Rehabilitation method affects behavior, welfare, and adaptation potential for subsequent release of orphaned white rhinoceros

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ABSTRACT

Poaching is the primary threat to the survival of rhinoceros' populations. One frequent consequence of poaching is the creation of orphan calves. If found, orphans are taken into captivity for rehabilitation and subsequent release. However, rehabilitation practices can influence their behavior and welfare, potentially compromising their post-release adaptation and survival. In this study, the effects of hands-off and hands-on rehabilitation methods on the behavior, welfare and adaptation potential of orphaned white rhinoceros (*Certatotherium simum simum*) were compared. To achieve these aims, 12 behavioral, one physiological, and four physical indicators of welfare and adaptation potential were measured non-invasively on 25 orphaned rhinos at two rehabilitation facilities in South Africa. Results indicated that although orphan welfare was not compromised under either rehabilitation method, the hands-off cohort showed fewer indicators of poor welfare and more indicators of good welfare. Regarding adaptation potential, hands-off rehabilitated rhinos showed the species' natural response to humans, and alert and defense behaviors were part of their behavioral repertoire. The hands-on cohort displayed fewer social interactions than the hands-off cohort, showed habituation to humans, and seldom expressed alert or defense behaviors, which could potentially compromise their survival and social integration after release. Post-release studies are required to confirm whether fitness is compromised in hands-on rehabilitated rhinos. Until then, we suggest to minimize anthropogenic exposure during rehabilitation in order to maximize welfare, and retain crucial behaviors for post-release adaptation and survival.

Keywords: Wildlife rehabilitation, conservation, captivity, habituation, behavioral competence, orphan rhino

INTRODUCTION

The recent surge in poaching of African rhino species has substantially increased the number of orphan calves arriving at rehabilitation centers. In South Africa alone, arrivals increased from 5 to 56 orphan rhino in the 2011-2016 period (unpublished data). Rhino orphans are kept under human care until they are old enough, typically over two years of age, to be returned to the wild. Under natural conditions animals are constantly stimulated by changes in their physical and social environment. However, when brought into captivity some stimuli are reduced, while

others are increased (Broom & Johnson, 2000). These abnormal changes in stimulation due to captivity can lead to apathy, stereotypies, and loss of the capacity to adapt to a new environment, (Mason & Latham 2004; Melfi 2009), ultimately compromising their welfare.

There is currently no consensus definition of welfare (Dawkins 1980; Swaisgood 2007). However, for the purpose of this study, we have made use of the definition of Broom (1988), who defines the welfare of an individual as its state related to its attempts to cope with the environment, understanding by coping the ability to successfully deal with the current circumstances. According to this definition, welfare varies on a continuum from very good when an animal is coping, to very poor when it has difficulty coping, or is failing to do so (Broom & Johnson 2000). Promoting good welfare in captive wildlife is a prerequisite to ex-situ conservation (Swaisgood 2007). Ethical reasons aside, poor welfare can negatively impact on core physiological functions, such as reproduction, immunity and growth (Moberg 1991), potentially compromising the ultimate aim of rehabilitation, which is to return animals to the wild as breeding individuals that function normally within a social system (Guy et al. 2013).

Aside from its effect on welfare, captivity can modify the behavior of an animal in ways that, although beneficial during the captive period, could be detrimental in the wild (Kleiman 1996). Many reintroduction programs where animals were sourced from captivity have failed due to a lack of behaviors essential for post-release survival. Examples are predator and human avoidance (Zidon et al. 2009; de Faria et al. 2018), or hunting and foraging skills (Beck et al. 1994; Vickery & Mason 2003), among others. This has prompted the International Union for the Conservation of Nature (IUCN) to recommend that candidates for release must exhibit these behaviors (IUCN/SSC 2013). Tameness, the lack of a flight tendency from humans (Hediger 1964), is a classic example of how behaviors developed during captivity can compromise post-release survival. White rhinoceros (*Ceratotherium simum simum*) under wild conditions show fear towards humans, which is manifested by alert behaviors and a flight response (Owen-Smith 1973). However, they can become human-imprinted if hand-raised (Trendler 2005). Released animals lacking an adequate behavior towards humans may become easier targets to poachers (e.g. Black rhino, *Diceros bicornis*: Matipano 2004; gorillas, *Gorilla g. graueri*: Kasereka et al. 2006; Barbary macaque, *Macaca sylvanus*: Ménard et al. 2014; birds: Samia et al. 2015). Additionally, habituated wildlife can become bolder and, or, more aggressive towards people

(e.g. Ikuta & Blumstein 2003; Webb & Blumstein 2005; Knight, 2009), sometimes leading to culling of the culprit animal if people are injured (white rhinos: Verdoorn, 1995; Grizzly bears, *Ursus arctos*: Mattson et al. 1992).

Despite the potential benefits of rehabilitation and release programs in counteracting the severe effects of poaching on rhino numbers, there is a paucity of information on their effectiveness. Possibly because standardization across facilities is often difficult (e.g. differences in age, time spent in captivity, enclosure size, etc.), publications on this topic consist of technical reports or case studies where the methodologies are described rather than being evaluated (Great horned rhino, *Rhinoceros unicornis*: Choudhury & Mainkar 2005; Barman 2014; white and black rhino, *Diceros bicornis*: Rogers 1993a). This lack of evidence is not limited to rhino species (e.g. Eagle owl, *Bubo bubo*: Zuberogoitia et al. 2003; green sturgeon, *Acipenser medirostris*: Thomas et al. 2013), thus calling the value of wildlife rehabilitation into question (Mullineaux 2014). Additionally, wildlife rehabilitation is generally costly (Clark et al. 2002; Guy et al. 2013); with an estimated cost of US$ 4000 – 10 000 calf/year, depending on extent of medical treatment and age of the calf (unpublished data). Yet, to date there is no scientific evidence that rhino rehabilitation is effective in terms of post-release performance.

