

# EFFECT OF BOMA CONFINEMENT ON HEMATOLOGIC AND BIOCHEMICAL VALUES IN FREE-RANGING WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*) IN KRUGER NATIONAL PARK, SOUTH AFRICA

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**ABSTRACT:** Boma adaptation is an important component of rhinoceros translocations to allow transition to new diets, restricted space, and quarantine for disease screening. However, up to 20% of recently captured white rhinoceros (*Ceratotherium simum*) do not adjust to captivity, resulting in early release or even death. The causes and physiologic consequences of maladaptation to boma confinement are poorly understood. The aim of this investigation was to evaluate hematologic and serum biochemical changes in maladapted rhinoceros compared to animals that adapted under the same boma conditions. Ninety-six white rhinoceros were captured between 2009 and 2011 in Kruger National Park, South Africa and placed in bomas prior to translocation. Weight, complete blood count, and serum biochemical panel results were recorded when rhinoceros were placed in the boma and repeated on the day of release. In this study, the mean duration of boma confinement for maladapted white rhinoceros was 13 d (range 8–16 d) compared to 89.9 d (range 39–187 d) for adapted animals. Mean weight loss between capture and release was significantly greater in maladapted rhinoceros (224.0 versus 65.9 kgs;  $P < 0.001$ ). Although adapted rhinoceros had statistically significant changes in some hematologic and biochemical values, most were not considered clinically relevant. In contrast, the maladapted rhinoceros had significant changes at the time of early release from the boma, including evidence of leukocytosis with left shift, lymphopenia, eosinopenia, decreased red blood cell count and hematocrit, increased serum creatine kinase, and decreased serum calcium, phosphorus, and magnesium values. Along with loss of body condition, these findings were consistent with a stress-associated catabolic response. These changes occurred in the first 2 wk of confinement, and the results provide a foundation for evaluating adaptation in white rhinoceros. Future studies should focus on factors that improve adaptation and welfare of recently confined free-ranging white rhinoceros.

**Key words:** Boma adaptation, *Ceratotherium simum*, hematology, immobilization, serum biochemistry, white rhinoceros.

## INTRODUCTION

Conservation programs for African rhinoceros depend on capture and translocation to maintain genetic diversity and restore populations (Emslie et al. 2009). With increasing losses due to poaching, it is crucial that animals be moved to safe locations. However, in some cases the novel environment can present new challenges, including availability of preferred vegetation, restricted space, and new social groups. To prepare for these changes and meet requirements such as

quarantine for disease screening and treatment of injured animals, free-ranging rhinoceros are often placed into bomas (holding enclosures) as an adaptation step, which can last for weeks to months (Emslie et al. 2009). However, the confinement of free-ranging animals, exposure to humans, novel food, and water sources can result in stress. Although the stress response is adaptive, persistent stress can lead to physiologic disturbances resulting in weight loss, immunocompromise, susceptibility to disease, and decreased reproductive capacity (Dantzer et

al. 2014; Fischer and Romero 2019). This may further complicate the success of treating rhinoceros with poaching injuries.

Adaptation to captivity may vary between individuals based on species, sex, age, origin, and other characteristics (Fischer and Romero 2019). As many as 10%–20% of free-ranging white rhinoceros (*Ceratotherium simum*) are unable to adapt to boma confinement (Emslie et al. 2009; Miller et al. 2016). In a previous study, a boma scoring system, which included daily evaluation of appetite, fecal consistency and volume, and behavior, was developed to provide a method to determine adaptation (Miller et al. 2016). Rhinoceros were considered maladapted if their overall scores remained below 15 out of 20 or showed a downward trend within the first 2 wk of confinement. Early release of maladapted rhinoceros has been recommended to prevent further morbidity and mortality (Rogers 1993; Kruger et al. 1999). Maladaptation results in loss of translocation candidates, along with the time and associated costs and, in some cases, death of the rhinoceros. Therefore, understanding the causes and identifying changes associated with maladaptation are necessary to prevent morbidity and mortality of recently confined white rhinoceros.

Previous studies have reported no obvious association of intrinsic (age, sex, body weight) or capture-related factors on probability of boma adaptation in white rhinoceros (Miller et al. 2022). Measurements of plasma cortisol and fecal glucocorticoid metabolites have shown that white rhinoceros have variable individual responses to capture and captivity (Kruger 2017). However, the physiologic effects of adaptability have not been investigated in boma-confined white rhinoceros. Therefore, our study aimed to evaluate changes in hematologic and serum biochemical values in boma-adapted and maladapted white rhinoceros in Kruger National Park, South Africa. This information will improve understanding of the consequences of confinement and identify changes that may require intervention.

