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Original Research Article

Individual stress responses of white rhinoceros (*Ceratotherium simum*) to transport: implication for a differential management

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ABSTRACT

Physiological stress in captive wild animals may be caused by stressors such as capture, handling, and transport. Continuous strong stimulation may induce a long-term physiological stress in captive wild animals after transport. Fecal Glucocorticosteroid Metabolites (FGM), vital signs and behavioral changes were used to establish stress responses of white rhinoceros during a translocation process. The result indicated that the overall FGM increased significantly ($p < 0.05$) during transport compared to FGM baseline concentration established in two rhino breeding centers. Respiratory rate, heart rate, and body temperature were significantly increased during capture and transport. Grouping and aggressive behavior increased after transport, reflecting the acclimatization to the new social environment. Feeding also increased probably due to increased energy consumption during transport. The overall FGM concentration increased during capture and transport but normalized within an average period of 32 days after transport. Individual differences were attributed to previous transport experience and the ability of intrinsic control through increased adrenaline levels. Recommendations to improve the management and welfare of captive white rhinoceros on transport are provided.

1. Introduction

Causes of physiological stress in wild animals retained in captivity are manifold, including stressors such as artificial lighting, exposure to loud or aversive sound, arousing odors, and uncomfortable temperatures or substrates (Morgana and Tromborg, 2007). Moreover, confinement-specific stressors such as the restriction of movements, limited retreat

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opportunities, forced proximity to humans, reduced feeding occasions, maintenance activities, capture, and transport are potential factors that may adversely affect wildlife living in captivity (Morgana and Tromborg, 2007). Especially transport for management reasons is known to elicit major behavioral and physiological stress responses (Cregier, 1982; Dembiec et al., 2004). Stress can be described as a complicated but comprehensive physiological response generated by both, autonomic nerves and steroid hormones (e.g. glucocorticoids) discharged when animals are confronted with severe changes in their environment. Fecal glucocorticosteroid metabolites (FGMs), produced in the adrenal glands, are usually applied to measure physiological stress in wild and domestic animals held in captivity (Sapolsky, 1990, 2005; Deng et al., 2014).

Several factors can affect the animals' stress response during transport, including flurry human activities, space limitation, and separation from conspecifics (Selye, 1946). Highest stress responses were reported when animals are transported for the first time in their life (Grandin, 1997). Individual stress responses depend on the animals' personal experience and its vital signs, but also on the length and duration of the transport (Dembiec et al., 2004). Individual personalities such as neuroticism, extraversion, conscientiousness, agreeableness, and openness were found to influence stress perceptions in captive wildlife (McCrae and Costa, 2008). An animals' personality (or temperament) is an inheritable trait known to influence the stress response of individuals substantially (McDougall et al., 2006), especially during translocations and reintroductions (Griffith et al., 1989; Wolf et al., 1998). Studies on voles, for example, suggest that 'boldness' involves a higher risk of predation when released into the wild (Woodroffe, 2003) while in 'shy' individuals predator-induced mortality did not increase after release (Banks et al., 2002). However, animals reared in captivity for several generations are decreasingly alerted by changes in their environment leading to reduced physiological stress responses (McPhee, 2004).

