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ORIGINAL PAPER

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 rhino 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 rhino 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 rhino. 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 In South Africa alone, arrivals increased from 5 to 56 orphan rhino in the 2011–2016 period (unpublished data).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 2 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 and 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 and 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

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(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 and 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 and 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 rhino (*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 and Blumstein 2003; Webb and Blumstein 2005; Knight 2009), sometimes leading to culling of the culprit animal if people are injured (white rhino: 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 (greater one-horned rhino, *Rhinoceros unicornis*: Choudhury and Mainkar 2005; Barman et al. 2014; white and black rhino: 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 4 years of age (Table A1) were included in the study.

Rhinos were housed in *bomas* (captive wildlife enclosure), which 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 (50×25 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 (15×15 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, which 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). Behavioral indicators of poor welfare included: frequent expression of discomfort behaviors (Broom and Johnson 2000), constant alertness (Mench and Mason 1997), lethargy (Broom and Johnson 2000; Swaisgood 2007), frequent aggression (Broom and Johnson 2000; Swaisgood 2007), presence of stereotypies (Broom and Johnson 2000; Mason and Latham 2004; Swaisgood 2007), proximity (as an indirect measure of social isolation, Price and Stoinski 2007; Salas et al. 2018), and activity budgets (detection of major deviations from those of wild counterparts, Carlstead and Shepherdson 2000; Hosey et al. 2009). As physical indicators of poor welfare, we used variables indicative of a health problem (Broom and Johnson 2000; Carlstead and 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 and 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, rhino 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 rhino 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 and Bateson 1993), all other behavioral data collection started 10 min later, 2 min longer than the average time taken for rhino 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 1 h. Frozen samples were lyophilized, pulverized, and sieved through a metal wire-mesh strainer to remove undigested material (Fieß et al. 1999). Between 0.1 and 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 were 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 rhino 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, Figs. 1 and 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

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. Italicized figures indicate significant p values ($p < 0.05$)

Indicator	Unit of analysis	Hands-off					Hands-on		
		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 (*Ascaris* sp.)	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

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 rhino (Fig. 2). Hands-off rehabilitated rhino spent almost 50% less time feeding than their wild counterparts and showed a more diverse activity budget than hands-on and free-ranging rhino. However, standing occupied

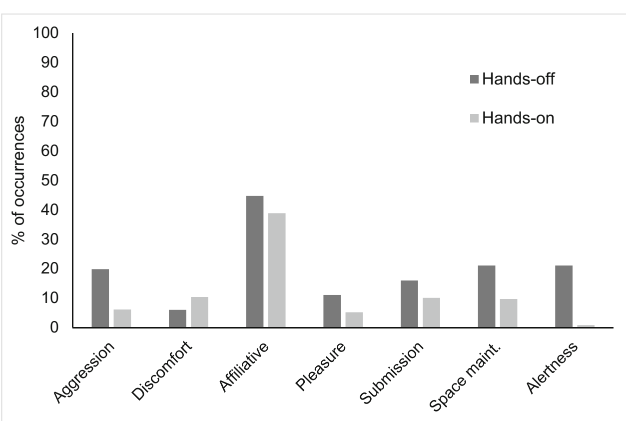

Fig. 1 Percentage of occurrences of the behavioral indicators gathered through focal continuous recording shown by orphan rhino rehabilitated under a hands-off and hands-on method

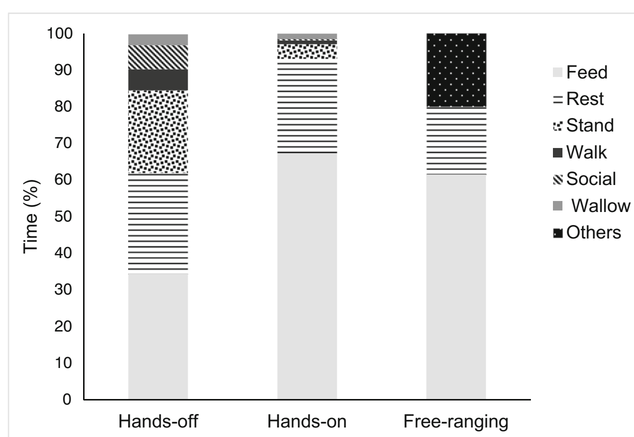

Fig. 2 Activity budgets in hands-off and hands-on rehabilitated orphan rhino, and those of free-ranging rhino for the same season and time of the day (Owen-Smith 1973). In free-ranging rhino, the categories stand, walk, social and others are included in the category "others"

22% of their total activity budget. The hands-on cohort spent similar time feeding compared to free-ranging rhino, but only 7% of their total time was dedicated to activities other than feeding and resting.

