary officials should occur promptly once a diagnosis is made. Consultation with the SSP veterinary advisor is also recommended.

### *Salmonellosis*

Salmonella infection can cause enteritis and fatal septicemia in captive and newly captured wild rhinoceroses (Windsor and Ashford, 1972; Kenny et al., 1997). In a retrospective survey of captive black, white, and greater Asian one-horned rhinos in the U.S., 11% reported positive cultures, usually associated with clinical signs (Kenney, 1999). Clinical infection may result secondary to transport, changes in diet, immobilization, concurrent disease, or exposure to a large number of organisms. Lethargy, anorexia, signs of colic, diarrhea, and death may be observed. Successful treatment using trimethoprim-sulfamethoxazole and supportive care is possible if initiated early. However, treatment of asymptomatic animals is NOT recommended.

### *Leptospirosis*

Leptospirosis usually presents with depression and anorexia. Other signs may include hemolytic anemia (not present in all cases), hemoglobinuria, signs of colic, and development of skin ulcers. Abortion has also been linked to infection with *Leptospira* in a greater Asian one-horned rhinoceros (Miller, 2003). Fatality rates are high in clinically affected black rhinos although successful treatment with trimethoprim-sulfamethoxazole and ceftiofur has been reported (Neiffer et al., 2001). Diagnosis is based on high antibody titers (microagglutination test – MAT) and confirmed by detection of leptospiral organisms in urine or tissues (fluorescent antibody test).

Preventive measures include possible vaccination of greater Asian one-horned rhinos with a multivalent large animal product, rodent and wildlife control programs, and good husbandry to minimize contamination of feed and water. Abscesses and systemic anaphylactic reactions, including hives and skin sloughing, have occurred following leptospirosis vaccination in the black rhinoceros. Those individuals with a history of systemic adverse reactions should forego regular vaccination.

### ***Gastrointestinal Infections***

See salmonellosis and GI torsion, impaction, and ulcers for other GI conditions.

### ***Encephalomyocarditis Infection (EMC)***

EMC viral infection usually results in acute death due to myocarditis (Gaskin et al., 1980). The southeastern/Gulf Coast states in the U.S. are considered an endemic area. Diagnosis is usually based on virus isolation at necropsy from heart, spleen, or other tissues (<http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/53600.htm>). Prevention should target rodent control, although experimental vaccines have been proposed. A commercial vaccine is not currently available.

### ***Fungal Pneumonia***

Fungal pneumonia is usually due to *Aspergillus spp.* and primarily observed in black rhinoceroses secondary to immunocompromise from concurrent disease, broad spectrum antibiotic therapy, or corticosteroid use (Weber and Miller, 1996). Clinical signs may include weakness, weight loss, epistaxis, or other signs consistent with pneumonia. Diagnosis is challenging, although serology and bronchoscopy, with cytology and fungal culture, may be useful. Long-term treatment with antifungal drugs (e.g., itraconazole) is expensive and has unknown efficacy. Definitive diagnosis is often made at necropsy.

### ***Anthrax***

Death due to anthrax has been observed in wild rhinoceroses. It has been implicated in a die-off of several Javan rhinos in Ujung Kulan National Park, though definitive diagnosis is often lacking in remote field regions (R. Radcliffe, pers. comm.). Most cases result in sudden death. Foamy discharge from the mouth and nostrils can be seen and may appear similar to EMC infection. Diagnosis is based on identification of anthrax bacilli in blood or tissue smears. Vaccination of ranches rhinos has been used in some endemic areas of Africa. Sporadic outbreaks in domestic livestock occur in the U.S., although there have been no reports of rhino mortality in these areas (e.g., Texas).

### ***Skin Conditions and Dermatopathy Syndromes***

- Neoplasia—A greater Asian one-horned rhino with squamous cell carcinoma of the horn has been successfully treated with surgery and radiation (Greer et al., 2010). Cutaneous melanomas have been reported in greater Asian one-horned rhinoceroses (Wack et al., 2010).
- Environmental—Dermatitis, especially in greater Asian one-horned rhinos, may be due to inadequate access to wallows and pools.

These cases illustrate the importance of biopsy and culture for identifying the etiology of and directing treatment in dermatologic cases in rhinos.

### ***Pododermatitis and Chronic Foot Problems***

Although all species of rhinoceros are susceptible to developing pododermatitis due to inappropriate substrate or other husbandry conditions in captivity, the greater Asian one-horned rhino (GAOHR) appears to be more susceptible to chronic foot problems. Studies comparing wild and European zoo GAOHR revealed 41% had vertical and/or horizontal cracks, 16% had pad fistulas and ulcerations, 69% had abraded side horn walls associated with an inflammatory process, and all the captive breeding bulls and at least half of the females had cracks between the central sole and adjacent pad (von Houwald, 2005). In contrast, foot anatomy of the wild GAOHR showed hard elongated and concave pads without lesions. The authors concluded that abrasive substrate, long-term indoor housing in northern climates, and limited access to a pool contributed to chronic foot trauma in captive rhinos. Over 28% of adult male captive *R. unicornis* are reported to have chronic pododermatitis (Atkinson et al., 2001). Management of the condition includes improvements in husbandry and medical/surgical interventions. Medical treatment may be in the form of oral antimicrobial medication and topical use of copper sulfate and oxytetracycline. Regular hoof trimming and surgical debridement of necrotic lesions, along with use of collagen products for granulation tissue stimulation, can lead to improvement in appearance and comfort of the animal. In addition to the factors mentioned above, nutritional imbalances (e.g., zinc) are also being investigated.

### ***Corneal Ulcers and Keratitis***

Corneal trauma and secondary infection can result in corneal ulceration and perforation. Surgical management of a melting corneal ulcer using a conjunctival graft has been described in a greater Asian one-horned rhino (Gandolf et al., 2000).

### ***Horn Problems***

Horn avulsion, cracks, or other trauma can occur as a result of acute or chronic rubbing, pressure, self-induced, or conspecific fighting. This may lead to myiasis, abscessation, osteomyelitis, or pain with behavioral changes. Radiographs, thermography, or fluoroscopy can be used to assess the extent of the damage. Treatment may involve debridement, antibiotics, wound treatment, and fly control (Suedmeyer, 2007). A squamous cell carcinoma of the horn in a greater Asian one-horned rhinoceros was diagnosed and managed with partial amputation and radiation therapy (Greer et al., 2010). The horn is attached to the basal dermal layer, not unlike the laminae of the horse's hoof wall, and therefore perturbations in blood flow can presumably lead to laminitis.

### ***Gastrointestinal Torsion, Impaction, and Ulcers and Other Conditions***

Torsion may result from abdominal trauma or severe GI disease. Severe torsion may lead to acute death; other signs are related to abdominal pain. Dietary changes, dehydration, ingestion of foreign material (i.e., sand), and inadequate fiber content may result in GI impaction. Gastric ulcers have been observed on gastroscopy and at necropsy in rhinos that have received long-term non-steroidal therapy or have concurrent disease. A 32-year old black rhinoceros was euthanized due to rapid onset of wasting secondary to perforation of a pyloric ulcer and peritonitis. Treatment depends on identifying the cause and site of the gastrointestinal condition. Surgical correction of torsion is usually recommended, although most cases are not diagnosed prior to necropsy. Impaction can be treated using rectal enemas, oral administration of psyllium, mineral oil, or other products to increase GI water content. Equine anti-ulcer medications such as omeprazole, sucralfate, and H2-blockers have been used in rhinos by scaling the equine dose. Prevention

includes adequate dietary fiber and fluid intake, and avoiding abdominal trauma and ingestion of foreign material. Esophageal dilation was observed in a black rhinoceros following ingestion of a foreign body; successful management involved diagnosis by endoscopy and a change in feeding practices to a low fiber diet (Radcliffe et al., 1998). Inflammatory bowel disease was confirmed at necropsy in a greater Asian one-horned rhinoceros that presented with weight loss and progressive diarrhea (Ferrell et al., 2010).

### ***Gingivitis and Dental Tartar***

All rhino species appear to accumulate dental tartar in captivity, especially if they are not given access to hard or course food items. Similar to horses, rhinoceros also develop dental points that may eventually create clinical mastication problems with age and require periodic dental flotation.

### ***Renal Disease***

Since 2001, chronic glomerulonephritis and/or renal failure have been recognized as a contributing cause of death in at least seven black rhinos. Mineralization of other tissues has been associated with chronic renal changes as well as three cases of accidental vitamin D toxicosis (Murnane et al., 1994; Fleming and Citino, 2003). Weight loss, decreased appetite, dermatitis, and signs of gastroenteritis may be present, including gastric ulcers. Significant changes in blood urea nitrogen, creatinine or urinalysis were not always evident in these cases, making diagnosis difficult until necropsy. However, two white rhinos (aged >40 years) both developed progressive chronic renal failure characterized by uremia, isosthenuria and hypercalcemia. Nutritional management using a high energy, low protein feed (Equine Senior, Purina Mills, St. Louis, MO, USA) was instituted in both cases and appeared to stabilize the renal disease (S. Ferrell and R. Radcliffe, pers. comm.). Further investigation into causes of renal disease (other than toxicosis-related) is needed.

### ***Creosote Toxicosis***

Seven fatalities occurred among a group of 20 black rhinoceroses captured and housed in bomas constructed with creosote-treated wood in Zimbabwe (Kock et al., 1994). Although five cases died after transport to the U.S. and Australia, they exhibited the same clinical signs and lesions as the initial cases. Animals were lethargic, partially anorectic, developed swollen limbs, and passed brown urine. Abnormal blood results included anemia, and elevated liver enzymes and bilirubin. Post-mortem findings included widespread hemorrhages; swollen, pigmented liver; and ulcers of the upper gastrointestinal tract. The presumptive cause of death was liver dysfunction due to creosote toxicosis. Exposure to creosote-treated housing materials should be avoided for all rhino species.

### **Common Injuries and Treatment: Skin Lacerations, Punctures, Wounds**

Trauma due to conspecific fighting, mating injuries, collision with obstacles in enclosures or in crates, interspecific conflict or, in free-ranging situations, poaching is relatively common. Common sense application of wound treatment principles apply, although the thick skin does not lend itself to primary closure and has a tendency to undermine abscesses along the fascial planes. Therefore, wounds are usually treated by second intention using debridement, hydrotherapy, topical and/or systemic antibiotics, analgesics, and the array of wound healing products that accelerate granulation. A recent article describes the use of vacuum-assisted healing of a surgical wound in a black rhinoceros to improve healing time (Harrison et al., 2011).

## **Necropsy Protocol**

Any rhinoceros that dies or is euthanized should have a complete necropsy performed and tissues submitted for histopathology. The Rhinoceros TAG/SSP Veterinary/Nutrition Blood and Tissue Collection **Protocol** for Rhinoceros should be consulted for current information on the species pathology advisors as well as specific sample **requests** for research and banking purposes.

## **Capture, Restraint, and Immobilization Techniques**

Management of captive rhinoceroses often requires procedures that involve handling of these large and powerful animals. Husbandry training techniques have advanced the application of preventive medical, diagnostic, and minor therapeutic procedures. However, intensive medical and surgical interventions may require physical and/or chemical restraint for safety and comfort of the patient and staff. Since new developments are continuously advanced in this field, the following recommendations should be used as guidelines, and the veterinary advisors and literature should be consulted for specific situations.

### ***Physical Restraint***

Advances have been made in facilities that include a variety of stall and chute designs for physical restraint of rhinoceros. These will be addressed in the Training and Enclosure Design chapters.

### ***Chemical Restraint***

Significant changes in options for chemical restraint of captive rhinoceroses have occurred during the last two decades. The degree of sedation, analgesia, or immobilization required will vary with the procedure to be performed. Health status and temperament of the individual animal, as well as facility design and staff experience may dictate preference for one technique over another.

## **Chemical Restraint and Anesthesia of Rhinoceros**

### ***Introduction***

Anesthesia of rhinoceroses requires adequate preparation, equipment, and experience. It is highly recommended that rhinoceros anesthesia should be attempted only with experienced personnel or after consulting knowledgeable practitioners. Contingency plans for emergencies are a key component of preparations. Clearly defined roles for all personnel should be outlined and reviewed. Adequate equipment for moving a large animal should also be available.

The choice of chemical restraint techniques will be determined by a number of factors, including physical facilities where immobilization will be performed, health status of the individual, procedures to be achieved and level of analgesia required, length of intended immobilization, and experience of the veterinary and animal care staff. Only captive rhinoceros anesthesia techniques will be covered in this section. For more information on immobilization of free-ranging rhinoceros, the reader is referred to specific references on this subject ([www.rhinosourcecenter.com](http://www.rhinosourcecenter.com)) (Miller, 2003; Bush et al., 2004; Portas, 2004; Kock et al., 2006; Radcliffe and Morkel, 2007; Wenger et al., 2007).

### ***Equipment***

With training and availability of physical restraint devices (“chutes”), drug delivery for chemical restraint can be more easily and accurately achieved, create less stress for the individual, and provide greater control of the induction. Standing restraint is also feasible in these situations.



For rhinoceros in stalls, pens or exhibits, drug delivery often requires use of darting equipment. Depending on the situation, pole syringes with robust needles may be used on selected occasions. Hand-injection is also easily achieved in conditioned animals, although care must be taken when using potent opioids to ensure human safety. Most darting systems can be used in captive situations, as long as a robust needle (minimum 40-60 mm x 2 mm needle) is used to penetrate the thick skin and deliver the drug into the muscle. Nylon darts (Teleinject<sup>®</sup>, Telinject USA, Inc., Agua Dulce, CA, USA; Dan-Inject<sup>®</sup>, Danwild LLC, Austin, TX, USA) are preferred in these situations since they are less traumatic than metal darts. Ideal sites for drug injection are just caudal to the ear on the lateral cervical area, upper caudal hindlimb, or shoulder. However, any site can be used if the dart is placed perpendicular to the skin and is adequate to penetrate muscle.

Additional equipment that should be available include a blindfold, ear plugs (cotton cast padding, stuffed socks), heavy ropes, and padding for recumbency. Inflated truck inner tubes can be used to reduce pressure points. Otherwise, heavy mats or padding should be used if the procedure will take place on a concrete floor. Immobilizations carried out in sandy or grassy areas may provide enough padding for shorter procedures.

Contingency plans for moving a rhino that becomes recumbent in a difficult position should also be made. Lining stalls with plywood sheeting to prevent the head/horn from getting stuck should be considered in areas with open bollards or poles.

Additional equipment should include the availability of oxygen and other emergency equipment. If a field procedure is being planned, an axe or chain saw and bolt cutter may be needed if there are trees/vegetation/other obstacles in the area where the rhino can get caught.

### ***Preparations***

Depending on the health status, environment, and procedure planned, removal of food and water prior to the procedure should be considered at least overnight. Consult the veterinarian for specific instructions.

### ***Positioning***

Rhinoceros are prone to developing myopathy and neuropathy after recumbency. The optimal position remains controversial and may be dictated by the planned procedure. Lateral positioning is often preferred since it provides the optimal circulation to the limbs, although sternal recumbency may provide better ventilation (Morkel et al., 2010). During the procedure, limbs should be “pumped” about every 20 minutes to encourage circulation (Radcliffe and Morkel, 2007). If the animal needs to be in sternal recumbency for the procedure, it is ideal to roll the animal into lateral recumbency whenever possible and pump the legs.

### ***Anesthetic Monitoring***

With the advent of new tools for monitoring physiological parameters in veterinary patients, it is imperative that monitoring be performed on immobilized rhinoceros to minimize complications. Ideally, an accurate weight or weight estimate will facilitate optimal drug calculation and prevent drug over- or under-dosing and associated complications (Adams et al., 2005). Thorough physical examination should be performed, along with regular recording of vital signs (respiratory rate and depth, heart rate, temperature, mucous membrane color and capillary refill time). Ideally, this should be assigned to dedicated personnel and measured at five-minute intervals throughout the procedure.

Pulse oximetry is a useful tool for monitoring trends in hemoglobin oxygen saturation but can be prone to false readings due to placement and the thick skin of the patient. Therefore, it should not be a substitute for basic clinical assessment. Sites for placement of the sensor include the ear pinnae (scraping can sometimes improve readings) and mucosal folds of the penis, vulva, or rectum. Side-by-side placement of sensor pads has been used in the conjunctival sac, gingival and nasal mucosa, and inside the rectum, vagina, or prepuce. Ideally, readings should be greater than 90%, but interpretation should be made in conjunction with the color of the mucous membranes, blood, and other clinical signs. Capnography may also enhance patient monitoring by enabling early detection of adverse respiratory events, including hypoxia. While pulse oximetry gives trends in patient oxygenation, capnography provides information about CO<sub>2</sub> production, pulmonary perfusion, alveolar ventilation, respiratory patterns, and elimination of CO<sub>2</sub> from the anesthesia circuit and ventilator.

### ***Standing Sedation***

Standing sedation should only be attempted under conditions that take into consideration animal and staff safety. The type of procedure, as well as the temperament of the animal, restraint device, and husbandry training of the individual, will determine the level of sedation and analgesia required. Excitement and environmental or painful stimuli can override drug effects. As a general rule, individuals that are acclimated to people and husbandry training and restraint practices tend to require lower doses of immobilizing agent. Opioids tend to have more potent effects than the tranquilizer/sedative classes of drugs. A combination of butorphanol and azaperone has proven effective for repeat procedures, inducing standing sedation and recumbency in the white rhinoceros (Radcliffe et al., 2000a). See Table 4.18 for suggested doses for standing sedation of captive rhinoceros.

Table 4.18. Standing chemical restraint doses for adult captive greater one-horned rhinoceros.

<b>Chemical Restraint Drug(s)</b>	<b>Reversal Agent(s)</b>
100 mg butorphanol + 100 mg azaperone IM	naltrexone at 2.5 mg/mg butorphanol
0.5-1.5 mg etorphine IM	naltrexone at 40 mg/mg etorphine

### ***Immobilization/General Anesthesia***

The primary class of drugs for general anesthesia in rhinoceros is the opioids. Etorphine is most commonly used, although carfentanil and more recently, combinations of etorphine and thiafentanil have also been administered to rhinos. Opioids are typically combined with azaperone, an alpha-2 agonist (e.g., xylazine, detomidine), or acepromazine to provide muscle relaxation and to counteract the hypertensive effect of the opioids (LeBlanc et al., 1987). Midazolam, diazepam, or guaifenesin infusion can also provide additional muscle relaxation. Greater one-horned rhinos appear to be more sensitive to the effects of opioids than black rhinos and exhibit muscle tremors, limb paddling, hypoxia, hypercapnia, and hypertension (Atkinson, 2001; Portas, 2004). Butorphanol has been administered to antagonize respiratory depressive effects in white rhino (10-20:1 butorphanol:etorphine in mgs); however, it may also lighten the plane of anesthesia (Miller et al., 2013). Other partial opioid agonist-antagonists are routinely used in the field and can be adapted for captive rhinos when available (e.g., nalbuphine). Butorphanol-azaperone and butorphanol-medetomidine/detomidine combinations have successfully induced recumbency in captive greater one-horned rhinos (Portas, 2004; Radcliffe et al., 2007; Waltzer et al., 2010).

Supplemental ketamine, opioids, guifanesin, propofol, or isoflurane can be used to deepen the level of anesthesia and lengthen immobilization as required (Ball et al., 2001). See Table 4.19 for recommended doses used for recumbent immobilization of rhinoceros.

Oxygen supplementation by intratracheal intubation or nasal insufflation (flow rates of 15-30 liters/minute) can increase oxygen saturation values (Morkel et al., 2010). Doxapram administration for apnea has also been used in rhinos but may only provide short-term relief. Partial or complete reversal should be considered in severe cases of hypoxia.

Table 4.19. Recumbent immobilizing doses for adult captive greater one-horned rhinoceros.

Drug(s)	Reversal Agent(s)
3.5-3.8 mg etorphine + 14 mg detomidine + 400 mg ketamine IM	naltrexone 50 mg/mg etorphine atipamezole 5 mg/mg detomidine
2.5 mg etorphine + 10 mg acepromazine IM	naltrexone 50 mg/mg etorphine ½ IV, ½ IM
0.7-1 mg carfentanil IM	naltrexone 100 mg/mg carfentanil
120 mg butorphanol + 80 mg detomidine IM	naltrexone 2.5 mg/mg butorphanol atipamezole 5 mg/mg detomidine

### ***Use of Tranquilizers/Sedatives for Transport and Other Uses***

There may be occasions other than medical procedures when rhinoceros need to be sedated for short or more extended periods of time, such as during crating and transport, or confinement for other reasons. With the advent of husbandry training, the need for drugs in captive rhinos has become more limited but should always be available as an option. Tranquilizer/sedative drugs may be used in rhinoceroses to relieve anxiety, reduce hostility, decrease motor activity, alleviate excitement, and to facilitate animal introductions. Drug choice is based on the desired duration of action and expected outcome. Long-acting neuroleptics (LANs) are typically administered in free-ranging rhino after capture for transport and boma acclimation, although they have also been used in captive rhino for longer duration tranquilization (see Table 3.18 in black rhinoceros Health chapter).

## ***NUTRITION***

Many of the health problems identified in captive rhinos are believed by some to be linked to nutritional factors. Rhinos consume a large number of species of plants with a diverse array of physical characteristics and nutrients. They represent a range of feeding strategies and, consequently, diet, from browsers (or selective feeders) to unselective grazers. Captive diets may have possible imbalances in dietary fats (particularly essential fatty acids) and soluble and insoluble carbohydrates, as well as minerals and vitamins for some species. This chapter outlines current dietary information for maintaining rhinos in captivity and includes a section on hand-rearing.

### **Nutritional Requirements**

Due to similarities in digestive tract morphology, the domestic horse still represents the best nutritional model for all rhinoceros species. Until further information is obtained, diets should be

formulated using current National Research Council (NRC; 2007) recommendations for horses of various physiological stages. Minimum nutrient requirements are listed in Table 4.20.

Table 4.20. Nutrient concentrations in total diets for horses and ponies (dry matter basis; modified from NRC, 2007).

Nutrient	Growing	Mature/ Maintenance	Pregnant/ Lactating
Dig. Energy (Mcal/kg)	2.45 - 2.90	2	2.25 - 2.60
Crude Protein (%)	12-15	8	10-13
Ca (%)	0.6	0.3	0.4
P (%)	0.3	0.2	0.3
Mg (%)	0.1	0.1	0.1
K (%)	0.3	0.3	0.4
Se (mg/kg)	0.1	0.1	0.1
Vit A (IU/kg)	2000	2000	3000
Vit D (IU/kg)	800	300	600
Vit E (IU/kg)	80	50	80

Good quality forages should provide primary nutrients for all herbivores, with concentrate feeds used to balance energy, protein, mineral or vitamin needs. Hay storage is particularly important for ensuring proper dietary management. Moldy or dusty hay may cause colic and/or heaves. Large amounts of poor-quality hay should not be fed to rhinos, as it may be so poorly digested that impaction and/or colic will result. Very high-quality legume or small-grain hay may be so readily digested that when fed with concentrates, loose feces or colic may result. The larger, grazing rhino species should have *ad libitum* access to grass hay and water. The concentrate portion of the ration should be given in at least two feedings daily for better utilization. When practical, a small feeding of hay should be encouraged prior to each concentrate feeding. Greater one-horned rhinos may also be fed some browse.

In studies of intake, digestion and passage in zoo herbivores, Foose (1982) measured dry matter intakes of approximately 1% of body mass when greater one-horned rhinos (n=3) were fed grass hays and slightly higher levels (1.2-1.6% of body mass) when fed alfalfa hay. More recent studies (Clauss et al., 2005a,b) report lower intake levels on hay-based diets of 0.5 to 1.3% of body weight and a tendency for the species to maintain weight even on low energy intakes. Thus, a guideline for as-fed diet quantity would be approximately 1.0 to 1.5% of body mass, but the exact quantity of high-fiber pellets will depend on their formulation. Offering sweet feeds in excess of 33% of the total calories in the diet is not advised. Greater one-horned rhinos have been shown to maintain weight even with no concentrates in the diet, but special attention must be paid to mineral nutrition. Larger pellets (>1.0 cm diameter) work well with grazing species.

Animals can sometimes be encouraged to consume less palatable forages if hays are soaked in water or sprinkled with molasses. Applesauce has proved to be helpful in administering unpalatable medications and/or supplements.

Produce items and browse often fed to greater one-horned rhinos are listed below (Table 4.21). The most common hays and concentrated pellets fed among greater one-horned rhinos also

are noted below (Table 4.21). Hay and browse often is fed in variable amounts, depending on the seasonal browse available. It is important to remember that all diets should be based on forages, not concentrates.

### **Feeding Location**

As with all zoo species, feed should be offered on a concrete pad or in livestock troughs or bins. Sand impaction has previously been documented in rhinos (Nouvel and Pasquier, 1946); therefore, feeding directly on the ground is not recommended. To reduce competition for food, individual feeding stations or adequate space at communal feeders is recommended.

### **Supplements**

Dietary supplements should be unnecessary in properly formulated rations. A possible vitamin-E deficiency has been suggested but not confirmed in zoo rhinos; current recommendations based on natural browse composition suggest that diets should contain 150 to 200 IU vitamin E/kg dry matter. Salt blocks and water should be available at all times. If grown in an area prone to soil selenium (Se) deficiency, forage should be tested routinely for determination of Se content in order to provide data needed for balancing rations (Table 4.20).

### **Problematic Diets**

High-quality alfalfa as an exclusive forage is unnecessary and may lead to mineral imbalances, colic and diarrhea. The consumption of fresh red maple browse has been associated with hemolytic anemia in horses and should therefore be avoided.



Table 4.21. Food items fed to Indian rhinoceros. Amounts (kg) reflect daily rations and some items are fed free choice (f.c.).

Produce	Amount	Browse/Grasses <sup>1</sup>	Amount	Hay	Amount	Pellets and Mixes	Amount
Apple	0.14-1.1	<i>Abies fraseri</i>	0-10	Alfalfa	2.81	Custom formula low energy pellet	6-12
Banana	0.14-0.82	Acacia	0-15	Alfalfa w/ brome w/ timothy	20	Grainland Select wheat bran	
Bell pepper	0.5	Acer <sup>1</sup>	0-f.c.	Alfalfa w/ coastal bermudagrass	f.c.	HMS ADF 16	1.15-9
Bok choy	0.14	<i>Alnus</i>		Alfalfa w/ coastal bermudagrass w/ timothy	32	Mazuri ADF 16	6-10
Broccoli	0.5	<i>Arundo</i>	0-11	Alfalfa w/ timothy	f.c.	Mazuri Wild Herbivore	0-7
Cantaloupe	0.14	Bamboo		Coastal bermudagrass	12-38	Purina Omolene 100	0.88
Carrot	0.14-0.82	<i>Caeltis laevigata</i>	0-10	Coastal bermudagrass w/ sudangrass	9.3-18.6	SDZ Global High Fiber ADF-25 Herbivore	10-12.8
Celery	0.14-0.5	<i>Celtis</i>	0-3	Coastal bermudagrass w/ timothy	45	TMR Custom Browser(Pennfield)	5
Cucumber	0.5	<i>Cersis</i>		Orchardgrass w/timothy	0-40	Toronto Zoo Formula ADF 22%	8
Grapes	0.67	<i>Coprosma repens</i>	9	Timothy	2.45-27	Zoo Nutrition Network Herbivore	4.08-6.81
Green beans	0.14	<i>Elaeagnus</i>	0-f.c.				
Honeydew	0.14	<i>Escallonia</i>	9				
Kale	0.25-0.5	<i>Ficus</i>	0-15				
Lettuce	0.14-0.5	<i>Fraxinus</i>	0-3				
Orange	0.5-0.75	<i>Gleditsia</i>	0-3				
Pear	0.14-0.5	<i>Gordonia lasianthus</i>	0-10				
Pumpkin	0.14	<i>Hibiscus</i>	5-15				
Squash	0.14	<i>Ilex coriacea</i>	0-10				
Sugar cane	0.14	<i>Ligustrum<sup>1</sup></i>	5-15				
Sweet potato	0.14-0.68	<i>Liquidambar</i>	0-10				
Watermelon	0.14-0.2	<i>Malus<sup>1</sup></i>	0-8				
		<i>Morus</i>	0-11				
		<i>Musa</i>	0-10				
		<i>Musaceae</i>	9				

Table 4.21. Continued.

Produce	Amount	Browse/Grasses <sup>1</sup>	Amount	Hay	Amount	Pellets and Mixes	Amount
<i>Myrica</i>			0-10				
<i>Napier grass</i>							
<i>Nyssa</i> <sup>1</sup>			0-10				
<i>Persea borbonia</i>			0-10				
<i>Phyllostachys</i>			0-11				
<i>Plantanus</i>			0-10				
<i>Poaceae</i>			9				
<i>Populus</i>			0-8				
<i>Pyrus calleryana</i>			0-10				
<i>Quercus</i> <sup>1</sup>			5-15				
<i>Robinia</i> <sup>1</sup>			f.c.				
<i>Salix</i>			0-f.c.				
<i>Sassafras</i>							
<i>Schefflera</i>			4-15				
<i>Ulmus</i>			0-10				
<i>Vitis</i>							

<sup>1</sup> Some browse species are potentially toxic when offered in large quantities. Variety and moderation are key. Animal managers should consult a nutritionist before feeding substantial quantities of any one browse item.

## Hand-Rearing

A limited number of rhino calves have been and are currently being raised using various formulas. Reports and published information must be carefully scrutinized for measures of success and methodology in milk-sample analysis. The following information uses the ungulate hand-rearing chapter in the AZA Infant Diet Notebook as a base for general feeding guidelines and formula selection (Reiter et al., 1994). This recommendation is to be used as a guideline for standardization of a hand-rearing diet.

### *Milk Composition and Formula Selection*

Based on available data, rhinoceros milk is more dilute than milks of other ungulate species. It is low in solids, low in protein, very low in fat and high in sugar compared with milk of equids, bovids and cervids (Oftedal, 1984). Formula selected should mimic mother's milk in composition as much as possible (Table 4.22). In Table 4.23, Formula One has been used to raise a calf to one year of age; Formula two more closely mimics mother's milk. In Europe, Mazuri® makes a rhino milk replacer ([www.mazuri.eu](http://www.mazuri.eu)). Land O'Lakes® Mare's Match® (Table 4.24) has been used to supplement a greater-one horned rhino calf at San Diego Zoo Safari Park and possibly could be used for hand-raising calves (powder:H<sub>2</sub>O = 1:6 but formula may need to be mixed at a more dilute ratio (1:8) for the first few days to avoid problems with constipation). The San Diego Zoo Safari Park has used a low fat cow's milk: nonfat cow's milk: lactose powder: water (27:9:1:1 by weight) (Blakeslee and Zuba, 2002).