The establishment and development of non-invasive sampling technics and the availability of FGM assays has substantially improved the monitoring of stress levels in wild animals (Wasser et al., 1997, 2000; Goymann et al., 1999; Millspaugh et al., 2001; Schatz and Palme, 2001; Creel et al., 2002; Dehnhard et al., 2003; Franceschini et al., 2008). This is particularly important for species that are regularly moved between zoos, breeding centers or protected areas (e.g. rhinos, ungulates; Wasser et al., 2000, Brown et al., 2001; Turner et al., 2002; Carlstead and Brown, 2005; Linklater et al., 2010). Transport stress in domestic livestock has been widely studied in species such as pigs (McGlone et al., 1993), cattle (Palme et al., 2000), horses (Schmidt et al., 2010), and sheep (Fisher et al., 2010), but also in a number of wild animals (Montane and Galvão, 2002; Dembiec et al., 2004; Viljoen et al., 2008; Carlstead and Brown, 2005; Linklater et al., 2010). Compared to domestic livestock species (Palme et al., 2000; Fisher et al., 2010), wild animals that experienced capture and transport show extended periods of physiological stress (Goymann et al., 1999; Terio et al., 1999; Dehnhard et al., 2003). A short period of physiological stress is helpful for animals to respond to sudden external environment change, but a continued exposure may substantially impair body functions (Sapolsky, 1990) such as metabolic disturbance (Jayo et al., 1993), gastrointestinal dysfunction (Taché et al., 1994), or decreasing immunity and productivity (Broom and Johnson, 1993; Brown et al., 2001; Baker et al., 2002). A fast recovery from physiological stress is thus imperative to ensure the health and welfare of wildlife species during prolonged transport.

White rhinoceros (*Ceratotherium simum*) naturally occur in the savannas of southern and eastern Africa. They were previously listed as 'Endangered' by the IUCN Red List but are now considered as 'Near Threatened' (Emslie, 2012). From 2006 to 2007, China imported a total of 117 white rhinoceros from South Africa to start commercial rhinoceros farming (Cota-Larson, 2013). Imported rhinoceros represented the foundation of a breeding program located in a private rhinoceros breeding center in Yunnan Province (The Breeding and Research Center of Wildlife). In 2012, the Yunnan population was supplemented by animals from another rhinoceros breeding project located on Hainan Island in southern China (White Rhino Breeding and Research Center), where the extraction of rhino horn from living animals was achieved for the first time in 2008 (Du and Jia, 2008). In 2015, the Yunnan breeding enterprise, imported another 40 white rhinoceros, hoping to expand the Yunnan population to 200 individuals within five years.

The keeping and breeding of white rhinoceros in captivity is linked to several problems, such as low reproduction rates (Emslie and Brooks, 1999; Carlstead and Brown, 2005) and chronic (Carlstead et al., 1999a, b; Carlstead and Brown, 2005) and even life-threatening stress (Letty et al., 2007; Teixeira and Okazaki, 2007). Brown et al. (2001) have validated a fecal glucocorticoid assay for black and white rhinoceros in North America and according to Turner et al. (2002), FGM from feces and serum of white rhinoceros were well correlated with glucocorticoid obtained from HPLC (High-Performance Liquid Chromatography) tests. Thus, fecal samples can be a reliable resource for monitoring stress related glucocorticoids in white rhinoceros. In white rhinoceros, FGM levels reflect the real status of adrenal activity and are commonly used to monitor stress reactions during transport (Brown et al., 2001; Carlstead and Brown, 2005). However, these are not real-time responses, but increased FGM levels occur usually with a certain time lag, i.e. 12–48 h after the actual stressor occurred (Brown et al., 2001; Möstl and Palme, 2002). While the negative impact of transport on white rhinoceros has been reported (Linklater et al., 2010), individual differences, the effect of personality as well as factors influencing an individuals' physiological stress during transport are not yet investigated.

Our study therefore aims to test for these factors in the white rhinoceros during and after transport:

(1) significant changes in vital signs during transport, i.e. respiratory rate, heart rate or body temperature;
(2) significant changes in the frequency of 15 behavior recorded before and after transport;
(3) differences in the overall FGM concentrations during different phases of the transport and the pre-transport FGM at two breeding centers (FGM baseline concentrations);

(4) individual differences in FGM concentrations during the capture (C) and transport (D) phase and the pre-transport FGM at two breeding centers (FGM baseline concentrations).