Wildlife rehabilitation will only make a significant contribution to conservation if rehabilitated animals survive and establish self-sustaining populations when returned to the wild (Mullineaux 2014). Captive animals that are unable to show the behavioral repertoire of their species after release (e.g. alertness, social behavior, territoriality) may not reproduce, or not adapt and subsequently die, compromising their welfare (Seddon et al. 2007) and the purpose of rehabilitation (Molony et al. 2006). Despite the methodological challenges associated with conducting research across rehabilitation facilities, the present study compared the behavior, welfare and adaptation potential of orphaned white rhino rehabilitated under a hands-off and a hands-on method.

METHODS

Subjects and study sites

The study took place between September 2016 and March 2017 (mainly summer months) at two rehabilitation facilities in Mpumalanga Province, South Africa. A total of 25 orphaned southern white rhinoceros, 12 males and 13 females ranging from 14 months to approximately four years of age (Table A1) were included in the study.

Rhinos were housed in *bomas* (captive wildlife enclosure), that were built on natural substrate and according to an industry-standard design (e.g. Rogers 1993b). Rhinos at Facility 1 were housed in four large bomas (50 x 25 m). Alfalfa (*Medicago sativa*) and teff hay (*Eragrostis teff*) were provided every day *ad libitum* in equal amounts on concrete slabs. Water was available in concrete troughs *ad libitum*. Rhinos at Facility 2 were housed in three smaller bomas (15 x 15 m) but had daily access to a 0.8 ha camp from 7:00 to 15:00 every day, where natural grazing was available and hay was provided. Alfalfa and teff hay (~1:3), and game pellets (Grazer game cubes 12%, Epol) were provided at the bomas. The quantity varied according to rhino age and whether or not they were still being bottle-fed (foal milk replacer, Denkavit).

Research design

Facility 1 used a *hands-off* method, where human contact was kept to a minimum throughout the entire rehabilitation process. Orphans were not bottle-fed (they were weaned upon arrival), and were housed together with a surrogate mother (wild-caught young adult female 5-7 years of age) in groups of 3-6 animals. Staff only entered the bomas for husbandry duties (i.e. cleaning and feeding) and only when the rhinos were moved to other boma compartments. Physical contact between rhinos and staff was not permitted.

At Facility 2 orphans were rehabilitated using a *hands-on* method during every stage of rehabilitation. Orphans were housed in groups of 2-7 individuals without a surrogate mother, and bottle-fed with milk formula and subsequently an electrolyte-enriched mixture until 18-24 months of age. Bottle-feeding was mainly carried out by temporary volunteers 2-5 times per day involving physical and auditory contact. Physical contact with staff and volunteers therefore occurred daily.

Each facility used a different rehabilitation method, making standardization of the research design difficult. Aside from the rehabilitation method, diet, average time in the bomas before the study commenced, and enclosure size were the main differences between facilities.

Assessment of animal welfare and adaptation potential

We used a comprehensive list of behavioral, physical and physiological indicators that had been previously used in the literature to assess captive wildlife welfare, that could be collected non-invasively (Tables A2, A3, A4). Because it is now widely accepted that good welfare is not simply the absence of negative experiences, but rather the presence of positive ones (for a review see Boissy et al. 2007), we used both indicators of poor (e.g. fear, disease, social isolation) and good welfare (e.g. pleasure or comfort behaviors). The behavioral indicators of poor welfare used were: frequent expression of discomfort behaviors (Broom & Johnson 2000), constant alertness (Mench & Mason 1997), lethargy (Broom & Johnson 2000, Swaisgood 2007), frequent aggression (Broom & Johnson 2000, Swaisgood 2007), presence of stereotypies (Broom & Johnson 2000, Mason & Latham 2004, Swaisgood 2007), proximity (as an indirect measure of social isolation, Price & Stoinski, 2007; Salas et al. 2018) and activity budgets (detection of major deviations from those of wild counterparts, Carlstead & Shepherdson 2000, Hosey et al. 2009). As physical indicators of poor welfare, we used variables indicative of a health problem (Broom & Johnson 2000, Carlstead & Shepherdson, 2000; Dawkins 2006): abnormal stools, fecal parasites (macroscopically visible), injuries (Table A4) and body condition. Finally, we used elevated concentrations of fecal glucocorticoid metabolites (fGCM) as a physiological indicator of poor welfare (e.g. Brown et al. 2001). As indicators of good welfare, we used the display of affiliative behaviors (Boissy et al. 2007), and behaviors that are related to pleasure or comfort (Broom & Johnson 2000, Boissy et al. 2007, Dawkins 2006).

To assess adaptation potential, we measured indicators that may be important for orphan post-release adaptation and survival. In particular, we measured the expression of social behaviors (affiliative, aggressive, submissive and space claim-related behaviors, Table A2), as being socially skilled is likely necessary to integrate into free-ranging rhino populations (Whitehead 2010; Brakes et al. 2019). Additionally, because poaching is one of the main threats to white rhino populations (Emslie 2012), we measured alert-related behaviors (Table A2) and the

response of orphans to humans (Table A4). Therefore, affiliative, aggressive and alert-related behaviors were used for the assessment of both welfare and adaptation potential.

