

Unforeseen consequences of conservation management practices: case study on herding rhino as an anti-poaching measure

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ABSTRACT

The current biodiversity crisis calls for effective conservation measures. However, some measures may have unforeseen negative consequences on individual species. Herding, a management practice currently used in some wildlife species to protect animals from poachers, can cause habituation to humans, which unintentionally might increase their risk of being poached at a later time. Herding could also negatively impact animal welfare and physiology when perceived as a stressor, with potential negative consequences at the individual and population level. In this study we tested the following hypotheses: 1) herding elicits habituation to humans in white rhino, and 2) this practice affects the behavior and physiological state of rhino, but they can habituate to it. To that aim, we investigated the effects of herding on the response of rhino to initially unfamiliar people, expression of aggressive and discomfort behaviors, and fecal glucocorticoid metabolite (fGCM) concentrations (a commonly used indicator of physiological stress) in two cohorts of six and 10 semi-wild orphan white rhino. Rhino did not interrupt their activity to an approaching person on foot (99%) or in a vehicle (96%), indicating habituation to humans. Aggressive and discomfort behaviors were displayed more often while being herded, and their frequency increased significantly with increasing herding pressure. Finally, overall mean fGCM concentrations were 16.1% higher under herding compared to non-herding conditions, but individual fGCM levels varied up to 17-fold under each scenario, suggesting that herding might not elicit a physiological stress response in these animals. While rhino security is paramount in the current poaching crisis, management practices must ensure that they do not impact negatively at the individual and population level.

Keywords: management practices, human habituation; animal welfare; fecal glucocorticoid metabolites; behavior

INTRODUCTION

Human activity is negatively affecting wildlife populations on a global scale. Examples of these effects are numerous, including habitat fragmentation that influences the demographic and genetic stability of wild populations (O'Grady *et al.*, 2006), human disturbance that is increasing the nocturnality of some mammal species (Gaynor *et al.*, 2018), and the introduction of invasive species that are becoming a major threat to global biodiversity (e.g. Schmitz & Simberloff 1997). Unfortunately, some human interventions that are intended to benefit conservation can also have negative consequences to the animals or populations that they are attempting to protect. For example, fencing protected areas can cause mass mortalities when migrations are cut off from critical water sources (e.g.

Hayward & Kerley, 2009), zoological parks can inadvertently introduce non-indigenous species (Fàbregas, Guillén-Salazar & Garcés-Narro, 2010), supplementary feeding in private reserves can increase aggression amongst individuals and alter home ranges (Cinková, Ganslosser & Kretzschmar, 2017), and eco-tourism, advocated as a tool to conserve species and habitats through educational and socio-economic benefits (Macfie & Williamson, 2010), can negatively impact animal welfare (e.g. Moorhouse *et al.*, 2015) and increase predation risk (Geffroy *et al.*, 2015). The recent surge in poaching of African rhinoceros (Milliken, Emslie & Talukdar, 2009) has encouraged protected areas to implement increased security measures (Duffy, 2014). Sanctuaries, one of the management systems under which rhino populations are protected (Leader-Williams *et al.*, 1997), are small areas of state-protected, private or communal land, in which rhino are deliberately confined, and where security units are deployed at a high density (Emslie & Brooks, 1999). Rhino at sanctuaries are usually classified as *semi-wild*, which, according to the African Rhino Specialist Group, is when animals are living in areas of less than 10 km², at compressed density and spacing, with high stocking densities at times, regular food supplementation, subject to veterinary procedures when necessary, and allowed to breed naturally (Leader-Williams *et al.*, 1997). This management system is often used for rehabilitated animals (either survivors of poaching or rescued orphans), and it is not limited to rhino species (e.g. elephants: The Sheldrick Wildlife Trust in Kenya).

As an additional security measure, herding, in the sense of directing a group of animals to an open area where they can graze during the day, and escorting them back at night to a safe area, is sometimes implemented in sanctuaries with rhino (e.g. Randle & Kiley-Worthington, 2004, Patton *et al.*, 2011, Barichievy *et al.*, 2017) and elephants (McKnight, 1995). Although effective against poachers (Barichievy *et al.*, 2017), this practice can have unintended consequences, such as habituation to humans on the herded animals (McKnight, 1995). Habituation is a behavioral response decrement that results from repeated stimulation that does not involve sensory adaptation or motor fatigue (Thompson & Spencer, 1966). The opposite phenomenon is termed sensitization: an increased behavioral responsiveness to a repeated or ongoing stimulus over time (Richardson *et al.*, 1995). Despite the advantages that habituation may provide for intense management systems (e.g. improved docility, decreased discomfort or risk of injuries for both humans and animals: Boivin *et al.*, 2003), it can have negative consequences on the animals when they transition into a less intensive management system. The most obvious consequence of habituation is a higher risk of poaching, as the animal is less prone to flee from humans (e.g. gorillas, *Gorilla g. graueri*: Kasereka *et al.*, 2006; Barbary macaque, *Macaca sylvanus*: Ménard *et al.*, 2014; birds: Samia *et al.*, 2015; several species: Geffroy