2. Materials and methods

2.1. Study area and objects

The study was carried out in two private white rhinoceros breeding centers, i.e. the 'White Rhinos Breeding and Research Center' in Tianya, near Sanya City in Hainan Province and the 'Breeding and Research Center of Wildlife' in Shilin County, Yunnan Province (hereafter referred to as Sanya and Shilin breeding centers). To date, both breeding centers hold a total stock of 89 white rhinoceros, 22 in Sanya and 67 in Shilin. Due to management requirements, 10 white rhinoceros were translocated from Sanya to Shilin in 2012. To monitor the stress response of translocated individuals, a total of 14 adult white rhinoceros (age six to 14 years) were encompassed in this study. Eight individuals (six females, two males) from Sanya were designated for translocation, another six adults (three females, three males) from Shilin were selected as a control group which did not receive any handling or treatment (Table 1). No other individual had transport experience within the past four years, except individuals Q0 and Q6. The housing, nutrition, and management operations were similar in both breeding centers. The housing cells of the two breeding centers are both 6 m*6 m. Rhinos are kept as groups and fed with alfalfa, and have the same active schedules (Fig. 1). The weather is cooler in Shilin than in Sanya, and the playground is much bigger in Shilin than in Sanya. All animals in this study were tested negative for gastro-intestinal parasites infection prior to translocation. To avoid any stress, it was ensured that study animals were not disturbed during the fecal sample collection. To facilitate habituation, crates for transport were placed in rhino enclosures seven days prior to translocation.

2.2. Sample collection

Samples were collected from 1 April 2012 to 5 September 2012. Test animals were sampled once every two days before and after transport, but once every day during transport. We collected the samples at 8:00am everyday to make sure all samples are fresh. Every rhino for transportation was housed solitary, so we can distinguish each one very well. Control individuals were sampled once every two days for the entire study period. In total 942 fecal samples were collected into labeled plastic bags and stored at −20 °C. Samples were sent to the laboratory at Beijing Forestry University for ELISA testing, using a mobile refrigerator to avoid interrupting the cooling chain.

2.3. Vital signs

Before, during, and after the transport, individuals' vital signs were assessed once every day by recording the respiratory rate, heart rate, and body temperature. Thoracic movements were counted for calculating the respiratory rates, venous pulse at the tail was measured for calculating the heart rate, and a handheld infrared thermometer gun (Omega, OS543, range: −20 to 500 °C, resolving power: 0.5 °C) was used to measure the forehead temperature. Due to technical problems, we abandoned recording the heart rate during transport. Body temperature was only measured during and post-transport. All individuals included in this study were habituated to the presence of humans, and underwent regular routine health checks. FGM measurements will therefore not be affected by measuring the vital signs.

2.4. Behavioral observations

All translocated individuals were observed before and after transport to record the frequencies of 15 different behaviors, except individual Q13. Independent behavior was defined as one individual not being accompanied by others within a range of 5 m, including Single Walking (SW), Single Standing (SS), and Single Resting (SR). Group behavior was defined as two or

Table 1
Age, sex and origin of translocated and control individuals included in this study.

Sanya			Shilin		
Transported individuals	Age	Sex	Control individuals	Age	Sex
Q34	6	F	D5	12	M
Q0	14	F	D83	7	M
Q13	14	M	D80	9	F
Q28	6	F	D46	7	M
Q19	7	M	D6	12	F
Q6	8	F	D7	11	F
Q25	6	F			
Q17	7	F			

Fig. 1. The environment of white rhino Breeding and Research Center in Sanya and Shilin.

more individuals performing the same behavior within a range of 5 m, including Group Walking (GW), Group Standing (GS), and Group Resting (GR). In addition, we recorded aggressive behaviors including Attacks (A), Feinted Attacks (FA), Horn fencing (HF), Snarling (SN) and Staring (S). Other behaviors recorded were Chewing (C), Feeding (F), Running (R), and Submissive (SU) behavior. Aggressive and other behaviors were recorded independent of whether performed in a group or as a single. Using scan-sampling in conjunction with time-interval sampling (Engel, 1996), the activity of each animal was recorded by one observer every 3 min for 6 h per day pre- and post-translocation coming to a total sampling period of 72 h per individual. The behavior frequency was then established as the number of observations per hour. (8:00am-11:00am; 13:00pm-16:00pm, other time the rhinos are housed in enclosures) for every individual in one enclosure. Sampling was carried out for six days (36 h) the control group, vital signs and behavioral observations were not recorded.