Adaptation potential indicators

Rhino 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 rhino 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 and Mendl 1993). This has prompted the use of multiple indicators when assessing animal welfare (Broom and Johnson 2000; Wielebnowski 2003; Hill and Broom 2009). However, there is still disagreement on how different indicators should be combined (Moberg and 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 and 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 and Latham 2004). Additionally, fecal parasites were rare, none of the rhino 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 rhino. This was also true for the display of affiliative behaviors (Boissy et al. 2007), and pleasure-related behaviors, including body scratching (Roosevelt 1910; Owen-Smith 1973) and horn rubbing. Although excessive horn rubbing is a common abnormality in captive rhino (Fouraker et al. 1996), the normal appearance of the horns in the studied animals suggests a beneficial rather than aberrant behavior.

Hands-off rehabilitated rhino 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 rhino (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 and Pellis 1987). As for many other species (Pellis and 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. Furthermore, it could be considered an indicator of behavioral competence.

In captivity, incongruent activity budgets can be an indicator of poor welfare (Carlstead and 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 and 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 and Johnson 2000; Melfi 2009). Evaluated rhino did not develop overt disease during the study, but rhino rehabilitated under a hands-on approach often presented with diarrhea. Diarrhea is fairly common in captive orphan rhino 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 and 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 rhino 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 rhino 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 and 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 and 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 rhino 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. Although fearfulness and a constant state of alertness negatively impact welfare (Mench and 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 rhino 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) and 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 for example, chicks reared with adults are more vigilant than hand-raised ones (Beani and Dessì-Fulgheri 1998; Valutis and 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 and 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 poor 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.

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

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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).

References

Badenhorst M, Otto M, Goot AC, Ganswindt A (2016) Stress steroid levels and the short-term impact of routine dehorning in female southern white rhinoceroses (*Ceratotherium simum simum*). Afr Zool 51(4):211–215

Barman R, Choudhury B, Ashraf NVK, Menon V (2014) Rehabilitation of greater one-horned rhinoceros calves in Manas National Park, a World Heritage Site in India. Pachyderm 55:78–88

Beani L, Dessì-Fulgheri F (1998) Anti-predator behaviour of captive grey partridges (*Perdix perdix*). Ethol Ecol Evol 10:185–196

Beck BB, Rapaport LG, Price MS, Wilson AC (1994) Reintroduction of captive-born animals. In: Olney PJ, Mace G, Feistner A (eds) Creative conservation. Springer, Dordrecht, pp 265–286

Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, Winckler C, Forkman B, Dimitrov I, Langbein J, Bakken M (2007) Assessment of positive emotions in animals to improve their welfare. Physiol Behav 92(3):375–397

Brakes P, Dall SR, Aplin LM, Bearhop S, Carroll EL, Ciucci P, Fishlock V, Ford JK, Garland EC, Keith SA, McGregor PK (2019) Animal cultures matter for conservation. Science 363(6431):1032–1034

Broom DM (1988) The scientific assessment of animal welfare. Appl Anim Behav Sci 20(1–2):5–19

Broom DM, Johnson KG (2000) Stress and animal welfare. Kluwer, Dordrecht

Brown J, Bellem A, Fouraker M, Wildt D, Roth T (2001) Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in North America by noninvasive endocrine monitoring. Zoo Biol 20:463–486

Carlstead K, Shepherdson DJ (2000) Alleviating stress in zoo animals with environmental enrichment. In: Moberg GP, Mench JA (eds) The biology of animal stress: basic principles and implications for animal welfare. CAB International, Wallingford, pp 337–354

Choudhury B, Mainkar K (2005) Rehabilitation of hand-reared rhino calves in Southern Africa: implications for the greater one-horned rhinoceros. In: Menon V, Ashraf NVK, Panda P, Mainkar K (eds)