## MATERIALS AND METHODS

### Animals and boma confinement

Free-ranging white rhinoceros ( $n=96$ ) were immobilized, weighed, and transported to temporary holding facilities (bomas) in Kruger National Park ( $23^{\circ}49'60''S$ ,  $31^{\circ}30'0''E$ ), South Africa during 2009–2011, prior to translocation, and immobilized for release from bomas as described (Miller et al. 2016, 2022). All rhinoceros were apparently healthy on physical examination and not injured at the time of capture. The study population consisted of 53 females and 43 males, with the majority being subadults (3–7 yr;  $n=73$ ), along with 22 adults ( $\geq 7$  yr) and one animal without a recorded age. Animals were caught and cared for according to the South African National Parks (SANParks) Standard Operating Procedures for the Capture, Transportation and Maintenance in Holding Facilities of Wildlife (SANParks 2005; approved by SANParks Animal Use and Care Committee). All rhinoceros received zuclopenthixol acetate (Clopixol-Acuphase, H. Lundbeck Pty. Ltd., North Riding, South Africa); total dose range 50–100 mg intramuscularly (IM) as a long-acting tranquilizer at the time they were off-loaded into the bomas. Rhinoceros were housed in enclosures approximately 25×50 m with ample shade, water, and food offered on cement slabs. Ad libitum feed consisted of a mixture of 50% tef (*Eragrostis tef*) and 50% high-quality lucerne (*Medicago sativa*) hay offered twice daily. Once an animal was eating and defecating normally, it was shifted to a smaller pen (10 m × 20 m). A maximum of two compatible animals were housed together in a boma, but most animals were kept individually. A normal low level of ticks was observed on all rhinoceros at the time of capture. Animals were treated with a topical ectoparasiticide (Flumethrin 0.5% m/v, Drastic Deadline, Bayer (Pty) Ltd. Animal Health Division, Isando, South Africa) at the time of capture as part of the protocol for translocation. Boma adaptation scoring was performed daily based on observations of appetite, fecal consistency/volume, and behavior, as previously reported (Miller et al. 2016). This was performed by an experienced boma manager each day the while rhinoceros were present in the bomas. Rhinoceros with an individual score of less than 15 (out of 20), or a declining score during the first 7–14 d of boma confinement, were considered maladapted. Rhinoceros with boma scores less than 15 were visually examined by a veterinarian, who decided if and when to release this individual. Capture and release weights were recorded using a hanging scale once the rhinoceros was in the crate.

### Sample collection and processing

Blood was collected from the radial vein into ethylenediaminetetraacetic acid (EDTA) and serum Vacutainers (BD Biosciences, Franklin Lakes, New Jersey, USA) during initial immobilization in the field and during immobilization for release from the bomas. Samples were transported on ice packs to the laboratory in Skukuza for processing within 8 h of collection. Manual white blood cell (WBC) and differential counts were performed on blood smears prepared with a commercial eosin-methylene blue stain (Kyro-Quick stain, Kyron Laboratories, Benrose, South Africa). An automated hematology analyzer (Vet ABC, Scil Animal Care Company, Gurnee, Illinois, USA) was used for red blood cell (RBC) counts, hemoglobin concentration, and hematocrit for samples collected in 2011; this was not available in 2009–2010. Serum was separated by centrifugation at  $800 \times G$  for 10 min, then 0.2 mL was added to a Large Animal chemistry rotor (ABAXIS, Inc., Union City, California, USA) placed into an ABAXIS VetScan2<sup>®</sup> chemistry analyzer (ABAXIS) as previously described (Mathebulu et al. 2012).

The length of time between the initial sample and release sample varied due to translocation logistics and reasons for release recorded. Early release (<30 d) occurred when rhinoceros were considered maladapted, based on a low daily boma score (<15; Miller et al. 2016) and inadequate appetite, abnormal defecation or behavior, or significant loss of body condition. Rhinoceros were released in their capture location and usually began eating immediately after release.

### Data analyses

Data were assessed for normality visually using a histogram with a density curve and more formally by using the Shapiro-Wilk test for normality. Hematologic and serum biochemical results (mean, SD, median and 25th and 75th percentiles) were calculated for samples collected at the time of capture and release and compared using paired *t*-tests or a Wilcoxon signed-rank test (according to data distribution). Results were also compared between boma-adapted and maladapted rhinoceros using medians and the Wilcoxon rank sum test. Stata Statistical Software (StataCorp 2009) version 11 was used to perform all data manipulation and statistical analysis. Statistical significance was set at  $P \leq 0.05$ .