et al., 2015). Unsurprisingly, rhino also become less vigilant when regularly exposed to people (Muntifering *et al.*, 2018). Habituated wildlife can also become bolder and more aggressive towards humans (e.g. Ikuta & Blumstein, 2003; Webb & Blumstein, 2005; Knight, 2009), sometimes leading to culling the animal if people are injured (Grizzly bears, *Ursus arctos*: Mattson, Blanchard & Knight, 1992; Southern white rhino, *Ceratotherium simum simum*: Verdoorn, 1995). Additionally, habituation can impact populations; for example, impairment of fitness-related traits such as reproduction or offspring provisioning have been reported in species belonging to very different taxa (e.g. Indo-Pacific bottlenose dolphin, *Tursiops sp.*: Bejder, 2005; Adélie penguins, *Pygoscelis adeliae*: Giese, 1996; bald eagles, *Haliaeetus leucocephalus*: Grubb & King, 1991). As such, it may decrease the chance that habituated animals will establish self-sustaining populations. Last but not least, the loss of behavioral traits in a species (fear from humans in this case) might compromise its adaptation to new environments, where such traits would have been advantageous (Caro & Sherman, 2012).

Aside from habituation to humans, herding could negatively impact animal welfare. Whereas there is no consensus on a definition of welfare (Moberg, 2000; Swaisgood 2007), for the purpose of this study, we have used the definition of Broom (1988), who defines the welfare of an individual as “the animal’s state related to its attempts to cope with the environment”, understanding by coping the ability to successfully deal with the current circumstances. Herding might be an aversive experience for herded animals, as it forces close proximity among individuals and deprives animals from moving freely. Aversion is probably accentuated in species where the physical characteristics and strength of the animals require an intense herding style (e.g. loud voice commands, whips, canes), such as in rhino or elephants. When an animal is subjected to an aversive activity, its welfare is negatively impacted (e.g. Dawkins 1980; Broom & Johnson, 2000). In domestic species, extensive research links poor human-animal relationships with lower reproductive rates (Pedersen, 1994; Hemsworth *et al.*, 1999); a link which has also been established in captive wildlife (Mellen, 1991; Bloomsmith *et al.*, 1997; Bayne, 2002; Wielebnowski *et al.*, 2002), including both African rhino species (Carlstead & Brown, 2005).

Finally, recurrent lack of control of movement, and forced social proximity could also be perceived as a stressor by herded animals, causing a state of perturbed homeostasis that can evoke manifold adaptive reactions, usually summarized as the stress response (e.g. Holsboer & Ising, 2010). Such a response involves physiological and behavioral changes to enable animals to cope with those intrinsic or extrinsic stimuli (i.e. stressors), including the

activation of the hypothalamic–pituitary–adrenocortical (HPA) axis, which increases the secretion of glucocorticoids (GC) by the adrenal glands (e.g. Palme, 2019). The secretion of GC stimulates rapid changes in the physiology of an animal (e.g. temporal hyperglycemia) allowing an efficient response to restore homeostasis (e.g. Romero, 2004). However, if the HPA axis is frequently activated, it can have pathophysiological effects (e.g. Sapolsky, 2005; McEwen & Wingfield, 2010), which in turn can negatively affect reproduction, immunity and growth (Sapolsky, Romero & Munk, 2000; Wingfield & Romero, 2001). Quantification of GC or its metabolites has become a widely accepted approach to monitor responses to stressors in a number of wildlife species, including white rhino (Brown *et al.*, 2001; Palme, 2019). However, when interpreting alterations in GC concentrations, variation related to season, sex, age, reproductive status, and neonatal exposure to stress should be taken into account (Romero, 2002, 2004; Bush & Hayward, 2009). In this regard, adding behavioral observations to GC data can be beneficial when interpreting results (Broom & Johnson, 2000; Dawkins, 2006). Behavioral signs that indicate an animal is trying to re-establish homeostasis include avoidance behaviors, aggression, and startle or defense responses, amongst others (Carlstead & Shepherdson, 2000; Cook *et al.*, 2000).

Effective conservation measures are necessary in the face of the current biodiversity decline. However, it is crucial to evaluate whether these interventions have negative consequences on the target species where they are employed (Dantzer *et al.*, 2014; Hampton & Hyndman, 2018). Here we use herding of rhino as a case study to investigate the potential short- and long-term effects of a management practice at individual and population levels. To that aim, we test the following hypotheses: (H1) herding of semi-wild orphan rhino sustains habituation towards humans, and (H2) this practice affects the behavior and physiological state of rhino, but they can habituate to it. Based on these hypotheses, we predict that: a) rhino will not show avoidance behavior towards humans or vehicles; b) rhino will display more aggression and discomfort with increasing herding pressure, but these behaviors will decrease over time due to habituation to the practice, c) fecal glucocorticoid metabolite (fGCM) concentrations will be higher under herding conditions compared to before herding had occurred, but they will decrease over time due to habituation to herding, and d) herding pressure will decrease over time as rhino become more compliant to the practice.

MATERIALS AND METHODS

Study area and subjects

The study took place between June 2016 and July 2017 at a rhino sanctuary in South Africa (location not disclosed for security reasons). The sanctuary included a 350 ha fenced-off area where rhino were released under semi-wild conditions. Anti-poaching measures included foot patrols, horse patrols, canine units, rhino guards and electrified fencing on the perimeter of the property.