Data collection

Data collection was alternated between facilities to avoid seasonal effects between cohorts. A total of 528 h of data were collected between the two facilities (approximately 21 h/rhino). To avoid biases due to time of the day, temperature, proximity to feeding time or other unknown factors, rhinos were observed in random order, using a Latin square design to ensure the equal distribution for number of observations and sessions. Behavioral data collection took place before 7:00 and after 16:00 to avoid human interference from activities such as cleaning and feeding. These are also the periods when wild rhinos are more active during daylight hours in summer months (Owen-Smith 1973). Focal sampling and continuous recording were used to record behavioral events (affiliative behaviors, discomfort, alertness, submission, space maintenance, pleasure-related behaviors, aggression, and stereotypies, Table A2). Group scans and instantaneous recording were used to determine proximity to others (< 2 m), behavioral states (subsequently used to establish activity budgets), and mud wallowing (Table A3). Unlike all the other pleasure-related behaviors in the study, mud-wallowing is a behavioral state, and not an event. It was thus recorded and analyzed separately. During each morning and afternoon session (120-130 min), each animal was individually observed for 10 min (focal continuous for behavioral events), with group scans every 5 min (for behavioral states and proximity). Response to humans (Table A4) was scored once per day, when the researcher (MF) arrived at the bomas. To avoid observer effect (Martin & Bateson 1993), all other behavioral data collection started 10 min later; 2 min longer than the average time taken for rhinos to resume their behavior after the appearance of a person in a previous pilot study (unpublished data).

Physical indicators and lethargy were recorded once per day, after each morning observation session (Table A4). Body condition score was assessed monthly from September to December 2016 using a scoring system of 1-5 (Keep 1971), where 1 indicates very poor and 5 excellent condition. All behavioral and physical data were collected by MF by direct observation, using paper spreadsheets and a stopwatch.

Fecal sample collection, steroid extraction and glucocorticoid metabolite concentration analysis

A total of 373 fecal samples were collected (median: 2.5 samples/week/animal; range 1-6). Feces were collected after the morning sessions and within 2 h of defecation. Once an animal had defecated, the position of the dropping and the time of defecation were recorded for subsequent collection. If another animal defecated on top of the identified dropping, or if more than 2 h had lapsed between defecation and the time of collection, the sample was not collected.

Approximately 50 g of homogenized fecal material was collected and immediately placed on ice and frozen at -20 °C within one hour. Frozen samples were lyophilized, pulverized, and sieved through a metal wire-mesh strainer to remove undigested material (Fieß et al. 1999). Between 0.1 - 0.11 g of fecal powder was then extracted with 80% ethanol in water (3 ml) according to the procedure described by Ganswindt et al. (2002). Resulting extracts were used to measure fGCM concentrations using an already established enzyme-immunoassay for white rhino (Badenhorst et al. 2016). Detailed assay characteristics, including full descriptions of the assay components and cross-reactivities are provided by Touma et al. (2003). Sensitivity of the assay at 90% binding was 2.4 ng/g fecal dry weight (DW). Intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low value quality controls were 6.6% and 6.7%, and 7.9% and 8.9%, respectively. All steroid extractions and hormone analyses were performed at the Endocrine Research Laboratory, University of Pretoria (South Africa).

Data analysis

Generalized linear mixed models (GLMM) were used to evaluate differences between rehabilitation methods for all variables with the exception of body condition score, where a Mann-Whitney U test was used to compare mean body scores for each rhino.

Rehabilitation approach, sex, and age (categorized in years) were included as fixed effects, and rhino and boma as random effects in all models. Boma was subsequently removed from models when the effect was not significant. “Session” was used as the unit of analysis for behavioral events (i.e. continuous variables). Since multiple observations in a session was a rare event, continuous variables were transformed into 0 (no occurrence) and 1 (any occurrence) and a binomial likelihood function was assumed. For physical indicators, lethargy, and response to humans, “day” was the unit of analysis (0 for absence, 1 for presence). A binomial distribution was also assumed in these models. Regarding response to humans, although this variable was

initially recorded using five categories, it was subsequently analyzed as two: no response (former category 1), and response (former categories 2 and 3). Categories 4 and 5 were never observed during the course of the study (Table A4).

For mud wallowing and proximity, "session" was used as the unit of analysis. For each session, the number of scans where the animal was observed wallowing (or in proximity of another rhino) was divided by the total number of scans in the session. A Poisson distribution and a log link function were used in the model. Finally, to compare fGCM concentrations, we included a first-order autoregressive correlation structure in the linear model to account for the repeated measures design.

To determine whether activity budgets were different to those of free-ranging rhino, we contrasted the activity budgets of the hands-off and hands-on cohorts to those reported by Owen-Smith (1973) for rhinos of similar age in iMfolozi Game Reserve (South Africa), during the same time of the day and season. Because the cited study only reported total percentages, formal statistical comparisons were not possible and results were presented descriptively.

All statistical tests were performed using SPSS 25 software (IBM Corp 2017), and statistical significance set at 0.05.

RESULTS

Animal welfare indicators

Boma was not statistically significant in any model and was subsequently removed. There were significant differences between facilities in seven welfare indicators. Hands-off rehabilitated rhinos were more alert ($F = 56.935$, $p < 0.001$), displayed more affiliative ($F = 6.698$, $p = 0.010$) and pleasant-related behaviors ($F = 13.239$, $p < 0.001$), including wallowing ($F = 5.338$, $p = 0.021$) compared to the hands-on cohort (Table 1, Fig 1, Fig 2). Aggression, both the display of aggressive behaviors and the presence of injuries (mainly in the form of minor facial abrasions) were also more frequently observed in the hands-off cohort (aggressive behaviors: $F = 35.369$, $p < 0.001$; injuries: $F = 14.704$, $p = 0.004$) (Table 1). Abnormal stools (i.e. diarrhea) were more prevalent in the hands-on cohort ($F = 22.470$, $p < 0.001$) (Table 1).