## RESULTS

White rhinoceros were confined in the bomas for up to 187 d prior to translocation.

The mean length of stay for boma-adapted rhinoceros ( $n=79$ ) was 89.9 d with a range of 39–187 d, which was significantly longer than for maladapted individuals ( $n=19$ ; 18 early release and one death;  $P<0.001$ ) with a mean length of stay of 13 d and a range of 8–16 d. Postmortem testing of the rhinoceros that died confirmed *Salmonella* gastroenteritis as the cause of death. Median daily individual boma adaptation scores for adapted rhinoceros were significantly greater than those of the maladapted rhinoceros starting at day 8 of confinement (13 versus 10,  $P=0.0012$ ). The adapted rhinoceros median scores continued to increase and plateaued at 16, whereas the scores for maladapted rhinoceros remained at 10 for the duration of confinement period. Although the initial mean ( $\pm$ SD) weights of adapted ( $1,460 \pm 314$  kg) and maladapted ( $1,526 \pm 287$  kg) rhinoceros were not statistically different ( $P=0.42$ ), the mean weight loss between capture and release of maladapted rhinoceros ( $224.0 \pm 61.1$  kg) was statistically greater ( $P<0.001$ ) than weight loss ( $65.9 \pm 77.8$  kg) in adapted animals. In addition, the weight loss occurred over a shorter time (8–16 d) for the maladapted rhinoceros.

Changes in hematologic and serum biochemical values that occurred during boma confinement (39–187 d) in adapted rhinoceros (capture and release data;  $n=63$  with hematologic,  $n=73$  with chemistry results) are shown in Tables 1 and 2, respectively. The mean total WBC counts ( $16.9 \times 10^9/L$  versus  $14.9 \times 10^9/L$ ;  $P=0.007$ ) and proportions of basophils declined (0.01 versus 0.0003;  $P=0.01$ ), along with a nearly 50% reduction in the proportions of eosinophils (0.162 versus 0.089;  $P<0.0001$ ) between capture and release, respectively. An increase in the proportion of neutrophils at release was also statistically significant (0.431 versus 0.512;  $P<0.0001$ ). There were no significant changes in proportions of lymphocyte or monocyte populations. Mean RBC counts ( $0.0075 \pm 0.0015 \times 10^{12}/L$  versus  $0.0080 \pm 0.0009 \times 10^{12}/L$ ), hemoglobin concentrations ( $156 \pm 37g/L$  versus  $152 \pm 17g/L$ ), and hematocrit values ( $0.443 \pm 0.086L/L$  versus  $0.474 \pm 0.053L/L$ ) in a subset of rhinoceros from 2011 were not significantly different

TABLE 1. Hematologic values of boma-adapted white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa, at capture and release.

Variable <sup>a</sup>	Mean	SD	n	P*
WBC ( $\times 10^9/L$ )				0.007
Capture	16.9	6.2	63	
Release	14.9	4.3	63	
Neutrophils**				<0.0001
Capture	0.431	0.102	63	
Release	0.512	0.078	63	
Lymphocytes**				0.32
Capture	0.255	0.087	63	
Release	0.249	0.091	63	
Monocytes**				0.30
Capture	0.130	0.074	63	
Release	0.147	0.059	63	
Eosinophils**				<0.0001
Capture	0.162	0.069	63	
Release	0.089	0.051	63	
Basophils**				0.01
Capture	0.010	0.030	63	
Release	0.0003	0.0017	63	
Bands**				0.21
Capture	0.0040	0.0146	63	
Release	0.0013	0.0061	63	

<sup>a</sup> WBC = white blood cell.

\* P was calculated by comparing means using paired *t*-tests.

\*\* Expressed proportions.

between capture and release, respectively ( $P>0.05$ ). Mean total protein, albumin, blood urea nitrogen (BUN), creatine kinase (CK), and aspartate aminotransferase (AST) were significantly increased at the time of release compared to capture values (Table 2;  $P<0.05$ ). Significantly decreased mean alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and magnesium (Mg) values were present at the time of release ( $P<0.05$ ).

Changes in hematologic and biochemical values that occurred during boma confinement (8–16 d) in maladapted rhinoceros (capture and release data;  $n=15$  with hematologic,  $n=17$  with chemistry results) are shown in Tables 3 and 4, respectively. There were significant increases between capture and release, respectively, in median total WBC count ( $15.4 \times 10^9/L$  versus  $21.1 \times 10^9/L$ ;  $P=0.045$ ), proportions of neutrophils (0.435 versus 0.550;  $P=0.0006$ ), and

TABLE 2. Serum biochemistry values for boma-adapted white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa, at capture and release.