The study comprised a herd of 16 white rhino, approximately 2.5 to six years old (Table 1). All animals were rehabilitated orphans that had been hand-raised and habituated to people (Fàbregas et al., 2020). Before release, rhino were kept in groups of 3-10 animals, and housed in *bomas* (captive wildlife enclosure) but with daily access to adjacent open areas (0.8 – 2 ha). The current study was approved by the University of Pretoria Animal Use and Care Committee (protocol V030-16).

As part of the sanctuary's management plan, rhino were released at two different times: Cohort 1 (n=10) was released in December 2016, and Cohort 2 (n=6) at the end of May 2017, nine days before data collection started. Daily, at 07:00, all rhino were herded into the 350 ha fenced area by 2-3 guards to graze in areas considered to be safe (i.e. away from the perimeter fence). At 16:00 rhino were herded back to an area adjacent to the bomas with a roofed section where *ad libitum* teff hay (*Eragrostis teff*) and water were available.

Rhino guards interacted with rhino in three main ways: directing their movement (herding to/from the grazing areas), preventing smaller units straying from the herd, and breaking up fights between rhino if contact aggression occurred. These actions were achieved by vocal commands (shouting, whistling), waving a cane, cracking a whip in the air, or physically pushing the rhino by hand from behind. For security reasons, rhino from both cohorts were managed as a single group at all times.

Behavioral data collection

Behavioral data collection took place between May and July 2017 for both Cohorts. Consequently, the data presented in this study corresponds to 6-8 months of herding for Cohort 1, and the first two months of herding for Cohort 2. Habituation to humans was evaluated as the response of each rhino to an initially unfamiliar person (i.e. the researcher) approaching within 20 m on foot, and the response to an approaching vehicle within 30 m. The response to a person on foot was evaluated every morning, as the researcher arrived for behavioral data collection. Response to vehicles was recorded opportunistically when a vehicle drove by. Rhino response was recorded using

five mutually exclusive categories: 1) behavior uninterrupted; 2) approach person/vehicle; 3) alert (head up, ears towards the stimulus: Owen-Smith, 1973) and resume behavior; 4) alert and walk away from the person/vehicle, and 5) alert and flee from the person/vehicle.

To evaluate the effect of herding on rhino behavior, study subjects were observed for 24 non-consecutive days (i.e. (total observation time: 63.75 h; median: 4.25 h/rhino, range: 2.83 – 4.58 h/rhino). Data collection was equally distributed into morning and afternoon sessions, which commenced 10 min before herding started, and continued for another 40 min until a total of 10 rhino had been observed in each session. The 10 minute-periods before herding started served as a baseline to compare the expression of the evaluated behaviors during herding and non-herding periods. The order in which rhino would be observed was established by randomized lists designed for each session. Each rhino within a session was observed for 5 min, using focal sampling and continuous recording (Martin & Bateson, 1993) to register aggression and discomfort behaviors (Table 2) as indicators of poor welfare (Broom & Johnson, 2000; Carlstead & Shepherdson, 2000; Cook *et al.*, 2000). Herding pressure was measured as the number of commands (auditory or physical) given by a guard to the herd to advance in a particular direction (counts/5-min block). Therefore, herding pressure was not dependent on the focal rhino, but on the behavior of the entire herd. Rhinos were observed for a total of 765 5-min block periods for the duration of the study (median: 51, range: 34 – 55 blocks/rhino).

Fecal sample collection, steroid extraction and glucocorticoid metabolite concentration analysis

A total of 314 fecal samples were collected during the study. Of these, 192 samples were collected before release, while animals were still in the bomas (median: 7.5 samples/rhino; range 2-24), and 122 samples were collected after release, when rhino were herded daily (median 8.5 samples/rhino, range: 4-11). Sample collection in the bomas took place between June and September 2016 for Cohort 1, and between September 2016 and March 2017 for Cohort 2.

All fecal samples were collected within 2 h of defecation. Approximately 30 g of fecal material was collected by removing pieces from the middle of 3-4 boli of a dropping. The sample was immediately placed on ice and frozen at -20 °C within one hour. Subsequently, frozen samples were lyophilized, pulverized, and sieved through a metal wire-mesh strainer to remove undigested material (Fieß, Heistermann & Hodges, 1999). Between 0.10 - 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). Concentrations of fGCM were measured in the extracts using an immunoreactive 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA detecting 3 β ,11 β -diol-CM, first described in Touma *et al.* (2003), and validated for white rhino by 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 2 400 μ g/g fecal dry weight (DW). Intra-assay coefficients of variation (CV), determined by repeated measurements of high and low value quality controls were 6.6% and 6.7%, respectively. Inter-assay CV, also determined by repeated measurements of high and low value quality controls were 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 formally test predictions regarding the expression of aggressive and discomfort behaviors, as well as variations in fGCM concentrations. To assess the effect of herding on aggression and discomfort, these variables were transformed into two categories prior to analysis, assuming a binomial likelihood function for the model. We used 0 for 5-min blocks where no aggression (n = 657) or discomfort occurred (n = 561), and 1 for blocks where at least one behavior was observed, irrespective of how often it/they occurred (aggression: n = 99, discomfort: n = 195). To evaluate how herding pressure impacted on the expression of aggressive and discomfort behaviors, percentiles were used to create an ordinal variable with equal numbers of observations (to the extent possible): no herding (0 commands/block, n = 261), mild herding (1-5 commands/block, n = 244) and intense herding (6-77 commands/ block, n = 251). Rhino ages (Table 1) were dichotomized as: juveniles (2-3 years of age) and young adults (4-6 years of age). In the aggression and discomfort models, sex, age, herding pressure, time of the day (morning or afternoon), the number of days from the start of herding (continuous variable “days herded”), cohort, and the interaction between days herded and cohort were included as fixed effects, with “rhino” as a random effect, to account for individual differences. A Spearman correlation was used to evaluate how herding pressure changed over time. In this correlation, herding pressure was used as the original variable (i.e. counts/5-min block), and time as the sequential study day.