Table 4.22. Nutrient compositions of rhino milk (Gregory et al., 1965) and recommended formulas (% as-fed basis).

Formula	Solids	Protein	Fat	Sugar
Rhino milk	8.8	1.4	0.2	6.6
Formula 1	10.3	3.3	0.3	5.9
Formula 2	8.3	1.7	0.2	6.6

Table 4.23. Compositions of rhino hand-rearing formulas.

Ingredients	Parts by Volume	
	Formula 1	Formula 2
Water	32	9
Skim milk	32	9
Karo Syrup	1	1

Table 4.24. Products used in hand-rearing diets.

Product	Manufacturer/Distributor
Mare's Match Milk Replacer Colostrum Replacement	Land O' Lakes Animal Milk Products Shoreview, MN 55126
Colostrx	Protein Technology, Inc. Minneapolis, MN 95403
Seramune Oral	Sera, Inc. Shawnee Mission, KS 66285
Replenish	Fermenta Animal Health Co. Distributor Kansas City, MO 64153
Fer-In-Sol Poly-Vi-Sol with Iron	Mead Johnson Nutritionals Bristol-Meyer Co. Evansville, IN 47721
Major Multi-Vita Drops	Major Pharmaceutical Corp. Distributor Chicago, IL 60612
Lixotinic	Revival Animal Health Distributor Orange City, Iowa 51041
Probios	Vet Plus, Inc. Menomonie, WI 54751
Lactaid	Lactaid, Inc. Pleasantville, NJ 08232

Though rhinoceros' milk is different from cow's milk, the latter may still be appropriate for hand-rearing rhinos if used in combination with other ingredients. Cow's milk is low in iron; consequently, an iron source such as Fer-In-Sol<sup>®</sup> (Table 4.24) should be added to the formula at two drops per 100 g of formula. In addition, infant vitamins, such as Major<sup>®</sup> Multi-Vita Drops<sup>®</sup> (Table 4.24), should also be added to the formula at two drops per 100 g of formula. Some infant vitamins, such as Mead Johnson<sup>®</sup> Poly-Vi-Sol with Iron<sup>®</sup> (Table 4.24), contain added iron. San Diego Zoo Safari Park uses Probios<sup>®</sup> (2 tbsp) and Lixotinic<sup>®</sup> (0.44 ml /kg body weight)(Table 4.24) as daily supplements added in the first bottle. The animal may also benefit from the addition of Lactaid<sup>®</sup> at one drop per 100 g of formula. Lactaid<sup>®</sup> (Table 4.24) aids in carbohydrate digestion and helps prevent possible gastrointestinal tract distress. If the neonate is less than 24 hours old, colostrum diluted 50% with water or an electrolyte solution for ungulates, such as Replenish<sup>®</sup> (Table 4.24), should be administered for the first 24 hours. Though species-specific colostrum is preferred, cow colostrum may be used. San Diego Zoo Safari Park uses Land O'Lakes<sup>®</sup> Colostrum

Replacement<sup>®</sup>(Table 4.24) in the first 24 hours after nursing followed by a mix of 50% colostrum and 50% formula during the next 24 hours, and then 100% formula until weaning. Products such as Colostrx<sup>®</sup> and Seramune<sup>®</sup> Oral may also be used (Table 4.24). To avoid gastrointestinal distress, a diluted formula should be offered beginning on day two. The formula can be gradually increased to full concentration depending on the animal's health, including weight gain and stool condition. Prior to feeding, the formula should be warmed to approximately 37°C (99°F). Rhinoceros calves prefer their milk cooler than many other ungulates.

### **Feeding Regimen**

The calf should be stabilized and hydrated before any feeding. Quantity fed should range from 10 to 13% of body weight (BW). Animals should be fed every two hours. Because infants suckle during daylight hours, feeding should be equally spaced in a 12 hour period not to exceed 3 to 4% of body weight at any one feeding. It is recommended that feeding begin with 10% of body weight split equally into 12 feeds one hour apart during daylight hours. The quantity of formula fed should be adjusted daily based on the animal's weight. Animals should be weighed at the same time each day. Fresh water should be available at all times.

Table 4.25. Example feeding regimen, provided by San Diego Zoo Safari Park..

Week 1 & 2	6am, 8, 10, 12, 2, 4, 6, 8pm	(18-20% BW) 8 feedings
Week 3 & 4	6am, 8, 10, 12, 2, 4, 6pm	(17-19% BW) 7 feedings
Week 5 & 6	6am, 8, 10:30, 1, 3:30, 6pm	(16-18% BW) 6 feedings
Week 7 & 8	6am, 9, 12, 3, 6pm	(14-16% BW) 5 feedings
Week 9 – 14	6am, 10, 2, 6pm	(12-14% BW) 4 feedings
Week 15	No more increases to daily volume	
Week 15 – 30	6am, 12, 6pm	(8-12% BW) 3 feedings
Week 30 – 40		(5-7% BW)
Week 41		(3-4% BW)
Week 52	Start dropping amount on all bottles	
Week 60	Weaned	

If diarrhea occurs, the quantity of formula fed should be decreased or the formula diluted until stool condition returns to normal. If diarrhea is persistent, an electrolyte solution can be used to dilute the formula, replacing some or all of the water. In addition, the number of feedings can be increased to lessen the quantity fed at any one time.

Formula can be prepared ahead of time and warmed as needed. Water should be boiled to decrease possible contamination due to pathogens and refrigerated before being added to the formula. The formula should be refrigerated and used within 72 hours. Prior to feeding, the formula should be warmed to the animal's body temperature. Calf nipples work well with large species. Bottles should be boiled before use. Diluted bleach may be used as a disinfectant. Formula left over from each feed should be discarded.

### **Weaning**

Weaning may begin as early as six months if necessary and should be completed in one year. Weaning is a slow process involving carefully monitoring of body weight and solid food consumption. Animals should have access to solid food at all times. A nutritionally complete pellet diet such as Calf Manna (Manna Pro Products, LLC., Chesterfield, MO, USA), horse feeds



or high fiber ungulate pellets, in addition to high-quality grass hay, is appropriate. Formula may be decreased by gradually eliminating the number of feeds or decreasing the amount offered per feed and gradually decreasing the number of feeds.

## RESEARCH

### Introduction and Top Research Priorities

Largely due to the rhino poaching crisis that far out-weighs any other challenge facing rhinos today, the top five priorities of the Rhino Research Council, listed below, are skewed towards *in situ* issues. It is important to note that these research priorities are not ranked in order of importance, so the first and only priority focused on the *ex situ* population is not considered more important than the others. The Rhino Research Council realizes that conservation of genetic diversity via cell/gamete rescue from remnant populations is really not a research issue and is more of an implementation challenge, but it was identified as an important action to emphasize at this time of crisis. Following this list of the highest research priorities, the chapter continues with important areas of *ex situ* research, including reproduction, health, nutrition, behavior/ecology, and genetics. For more detailed discussions of *in situ* research needs, the reader is referred to the Rhino Research Council's 2014 Rhino Research Masterplan.

### *Investigate Major Factors Affecting Health and Reproduction Ex Situ*

- Epidemiology of browser rhino health issues—Health concerns and unusual syndromes continue to impact black rhinos in captivity, but the incidence of various conditions shifts over time, and patterns require constant monitoring and re-evaluation to track current trends and conditions. Otherwise, outdated problems and historic records continue to be referenced at the cost of realizing emerging trends and realities. One example is the significant increase in the number of black rhino deaths due to renal disease compared to five years ago and a decreased incidence of mortality due to hemolytic anemia compared to 20 years ago. It would be extremely useful to secure ongoing support for a point person whose priority is to evaluate the historical data and current situations to keep abreast of the problems and factors associated with those health issues.
- Iron overload syndrome (significance, detection, treatment, prevention)—Evidence that hemosiderosis is occurring in browsing rhinos is solid, but there are still many questions surrounding the impact of this condition on rhino health, whether it is primary or secondary, why it is occurring, and what can be done about it. In the past decade, relatively little progress has been made. Although there has been some progress in developing oral iron chelators for humans, the efficacy and safety of such medications are unknown in rhinos, and they are cost prohibitive. Research on dietary tannins as iron chelators has produced inconclusive results. Recent studies on the impact of large volume, regular phlebotomies for reducing iron marker values has shown promise but is unlikely to be adopted as a long-term management strategy at many institutions. Research in any of the four areas—cause, detection, prevention and treatment—could be valuable in producing insight on how to manage/prevent this condition.
- Obesity/body condition scoring—Overconditioning in captive rhinos can lead to a multitude of health problems, including musculoskeletal, foot, and reproductive problems. One example is the almost certain exacerbation of pododermatitis associated with captive husbandry conditions in the greater one-horned rhino. Research on standardizing body condition scores for all rhino species, along with improved nutritional management, should be a priority for captive health.

- Sub-optimal reproduction (stillbirths, embryo loss, phytoestrogens, pathology)—Although reproductive success has improved in white and greater one-horned rhinos by changing management strategies, providing larger spaces and more complex social groups that include experienced and inexperienced individuals, and allowing mate choice options, there are a few reproductive challenges that remain unresolved and challenge our ability to develop self-sustaining populations. For example, < 50% of all captive white rhinos are reproductively successful, almost 50% of greater one-horned rhino calves are stillborn, and as our ability to evaluate larger numbers of female rhinos has improved, incidence of known embryo loss and reproductive pathology continue to increase.

### ***Improve Rhino Identification and Monitoring***

- Optimize/standardize survey methodologies—Given the current poaching crisis, research that can improve our ability to monitor and survey wild populations is extremely important. Post-release monitoring is our primary method of determining the success of translocation, dehorning and many other conservation efforts. Data compiled from tracking individuals after translocation can be used to develop specific criteria that individual rhinos should meet (sex, age, reproductive status) prior to translocation in order to improve the success rate. Better methods for signal transduction and reception would be helpful for forest-dwelling animals. Creative methods of transmitter attachment are needed since horn transmitters are difficult to insert in de-horned animals, and radiocollars are a challenge given the neck morphology of rhinos. Also, lesions can occur with collars. Given the abundance of research on this topic in the military field, adaptation and application of this newer technology may be useful for rhinos.
- Genetic census—Advances in genetic analyses of fecal samples, hair, environmental-DNA and microbiomes could be used to determine the genetic diversity among wild populations, including sex ratios, number of individuals and extent of inbreeding.

### ***Identify Most Important Factors in Translocation Success***

Translocations have proven extremely valuable in saving rhinos from poaching, re-populating parks/ranches, bolstering dwindling populations and establishing new populations. However, there is room for improvement, and given the importance of this action for long-term rhino survival, research is needed to enhance survival and reproductive success of every rhino moved. Studies might focus on the following example topics: white rhino boma maladaptation, mortality rates in different habitats, impacts of dehorning, impact on source populations, and identifying the type of individuals most likely to succeed.

### ***Determine Ecological Factors Impacting Rhino Populations***

To choose the best locations for rhinos and provide guidance for those who want to improve their land in support of rhino populations, more information is required regarding specific ecological traits that impact rhino population survival and ability to thrive. Spatial ecology is especially important and is integrated with habitat quality, usage and preference.

### ***Conserve Genetic Diversity via Cell/Gamete Rescue from Remnant Populations (small/sub-populations and highly endangered)***

Given the poaching crisis in Africa and India and the rapid decline of Sumatran rhinos throughout their range, it seems prudent that every effort should be made to preserve cell lines and gametes from all genetically valuable rhinos/populations. The methodologies already exist but may

need to be modified for the field conditions encountered in the African savannah and the tropical forests of Asia.

## **Areas in Need of Research *Ex Situ***

### ***Reproduction***

There are several options for minimizing aggression when trying to breed greater one-horned rhinos: 1) move aggressive animals to a very large enclosure where the females can get away from the males, and the rhinos will usually work through their differences with minimal injury; 2) pair experienced males with inexperienced females and inexperienced males with experienced females; 3) use urinary hormone monitoring and behavioral observations to ensure introductions coincide with the female's estrus; or 4) conduct artificial insemination, which has now proven successful in this rhino species. If these approaches are utilized and successful, this challenge will be resolved and will be dropped from future masterplans.

Stillborn calves, however, continue to be a big problem for greater one-horned rhinos, and stillbirth the cause associated with 30% of all greater one-horned rhino mortalities in North America and Europe. Although first thought to be primarily occurring with primiparous cows, it is not uncommon in multiparous cows as well. A study is underway to determine if fetal presentation or amniotic sac rupture during delivery are associated with live/dead outcome. Other important areas of reproduction research are as follows:

- Early embryonic death (EED)—Repeated documentation of EED in all captive rhino species is puzzling. In the past 15 to 20 years, there have been at least six confirmed cases of EED in white rhinos at four different institutions, five confirmed cases of EED in greater one-horned rhinos at three different locations, and seven EEDs confirmed in two Sumatran rhinos at two locations. Few rhinos are monitored closely enough to diagnose EED, so considering the number of confirmed cases, it appears to be a common occurrence in the captive population. It has also been reported in at least one field study in wild black rhinos. However, in most field studies females breed only once or twice before conceiving and carrying pregnancies to term, so the high incidence of EED in captivity seems aberrant. In addition to EED, abortions later in gestation have also been reported. There are many potential causes of EED and abortion in rhinos, including uterine infection, uterine scarring from a previous dystocia, endometrial hyperplasia, reproductive pathology, hormonal insufficiency, dietary insufficiency or toxicity, social/behavioral influence of enclosure co-inhabitants or other health-related conditions of the female rhinos. It is quite possible EED is occurring in many females that are breeding repeatedly without producing calves. Further investigation into the prevalence and potential causes of EED and abortion is warranted.
- Reproductive pathology—Reproductive tract pathology in female rhinos has been reported in all captive rhino species and prevalence can be as high as 50% in females over 15 years of age (white rhinos). It can range from small, inconsequential cysts that do not affect fertility to large, invasive tumors that jeopardize the life of the rhino. There is general agreement that these pathologies typically develop in rhinos that spend the majority of their lives in a non-pregnant state and could be related to repeated exposure to hormone fluctuations, a condition not natural in wild rhinos that spend much of their time pregnant. However, there are some younger rhinos that also develop severe pathology, and more recent research has revealed that a fairly substantial proportion of white and greater one-horned rhinos develop pathologies in their early teens. Understanding the etiology of this pathology and identifying hormone therapies that might prevent it would be very useful. Furthermore, criteria for

determining when the degree and types of pathology observed during an exam can be tolerated without loss of fertility versus those that render a female infertile would be very useful to establish so that appropriate management recommendations could be made.

- Silent estrus—Behaviorally silent estrous cycles have now been documented in several greater one-horned rhinos. This phenomenon challenges efforts to breed this species in zoos because introductions often are made based on behavior. In greater one-horned rhinos, it can be overcome by monitoring urinary hormones and introducing the pair based on hormonal results.
- Early pregnancy marker—Pregnancy can be diagnosed in rhinos through progesterone monitoring and ultrasound. It would be useful for management and for learning more about EED if a pregnancy-specific marker could be identified in rhinos not trained for ultrasound, but this is not a high priority.
- Low libido/lack of reproductive activity—For unknown reasons, some male greater one-horned rhinos fail to exhibit appropriate mating behaviors when in the presence of an estrous female.

The use of assisted reproduction has the potential of becoming a very useful tool for regional and metapopulation management of rhinos. Additionally, assisted reproduction may provide a means of overcoming physical and/or behavioral problems that currently prevent reproduction in specific individuals. Progress towards these goals has been impressive, and it is now time to look at priorities considered feasible to overcome during the next five years.

- Semen collection—Semen collection by electroejaculation (EEJ) has become more reliable and has been successful in all four captive rhino species. However, there are differences in the quality of samples collected with greater one-horned rhinos being the most reliable in producing a high quality, concentrated sample. In black, white and Sumatran rhinos, samples often are more dilute and more likely to be contaminated with urine or red blood cells. Samples are often adequate for cryopreservation, artificial insemination (AI) or in vitro fertilization but often are not able to withstand more complex processing like sperm sorting. More research into the effects of different anesthetic protocols and attempts at penile catheterization may prove valuable. Chemical induction of ejaculation has been attempted but has not proven very effective yet.
- In vitro fertilization (IVF)—To date, IVF success has been minimal (one IVF attempt produced one embryo in a black rhino and one IVM/IVF attempt produced one embryo in a black rhino) and more research is needed. Success has been minimal, in part, because of limited opportunities.
- Sperm sorting—Since 2005, 25 EEJ attempts across three species (n=10 black, n=9 white, n=6 greater one-horned) have been conducted for sperm sorting. Inconsistent ejaculate quality is the greatest challenge to application of sorting technology and integration into AI programs for each species. Only 36% of collections were good enough for sorting. Greater one-horned rhino sperm has a unique problem: an interaction between the seminal plasma and egg yolk that prevents staining of X and Y populations. Most progress has been with white rhino where sorted, cryopreserved samples are now available for use in AI procedures.
- Artificial insemination—Successful AI protocols using cryopreserved semen now exist for white rhinos (five term pregnancies) and greater one-horned rhinos (three term pregnancies). AI has not yet been successful in Sumatran or black rhinos. Additional research is needed in developing successful procedures for black rhinos and Sumatran rhinos, and research to improve efficiency/success rates in all rhinos is also needed to ensure the technology is inte-

grated into the captive management plan. Some challenges include: breaking down intact hymens in females that have never mated, determining ovulatory versus anovulatory cycles in greater one-horned rhinos, and controlling the cycle for planned, timed AI to improve feasibility of use.

- Estrous cycle manipulation—In Europe, significant progress has been made with white rhinos using synthetic progestin and human chorionic gonadotropin or gonadotropin releasing hormone. Similar methodologies are being tested in the U.S. An ovulation induction protocol has been established for Sumatran rhinos. Research is still needed on effective methods for controlling the cycle in greater one-horned rhinos so that specifically-timed AI procedures can be planned and implemented.
- Gamete rescue—Protocols for rescuing sperm post-mortem are well established but protocols for oocyte rescue are not. A few attempts have been made with oocyte rescue, and one two-cell embryo was produced following maturation and IVF with frozen-thawed sperm. However, all protocols still need substantial research before they will be applicable for management purposes.

### **Health**

- Obesity/body condition index – Overconditioning in captive rhinos leads to a multitude of health problems, including musculoskeletal, foot, and reproductive problems. This has particularly been associated with pododermatitis along with captive husbandry conditions in this species of rhino. Research examining methods of standardizing body condition scores for all rhino species, along with improved nutritional management, should be a priority for captive health. Methods applied to domestic livestock, and more recently elephants, should be considered for use in rhinos. Also, is the inclusion of cereal-based concentrates in rhino diets detrimental?
- Gastrointestinal and cardiovascular problems—These problems might be underreported in this species. These were significant contributors to adult mortality in a recently-reported review of necropsy reports. Research to investigate the presence of health issues and contributing factors (nutrition, management, stress, etc.) should be considered for this species.
- Quantifying stress (especially chronic stress)—Develop laboratory and behavioral markers of stress. What is the health impact, for instance, on the development of gastrointestinal ulcers, increased iron stores, etc.? New ideas include evaluation of neutrophil function using a portable luminometer and the analyses of five markers in fecal samples including thyroid hormones.
- Disease risk analyses—Translocations within and between range states or from abroad present risks for introduction of disease. Research into diseases that present potential risk to rhinos and into logistically-appropriate diagnostic techniques for screening and their incorporation into protocols should be considered part of the risk analysis and translocation process.
- Pharmacokinetics/dynamics of commonly used antibiotics and analgesics—Little scientific work has been done in rhinoceros species on therapeutic drugs. Non-empirical use may lead to inadequate or potentially adverse effects. With the advent of husbandry training and restraint devices, sample collection is now possible for these types of studies.



## **Nutrition**

Body condition scoring systems have been established for the African rhino species, however preliminary trials have proven that these scoring systems are very subjective if utilized by staff at each institution. Furthermore, efforts to control for bias by sending images of animals from different institutions to one person for evaluation were equally as difficult because of the variation in animal appearance based on photo angle/quality. Although similar scoring systems should be developed for Asian rhino species, less subjective methods for all species are needed. Perhaps physiological values for leptin, glucose, insulin, etc., could provide a more solid method of evaluating body condition in addition to computerized assessments, body measurements matched with weights and/or characterizing body types. This is an important issue since body condition has now been suggested as a factor involved in iron storage problems, disease, skewed sex ratio of calves and reproductive failure of captive-born white rhinos.

There are often requests for browse lists. Many papers exist on the browse chosen by wild rhinos as well as the nutritional components of the browse. However, the fact is that most zoos will feed what browse they can get locally and with the least amount of cost/effort. Regional browse studies on nutritional value and palatability of local species are helpful as general guides. Results of such studies have been reported for East coast species and a similar study on West coast species (in particular acacia) is ongoing. In addition, a study comparing sumac and willow was also recently reported. San Diego Zoo Global is studying the nutrient composition of elephant grass (*Pennisetum purpureum*) following one to six months of regrowth. Institutions with black or Sumatran rhino are encouraged to develop browse farms to help provide better diets for their animals, and these regional studies are a great reference when initiating a browse farm. Other important topics for nutritional research are as follows:

- Research needs related to reproduction include determining if there is an impact of diet on calf sex ratio, evaluation of new hand-rearing formulas for supplementation and complete rearing, determining milk production, and estimating energy requirements of lactation.
- Minimum dietary nutrient concentrations for rhinos need to be established for maintenance, gestation and breeding to determine if utilizing the horse guidelines are adequate.
- Science-based recommendations need to be developed regarding the use of alfalfa for rhinos.
- The use of fecal DNA analyses as a means of defining wild rhino diets should be investigated.
- The cause of tooth overgrowth could be related to diet (nutritional and/or mechanical), and further investigation could be helpful. There have been several cases of rhinos requiring repeated teeth floating procedures. Additional browse could be helpful since silica is known to reduce tooth growth.
- Recent investigations of trace minerals are ongoing, especially with copper, which is an important anti-oxidant that could combat iron. One study has changed the diet to enhance copper. Appropriate dietary concentrations of zinc and copper need to be determined as both are being supplemented for both hoof health and to bind with iron.

## **Behavior and Ecology**

Large-scale manipulative experiments to test hypotheses relating to social environment, stress, foraging/nutrition, movement patterns, etc. on reproduction, health and survival are encouraged. To achieve this goal, multiple large, naturalistic enclosures will need to be established and experimental groups assigned to different treatment conditions in a way that establishes multiple experimental replicates to address each question. Such a systematic approach, on a more limited

number of variables of interest, will advance understanding of the factors governing whether captive (and wild) populations thrive or fail. Areas of *ex situ* research might include:

- Role of communication (olfactory, acoustic) in reproduction, maintaining social relationships, endocrinology (stress and reproduction), and overall social organization (consider experimental manipulations, such as with chemical signals and acoustic playbacks)
- Evaluate social processes (courtship, aggression) leading to successful copulation
- How social mechanisms lead to establishment of social outcomes, such as territoriality, dominance, group composition, etc., and how these change through time as new animals move in and out of the group
- How do changes in ecological parameters affect social behavior, endocrinology (stress and reproduction) and organization?
- Role of climatic variables (temperature/rainfall/photoperiod) on social organization and behavior
- Development of methods to reduce bodily injuries in male-male conflict and inter-sexual conflict that inhibits reproduction
- Behavioral, hormonal, and health perspectives regarding social stress
- Manipulate social variables such as density, group size, and age-sex ratios to determine outcomes for social behavior, organization, stress, health, reproduction, and survival
- Avoidance and attraction patterns
- Social density and composition effects

## **Genetics**

The potential benefits of employing genetic tools for studying rhino conservation and population genetics are tremendous, but considerable research and development is still needed in many areas before direct, efficient, reliable application will be possible. Areas where conservation genetics could be most valuable include studies on genetic structure /connectivity of surviving populations, dispersal, paternity, and census. The potential methodologies would include analyses of mitochondrial DNA, microsatellites, genetic sexing, single nucleotide polymorphisms, immunogenetics and the microbiome. Over the next five years, there needs to be an emphasis on developing appropriate methodologies, including:

- Microsatellite analysis—Microsatellites are powerful if they can be amplified from DNA from feces and should be a priority.
- Next generation sequencing (NGS)—Since fecal DNA typing is difficult and genetic diversity in some rhinoceros species is low, NGS may be of great value in providing the initial screening for polymorphisms needed to develop markers to examine rhino population genetics.
- Standardization—Different labs using various loci are reporting different results. There needs to be some consistency, or the techniques may wrongly come to be considered unreliable for answering conservation/population questions.
- Shorter amplicons—Amplicons of 75-150 base pairs need to be targeted by genetic markers and made available for fecal DNA studies since degradation can occur rapidly in fecal material.
- Fecal microbiome analysis—Fecal microbiome analysis may be useful in some cases as an alternative to analysis of an individual's own genetic material. Microbial DNA is more plentiful and in better condition when excreted in the feces, and each individual has their own

microbial profile. This was the methodology employed to determine that the fecal samples from the Viet Nam Javan rhino were all from the same individual.

- Immunogenetic variability—Immunogenetic variability and its relationship to mate choice would be interesting to investigate. If pre-testing for mate compatibility or interest could occur prior to animal transport, it would be very valuable to animal managers in zoos and managed reserves/ranches.
- Environmental DNA—eDNA is an emerging tool in genetic studies but may be more challenging under tropical conditions.

Ongoing priority genetic studies that might include *ex situ* components include:

- Major Histocompatibility Complex (MHC) variability—MHC analyses are being planned and, once established, will help significantly when analyzing populations for variation in genes that are under selection. This is particularly important information for species that appear to have low neutral genetic variation, for example the greater one-horned and white rhinoceros.
- Fecal DNA analyses—Some work has been done, but the method needs to be optimized so that individual genotypes from fecals can be used for census work and assessing and studying breeding strategies, dispersal, etc.
- Study of disease history and parasite load—A critical emerging arena to which genetics can contribute is disease risk assessment of different rhino populations. This is particularly relevant in light of the tragic loss of five captive Sumatran rhino in Malaysia. A particular avenue worth pursuing is polymerase chain reaction techniques that can determine presence and load of different pathogens from tissue collections and fecals.

## **BEHAVIOR**

### **Ranging Behavior and Sociality**

Asian rhinoceros species are the most solitary of the extant rhino species (Ripley, 1952; Hutchins and Kreger, 2006), but temporary groups of subadults might be observed in the wild (Fouraker and Wagener, 1996). Greater one-horned, or Indian, rhinos also have the greatest affinity for water (Hutchins and Kreger, 2006). Wild greater one-horned rhinos feed on grasses in moist, riverine forest and khair-sissoo forest during the cooler season, and in tallgrass floodplain during the hot season (Steinheim et al., 2005). Both in the wild and in managed settings, male and female rhinos defecate almost exclusively at dung piles (88% of the time; Laurie, 1982). Home range boundaries are delineated by scent marks left by males during spray-urination (Ripley, 1952), dung-kicking, scraping with the fore- and/or hindlegs, and horn-scraping (Table 4.26; Owen-Smith, 1988). Home ranges of males overlap; therefore males must defend estrous females from other males (Laurie, 1982).

### **Reproductive Behavior**

It is recommended that males and females of this species should be kept separately and introduced only for breeding purposes (Fouraker and Wagener, 1996). While the most common vocalizations are “grunts” or “low bellows” (Table 4.26), females will use a high, penetrating, whistling vocalization to communicate their sexual receptivity (Ripley, 1952) (and their location in the wild) beginning six to ten hours before breeding (Hutchins and Kreger, 2006). This can attract more than one male in the wild, leading to violent male-male interactions (Laurie, 1982). Precopulatory behavior in this species also can be aggressive, including chasing up to 2 km (1.2 mi)

and biting by both sexes (Hutchins and Kreger, 2006). Laurie (1982) suggested that this behavior might guarantee that a female mates with the most dominant male in the vicinity, and females will avoid undesirable males by running away or fighting when cornered (Hutchins and Kreger, 2006).

As the female rhino approaches peak estrus, indicated by behaviors such as increased urine spraying, inappetance, increased vocalizations and vulva winking (Table 4.27), an introduction should be attempted. It has been noted that peak estrus lasts approximately 24 hours, and optimal conception chances occur between hours eight and 12 (Fouraker and Wagener, 1996). Eventually, preliminary mounts will be tolerated until the female eventually stands with her tail curled for full penetration (Fouraker and Wagener, 1996). Greater one-horned rhinos generally have been reported to mount before an erection occurs (Fouraker, and Wagener 1996). At full copulation, rhinos may remain coupled for 30 minutes to one hour, with ejaculations every few minutes (Fouraker and Wagener, 1996). It is a good idea to keep a record of all observations of sexual behavior, and suggested instructions for doing so, as well as a data sheet, are provided in Appendices E and G, respectively.

Cows will become aggressive to their older calves approximately one week prior to the birth of the next calf (Laurie, 1982), but termination of the relationship ranges from gradual to immediate (Hutchins and Kreger, 2006). Prior to parturition, zoo-managed female greater one-horned rhinos might pace within the enclosure (Hutchins and Kreger, 2006). Parturition usually lasts ten to 12 hours from water-break, though first-time mothers may take longer to calve (Fouraker and Wagener, 1996). Infants may nurse hourly while older calves nurse approximately every couple of hours (Fouraker and Wagener, 1996). It has been reported that as the calf ages, nursing will usually decrease in frequency (Fouraker and Wagener, 1996). Young calves under two months of age may be left alone while their mother moves up to 800 m (2,625 ft) away to forage, but they remain close to their mother when older than two months (Laurie, 1982). If the facility is able to house multiple female greater one-horned rhinos together, the cow and calf should not be introduced to the others for approximately four months following birth (Fouraker and Wagener, 1996).

A common behavioral abnormality observed in captive rhinos is excessive horn rubbing (Hutchins and Kreger, 2006), which might indicate boredom or distress.

Table 4.26. Ethogram to assist with general rhino management (adapted from Fouraker and Wagener, 1996).

General Behaviors	Definition
Locomote	Move about covering ground
Resting	Recumbent on the ground
Wallow	Roll, lay down, or move about in an area that is wet or muddy
Forage	Search for and consume food
Object toss	Tilt or lift inanimate object off the ground
Horn rub	Rub horn against an object; often occurs if horn becomes wet but also occurs when dry
Head sweep	Head swings laterally relative to the ground, rooting the air with the horn
Mouthing	Repeated chewing or gumming motion with mouth open; not associated with eating
Nurse	Calf becomes still and suckles; tail may wag

Table 4.26. Continued.