Variable <sup>a</sup>	Mean	SD	n	P*
Albumin (mmol/L)				<0.0001
Capture	0.0376	0.0060	73	
Release	0.0466	0.0090	73	
ALP (U/L)				<0.0001
Capture	73.7	19.1	73	
Release	56.1	17.8	73	
AST (U/L)				0.004
Capture	68.9	17.5	73	
Release	78.2	30.7	73	
Calcium (mmol/L)				0.19
Capture	2.99	0.20	73	
Release	3.04	0.22	73	
GGT (U/L)				0.007
Capture	14.3	4.8	73	
Release	12.8	2.9	73	
Total protein (g/L)				0.001
Capture	98	13	73	
Release	104	10	73	
Globulin (g/L)				0.43
Capture	74	9	73	
Release	73	11	73	
BUN (mmol/L)				<0.001
Capture	3.93	1.07	73	
Release	6.68	1.43	73	
CK (U/L)				<0.001
Capture	200.8	72.2	73	
Release	298.3	174.5	73	
Phosphorus (mmol/L)				0.20
Capture	1.48	0.26	77	
Release	1.55	0.26	73	
Magnesium (mmol/L)				<0.001
Capture	1.27	0.16	77	
Release	1.15	0.16	73	

<sup>a</sup> ALP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; BUN = blood urea nitrogen; CK = creatine kinase.

\* P was calculated by comparing means using paired *t*-tests.

decline in proportions of lymphocytes (0.260 versus 0.230;  $P=0.029$ ). There was also an increase in band neutrophils, although this was not statistically significant (0 to  $0.044 \pm 0.077$ ;  $P=0.12$ ). In 2011 samples, there were decreases in mean RBC counts ( $0.0085 \pm 0.0017 \times 10^{12}/L$  versus  $0.0059 \pm 0.0015 \times 10^{12}/L$ ;  $P=0.15$ ) and hematocrit values ( $0.550 \pm 0.149/L$  versus

TABLE 3. Hematologic values of boma-maladapted white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa, at capture and release.

Variable <sup>a</sup>	Median	p25	p75	n	P*
WBC ( $\times 10^9/L$ )					0.045
Capture	15.4	11	21.2	15	
Release	21.1	15.7	23.6	15	
Neutrophils**					0.0006
Capture	0.435	0.360	0.490	15	
Release	0.550	0.510	0.630	15	
Lymphocytes**					0.029
Capture	0.260	0.230	0.290	15	
Release	0.230	0.060	0.280	15	
Monocytes**					0.69
Capture	0.125	0.120	0.140	15	
Release	0.140	0.060	0.170	15	
Eosinophils**					0.42
Capture	0.145	0.110	0.180	15	
Release	0.050	0	0.0090	15	
Basophils**					0.25
Capture	0	0	0	15	
Release	0	0	0	15	
Bands**					0.12
Capture	0	0	0	15	
Release	0	0	0.130	15	

<sup>a</sup> p25 = 25th percentile; p75 = 75th percentile; WBC = white blood cell.

\* *P* was calculated by comparing medians using Wilcoxon signed-rank tests.

\*\* Expressed proportions.

$0.372 \pm 0.102L/L$ ;  $P=0.07$ ) from capture to release, respectively, although these were not statistically significant ( $n=4$ ). Median biochemical values in maladapted rhinoceros had statistically significant changes between capture and release, respectively, including increases in BUN and CK and decreases in ALP, GGT, and Mg (Table 4;  $P<0.05$ ).

Comparisons of median hematologic and biochemistry values at the time of release from boma-adapted and maladapted rhinoceros are shown in Tables 5 and 6. Maladapted rhinoceros had a significantly higher median WBC count, proportion of neutrophils, and a decreased proportion of eosinophils. In addition, for 2011 samples there was a decreased median RBC count ( $5.9 \times 10^9/L$  versus  $7.9 \times 10^9/L$ ;  $P=0.002$ ) and hematocrit value ( $0.379L/L$  versus  $0.476L/L$ ;  $P=0.014$ ) in mal-

adapted compared to adapted animals. There was also a significantly higher median CK and lower serum albumin, AST, BUN, calcium (Ca), phosphorus (P), and Mg values in the maladapted rhinoceros (all comparisons  $P<0.05$ ).