To assess the effect of herding on fGCM concentrations, daily herding (no herding before release vs. herding after release), number of days herded (continuous), sex, age, cohort, and an interaction between cohort and days herded

were introduced as fixed effects in the GLMM. Additionally, we included the age at which rhino were orphaned as a fixed effect, since stress early in life can affect the functioning of the HPA axis later in life (Romero, 2004). Since white rhino start eating grass at 2 months of age while still completely dependent on milk (Owen-Smith, 1973), we used three categories for the variable “age when orphaned”: 2 months (mo), 3-8 mo, and older than 8 mo. By 8 mo, rhino calves graze *ca.* 90% of the time that their mothers graze (Owen-Smith, 1973). Rhino was introduced as a random effect. The model adjusted for the repeated measures design by including a first-order autoregressive covariance term (Simpson et al., 2010).

Correlation coefficients were calculated between all model covariates and coefficients > 0.7 or < -0.7 were further evaluated by calculating variance inflation factors (VIF) with $VIF > 5$ considered indicative of substantial collinearity requiring the exclusion of one of the predictors from the models. Scatter plots of residuals versus predicted values were created to evaluate the assumptions of independence, homoscedasticity, and normality for the models assessing fGCM concentrations. Statistical analyses were performed with SPSS 26 (IBM Corp, 2019), and statistical significance set at 0.05.

RESULTS

Habituation to humans

We recorded 1306 responses of rhino to humans: 734 responses to an approaching person on foot, and 572 to an approaching vehicle. The main response in both cases was uninterrupted behavior (person on foot: 94%, vehicle: 99%). In the remaining 6% of the cases of a person approaching, the rhino response was to approach the person. Given the homogeneity of the results, statistical confirmation of the differences between the five response categories was not performed.

Effects of herding on behavior and fGCM concentrations

Rhino showed significantly more aggression and discomfort with than without herding (Table 3); the frequency of these behaviors was positively associated with herding pressure in both cohorts (aggression: no herding mean \pm SD = 0.04 ± 0.33 counts/5-min block, mild herding mean \pm SD = 0.11 ± 0.37 counts/5-min block, intense herding mean \pm SD = 0.5 ± 1.1 counts/5-min block; discomfort: no herding mean \pm SD = 0.15 ± 0.90 counts/5-min block , mild

herding mean \pm SD = 0.54 ± 1.31 counts/5-min block, intense herding mean \pm SD = 1.63 ± 2.91 counts/5-min block) (Fig. 1). Cohort and sex were not predictive for the expression of aggressive responses (Table 3), but age was, with juveniles (i.e. 2-3 years of age) showing less aggression than young adults (juveniles mean \pm SD = 0.11 ± 0.41 counts/5-min block, young adults mean \pm SD = 0.42 ± 1.03 counts/5-min block). Additionally, aggression was less frequent in the afternoon than in the morning (morning mean \pm SD = 0.27 ± 0.84 , afternoon mean \pm SD = 0.17 ± 0.52 counts/5-min block). Aggression decreased during the study period in Cohort 2 but not in Cohort 1 (Table 3). Likewise, discomfort behaviors were less frequent in younger animals (juveniles mean \pm SD = 0.69 ± 1.78 counts/5-min block, young adults mean \pm SD = 0.91 ± 2.36 counts/5-min block) and during the afternoon sessions (morning mean \pm SD = 0.99 ± 2.49 counts/5-min block, afternoon mean \pm SD = 0.54 ± 1.32 counts/5-min block), while sex was not predictive for the expression of discomfort. Cohort 2 exhibited significantly more discomfort behaviors than Cohort 1 (Cohort 1 mean \pm SD = 0.67 ± 2.07 , Cohort 2 mean \pm SD = 0.98 ± 1.85 counts/5-min block). Discomfort behaviors increased over time in Cohort 1 but decreased in Cohort 2 (Table 3). Lastly, herding pressure showed a small but significant increase during the study period (Spearman Rho = 0.124, $p = 0.001$).

There were marked individual differences in fGCM concentrations among rhino (Fig. 2). However, overall mean fGCM concentrations were higher during the herding period (mean \pm SD = 0.65 ± 0.16 $\mu\text{g/g DW}$) than in the pre-release period (mean \pm SD = 0.56 ± 0.19 $\mu\text{g/g DW}$, Table 3). Respective fGCM concentrations decreased over time in both cohorts (Fig. 2), but the rate of decrease was greater in Cohort 2 compared to Cohort 1 (Table 3). Sex, age, age when orphaned and cohort were not predictive of fGCM concentrations in the studied rhino.