<b>Elimination Behavior</b>	<b>Definition</b>
Urinate	Discharge or pass urine in a stream
Defecate	Discharge fecal material
Hind foot kicking	Rapid alternation of hind feet against ground while remaining stationary; often associated with elimination by the male
Hind leg drag	Walk with hind legs stiff and straight, producing a scrape-mark on the ground (usually performed by males during scent marking before or after urine spraying)
Urine spray/squirt	Project urine in a strong spray (usually male) or in distinct squirts (usually female); may be directed on a substrate
Urine/feces investigation	Smell or taste urine pool or feces; flehmen often exhibited
Defecation on pile	Defecate on an area that has been used repeatedly as a dung pile
<b>Social Behaviors</b>	<b>Definition</b>
Proximity	Rhinos are within one body length of each other
Face-to-face stare	Rhinos stand less than one body length from and facing each other
Affiliative physical contact	Touch, rub, or lick other animal; often nasonasal contact
Horn wrestling	Slow lateral movements of the head with the horns pressed together.
Follow	Locomote to remain within close proximity of another animal
Charge	Locomote rapidly toward another animal with the head lowered
Chase	Locomote rapidly in pursuit of another animal
Open-mouth threat	Display in which one rhino faces another with open mouth and bellowing; also associated with charge/chase behavior
Spar	Rhinos use horns in offensive or defensive manner; may contact heads/horns and use body weight against each other
Horn strike	Strike another rhino with horn
Gore	Pierce or wound another rhino with horn
<b>Reproductive Behaviors</b>	<b>Definition</b>
Flehmen	Raise head and curl underside of upper lip upward; often seen in males in response to female urine
Anal and genital investigation	Sniff anogenital region of another animal
Erection	Penis in erect position
Penis unsheathed	Penis is dropped from the sheath, possibly in a partial erection



Table 4.26. Continued.

<b>Reproductive Behavior Cont.</b>	<b>Definition</b>
Stand	Female remains stationary during a chin-rest or mount
Chin-rest	Male rests chin on female's back or hindquarters in preparation for copulation
Mount	Male's weight is on hind legs, head over female's withers; copulation posture
Mis-mount	Male mounts female but not in proper orientation for copulation
Copulation	Penile penetration of vagina with successful ejaculation
Vulva wink	Rapid contractions of vulva, exposing clitoris
Vulva swelling	Swelling, color change, and/or dilation of the vulva
<b>Vocalizations</b>	<b>Definition</b>
Snort	Vocalization in which air is gruffly forced through the nasal passages, usually aggressive or defensive in context as a space-maintenance or –increasing signal
Honk	Loud, low-pitched guttural vocalization of metallic echoing nature emitted in a single burst or in a series; often follows an initial snort as a space-maintenance or –increasing signal, sudden alarm, or aggression
Bleat	Loud blaring single tone vocalization similar to the prolonged lowing of domestic cattle; made with head low, mouth open, tusks bared, and ears pinned
Roar	Similar to the bleat but louder and more forceful; common during face-to-face agonistic encounters by the submissive individual or when fleeing from a dominant adult male
Shriek	Very loud high-pitched vocalization, variable in tone and reminiscent of elephant trumpeting or dogs squealing in pain; only used during intense aggression
Squeak-Pant	Highly variable, sharp, squeaking noise similar to squeaking machinery, followed by a panting noise made by a sharp exhalation; usually given by adult males when chasing another rhino or by estrous females
Moo-grunt	Short, gruff grunt to a long, squeaky grunt like a rusty hinge; made deep inside the throat by calves with mouth open or closed, usually when separated or returning to mother

Table 4.27. General reproductive behaviors observed during estrus and courtship (Fouraker and Wagener, 1996).

Female Behaviors	Male Behaviors
Vocalizations <sup>a</sup>	Vocalizations
Urine squirt/spraya <sup>a</sup>	Frequent urination; urine spray
Urogenital changes (e.g., vulva swelling)	Erection
Vulva “winking”	Genital inspection of female
Vaginal discharge <sup>a</sup>	Flehmen response
Aggression toward male	Charge/chase female
Maintains proximity to male	Maintains proximity to female
Nuzzles male’s belly and/or genitals	Follows female
Stands for male	Chin-rest
Inappetance	Mounts female

<sup>a</sup>Estrous behaviors in the absence of a male are often difficult to distinguish. In general, increased activity, agitation, vocalizations, spray-squirting urine, and vaginal discharge have been cited. As the female approaches peak estrus, these behaviors usually increase in frequency. Some females have been reported to successfully breed without exhibiting any overt behavioral signs of estrus.

### Behavior During Introductions (Fouraker and Wagener, 1996)

Male and female greater one-horned rhinos should be introduced only during the female’s estrous period. Introductions often result in aggression, and it should be noted that rhinos of both sexes have been the aggressors. Behaviors that have been noted during rhino introductions are listed in Table 4.28. Territorial defense is often limited to ritualized confrontations, in which two rhinos advance toward each other but stop and engage in a horn-to-horn stare (Tables 4.26 and 4.28). Also as part of this ritual, the two individuals may touch horns, back apart, and wipe their horns on the ground (Nowak, 1991). More intensive conflicts (Table 4.29) involve charges and the infliction of injuries by horning or ramming. It is important to note that what is often perceived as serious or dangerous aggression between rhinos is, in fact, normal behavior requiring no intervention of any kind. Along with increased size and thick skin comes decreased vulnerability compared with many other animals.

In some cases, aggression may proceed to a point at which management should intervene to prevent serious injury. Captive managers should allow some aggression during an introduction but be prepared to intervene in the event that aggression threatens the lives of one or more rhinos. Guidelines for intervening may vary across institutions, but in general, careful consideration should be given to intervening in an introduction before aggression reaches Level 5 (Table 4.29). Stopping an introduction at a level prior to this will not lessen aggression during a subsequent introduction attempt. Animals that are allowed to “settle their differences” will establish some territorial boundaries and will usually not engage in serious aggression again, with the exception of a male attempting to approach an estrous female. In sum, moderate aggression is commonplace in any rhino introduction; sparring and fighting will occur and result in minor injuries (cutaneous wounds). However, in most cases, aggression levels prior to Level 5 may be allowed to continue using the discretion of management.

Table 4.28. Behaviors noted during rhino introductions (Fouraker and Wagener, 1996).

Non-aggressive Behaviors	Ritualized Confrontations	Potential Stress-Related Behaviors	Aggressive Behaviors
Follow	Head sweep	Pacing	Charge/chase
Touch/rub/lick	Face-to-face stare	Running (excessive)	Open-mouth threat
Anal or genital investigation		Space-maintenance and threat vocalizations (excessive)	Sparring
		Diarrhea	Goring

Table 4.29. Levels of aggression in rhinos (Fouraker and Wagener, 1996).

Level of Aggression	Definition
1	Rhinos are charging each other but do not make physical contact
2	Rhinos are charging each other with physical contact resulting in some cuts and scrapes to the facial area
3	Rhinos are charging each other with physical contact resulting in cuts and scrapes to the facial area and body
4	Charging and/or pursuit <sup>a</sup> proceeds to the point that one or both rhinos are knocked down at least once. Scrapes and cuts are deeper and more numerous.
5	Aggression and pursuit proceed to the point that one or both rhinos have subcutaneous wounds or arterial blood flow

<sup>a</sup> It should be noted that one animal might break away from the confrontation and attempt to escape. The aggressor often will pursue and begin horn-prodding the underbelly of the escapee as the two run around the enclosure. Often a rear leg is hooked and held aloft while pursuit continues. If the escapee does not stop and resume a defensive posture, the animals might continue until heat or exhaustion becomes a critical factor. Aggression at this point is more serious.

Animal personality and disposition should always be considered in introductions. A subordinate animal should be introduced to a more dominant animal in an enclosure familiar to the subordinate. In the case of multiple-animal introductions, the most subordinate animal should be introduced to the next most subordinate, and so on up the dominance hierarchy. Greater aggression may be noted in some individuals in the presence of an estrous female; therefore, any introduction attempt at this time should be especially well-monitored. Aggression may be meaningfully reduced by allowing individuals to become familiar with each other through protected contact (e.g., through enclosure bars) or, possibly, by exposing them to one another's odors for a period prior to introduction.

# PART V

## SUMATRAN RHINOCEROS

### TAXONOMY AND STATUS IN CAPTIVITY

#### Scientific Name and Origin

*Dicerorhinus sumatrensis*

*Dicerorhinus*: Greek *di*, meaning “two”; *cero*, meaning “horn”; and *rhinus*, meaning “nose”

*sumatrensis*: referring to Sumatra (with the Latin *-ensis*, meaning a locality)

#### Common Names

Sumatran rhinoceros

Asian two-horned rhinoceros: the only two-horned rhino in the Asian region

Hairy rhinoceros: refers to the long, shaggy hair found on the species in contrast to the other nearly hairless species

#### Distribution and Habitat

Southeast Asia (primarily Indonesia and Malaysia)

Montagne rain forests

#### Size

600 to 800 kg (1,300 to 1,700 lb)

0.09 to 1.5 m (3 to 5 ft) tall at shoulder

Primary horn 25 to 79 cm (10 to 31 in)

#### Physical Description

Reddish-brown coat sparsely covered with long hair, pronounced in younger animals

Fringed ears

Two horns

#### Life History Characteristics

Browser

Solitary with the exception of females with calves; males solitary but visit female territories to mate

Sexually mature at 5.5 and 6.5 years of age (females and males, respectively)

Gestation period 473 to 480 days; interbirth interval of three years

Sumatran rhinos in captivity in North America and SE Asia historically did not fare well, but improvements in husbandry, especially diet, have greatly reduced mortality rates over the last ten to 15 years. Only seven Sumatran rhinos have been imported to North America for the captive breeding program. All originated from Indonesia and are the *Dicerorhinus sumatrensis sumatrensis* subspecies. Today the North American Sumatran rhino population consists of two animals (two F1 offspring), and it is likely the population will remain quite small. Only two pairs of Sumatran

rhinos have bred successfully in captivity in the last century. The first successful pair that produced three calves was maintained and bred in North America at the Cincinnati Zoo & Botanical Garden; a second breeding pair lives at the Sumatran Rhino Sanctuary in Way Kambas National Park, Indonesia. Our knowledge of Sumatran rhino husbandry is vastly improved from what it was in the early 1990's when the animals first arrived in North America, but there is still much to be learned. Health, nutrition and research concerns for Sumatran rhinos are described in their respective chapters. Sumatran rhino-specific information regarding group composition, introductions, breeding, calf development, shipping and exhibit design is described below.

## **MANAGEMENT**

### **Group Composition in Captivity**

Sumatran rhinos are generally considered solitary outside of breeding. Females are thought to be territorial and to avoid one another. Males are also thought to be solitary but to visit the territories of females and possibly fight over them after calves are weaned. Prior to weaning, females are commonly found with their offspring (Van Strien, 1986).

Based on these data on Sumatran rhino social organization as well as collective experience in captivity, it is recommended that adult animals be housed separately and introduced only for breeding purposes (Tables 5.1 and 5.2). It is perfectly acceptable for the rhinos to share a barn and adjacent enclosures since being within hearing and olfactory range of each other is not detrimental, but they should not be allowed in the same space at the same time due to aggression (unless they are introduced during estrus for mating).

Table 5.1. Possibilities for rhino social groupings in captivity (same exhibit).

Rhino species	Multiple Animals of Same Sex		Multiple Animals of Opposite Sexes
	Adult males	Adult females	
Sumatran	Not recommended	Not recommended	Possible ONLY when female is receptive for breeding (~24 hour period)

Table 5.2. Recommended numbers for institutional holding.

Rhino species	Recommended Minimum Groupings for Breeding	Preferred Optimal Holding for a Breeding Institution	Exhibit Only (per institution)
Sumatran	1.1	1.1	0.1 or 1.0

### **Introductions**

Because of the relatively more aggressive, territorial nature of Sumatran rhinos, and their solitary nature in the wild, introductions should be attempted only for breeding purposes (one male to one female). With the possible exception of very large facilities and when cows are nursing calves, individual rhinos should be held separately in all other situations.



## Introductions for Mating

Determining when to introduce Sumatran rhinos for mating requires diligent ultrasound evaluations of the ovaries and/or serum hormone monitoring because this species rarely exhibits behavioral signs of estrus, and those individuals that do, are inconsistent in their displays of such behavior. Unlike all other rhinos studied to date, Sumatran rhinos are induced ovulators, meaning they ovulate only if introduced to a male for mating. The appropriate day for introducing a female to a male should be based on ultrasound results that reveal a recently developed, pre-ovulatory follicle that has grown to approximately 20 mm in diameter. Alternatively, when serum progesterone levels drop to very low (<50 pg/ml) concentrations, the female typically is in estrus, though using hormone levels alone is less accurate than conducting ultrasound exams as basal hormone concentrations can differ among individuals. If the female is mating and thus ovulating, estrus can be expected approximately every 21 days (19-22) if the female does not conceive. If the female does not breed and ovulate, the reproductive cycle might become more irregular and less predictable (Roth et. al., 2001).

Once it is determined that the female should be receptive for mating, the pair should be introduced in an outdoor breeding enclosure (see Enclosure Design chapter for specifications on this enclosure). The female should already be familiar with the enclosure and should be moved into the enclosure first followed by the male. The initial behavior between the two rhinos when the timing of the introduction is correct can vary significantly from one introduction to the next.

The following are normal behaviors that can be observed when the pair is introduced prior to mating, in addition to those listed in Table 5.3:

- 1) It is very common for the male to chase the female as soon as he is allowed in her space, and she will typically run from him. This chasing can go on for quite some time, but the pace typically slows over time. It also might stop for a while and then start up again.
- 2) If the female does not run from the male and stands her ground, sparring will likely take place. This also can go on for quite some time. Eventually it will slow, the animals will back away from each other and the female will eventually turn her back on the male so that he can follow her.
- 3) During some introductions, the male does not chase the female. Instead, he slowly follows the female while vocalizing (whistle-tooting). The female may respond by slowly walking in front of him or by cantering/trotting around the enclosure keeping her distance from him for some time before allowing him to catch up to her. When the male is peaceful like this the female might also stop and lay down in front of him.

Table 5.3. Behaviors noted during rhino introductions for mating.

Non-Aggressive Behaviors	Ritualized Mating Behaviors That Could Be Aggressive	Aggression Behaviors
Follow (walking)	Head-to-head sparring	Charge/chase at high speed
Chin rest	Chasing (low to medium speed)	Charging and hitting hard
Whistle tooting		Squealing loudly (usually while running)
Laying down (females)		Open mouth slashing with canine teeth

If the timing of the introduction is not correct, the male will typically pursue the female at very high speed (often with the female squealing loudly). The vocalizations will be predominantly squeals and snorts without the whistle-tooting. It is very difficult to distinguish between the mating ritual behavior and true aggression. Only through extensive experience and knowledge of the rhinos' behaviors and vocalizations is it possible to distinguish between the two behaviors and to decide if the rhino pair should be separated. Even when the timing is correct, the pre-mating ritual can lead to injury.

There have been several approaches to separating aggressive rhinos, none of which is ideal. Sometimes the attention of the chasing male can be diverted with the offering of favorite food items, and sometimes a gate can be shut between the male and female once the female puts enough distance between herself and the male. Yelling and water hoses have not been very effective.

### ***Reintroduction of a Post-Partum Female (Without Calf) to a Male***

Breeding a post-partum Sumatran rhino prior to weaning a calf is not advised. Despite the fact that the Sumatran rhino exhibits ovarian activity within approximately five months of giving birth, the lactating cow loses considerable weight and body condition and should be given ample time to regain body condition after weaning her calf before conceiving again.

## **Mating**

Typically the male will mount the female several times before achieving an erection (Table 5.4). During the first few mountings, it is not uncommon for the female to run out from under the male. Such action is often followed by more chasing. When the female stands for the male, he will start to get an erection, and it usually takes several more mountings before copulation is achieved. Copulation typically lasts 20 to 45 minutes but could be shorter or longer and is accompanied by several periods of male thrusting (presumably ejaculation). If copulation lasts less than 20 minutes, it is likely that another copulation will take place if the pair is left together. If there was a lot of pre-mating behavior and the copulation lasted 30-45 minutes, the pair should be separated and the mating period for that estrus considered to be finished. The time from introduction to copulation can range from one to two hours to up to 12 hours.

Table 5.4. General reproductive behaviors observed during estrus and courtship in Sumatran rhinos.

<b>Female</b>	<b>Male</b>
Vocalizations and snorting	Vocalizations (whistle-toot)
Urine spray/squirt	Frequent urination; urine spray/squirt
Tail held to the side or up	Erection
Stands for male	Genital inspection of female (A/G investigation)
Spars with male	Charge/chase female
Runs from male or just canters in a circle	Chin rest
Lays down in front of male	Mounts female
Runs out from under male	Follows female
Maintains proximity to male	Maintains proximity to female

## Pregnancy and Parturition

A Sumatran rhino that conceives in good body condition should only gain 100-150 lb during gestation as calves weigh approximately 34 kg (75 lb; Table 5.5) at birth. Pregnancy can be diagnosed and monitored by ultrasound and/or hormone analyses. Early pregnancy loss (within the first 90 days) has been documented in two females and is probably not uncommon in this species. Sumatran rhino mammary development is modest, but distinct changes can be noted if cows are closely monitored (Table 5.6).

Table 5.5. North American Sumatran rhino reproductive statistics (Roth et. al., 2004).

Gestation	16 months
Breeding season	All year
Birth intervals	33 and 34 months (~3 years)
Estrus cycle length	21 days (19-22 days)
Weight of newborn calf	~34 kg (65-80 lb)

Table 5.6. Physiological and behavioral indicators of impending parturition in female Sumatran rhinos.

30 Days Prior to Birth	2 Weeks Prior to Birth	24 Hours Prior to Birth
Increase in teat size	Nipples/mammary enlarge Mucus plug ~1wk prior Vulva loosens and dilates, especially when cow lays down	Increased restlessness, lies down/gets up often Sprays urine often Increased vocalizations and horn rubbing

## Calf Development

Neonatal milestones, behavior and growth rate of healthy Sumatran rhino calves have been fully detailed by Plair et al. (2012). Briefly, healthy calves can be expected to stand within one to 1.5 hours, to walk within 1.5 to 2.5 hours, and nurse within five hours of delivery. During the first week, calves will nurse every one to three hours for two to five minutes at a time. Calves typically will urinate within the first 24 hours, but do not defecate for over two weeks (16-18 days). Calves gain weight quickly the first week, averaging about 4.5 lb/day. Following that first week, calves typically gain two to 2.5 lb per day throughout the first year until they are weaned. Therefore, a Sumatran rhino calf will weigh between 800 to 900 lb at one year of age. Calves begin mouthing leaves very early in life. It is not unusual to see a week-old calf with leaves in its mouth. Often, the calf will chew on the twigs extending from its mother's mouth which could explain how wild calves learn what browse they should be eating in the forest.

It is estimated that wild calves leave their mothers at 480 to 540 days of age (16-18 mo; Van Strien, 1986). Captive calves have been weaned at 380 to 490 days (12.5-16.5 mo) with no ill side effects. Calves may pace and vocalize for up to one week after being separated from their mother. Preparing the calf for weaning by separating it from its mother for increasing intervals of time leading up to the weaning date facilitates the transition. A calf's weight may plateau for one to two

months before it starts to gain weight again, albeit at a slower rate than when it was nursing (15-20 lb/month), for the following year.

## **Identification**

Although physical characteristics such as horn size and shape make individual rhinos fairly easy to distinguish from one another, sound rhino management requires that animals be identified through permanent and reliable methods. Trovan® transponders (Electronic Identification Devices, Ltd., Santa Barbara, CA), implanted at the base of the left ear during post-natal examination or as soon after birth as possible, are one means of permanent primary identification for all individuals. Adults should be transpondered opportunistically. Transponder numbers need to be reported to the studbook keeper. In addition to a transponder, each individual should have a secondary visual means of identification, such as an ear tag or ear notch. Photographs or sketches in the animal's records may also serve this purpose.

## **Keeper Training and Interaction**

As with any position involving the management of large animals, rhinoceros keepers should have as much formalized training and experience as possible and should be familiar with rhino behavior and husbandry. In order to ensure safety and to properly meet the requirements of management, it is recommended that more than one keeper be responsible for the care of these animals on a daily basis. Keeper interaction should be restricted to designated areas and should be conducted in accordance with institutional protocols. Finally, consistency of routine is vital in daily interaction.

There are no conclusive data to indicate the effects of different styles of keeper interaction on rhinoceros behavior or reproductive success in a managed breeding situation. Interaction styles range from no contact at all to daily hands-on contact. In an effort to create an environment patterned after the wild, however, at no time should relationships with keepers substitute for natural interaction among individuals. It is important that rhinoceros personnel keep a daily log, noting any unusual behavioral changes. It is the responsibility of management to supply all pertinent data to the studbook keeper.

## **Daily Regimen**

Fresh water should be available at all times and should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset, spillage or leakage. Rhinos need access to water pools and/or mud baths to keep their skin healthy; mud wallows should be renovated periodically to prevent contamination (see Enclosure Design chapter for more information on pools and wallows).

Natural substrates should be spot-cleaned and raked daily, and hard-surfaced areas not exposed to the elements should be dry-cleaned or hosed daily and disinfected at least weekly. Indoor housing surfaces, as well as walls and rub areas, should be cleaned daily. Additionally, the daily hosing or showering of rhinos in the barn with temperature-controlled water is recommended to promote healthy skin during the winter months.

In order to facilitate participation in many research programs involving rhinos, it is recommended that institutions implement training programs following the protocols recommended in the Training chapter. Additionally, it is recommended that some form of environmental/behavioral enrichment be integrated into daily rhino management.

# REPRODUCTION

## Seasonal Changes in Physiology or Behavior Associated with Reproduction and Management Implications

The mature female Sumatran rhinoceros exhibits reproductive activity and will breed throughout the year. No apparent seasonal effects on reproductive activity have been documented, even when the species is maintained in a more temperate environment with distinct seasons (Roth et al., 2001). Similarly, the male Sumatran rhinoceros produces semen and will mate with females any time throughout the year. Fecal testosterone metabolite concentrations in mature male rhinos remain relatively constant from one season to the next, providing further evidence that there are no seasonal effects on male reproduction.

### Reproductive Cycle

Because the female Sumatran rhino is an induced ovulator, her cycle can be highly variable when she is not being introduced to a male for mating. Although a surge in luteinizing hormone (LH) induces ovulation, the stimulus for that LH surge is unknown and is not dependent on successful copulation with a male, but interaction with a male is required. If the female is introduced to a male for breeding and ovulates but does not conceive, she will typically come into estrus again in about 21 days (19-23 days). If the female does not ovulate, her cycle could be shorter or longer than the normal 21 days (Roth et al., 2001).

### Peak Breeding Season for Captive Rhinos in North America

There have only been three captive Sumatran rhino births in North America and one in Sumatra. These events occurred in April, June, July and September. Because of the low numbers of births, a peak breeding season cannot be determined.

### Reproductive Monitoring and its Use in Timing Introductions for Mating

Reproductive monitoring is essential for breeding Sumatran rhinos in captivity because behavioral signs of estrus often are unreliable, and the rhinos can be very aggressive with each other if the introduction is not timed during the rhino's estrus. Longitudinal ultrasound monitoring of ovarian activity is the most effective method for timing introductions for breeding. Introductions should be initiated when a new follicle grows to pre-ovulatory size (19-23 mm in diameter) and not when the follicle is smaller or larger than that size range (Roth et al., 2001). Serum progesterone can also be useful in timing introductions. Once the baseline concentration of progesterone is determined for an individual, introductions on the days when progesterone is at baseline are likely to coincide with the rhino's estrus. However, ultrasonography remains the most reliable and accurate methodology for timing introductions in this species. Several hormones and their metabolites have been employed for monitoring reproductive activity in the Sumatran rhinoceros (Table 5.7).

Table 5.7. Reproductive hormones monitored in the Sumatran rhinoceros (adapted from Roth, 2006).

Sample	Estrogen	Progesterone	Testosterone	LH	Other
Urine	♀ <sup>1</sup>			♀ <sup>4</sup>	
Feces		♀ <sup>1,2</sup>	♂ <sup>5,*</sup>		
Serum		♀ <sup>2,3</sup>	♂ <sup>*</sup>	♀ <sup>4,2</sup>	♀ <sup>2</sup> Prolactin, Relaxin

<sup>1</sup> Heistermann et al., 1998; <sup>2</sup>Roth et al., 2001; <sup>3</sup>Roth et al., 2004; <sup>4</sup>Roth et al., 2011; <sup>5</sup>Roth et al., 2013; \*Roth and Stoops, unpublished data



## **Pregnancy Detection and Loss**

Early pregnancy in the Sumatran rhino can be reliably detected by a rectal ultrasound examination. The embryonic vesicle may be visible in the uterus as early as 14 days after a successful mating, but it is best to wait until 16 days after mating to diagnose a pregnancy. By 16 days, the embryonic vesicle is about 10 mm in diameter and unlikely to be missed (Roth et al., 2001). Because the developing fetus drops over the pelvic brim at approximately 75 to 85 days of gestation, transabdominal ultrasound is more useful for diagnosing pregnancy later in gestation. Pregnancy can also be detected by monitoring serum progesterone concentrations, which exceed luteal levels after about three months of gestation (Roth et al., 2004).

Early embryonic loss has been confirmed by ultrasound multiple times in two captive, nulliparous Sumatran rhinos (Roth et al., 2001). Anecdotal evidence suggests that administration of altrenogest (synthetic progesterone) may help the female to maintain the pregnancy (Roth et al., 2004). No harmful side effects from administering the hormone throughout gestation have been observed.

## **Reproductive Technologies (Semen Collection, Artificial Insemination, etc.)**

Viable Sumatran rhino semen has been collected post-coitally from a female bred naturally and from males by electroejaculation, however the quality and sperm concentrations in these samples have not been high. Protocols for cryopreserving the sperm appear to be effective (O'Brien and Roth, 2000).

In the past few years, there has been a surge in the development of reproductive technologies for the female Sumatran rhino. One attempt to rescue, mature and fertilize oocytes post-mortem resulted in some oocytes maturing in vitro, but no embryos were produced (Stoops et al., 2011). Attempts to stimulate ovarian activity with exogenous hormone treatment in older, reproductively inactive females have not been successful (Patton et al., 1998; Payne and Hildebrandt, pers. comm.). In preparation for artificial insemination in potentially fertile females that cannot be mated naturally, an ovulation induction protocol was established (Roth et al., 2011). Since then, AI has been performed on three female Sumatran rhinos, one each in the U.S., Sabah, Malaysia and Sumatra, but no pregnancies have resulted. Efforts to develop AI methodologies for this species are ongoing.

## **Fertility Assessment**

Male fertility assessments should include: 1) an ultrasound examination of the testicles to evaluate tissue consistency and size, 2) electroejaculation to confirm sperm production, and 3) serum or fecal testosterone analysis. It is important to note that a positive result from electroejaculation is very meaningful, but a negative result is not diagnostic of male infertility because electroejaculation is not always successful in producing good quality sperm samples, even from proven bulls.

Female rhinos should be examined by rectal ultrasound to determine if any pathologies (masses or cysts) exist in the vagina or uterus. These types of pathologies have been reported in several female Sumatran rhinos (Schaffer et al., 1994) but do not develop in every older rhino. Ultrasonography can also be used to determine if the ovaries are active. Although a reproductively active Sumatran rhino almost always exhibits some follicular development on her ovaries, it may be necessary to perform multiple exams before concluding that a female is or is not exhibiting ovarian activity. Because a female Sumatran rhino that is not being introduced to a male is unlikely

to be ovulating, hormone monitoring may not be very informative for assessing potential fertility. However, if serum progesterone concentrations are fluctuating over time, it is likely the female is at least exhibiting some reproductive activity.

## **Genome Resource Banking**

Due to the very low numbers of Sumatran rhinos remaining in the world, genome resource banking is recommended for all captive Sumatran rhinos. Fibroblast cell lines have been established from the Sumatran rhinos in North America and are cryopreserved at the San Diego Zoo's Institute for Conservation Research. Similarly, sperm has been collected and cryopreserved at Cincinnati Zoo's Center for Conservation and Research of Endangered Wildlife.

## **Challenges**

Breeding the Sumatran rhino in captivity proved to be extremely difficult because of their solitary nature, aggressive tendencies towards each other and the lack of understanding about their reproductive physiology. Therefore, the breeding program was considered a failure for many years. However, there is now a much better understanding of their reproductive mechanisms, and breeding has been successful. The template for monitoring females and introducing males to females for mating now exists. Unfortunately, the number of animals in a managed breeding situation is much smaller than that needed to develop a self-sustaining population. Additional animals with new gene diversity need to be captured and infused into the population to avoid multiple years of severe inbreeding. Furthermore, there is now great resistance to moving rhinos between countries, an action that is necessary to ensure genetically diverse pairings and to maximize the program's success. The development of artificial insemination in conjunction with sperm banking may help to overcome this political hurdle in the short term, but it is unlikely to serve as an adequate substitute for natural breeding.

## ***ENRICHMENT***

A planned and implemented enrichment program can contribute to better health by providing animals opportunities to exert some form of control over their environment (Carlstead and Shepherdson, 1994; Baser, 1998). As it pertains to zoo-managed rhinos, we strive to encourage species-specific behaviors in rhinos while providing them response options to environmental change. This will ultimately result in their mental stimulation and the development of naturalistic behaviors (Swaisgood and Shepherdson, 2005).

Each institution may have its own set of goals and criteria for its program, which fundamentally should start with an examination of the animal's natural history, activity patterns and behaviors seen in the wild. For example, providing a diversity of browse items throughout the exhibit gives a Sumatran rhino the opportunity to forage (i.e., search for and choose its diet). Since rhinos spend the majority of their day eating, keepers could look at modifying feeding schedules or providing enrichment throughout the day rather than just once a day.