There were no significant differences between cohorts for seven welfare indicators. Mean body condition score was nearly identical (3.87 and 3.86 in hands-off and hands-on cohorts, respectively; $U = 57.000$, $p = 0.270$), proximity to other rhino scored high (mean hands-off and hands-on 79% and 86% respectively; $F = 0.048$, $p = 0.827$), lethargy ($F = 0.041$, $p = 0.840$) and intestinal worms ($F = 0.263$, $p = 0.608$) were seldom recorded within either cohort, and the display of discomfort behaviors occurred in only 5.49% (hands-off) and 10.35% (hands-on) of the sessions ($F = 2.378$, $p = 0.123$) (Fig. 1). Stereotypies were never observed during the course of the study. Regarding fGCM, overall individual concentrations varied descriptively (hands-off; median: 0.57 µg/g DW, range: 0.31-1.26; hands-on; median: 0.55 µg/g DW, range: 0.05-1.03) (Table A5), but no significant differences were identified between cohorts ($F = 3.260$, $p = 0.075$).

Activity budgets could not be assessed statistically. Descriptively, both cohorts showed different activity budgets to those of free-ranging rhinos (Fig. 2). Hands-off rehabilitated rhinos spent almost 50% less time feeding than their wild counterparts, and showed a more diverse activity budget than hands-on and free-ranging rhinos. However, standing occupied 22% of their total activity budget. The hands-on cohort spent similar time feeding compared to free-ranging rhinos, but only 7% of their total time was dedicated to activities other than feeding and resting.

Adaptation potential indicators

Rhinos at the hands-off facility were more social than those at the hands-on facility, as indicated by higher frequencies in the display of affiliative, space maintenance ($F = 17.266$, $p < 0.001$) and aggressive behaviors. Submissive behaviors were not different from the hands-on cohort ($F = 2.449$, $p = 0.118$) (Fig. 1, Table 1). Additionally, the hands-off cohort reacted to the presence of humans ($F = 114.762$, $p < 0.001$), and expressed alert behaviors more often than the hands-on cohort (Fig. 1). Hands-on rehabilitated rhinos seldom reacted to human presence (0.19% of the times) (Table 1), and alert behaviors were rarely observed (0.82%).

Effect of sex and age

In general, sex and age had no effect on the indicators used in this study. Exceptions were the expression of alert behaviors ($F = 5.684$, $p = 0.017$) and response to humans ($F = 0.252$, $p =$

0.002) that were displayed less often in females than in males. Age had an effect in the prevalence of abnormal stools ($F = 6.011$, $p = 0.001$), where 2 and 3-year old had lower incidence of diarrhea than 1-year old rhinos ($t = -3.226$, $p = 0.01$ and $t = -3.453$, $p = 0.01$, respectively). Finally, females had lower fGCM than males ($F = 9.989$, $p = 0.002$), with age also having an influence on this indicator ($F = 3.485$, $p = 0.019$), where 1 and 2-year-old had lower fGCM than 4-year old rhinos (1-year-old: $t = -2.610$, $p = 0.011$; 2-year-old: $t = -2.459$, $p = 0.016$).

DISCUSSION

Each facility where data collection took place used a different rehabilitation method, making standardization of the research design difficult. Aside from rehabilitation method, diet, average time spent in the bomas, and enclosure size differed between facilities. Although lack of standardization is common in zoo and rehabilitation research (particularly when different institutions are compared), the outcome of this study should be evaluated in consideration of the potential effects that these factors might have had on presented results.

Orphan welfare during rehabilitation

It is generally recognized that no single indicator of welfare is adequate on its own and can give conflicting results if considered independently (e.g. Dawkins 1980; Mason & Mendl 1993). This has prompted the use of multiple indicators when assessing animal welfare (Broom & Johnson 2000; Wielebnowski 2003; Hill & Broom 2009). However, there is still disagreement on how different indicators should be combined (Moberg & Mench 2000; Swaisgood 2007) and which ones should be given priority (Dawkins 1998). In the current study, isolation was not observed in either cohort (as indicated by the high proximity levels), lethargy was only occasional across study samples, and stereotypies, one of the most important indicators of long-term welfare problems (e.g. Broom & Johnson 2000; Swaisgood 2007), were never recorded; although stereotypies *per se* are not an unequivocal welfare indicator, they correlate well with other indicators of welfare (Mason & Latham 2004). Additionally, fecal parasites were rare, none of the rhinos in the study required veterinary treatment, and the body condition score (Keep 1971) was good at both cohorts. The low frequency or complete absence in the expression of these

indicators supports that welfare was not compromised with either rehabilitation method (Dawkins 1998; Swaisgood 2007).

However, good welfare is not simply the absence of negative experiences such as social isolation or disease, but also the presence of positive ones, like pleasure, comfort or contentment (Boissy et al. 2007). Mud wallowing, an important behavior for thermoregulation and control of ectoparasites (Owen-Smith 2013), was more frequently observed in hands-off rehabilitated rhinos. This was also true for the display of affiliative behaviors (Boissy et al. 2007), and pleasure related behaviors, including body scratching (Roosvelt 1910; Owen-Smith 1973) and horn rubbing. Although excessive horn-rubbing is a common abnormality in captive rhinos (Fouraker et al. 1996), the normal appearance of the horns in the studied animals suggests a beneficial rather than aberrant behavior.

Hands-off rehabilitated rhinos were significantly more social than the hands-on group, as indicated by higher frequencies in the display of three of the four social behaviors analyzed, including aggression. Whereas affiliative behaviors are regarded as unequivocal indicators of good welfare (Boissy et al. 2007), increases in agonistic behavior have been associated with stress in captive rhinos (Meister 1997). However, aggression is an adaptive behavior that forms part of the behavioral repertoire of virtually all mammalian species (Veenema 2009). In young animals, play-fighting is expressed frequently and it is essential for the appropriate development and use of adult aggression (Pellis & Pellis 1987). As for many other species (Pellis & Pellis 1987), there is no individual criterion that distinguishes play-fighting from adult-fighting for rhino. Similar fGCM to those of free-ranging rhino (Badenhorst et al. 2016), lack of serious injuries, and infrequent discomfort behaviors in the hands-off cohort supports that the observed aggression did not negatively affect their welfare, and could also be considered an indicator of behavioral competence.