Back to the wild: studies in wildlife rehabilitation. Conservation Reference Series 2. Wildlife Trust of India, New Delhi, pp 163–170
Clark JD, Huber D, Servheen C (2002) Bear reintroductions: lessons and challenges. Ursus 1:335–345
Cook CJ, Mellor DJ, Harris PJ, Ingram JR, Mathews LR (2000) Hands-on and hands-off measurement of stress. In: Moberg GP, Mench JA (eds) The biology of animal stress: basic principles and implications for animal welfare. CAB International, Wallingford, pp 123–146
Dawkins MS (1980) Animal suffering: the science of animal welfare. Chapman & Hall, London
Dawkins MS (1998) Evolution and animal welfare. Q Rev Biol 73(3): 305–328
Dawkins MS (2006) A user's guide to animal welfare science. Trends Ecol Evol 21:77–82
de Faria CM, de Souza SF, Costa DDL, da Silva MM, da Silva BC, Young RJ, de Azevedo CS (2018) Captive-born collared peccary (*Pecari tajacu*, Tayassuidae) fails to discriminate between predator and non-predator models. Acta Ethol 21(3):175–184
Ellis DH, Gee GF, Hereford SG, Olsen GH, Chisolm TD, Nicolich JM, Sullivan KA, Thomas NJ, Nagendran M, Hatfield JS (2000) Post-release survival of hand-reared and parent-reared Mississippi Sandhill cranes. Condor 102:104–112
Emslie R (2012) *Ceratotherium simum*. The IUCN Red List of Threatened Species 2012: e.T4185A16980466. https://doi.org/10.2305/IUCN.UK.2012.RLTS.T4185A16980466.en
Fieß M, Heistermann M, Hodges JK (1999) Patterns of urinary and fecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). Gen Comp Endocrinol 115:76–89
Fouraker M, Wagener T, Emery H (1996) AZA rhinoceros husbandry manual. Fort Worth Zoological Park, Fort Worth
Ganswindt A, Heistermann M, Borragan S, Hodges JK (2002) Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. Zoo Biol 21: 27–36
Geffroy B, Samia DS, Bessa E, Blumstein DT (2015) How nature-based tourism might increase prey vulnerability to predators. Trends Ecol Evol 30(12):755–765
Guy AJ, Curnoe D, Banks P (2013) A survey of current mammal rehabilitation and release practices. Biodivers Conserv 22(4):825–837
Hediger H (1964) Wild animals in captivity. Dover publications, New York
Hill SP, Broom DM (2009) Measuring zoo animal welfare: theory and practice. Zoo Biol 28(6):531–544
Hillman-Smith AKK, Owen-Smith N, Anderson JL, Hall-Martin AJ, Selaladi JP (1986) Age estimation of the white rhinoceros (*Ceratotherium simum*). J Zool 210(3):355–377
Hosey GR, Melfi VA, Pankhurst SJP (2009) Zoo animals: behaviour, management and welfare. University of Oxford Press, Oxford
IBM Corp (2017) IBM-SPSS statistics for Windows, version 25.0. IBM Corp, Armonk
Ikuta LA, Blumstein DT (2003) Do fences protect birds from human disturbance? Biol Conserv 112(3):447–452
IUCN/SSC (2013) Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. IUCN Species Survival Commission, Gland
Kasereka B, Muhigwa J, Shalukoma C, Kahekwa J (2006) Vulnerability of habituated Grauer's gorilla to poaching in the Kahuzi-Biega National Park, DRC. Afr Stud Monogr 27:15–26
Keep ME (1971) Observable criteria for assessing the physical condition of the white rhinoceros *Ceratotherium simum* in the field. Lammergeyer 13:15–28
Kleiman DG (1989) Reintroduction of captive mammals for conservation. BioSci 39(3):152–161
Kleiman DG (1996) Reintroduction programs. In: Kleiman DG, Allen ME, Thompson KV, Lumpkin S (eds) Wild mammals in captivity: principles and techniques. University of Chicago Press, Chicago, pp 297–305