Holding facilities, exhibit spaces and/or any constraints that may be unique to the facility should be examined since these areas will directly impact options for enrichment. The temperament and behavior of the specific individual(s) also needs to be taken into consideration. Once these criteria have been reviewed, a set of goals can be established. Generally speaking the primary goal should be to "promote opportunities for the expression of species-appropriate behaviors" (Joseph and Sevenich, 1999). The Rhinoceros Husbandry Resource Manual (Fouraker and Wagener, 1996)

clearly delineated the various functions enrichment can serve, such as (1) improving the well-being of the animal by increasing exercise, satisfying behavioral needs, and optimizing the level of stimulation that animals receive; (2) educating zoo visitors by increasing the levels of natural and interesting behaviors, visibility and activity levels; and (3) conserving endangered species by improving the success of breeding and reintroduction programs. The second goal of enrichment can be loosely defined as creating mental stimulation for the animal(s).

A successful enrichment program can be briefly summarized as one that:

- a) Establishes goals for the program
- b) Creates an enrichment approval form
  - States the purpose/goal of this enrichment
  - Provides a detailed description of enrichment items (construction material, thickness, dimensions, size of holes, etc.)
  - Identifies and addresses any facility or exhibit constraints
  - Identifies and addresses safety concerns
  - Cost estimates
- c) Identifies approval protocols for enrichment submissions
- d) Creates an enrichment calendar (monthly or weekly) to ensure implementation schedule
- e) Determines how staff will document and/or track animal response to enrichment offered

To accomplish the goals mentioned above, an enrichment plan should be tailored with a set of criteria for either the individual or the species in general. The final proposal should then be submitted through the appropriate channels for approval. Ideally, an enrichment approval system should be set up to allow keepers, managers, and veterinarians the ability to assess the proposed enrichment and approve/reject it. Institutions can alter their enrichment scheduling as well as vary the type of enrichment offered (e.g., toy, food, sensory, environmental, behavioral and social) and keep track through their record-keeping or on a barn calendar (Connett, 2009). One of the most intimidating aspects of enrichment is allocating the time to document animal behavior and/or responses to the enrichment offered, however observations of responses can either be done in a direct or indirect manner as time allows. This data will enable evaluation as to whether the enrichment goals have been met. Since enrichment is dynamic in nature, adjustments can be made at any time so that the most effective enrichment is offered to the animal(s).

Enrichment can correlate aspects of ethology, psychology and animal husbandry to create a more stimulating environment for the animal (Mellen and Ellis, 1996). This has led to the inclusion of enrichment options in exhibit designs. Exhibit enrichment can be done by varying topography, landscaping, utilizing deadfall and trees, creating dirt mounds, planting vegetation, providing a wallow, and alternating the substrate (dirt, leaf litter, mulch, etc.). Other options (public view versus privacy, shaded area versus sun, etc.) can all provide the animal some control over their environment and the ability to make choices throughout the day. The aforementioned exhibit variables can contribute to effective enrichment, especially when used in conjunction with other approved enrichment activities. For example, novel scents/perfumes/extracts can be used to create a “trail” throughout the exhibit, while holes drilled in deadfall can serve as an anchoring point for browse, and either one can provide the potential for exploration and create options for the animal. The American Association of Zookeepers (AAZK) has created The Enrichment Notebook (Chan, 2004) that provides suggested guidelines and contains information on exhibit enrichment, dietary enrichment and a section on safety considerations. When using enrichment devices that are

awkward and heavy, it is important to secure these items safely, not only for the animals, but keeper staff as well. Fortunately, these somewhat bulky items can be secured safely by using pulley systems and/or other equipment can be used to hoist devices to enable hanging them higher. A synopsis of rhino enrichment options that are currently being used at zoological facilities can be found in Table 5.8. This table gives suggestions as to the primary area of use as well as its presentation. Note that food items, especially biscuits, treats, and produce, should be fed sparingly. Examples of enrichment type and some options that can be used are outlined below. These options must be used in compliance with a facility’s enrichment protocol, regulations and safety considerations.

Toy:

- Boomer Ball (Boomer Ball, Grayslake, Illinois)
- Weeble (Otto Environmental, LLC., Milwaukee, Wisconsin)
- Suspended log

Food:

- Scatter food around exhibit to stimulate grazing/foraging
- Place food items in enrichment devices to be randomly dispensed
- Fruits and vegetables frozen in bucket of water

Sensory:

- Use conspecific’s dung for smell (olfaction)
- Play different animal vocalizations or hang bamboo “chimes” for hearing (audition)
- Mount street sweeper brush for touch (tactition)

Environmental:

- Changing substrate (leaf litter, dirt, mulch, etc.)
- Altering daily routine
- Rotating to different enclosure (pen)

Behavioral:

- Training new behaviors
- Training for veterinary procedures

Social:

- Creating mixed species exhibit

Training for husbandry behaviors is not only stimulating for the animal, but it also can provide an invaluable opportunity for the veterinarian to perform diagnostic procedures with potentially less stress and more cooperation from the animal(s) (Dover et al., 1994). The chapter for training will delve more deeply into this topic.

Table 5.8. Synopsis of rhinoceros enrichment ideas.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
<i>Amazing Graze</i> <sup>1</sup> (with biscuits/treats/produce)	X	X		X
Audio clips of vocalizations (rhino, other sp.)		X	X	
Beer keg		X	X	X
Biscuits (gorilla-sized leaf eater; horse treats)	X	X		X
Boat mooring buoy		X	X	
<i>Boomer Ball</i> <sup>2</sup>	X	X	X	X
Bowling ball		X		X
Branch/twig with produce skewered on it	X	X	X	X

Table 5.8. Continued.

Enrichment Option	Area of Use <sup>a</sup>		Presentation <sup>b</sup>	
	Exhibit	Holding	Suspended	Ground
Browse or approved plants	X	X	X	X
Brush	X	X	X	
Brushing by keeper		X		
Cardboard box (with or without produce)		X		X
Firehose “wall”		X	X	
Ice block (with or without flavor/produce)	X	X		X
Large cardboard tube		X		X
Log, stump, rootball	X	X	X	X
Melon (whole or chunks)	X	X		X
Mirror		X	X	
Mister	X	X	X	
Mud wallow	X	X		
Non-radial tire (with cuts in sidewall for safety)		X	X	
Oblong stone	X	X		X
Painting with non-toxic finger paint		X		
Paper grain bag filled with hay or produce		X	X	X
Paper mache ball	X	X	X	X
Peanut butter (on toys/walls/“furniture”)	X	X		
Planter bucket	X	X	X	X
Plastic soda concentrate container		X	X	X
Plastic drum (55 gal.)		X	X	X
Plastic ice block or iceberg toy		X		X
Plastic jug feeder		X	X	
Produce	X	X	X	X
PVC or bamboo chimes		X	X	
PVC tube with bells suspended inside		X	X	
Rolling treat bucket/feeder		X		X
Scent, flavoring, or extract	X	X		X
Snow-pile, -people (with or without fruit mix)		X		X
Substrate pile or change (dirt/gravel/sand/leaf)	X	X		
Traffic cone or pylon		X	X	
Training with keeper	X	X		
Triangle rattle		X	X	
Urine, feces, or soiled bedding from others	X	X		X
<i>Weeble</i> <sup>3</sup>	X	X		X
<i>Weeto</i> <sup>3</sup>	X	X		X

<sup>a</sup> Area of use may be dictated by institutional guidelines and criteria

<sup>b</sup> Presentation format is based on that which is most commonly used

<sup>1</sup> Sanctuary Supplies, Rootstown, OH; sanctuarysupplies.com

<sup>2</sup> Boomer Ball, Grayslake, IL; boomerball.com

<sup>3</sup> Otto Environmental, LLC., Milwaukee, WI; ottoenvironmental.com



# TRAINING

A husbandry training program is considered an integral component of progressive animal husbandry programs in zoos today. Providing zoo-managed animals with choices and behavioral opportunities in their environments through a training program can result in healthier animals and better educational experiences for the zoo visitors. Training rhinos leads to a wide variety of animal management opportunities, from shifting to safer, less stressful medical procedures.

The purpose of this chapter is to give trainers an idea of the types of behaviors that can be trained with rhinos (Appendix B). This chapter will also give direction on starting a training program, ideas for shaping techniques, possible reinforcements, specific challenges to training rhinos, and resources for additional training information.

## Setting Up a Training Program

A well-planned, consistently-delivered training process is critical to the success of any program. To achieve this type of program many facilities utilize a framework that is taught in an Association of Zoos and Aquariums (AZA) course, Managing Animal Enrichment and Training Programs. This framework is called the “SPIDER” model. Steps in this framework include: Setting goals, Planning, Implementation, Documentation, Evaluation, and Re-adjusting. For more information on this please see [www.animaltraining.org](http://www.animaltraining.org). It is beneficial to start a training program by determining the overall behavioral goals (i.e., detailing the specific behaviors to be trained). During this goal development process it is important to include all parties involved with the management of the animals. This may include meeting with and seeking feedback from keepers, veterinary staff, nutritionists, behavioral husbandry staff, curators, and managers. Having everyone on the same page with clearly laid-out plans, assignments, and timelines helps to facilitate a smooth process. Defining roles and creating clear channels of communication and decision-making authority among all participants is also important. This can be accomplished through regularly scheduled team meetings, a consistent method of documentation, and continual communication among all staff involved in training. Facility design can have an effect on setting up a training program. Discussions must also include how the rhino will be reinforced. The next two sections will discuss both of these topics further.

### *Facility*

When beginning a training program, it is important to start training in an area that is safe for the animal care staff and the animal, and where the rhino is comfortable. This is usually the night quarters or holding area (Fig. 5.1). Training can also be done in barn stalls, outdoor barn paddocks, training chutes, or even open exhibit areas. Because all facility designs are different, training staff will have to be creative and utilize the space available. For examples of possible body positioning behaviors, see Appendix B. For many of these behaviors trainers will require areas with no or minimal obstructions, which allow safe access to the desired body parts. It is important to remember that a fancy, expensive facility is not necessary to accomplish a successful training program, just a creative mind.

### *Reinforcement*

A critical component in training is finding a positive reinforcement or reward for which an animal is willing to work. In most cases, the rhino’s regular diet can be utilized for training. Any manner of storage container for the reinforcement can be used. Reinforcement items just need to be

easily retrieved and delivered as soon as possible after the desired behavior occurs. Reinforcement can be delivered by tossing it through posts or by hand feeding if it can be safely accomplished.



Fig. 5.1. Indoor chute for training and procedures (courtesy of C. Nordin, St. Louis Zoo).

## Training Methods

There are several steps to creating a husbandry training program for rhinos. After the behavioral goals are set, a safe facility to work the animals is identified, reinforcement type and how the reinforcement will be delivered has been determined, the next steps are learning about the animal to be trained, building a relationship with that animal, and designing a training plan. An overview on learning and training can be found in Mellen and Ellis (1996).

### *Natural History/Individual History*

An understanding of the natural history of rhinos, paired with information about an individual animal's specific background and level of experience, play an integral part in a husbandry training program. For example, wild-caught or hand-reared rhinos may react differently to trainers when compared to captive, parent-reared individuals. It is helpful to start training in a location where the animal is most comfortable. Some animals need to have more space available around them to feel comfortable within the training environment. The personal space needs for a rhino often center around the size of the enclosure in which the animal is being trained. Other relevant issues regarding an animal's space requirement may include proximity to conspecifics, other species, and issues such as noise levels and extraneous activity. Keeping training sessions short and moving at a comfortable pace for the rhino may help keep their focus. Tactile interaction is important—most rhinos enjoy being scratched. Care must be taken to avoid startling the animal or putting yourself in the danger zones—areas where the rhino is able to horn, kick, or injure someone. When desensitized, rhinos will interact positively with their keepers and will seek out tactile interaction. Keepers need to always maintain awareness and caution as startled rhinos can react violently and quickly. Studying the animal's natural history helps to gain insight into the animal's behavior. For more information on rhino natural history, see the Behavior chapter of this manual. Insight can also be gathered from staff, the animal's ZIMS records, or other institutions about the animal's individual

history. There is no substitute for simple observations—just watching the rhino’s behavior in different situations, for example, what it looks like when the rhino is calm or aggressive. Watch the rhino on and off exhibit, at different times throughout the day, and when the rhino is unaware it is being observed. This will greatly help understanding and interpretation of the rhino’s behavior during a training session.

### ***Relationship***

Developing a trusting relationship between the trainer and the animal being worked with can be very beneficial to training. If the animal is not comfortable being in close proximity to people, the rhino might back away from the trainer or try to horn the trainer. Developing a relationship can help address these issues. A relationship can be developed through normal daily animal care, such as feeding, observing, regular cleaning routines, and avoiding negative interactions. The more positive interactions the animal has with staff, the more comfortable the rhino will be. For example, instead of just putting the diet in the enclosure, the trainer can hand-feed the diet. If the animal is particularly skittish, the trainer may start by placing or tossing the food in the enclosure and sitting nearby while the rhino eats. As the animal becomes habituated to the trainer’s presence, s/he can begin to hand-feed and work their positioning closer and closer to the animal.

### ***Training Plans***

Creating a training plan can be a good process to help trainers think through what steps they are going to take to train a behavior. One way to do this is to establish what the final behavior will look like, and then break-down the behavior into a series of small steps called “successive approximations”. Examples of training plans can be found in Appendix C.

### ***Bridging Stimulus***

Utilizing a bridging stimulus can be a very useful tool in training rhinos but not a necessity. A bridge is a signal that pinpoints the exact moment in time the behavioral criterion was met. First a trainer must select a bridge. Common bridges used are clickers or whistles. Once selected, the trainer needs to associate the sound with the delivery of reinforcement. For example, if the bridge selected was the clicker, the trainer would click and feed, click and feed, and continue this for several sessions with the rhino. Once the association has been established, the sound of the clicker with a food reward, the trainer can begin to use the bridge to shape behaviors. For more information on a bridging stimulus see Pryor (1984), Mellen and Ellis (1996), and Ramirez (1999)

### ***Shaping Behavior***

For consistency, one trainer should shape new behaviors with an animal. Once the behaviors are trained and performed on cue consistently, other trainers can work with the animal. A shaping technique that works well with rhinos is using the sight of food as a lure. In most cases, rhinos focus on the food and follow the food wherever it is placed. Trainers can also use their body positioning when shaping behaviors. The animal will, on many occasions, shift its body position when the trainer moves. For example, if a rhino is facing a trainer and the trainer takes a step to the left, many times the rhino will follow and shift its body to the left as well. Another useful tool in the shaping process is a target. A target is an object to which an animal is trained to touch a part of its body (Mellen and Ellis, 1996). Training a rhino to target different parts of its body is a good method of shaping many body-positioning behaviors such as standing, presenting a side of the body, or presenting a foot (Appendix C). When starting a program it is best to begin by training some basic body-positioning behaviors such as open mouth, target, and back before moving on to more complex behaviors like standing for blood collection or other veterinary procedures.

## **Record Keeping**

It is important for trainers to keep records of all sessions. Trainers can go back and look for patterns in behavior, which helps keep consistency among trainers and leaves a historical record for others. For an example of a documentation form, please see Appendix D.

## **Safety**

Establishing a safety protocol is another valuable component to a rhino training program. These protocols will allow a facility and training team to set clear guidelines to ensure the safety and well-being of the trainers and the animals. The disposition of the individual rhinos should be taken into account during any training session, and the ability to read the animal's body language and temperament will allow the trainer to know when the rhino is agitated and the session needs to end. In general, it is advisable to end a session on a positive note and not wait until an animal is agitated.

In any training session where the trainer is in close proximity to the rhino, as in blood-draws, it is important for keepers to work in groups of two or more. If there are multiple keepers involved in a session, then there are multiple people that are able to watch the keeper that is working close to the rhino, the rhino's behavior, and the location of its head and horn(s).

## **Summary and Resources**

The purpose of this chapter is to give trainers an idea of the type of behaviors that can be trained with rhinos and what these behaviors might look like. This chapter provides direction on starting a training program, ideas for shaping techniques and reinforcement, some specific challenges to training rhinos, and resources for additional training information. This chapter is meant as a reference for basic training information and contains just a small amount of the information that is available.

The following is a list of additional resources that can be helpful in developing a training program:

- *Animal Keeper's Forum*, a publication of the American Association of Zoo Keepers
- [www.animaltraining.org](http://www.animaltraining.org)
- Animal Training Organizations –
  - IMATA (International Marine Animal Trainers Association)
  - AAZK (American Association of Zoo Keepers)
  - IAATE (International Association of Avian Trainers and Educators)
  - ABMA (Animal Behavior Management Alliance)
  - PEM (Principles of Elephant Management)
- International Rhino Keeper Association (IRKA)
  - [www.rhinokeeperassociation.org](http://www.rhinokeeperassociation.org)

# **TRANSPORTATION**

Sumatran rhinos are easily conditioned through positive reinforcement to enter into and travel in a crate. Conditioning should begin about one month prior to the transport date. These rhinos are very calm travelers if they have had time to adjust to spending time eating in a crate, so no anesthetics or sedatives are necessary when moving Sumatran rhinos. The crate should be at least 0.3 m (1 ft) wider and 0.6 m (2 ft) longer than the rhino.

## Pre-shipment Medical Procedures

Communication at the veterinary level between receiving and shipping institutions prior to rhino translocations is essential in order to discuss specific institutional and/or state requirements. Standard medical procedures for all moves should include the following: (1) a tuberculosis test within 30 days to six months of shipment (depending on where the rhino is being shipped) or as particular state, federal or international requirements dictate, (2) brucellosis serology if dictated by particular state or international requirements, (3) a physical examination, (4) three negative fecal screens 30 days prior to shipment, and (5) a review and update of vaccinations (see Health chapter). In addition, medical or research **protocols** defined by the SSP should be reviewed during the planning process.

## Crating

Crating is the recommended transport method, although transport in trailer stalls has also been successful. It is important in the latter case that the trailer is well reinforced. In all situations, the animal's behavior and conditions should be constantly monitored. Typical problems that can occur during shipping include the following: (1) animals destroying and/or climbing out of the crate top; (2) animals becoming inverted in the crate and unable to right themselves; (3) animals destroying end panels or doors, resulting in eye, horn or facial injuries; and (4) prolonged, excessive exertion resulting in hyperthermia and/or myopathy.

## Design

The International Air Transport Association (IATA) crate design specifications are listed in Table 5.9. Crates are usually constructed of wood, metal, or wood with steel reinforcements. Crate dimensions should be determined by the animal's size (Table 5.9), but a general principle is that the crate should be 0.3 m (1 ft) wider and 0.6 m (2 ft) longer than the animal when it is lying on its side. Crates with vertical bars situated at the head end will decrease injuries to the head and face but must be spaced correctly with at least 10-15 cm (4-6 in) gaps. Horizontal bars at the head end should be avoided, as they tend to cause horn breakage and/or damage. Crates with bars and doors at both ends are optimal.

Table 5.9. Approximate crate dimensions by species (modified from IATA, 1995).

Species	Length	Height
Black rhino	271 cm (107 in)	191 cm (75 in)
White rhino	475 cm (187 in)	221 cm (87 in)
Greater one-horned rhino	335 cm (132 in)	201 cm (79 in)

## Principles of Design

Frames should be constructed of strong metal with sides of solid hardwood. Vertical metal bars should be bolted in place at the front and back with sliding or hinged wooden doors to the exterior of the bars. The upper third of the wooden doors must have ventilation spaces or openings. There are several new requirements for lower ventilation as well. IATA specifies that the roof must be solid over the animal's head and slatted over the loin and hindquarters for ventilation. For



ground transportation, however, removable panels or hinged doors over the animal's head can be useful for administering to medical needs and monitoring the animal. Hatches also allow for more ventilation when an animal is standing calmly.

The interior must be smooth with no projections. Wooden crates have often been modified to include solid metal sheets at the head so that the horn deflects and cannot damage the wood. Only nuts and bolts should be used in the container. Entry and exit doors must be closed and bolted in strategic places to be strong enough to resist the animal, and to withstand the rigors of the equipment necessary to move the crate and rhino. The floor must be at least 2.5 cm (1 in) thick and be a non-slip surface. Some crate floors are slatted so that urine and feces can flow through so that the animal is not standing in excreta. For international and air transport, the container must be constructed in such a way that the floor and lower sides are leak-proof. In view of the diversity in size, strength and temperament of rhinos, the size and strength of the container must be sufficient to restrict the movement of and restrain the animal. Dimensions must be large enough to prevent cramping without allowing unnecessary movement. In general, the crate should be 0.3 m (1 ft) wider and 0.6 m (2 ft) longer than the animal when it is lying on its side.

At the front of the container, there must be provisions for water and food access at the base of the door and between the bars, if present. For air transport, this access point must be clearly marked FEEDING and be adequately secured when not in use. A water container must be provided and must be sufficiently large for the entry of the animal's muzzle. Some rhinos will not drink from a tub, and so offering a hose directly into the crate (either into the animal's mouth or just puddling in front of the rhino) is sufficient to provide water. Entrance and exit must be clearly indicated on crates used for air transport. Many crates are designed to be used in either direction. The above recommendations are modified from IATA standards to include specifications for ground transport. Before shipping by air, consult the current IATA specifications and/or the airline.

## **Acclimation Training**

Crate acclimation can require two to six weeks, although several zoos have crate-trained rhinos in seven days or less. Training should be completed by a method of approximation (with reinforcement given as rhinos demonstrate progress towards the desired behaviors). The first step is to introduce the crate as a non-interactive part of the animal's environment. Gradually, the food is moved toward and finally into the crate. If the animal acclimates to the point of completely entering the crate and will allow the door to be shut, the door should be left closed for short acclimation periods under close observation. It is not advisable to close the animal into the crate if the training period is short. No rhino likes to be closed into the crate, and even the best-trained rhinos will react negatively to being locked in. If there is not adequate time to train the rhino to acclimate to a closed crate, closing him/her in will only make the experience a negative one, and there will be a major setback to the ability to close the animal in for shipment. If the rhino does not completely acclimate to entering the crate, partial immobilization (standing restraint) may need to be utilized for shipping. Forced crating without training or immobilization is strongly discouraged.

## **Crating with Chemical Immobilization**

Immobilization offers a fairly simple way of crating a rhino. First, it should be noted that the usual pre-immobilization procedures (e.g., fasting, detainment in an adequate holding area, etc.) should be observed for any procedure requiring the use of chemical immobilization/tranquilization agents. For rhinos, etorphine (M-99) remains the drug of choice, although several alternatives

are available. For specific drugs and dosages, refer to the Health chapter of this publication. The duration of immobilization without the administration of an antagonist may range from 30 minutes to two hours.

Following crating, all rhinos should be held for 24 hours at the loading location for observation or accompanied by a veterinarian during transport. This step is necessary because renarcotization is common in hoofed animals, especially rhinos, given opioids. This step, however, may not be necessary if the butorphanol/detomidine anesthetic protocol is used (P. Morris, pers. comm.). Trained personnel should be present to administer the correct reversal agent(s) in the event of a renarcotization. Any other complications of crating can be managed more easily and effectively before departure rather than en route.

## **Transport**

Options for transporting rhinos are available. Each method has its advantages, and each should be scrutinized by evaluating the distance to be traveled, the personnel needed and the temperatures to which the animals will be subjected. A flat-bed truck and open trailer is temperature-restrictive. A crate within an open trailer should be protected from excessive wind, rain and sun. Enclosed trucks or trailers are other options that are necessary in extreme hot or cold temperatures. In any case, the transport vehicles must be climate-controlled if shipping in inclement weather (either hot or cold). If weather conditions and ventilation are appropriate, many rhinos have been moved in enclosed trailers without climate-control. Air transport, rather than ship transport, is the preferred option for any transoceanic translocation. Transport by ship is undesirable because of the excessive time at sea, variable conditions and more intensive personnel requirements. When transporting by air, it should be noted that some airlines may require the rhino crate to be placed in an aluminum air cargo box, which can restrict ventilation and subject the rhino to excessive heat buildup during both the airplane-loading process and transport.

During all rhino shipments, the shipper must be aware that any animal that has been immobilized (and to a lesser extent, some that have not) will be less capable of maintaining thermal homeostasis than a normal animal, and appropriate accommodations for this are necessary (e.g., ventilation, climate-control). If during the course of a transport procedure a situation arises in which the safety of the animal is jeopardized, a decision should be made through the appropriate channels to postpone or cancel the shipment. Leaving the decision of whether to transport an animal to the transporter or the recipient may be disadvantageous to the animal's welfare. The transporter is not familiar with pertinent medical and practical husbandry information, and recipients are at a disadvantage because they are often not present.

## ***ENCLOSURE DESIGN***

Whether an institution wishes to maintain rhinos only for exhibit or for breeding will determine the design of rhino enclosures. Whenever possible, institutions are encouraged to plan for breeding capabilities, but the various SSP coordinators and RAG chairs recognize the need for display-only exhibits, which can facilitate education and/or research. These exhibits serve rhino management programs by holding non-reproductive and single-sex specimens. The following section outlines design considerations for indoor and outdoor rhino facilities, as well as aspects of chute design for rhino restraint. Table 5.10 lists the recommended enclosure types and sizes for captive Sumatran rhinos.

Table 5.10. Recommended enclosure types and sizes for captive Sumatran rhinos [in sq m and (sq ft)].

Individual Holding (per rhino)		Breeding/Communal	
Indoor	Outdoor	Indoor	Outdoor
18.6 (200)	371.6 (4,000)	Not recommended	Minimum 465 (5,000)

## Outdoor Enclosure

The essential elements of an outdoor Sumatran rhino enclosure include:

- 1) shade over the entire exhibit
- 2) a mud wallow (whenever possible given weather restrictions)
- 3) a pool (whenever possible given weather restrictions)
- 4) rubbing posts or trees
- 5) clean feeding area
- 6) natural (non-concrete) substrate

In many respects, the critical enclosure characteristic is the availability of escape routes and visual barriers, which serve to hide or prevent access to an animal that is being pursued. Gates may be used as escape routes, provided that care is taken to prevent dead-end corners and to create “run-arounds” (brush piles, earth, or boulders) so that an animal can enter or leave the yard without an aggressor blocking or guarding the only exit. Posts or trees in the enclosure will provide the rhinos a necessary element for eliciting natural behaviors that include body rubbing, horn rubbing and territorial marking via urine spraying. It is recommended that enclosures be designed such that animals may be kept outdoors in shaded enclosures as much as is feasible within the following temperature constraints. Sumatran rhinos should not be locked outside when the temperature is below 10°C (50°F); sun, wind chill and rain should be considered in calculating temperature. During extremely cold weather, rhinos should not have access to pools or mud wallows; pools should be drained and mud wallows filled with substrate. Animals should not be let out if enclosures are icy. Temporary exposure to temperatures below 10°C (50°F) for cleaning is left to the discretion of management. Localities that experience average daily temperatures below 10°C (50°F; average of high and low temperatures over a 24-hr period) should provide heated facilities capable of maintaining a minimum temperature of 13°C (55°F).

### Primary Barriers

The barrier between rhinos and the viewing public is a critical element in the design of the outdoor exhibit. This primary barrier should allow visitors a clear view of the animals from a safe location. One consideration in choosing fence type should be the size of the enclosure. For example, smaller exhibits should be constructed with barriers that provide as much visual exposure as possible. Sumatran rhinos are excellent climbers and swimmers. Solid vertical primary barriers are best. Horizontal bar fencing is not recommended because these rhinos will climb on the bars and in doing so would be at risk of getting a leg stuck/broken between bars. Hot wire can be used as a secondary barrier to protect planters and deter climbing if there are some low spots in the primary barrier. As with other rhino species, a primary barrier should be a minimum of 1.5 m (5 ft) high and non-climbable. A moat would not be considered a barrier of any type for Sumatran rhinos.

## **Fencing**

In small enclosures, particular attention should be given to the climbing ability of rhinos and to the need for separating aggressive animals. A secondary barrier or a taller primary barrier may serve to counter these problems. It is important to consider fence spacing and keeper access/exit in the event of an emergency as well.

Recommended materials for primary fencing include solid concrete or rock walls and vertical pipe or posts spaced 25 to 30 cm (10-12 in) apart. The size of the exhibit to be fenced will determine the strength and type of fencing material used, as each type has both advantages and disadvantages. Concrete surfaces and bare steel cable create surfaces that may encourage rhinos to horn-rub excessively, causing abnormal horn wear. If necessary, surfaces should be covered with a non-abrasive material; one solution is to insert steel cable through plastic pipe, or concrete surfaces can be covered with non-toxic wood.

If poles are used, each should be approximately 30 cm (12 in) in diameter and set in concrete with approximately 1.8 m (6 ft) underground. Poles should be spaced as closely together as possible to prevent rhinos from getting their horns through and uprooting the fence. ***Creosote-treated poles, which are dangerous to rhinos, should not be used.***

Rocks or a rock apron can be utilized to protect the poles or other objects in the exhibit from damage. A rock apron should extend 1.8 m (6 ft) from the leading edge of the object to offer adequate protection. If small rocks are used, they should be several layers thick; otherwise, a single layer of very large rocks is probably adequate.

## **Secondary Barriers**

Though not critical to the design of outdoor enclosures, secondary barriers may protect exhibit features or lessen stress on primary barriers. Recommended types are butt rails, vertical poles and electrically charged, or “hot,” wire. Plantings can also serve as a secondary barrier when used to create a visual screen. For example, plantings that extend above a low wall can give the appearance of a bigger wall [although the primary barrier height minimum of 1.5 m (5 ft) still applies]. Electric fencing can deter animals from destroying plantings, trees and other secondary barriers. Rock aprons may also be used around trees and fence lines as secondary barriers.

## **Gates**

Enclosure gates can be the weakest points of the exhibit; therefore, adequate hinge and lock strengths are very important. Interior doors are usually constructed of heavy-gauge steel or pipe that is hinged or sliding. Sliding gates are optimal, as they have the ability for partial opening, and should be a minimum of 1.8 m (6 ft) wide and 2 m (6 ft 8 in) high. If the gate uses a track, care should be taken in the construction of the track to avoid injuring the feet of the animals as they run through gates during introductions. Exterior building doors may be made of steel or wood reinforced with steel, with the lower part covered by a steel plate to minimize damage. Gates should be constructed to allow keepers to open and close them without entering rhino space. Also, where appropriate, vehicle access to an enclosure should be provided.

There are a variety of options available to operate gates. The simplest systems are manually operated, either push-pull or cable driven. Rhino-size doors can be heavy and difficult to move. Mechanical systems can make this easier, using electric motors or hydraulic or pneumatic pressure to move the doors. These systems require some form of back-up system in case of a mechanical or power failure. Mechanical systems can generate significant forces that can injure or even kill an

animal. Safety measures have to be incorporated into the design of the gate operating system to prevent accidents.