In captivity, incongruent activity budgets can be an indicator of poor welfare (Carlstead & Shepherdson 2000, Hosey et al. 2009). However, deviations from wild-type behaviors may also be appropriate responses in a captive environment (Mathews et al. 2005). Activity budgets at both facilities were different to those of free-ranging rhinos. The hands-off cohort spent 50% less time feeding compared to their wild counterparts (Owen-Smith 1973), and the hands-on cohort

spent over 90% of their time feeding or resting, which could point towards a decreased complexity of behavior, often associated with poor welfare (Carlstead & Shepherdson 2000). However, the decrease in time spent feeding by the hands-off cohort could be explained by the unlimited nutrient-rich food supply (Mathews et al. 2005). The lack of stereotypies and apathy in both cohorts, good body condition scores, and fGCM concentrations within the species range support that the observed deviations from wild rhino activity budgets had no welfare implications.

Health problems are classical signs of compromised welfare (e.g. Broom & Johnson 2000; Melfi 2009). Evaluated rhinos did not develop overt disease during the study, but rhinos rehabilitated under a hands-on approach often presented with diarrhea. Diarrhea is fairly common in orphan rhinos and may be caused by overfeeding milk (Rogers 1993b), deciduous tooth eruption (Wallach 1969), abnormal microflora or protozoan and bacterial infections (*Giardia lamblia* and *Campylobacter spp*, Wagner & Edwards 2002), weaning (P Nieuwoudt, personal com), or nutritional imbalances. All rhinos in this study were over 12 months of age and therefore deciduous tooth eruption should have been complete (Hillman-Smith et al. 1986). Since weaned rhinos also suffered from diarrhea, tooth eruption and the overfeeding of milk were unlikely to be the primary causes of this condition. Diet and husbandry routines (e.g. cleaning regimes, frequent hand-to-mouth contact with volunteers while bottle-feeding) were confounding variables, therefore our results cannot determine the cause of this condition. However, its high prevalence in hands-on rehabilitated rhinos warrants further investigation.

Even though adrenocortical activity is one of the most commonly used physiological welfare indicators (Wasser et al. 2000), it must be interpreted in the context of other indicators (Dawkins 2006; Swaisgood 2007), as animals subjected to long-term chronic stressors may have similar or lower corticoid levels to non-stressed animals (Sakellaris & Vernikos-Danellis 1975; Linklater et al. 2010). Possible signs of stress include fright, frequent defense responses, decreased appetite, increased aggression, stereotypic behaviors, apathy, and decreased complexity of behavior (Carlstead & Shepherdson 2000, Cook et al. 2000). When such responses are sustained, there is a risk of poor welfare. The behavioral and physical welfare indicators evaluated in this study, along with fGCM concentrations that fell within the normal range for the species (Badenhorst et

al. 2016), supported the conclusion that the studied rhinos were unlikely suffering from chronic stress, and therefore poor welfare.

Potential consequences of the rehabilitation method on behavioral competence and post-release survival

Hands-on rehabilitated orphans seldom showed alert behaviors in response to stimuli (human or otherwise), and defense postures were never observed, indicating habituation to humans. Interestingly, this cohort had spent less time in captivity than the hands-off cohort. These results suggest that the rehabilitation method, and not the captive period is mainly responsible for the habituation, at least for the time frame the studied rhinos had spent in captivity. However, this hypothesis needs formal testing. Further research on this topic would be informative from a management point of view to determine which factor (i.e. rehabilitation method, or the captive period) has a stronger effect on habituation.

Although fearfulness and a constant state of alertness negatively impacts welfare (Mench & Mason 1997), a complete absence of these behaviors can compromise post-release survival (Kleiman 1989; Kasereka et al. 2006; Zidon et al. 2009; Ménard et al. 2014; Geffroy et al. 2015). Loss of fear of humans is one of the most important challenges of captive-release programs, and this is more worrisome for frequently poached species (Samia et al. 2015). Our results indicate that orphan white rhinos become less vigilant when rehabilitated under a hands-on method, most likely due to regular contact with humans. For example, decreased alertness due to human exposure has been documented for black rhino, both in mother-raised free-ranging animals (Muntifering et al. 2018), as well as in hand-raised orphans after release (Matipano 2004).

Hands-on rehabilitated rhino showed fewer social interactions than the hands-off cohort, despite being kept in smaller enclosures. However, the former readily engaged in physical contact with humans, even unfamiliar people. Frequent affectionate interactions between volunteers and orphans likely led to socialization with the human species (Scott 1992; Raussih et al. 2003), potentially explaining the lower sociability to their own kind. Similarly, calves (*Bos taurus*) that had received additional human contact (stroking, talking, suckling) interacted more with people (sniffing, licking or touching) than those that had minimal human contact (Raussih et al. 2003). Socialization with humans and decreased social interactions with conspecifics could make

orphans more susceptible to poaching, increase the chances of human-wildlife conflict and negatively influence their integration into wild populations.

Limitations of the study

The main limitations of this study are the lack of standardization across the two facilities, and the lack of replication for each rehabilitation method. Although we have discussed the results under this light, further research at other facilities and under more controlled settings, if possible, is needed. From a data analysis standpoint, an additional limitation is that multiple independent statistical tests were performed, and this has the possibility of increasing the likelihood of at least one individual false-positive result (type I error). Also, data had a hierarchical structure with rhino nested within boma which was nested within location (i.e. rehabilitation method). However, the assignment of rhino to bomas was a management decision of the location (primary exposure of interest) rather than a random event and boma was subsequently removed from statistical models because the term was not significant. The analytical approach to these data is therefore another potential limitation.