2.5. Hormone extraction and determination

FGM were extracted and FGM concentrations were established following procedures described in Brown et al. (2001) and Carlstead and Brown (2005). For details see the supplementary material.

2.6. Time settings

Turner et al. (2002) reported that increased FGM concentrations of white rhinoceros recovered to normal in four to six weeks after transport. Due to the gut passage time, FGM occurs usually with 12 h delay (Deng et al., 2014)—dates used in our analysis were therefore back-dated to the real date within the translocation process. We divided the translocation process into three phases: B) the normal pre-transport period (baseline level; 28.03. to 28.04.2012), C) the capture period (28.04. to 03.05.2012), and D) the transport period (04.05—07.05.2012). According to Linklater et al. (2010), both males and females showed a strong increase in FGM immediately after capture lasting for four to 16 days. To test for hormonal fluctuations during the post-transport period, we extended the recovery sampling time to a total of 102 days. The post-transport period was divided into seven phases (AS1-7), each lasting 15 ± 5 days. The recovery period included two stages, i.e. AS1 (7 May to 22 May, 2012) and AS2 (24 May to 10 June, 2012), normal period includes five stages: AS3 (11 June to 26 June, 2012), AS4 (27 June to 11 July, 2012), AS5 (13 July to 27 July, 2012), AS6 (29 July, 12 August) and AS7 (14 August to 5 September, 2012).

2.7. Data analysis

To achieve a normal distribution, all data were square root transformed prior to statistical testing using SPSS vs17.0 (SPSS Inc., 2008; Chicago, IL, USA). Subsequently, all vital signs and behavioral measurements met normal distribution and homoscedasticity requirements. To test for differences in heart and respiratory rate between pre- and post-transport a Mann-Whitney *U* test was used. To test for differences in body temperature before, during and after transport, a One-way ANOVA was conducted. A pair-wise multiple comparison procedure (LSD test) was applied to unravel where differences occur. To test for differences in 15 behavior recorded before and after transport, a Mann-Whitney *U* test was applied. A Mann-Whitney *U* test was also used to test for differences between the FGM baseline concentration in both breeding centers and the different periods of the translocation process (C, D, AS1, 2 and AS3-7). Individual FGM concentrations during different periods of the translocation (C, B, AS1, 2, AS3-7) were also compared to FGM baseline concentrations derived from Sanya and Shilin breeding centers using a Mann-Whitney *U* test.

3. Results

3.1. Vital signs

The mean respiratory rate significantly increased from 18.95 ± 0.62 to 21.94 ± 1.67 during post-transport (Mann-Whitney *U* test: $N_{Sanya} = 4$, $N_{Shilin} = 3$, $p < 0.05$; Table 2). In addition, the average heart rate significantly increased from 36.13 ± 0.85 to 44.00 ± 2.04 during post-transport (Mann-Whitney *U* test: $N_{Sanya} = 3$, $N_{Shilin} = 8$, $p < 0.05$; Table 2). Individual heart rates of Q34, Q17, and Q19 accelerated notably between pre- and post-transport, while heart rates of individual Q6, Q28 and Q0 declined slightly. Average body temperature significantly increased during transport, but decreased again after transport (One-way ANOVA: $F = 19$, $N_{Sanya} = 2$, $N_{Shilin} = 9$, $p < 0.01$; Table 2).