### ***Substrate***

The outdoor enclosure should have a well-drained surface that provides adequate footing for rhinos. Although the feet of Sumatran rhinos do not exhibit the extensive problems observed in captive Indian rhinos, they fare better on natural substrate, especially with mud exposure, instead of concrete. Rhinos should be carefully observed upon introduction to a new substrate, as excessive ingestion of the substrate from feeding on the ground has caused impaction in other hind-gut digesters.

### ***Water***

Fresh, potable water should be available at all times. Water should be changed daily or supplied by an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be substantially constructed to prevent injury, upset, spillage or leakage.

### ***Mud Wallows***

All rhinos need access to pools and/or mud wallows for skin health, temperature regulation and behavioral enrichment. The size of the mud wallow should be adequate for a single rhino or a cow and calf to wallow comfortably and should be deep enough that the rhinos can completely cover their bodies in mud by submerging themselves or rolling in the wallow. It should be noted that given a start, rhinos may construct their own mud wallows. Construction of wallows varies by institution and exhibit. Some facilities use a concrete basin or pool filled with substrate, while others simply dig out a section of the enclosure. If a concrete pool is used, it should have the ability to be drained when needed, but care should also be taken to protect the drain line from being filled with substrate. If a wallow is dug on exhibit, it should be situated in an area that will allow adequate drainage away from the wallow. It is also important that a good source of water be nearby to keep the area wet. Wallows are much easier to manage if the enclosure allows access for a skid steer or tractor with front loading bucket.

Mud wallows must be actively managed. The type of soil used in the wallow is important. Clean, shredded topsoil is a good choice. If the local soil does not create mud of a satisfying consistency, it can be adjusted with the addition of topsoil, clay or sand. Standing water in an animal area can be a potential USDA compliance issue. The wallow should be monitored for the growth of algae. If the algae cannot be removed by raking or scraping, it may be necessary to fill the area in to allow it to dry completely for several days, and then dug out again. Depending on the local environment, the substrate may need to be changed several times during a year to prevent contamination. Institutions in northern climates may need to fill in the wallow during winter months.

### ***Pools***

Sumatran rhinos preferentially defecate in water. By providing a pool in addition to the wallow, the rhinos will defecate in the pool and the wallow will remain relatively clean and require less upkeep. However, the filtration system of the pool needs to be adequate to handle rhino feces on a daily basis. The recommended size is 46.45 sq m (500 sq ft) and about 1 m (3-4 ft) deep. Ramps are preferable to steps for entry into the pool. They should be placed in at least two or three locations around a pool, if not the entire perimeter, to ensure safe in-and-out access. Ramp slopes should be no greater than 15 to 20°. If steps are used rather than ramps, they should have a 20- to



25-cm (8-10-in) rise with a 41- to 61-cm (16-24-in) step. Multiple entries to a pool prevent it from being a “dead end” in the enclosure. Keeper access for cleaning should be considered in the design of slopes or steps. The pool substrate should be broom-swept concrete.

### **Shade**

Access to shade is a USDA requirement, but it is not enough to simply provide Sumatran rhinos with shade. The entire exhibit should be protected from direct sunlight during the day. Southeast Asian forests filter out >95% of UV radiation, and the eyes of these rhinos are very sensitive to too much sun, basically becoming sunburned when exposed too long. Exactly how much sun they can tolerate and for how long is unknown, but the safest approach is to keep the species protected from sun at all times. The rhinos do not know to seek out shade and will often stand directly in the sunlight, especially in cooler temperatures, so simply providing a shaded area within the enclosure is inadequate.

### **Indoor Enclosure**

Indoor exhibit elements must include:

- 1) water source
- 2) mats
- 3) scale
- 4) chute that provides access to the animal for blood collection/medical procedures/  
ultrasound exams

Indoor housing is recommended for additional separation capabilities (beyond the primary enclosure) and is critical for most institutions since colder nights can be experienced, even in southern states. At no time should rhinos be forced to endure temperatures below freezing for any length of time; animals may go out for short periods when temperatures are below freezing, but they should have access to radiant heat or heated enclosures during these times. An indoor facility in the winter should be heated to a minimum of 13°C (55°F) with the capability of maintaining some areas of the barn at 23.9°C (75°F). Supplemental heat may be needed when dealing with infants or with sick or older animals. Note that some acclimation may be necessary before moving animals from a warm barn to the outdoors during winter months. The humidity level should be maintained at 40 to 70%. Shower sprays or water baths should be offered in areas of relatively low humidity. Indoor facilities should be maintained with a negative air pressure, and ventilation should be provided to accommodate at least four air exchanges per hour (USDA recommendations for a cold-weather heated barn). Institutions are encouraged to check with their local authorities for air-exchange requirements when the public or personnel occupy the facility.

Within any indoor facility, areas must be provided for food and water. Fresh water should be available at all times and should be changed daily or be supplied from an automatic-fill or continuous-flow device. Regular cleaning and disinfecting should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be constructed to prevent upset, spillage or leakage.

### **Separation Capabilities, Substrate and Special Features**

Isolated stalls are essential for Sumatran rhinos. The indoor enclosure should include a minimum of 18.6 sq m (200 sq ft) per rhino. Stalls should have concrete floors that are easy to clean and have proper drainage, but rubber mats or proper bedding should be provided to protect the

rhinos' feet. Dirt is not an acceptable substrate indoors. The male rhinos spray their alkaline urine 3 to 5 m (12-15 ft) into the air frequently throughout the day and night, so this should be taken into consideration when designing the indoor holding area. A mounted log for rubbing should also be included when possible.

In addition to standard internal enclosure design requirements, due to the fragility of this species and its unusual, particular diet, a scale should be installed so that the rhinos can be weighed weekly at a minimum to closely monitor body condition. In addition, blood should be collected regularly for health screens, and this is best accomplished by conditioning the rhinos to tolerate the procedure while standing in a chute while receiving positive reinforcement. If an institution plans to breed the species, the chute must be built to accommodate regular rhino rectal ultrasound exams.

Normal light cycles seem to be adequate for rhinos. However, if an animal is to be held indoors for more than 12 hours (e.g., winter at cold-climate institutions), facilities should provide artificial or natural light sources to simulate natural cycles. Fluorescent lighting is an efficient light source that provides broad-spectrum illumination; however, skylights should be included whenever possible.

## **Breeding Enclosure**

Breeding enclosure should include:

- 1) solid wall perimeter (not hot wire)
- 2) perimeter shape free of any corners where animals could get trapped
- 3) divider so that aggressive animals can be separated (adjoining enclosures work well)
- 4) full visibility of all areas by animal staff

For breeding, it is best if a large area is available because the rhinos often chase each other when introduced. However, solid perimeter barriers usually do not enclose large areas. Therefore, two smaller adjacent enclosures with gates between them work well because animals can be separated if necessary. A rounded perimeter is ideal because rhinos cannot become trapped by one another in a corner. Regardless of the size or shape of the enclosure, there must be an exit strategy in place for a rhino being chased by another (i.e., gate into another enclosure, door into the barn, etc.). A rhino pair should not have access to a barn while together as the space inside the barn would be small and could serve as a trap for one of the animals. A rhino pair in a small enclosure should never be left unattended, and the attending staff needs to keep the rhinos in sight at all times. Because Sumatran rhinos sometimes exhibit many hours of mating behavior before copulating, outdoor lighting may be necessary in outdoor enclosures for the observation of breeding at night.

## **Physical Restraint Designs**

Numerous institutions have constructed permanent physical devices to restrain their rhinos when necessary. Such "chutes" can be very valuable for physical exams as well as nutritional, reproductive or veterinary research. In addition to the following general information, please consult the Health chapter of this publication as well as Schaffer (1993) and Eyres et al. (1995). Institutions in the United States that currently have chutes and may be able to provide additional information include Henry Vilas, Saint Louis, Sedgwick County, Oklahoma City, Henry Doorly, Cincinnati, Caldwell, and Milwaukee County Zoos, Fossil Rim Wildlife Center, and the Wilds. Companies that may assist in chute design and construction include Animar Systems, Inc. (Springfield, MO, USA),

Cummings and Son, Inc. (Garden City, KS, USA), Mark McNamara of Fauna Research (Tamer, Fauna Research, Inc., Red Hook, NY, USA). In general, institutions modifying rhino exhibits or constructing new ones should incorporate a physical restraint area or device into their design.

Several physical restraint designs are effective for rhinos. These range from a small restricted area in which to contain the animal to an area that contains one or more hydraulics that will “squeeze” together to restrict an animal’s movement. In general, major restraint chute design considerations include strength, durability, type and function. It should be noted, however, that available space and animal size and disposition vary across institutions and should be individually addressed.

In general, both zoological managers and researchers emphasize that the general restraint area should be an active component of daily rhino management. Methods to accomplish this vary. A restraint chute or restraint area can be designed so that the rhinos must pass through it to exit the barn into their yard. If rhinos are fed indoors, part of the feed (e.g., produce, grain) can be offered in the chute area. Finally, more extensive conditioning (see Training chapter) can be particularly effective in habituating rhinos to physical restraint. Such a program should be attempted prior to detaining a rhino in a chute for an exam.

Rhino chutes should be manufactured out of steel or a combination of steel and steel-reinforced wood. Some institutions have also used steel-strength aluminum (6061-T52 aluminum). Aluminum of this type is lighter and more maneuverable than steel, as well as potentially less stressful to rhinos because of “deader” sound properties than steel (i.e., when metal scrapes metal).

Permanent pass-through indoor restraint chutes (similar to those constructed for elephants) are especially effective for rhinos. With training, this type of chute may allow for daily rhino observations. Furthermore, inclement weather will not affect the use of an indoor restraint chute. The chute should allow restraint of the animal when it is passing through in either direction so that the shifting routine of the animal is not interrupted (Schaffer, 1993). The width of the chute should limit side-to-side movement while still allowing the animal to comfortably lie down. Animals can become wedged in tight-fitting chutes if the sides cannot be released. To alleviate excessive forward movement of the animal when it lowers its head, two vertical bars that push in from the sides of the chute to the shoulders of the rhino may be utilized. Quick release of these shoulder bars often relieves agitated animals without having to release them completely.

High-walled chutes or bars over the top of the chute keep the animal from climbing or rearing up. Horizontal bars in the chute’s entry gates and sides are hazardous for examiners when the animal lies down. Vertical bars on the sides can trap researchers’ arms if the animal can move forward. If the animal’s forward and side-to-side mobility can be limited, vertical bars or walls on all sides are recommended. The distance between these bars along the sides of the chute should be great enough to prevent the animal’s foot from becoming wedged if the animal rolls on its side in the chute. For personal safety, this distance can be divided with removable vertical bars.

Rhinos may slam swinging doors; thus, sliding or guillotine gates are safer. A rectangular opening in these gates so that a palpation can be performed should not pin the arm of an examiner when the animal is shifting. The distance between the vertical sides of this rectangular opening must be wide enough to provide for staff safety while still limiting the space through which a rhino could squeeze. Also, the horizontal bottom bar of this rectangle should be only a few inches from the ground, as animals frequently lie down. Solid doors on the outside of these gates can be used to stop rhinos, as they may attempt to charge even small openings. Additionally, good lighting and accessible electrical sources are useful.

A closed chute (Fig. 5.2) is another option that has been used successfully for the treatment of a rhino with a urinary-tract infection and another with infected lesions on its foot (Eyres et al., 1995). As noted in Figure 5.2, a typical closed chute has both front and back gates. The back gate restricts the rhino's movement by sliding forward. Additionally, the hind end of the rhino is supported by a V-design that prevents it from lying down. This design also allows additional safety for staff while working with the animal. In many respects, a closed chute does not depend as strongly on conditioning of the rhinos as does a squeeze chute, although acclimation is recommended prior to attempting any treatments within the chute. The design of a closed chute might necessitate an outdoor location in most cases; therefore, the use of this type of chute may be limited by weather.

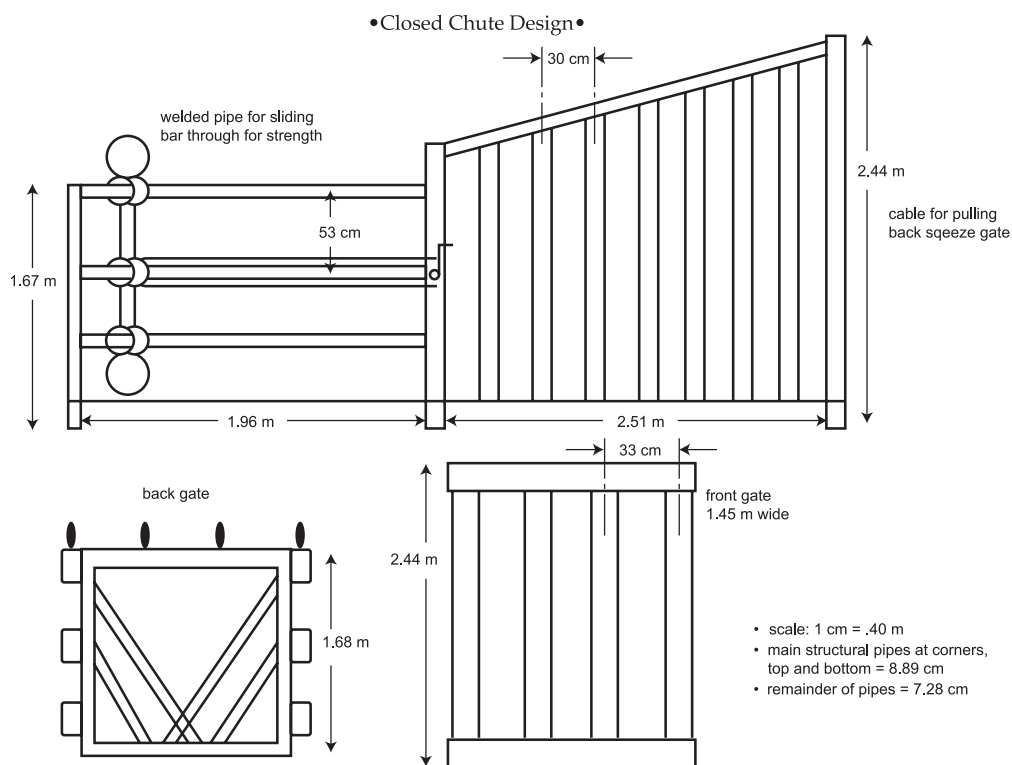


Fig. 5.2. Closed stall rhino restraint chute. Note that a typical closed chute has gates that restrict the rhino's movement and prevent it from lying down. The advantage of a closed chute is that it does not depend as strongly on conditioning as does a squeeze or free-stall chute (Eyres et al., 1995).

A free-stall chute can be used for animals more sensitive to a confined enclosure (Fig. 5.3). The design of this type of chute allows the rhino to enter or exit at its will and thus may help to keep rhinos calmer during procedures. Because there is free access, however, rhinos must be conditioned to target or stand still; thus, relatively non-invasive procedures also work best. Procedures that have been accomplished with a conditioned rhino in a free-stall include ultrasound and serial collection of blood and feces (Eyres et al., 1995).

A free-stall design can easily be incorporated into an existing pen or stall, indoors or outdoors. The open back of this type of chute allows the animal to enter and leave the structure at will. Protection of staff when working with the rhino is critical; a partial back wall constructed of vertical pipes allows staff to step out of the way (Fig. 5.3).

### •Free-Stall Chute Design•

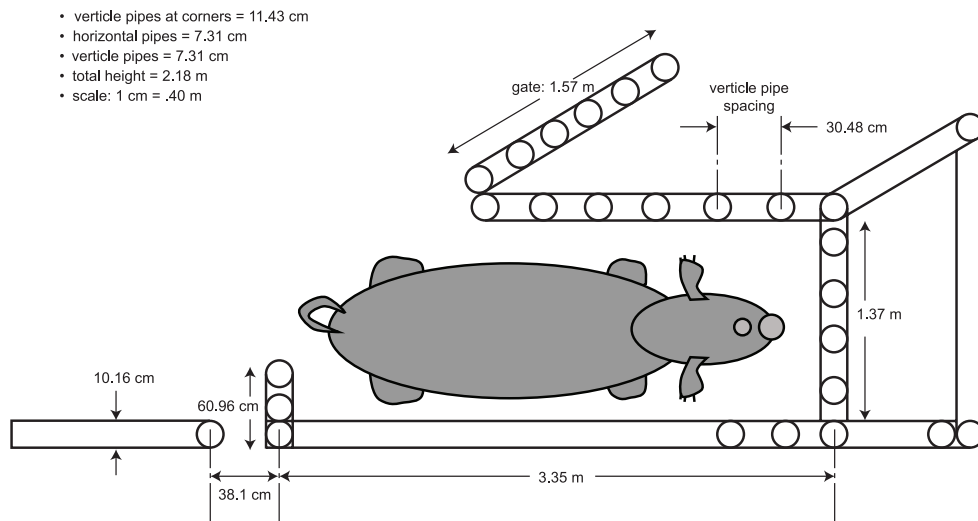


Fig. 5.3. Free-stall rhino restraint chute. The design of a free-stall chute allows the rhino to enter or exit at will and can be used for animals more sensitive to a confined enclosure. This type of restraint chute, however, is best used for relatively non-invasive procedures and with rhinos that have been conditioned to target or stand still (Eyres et al., 1995).

## HEALTH

### Introduction

This section provides a general overview of preventive medical and disease management, and chemical restraint of captive rhinoceroses. More detailed information for many of these topics is available in the references provided as well as through the Rhino Resource Center ([www.rhinosourcecenter.com](http://www.rhinosourcecenter.com)).

### Physiological Normals and Sample Collection

With the increased use of training for husbandry and medical procedures, resting values for heart rate, respiratory rate, temperature and other values have been obtained for non-restrained black and white rhinos. The various species appear to be similar with heart rates of 30 to 40 beats per minute and respiratory rates of six to 12 breaths per minute. Rectal temperatures are typically 34.5-37.5°C (94-99.5°F), although temperatures may be higher in anesthetized rhinos (37-39°C; 98.6-102°F) due to exertion or muscle tremors (Miller, 2003; Radcliffe and Morkel, 2007; Morkel et al., 2011). Values are comparable to domestic horse ranges. Limited information about electrocardiography (ECG) is available in rhinos (Jayasinge and Silva, 1972). Indirect blood pressure has been measured in unsedated black and white rhinoceros using a human blood pressure cuff around the base of the tail. Mean values reported for unanesthetized white rhino are  $160 \pm 2.9$  mm Hg (systolic),  $104 \pm 2.3$  mm Hg (diastolic), and  $124 \pm 2.2$  mm Hg (mean blood pressure) (Citino and Bush, 2007). In anesthetized animals, etorphine can cause hypertension, although variable mean blood pressure measurements (107-280 mm Hg) have been observed, depending on drugs used and time values measured.



Hematologic, biochemical, mineral, protein electrophoresis, and blood gas values have been previously published (Tables 5.11-5.13; Flesness, 2002; Miller, 2003). Although most parameters can be generally interpreted similar to other perissodactyls, there are several differences that appear to be normal in rhinos. Total protein and globulins tend to be higher than in domestic horses. Other values that differ include lower sodium and chloride. Free-ranging rhinos tend to have higher creatine phosphokinase (CPK) than captive animals (this may be biased by immobilization technique)(Kock et al., 1990; Mathebula et al., 2012).

Table 5.11. Mean hematology values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
WBC x 10 <sup>3</sup> / $\mu$ l	8.42 (2.48)	9.30 (2.46)	7.20 (1.33)	8.27 (1.55)
RBC x 10 <sup>6</sup> / $\mu$ l	4.01 (0.88)	5.77 (1.28)	6.43 (0.86)	5.32 (1.09)
HBG g/dl	12.0 (2.0)	13.8 (3.8)	13.4 (1.5)	12.4 (1.6)
Hct %	33.4 (5.7)	36.9 (9.3)	37.0 (4.6)	36.9 (4.2)
MCV FL	85.7 (9.0)	63.8 (7.8)	57.8 (4.9)	71.5 (11.2)
MCH pg/cell	30.5 (3.3)	23.5 (1.9)	21.3 (3.0)	23.9 (3.8)
MCHC g/dl	35.7 (2.7)	37.9 (7.3)	36.3 (3.2)	33.5 (2.1)
Platelets x 10 <sup>3</sup> / $\mu$ l	284 (83)	378 (103)	178 (53)	198 (135)
Nucleated RBC/100 WBC	0	1 (1)	0	–
Reticulocytes %	1.6 (2.9)	–	–	–
Neutrophils x 10 <sup>3</sup> /ml	5.24 (2.18)	5.42 (2.05)	5.13 (1.24)	4.86 (1.16)
Lymphocytes x 10 <sup>3</sup> /ml	2.48 (1.1)	2.35 (1.15)	1.74 (0.67)	2.52 (0.90)
Monocytes x 10 <sup>3</sup> /ml	0.43 (0.32)	0.65 (0.55)	0.22 (0.15)	0.36 (0.22)
Eosinophils x 10 <sup>3</sup> /ml	0.25 (0.22)	0.54 (0.59)	0.32 (0.31)	0.37 (0.21)
Basophils x 10 <sup>3</sup> /ml	0.17 (0.09)	0.10 (0.05)	0.13 (0.05)	0.08 (0.01)
Neutrophilic bands x 10 <sup>3</sup> /ml	0.27 (0.35)	0.71 (1.18)	0.22 (0.20)	0.31 (0.24)

Table 5.12. Mean blood chemistry values in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
BUN mg/dl	13 (3)	16 (3)	3 (2)	6 (2)
Creatinine mg/dl	1.1 (0.2)	1.8 (0.4)	1.3 (0.2)	0.9 (0.1)
Uric acid mg/dl	0.5 (0.2)	0.9 (0.8)	0.3 (0.2)	-
Bilirubin mg/dl	0.3 (0.1)	0.3 (0.3)	0.4 (0.3)	0.2 (0.1)
Glucose mg/dl	69 (21)	97 (39)	82 (25)	76 (13)
Cholesterol mg/dl	102 (37)	93 (26)	53 (21)	48 (21)
CPK IU/L	255 (248)	409 (722)	260 (203)	617 (398)
LDH IU/L	595 (427)	537 (320)	267 (149)	231 (38)
Alk Phos IU/L	80 (55)	92 (51)	80 (41)	17 (6)
ALT IU/L	16 (7)	16 (9)	7 (7)	6 (3)
AST IU/L	85 (27)	71 (25)	61 (27)	39 (9)
GGT IU/L	27 (18)	19 (14)	18 (16)	6 (2)
Total protein g/dl	7.6 (0.9)	8.5 (1.0)	7.5 (0.9)	7.5 (0.4)
Globulin g/dl (electrophoresis)	4.9 (0.9)	5.3 (0.8)	4.5 (0.7)	3.8 (0.7)
Albumin g/dl (electrophoresis)	2.6 (0.4)	3.2 (0.5)	2.9 (0.5)	3.6 (0.6)
Fibrinogen mg/dl	104 (195)	101 (241)	350 (84)	324 (85)

Table 5.13. Mean serum mineral values and blood gases in rhinoceros ( $\pm$ SD).

Parameter	Black	White	Greater Asian One-Horned	Sumatran
Ca mg/dl	12.7 (1.0)	11.8 (0.9)	11.4 (0.8)	13.3 (1.1)
P mg/dl	4.8 (1.1)	4.0 (0.9)	4.0 (0.9)	3.7 (0.7)
Na mEq/L	133 (3)	134 (5)	132 (3)	133 (4)
K mEq/L	4.7 (0.6)	4.7 (0.8)	4.1 (0.4)	4.6 (0.6)
Cl mEq/L	96 (0.3)	95 (4)	91 (3)	100 (3)
Bicarb mEq/L	23.3 (4.2)	18 (0)	27.0 (0)	-
CO <sub>2</sub> mEq/l	25.4 (9.9)	25.3 (8.8)	27.3 (3.7)	22.8 (2.4)
Iron $\mu$ g/dl	227 (66)	176 (67)	152 (70)	-
Mg mg/dl	3.34 (3.45)	118.2 (232.5)	7.95 (8.56)	-

Venipuncture can be routinely performed on awake captive rhinoceroses using training and/or restraint devices (Fig. 5.4). The most commonly used sites for blood collection are the ear (auricular) vein, metacarpal vein (lower inside forelimb), and radial vein (inside of forelimb crossing the carpus)(Fig. 5.4-5.6). The tail (coccygeal) vein has also been used, particularly for Sumatran rhinos, and is approached from the ventral aspect similar to the technique for blood collection in domestic cattle. Arterial access is available for blood gas sampling using the medial

auricular artery (inside of the ear)(Fig. 5.7). Large volumes of blood can be collected from the radial or metacarpal veins for diagnostic testing, plasma/serum banking, or for therapeutic phlebotomy (Mylniczenko et al., 2012). One to eight liters have been obtained from trained standing rhinoceros (Fig. 5.8; V. Clyde, pers. comm.). Rhinoceros blood cells resemble those of domestic horses. Nucleated red blood cells (NRBC) and reticulocytes may be observed in anemic animals. Elevated total white blood cell counts (wbc) and eosinophil numbers have been observed in wild rhinoceroses, presumably due to the response to capture and parasite loads, respectively.



Fig. 5.4. Voluntary blood collection from auricular vein in awake rhinoceros.



Fig. 5.5. Blood collection from the metacarpal vein in a standing rhinoceros.





Fig. 5.6. Blood collection from radial vein in immobilized rhinoceros.



Fig. 5.7. Arterial blood sample collection from medial auricular artery.



Fig. 5.8. Therapeutic phlebotomy in a trained rhinoceros (courtesy of V. Clyde).

Urinalysis panels in captive rhinos are similar to horses with large numbers of calcium carbonate crystals, creating a normal milky yellow appearance to the urine. Calcium oxalate, phosphate, and ammonium crystals may also occur, depending on the diet. Occasionally dark discoloration of urine associated with pigmentation of certain browse species (e.g. ash, mulberry) can be mistaken for blood or myoglobin. However, analysis should be performed to rule-out any abnormalities. Normal values for the different rhino species have been published (Table 5.14; Haffey et al., 2008).

Table 5.14. Reported range of mean urinalysis results from three captive rhinoceros species.

	pH	Specific Gravity
Greater Asian one-horned rhinoceros	8.08-8.70	1.019-1.031
Sumatran rhinoceros	8.16-8.32	1.010-1.021
Black rhinoceros	8.10-8.26	1.010-1.012

Due to size, cerebral spinal fluid collection has not been successful except in a few rhino calves (S. Citino, pers. comm.). Therefore, extrapolation of normal values from domestic horses and other perissodactyls should be used for interpretation.

Incorporation of scales into rhino facilities has permitted monitoring of body weights and physical condition. Regular weighing is recommended to observe trends associated with growth, diet changes, and disease. Average range for adult body weight is 600-800 kg (1,323-1,764; Miller, 2003).



## Preventive Medical Health Programs

Routine health monitoring should be performed on all rhinoceros on an ongoing basis. Animals should be trained to permit sampling and examination. The following **protocol** advises that specific laboratory tests be performed for the purpose of evaluating current health status. Additional tests are recommended to increase baseline information on other diseases to determine their significance to rhinoceros health. The final decision for specific procedures should be made by the animal care and veterinary staff based on individual circumstances.

- Physical exam by a veterinarian familiar with rhinoceros health problems. This should include a review of all systems (if performed in a restraint device, exam may be limited by training and temperament of the individual and design of facility).
- Body weight—Actual weight should be recorded whenever possible; body scores and/or digital photos can be used when scales are not available.
- Blood collection for complete blood count (CBC), serum chemistry panel, fibrinogen, serum protein electrophoresis and extra serum/plasma for banking (minimum of 10-20 ml). Blood smears should be carefully screened for the presence of hemoparasites, especially in recently captured or imported animals. The current Rhino SSP/TAG tissue/sample collection **protocol** should be consulted for additional samples that may be **requested** for research, disease screening, etc.
- Fecal samples should be collected semiannually or at least annually (depending on management system) for direct, flotation, and sedimentation to detect internal parasites.
- Annual enteric pathogen screening may be included, especially for animals in intensive management situations (e.g., breeding). Aerobic culture of feces for enteric pathogens should include special media for the detection of *Salmonella* spp. Because *Salmonella* organisms may be shed intermittently, at least three to five fecal cultures should be performed (may be done on consecutive days).
- Vaccinations—Vaccination for rabies, tetanus, and arboviruses (EEE/WEE/WNV) may be considered if the diseases are considered endemic in the area or increased risk factors are identified. There have been isolated cases of rabies and tetanus documented in rhinoceros (Jones, 1978; Mukherjee et al., 1984; Selvam et al., 2003). Check with the SSP veterinary advisor for the most current recommendations on species-specific vaccination protocols.

Additional preventive health recommendations have been included for consideration when performing examinations.

- Serological screening for leptospirosis (multiple serovars) and West Nile Virus (WNV). Although these tests are not species-specific and have not been validated for rhinoceros, they may detect cross-reactive antibodies in exposed animals. The presence of antibodies does not necessarily denote current infection or disease. Antibodies to leptospirosis have been detected in vaccinated rhinoceros and may be used to monitor response and possibly determine frequency of vaccination, although data is insufficient to determine protective titers. Insufficient data is available at this time to determine the significance of WNV antibodies in rhinoceroses; although it is important to note that a greater one-horned rhinoceros with clinical signs developed a WNV titer during a period of known exposure (P. Calle, pers. comm.). One study in greater Asian one-horned rhinoceroses did not find seroconversion in response to vaccination with a commercial equine WNV vaccine (Wolf et al., 2008).