Finally, we were unable to establish whether lack of alertness in hands-on rehabilitated orphans is due to intense human contact, another factor such as the lack of a surrogate mother, or a combination of both. In certain captive birds, chicks reared with adults are more vigilant than hand-raised ones (Beani & Dessì-Fulgheri 1998; Valutis & Marzluff 1999; Kreger et al. 2004). Whether this applies to rhino remains unknown. Although the benefits of an adult figure during development might seem obvious, it is not always necessarily the case; hand-reared juveniles of certain birds are equally likely to survive (van Heezik & Seddon 1998), or even show a higher post-release survival rate than parent-reared animals (Ellis et al. 2000). In the case of surrogate mothers, bringing a wild rhino female into captivity can only be justified (and recommended) if there is empirical evidence demonstrating that she confers appreciable advantages to the orphans, either during the rehabilitation process or post-release.

Conclusions

Although the present study has limitations, to the best of our knowledge, this is the first study in any mammalian species where the effects of different rehabilitation methods on behavior and

welfare are compared, and therefore we believe it is of value. Rhino welfare was not obviously compromised at either facility. However, orphans rehabilitated under a hands-off method showed more indicators of good welfare and less indicators of bad welfare. Hands-on rehabilitated orphans on the other hand were less social, lacked avoidance to people and seldom showed alert or defense behaviors. These deficiencies could jeopardize their survival after release, increase the chances of human-wildlife conflict, and hamper their integration into free-ranging rhino populations.

So far, none of the animals observed in this study have been released. As such, it is unknown whether their behavior will change once human contact is discontinued. However, considering the results obtained with other species, including black rhino, and the recommendations of the IUCN, a rehabilitation approach where those behaviors that are crucial for survival and social integration are maintained throughout the rehabilitation process is advised until further research has been conducted.

Ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed in this study were in accordance with the ethical standards of the University of Pretoria Animal Ethics Committee (V030-16).

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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FIGURE CAPTIONS

Fig 1 Percentage of occurrences of the behavioral indicators gathered through focal continuous recording shown by orphan rhinos rehabilitated under a hands-off and hands-on method

Fig 2 Activity budgets in hands-off and hands-on rehabilitated orphan rhino, and those of free-ranging rhino for the same season and time of the day (Owen-Smith 1973). In free-ranging rhinos the categories stand, walk, social and others are included in the category “others”

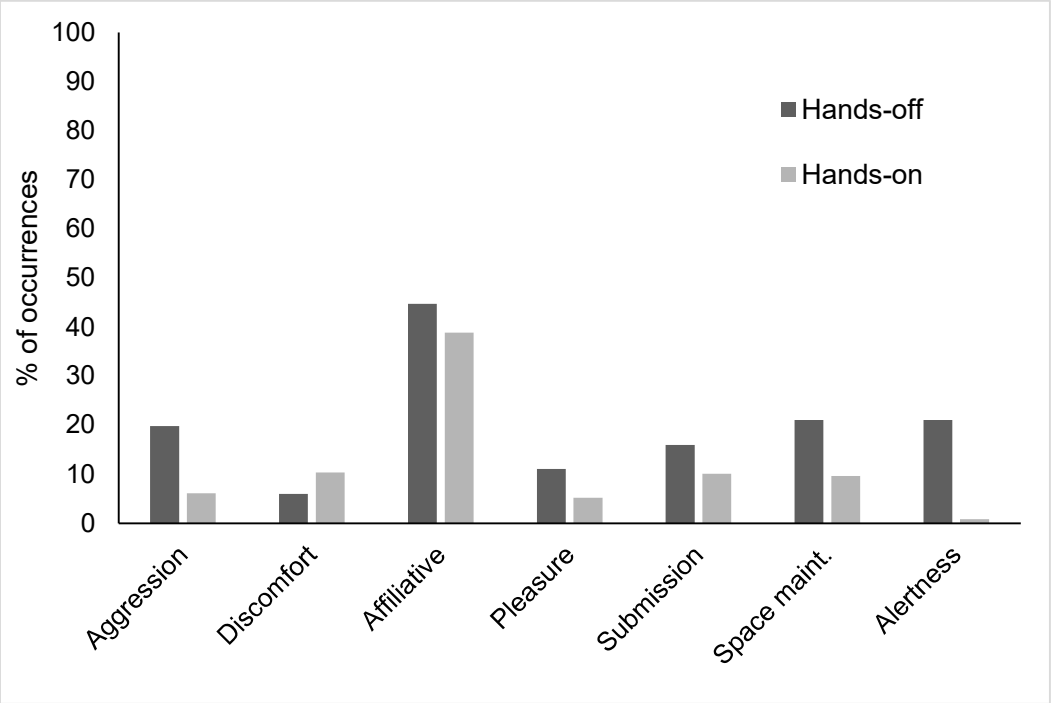
TABLE HEADINGS

Table 1 Test results and percentage of occurrences of the welfare and adaptation potential indicators analyzed with generalized linear mixed models under a hands-off and a hands-on method. Bold figures indicate significant p-values ($p < 0.05$)