## DISCUSSION

Confinement of recently captured wild animals is recognized to cause stress and may be complicated by effects of capture, adjustment to unfamiliar dietary items, and potential anorexia associated with long-term tranquilizers (Linklater et al. 2009; Fischer and Romero 2019). In this study, apparently healthy free-ranging white rhinoceros, placed into bomas prior to translocation, showed weight loss and changes in hematologic and serum biochemical values in both adapted and maladapted animals. There was a significant difference in the time between capture and release samples between the adapted and maladapted rhinoceros, with a relatively long period between blood collections in the adapted rhinoceros, which could have influenced these findings. The trends in the adapted rhinoceros were suggestive of adjustment because many of the changes were not clinically relevant or were considered to show a return toward reference values, such as a decrease in WBC count. In contrast, weight loss and changes in blood values in maladapted rhinoceros were significantly greater and were consistent with a decline in condition or catabolic state, despite the shorter duration of confinement.

Hematologic profiles provide insight into the health status of the individual, with trends suggesting either improvement or deterioration. The decrease in mean WBC count in adapted rhinoceros was consistent with a decreased stress leucocytosis, although both values were within ranges previously reported in white rhinoceros (Miller and Buss 2015). There was no evidence of lymphopenia or anemia associated with boma confinement. The decrease in proportion of eosinophils was most probably due to the parasiticide treat-



TABLE 4. Serum biochemistry values in boma-maladapted white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa, at capture and release.

Variable <sup>a</sup>	Median	p25	p75	n	P*
Albumin (mmol/L)					0.09
Capture	0.04	0.04	0.04	17	
Release	0.04	0.04	0.05	17	
ALP (U/L)					0.016
Capture	66	56	83	17	
Release	61	48	66	17	
AST (U/L)					0.83
Capture	53	42	59	17	
Release	51	38	71	17	
Calcium (mmol/L)					0.06
Capture	2.99	2.92	3.12	17	
Release	2.92	2.79	3.04	17	
GGT (U/L)					0.02
Capture	12	10	16	17	
Release	12	9	14	17	
GGT (nkat/L)					0.02
Capture	200	167	267	17	
Release	200	150	233	17	
Total protein (g/L)					0.31
Capture	96	94	101	17	
Release	102	92	106	17	
Globulin (g/L)					0.72
Capture	70	67	73	17	
Release	70	67	74	17	
BUN (mmol/L)					0.003
Capture	3.21	2.5	3.93	17	
Release	4.64	3.93	7.14	17	
CK (U/L)					0.0056
Capture	171	149	200	17	
Release	312	184	726	17	
Phosphorus (mmol/L)					0.10
Capture	1.32	1.23	1.48	17	
Release	1.23	1.00	1.42	17	
Magnesium (mmol/L)					0.0002
Capture	1.23	1.15	1.32	17	
Release	1.07	0.74	1.11	17	

<sup>a</sup> p25 = 25th percentile; p75 = 75th percentile; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; BUN = blood urea nitrogen; CK = creatine kinase.

\* P was calculated by comparing medians using Wilcoxon signed-rank tests.

ment administered at the time of capture and was consistent with the decreased tick load observed at release. The relative changes in basophil and neutrophil populations were physiologically inconsequential, and values remained within previously published ranges (Miller and Buss 2015). The shifts in hematologic values were similar to those observed

in other wild mammals during their adjustment to captivity (St. Auburn et al. 1989; Sánchez-Sarmiento et al. 2015). The minor changes observed at the time of release were not considered to be clinically significant and reflected the level of adjustment achieved during the longer boma confinement period (39–187 d).

TABLE 5. Hematologic values at release for boma-adapted and maladapted white rhinoceros (*Certhium simum*) in Kruger National Park, South Africa.

Variable <sup>a</sup>	Median	p25	p75	n	P*
WBC ( $\times 10^9/L$ )					0.0014
Adapted	14.2	12	18.4	63	
Maladapted	21.1	15.7	23.6	15	
Neutrophils**					0.042
Adapted	0.500	0.470	0.540	63	
Maladapted	0.550	0.510	0.630	15	
Lymphocytes**					0.12
Adapted	0.250	0.190	0.310	63	
Maladapted	0.230	0.060	0.280	15	
Monocytes**					0.41
Adapted	0.140	0.110	0.170	63	
Maladapted	0.140	0.060	0.170	15	
Eosinophils**					0.009
Adapted	0.090	0.040	0.120	63	
Maladapted	0.050	0	0.090	15	
Basophils**					1.0
Adapted	0	0	0	63	
Maladapted	0	0	0	15	
Bands**					0.028
Adapted	0	0	0	63	
Maladapted	0	0	0.130	15	

<sup>a</sup> p25 = 25th percentile; p75 = 75th percentile; WBC = white blood cells.