- Serum/plasma vitamin E levels should be checked on a regular basis to assess adequacy of diet and supplementation protocols (see Nutrition chapter). See the most current Rhino TAG/SSP preventive health protocol for recommended laboratories.
- Reproductive tract examination—When feasible, a complete reproductive examination should be conducted to include transrectal ultrasound, semen collection and analysis, and serum or fecal collection for hormone analysis (Radcliffe et al., 1997). Uterine leiomyomas, cystic ovaries, and irregular cycling have been observed in captive animals (Hermes et al., 2004). Since these conditions can have potentially significant effects on reproduction, a careful evaluation is warranted if the animal is being considered for breeding. Standing laparoscopy enabled visual examination and uterine biopsy of a leiomyoma in a southern white rhinoceros (Radcliffe et al., 2000b). A number of publications describe the technique for ultrasonography in rhinoceroses and normal reproductive biology. The reader is referred to the Rhino Resource Center for further information ([www.rhinoresourcecenter.com](http://www.rhinoresourcecenter.com)).
- Urinalysis should include both fluid and sediment evaluation of a clean voided sample. Microbial culture should be considered if there is evidence of blood cells or bacteria.
- Radiographs of feet are strongly recommended if any signs of pododermatitis or nail cracks are observed (Atkinson et al., 2002). Regular foot trimming and care may require immobilization in certain individuals (i.e., those that have a history of chronic foot problems). See section on pododermatitis.
- Diagnostic tests for tuberculosis—Periodic testing for tuberculosis in rhinoceroses should be considered, especially if there has been a history in the institution or herd. Intradermal testing can be performed using bovine PPD (0.1 ml ID) in the eyelid, behind the ear, caudal tailfold, or axillary region. Both false positive and false negative results have been found when performing intradermal tuberculin testing in rhinos (Godfrey et al., 1990). Ancillary tests such as nasal swabs, tracheal washes, and gastric lavages for mycobacterial culture have also been used. Serological tests [ElephantTB STAT-PAK®, MAPIA, DPP (dual pathway platform); Chembio Diagnostic Systems, Inc.] are being investigated for use in rhinos. See section on tuberculosis.
- Other vaccination regimens will depend on regional requirements and exposure risks (consider multivalent vaccination for Clostridial diseases). Contact the SSP veterinary advisor for the most up-to-date information.

Animals being moved between institutions should receive a pre-shipment examination and testing. This is similar to the routine health procedures but should also include screening for tuberculosis and other requirements dictated by the receiving institution (see quarantine section below).

## Neonatal Examinations

Ideally, neonates should be examined within 24 to 72 hours of birth to detect any congenital defects. Often the dam can be separated while the calf is manually restrained for a brief, but thorough exam, including body weight, and if feasible, blood collection. Complete blood count, biochemical panel, tests for passive transfer of immunoglobulins [e.g. glutaraldehyde coagulation, zinc sulfate turbidity, radial immunodiffusion plates (equine plates work well in rhinos and can be standardized by testing healthy rhinos), and serum protein electrophoresis], vitamin E level, and banking should be performed. A microchip may be placed behind the left ear for future identification. Particular attention during the exam should be paid to the umbilical stalk for signs

of infection, urine leakage, or hernia; passage of meconium, normal rectal/anal anatomy and tone; suckling reflex and neurological status. Regular weights and developmental progress should be documented. Lack of weight gain may be due to inadequate maternal milk production and care, or an indication of health or developmental problems in the calf. Greater Asian one-horned, white and black rhinoceros calves have been successfully hand-reared (covered under Nutrition chapter). In one case, a white rhinoceros calf was orphaned when its dam died, and the calf was taught to drink from a bucket at the age of six weeks. Weakness or other problems resulting in prolonged recumbency can cause decubital ulcers. Treatment of two neonatal white rhinos has been described using serial sedation with butorphanol alone or in combination with detomidine for restraint (Gandolf et al., 2006). Rectal prolapse has been reported in black rhinoceros calves, with surgical intervention required in at least two cases (Pearson et al., 1967; Abou-Madi et al., 1996).

## Parasites

Internal parasites are more commonly found in free-ranging rhinos than in captivity. Parasites reported include nematodes (*Diceronema versterae*, *Parabronemia roundi*—associated with stomach nodules), *Draschia megastoma*, multiple species of *Kiluluma*, *Khalilia rhinocerotis* and pinworms (*Oxyuris karamoja*). Tapeworms (*Anoplocephala* sp.) can cause asymptomatic infestation in both captive and wild rhinos (Miller, 2003). Stomach flukes (*Paramphistoma* sp.) have been identified in Sumatran rhinoceroses in Indonesia (R. Radcliffe, pers. comm.). One case of neosporosis led to acute fatal myocarditis in a neonatal white rhino calf and abortion in another case (Williams et al., 2002; Sangster et al., 2010).

Ticks are regularly found on free-ranging rhinoceroses, and imported animals should be carefully examined and treated. Vector-borne parasitic infections occur in endemic areas. Sumatran rhinoceroses appear to be particularly susceptible to trypanosome infection and suffer high mortality, presumably because the disease originated in Africa and was introduced relatively recently to the Asian continent with the movement of cattle. Trypanosomiasis was implicated in the death of five animals at a sanctuary in Malaysia, ending that nation's captive breeding efforts (Mohamad et al., 2004).

Capture and translocation or importation may exacerbate potential parasitic infestations and increase the risk of introduction of novel pathogens to a new environment. Therefore, careful screening for external and internal parasites, prophylactic treatment of wounds with fly repellent, pour-on tick treatments (coumaphos, flumethrin), and judicious use of antiparasitic medications (pyrantel pamoate, fenbendazole, ivermectin, praziquantel) should be considered. Check with the SSP veterinary advisor for current recommendations.

## Quarantine

Due to the size, strength, and temperament of a rhinoceros, it may be logistically difficult to maintain isolation from other animals during arrival and quarantine. The Rhinoceros TAG/SSP Recommended Preshipment Protocol for Rhinoceros lists a comprehensive battery of tests for health assessment prior to shipment. Since most zoological institutions will not have the facilities available to safely house and manage a newly arriving rhinoceros, it is important that the receiving institution work closely with the sending institution to ensure that all (or as many as possible) of the listed tests are conducted and results reviewed before shipment. Following the preshipment protocol may help compensate for some of the quarantine compromises that may be required. Regardless of preshipment test results, every attempt should be made to maintain some degree of physical separation of the incoming animal from the resident rhinos after arrival.

Current quarantine practices recommend a minimum 30 to 90 day quarantine period for most mammalian species in zoos and aquaria. Social concerns, physical facility design, and availability of trained rhino staff may dictate a modified quarantine protocol. Specific quarantine guidelines and protocols at each institution should be reviewed jointly and decisions made by the veterinary and animal management staff. Recommended **procedures** to consider as part of a comprehensive plan for rhinoceros quarantine include:

- Thorough physical examination including a review of all organ systems.
- Blood collection for CBC (including blood smear examination for hemic parasites), serum chemistry panel, fibrinogen, serum protein electrophoresis, and serum bank.
- Fecal collection for parasite screening (direct, flotation, sedimentation) conducted weekly for the first three weeks.
- Fecal cultures for *Salmonella* spp. conducted at least weekly for the first three weeks.
- Any procedures that were not completed prior to transport or that may be due, such as vaccination, serologic screening, or TB testing.

It should be emphasized that the quarantine test requirements should be strongly considered regardless of the results of preshipment testing. The stress of transport and quarantine may result in health changes (for example, *Salmonella* shedding) that were not detected during testing at the sending institution.

## **Hospitalization and Critical Care**

In most cases, hospitalization is impractical with adult rhinoceroses. Most animals will be treated in their holding areas. Rhino calves may be hospitalized in adequate large animal holding facilities if the severity of their medical condition warrants. Barns or holding areas that incorporate species-appropriate restraint devices facilitate medical treatment. Some of the following medical problems require active intervention, including sedation, immobilization, injectable drug therapy, and/or fluid therapy. Although it has been achieved, fluid therapy in rhinoceros presents logistical challenges. In addition to the intravenous route, it is possible to improve hydration using rectal enemas with warm physiologic solutions or even tap water. Animal and staff safety should be a priority in any planned intervention.

## **Diseases**

### ***Tuberculosis***

*Mycobacterium bovis* and *M. tuberculosis* have caused infections in captive rhinoceroses (Stetter et al., 1995; Miller, 2008). Initial infection may be asymptomatic or result in progressive weight loss and emaciation, with coughing and dyspnea occurring in the terminal stages. Nasal discharge may be present but is not a consistent sign. Most infections are pulmonary. Antemortem testing includes intradermal tuberculin test, tracheal and/or gastric lavage for mycobacterial culture, and serological tests (see preventive health section for details). Retrospective analyses of serum from *M. tuberculosis*-infected black rhinos showed positive results using the ElephantTB Stat-Pak® (Duncan et al., 2009). Treatment has been attempted using isoniazid, rifampin, ethambutol, and pyrazinamide (Barbiers, 1994; Duncan et al., 2009). However, assessment of successful response is limited. Concerns for other collection animals, staff and public health need to be considered prior to initiation of therapy. Since this is a reportable disease, notification of the appropriate state veterinary officials should occur promptly once a diagnosis is made. Consultation with the SSP veterinary advisor is also recommended.

## **Salmonellosis**

Salmonella infection can cause enteritis and fatal septicemia in captive and newly captured wild rhinoceroses (Windsor and Ashford, 1972; Kenny et al., 1997). In a retrospective survey of captive black, white, and greater Asian one-horned rhinos in the U.S., 11% reported positive cultures, usually associated with clinical signs (Kenney, 1999). Clinical infection may result secondary to transport, changes in diet, immobilization, concurrent disease, or exposure to a large number of organisms. Lethargy, anorexia, signs of colic, diarrhea, and death may be observed. Successful treatment using trimethoprim-sulfamethoxazole and supportive care is possible if initiated early. However, treatment of asymptomatic animals is NOT recommended.

## **Leptospirosis**

Leptospirosis usually presents with depression and anorexia. Other signs may include hemolytic anemia (not present in all cases), hemoglobinuria, signs of colic, and development of skin ulcers. Fatality rates are high in clinically affected black rhinos although successful treatment with trimethoprim-sulfamethoxazole and ceftiofur has been reported (Neiffer et al., 2001). Diagnosis is based on high antibody titers (microagglutination test – MAT) and confirmed by detection of leptospiral organisms in urine or tissues (fluorescent antibody test). Preventive measures include rodent and wildlife control programs, and good husbandry to minimize contamination of feed and water.

## **Gastrointestinal Infections**

See salmonellosis and GI torsion, impaction, and ulcers for other GI conditions.

## **Encephalomyocarditis Infection (EMC)**

EMC viral infection usually results in acute death due to myocarditis (Gaskin et al., 1980). The southeastern/Gulf Coast states in the U.S. are considered an endemic area. Diagnosis is usually based on virus isolation at necropsy from heart, spleen, or other tissues (<http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/53600.htm>). Prevention should target rodent control, although experimental vaccines have been proposed. A commercial vaccine is not currently available.

## **Fungal Pneumonia**

Fungal pneumonia is usually due to *Aspergillus spp.* and primarily observed in black rhinoceroses secondary to immunocompromise from concurrent disease, broad spectrum antibiotic therapy, or corticosteroid use (Weber and Miller, 1996). Clinical signs may include weakness, weight loss, epistaxis, or other signs consistent with pneumonia. Diagnosis is challenging, although serology and bronchoscopy, with cytology and fungal culture, may be useful. Long-term treatment with antifungal drugs (e.g., itraconazole) is expensive and has unknown efficacy. Definitive diagnosis is often made at necropsy.

## **Anthrax**

Death due to anthrax has been observed in wild rhinoceroses. It has been implicated in a die-off of several Javan rhinos in Ujung Kulan National Park, though definitive diagnosis is often lacking in remote field regions (R. Radcliffe, pers. comm.). Most cases result in sudden death. Foamy discharge from the mouth and nostrils can be seen and may appear similar to EMC infection. Diagnosis is based on identification of anthrax bacilli in blood or tissue smears. Vaccination of ranches rhinos has been used in some endemic areas of Africa. Sporadic outbreaks



in domestic livestock occur in the U.S., although there have been no reports of rhino mortality in these areas (e.g., Texas).

### ***Skin Conditions and Dermatopathy Syndromes***

There are several distinct syndromes reported in captivity.

- Superficial necrolytic dermatopathy—This has also been called ulcerative skin disease, vesicular and ulcerative dermatopathy, and mucosal/cutaneous ulcerative syndrome and is observed in black rhinoceros (Munson and Miller, 1999; Dennis et al., 2007). Initial signs are epidermal plaques or vesicles that progress to ulcers, often over pressure points, ear margins, coronary bands and tail tip. Oral or nasal ulcers may develop concurrently. Clinical signs are intermittent and, during episodes, rhinos may also be anorexic, depressed, lame, have oral or nasal bleeding, and lose weight. Affected animals may have decreased albumin and hematocrits. In most cases, the skin lesions are associated with other concurrent health issues, including GI and respiratory disease. Research suggests that the underlying cause may be an imbalance of dietary essential micronutrients with related metabolic changes. Management includes symptomatic treatment such as topical or systemic antibiotics, topical ointments, and hydrotherapy. If lesions become extensive, it can be fatal. Treatments with cryotherapy and steroids have been successful, but rhinos treated with systemic steroids can develop fatal fungal infections. Occasionally, lesions may resolve spontaneously.
- Eosinophilic granuloma syndrome—This syndrome usually presents with oral and nasal non-healing ulcers and granulomas in black rhinoceros (Pessier and Munson, 2004). Cytology shows a predominance of eosinophils associated with lysis of collagen and mineralization. Development of significant ulceration may lead to epistaxis (nose bleeds) or oral bleeding. A similar syndrome has been described in domestic cats. Although lesions may resolve spontaneously, usually over one to seven months, they may recur. Treatment has included corticosteroid therapy, which can result in fungal pneumonia, or local cryotherapy. Laser therapy has been used to treat ulcers but may exacerbate hemorrhage. Supportive care is recommended in severe cases. Eosinophilic granulomas in wild rhinos are typically associated with *Stephanofilaria dinniki*.
- Neoplasia—Squamous cell carcinoma (SCC) has been treated by surgical excision in a captive white rhino (Goodman et al., 2007). A greater one-horned rhino with SCC of the horn has also been successfully treated with surgery and radiation (Greer et al., 2010). Cutaneous melanomas have been reported in both black and greater Asian one-horned rhinoceroses (Wack et al., 2010).
- Environmental—Dermatitis, especially in Sumatran rhinos, may be due to inadequate access to wallows and pools.

These cases illustrate the importance of biopsy and culture for identifying the etiology of and directing treatment in dermatologic cases in rhinos.

### ***Pododermatitis and Chronic Foot Problems***

All species of rhinoceros are susceptible to developing pododermatitis due to inappropriate substrate or other husbandry conditions in captivity. Abrasive substrate, long-term indoor housing in northern climates, and limited access to a pool contribute to chronic foot trauma in captive rhinos (von Houwald, 2005). Management of the condition includes improvements in husbandry and medical/surgical interventions. Medical treatment may be in the form of oral antimicrobial medication and topical use of copper sulfate and oxytetracycline. Regular hoof trimming and

surgical debridement of necrotic lesions, along with use of collagen products for granulation tissue stimulation, can lead to improvement in appearance and comfort of the animal. In addition to the factors mentioned above, nutritional imbalances (e.g., zinc) are also being investigated.

### ***Corneal Ulcers and Keratitis***

Corneal trauma and secondary infection can result in corneal ulceration and perforation. Surgical management of a melting corneal ulcer using a conjunctival graft has been described in a greater Asian one-horned rhino (Gandolf et al., 2000). Exposure keratitis is a relatively common sequelae to North American captivity in captive Sumatran rhinos that may lack access to adequate shade. However, there is evidence that overexposure to harmful ultraviolet radiation may not be the inciting factor and an autoimmune component is suspected in at least some recurrent cases (S. Citino, pers. comm.). Medical and surgical treatment is similar to that for equine corneal conditions, although it may require multiple sedation or immobilization events to achieve adequate resolution of the lesion.

### ***Horn Problems***

Horn avulsion, cracks, or other trauma can occur as a result of acute or chronic rubbing, pressure, self-induced, or conspecific fighting. This may lead to myiasis, abscessation, osteomyelitis, or pain with behavioral changes. Radiographs, thermography, or fluoroscopy can be used to assess the extent of the damage. Treatment may involve debridement, antibiotics, wound treatment, and fly control (Suedmeyer, 2007). A squamous cell carcinoma of the horn in a greater Asian one-horned rhinoceros was diagnosed and managed with partial amputation and radiation therapy (Greer et al., 2010). The horn is attached to the basal dermal layer, not unlike the laminae of the horse's hoof wall, and therefore perturbations in blood flow can presumably lead to laminitis. A male Sumatran rhinoceros suffered an acute episode of horn laminitis following exposure to herbicides traced to the feeding of browse from a nearby banana plantation. The horn separated from the dermis around the base with serosanguinous fluid discharge (D. Candra and R. Radcliffe, pers. comm.).

### ***Gastrointestinal Torsion, Impaction, and Ulcers and Other Conditions***

Gastrointestinal torsion has resulted in death of captive Sumatran rhinos. Torsion may result from abdominal trauma or severe GI disease. Severe torsion may lead to acute death; other signs are related to abdominal pain. Dietary changes, dehydration, ingestion of foreign material (i.e., sand), and inadequate fiber content may result in GI impaction. Gastric ulcers have been observed on gastroscopy and at necropsy in rhinos that have received long-term non-steroidal therapy or have concurrent disease. A 32-year old black rhinoceros was euthanized due to rapid onset of wasting secondary to perforation of a pyloric ulcer and peritonitis. Treatment depends on identifying the cause and site of the gastrointestinal condition. Surgical correction of torsion is usually recommended, although most cases are not diagnosed prior to necropsy. Impaction can be treated using rectal enemas, oral administration of psyllium, mineral oil, or other products to increase GI water content. Equine anti-ulcer medications such as omeprazole, sucralfate, and H<sub>2</sub>-blockers have been used in rhinos by scaling the equine dose. Prevention includes adequate dietary fiber and fluid intake, and avoiding abdominal trauma and ingestion of foreign material. Esophageal dilation was observed in a black rhinoceros following ingestion of a foreign body; successful management involved diagnosis by endoscopy and a change in feeding practices to a low fiber diet (Radcliffe et al., 1998). Inflammatory bowel disease was confirmed at necropsy in a greater Asian one-horned rhinoceros that presented with weight loss and progressive diarrhea (Ferrell et al., 2010).

### ***Gingivitis and Dental Tartar***

All rhino species appear to accumulate dental tartar in captivity, especially if they are not given access to hard or course food items. However, black rhinoceros appear to develop severe proliferative gingivitis, not always directly associated with the degree of accumulation of calculus (Beagley et al., 2010). Lesions are vascular and periodically hemorrhage, causing blood-tinged saliva. The gross appearance is similar to raw ground meat. The cause of this condition is unknown but may be related to lack of browse in the diet. Similar to horses, rhinoceros also develop dental points that may eventually create clinical mastication problems with age and require periodic dental flotation.

### ***Renal Disease***

Since 2001, chronic glomerulonephritis and/or renal failure have been recognized as a contributing cause of death in at least seven black rhinos. Mineralization of other tissues has been associated with chronic renal changes as well as three cases of accidental vitamin D toxicosis (Murnane et al., 1994; Fleming and Citino, 2003). Weight loss, decreased appetite, dermatitis, and signs of gastroenteritis may be present, including gastric ulcers. Significant changes in blood urea nitrogen, creatinine or urinalysis were not always evident in these cases, making diagnosis difficult until necropsy. However, two white rhinos (aged >40 yr) both developed progressive chronic renal failure characterized by uremia, isosthenuria and hypercalcemia. Nutritional management using a high energy, low protein feed (Equine Senior, Purina Mills, St. Louis, MO, USA) was instituted in both cases and appeared to stabilize the renal disease (S. Ferrell and R. Radcliffe, pers. comm.). Further investigation into causes of renal disease (other than toxicosis-related) is needed.

### ***Hemosiderosis/Hemochromatosis (Iron Overload Disorder)***

Serum iron and ferritin levels in captive Sumatran rhinoceros kept in North America are significantly higher than those measured in animals kept in sanctuaries in Sumatra (Candra et al., 2012). Levels are related to the amount of browse fed. In contrast, values for ferritin and tissue iron do not appear to be elevated in captive white or greater Asian one-horned rhinos (Smith et al., 1995; Paglia et al., 2001). Although hemolytic anemia, vitamin E deficiency, and hereditary disorders have all been proposed as potential causes, it is now believed to be related to dietary factors in the captive browser species. Hemosiderosis (tissue iron accumulation) is a common finding in multiple organs in black rhinoceros, although inflammation and lesions associated with these changes may also be observed (hemochromatosis). A fatality in a captive Sumatran rhino was associated with multi-organ hemochromatosis. Recommendations to minimize accumulation and reduce iron load include low iron diets, provision of browse, therapeutic phlebotomy (regular large-volume blood collection) and treatment with iron chelating agents in those individuals with suspected clinical disease. Recent success with large-volume phlebotomy and low iron diets have shown promising results in lowering elevated ferritin levels in captive black rhinos (Mylniczenko et al., 2012). Captive browsing rhinoceroses may be best managed in native habitat that can offer appropriate nutrition; for instance, Sumatran rhinos appear to have fewer problems with iron overload syndrome when managed in captive situations in range countries (D. Candra, pers. comm.).

### ***Creosote Toxicosis***

Seven fatalities occurred among a group of 20 black rhinoceroses captured and housed in bomas constructed with creosote-treated wood in Zimbabwe (Kock et al., 1994). Although five cases died after transport to the U.S. and Australia, they exhibited the same clinical signs and lesions as the initial cases. Animals were lethargic, partially anorectic, developed swollen limbs,

and passed brown urine. Abnormal blood results included anemia, and elevated liver enzymes and bilirubin. Post-mortem findings included widespread hemorrhages; swollen, pigmented liver; and ulcers of the upper gastrointestinal tract. The presumptive cause of death was liver dysfunction due to creosote toxicosis. Exposure to creosote-treated housing materials should be avoided for all rhino species.

## **Common Injuries and Treatment: Skin Lacerations, Punctures, Wounds**

Trauma due to conspecific fighting, mating injuries, collision with obstacles in enclosures or in crates, interspecific conflict or, in free-ranging situations, poaching is relatively common. Common sense application of wound treatment principles apply, although the thick skin does not lend itself to primary closure and has a tendency to undermine abscesses along the fascial planes. Therefore, wounds are usually treated by second intention using debridement, hydrotherapy, topical and/or systemic antibiotics, analgesics, and the array of wound healing products that accelerate granulation. A recent article describes the use of vacuum-assisted healing of a surgical wound in a black rhinoceros to improve healing time (Harrison et al., 2011).

## **Necropsy Protocol**

Any rhinoceros that dies or is euthanized should have a complete necropsy performed and tissues submitted for histopathology. The Rhinoceros TAG/SSP Veterinary/Nutrition Blood and Tissue Collection **Protocol** for Rhinoceros should be consulted for current information on the species pathology advisors as well as specific sample **requests** for research and banking purposes.

## **Capture, Restraint, and Immobilization Techniques**

Management of captive rhinoceroses often requires procedures that involve handling of these large and powerful animals. Husbandry training techniques have advanced the application of preventive medical, diagnostic, and minor therapeutic procedures. However, intensive medical and surgical interventions may require physical and/or chemical restraint for safety and comfort of the patient and staff. Since new developments are continuously advanced in this field, the following recommendations should be used as guidelines, and the veterinary advisors and literature should be consulted for specific situations.

### ***Physical Restraint***

Advances have been made in facilities that include a variety of stall and chute designs for physical restraint of rhinoceros. These will be addressed in the Training and Enclosure Design chapters.

### ***Chemical Restraint***

Significant changes in options for chemical restraint of captive rhinoceroses have occurred during the last two decades. The degree of sedation, analgesia, or immobilization required will vary with the procedure to be performed. Health status and temperament of the individual animal, as well as facility design and staff experience may dictate preference for one technique over another.

## **Chemical Restraint and Anesthesia of Rhinoceros**

### ***Introduction***

Anesthesia of rhinoceroses requires adequate preparation, equipment, and experience. It is highly recommended that rhinoceros anesthesia should be attempted only with experienced

personnel or after consulting knowledgeable practitioners. Contingency plans for emergencies are a key component of preparations. Clearly defined roles for all personnel should be outlined and reviewed. Adequate equipment for moving a large animal should also be available.

The choice of chemical restraint techniques will be determined by a number of factors, including physical facilities where immobilization will be performed, health status of the individual, procedures to be achieved and level of analgesia required, length of intended immobilization, and experience of the veterinary and animal care staff. Only captive rhinoceros anesthesia techniques will be covered in this section. For more information on immobilization of free-ranging rhinoceros, the reader is referred to specific references on this subject ([www.rhinosourcecenter.com](http://www.rhinosourcecenter.com)) (Miller, 2003; Bush et al., 2004; Portas, 2004; Kock et al., 2006; Radcliffe and Morkel, 2007; Wenger et al., 2007).

### ***Equipment***

With training and availability of physical restraint devices (“chutes”), drug delivery for chemical restraint can be more easily and accurately achieved, create less stress for the individual, and provide greater control of the induction. Standing restraint is also feasible in these situations.

For rhinoceros in stalls, pens or exhibits, drug delivery often requires use of darting equipment. Depending on the situation, pole syringes with robust needles may be used on selected occasions. Hand-injection is also easily achieved in conditioned animals, although care must be taken when using potent opioids to ensure human safety. Most darting systems can be used in captive situations, as long as a robust needle (minimum 40-60 mm x 2 mm needle) is used to penetrate the thick skin and deliver the drug into the muscle. Nylon darts (Teleinject<sup>®</sup>, Telinject USA, Inc., Agua Dulce, CA, USA; Dan-Inject<sup>®</sup>, Danwild LLC, Austin, TX, USA) are preferred in these situations since they are less traumatic than metal darts. Ideal sites for drug injection are just caudal to the ear on the lateral cervical area, upper caudal hindlimb, or shoulder. However, any site can be used if the dart is placed perpendicular to the skin and is adequate to penetrate muscle.

Additional equipment that should be available include a blindfold, ear plugs (cotton cast padding, stuffed socks), heavy ropes, and padding for recumbency. Inflated truck inner tubes can be used to reduce pressure points. Otherwise, heavy mats or padding should be used if the procedure will take place on a concrete floor. Immobilizations carried out in sandy or grassy areas may provide enough padding for shorter procedures.

Contingency plans for moving a rhino that becomes recumbent in a difficult position should also be made. Lining stalls with plywood sheeting to prevent the head/horn from getting stuck should be considered in areas with open bollards or poles.

Additional equipment should include the availability of oxygen and other emergency equipment. If a field procedure is being planned, an axe or chain saw and bolt cutter may be needed if there are trees/vegetation/other obstacles in the area where the rhino can get caught.

### ***Preparations***

Depending on the health status, environment, and procedure planned, removal of food and water prior to the procedure should be considered at least overnight. Consult the veterinarian for specific instructions.

### ***Positioning***

Rhinoceros are prone to developing myopathy and neuropathy after recumbency. The optimal position remains controversial and may be dictated by the planned procedure. Lateral positioning is



often preferred since it provides the optimal circulation to the limbs, although sternal recumbency may provide better ventilation (Morkel et al., 2010). During the procedure, limbs should be “pumped” about every 20 minutes to encourage circulation (Radcliffe and Morkel, 2007). If the animal needs to be in sternal recumbency for the procedure, it is ideal to roll the animal into lateral recumbency whenever possible and pump the legs.

### ***Anesthetic Monitoring***

With the advent of new tools for monitoring physiological parameters in veterinary patients, it is imperative that monitoring be performed on immobilized rhinoceros to minimize complications. Ideally, an accurate weight or weight estimate will facilitate optimal drug calculation and prevent drug over- or under-dosing and associated complications (Adams et al., 2005). Thorough physical examination should be performed, along with regular recording of vital signs (respiratory rate and depth, heart rate, temperature, mucous membrane color and capillary refill time). Ideally, this should be assigned to dedicated personnel and measured at five-minute intervals throughout the procedure.

Pulse oximetry is a useful tool for monitoring trends in hemoglobin oxygen saturation but can be prone to false readings due to placement and the thick skin of the patient. Therefore, it should not be a substitute for basic clinical assessment. Sites for placement of the sensor include the ear pinnae (scraping can sometimes improve readings) and mucosal folds of the penis, vulva, or rectum. Side-by-side placement of sensor pads has been used in the conjunctival sac, gingival and nasal mucosa, and inside the rectum, vagina, or prepuce. Ideally, readings should be greater than 90%, but interpretation should be made in conjunction with the color of the mucous membranes, blood, and other clinical signs. Capnography may also enhance patient monitoring by enabling early detection of adverse respiratory events, including hypoxia. While pulse oximetry gives trends in patient oxygenation, capnography provides information about CO<sub>2</sub> production, pulmonary perfusion, alveolar ventilation, respiratory patterns, and elimination of CO<sub>2</sub> from the anesthesia circuit and ventilator.

### ***Standing Sedation***

Standing sedation should only be attempted under conditions that take into consideration animal and staff safety. The type of procedure, as well as the temperament of the animal, restraint device, and husbandry training of the individual, will determine the level of sedation and analgesia required. Excitement and environmental or painful stimuli can override drug effects. As a general rule, individuals that are acclimated to people and husbandry training and restraint practices tend to require lower doses of immobilizing agent. Opioids tend to have more potent effects than the tranquilizer/sedative classes of drugs. A combination of butorphanol and azaperone has proven effective for repeat procedures, inducing standing sedation and recumbency in the white rhinoceros (Radcliffe et al., 2000a). See Table 5.15 for suggested doses for standing sedation of captive rhinoceros.

Table 5.15. Standing chemical restraint doses for adult captive Sumatran rhinoceros.

Chemical Restraint Drug(s)	Reversal Agent(s)
25-40 mg butorphanol IM (use azaperone in longer procedures)	naltrexone at 2.5 mg/mg butorphanol
120-150 mg butorphanol + 5-7 mg medetomidine IM (use 1-2 mg nalorphine IV to keep standing)	naltrexone at 1 mg/mg butorphanol atipamezole at 5 mg/mg medetomidine
0.8-1.5 mg etorphine IM	naltrexone at 40 mg/mg etorphine

### ***Immobilization/General Anesthesia***

The primary class of drugs for general anesthesia in rhinoceros is the opioids. Etorphine is most commonly used, although carfentanil and more recently, combinations of etorphine and thiafentanil have also been administered to rhinos. Opioids are typically combined with azaperone, an alpha-2 agonist (e.g., xylazine, detomidine), or acepromazine to provide muscle relaxation and to counteract the hypertensive effect of the opioids (LeBlanc et al., 1987). Midazolam, diazepam, or guaifenesin infusion can also provide additional muscle relaxation. White and Indian rhinos appear to be more sensitive to the effects of opioids than black rhinos and exhibit muscle tremors, limb paddling, hypoxia, hypercapnia, and hypertension (Weber and Miller, 1996; Portas, 2004). Butorphanol has been administered to antagonize respiratory depressive effects in white rhino (10-20:1 butorphanol:etorphine in mgs); however, it may also lighten the plane of anesthesia (Miller et al., 2013). It should be used with caution in black rhinos since they appear to be more sensitive and can suddenly get to their feet (Portas, 2004). Other partial opioid agonist-antagonists are routinely used in the field and can be adapted for captive rhinos when available (e.g., nalbuphine). Butorphanol-azaperone and butorphanol-medetomidine/detomidine combinations have successfully induced recumbency in captive Sumatran rhinos (Radcliffe et al., 2002; Portas, 2004; Waltzer et al., 2010). Supplemental ketamine, opioids, guaifenesin, propofol, or isoflurane can be used to deepen the level of anesthesia and lengthen immobilization as required (Ball et al., 2001). See Table 5.16 for recommended doses used for recumbent immobilization of rhinoceros.