Table 1

		Hands-off					Hands-on		
Indicator	**Unit of analysis**	**Observed units (n)**	**Units where the indicator occurred**	**% of occurrences**	**Test statistic (F)**	**p-value**	**Observed units (n)**	**Units where the indicator occurred**	**% of occurrences**
Aggression	Session	651	129	19.82	35.369	**< 0.001**	734	45	6.13
Discomfort	Session	651	39	5.49	2.378	0.123	734	76	10.35
Stereotypies	Session	651	0	0	N/A	N/A	734	0	0
Lethargy	Day	383	2	0.52	0.041	0.840	531	8	1.51
Affiliative	Session	651	291	44.70	6.698	**0.010**	734	285	38.83
Pleasant	Session	651	72	11.06	13.239	**< 0.001**	734	38	5.18
Submission	Session	651	104	15.98	2.449	0.118	734	74	10.08
Space maintenance	Session	651	137	21.04	17.266	**< 0.001**	734	71	9.67
Abnormal stools	Day	202	23	11.39	22.470	**< 0.001**	249	99	39.76
Fecal parasites	Day	200	4	2	0.263	0.608	249	0	0
Injuries	Day	351	73	20.80	14.704	**< 0.001**	529	29	5.48
Alertness	Session	651	137	21.04	56.935	**< 0.001**	734	6	0.82
Response to humans	Day	383	148	38.64	114.762	**< 0.001**	537	1	0.19

Figure 1


100
90
80
70
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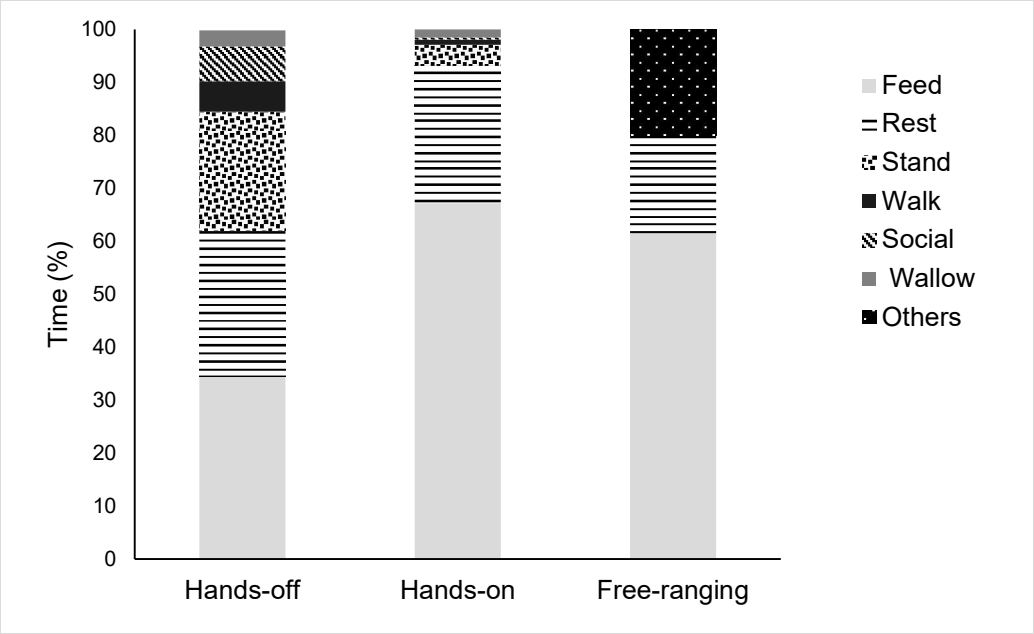
0
% of occurrences
Hands-off
Hands-on
Aggression
Discomfort
Affiliative
Pleasure
Submission
Space maint.
Alertness

Figure 2


100
90
80
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60
50
40
30
20
10
0
Time (%)
Hands-off
Hands-on
Free-ranging
Feed
Rest
Stand
Walk
Social
Wallow
Others

Table A1 Orphan rhinos participating in the study. Estimated age and time in the boma correspond to the date when the study started.

Rehabilitation method	Rhino	Sex	Age (years)	Time in boma (months)
Hands-off (Facility 1)	1	M	1	1
	2	M	3	22
	3	M	3	2
	4	F	4	1
	5	M	2	7
	6	F	2	7
	7	F	2	3
	8	M	2	1
	9	M	1	0
	10	F	1	0
	11	F	1	0
	12	F	1	3
Mean			1.92	3.92
SE			0.29	1.8
Hands-on (Facility 2)	13	F	2	21
	14	M	2	22
	15	F	2	16
	16	M	1	14
	17	M	2	14
	18	M	1	7
	19	F	1	5
	20	F	2	27
	21	F	2	24
	22	F	3	30
	23	F	2	17
	24	M	3	23
	25	M	2	24
Mean			1.92	18.27
SE			0.18	2.43

Rehabilitation method affects behavior, welfare, and adaptation potential for subsequent release of orphaned white rhinoceros

Acta Ethologica

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Table A2 Ethogram showing the behavioral events and their classification into categories. Descriptions partly based on Owen-Smith (1973), and Metrione et al. (2007)