DISCUSSION

Habituation to humans

Human approach or human presence generally triggers antipredatory behavior in wildlife (Frid & Dill, 2002; Knight, 2009). White rhino are no exception and, under wild conditions, fear is manifested by alert behaviors and a flight response towards humans (Owen-Smith, 1973). Here, we show that herded rhino did not demonstrate such fearful responses. Over the course of the study period, rhino behavior was seldom interrupted when a person or vehicle approached, and on the few occasions that it was (6% and 1% respectively), the main response was for the rhino to approach the person or vehicle. These results support our hypothesis that, under herding conditions, rhino orphans remain habituated to humans. Considering the constant human-animal interaction required for herding, these results

were intuitive. Contrary to what has been reported for other wild ungulates (Stankovich, 2008), humans on foot did not elicit more avoidance in the rhino than a vehicle. Because guards were permanently on foot, a different human being was probably not perceived as a threat. It is currently unclear whether rhinos are able to distinguish between different groups of people, such as guards and researchers. For example, elephants (*Loxodonta africana*) distinguish hunters (Maasai men) from non-hunters (Maasai women and children) based on the sounds of their voices (McComb *et al.*, 2014). However, tourist-habituated gorillas do not distinguish between poachers and tourists, as indicated by not readily attacking or hiding when a poacher approaches, as non-habituated gorillas do (Kasereka *et al.*, 2006). If herded rhinos cannot differentiate between guards and poachers, as they seem to not differentiate between guards and researchers, they will be at higher risk of poaching when transitioned to a less intensive surveillance and management system.

The rhinos in this study were already habituated to humans prior to release. Whether these animals would show a natural response to humans (i.e. alert and flight) if they were not subjected to herding could not be determined in our study. What is clear from our results is that rhinos remain habituated under herding conditions. A study documenting the release of a zoo-born white rhino in Etosha National Park (Namibia) reported that the animal, which used to approach people at the zoo and during the boma period, no longer approached humans or responded to calls two months after release, when human contact ceased (Böer *et al.*, 1999). This result indicates that spontaneous recovery, defined as the recovery of a response once the stimulus the animal had habituated to has been withheld (Thompson & Spencer, 1966), can occur in this species given that human contact is interrupted. In contrast, in a study on black rhino orphans (*Diceros bicornis*) Matipano (2004) reported that following release, orphans spent more time in areas with than without humans, allowing researchers to approach them within a few meters. Whether the difference in response between these studies is species specific (i.e. black vs. white), a consequence of having been orphaned, or due to different timing, is worthy of further investigation. In light of the conservation potential of rehabilitated animals and the detrimental consequences that wildlife habituation may have at the individual level (e.g. higher poaching risk: Geffroy *et al.*, 2015; culling due to increased boldness or aggressiveness towards humans: Verdoorn, 1995), further research in this area is warranted. At the population level, if herding is sustained over generations, the risk of losing behavioral attributes should be considered a likelihood (Caro & Sherman, 2012). From a conservation standpoint, maintaining behavioral attributes that have enabled a species to persist into the present era is essential to ensure their long-term survival (Redford *et al.*, 2011). Moreover, conservation aside, the

question remains whether it is ethically acceptable to conserve (and consciously promote through management practices) a partially artificial version of a species; that is without the behavioral repertoire shaped by evolution to survive in its environment. This question, in our view, requires deep consideration.

Effects of herding on rhino welfare and fGCM concentrations

As predicted, aggression and discomfort were more frequently observed during herding, showing a positive association between their frequency and herding pressure. Higher rates of aggression and discomfort indicate that rhino perceived herding as an aversive stimulus and, as such, it negatively impacted on their welfare (Dawkins, 1988; Broom & Johnson, 2000). In fact, forced close proximity also elicited aggression in a herded group of orphan black rhino (Randle & Kiley-Worthington, 2004). Unpleasant human-animal contact in production animals has been associated with changes in fitness-related traits such as reduced testicular size, mating behavior, and pregnancy rate (Hemsworth, Barnett & Hansen, 1981; Zulkifli, 2013). In the Randle & Kiley-Worthington (2004) study, the authors attributed rhino lack of breeding to the increased aggression observed in the herd, although this hypothesis was not formally tested. None of the females in our study had mated by June 2020 (pers. obs.), despite most of them having reached sexual maturity (unpub. data). Although the impact of herding on reproduction could not be tested in our study, long-term data collection is strongly encouraged given the importance of reproduction in the establishment of self-sustaining populations.

In the current study, fGCM concentrations during the herding period were slightly but significantly higher than before herding started. Although these results could indicate an effect of the herding practice on rhino adrenocortical activity, overall fGCM means were comparable to those reported for free-ranging as well as semi-wild rhino (Badenhorst *et al.*, 2016). Moreover, the pronounced individual variation observed in the study sample demonstrates complexity in pinpointing an actual cause-effect relationship between herding and increased fGCM concentrations. Another point that stands out is the rise in fGCM concentrations seen for Cohort 2 during the last month of data collection in the bomas. There were no apparent changes in the husbandry of the animals during this time, and our field notes do not provide any explanation for this increase. Frequent long-term monitoring of herded rhino would be necessary to, on one hand, confirm or reject a cause-effect relationship between herding and fGCM and, on the other hand, to determine whether the subtle increase in fGCM observed in herded rhino actually has a negative

impact on core biological functions such as reproduction, immunity or growth (Sapolsky *et al.*, 2000; Wingfield & Romero, 2001).