Oxygen supplementation by intratracheal intubation or nasal insufflation (flow rates of 15-30 liters/minute) can increase oxygen saturation values (Morkel et al., 2010). Doxapram administration for apnea has also been used in rhinos but may only provide short-term relief. Partial or complete reversal should be considered in severe cases of hypoxia.

Table 5.16. Recumbent immobilizing doses for adult captive Sumatran rhinoceros.

Drug(s)	Reversal Agent(s)
30-50 mg butorphanol + 50-60 mg azaperone IM	naltrexone 2.5 mg/mg butorphanol IM/IV
1 mg etorphine + 60 mg azaperone IM	naltrexone 50 mg/mg etorphine ½ IV, ½ IM
10 mg butorphanol + 10 mg detomidine IM, wait 20 min then 1.2 mg etorphine + 5 mg acepromazine IM	naltrexone 150 mg IV + atipamezole 20 mg IV

## ***Use of Tranquilizers/Sedatives for Transport and Other Uses***

There may be occasions other than medical procedures when rhinoceros need to be sedated for short or more extended periods of time, such as during crating and transport, or confinement for other reasons. With the advent of husbandry training, the need for drugs in captive rhinos has become more limited but should always be available as an option. Tranquilizer/sedative drugs may be used in rhinoceroses to relieve anxiety, reduce hostility, decrease motor activity, alleviate excitement, and to facilitate animal introductions. Drug choice is based on the desired duration of action and expected outcome. Long-acting neuroleptics (LANs) are typically administered in free-ranging rhino after capture for transport and boma acclimation, although they have also been used in captive rhino for longer duration.

### ***Sumatran Rhinoceros***

Few reports of Sumatran rhinoceros anesthesia exist since captive specimens are rare. Etorphine (0.98-1.23 mg or 1 mg) combined with acepromazine (4-5 mg) or azaperone (60 mg) has been used to anesthetize captive Sumatran rhinoceroses (Portas, 2004; Radcliffe and Morkel, 2007). One adult male was immobilized on two occasions using a two-stage darting protocol. The first doses were considered inadequate and the authors subsequently recommended 10 mg butorphanol plus 10 mg detomidine IM followed 20 minutes later with 1.2 mg etorphine and 5 mg acepromazine IM, plus 50 mg supplemental doses of ketamine IV to extend the anesthesia period (Waltzer et al., 2010). Darting of this animal likely contributed to long induction times (up to 40 minutes)—Sumatran rhinoceros are easily conditioned for hand injection in a chute. As with the African species, muscle rigidity and cardiopulmonary depression are common with use of the potent opioids, and preanesthetic administration of a tranquilizer to limit muscle tremors and improve respiratory function is prudent. Total azaperone doses should be kept under 100 mg as ataxia has been noted upon recovery with higher doses in this species. Butorphanol has been combined with detomidine for standing sedation while a mixture of butorphanol and azaperone for standing sedation and full recumbent procedures has also been routinely used (Radcliffe et al., 2002).

As with the African species, butorphanol combinations are preferred in captive Sumatran rhinoceros to preclude the adverse cardiopulmonary changes associated with use of more potent opioids. For adult animals, butorphanol at a dose of 60-80 micrograms/kg with azaperone at 80-100 micrograms/kg and a range of 30-50 mg and 50-60 mg butorphanol and azaperone, respectively, is recommended with higher butorphanol doses being used on occasion to induce recumbency. Antagonism of the butorphanol effects is accomplished with naltrexone at a dose of 2.5 times the dose of butorphanol (Radcliffe et al., 2002). Other tranquilizers may be used in place of azaperone, such as the alpha-2 agonists, but care should be exercised as hypoxemia has been reported with use of these sedatives. Local anesthetics may facilitate invasive procedures, however, use of more potent narcotics such as etorphine or other pharmacologic agents such as ketamine and medetomidine may be indicated to induce surgical anesthesia.

## ***NUTRITION***

### **Nutritional Requirements**

A formal nutrition program is recommended to meet the nutritional and behavioral needs of all Sumatran rhinos (AZA Accreditation Standard 2.6.2; AZA, 2014). Diets should be developed using the recommendations of nutritionists, the Nutrition Scientific Advisory Group (NAG) feeding guidelines ([http://www.nagonline.net/Feeding%20Guidelines/feeding\\_guidelines.htm](http://www.nagonline.net/Feeding%20Guidelines/feeding_guidelines.htm)), and veterinarian's as well as AZA Taxon Advisory Groups (TAGs), and Species Survival Plans (SSP)

Programs. Diet formulation criteria should address the animal’s nutritional needs, feeding ecology, as well as individual and natural histories to ensure that species-specific feeding patterns and behaviors are stimulated.

Sumatran rhinos consume large quantities (50 kg) of fresh “browse” or leaves and stems from broad-leaved trees, shrubs, and herbs (Dierenfeld et al., 1994). Not much is known about their gastrointestinal tract, but similarities are expected between Sumatran rhinos and other rhinos and horses of similar size [Lintzenich and Ward, 1997; National Research Council (NRC), 2007]. Table 5.17 represents the target range set for this species.

Table 5.17. Target nutrient range for the Sumatran rhino on a dry matter basis.

Nutrient	Target Nutrients <sup>a</sup>	Target Nutrients–Horse <sup>b</sup>
Protein, %	15.54-19.98	5.99-17.04
Vitamin A, IU/g	1.11-3.85	0.84-3.33
Vitamin D, IU/g	0.22-0.55	0.37-0.68
Vitamin E, mg/kg	120-350	33.9-100.9
Thiamin, mg/kg	2.22-4.99	1.4-6.9
Riboflavin, mg/kg	2.22	0.93-2.7
Calcium, %	0.22-0.72	0.22-0.65
Phosphorus, %	0.16-0.38	0.16-0.42
Magnesium, %	0.077-0.11	0.04-0.14
Potassium, %	0.29-0.42	0.12-0.59
Sodium, %	0.099-0.29	0.05-0.46
Iron, mg/kg	39-49	23.3-69.3
Zinc, mg/kg	39.9	18.7-55.5
Copper, mg/kg	9.99	4.7-13.8
Manganese, mg/kg	39.9	18.7-55.5
Selenium, mg/kg	0.099	0.05-0.14
Iodine, mg/kg	0.099-0.59	0.17-0.49

<sup>a</sup>Lintzenich and Ward, 1997.

<sup>b</sup>Horse NRC, 2007 for a 600 kg and a 900 kg horse assuming 2% body mass consumption converted to 100% dry matter diet.

## Diets

The formulation, preparation, and delivery of all diets must be of a quality and quantity suitable to meet the animal’s psychological and behavioral needs (AZA Accreditation Standard 2.6.3; AZA, 2014). Food should be purchased from reliable, sustainable and well-managed sources. The nutritional analysis of the food should be regularly tested and recorded. Food preparation must be performed in accordance with all relevant federal, state, or local regulations (AZA Accreditation Standard 2.6.1; AZA, 2014). The appropriate hazard analysis and critical control points (HACCP) food safety protocols for the diet ingredients, diet preparation, and diet administration should be established. Diet preparation staff should remain current on food recalls, updates, and regulations per USDA/FDA. Remove food within a maximum of 24 hours of being offered unless state or federal regulations specify otherwise and dispose of per USDA guidelines.

The Sumatran rhino was brought into captivity to maintain and breed if possible. However, proper diet and nutrition are required for reproduction (Dierenfeld et al., 2000). Issues with stool consistency, gastric torsion, and metabolic imbalances might be related to diet (M. Campbell, pers. comm.). Recommendations for feeding had included high-fiber pellets, mixed grass/legume hay and available browses that most closely duplicated the wild diet. The diet that was chosen, outlined in Table 5.18, has maintained animals and supported reproduction (Dierenfeld et al., 1994). Updated in 2000, the Cincinnati Zoo and Botanical Garden offered a mixture in the diet of fresh browse (from California), hay, and a nutritionally complete food (Dierenfeld et al., 2000). The major portion of the diet was fresh browse.

Table 5.18. Diet outlined for the Sumatran rhino species at Bronx and Cincinnati Zoos (Dierenfeld et al., 1994).

Food Category	Description	Quantity
Hay	Orchard grass/legume	Ad libitum (intake of 10 kg)
Nutritionally Complete Pellet	Pellets developed for moose	3.2 kg
Browse	Honeysuckle, willow, mulberry	No amount given

Since browse plants are a major portion of the animal's diet, all plants must be identified and assessed for safety. The responsibility for approval of plants and oversight of the program should be assigned to at least one qualified individual (AZA Accreditation Standard 2.6.4; AZA, 2014) in addition to a team of nutrition, veterinary and animal management staff. The program should identify if the plants have been treated with any chemicals or grown near any point-sources of pollution and if the plants are safe for the rhinos. If animals have access to plants in and around their exhibits, there should be a staff member responsible for ensuring that toxic plants are not available. Several references are available, including Toxic Plants of North America (Burrows and Tyrl, 2001).

The nutrient content of browse plants to the total diet should be determined. Browse plants contribute to the major fiber level, but secondary plant compounds such as tannins, alkaloids and saponins also may be present within these plants. The browse plants also should be offered in an appropriate manner.

The diets for three Sumatran rhinos at two institutions are listed in Table 5.19, and Table 5.20 shows how those diets compare with the recommended nutrient targets.

## Nutritional Evaluations

The abnormal accumulation of iron within organ systems has been described previously in captive Sumatran rhinoceros. This pathological condition remains a challenge to prevent, diagnose antemortem, and treat effectively. The etiology for this process remains unclear but could have a nutritional component. A review of six post-mortem exams (gross and histopathology) revealed varying degrees of hemosiderosis in multiple organs, including small intestine, lung, spleen, kidney, liver, lymph nodes and bone marrow. One adult female died as a result of hepatic cirrhosis due to large quantities of iron being deposited in her liver (M. Campbell, pers. comm.).



**Table 5.19. Sumatran rhino diets as fed.**

Rhino	Grams							g/wk	% in diet
	Sun	Mon	Tues	Wed	Thurs	Fri	Sat		
<b>Suci</b>									
Food Item	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	g/wk	% in diet
Banana	272	1816	272	272	1816	272	1816	6536	2.26
Apple	2270	2270	2270	2270	2270	2270	2270	15890	5.49
Pear	1544			1544				3088	1.07
Papaya						1544		3088	1.07
Carrot	454	454	454	454	454	454	454	3178	1.10
Vitamin E – Emcelle	6	6	6	6	6	6	6	42	0.01
Browse <sup>1</sup>	34050	34050	34050	34050	34050	34050	34050	238350	82.41
Orchard grass/Alfalfa	2724	2724	2724	2724	2724	2724	2724	19068	6.59
<b>Total</b>	<b>41320</b>	<b>41320</b>	<b>41320</b>	<b>41320</b>	<b>41320</b>	<b>41320</b>	<b>41320</b>	<b>289240</b>	<b>100.00</b>
<b>Ipuh</b>									
Food Item	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	g/wk	% in diet
Banana	272	1816	272	272	1816	272	1816	6536	2.15
Apple	2270	2270	2270	2270	2270	2270	2270	15890	5.15
Pear	1544			1544				3088	1.00
Papaya						1544		3088	1.00
Carrot	454	454	454	454	454	454	454	3178	1.03
Vitamin E – Emcelle	6	6	6	6	6	6	6	42	0.01
Browse <sup>1</sup>	34050	34050	34050	34050	34050	34050	34050	238350	77.31
ZNN cubes <sup>2</sup>	2724	2724	2724	2724	2724	2724	2724	19068	6.18
Orchard grass/Alfalfa	2724	2724	2724	2724	2724	2724	2724	19068	6.18
<b>Total</b>	<b>44044</b>	<b>44044</b>	<b>44044</b>	<b>44044</b>	<b>44044</b>	<b>44044</b>	<b>44044</b>	<b>308308</b>	<b>100.00</b>
<b>Harapan at Cincinnati</b>									
Food Item	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	g/wk	% in diet
Browse <sup>3</sup>	45400	45400	45400	45400	45400	45400	45400	317800	93.46
Mazuri Wild Herbivore <sup>4</sup>	2724	2724	2724	2724	2724	2724	2724	19068	5.61
Beet Pulp (soaked)	450	450	450	450	450	450	450	3150	0.93
<b>Total</b>	<b>48574</b>	<b>48574</b>	<b>48574</b>	<b>48574</b>	<b>48574</b>	<b>48574</b>	<b>48574</b>	<b>340018</b>	<b>100</b>
<b>Harapan at White Oak</b>									
Food Item	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	g/wk	% in diet
Browse <sup>5</sup>	37500	37500	37500	37500	37500	37500	37500	262500	75.0
Produce <sup>6</sup>	6000	6000	6000	6000	6000	6000	6000	42000	12.0
Mazuri Wild Herbivore	4500	4500	4500	4500	4500	4500	4500	31500	9.0
Hay <sup>7</sup>	2000	2000	2000	2000	2000	2000	2000	14000	4.0
<b>Total</b>	<b>50000</b>	<b>50000</b>	<b>50000</b>	<b>50000</b>	<b>50000</b>	<b>50000</b>	<b>50000</b>	<b>350000</b>	<b>100</b>

<sup>1</sup> Browse for Suci and Ipuh is different ficus species obtained from San Diego Zoo Global.

<sup>2</sup> ZNN cubes are a Zoo Nutrition Network product that is a specially-formulated product for the Cincinnati Zoo & Botanical Garden.

<sup>3</sup> Browse for Harapan contains ficus sp., elm, mulberry, and Kafir plum.

<sup>4</sup> While housed at White Oak Conservation, Harapan's browse included Abizia juulibrissin, Betula, Caeltis laevigata, Celtis spp., Cornus spp., Eleagnus, Ficus spp., Gordonia lasianthus, Liquidambar, Lex, Morus, Musa, Myrica, Nyssa, Persea borbonia, Phyllostachys, Pittisporum, Plantanus, Pyrus calleryana, Tilia, Ulmus, and Vitis spp.

<sup>5</sup> Apple, banana, cantaloupe, carrot, honeydew, papaya, pear, pineapple, strawberries, sweet potato, and watermelon.

<sup>6</sup> Alfalfa and coastal bermudagrass fed together.

Table 5.20. Nutrient analysis of the Sumatran rhino diets on a dry matter basis compared to the targets.

Nutrient	Suci	Ipuh	Harapan	Target Target Nutrients <sup>a</sup>	Nutrients – Horse <sup>b</sup>
Protein, %	10.2	11.52	11.1	15.54-19.98	5.99-17.04
Vitamin A, IU/g	9.15	8.85	0.95	1.11-3.85	0.84-3.33
Vitamin D, IU/g	No data	0.19	0.18	0.22-0.55	0.37-0.68
Vitamin E, mg/kg	132	179	46.70	120-350	33.9-100.9
Thiamin, mg/kg	0.1	1.71	0.78	2.22-4.99	1.4-6.9
Riboflavin, mg/kg	0.13	2.54	6.76	2.22	0.93-2.7
Calcium, %	1.44	1.42	1.63	0.22-0.72	0.22-0.65
Phosphorus, %	0.18	0.26	0.17	0.16-0.38	0.16-0.42
Magnesium, %	0.23	0.23	0.20	0.077-0.11	0.04-0.14
Potassium, %	2.29	2.14	1.70	0.29-0.42	0.12-0.59
Sodium, %	0.11	0.14	0.13	0.099-0.29	0.05-0.46
Iron, mg/kg	112	139	114	39-49	23.3-69.3
Zinc, mg/kg	18.3	37.52	37.88	39.9	18.7-55.5
Copper, mg/kg	7.8	9.61	7.94	9.99	4.7-13.8
Manganese, mg/kg	33.1	42.88	25.93	39.9	18.7-55.5
Selenium, mg/kg	No data	0.08	0.07	0.099	0.05-0.14
Iodine, mg/kg	No data	0.32	0.30	0.099-0.59	0.17-0.49

<sup>a</sup>Lintzenich and Ward, 1997.

<sup>b</sup>Horse NRC, 2007 for a 600 kg and a 900 kg horse assuming 2% body mass consumption converted to 100% dry matter diet.

## Hand-Rearing

A limited number of rhino calves have been and are currently being raised using various formulas, but no Sumatran rhino calves have been hand-reared to date. Reports and published information must be carefully scrutinized for measures of success and methodology in milk-sample analysis. The following information uses the ungulate hand-rearing chapter in the AZA Infant Diet Notebook as a base for general feeding guidelines and formula selection (Reiter et al., 1994). This recommendation is to be used as a guideline for standardization of a hand-rearing diet.

### *Milk Composition and Formula Selection*

Based on available data, rhinoceros milk is more dilute than milks of other ungulate species. It is low in solids, low in protein, very low in fat and high in sugar compared with milk of equids, bovids and cervids (Oftedal, 1984). Formula selected should mimic mother's milk in composition as much as possible (Table 5.21). In Table 5.22, Formula One has been used to raise a calf to one year of age; Formula Two more closely mimics mother's milk. In Europe, Mazuri<sup>®</sup> makes a rhino milk replacer for white rhinos ([www.mazuri.eu](http://www.mazuri.eu)). Land O'Lakes<sup>®</sup> Mare's Match<sup>®</sup> (Table 5.23) has been used to supplement a greater-one horned rhino calf at San Diego Zoo Safari Park and possibly could be used for hand-raising rhino calves (powder:H<sub>2</sub>O = 1:6 but formula may need to

be mixed at a more dilute ratio (1:8) for the first few days to avoid problems with constipation). The San Diego Zoo Safari Park has used a low fat cow's milk: nonfat cow's milk: lactose powder: water (27:9:1:1 by weight)(Blakeslee and Zuba, 2002).

Table 5.21. Nutrient compositions of rhino milk (Gregory et al., 1965) and recommended formulas (% as-fed basis).

Formula	Solids	Protein	Fat	Sugar
Rhino milk	8.8	1.4	0.2	6.6
Formula 1	10.3	3.3	0.3	5.9
Formula 2	8.3	1.7	0.2	6.6

Table 5.22. Compositions of rhino hand-rearing formulas.

Ingredients	Parts by Volume	
	Formula 1	Formula 2
Water	32	9
Skim milk	32	9
Karo Syrup	1	1

Though rhinoceros' milk is different from cow's milk, the latter may still be appropriate for hand-rearing rhinos if used in combination with other ingredients. Cow's milk is low in iron; consequently, an iron source such as Fer-In-Sol® (Table 5.23) should be added to the formula at two drops per 100 g of formula. In addition, infant vitamins, such as Major® Multi-Vita Drops® (Table 5.23), should also be added to the formula at two drops per 100 g of formula. Some infant vitamins, such as Mead Johnson® Poly-Vi-Sol with Iron® (Table 5.23), contain added iron. San Diego Zoo Safari Park uses Probios® (2 tbsp) and Lixotinic® (0.44 ml /kg body weight)(Table 5.23) as daily supplements added in the first bottle. The animal may also benefit from the addition of Lactaid® at one drop per 100 g of formula. Lactaid® (Table 5.23) aids in carbohydrate digestion and helps prevent possible gastrointestinal tract distress. If the neonate is less than 24 hours old, colostrum diluted 50% with water or an electrolyte solution for ungulates, such as Replenish® (Table 5.23), should be administered for the first 24 hours. Though species-specific colostrum is preferred, cow colostrum may be used. San Diego Zoo Safari Park uses Land O'Lakes® Colostrum Replacement® (Table 5.23) in the first 24 hours after nursing followed by a mix of 50% colostrum and 50% formula during the next 24 hours, and then 100% formula until weaning. Products such as Colostrx® and Seramune® Oral may also be used (Table 5.23). To avoid gastrointestinal distress, a diluted formula should be offered beginning on day two. The formula can be gradually increased to full concentration depending on the animal's health, including weight gain and stool condition. Prior to feeding, the formula should be warmed to approximately 37°C (99°F). Rhinoceros calves prefer their milk cooler than many other ungulates.

Table 5.23. Products used in hand-rearing diets.

<b>Product</b>	<b>Manufacturer/Distributor</b>
Mare's Match Milk Replacer Colostrum Replacement	Land O' Lakes Animal Milk Products Shoreview, MN 55126
Colostrx	Protein Technology, Inc. Minneapolis, MN 95403
Seramune Oral	Sera, Inc. Shawnee Mission, KS 66285
Replinish	Fermenta Animal Health Co. Distributor Kansas City, MO 64153
Fer-In-Sol Poly-Vi-Sol with Iron	Mead Johnson Nutritionals Bristol-Meyer Co. Evansville, IN 47721
Major Multi-Vita Drops	Major Pharmaceutical Corp. Distributor Chicago, IL 60612
Lixotinic	Revival Animal Health Distributor Orange City, Iowa 51041
Probios	Vet Plus, Inc. Menomonie, WI 54751
Lactaid	Lactaid, Inc. Pleasantville, NJ 08232

### ***Feeding Regimen***

The calf should be stabilized and hydrated before any feeding. Quantity fed should range from 10 to 13% of body weight (BW). Animals should be fed every two hours. Because infants suckle during daylight hours, feeding should be equally spaced in a 12-hour period not to exceed 3 to 4% of body weight at any one feeding. It is recommended that feeding begin with 10% of body weight split equally into 12 feeds one hour apart during daylight hours. The quantity of formula fed should be adjusted daily based on the animal's weight. Animals should be weighed at the same time each day. Fresh water should be available at all times.

If diarrhea occurs, the quantity of formula fed should be decreased or the formula diluted until stool condition returns to normal. If diarrhea is persistent, an electrolyte solution can be used to dilute the formula, replacing some or all of the water. In addition, the number of feedings can be increased to lessen the quantity fed at any one time.

Formula can be prepared ahead of time and warmed as needed. Water should be boiled to decrease possible contamination due to pathogens and refrigerated before being added to the formula. The formula should be refrigerated and used within 72 hours. Prior to feeding, the formula should be warmed to the animal's body temperature. Calf nipples work well with large species. Bottles should be boiled before use. Diluted bleach may be used as a disinfectant. Formula left over from each feed should be discarded.

Table 5.24. Example feeding regimen, provided by San Diego Zoo Safari Park.

Week 1 & 2	6am, 8, 10, 12, 2, 4, 6, 8pm	(18-20% BW) 8 feedings
Week 3 & 4	6am, 8, 10, 12, 2, 4, 6pm	(17-19% BW) 7 feedings
Week 5 & 6	6am, 8, 10:30, 1, 3:30, 6pm	(16-18% BW) 6 feedings
Week 7 & 8	6am, 9, 12, 3, 6pm	(14-16% BW) 5 feedings
Week 9 – 14	6am, 10, 2, 6pm	(12-14% BW) 4 feedings
Week 15	No more increases to daily volume	
Week 15 – 30	6am, 12, 6pm	(8-12% BW) 3 feedings
Week 30 – 40		(5-7% BW)
Week 41		(3-4% BW)
Week 52	Start dropping amount on all bottles	
Week 60	Weaned	

### **Weaning**

Weaning may begin as early as six months if necessary and should be completed in one year. Weaning is a slow process involving carefully monitoring of body weight and solid food consumption. Animals should have access to solid food at all times. A nutritionally complete pellet diet such as Calf Manna (Manna Pro Products, LLC., Chesterfield, MO, USA), horse feeds or high fiber ungulate pellets, in addition to high-quality grass hay, is appropriate. Formula may be decreased by gradually eliminating the number of feeds or decreasing the amount offered per feed and gradually decreasing the number of feeds.

## **RESEARCH**

### **Introduction and Top Research Priorities**

Largely due to the rhino poaching crisis that far out-weighs any other challenge facing rhinos today, the top five priorities of the Rhino Research Council, listed below, are skewed towards *in situ* issues. It is important to note that these research priorities are not ranked in order of importance, so the first and only priority focused on the *ex situ* population is not considered more important than the others. The Rhino Research Council realizes that conservation of genetic diversity via cell/gamete rescue from remnant populations is really not a research issue and is more of an implementation challenge, but it was identified as an important action to emphasize at this time of crisis. Following this list of the highest research priorities, the chapter continues with important areas of *ex situ* research, including reproduction, health, nutrition, behavior/ecology, and genetics. For more detailed discussions of *in situ* research needs, the reader is referred to the Rhino Research Council's 2014 Rhino Research Masterplan.



### ***Investigate Major Factors Affecting Health and Reproduction Ex Situ***

- Epidemiology of browser rhino health issues—Health concerns and unusual syndromes continue to impact black rhinos in captivity, but the incidence of various conditions shifts over time, and patterns require constant monitoring and re-evaluation to track current trends and conditions. Otherwise, outdated problems and historic records continue to be referenced at the cost of realizing emerging trends and realities. One example is the significant increase in the number of black rhino deaths due to renal disease compared to five years ago and a decreased incidence of mortality due to hemolytic anemia compared to 20 years ago. It would be extremely useful to secure ongoing support for a point person whose priority is to evaluate the historical data and current situations to keep abreast of the problems and factors associated with those health issues.
- Iron overload syndrome (significance, detection, treatment, prevention)—Evidence that hemosiderosis is occurring in browsing rhinos is solid, but there are still many questions surrounding the impact of this condition on rhino health, whether it is primary or secondary, why it is occurring, and what can be done about it. In the past decade, relatively little progress has been made. Although there has been some progress in developing oral iron chelators for humans, the efficacy and safety of such medications are unknown in rhinos, and they are cost prohibitive. Research on dietary tannins as iron chelators has produced inconclusive results. Recent studies on the impact of large volume, regular phlebotomies for reducing iron marker values has shown promise but is unlikely to be adopted as a long-term management strategy at many institutions. Research in any of the four areas—cause, detection, prevention and treatment—could be valuable in producing insight on how to manage/prevent this condition.
- Obesity/body condition scoring—Overconditioning in captive rhinos can lead to a multitude of health problems, including musculoskeletal, foot, and reproductive problems. One example is the almost certain exacerbation of pododermatitis associated with captive husbandry conditions in the greater one-horned rhino. Research on standardizing body condition scores for all rhino species, along with improved nutritional management, should be a priority for captive health.
- Sub-optimal reproduction (stillbirths, embryo loss, phytoestrogens, pathology)—Although reproductive success has improved in white and greater one-horned rhinos by changing management strategies, providing larger spaces and more complex social groups that include experienced and inexperienced individuals, and allowing mate choice options, there are a few reproductive challenges that remain unresolved and challenge our ability to develop self-sustaining populations. For example, < 50% of all captive white rhinos are reproductively successful, almost 50% of greater one-horned rhino calves are stillborn, and as our ability to evaluate larger numbers of female rhinos has improved, incidence of known embryo loss and reproductive pathology continue to increase.

### ***Improve Rhino Identification and Monitoring***

- Optimize/standardize survey methodologies—Given the current poaching crisis, research that can improve our ability to monitor and survey wild populations is extremely important. Post-release monitoring is our primary method of determining the success of translocation, dehorning and many other conservation efforts. Data compiled from tracking individuals after translocation can be used to develop specific criteria that individual rhinos should meet (sex, age, reproductive status) prior to translocation in order to improve the success rate. Better methods for signal transduction and reception would be helpful for forest-dwelling animals. Creative methods of transmitter attachment are needed since horn transmitters

are difficult to insert in de-horned animals, and radiocollars are a challenge given the neck morphology of rhinos. Also, lesions can occur with collars. Given the abundance of research on this topic in the military field, adaptation and application of this newer technology may be useful for rhinos.

- Genetic census—Advances in genetic analyses of fecal samples, hair, environmental-DNA and microbiomes could be used to determine the genetic diversity among wild populations, including sex ratios, number of individuals and extent of inbreeding.

### ***Identify Most Important Factors in Translocation Success***

Translocations have proven extremely valuable in saving rhinos from poaching, re-populating parks/ranches, bolstering dwindling populations and establishing new populations. However, there is room for improvement, and given the importance of this action for long-term rhino survival, research is needed to enhance survival and reproductive success of every rhino moved. Studies might focus on the following example topics: white rhino boma maladaptation, mortality rates in different habitats, impacts of dehorning, impact on source populations, and identifying the type of individuals most likely to succeed.

### ***Determine Ecological Factors Impacting Rhino Populations***

To choose the best locations for rhinos and provide guidance for those who want to improve their land in support of rhino populations, more information is required regarding specific ecological traits that impact rhino population survival and ability to thrive. Spatial ecology is especially important and is integrated with habitat quality, usage and preference.

### ***Conserve Genetic Diversity via Cell/Gamete Rescue from Remnant Populations (small/sub-populations and highly endangered)***

Given the poaching crisis in Africa and India and the rapid decline of Sumatran rhinos throughout their range, it seems prudent that every effort should be made to preserve cell lines and gametes from all genetically valuable rhinos/populations. The methodologies already exist but may need to be modified for the field conditions encountered in the African savannah and the tropical forests of Asia.

## **Areas in Need of Research *Ex Situ***

### ***Reproduction***

Hormone and/or ultrasound monitoring have facilitated the Sumatran rhino breeding program by allowing managers to pair animals when the female is in estrus, thereby minimizing the odds of severe conflict between the pair. If this approach is utilized and successful, the challenge of minimizing aggression when pairing for breeding will be resolved and will be dropped from future masterplans. Other important areas of reproduction research are as follows:

- Early embryonic death (EED)—Repeated documentation of EED in all captive rhino species is puzzling. In the past 15 to 20 years, there have been at least six confirmed cases of EED in white rhinos at four different institutions, five confirmed cases of EED in greater one-horned rhinos at three different locations, and seven EEDs confirmed in two Sumatran rhinos at two locations. Few rhinos are monitored closely enough to diagnose EED, so considering the number of confirmed cases, it appears to be a common occurrence in the captive population. It has also been reported in at least one field study in wild black rhinos. However, in most field studies females breed only once or twice before conceiving and carrying pregnancies to term,

so the high incidence of EED in captivity seems aberrant. In addition to EED, abortions later in gestation have also been reported. There are many potential causes of EED and abortion in rhinos, including uterine infection, uterine scarring from a previous dystocia, endometrial hyperplasia, reproductive pathology, hormonal insufficiency, dietary insufficiency or toxicity, social/behavioral influence of enclosure co-inhabitants or other health-related conditions of the female rhinos. It is quite possible EED is occurring in many females that are breeding repeatedly without producing calves. Further investigation into the prevalence and potential causes of EED and abortion is warranted.