\* P was calculated by comparing medians using Wilcoxon rank sum test.

\*\* Expressed proportions.

Although there were several statistically significant changes in serum biochemical values of adapted rhinoceros between capture and release, many of these changes were relatively minor and reflected a trend toward return to normal reference values. The mild increases in total protein and albumin could be associated with feed quality and quantity in the bomas, or possibly decreased water intake, which was supported by a mild increase in BUN. The increases in AST and CK, along with BUN, also were consistent with a mild catabolic state, which was manifested as weight loss between capture and release. However, overall, changes were relatively small, with most remaining within previously reported serum biochemical values (Mathebula et al. 2012; Miller and Buss 2015). Therefore, although this group of rhinoceros

was categorized as adapted, there were measurable physiologic changes which indicated that they had not completely achieved the same condition that they were in at the time of capture, i.e., as a group, they were in a mild-moderate negative energy balance with possible mild dehydration. Adaptation appears to take a minimum of 3 wk, based on scores reaching a plateau (Miller et al. 2016); it is recommended that white rhinoceros put into bomas be given more time (8–12 wk) to regain condition prior to being transported.

The hematologic changes in maladapted rhinoceros between capture and release were consistent with a stress response (Kock et al. 1999; Turner et al. 2002). During the relatively short period of captivity, these rhinoceros developed a stress leukocytosis with left shift, lymphopenia, eosinopenia, and anemia. Changes in WBC counts with a shift in the neutrophil:lymphocyte ratio have been associated with immune consequences of stress in several species and may lead to greater susceptibility to disease (Rogers 1993; Fischer and Romero 2019). Cases of enterocolitis caused by *Clostridium* and *Salmonella* spp. have been reported in white rhinoceros put into stressful conditions, and can lead to death, which occurred in one rhinoceros in this study (Kruger et al. 1999; M.A.M. pers. comm.). There was also a trend toward increased proportion of band neutrophils, suggesting the presence of a systemic inflammatory response (Mare et al. 2015). Anemia may be a result of bone marrow suppression due to stress or, more likely, blood loss associated with gastrointestinal ulceration. Stress is a recognized contributor to gastrointestinal tract changes in motility, shifts in microbiome, and ulceration (Caso et al. 2008). Evidence of gastritis as well as punctate ulcers has been observed by endoscopic examination of recently captured white rhinoceros (M.A.M., pers. comm.). Similar changes in hemograms have been observed in translocated black rhinoceros (Kock et al. 1999). Overall, the hematologic changes were consistent with a negative nitrogen balance or catabolic state. This hypothesized catabolic condition associated with inappetence and

TABLE 6. Serum biochemistry values at release in boma-adapted and maladapted white rhinoceros (*Certotherium simum*) in Kruger National Park, South Africa.

Variable <sup>a</sup>	Median	p25	p75	n	P*
Albumin (mmol/L)					0.028
Adapted	0.050	0.045	0.053	73	
Maladapted	0.042	0.038	0.050	17	
ALP (U/L)					0.57
Adapted	54	43	65	73	
Maladapted	61	48	66	17	
AST (U/L)					0.003
Adapted	74	63	85	73	
Maladapted	51	38	71	17	
Calcium (mmol/L)					0.01
Adapted	3.07	2.94	3.17	73	
Maladapted	2.92	2.79	3.04	17	
GGT (U/L)					0.23
Adapted	13	11	15	73	
Maladapted	12	9	14	17	
Total protein (g/L)					0.11
Adapted	104	101	110	73	
Maladapted	102	92	106	17	
Globulin (g/L)					0.78
Adapted	71	66	79	73	
Maladapted	70	67	74	17	
BUN (mmol/L)					0.014
Adapted	6.78	6.43	7.50	73	
Maladapted	4.64	3.93	7.14	17	
CK (U/L)					0.34
Adapted	246	190	344	73	
Maladapted	312	184	726	17	
Phosphorus (mmol/L)					0.0001
Adapted	1.55	1.42	1.71	73	
Maladapted	1.22	1.00	1.42	17	
Magnesium (mmol/L)					0.0042
Adapted	1.15	1.03	1.23	73	
Maladapted	1.07	0.74	1.11	17	

<sup>a</sup> p25 = 25th percentile; p75 = 75th percentile; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; BUN = blood urea nitrogen; CK = creatine kinase.