Interestingly, and contrary to many other species, age and sex (e.g. Reeder & Krammer, 2005; Datnzer *et al.*, 2014) had no effect on fGCM concentrations in the studied rhino. However, our sample was mostly comprised of young animals (i.e. juveniles and very young adults). The age at which rhino calves were orphaned also had no effect on fGCM concentrations. These rhino lost their mothers between 1-10 months of age due to poaching, an undeniably severe stressor. Early neonatal stress can impact the stress response later on in life (Romero, 2004). When such stressors are severe, this can cause life-long hypersecretion of GCs to moderate stressors (rats: Plotsky & Meaney, 1993; Shanks, Larocque & Meaney, 1995). Although the age at the time of losing their mothers did not impact fGCM in the studied animals, a similar study comparing orphans and mother-raised calves in an experimental setting would be required to determine whether being involved in a poaching incident at a young age subsequently alters the adrenocortical response of rhino to stressors.

Habituation of rhino to herding

Contrary to our prediction, herding pressure did not decrease over time, but showed a small but significant increase during the study period, suggesting that rhino did not become more compliant to the practice. However, there was variation among cohorts. In Cohort 1, herded for 6 months before the study commenced, aggression remained constant while discomfort increased in frequency. Whereas in Cohort 2, herded for only 9 days prior to the start of the study, both discomfort and aggression decreased during those two months. Considering that discomfort was substantially higher in the short-term (Cohort 2) than in the long-term herded rhino (Cohort 1), but aggression was comparable between cohorts, these results suggest an initial habituation to herding (months 0-2), but in the mid-long term (after 6 months of exposure) rhino still react with aggression when herded, while their discomfort gradually increases. These differences in response to the same stimuli over time emphasize the importance of rigorously collecting longitudinal measurements when assessing habituation (Nisbet, 2000), and not just the response to a stimulus at a certain point in time. Failure to do so could lead to the premature conclusion that any difference in responsiveness observed between groups or individuals constitutes habituation or sensitization (Bejder *et al.*, 2009).

Limitations of the study

This study had some limitations. All data were collected on the same reserve and different guards were used for herding, though they probably had similar herding styles. Statistical analysis could have been performed differently including fitting independent models pre- and post-herding rather than the presented combined model. Within our modelling approach, the ‘days herded’ variable was correlated with the variables “daily herding’ and “herding pressure’ but further assessment did not identify substantial collinearity among these predictors. These issues and the small sample size of 16 animals should be taken into account in the generalizability of findings. Furthermore, age was a confounding variable, as Cohort 2 only had young animals, which resulted in poor precision in some estimates due to model complexity. Further studies in other reserves with a research design that avoids potentially confounder factors (e.g. cohorts with animals representing all categories of each predictor variable) is strongly encouraged.

Management implications

Our results indicate that herding keeps orphan rhino habituated to humans, negatively impacts their welfare, and herded rhino do not habituate to the practice in the mid-long term. These effects could impact survival of rhino transitioned to a less intensive protection system. Since herding is also practiced with other species, including elephants, evaluating its impact on animals subjected to this practice is strongly advised.

Despite the negative consequences of herding on the animals, individual guarding systems are indeed effective anti-poaching measures (Barichievy *et al.*, 2017). In the Ziwa Rhino Sanctuary (Uganda), for example, guards passively accompany rhino without interfering with their movement (e.g. Patton *et al.*, 2011), which possibly eliminates the effects of herding on welfare. However, the animals likely remain habituated to humans. Moreover, another important aspect that should not be dismissed in any conservation activity is the financial cost. Poaching has required a dramatic increase in economic resources for security (Lindsey & Taylor, 2011), and this investment has put significant financial pressure on protected areas (Taylor *et al.*, 2014). Since conservation agencies are notoriously underfunded (Margules & Pressey, 2000), investments in biodiversity conservation must be strategically allocated (Naidoo & Ricketts, 2006). In this regard, individual guarding systems, such as herding, could be diverting resources from other conservation activities (e.g. habitat restoration), including those targeted at combatting poaching, such as community relations in protected areas, or intelligence networks.

While acknowledging that security surveillance remains paramount under the current poaching crisis, other more cost-effective measures could be implemented to ensure rhino protection while avoiding negative effects on the species. For example, intense surveillance (from rangers), particularly foot patrols, have proven to be a deterrent to poachers in small areas where species such as elephants and rhino are under a high poaching threat (Leader-Williams, Albon & Berry, 1990; Jachmann & Billiouw, 1997). Strategic militarization in deploying surveillance personnel, and an increase in intelligence networks could further improve the efficacy of this method (Barichiev *et al.*, 2017). Other anti-poaching measures include the use of Remotely Piloted Aircraft Systems (i.e. drones when used by the military) (Mulero-Pázmány *et al.*, 2014; Penny *et al.*, 2019), canine units, intelligent fence intrusion detection systems and real-time anti-poaching tags (Donoghue & Rutz, 2016).