Knight J (2009) Making wildlife viewable: habituation and attraction. Soc Anim 17(2):167–184
Kreger MD, Estevez I, Hatfield JS, Gee GF (2004) Effects of rearing treatment on the behavior of captive whooping cranes (*Grus americana*). Appl Anim Behav Sci 89:243–261
Linklater WL, MacDonald EA, Flamand JRB, Czekala NM (2010) Declining and low fecal corticoids are associated with distress, not acclimation to stress, during the translocation of African rhinoceros. Anim Conserv 13(1):104–111
Martin P, Bateson P (1993) Measuring behaviour: an introductory guide, 2nd edn. Cambridge University Press, Cambridge
Mason GJ, Latham NR (2004) Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Anim Welf 13:S57–S69
Mason G, Mendl M (1993) Why is there no simple way of measuring animal welfare? Anim Welf 2:301–320
Mathews F, Orros M, McLaren G, Gelling M, Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biol Conserv 121(4):569–577
Matipano G (2004) Post-release ranging behaviour of hand-raised black rhinoceros, *Diceros bicornis*, in Matusadona National Park, Zimbabwe with recommendations for management of introduction to the wild. Koedoe 47(1):89–101
Mattson DJ, Blanchard BM, Knight RR (1992) Yellowstone grizzly bear mortality, human habituation, and whitebark pine seed crops. J Wildl Manag 56:432–442
Meister J (1997) Untersuchungen zum Sozial-und Reproduktionsverhalten von Breitmaulnashörnern (*Ceratotherium simum*) in zoologischen Einrichtungen. Dissertation, University of Erlangen-Nürnberg
Melfi VA (2009) There are big gaps in our knowledge, and thus approach, to zoo animal welfare: a case for evidence-based zoo animal management. Zoo Biol 28(6):574–588
Ménard N, Foulquier A, Vallet D, Qarro M, Le Gouar P, Pierre JS (2014) How tourism and pastoralism influence population demographic changes in a threatened large mammal species. Anim Conserv 17(2):115–124
Mench J, Mason G (1997) Behaviour. In: Appleby MC, Hughes B (eds) Animal welfare. Center for Agriculture and Bioscience International, New York, pp 127–141
Moberg GP (1991) How behavioral stress disrupts the endocrine control of reproduction in domestic animals. J Dairy Sci 74:304–311
Moberg G, Mench J (2000) The biology of animal stress: basic principles and implications for animal welfare. CAB International, Wallingford
Molony SE, Dowding CV, Baker PJ, Cuthill IC, Harris S (2006) The effect of translocation and temporary captivity on wildlife rehabilitation success: an experimental study using European hedgehogs (*Erinaceus europaeus*). Biol Conserv 130(4):530–537
Mullineaux E (2014) Veterinary treatment and rehabilitation of indigenous wildlife. J Small Anim Pract 55:293–300
Muntifering JR, Linklater WL, Naidoo R, Uri-Khob S, Preez PD, Beytell P, Jacobs S, Knight AT (2018) Sustainable close encounters: integrating tourist and animal behaviour to improve rhinoceros viewing protocols. Anim Conserv 22:189–197. https://doi.org/10.1111/acv.12454
Owen-Smith RN (1973) The behavioural ecology of the white rhinoceros. Doctoral dissertation, University of Wisconsin
Owen-Smith RN (2013) *Ceratotherium simum*, white rhinoceros. In: Kingdon J, Hoffmann M (eds) Mammals of Africa, volume V: Carnivores, pangolins, Equids and Rhinoceroses. Bloomsbury Publishing, London, pp 446–454
Pellis SM, Pellis VC (1987) Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat *Rattus norvegicus*. Aggr Behav 13(4):227–242