Category	Behavior	Description
Alertness	Alert	Head lifted rapidly, ears scanning, body remains stationary
	Tail up	Tail is curled up without defecation or urination following
	Defense formation	Rhinos stand with their rear ends together facing outwards in different directions
	Flee	Trot or run away after a disturbance. Tail curled up or not
	Disturbed by researcher	A rhino discontinues the behavior it was performing when a person approached, moved or made a noise. It immediately looked in the direction of the person, adopted an alert posture, and may or may not have resumed its previous behavior
	Disturbed by others	A truck, a guard, a generator, a loud noise. Snorting and panting may happen while fleeing from the disturbance with or without tail up
Affiliative	Rub	Rhino rubs its head/body against other rhino. Rub is recorded whether the focal animal is the actor or the receptor of the behavior
	Follow surrogate	Walking behind a surrogate mother while she is walking. Both animals are in movement
	Follow orphan	Walking behind another orphan while the latter is walking. Both animals are in movement
	Follow group	Walking behind two or more rhinos while they walk
	Naso-nasal contact	Two rhinos move slowly towards each other, eventually allowing noses to meet. Movements slow and relaxed
	Head fling	Play invitation and indication of excitement. Head swung up and down rapidly in the vicinity of other rhino
Discomfort	Whine	A thin mewing tone that raises and falls in pitch
	Squeak	Calf distress signal. Abrupt and high pitch
Submission	Yield	Relinquishing of ground or food
	Presenting side	Turning the side of the body towards another rhino and the head away
Space maintenance	Snarl Chase	A gruff roar, brief or rumbling, made with the mouth open, head thrust back, and ears laid back. The vocalization is accompanied by a rapid movement by the actor where it turns around rapidly in the direction of the other rhino. The actor might move a few steps in the direction of the recipient
	Snort	Mild “keep away” warning. Nasal ex- or inhalation
	Charge	Rapid advance against other rhino or person
Agonistic	Horn wrestle	Horn lowered parallel to the ground then hit sideways against horn of the recipient repeatedly
	Horn against horn stare	Horns of two bulls pressed together with heads raised and ears forward
	Attack	Horn jabbing movements directed toward body of recipient. Not reciprocal
	Fight	Two rhinos press horns to each other’s while advancing/retreating towards/from the adversary. Attack gestures made by both opponents while trying to drive each other away, including hitting the adversary with the horn. Fights are considered two independent events if separated by 5 minutes or more. Otherwise it is considered the same event
Pleasure-related	Scratch	Rhino rubs any part of its body but the horn against an object (e.g. post, tree stump or trunk) repetitively. Frequently preceded by mud wallowing.
	Horn rub	Horn rubbed against a hard object repetitively
Stereotypies	Backing	Walking, but instead of feet swinging forward in stride they swing backwards, motion must be sustained long enough so that it is not merely used to change the direction of an animal’s body, but without evident direction or purpose in the locomotion
	Bar biting	Biting of metal bars or boma poles

	Pacing	Repetitive locomotion in a specific area without apparent purpose. It can be a repetitive path along the boma perimeter, a small circle, or just going back and forth along a wall
	Foot dragging	While walking or standing, lifting feet and swinging either forward or from side to side; feet not fully clearing the ground so that they scrape across the ground with the motion. Animal walking with hind legs stiff and straight behind
	Head swiping	Rhino has head to ground moving it laterally, rooting air with horn or horning air, dirt or ground
	Mouthing	Rhino makes repeated chewing or gumming motion with open mouth, not associated with eating or flehmen

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Table A3 Ethogram of the behavioral states used in this study. Descriptions partly based on Owen-Smith (1973)

Behavior	Description
Feeding	Major attention devoted to ingesting food, sometimes coupled with directed movement
Walking	Sustained locomotion with little or no feeding
Standing	Remaining stationary but alertly aware of the surroundings
Resting	Either lying down or standing drowsily with head low, displaying diminished attention to the environment
Wallowing	Rolling in the mud. When they rub against a tree or pole afterwards it is recorded as an event
Social	Engaged in social interactions with other group members, whether the observed rhino is the actor or the receptor of the interaction

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Table A4 Physical and behavioral variables assessed once per day used to assess orphan rhino welfare. Response to humans was used in the evaluation of adaptation potential of the study animals

Variable	Description
Abnormal stools	Diarrhea or very soft feces
Fecal parasites	Gross visual presence of parasitic worms of the genus *Ascaris* in the feces
Injuries	Minor external injuries such as cuts or scratches (only if blood is visible)
Lethargy	Rhino appears dull, drowsy, listless
Response to humans	1: Ignores human presence. Current behavior is not disrupted by human presence/approach. 2: Current behavior is interrupted. Adopts an alert posture. Eventually (less than 5 min) resumes the behavior previously performed 3: Current behavior is interrupted. Adopts an alert posture (sometimes defense formation with other group members) and walks or trots away from person. Alert posture might be performed several times every time it moves away from person 4: Trots away, mock charges against poles/gates 5: Trotting and running around boma, charges against poles/gates

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Table A5 Individual fecal glucocorticoid metabolite concentrations (fGCM) of rhinos in the hands-off (mean= 0.61, SE = 0.03) and hands-on (mean = 0.54, SE = 0.02) cohorts. Statistics are expressed in µg/g of dry weight

Rhino	Rehabilitation method	n	Mean	Median	Minimum	Maximum
1	Hands-off	16	0.50	0.50	0.35	0.65
2		20	0.62	0.57	0.45	0.98
3		12	0.68	0.68	0.39	1.08
4		13	0.82	0.83	0.43	1.26
5		5	0.57	0.59	0.49	0.65
6		15	0.67	0.67	0.53	0.83
7		13	0.63	0.64	0.50	0.79
8		12	0.48	0.50	0.31	0.68
9		2	0.66	0.66	0.50	0.83
10		5	0.49	0.48	0.43	0.55
11		5	0.50	0.46	0.39	0.66
12		5	0.65	0.64	0.50	0.86
13	Hands-on	11	0.61	0.58	0.25	0.81
14		14	0.47	0.46	0.21	0.73
15		14	0.64	0.72	0.30	0.87
16		24	0.51	0.51	0.18	0.81
17		26	0.49	0.51	0.18	0.74
18		10	0.58	0.67	0.13	0.82
19		7	0.44	0.46	0.11	0.76
20		22	0.57	0.61	0.10	0.73
21		20	0.62	0.64	0.23	1.03
22		25	0.57	0.56	0.25	0.89
23		25	0.57	0.59	0.05	0.94
24		24	0.56	0.56	0.27	0.80
25		28	0.41	0.42	0.08	0.65

Rehabilitation method affects behavior, welfare, and adaptation potential for subsequent release of orphaned white rhinoceros

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