This study serves to illustrate how management practices may hinder, rather than assist in the conservation of a species, emphasizing the importance of appropriate biological management in wild and captive populations (Walpole, 2002). A clear understanding of the potential effects of any conservation action will assist in developing better conservation programs and prevent unforeseen consequences. When the negative effects of a given conservation measure are unavoidable, or alternative practices do not exist, its benefits must be weighed against its negative effects, both at the individual as well as the population level.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. White rhino participating in the study. Ages are approximate and refer to the age of each animal at the onset of the study.

Cohort	Rhino	Sex	Age (y)
Cohort 1	1	M	4
	2	F	3.5
	3	F	4
	4	F	6
	5	M	6
	6	M	4
	7	M	4
	8	F	3
	9	F	4.5
	10	F	5
Sex ratio/mean age		0.67	4.4
Cohort 2	11	F	3
	12	M	3
	13	F	2.5
	14	M	3
	15	F	3
	16	F	3.5
Sex ratio/mean age		0.5	3

Table 2. Partial ethogram describing aggressive and discomfort behaviors in white rhinos. Descriptions partly based on Owen-Smith (1973), and Metrione, Penfold & Waring (2007).

Category	Behavior	Description
Discomfort	Whine	A thin mewling tone that raises and falls in pitch
	Squeak	Calf distress signal. Abrupt and high pitch
Aggression	Snarl chase	A gruff roar, brief or sustained, made with the mouth open, head thrust back, and ears laid back. The vocalization is accompanied by a rapid movement by the aggressor where it turns around suddenly in the direction of the other rhino. The aggressor might move a few steps in the direction of the recipient
	Snort	Mild “keep away” warning. Nasal ex- or inhalation
	Charge	Rapid advance towards another rhino or person
	Horn wrestle	Horn lowered parallel to the ground then hit sideways against horn of the recipient repeatedly
	Horn against horn stare	Horns of two rhino pressed together with heads raised and ears forward
	Attack	Horn jabbing movements directed towards the body of another rhino. Not reciprocated
	Fight	Two rhinos engage 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

Table 3. Generalized Linear Model results explaining the variation in aggression, discomfort, and faecal glucocorticoid metabolite concentrations (fGCM) in two cohorts of herded orphan white rhino. P-values < 0.05 are presented in bold. Age is categorized into juveniles (2-3 years of age) and young adults (4-6 years of age). “mo” stands for “months old”

Variable	Predictor (Baseline)	Coefficient	t-value	p-value	Effect measure (95% CI)
Aggression*	Mild herding (No herding)	2.07	3.98	< 0.001	7.92 (2.9 – 22)
	Intense herding (No herding)	3.11	6.405	< 0.001	22.45 (8.7 – 58)
	Cohort 2 (Cohort 1)	1.33	0.898	0.369	3.76 (0.21 – 68)
	PM (AM)	-0.56	-2.227	0.026	0.57 (0.35 – 0.94)
	Juvenile (young adult)	-0.93	-2.593	0.010	0.40 (0.20 – 0.80)
	Female (Male)	-0.33	-1.198	0.273	0.72 (0.40 – 1.3)
	Days herded	<0.01	0.233	0.816	1.00 (0.99 – 1.0)
	Days herded*Cohort	-0.04	-2.092	0.040	0.96 (0.93 – 0.99)
Discomfort*	Mild herding (No herding)	2.04	5.935	< 0.001	7.65 (3.9 – 15)
	Intense herding (No herding)	3.09	9.398	< 0.001	21.95 (12 – 42)
	Cohort 2 (Cohort 1)	7.50	5.405	< 0.001	1804 (118 – 27*10 ³)
	PM (AM)	-0.54	-2.613	0.009	0.58 (0.39– 0.87)
	Juvenile (young adult)	-0.85	-2.432	0.015	0.43 (0.22 – 0.85)
	Female (Male)	0.13	0.462	0.644	1.14 (0.66 – 2.0)
	Days herded	0.03	4.362	< 0.001	1.03 (1.0 – 1.1)
	Days herded*Cohort	-0.05	-3.763	< 0.001	0.95 (0.93 – 0.98)
fGCM†	Daily herding (No daily herding)	0.24	4.377	< 0.001	(0.13 – 0.35)
	Cohort 2 (Cohort 1)	-0.09	-1.443	0.150	(-0.22 – 0.03)
	Female (Male)	0.04	0.982	0.327	(-0.04 – 0.13)
	Juvenile (young adult)	-0.08	1.518	0.130	(0.03– 0.19)
	Orphaned between 2-8 mo (Orphaned at 2 mo or younger)	0.01	0.205	0.838	(-0.09 – 0.11)
	Orphaned at 8 mo or older (Orphaned at 2 mo or younger)	0.01	0.099	0.921	(-0.14 – 0.16)
	Days herded	<-0.01	-3.146	0.002	(-0.002 – 0.000)
	Days herded*Cohort	-0.01	-3.542	< 0.001	(-0.009– -0.002)