\* P was calculated by comparing medians using Wilcoxon rank sum test.

stress response is supported by the significant mean weight loss between capture and release ( $224 \pm 61.1$  kgs). This is an especially important finding since the duration of confinement was short (8–16 d).

The serum biochemical values that significantly changed between capture and release of maladapted rhinoceros included increased BUN and decreased Mg values. Although there was a large increase in mean CK, it was not a statistically significant change, probably

due to the small sample size. The decrease in serum Mg concentration was probably due to decreased food intake and, along with increased BUN and significant weight loss, suggests that the maladapted rhinoceros were in a severe catabolic state, which could lead to death if early release did not occur (Kruger et al. 1999; Miller et al. 2016). Although long-term monitoring after release was not performed, immediate observations after release revealed that the rhinoceros started grazing



almost immediately, suggesting that they might be able to recover.

The significance of the changes during boma confinement was even more apparent when differences in blood results between adapted and maladapted rhinoceros were compared at the time of release. Maladapted rhinoceros had stress hemograms, with significantly decreased mean hematocrit, Ca, P, and Mg levels, and clinical evidence of catabolism supported by the elevated CK levels and rapid weight loss. The mean length of boma confinement of maladapted rhinoceros was 13 d, which agrees with previous reports in which the first 10–14 d are considered the critical time in which white rhinoceros need to be monitored (Rogers 1993; Kruger et al. 1999; Miller et al. 2016). Significant weight loss as well as hematologic and biochemical abnormal values should prompt immediate evaluation of the rhinoceros for intervention, which could include release, supportive treatment with fluid therapy, appetite stimulants, gastroprotection, anti-inflammatory and antimicrobial drugs, or further tranquilization.

There were several limitations in this study that may have influenced the reported results. Blood samples used to assess changes that occurred during boma confinement were only taken at the time of release. Therefore, there was a significantly shorter time period between capture and release blood samples for maladapted compared to adapted rhinoceros (mean 13 d versus 89.9 d, respectively). It is unknown whether the adapted rhinoceros had similar early changes in weight and hematologic and biochemical results, but with the longer duration of confinement, these changes began to return to more-normal values over time. Another limitation is the small sample size of maladapted rhinoceros, which affected power and the ability to declare differences statistically significant. Due to logistic limitations, observations of details in management that might have affected maladaptation were unavailable. More-detailed observations of activity, feeding, and social behavior, evaluation of diet fed, and responses associated with presence of people would be useful for comparing and assessing differences between

maladapted and adapted rhinoceros. Interventions such as providing fresh-cut grass, separating incompatible animals, and administration of tranquilizers could be considered in individual rhinoceros showing signs of maladaptation. Because demographic and capture-related factors have been investigated in this cohort of rhinoceros and were not associated with maladaptation (Miller et al. 2022), further studies are needed to explore management and other variables that could predispose white rhinoceros to failed adjustment to boma confinement.

The results of this study suggest that there may be individual differences in the response of white rhinoceros to boma confinement that could be quantified. The tools used (boma scoring system, serial hematologic and serum biochemical analyses, weights) should be applied to other wildlife species that undergo translocation or confinement for treatment. These could be easily adapted to black rhinoceros boma management as well as individual or small groups of ungulates or carnivores that are captured. Identifying species-specific changes in hematologic and biochemical values during confinement would be a useful foundation to improve wildlife capture and to inform whether interventions are required as well as the effect of treatments. This system could also be applied to human-managed wildlife that are undergoing transport, treatment, or other changes in management.

In summary, findings in this study provide information on the effects of boma confinement on selected physiologic variables in recently captured white rhinoceros and identify changes that can be used to understand potential effects on health. As previously reported, the first 1–2 wk were crucial for evaluating individual rhinoceros' adjustment and determining when intervention was required prior to the development of serious consequences (Kruger et al. 1999; Miller et al. 2016). Ideally, serial hematologic and serum biochemical analyses would provide insight into the current health status of each rhinoceros; however, repeated immobilizations may exacerbate the stress associated with boma

confinement. Future studies should investigate whether this could be useful information for interventions and decision-making. Although adapted rhinoceros exhibited some negative energy balance during confinement, there was a significant and probably stress- and inappetence-associated catabolic change in maladapted rhinoceros. The boma scoring system previously described (Miller et al. 2016), along with changes in hematologic and serum biochemical values, provide additional evidence for decisions regarding intervention when managing rhinoceros in bomas. Future studies should continue to investigate predisposing factors and effective preventive and therapeutic techniques to improve adaptation and welfare of recently captured white rhinoceros. This is especially important as increasing numbers of compromised rhinoceros are being housed for treatment of poaching injuries.