- **Reproductive pathology**—Reproductive tract pathology in female rhinos has been reported in all captive rhino species and prevalence can be as high as 50% in females over 15 years of age (white rhinos). It can range from small, inconsequential cysts that do not affect fertility to large, invasive tumors that jeopardize the life of the rhino. There is general agreement that these pathologies typically develop in rhinos that spend the majority of their lives in a non-pregnant state and could be related to repeated exposure to hormone fluctuations, a condition not natural in wild rhinos that spend much of their time pregnant. However, there are some younger rhinos that also develop severe pathology, and more recent research has revealed that a fairly substantial proportion of white and greater one-horned rhinos develop pathologies in their early teens. Understanding the etiology of this pathology and identifying hormone therapies that might prevent it would be very useful. Furthermore, criteria for determining when the degree and types of pathology observed during an exam can be tolerated without loss of fertility versus those that render a female infertile would be very useful to establish so that appropriate management recommendations could be made.
- **Measuring estrogen**—Quantifying changes in estrogen levels that correlate with meaningful reproductive events has been difficult in several of the rhino species and continues to be challenging. A more accurate and meaningful way to assess estrogen concentrations could be useful for monitoring cyclicity, timing introductions and assessing other reproductive characteristics.
- **Early pregnancy marker**—Pregnancy can be diagnosed in rhinos through progesterone monitoring and ultrasound. It would be useful for management and for learning more about EED if a pregnancy-specific marker could be identified in rhinos not trained for ultrasound, but this is not a high priority.

The use of assisted reproduction has the potential of becoming a very useful tool for regional and metapopulation management of rhinos. Additionally, assisted reproduction may provide a means of overcoming physical and/or behavioral problems that currently prevent reproduction in specific individuals. Progress towards these goals has been impressive, and it is now time to look at priorities considered feasible to overcome during the next five years.

- **Semen collection**—Semen collection by electroejaculation (EEJ) has become more reliable and has been successful in all four captive rhino species. However, there are differences in the quality of samples collected with greater one-horned rhinos being the most reliable in producing a high quality, concentrated sample. In black, white and Sumatran rhinos, samples often are more dilute and more likely to be contaminated with urine or red blood cells. Samples are often adequate for cryopreservation, artificial insemination (AI) or in vitro fertilization but often are not able to withstand more complex processing like sperm sorting. More research into the effects of different anesthetic protocols and attempts at penile catheterization may prove valuable. Chemical induction of ejaculation has been attempted but has not proven very effective yet.

- In vitro fertilization (IVF)—To date, IVF success has been minimal (one IVF attempt produced one embryo in a black rhino and one IVM/IVF attempt produced one embryo in a black rhino) and more research is needed. Success has been minimal, in part, because of limited opportunities.
- Sperm sorting—Since 2005, 25 EEJ attempts across three species (n=10 black, n=9 white, n=6 greater one-horned) have been conducted for sperm sorting. Inconsistent ejaculate quality is the greatest challenge to application of sorting technology and integration into AI programs for each species. Only 36% of collections were good enough for sorting. Greater one-horned rhino sperm has a unique problem: an interaction between the seminal plasma and egg yolk that prevents staining of X and Y populations. Most progress has been with white rhino where sorted, cryopreserved samples are now available for use in AI procedures.
- Artificial insemination—Successful AI protocols using cryopreserved semen now exist for white rhinos (five term pregnancies) and greater one-horned rhinos (three term pregnancies). AI has not yet been successful in Sumatran or black rhinos. Additional research is needed in developing successful procedures for black rhinos and Sumatran rhinos, and research to improve efficiency/success rates in all rhinos is also needed to ensure the technology is integrated into the captive management plan. Some challenges include: breaking down intact hymens in females that have never mated, determining ovulatory versus anovulatory cycles in greater one-horned rhinos, and controlling the cycle for planned, timed AI to improve feasibility of use.
- Estrous cycle manipulation—In Europe, significant progress has been made with white rhinos using synthetic progestin and human chorionic gonadotropin or gonadotropin releasing hormone. Similar methodologies are being tested in the U.S. An ovulation induction protocol has been established for Sumatran rhinos. Research is still needed on effective methods for controlling the cycle in greater one-horned rhinos so that specifically-timed AI procedures can be planned and implemented.
- Gamete rescue—Protocols for rescuing sperm post-mortem are well established but protocols for oocyte rescue are not. A few attempts have been made with oocyte rescue, and one two-cell embryo was produced following maturation and IVF with frozen-thawed sperm. However, all protocols still need substantial research before they will be applicable for management purposes.

### **Health**

- Disease risk analyses—Translocations within and between range states or from abroad present risks for introduction of disease. Research into diseases that present potential risk to rhinos and into logistically-appropriate diagnostic techniques for screening and their incorporation into protocols should be considered part of the risk analysis and translocation process.
- Iron overload syndrome—There is a need for thorough pathology review of all captive Sumatran rhino deaths to better understand the historical extent and impact of confinement and stress in relation to this syndrome.
- Nutrition of Asian rhino browsers—Problems of captive diets due to low browse selection and availability should be researched. Studies on browse transport and degradation factors due to necessary refrigeration, storage and handling should be conducted. Also, is the inclusion of cereal-based concentrates in rhino diets detrimental?

- Protocol development—Protocols are needed for animal movements among institutions, including government requirements, vaccination strategies, etc.
- Establish normal biological and health parameters for captive and wild animals—More normal data is needed to better understand the health of captive animals or those moved for future translocation programs.
- Quantifying stress (especially chronic stress)—Develop laboratory and behavioral markers of stress. What is the health impact, for instance, on the development of gastrointestinal ulcers, increased iron stores, etc.? New ideas include evaluation of neutrophil function using a portable luminometer and the analyses of five markers in fecal samples including thyroid hormones.
- Pharmacokinetics/dynamics of commonly used antibiotics and analgesics—Little scientific work has been done in rhinoceros species on therapeutic drugs. Non-empirical use may lead to inadequate or potentially adverse effects. With the advent of husbandry training and restraint devices, sample collection is now possible for these types of studies.

### **Nutrition**

Body condition scoring systems have been established for the African rhino species, however preliminary trials have proven that these scoring systems are very subjective if utilized by staff at each institution. Furthermore, efforts to control for bias by sending images of animals from different institutions to one person for evaluation were equally as difficult because of the variation in animal appearance based on photo angle/quality. Although similar scoring systems should be developed for Asian rhino species, less subjective methods for all species are needed. Perhaps physiological values for leptin, glucose, insulin, etc., could provide a more solid method of evaluating body condition in addition to computerized assessments, body measurements matched with weights and/or characterizing body types. This is an important issue since body condition has now been suggested as a factor involved in iron storage problems, disease, skewed sex ratio of calves and reproductive failure of captive-born white rhinos.

There are often requests for browse lists. Many papers exist on the browse chosen by wild rhinos as well as the nutritional components of the browse. However, the fact is that most zoos will feed what browse they can get locally and with the least amount of cost/effort. Regional browse studies on nutritional value and palatability of local species are helpful as general guides. Results of such studies have been reported for East coast species and a similar study on West coast species (in particular acacia) is ongoing. In addition, a study comparing sumac and willow was also recently reported. San Diego Zoo Global is studying the nutrient composition of elephant grass (*Pennisetum purpureum*) following one to six months of regrowth. Institutions with black or Sumatran rhino are encouraged to develop browse farms to help provide better diets for their animals, and these regional studies are a great reference when initiating a browse farm. Other important topics for nutritional research are as follows:

- Research needs related to reproduction include determining if there is an impact of diet on calf sex ratio, evaluation of new hand-rearing formulas for supplementation and complete rearing, determining milk production, and estimating energy requirements of lactation.
- Minimum dietary nutrient concentrations for rhinos need to be established for maintenance, gestation and breeding to determine if utilizing the horse guidelines are adequate.
- Science-based recommendations need to be developed regarding the use of alfalfa for rhinos.
- The use of fecal DNA analyses as a means of defining wild rhino diets should be investigated.



- The cause of tooth overgrowth could be related to diet (nutritional and/or mechanical), and further investigation could be helpful. There have been several cases of rhinos requiring repeated teeth floating procedures. Additional browse could be helpful since silica is known to reduce tooth growth.
- Recent investigations of trace minerals are ongoing, especially with copper, which is an important anti-oxidant that could combat iron. One study has changed the diet to enhance copper. Appropriate dietary concentrations of zinc and copper need to be determined as both are being supplemented for both hoof health and to bind with iron.

### ***Behavior and Ecology***

Large-scale manipulative experiments to test hypotheses relating to social environment, stress, foraging/nutrition, movement patterns, etc. on reproduction, health and survival are encouraged. To achieve this goal, multiple large, naturalistic enclosures will need to be established and experimental groups assigned to different treatment conditions in a way that establishes multiple experimental replicates to address each question. Such a systematic approach, on a more limited number of variables of interest, will advance understanding of the factors governing whether captive (and wild) populations thrive or fail. Areas of *ex situ* research might include:

- Role of communication (olfactory, acoustic) in reproduction, maintaining social relationships, endocrinology (stress and reproduction), and overall social organization (consider experimental manipulations, such as with chemical signals and acoustic playbacks)
- Evaluate social processes (courtship, aggression) leading to successful copulation
- How social mechanisms lead to establishment of social outcomes, such as territoriality, dominance, group composition, etc., and how these change through time as new animals move in and out of the group
- How do changes in ecological parameters affect social behavior, endocrinology (stress and reproduction) and organization?
- Role of climatic variables (temperature/rainfall/photoperiod) on social organization and behavior
- Development of methods to reduce bodily injuries in male-male conflict and inter-sexual conflict that inhibits reproduction
- Behavioral, hormonal, and health perspectives regarding social stress
- Manipulate social variables such as density, group size, and age-sex ratios to determine outcomes for social behavior, organization, stress, health, reproduction, and survival
- Avoidance and attraction patterns
- Social density and composition effects

### ***Genetics***

The potential benefits of employing genetic tools for studying rhino conservation and population genetics are tremendous, but considerable research and development is still needed in many areas before direct, efficient, reliable application will be possible. Areas where conservation genetics could be most valuable include studies on genetic structure /connectivity of surviving populations, dispersal, paternity, and census. The potential methodologies would include analyses of mitochondrial DNA, microsatellites, genetic sexing, single nucleotide polymorphisms,

immunogenetics and the microbiome. Over the next five years, there needs to be an emphasis on developing appropriate methodologies, including:

- Microsatellite analysis—Microsatellites are powerful if they can be amplified from DNA from feces and should be a priority.
- Next generation sequencing (NGS)—Since fecal DNA typing is difficult and genetic diversity in some rhinoceros species is low, NGS may be of great value in providing the initial screening for polymorphisms needed to develop markers to examine rhino population genetics.
- Standardization—Different labs using various loci are reporting different results. There needs to be some consistency, or the techniques may wrongly come to be considered unreliable for answering conservation/population questions.
- Shorter amplicons—Amplicons of 75-150 base pairs need to be targeted by genetic markers and made available for fecal DNA studies since degradation can occur rapidly in fecal material.
- Fecal microbiome analysis—Fecal microbiome analysis may be useful in some cases as an alternative to analysis of an individual's own genetic material. Microbial DNA is more plentiful and in better condition when excreted in the feces, and each individual has their own microbial profile. This was the methodology employed to determine that the fecal samples from the Viet Nam Javan rhino were all from the same individual.
- Immunogenetic variability—Immunogenetic variability and its relationship to mate choice would be interesting to investigate. If pre-testing for mate compatibility or interest could occur prior to animal transport, it would be very valuable to animal managers in zoos and managed reserves/ranches.
- Environmental DNA—eDNA is an emerging tool in genetic studies but may be more challenging under tropical conditions.

Ongoing priority genetic studies that might include *ex situ* components include:

- Major Histocompatibility Complex (MHC) variability—MHC analyses are being planned and, once established, will help significantly when analyzing populations for variation in genes that are under selection.
- Fecal DNA analyses—Some work has been done, but the method needs to be optimized so that individual genotypes from fecals can be used for census work and assessing and studying breeding strategies, dispersal, etc. The analyses are especially needed for the Javan and Sumatran rhinos since it is the only material available for analyzing populations and learning more about animal numbers, locations, sex, relatedness, etc. Progress has been made towards this goal. Recently, eight Javan rhino microsatellite loci and 22 non-Javan rhino microsatellites have been optimized to amplify Javan rhino DNA. A project to optimize some of the loci that work better on Javan and Sumatran rhino tissue, along with new NGS loci to amplify rhino DNA from the feces of both these species, has been initiated.
- Study of disease history and parasite load—A critical emerging arena to which genetics can contribute is disease risk assessment of different rhino populations. This is particularly relevant in light of the tragic loss of five captive Sumatran rhino in Malaysia. A particular avenue worth pursuing is polymerase chain reaction techniques that can determine presence and load of different pathogens from tissue collections and fecals.

# BEHAVIOR

## Ranging Behavior and Sociality

Asian rhinoceros species, including Sumatran rhinos, are the most solitary of the extant rhino species (Hutchins and Kreger, 2006). Males and females probably only come together for breeding, and females are only accompanied by their offspring (Van Strien, 1986). When contact with another rhino is made, females can make vocalizations that range from a “squeal” to a “snort” (Table 5.25) to a “blow” (Zainal Zahari et al., 2005). Home ranges are delineated by scent marks left by males during spray-urination and defecation (Table 5.25; Owen-Smith, 1988; Zainal Zahari et al., 2005; Hutchins and Kreger, 2006). Unlike the African rhinos, Sumatran rhinos do not scrape their feet through the dung piles to spread odors (Hutchins and Kreger, 2006). Sumatran rhinos also do not range as widely as the African species and prefer to utilize a system of trails through their forest habitat (Hutchins and Kreger, 2006). They browse on a variety of vegetation throughout this habitat, including fruit (Hutchins and Kreger, 2006).

Sumatran rhinos are reported to be very sensitive to human disturbance in the wild, running long distances through the most difficult patches of forest (Hutchins and Kreger, 2006). A common behavioral abnormality observed in zoo-housed rhinos is excessive horn rubbing (Hutchins and Kreger, 2006), which might indicate boredom or distress.

## Reproductive Behavior

Females do not exhibit overt behavioral changes during estrus, thus reproductive hormone analysis or ultrasonography (Roth et al., 2001, 2004) might be necessary to assist managers in determining when to pair males and females for breeding purposes (Hutchins and Kreger, 2006). Observations of reproductive behavior in captive Sumatran rhinos (n=2 females and 2 males) were conducted during one to two hour daily introductions (Zainal Zahari et al., 2005). During this time, males explored females by smelling their hindquarters and urine, but the majority of physical contacts during initial introductions were onto the head and neck areas (Zainal Zahari et al., 2005). After smelling investigations of the female or her urine, the male might expose the tip of his tongue and exhibit flehmen (Table 5.25; Zainal Zahari et al., 2005). Contact to the hindquarters resulted in a snort or squeal from the female, and contact with her perineum resulted in the female bobbing her head, locomoting in reverse, and rapidly moving her tail (Zainal Zahari et al., 2005). The female made physical contact with the male as well during introductions, usually to the back and head (Zainal Zahari et al., 2005). The male might follow the female throughout the enclosure while using a whistle-tooting vocalization. If the timing of the introduction is incorrect, squealing and snorting will predominate and not whistle-tooting (as noted in Management, extensive experience is necessary to distinguish between the mating ritual and true aggression). During sparring, head swinging by the female appeared to initiate a charge from the male (Zainal Zahari et al., 2005). Other aggressive behaviors included chasing, biting, head butting, and horn clashes (Zainal Zahari et al., 2005). The day before estrus, both the male and female were observed to tail-raise or tail-swing more frequently, and the female squirted urine (Table 5.25; Zainal Zahari et al., 2005). The day of estrus was characterized by chin-resting, penile erection, mounting, and an increase in the number of anogenital contacts (Table 5.25) as the female reversed towards the male (Zainal Zahari et al., 2005). Sniffing, licking, and biting of the female’s perineum were noted (Zainal Zahari et al., 2005). Estrus lasted approximately 24 hours (Zainal Zahari et al., 2005). Copulation usually lasts 20 to 45 minutes, and multiple copulations can occur if the duration of the first is less than 20 minutes (see Management).

## Behavior During Introductions (Fouraker and Wagener, 1996)

Behaviors that have been noted during introductions are listed in Table 5.26 and in the Management section. Introductions often result in aggression, and it should be noted that rhinos of both sexes have been the aggressors. Intensive conflicts (Table 5.27) can occur, particularly if an introduction is attempted when the female is not in estrus. Extensive experience is necessary to distinguish between the mating ritual and true aggression in Sumatran rhinos. In some cases, aggression may proceed to a point at which management should intervene to prevent serious injury. Managers should allow some aggression during an introduction but be prepared to intervene in the event that aggression threatens the lives of one or both rhinos. Protocols for intervening may vary across institutions, but in general, careful consideration should be given to intervening in an introduction.

Table 5.25. Ethogram to assist with general rhino management (adapted from Fouraker and Wagener, 1996).

General Behaviors	Definition
Locomote	Move about covering ground
Resting	Recumbent on the ground
Wallow	Roll, lay down, or move about in an area that is wet or muddy
Forage	Search for and consume food
Object toss	Tilt or lift inanimate object off the ground
Horn rub	Rub horn against an object; often occurs if horn becomes wet but also occurs when dry
Head sweep	Head swings laterally relative to the ground, rooting the air with the horn
Hind leg drag	Walk with hind legs stiff and straight (usually performed by males during scent marking)
Mouthing	Repeated chewing or gumming motion with mouth open; not associated with eating
Nurse	Calf becomes still and suckles; tail may wag
Elimination Behavior	Definition
Urinate	Discharge or pass urine in a stream
Defecate	Discharge fecal material
Hind foot scrape	Rapid alternation of hind feet against ground while remaining stationary; often associated with elimination by the male
Urine spray/squirt	Project urine in a strong spray (usually male) or in distinct squirts (usually female); may be directed on a substrate
Urine/feces investigation	Smell or taste urine pool or feces; flehmen often exhibited
Defecation on pile	Defecate on an area that has been used repeatedly as a dung pile
Social Behaviors	Definition
Proximity	Rhinos are within one body length of each other
Face-to-face stare	Rhinos stand less than one body length from and facing each other

Table 5.25. Continued.

<b>Social Behaviors Cont.</b>	<b>Definition</b>
Affiliative physical contact	Touch, rub, or lick other animal
Follow	Locomote to remain within close proximity of another animal; indicative of estrus
Charge	Locomote rapidly toward another animal with the head lowered
Chase	Locomote rapidly in pursuit of another animal; male will chase estrous female
Open-mouth threat	Display in which one rhino faces another with open mouth; might be accompanied by slashing with canine teeth
Spar	Rhinos use horns in offensive or defensive manner; may contact heads/horns and use body weight against each other
Horn strike	Strike another rhino with horn
Gore	Pierce or wound another rhino with horn
<b>Reproductive Behaviors</b>	<b>Definition</b>
Flehmen	Raise head and curl underside of upper lip upward; often seen in males in response to female urine
Anal and genital investigation	Sniff anogenital region of another animal
Erection	Penis in erect position
Penis unsheathed	Penis is dropped from the sheath, possibly in a partial erection
Canter in circle	Female may flee from male or canter in a circle during estrus
Stand	Female remains stationary during a chin-rest or mount
Chin-rest	Male rests chin on female's back or hindquarters in preparation for copulation
Mount	Male's weight is on hind legs, head over female's withers; copulation posture
Mis-mount	Male mounts female but not in proper orientation for copulation
Copulation	Penile penetration of vagina with successful ejaculation
Vulva wink	Tail is held to the side or up with rapid contractions of vulva, exposing clitoris
Vulva swelling	Swelling, color change, and/or dilation of the vulva
<b>Vocalizations</b>	<b>Definition</b>
Whistle-tooting	Vocalization used by the male during courtship
Snort	Vocalization in which air is gruffly forced through the nasal passages, usually aggressive or defensive in context but also used by the female during estrus
Squealing	Vocalization used during a chase



Table 5.26. Behaviors noted during rhino introductions (Fouraker and Wagener, 1996; also see Management in this manual).

Non-aggressive Behaviors	Ritualized Confrontations	Potential Stress-Related Behaviors	Aggressive Behaviors
Follow	Head sweep	Pacing	Charge/chase
Chin-rest	Head-to-head spar	Excessive running	Squealing
Touch/rub/lick	Face-to-face stare	Excessive snorting or squealing	Open-mouth threat
Laying down	Low-speed chase	Diarrhea	Forceful spar
Anal or genital investigation			Goring or slashing with canines
Whistle-tooting			

Table 5.27. Levels of aggression in rhinos (Fouraker and Wagener, 1996).

Level of Aggression	Definition
1	Rhinos are charging each other but do not make physical contact
2	Rhinos are charging each other with physical contact resulting in some cuts and scrapes to the facial area
3	Rhinos are charging each other with physical contact resulting in cuts and scrapes to the facial area and body
4	Charging and/or pursuit <sup>a</sup> proceeds to the point that one or both rhinos are knocked down at least once. Scrapes and cuts are deeper and more numerous.
5	Aggression and pursuit proceed to the point that one or both rhinos have subcutaneous wounds or arterial blood flow

<sup>a</sup> It should be noted that one animal might break away from the confrontation and attempt to escape. The aggressor often will pursue and begin horn-prodding the underbelly of the escapee as the two run around the enclosure. Often a rear leg is hooked and held aloft while pursuit continues. If the escapee does not stop and resume a defensive posture, the animals might continue until heat or exhaustion becomes a critical factor. Aggression at this point is more serious.

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# APPENDIX A

## *Javan Rhinoceros*

### Scientific Name and Origin

*Rhinoceros sondaicus*

*Rhinoceros*: Greek *rhino*, meaning “nose” and *ceros*, meaning “horn”

*Sondaicus*: Probably referring to the Sunda islands in Indonesia (Latin—*icus* indicates a locality). “Sunda” means “Java”.

### Common Names

Javan rhinoceros

Asian lesser one-horned rhinoceros: In contrast to the greater one-horned rhino because of the single, comparatively smaller horn.

### Distribution and Habitat

Indonesia (extinct in Vietnam as of 2010)

Lowland tropical rainforests

### Size

900 to 1,400 kg (2,000 to 3,000 lb)

1.5 to 1.7 m (5 to 5.5 ft) tall at shoulder

Single horn 25 cm (10 in) long

### Physical Description

Gray, hairless; lesser, but still apparent, armor plating

One horn

### Life History Characteristics

Mostly a browser but some grazing

Generally solitary except for mothers and young or mating pairs

Females sexually mature at three to four years of age; males at six years

Gestation period approximately 16 months; interbirth interval of four to five years

## APPENDIX B

# *Trained Behaviors*

The following is a list of behaviors and their criteria that have been successfully trained with rhinos. The list is in alphabetical order and is not exhaustive. Basic behaviors that can facilitate rhino management include training to shift on and off exhibit, shifting into a transfer crate, and stationing alongside the barn or exhibit bollards or fencing for a body inspection.

- **Back:** Rhino moves backward at least one step and stops when bridged.
- **Come:** Rhino comes to the trainer and stands in front of the trainer.
- **Crate:** Rhino enters the transfer crate and remains calm while the door to the crate is shut.
- **Foot (with back feet):** The trainer taps the front of the foot, and the rhino picks up the foot. Trainer guides the foot to the desired position.
- **Foot (with front feet):** The trainer taps the back of the foot, and the rhino picks up the foot. The trainer guides it to the desired position.
- **IM/SQ injection:** One trainer holds the rhino “steady” while its body is lined up next to the bollards, and a second person gives an IM injection.
- **IV Blood Draw:** A trainer holds the rhino “steady” while its body is lined up next to the bollards, and a second trainer draws blood from the metatarsal area of front legs.
- **Move Up:** Rhino moves forward with at least one foot and stops when bridged.
- **Open Mouth:** Rhino opens mouth and holds mouth open. Thoroughness of the examination will be achieved through extended duration of the behavior.
- **Over:** Rhino moves its hind quarters in a lateral movement (left or right), until its body is lined up next to the bollards.
- **Scale Training:** Rhino stands “steady” on scale for weight reading.
- **Shift:** Rhino goes into desired stall or training chute with no baiting.
- **Shift off exhibit:** After cue rhino moves off exhibit into off-show (night) holding.
- **Steady (on target):** Rhino holds desired body part completely still on target.
- **Steady:** Rhino holds completely still.
- **Tactile Desensitization:** Rhino stands steady while trainers touch various locations.
- **Target:** Rhino touches the base of the horn or lips to the target.
- **Udder palpation:** One trainer has the rhino hold “steady” while a second trainer palpates the udders.

# **APPENDIX C**

## ***Example Training Plans***

### ***(DISNEY'S ANIMAL KINGDOM)***

#### **Target**

Final behavior: Rhino calmly approaches target (pool float on a stick) and touches lips to target and holds until bridged.

1. Show animal target (desensitize to presence if fear is apparent).
2. Reinforce any interest in target, touching or otherwise.
3. Use baiting if needed to entice animal to approach target and reinforce approach.
4. Reinforce animal for touching muzzle to target calmly (non-aggressively, with mouth closed).
5. Pair verbal cue, 'Target', with visual cue (show target) and reinforce for touching it with muzzle.
6. Move target to various locations and give cue (visual and verbal). Reinforce for calm approach and touching target with muzzle.

#### **Blood Collection**

Final behavior: Rhino remains calm and steady while blood is collected.

1. Train target and over "left" or "right" (as needed to get the rhino's body in position).
2. Train "steady."
3. Desensitize to manipulation of site (metatarsal for blood collection).
4. Desensitize to washing of the puncture site with water and then rub with Nolvasan®.
5. Desensitize to needles starting with blunt objects like a paper clip and gradually moving towards needles.
6. Animal stands calm and steady while blood is collected.

**APPENDIX D**  
**Training Documentation Form**  
**(DISNEY'S ANIMAL KINGDOM)**

Behavior: \_\_\_\_\_

Goal of behavior: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

Accession # and species of animal involved:

Steps involved in training this behavior: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Comments (reinforcement, bridge):

\_\_\_\_\_  
\_\_\_\_\_

Trainers involved: \_\_\_\_\_

Date training started: \_\_\_\_\_

Date goal achieved: \_\_\_\_\_

Trainers' daily rating of individual behaviors (trainer's perceptions of how well the animal performed that behavior during a specific training session)

- 1 = Unresponsive to cue
- 2 = Incorrect response to cue
- 3 = Partial response to cue
- 4 = Correct response to cue, but slow
- 5 = Correct response to cue, with no latency

Trainers' rating of animals' attitude during a training session

- 1 = Ignored trainer
- 2 = Somewhat attentive to trainer
- 3 = Moderately attentive to trainer
- 4 = Attentive to trainer
- 5 = Total attention to trainer (very focused)

Aggression during session

\_\_\_ Yes

\_\_\_ No

Progress to Goal

- 1=earliest stage of learning
- 10= behavior completed



# **APPENDIX E**

## ***Instructions for Rhinoceros Estrus Record-Keeping System***

*(San Diego Zoo Safari Park)*

Each day the person or persons that spend the most time working with the rhinos should fill out an estrus data sheet for each female >2 years old in the collection. These “observers” need not spend an excessive amount of time simply observing the rhinos. Rather, during the course of their daily routine, they should make a note of the occurrence of any behaviors related to estrus or courtship. At the end of the day, they should record these observations on the datasheet provided. Two datasheets cover a month period.

### **Header Information**

Please fill out the header information at the top of each datasheet. For “**Avg. observation time**” estimate the number of hours that the observers have visual access to the rhinos each day.

### **Behavior Scoring Scale**

- Note that all behaviors with male (M) apply only to fully adult males (>5 yr old).
- The scale for frequency of occurrence of behaviors is from 0 to 3, in increments of 0.5.
- If a particular behavior or condition was not observed that day, despite a “normal effort” to observe that animal, record a “0.” If a behavior/condition occurs infrequently, record a “1.” For example, if the appetite is a little decreased, the vulva are a little more swollen, or an occasional hiccups approach is seen, record a “1.” Record a “2” if the behavioral changes are moderately elevated (or decreased in the case of appetite), and a “3” if they are dramatically elevated, that is, close to the maximum you have observed for this behavior/condition.
- If you do not know whether a particular behavior or condition was present because of insufficient observation time (i.e., less than normal), do not guess. Leave it blank for that entry to indicate that you do not know. “0’s” and blanks mean something very different.
- If it was impossible for a particular behavior or condition to occur on that day, record a “X” for that entry. For example, if a female was not housed with a male, she would not be capable of showing any interest in a male, and males would not be capable of showing any interest in her.

- Guidelines for ranking **female interest in male:**
  - 0 = female shows little or no interest in being near or interacting with the male(s)
  - 1 = female is more tolerant of male's proximity, allowing him to approach and interact
  - 2 = female even more tolerant of male's proximity and may approach, follow and make contact with the male more frequently
  - 3 = female very tolerant of male's proximity, allowing substantial body contact such as anogenital investigation and chin-resting, and may approach, follow and make contact with the male even more frequently
  
- Guidelines for **male interest in female:**
  - 0 = male shows little or no interest in being near or interacting with the female
  - 1 = male approaches, follows and/or interacts with female occasionally; male is also tolerant of female's approach, etc.
  - 2 = male approaches, follows, and/or interacts with female more frequently. Male is persistent in seeking contact, such as nose-to-nose greet, gentle horn wrestling, chin-resting and anogenital investigation.
  - 3 = male devotes almost all his time and energy to maintaining proximity and interacting with and courting the female; there should be evidence of sexual arousal, such as an erection and mounting attempts.
  
- Guidelines for **aggression with male/aggression between male and the female's social partner\***:
  - 0 = little or no aggression
  - 1 = infrequent and mild non-contact aggression, such as aggressive vocalization (snarl display in white rhino, roar and bleat in greater one-horned rhino), horn display, lunging, charging, and chasing
  - 2 = non-contact aggression is more frequent and/or some mild contact aggression occurs, such as horn clash and horn-to-body
  - 3 = escalated contact and non-contact aggression (i.e., fighting), which may lead to injuries.

\* Social partner refers to the rhino(s) that is/are commonly associated with the rhino whose datasheet you are filling out; that is, they are almost always found together.