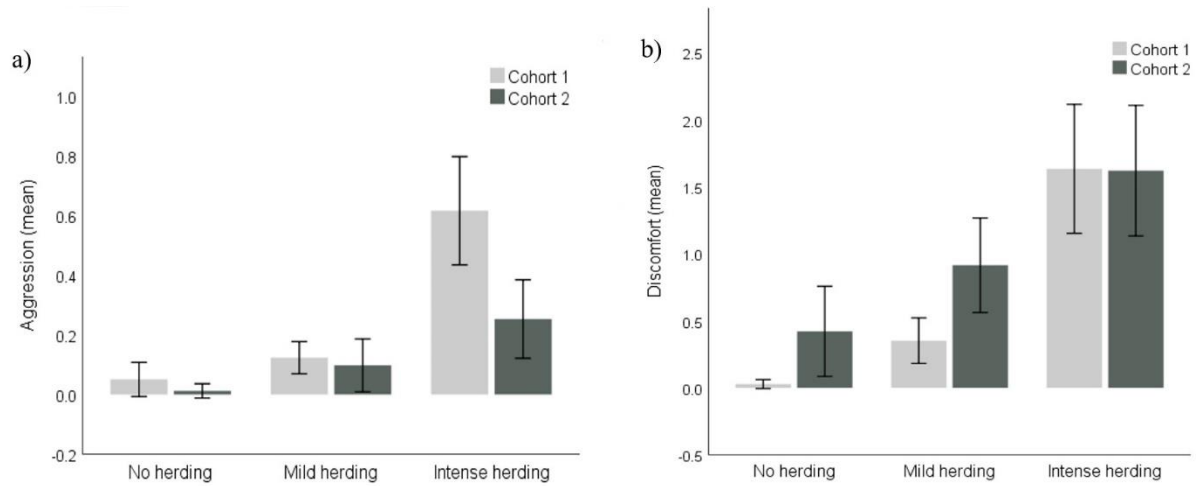


Fig. 1 Effect of herding pressure on the expression of aggressive (a) and discomfort (b) behaviors (mean count /5-min observation block) by two cohorts of orphan white rhino under herding conditions. Note the difference in scale between panels a) and b). Bars represent 95% confidence intervals.

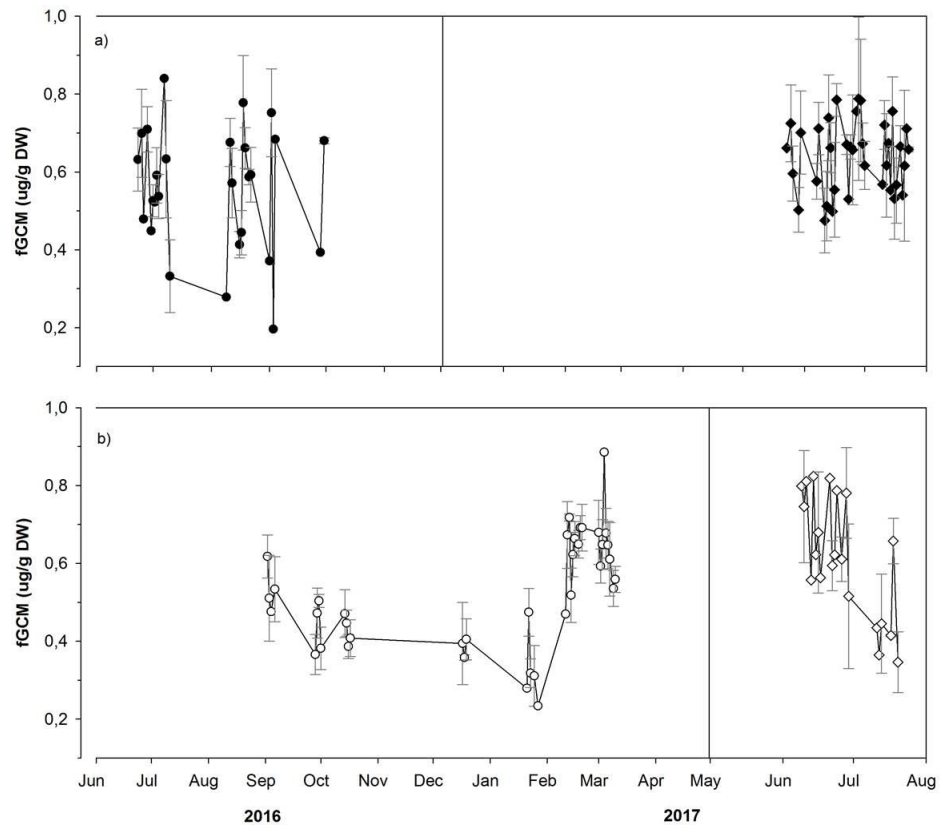


Fig. 2 Individual fecal glucocorticoid metabolite (fGCM) concentrations in two cohorts of orphan white rhino before and during daily herding by anti-poaching guards. Pre-herding fecal sample collection for Cohort 1 (a) took place between June and end of September 2016, and for Cohort 2 (b) between September 2016 and March 2017. A vertical line across each of the panels indicates the date of release and therefore the onset of the herding practice. Fecal sample collection during the herding period took place between May and end of July 2017 for both cohorts.