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#### LITERATURE CITED

- Caso JR, Leza JC, Menchén L. 2008. The effects of physical and psychological stress on the gastrointestinal tract: Lesson from animal models. *Curr Molec Med* 8:299–314.
- Dantzer B, Fletcher QE, Boonstra R, Sheriff MJ. 2014. Measures of physiological stress: A transparent or opaque window into the status, management and conservation of species? *Conserv Physiol* 2:cou023.
- Emslie RH, Amin R, Kock R. 2009. Guidelines for the in situ re-introduction and translocation of African and Asian rhinoceros. *International Union for Conservation of Nature*, Gland, Switzerland, 115 pp.
- Fischer CP, Romero LM. 2019. Chronic captivity stress in wild animals is highly species-specific. *Conserv Physiol* 7:coz093.
- Kock RA, Mihok SRO, Wambua J, Mwanzia J, and Saigawa K. 1999. Effects of translocation on hematologic parameters of free-ranging black rhinoceros (*Diceros bicornis michaeli*) in Kenya. *J Zoo Wildl Med* 30:389–396.
- Kruger M. 2017. *Capture and boma stress responses in white rhinoceros (Ceratotherium simum)*. PhD Thesis, Faculty of Science, University of the Witwatersrand, Johannesburg, South Africa, 93 pp.
- Kruger M, Grobler DG, Malan JH. 1999. Boma management and translocation of white rhino in the Kruger National Park. In: *Proceedings of the first rhino keepers' workshop*, Orlando, Florida, 7–8 May; International Rhino Foundation, Fort Worth, Texas, pp. 16–35.
- Linklater WL, MacDonald EA, Flaman JRB, Czekala NM. 2009. Declining and low fecal corticoids are associated with distress, not acclimation to stress, during the translocation of African rhinoceros. *Anim Conserv* 13:104–111.
- Mare TA, Treacher DF, Shankar-Hari M, Beale R, Lewis SM, Chambers DJ, Brown KA. 2015. The diagnostic and prognostic significance of monitoring blood levels of immature neutrophils in patients with systemic inflammation. *Critical Care* 19:57.
- Mathebula N, Miller M, Buss P, Joubert J, Martin L, Kruger M, Hofmeyr M, Olea-Popelka F. 2012. Biochemical values in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *J Zoo Wildl Med* 43:530–538.
- Miller M, Buss P. 2015. Rhinocerotidae (Rhinoceroses). In: *Zoo and wild animal medicine, Current Therapy*, 8th Ed., Miller RE, Fowler ME, editors. Elsevier, St. Louis, Missouri, pp. 538–547.
- Miller M, Kruger M, Kruger M, Olea-Popelka F, Buss P. 2016. A scoring system to improve decision-making and outcomes in the adaptation of recently captured white rhinoceros (*Ceratotherium simum*) to captivity. *J Wildl Dis* 52 (2 Suppl):S78–S85.
- Miller M, Olea-Popelka F, Buss P. 2022. Effect of capture and immobilization on boma adaptation in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *J Wildl Dis* 58: In press.
- Rogers PS. 1993. Care of the white rhinoceros *Ceratotherium simum* in captivity. In: *The capture and care manual: Capture, care, accommodation and transportation of wild African animals*, McKenzie AA, editor. Wildlife Decisions Support Services CC, Lynnwood Ridge, South Africa, pp. 546–553.
- Sánchez-Sarmiento AM, Zwarg T, Fernandes-Santos RC, Guimarães-Luiz T, Genoy-Puerto A, Matushima ER. 2015. Hematological parameters and the variations resulting from stress of *Alouatta caraya* during a

- wildlife rescue program in Brazil. *Am J Primatol* 77: 246–253.
- SANParks (South African National Parks). 2005. *Standard operating procedures for the capture, transportation and maintenance in holding facilities of wildlife*. SANParks, Pretoria, South Africa, 64 pp.
- St. Aubin DJ, Geraci JR. 1989. Adaptive changes in hematologic and plasma chemical constituents in captive beluga whales, *Delphinapterus leucas*. *Can J Fish Aquat Sci* 46:796–803.
- StataCorp LLC. 2009. *Stata statistical software* version 11. StataCorp LLC, College Station, Texas.
- Turner JW Jr, Tolson P, Hamad N. 2002. Remote assessment of stress in white rhinoceros (*Ceratotherium simum*) and black rhinoceros (*Diceros bicornis*) by measurement of adrenal steroids in feces. *J Zoo Wildl Med* 33:214–221.

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