Price EE, Stoinski TS (2007) Group size: determinants in the wild and implications for the captive housing of wild mammals in zoos. Appl Anim Behav Sci 103:255–264

Raussih S, Lensink BJ, BoissyA PM, Veissier I (2003) The effect of contact with conspecifics and humans on calves' behaviour and stress responses. Anim Welf 12(2):191–202

Rogers PS (1993a) Hand-raising of orphaned rhinoceros calves. In: MacKenzie AA (ed) The capture and care manual: capture, care, accommodation and transportation of wild African animals. Wildlife Decision Support Services and The South African Veterinary Foundation, Pretoria, pp 562–569

Rogers PS (1993b) Accommodation of the white rhinoceros *Ceratotherium simum* and black rhinoceros *Diceros bicornis*. In: MacKenzie AA (ed) The capture and care manual: capture, care, accommodation and transportation of wild African animals. Wildlife Decision Support Services and The South African Veterinary Foundation, Pretoria, pp 540–547

Roosevelt T (1910) African game trails: 105. Charles Scribners' Sons, New York

Sakellaris PC, Vernikos-Danellis J (1975) Increased rate of response of the pituitary-adrenal system in rats adapted to chronic stress. Endocr 97:597–602

Salas M, Manteca X, Abáigar T, Delclaux M, Enseñat C, Martínez-Nevado E, Quevedo MA, Fernández-Bellon H (2018) Using farm animal welfare protocols as a base to assess the welfare of wild animals in captivity—case study: Dorcas gazelles (*gazella dorcas*). Animals. 8. https://doi.org/10.3390/ani8070111

Samia DS, Nakagawa S, Nomura F, Rangel TF, Blumstein DT (2015) Increased tolerance to humans among disturbed wildlife. Nat Commun 6:8877

Scott JP (1992) The phenomenon of attachment in human and non-human relationships. In: Davis H, Belfour D (eds) The inevitable bond: examining scientist-animal interactions. Cambridge University Press, Cambridge, pp 72–92

Seddon PJ, Armstrong DP, Maloney RF (2007) Developing the science of reintroduction biology. Conserv Biol 21(2):303–312

Swaisgood RR (2007) Current status and future directions of applied behavioral research for animal welfare and conservation. Appl Anim Behav Sci 102(3):139–162

Thomas MJ, Peterson ML, Friedenberg N, Van Eenennaam JP, Johnson JR, Hoover JJ, Klimley AP (2013) Stranding of spawning run green sturgeon in the Sacramento river: post rescue movements and potential population-level effects. N Am J Fish Manag 33:287–297

Touma C, Sachser N, Möstl E, Palme R (2003) Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 130:267–278

Trendler K (2005) The principles of care and rehabilitation of orphaned wild mammals. In: Menon V, Ashraf NVK, Panda P, Mainkar K (eds) Back to the wild, studies in wildlife rehabilitation, Conservation reference series, vol 2. Wildlife Trust of India, New Delhi, pp 46–53

Valutis LL, Marzluff JM (1999) The appropriateness of puppet-rearing birds for reintroduction. Cons Biol 13:584–591

van Heezik Y, Seddon PJ (1998) Ontogeny of behavior of hand-reared and hen-reared captive houbara bustards. Zoo Biol 17:245–255

Veenema AH (2009) Early life stress, the development of aggression and neuroendocrine and neurobiological correlates: what can we learn from animal models? Front Neuroendocrinol 30(4):497–518

Verdoorn G (1995) Release criteria for rehabilitated wild animals. In: Penzhorn BL (ed) Proceedings of the SASOL symposium on wildlife rehabilitation. South African Veterinary Association and ARC (Animal Rehabilitation Centre), Pretoria, pp 89–94

Vickery SS, Mason GJ (2003) Behavioural persistence and its implication for reintroduction success. Ursus 14:35–46

Wagner DC, Edwards MS (2002) Hand-rearing black and white rhinoceroses: a comparison. Proceedings of the Second Rhino Keepers' Workshop 2001, Zoological Society of San Diego May 7–10, 2001. San Diego Zoological Society, San Diego, pp 18–27

Wallach JD (1969) Hand-rearing and observations of a white rhinoceros *Diceros s. simus*. Int Zoo Yearb 9(1):103–104

Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh JJ, Larson S, Monfort SL (2000) A generalized fecal glucocorticoid assay for use in a diverse array of non-domestic mammalian and avian species. Gen Comp Endocrinol 120:260–275

Webb NV, Blumstein DT (2005) Variation in human disturbance differentially affects predation risk assessment in western gulls. Condor 107(1):178–181

Whitehead H (2010) Conserving and managing animals that learn socially and share cultures. Learn Behav 38(3):329–336

Wielebnowski N (2003) Stress and distress: evaluating their impact for the well-being of zoo animals. J Am Vet Med Assoc 223:973–977

Zidon R, Saltz D, Shore LS, Motro U (2009) Behavioral changes, stress, and survival following reintroduction of Persian fallow deer from two breeding facilities. Conserv Biol 23(4):1026–1035

Zuberogoitia I, Torres JJ, Martínez JA (2003) Population reinforcement of Eagle Owl *Bubo bubo* in Biscay (Spain). Ardeola 50:237–244