3.2. Behavioral observations

Only two aggressive behavior significantly increased after transport i.e. HF and S (Mann-Whitney *U* test: $N_{Sanya} = 3$, $N_{Shilin} = 7$, $p < 0.05$; Fig. 1). Independent behaviors such as SS and SR decreased significantly after transport (Mann-Whitney *U* test: SS: U = 4, $p < 0.01$; SR: U = 6, $p < 0.05$; Fig. 2). Compared to pre-transport, only GW increased significantly after transport (Mann-Whitney *U* test: U = 7, $p < 0.05$; Fig. 1). All other independent and group behaviors did not change after transport. F and C increased significantly after transport (Mann-Whitney *U* test: F: U = 0, $p < 0.01$; C: U = 4, $p < 0.01$; Fig. 1). Individual differences were found in the frequency of aggressive behavior (e.g. HF) in Q34, Q19, and Q28 who clearly increased the frequency compared to that of pre-transport. Group behavior increased in Q0, Q19, Q25, Q6, and Q28 while solitary behavior increased in Q17 and Q34. Moreover, the frequency of F increased in all individuals after transport (Fig. 2).

3.3. Total fecal glucocorticosteroid metabolite (FGM) analysis

The FGM baseline concentration of white rhinoceros in the Sanya breeding center was 14.81 ± 1.37 ng/g feces, and thus significantly higher than that measured in the Shilin breeding center (7.39 ± 2.48 ng/g feces; Mann-Whitney *U* test: $N = 170$, $p < 0.01$). The overall FGM level increased slightly during the capture (C) phase, but was not significantly different from the FGM baseline concentration measured in the Sanya breeding center (Mann-Whitney *U* test: $N = 46$, $p > 0.05$; Fig. 3, Table 3). During the transport (D) phase, however, the mean FGM concentration was significantly increased compared to the FGM baseline concentrations observed in Sanya (Mann-Whitney *U* test: $N = 21$, $p < 0.01$) and Shilin breeding centers ($p < 0.01$; Fig. 3, Table 3). During the recovery period (AS1, 2) as well as during the normalized period after transport (AS3-7), the average FGM concentration showed no differences to Shilin and Sanya baseline values (Mann-Whitney *U* test: $N = 341$, $U = 6705$, $p > 0.05$; Fig. 3, Table 3).

3.4. Individual FGM concentrations analysis

We also tested individual stress responses to capture (C)、transport (D) and post-transportation (AS 1-7) in relation to the FGM concentration prior to transport (B). FGM concentration has increased dramatically in transport (D) which is significant differences compared to its own FGM baseline in phase B (Mann-Whitney *U* test: $N = 8$, $p < 0.05$) except for Q34 and Q0 (Fig. 4). Only Q34 have significant difference hormone changes in the capture phase compared with prior to transport (B) (Mann-Whitney *U* test: $N = 8$, $p < 0.05$).

After transportation, three individuals, including Q19 (Mann-Whitney *U* test: $N = 8$, $p > 0.05$), Q6 (Mann-Whitney *U* test: $N = 8$, $p > 0.05$) and Q17 (Mann-Whitney *U* test: $N = 9$, $p > 0.05$), hormone level drops rapidly, restore to the FGM baseline concentration (Fig. 4); three individuals, including Q34 (Mann-Whitney *U* test: $N = 8$, $p > 0.05$), Q13 (Mann-Whitney *U* test: $N = 8$, $p > 0.05$) and Q28 (Mann-Whitney *U* test: $N = 9$, $p > 0.05$), restore to the FGM baseline concentration in the recovery phase (AS1-2), but then, hormone levels produced a significant change (Mann-Whitney *U* test: $N = 8$, $p < 0.05$) in AS3-7

Table 2
Respiratory rate, heart rate and body temperature changes in eight white rhinos pre- and post-transport. Note that **: $p < 0.05$; *: $p < 0.01$.

Individuals	Respiratory rate(mean ± SE)			Heart rate(mean ± SE)			Body temperature(mean ± SE)		
	Pre	During	Post	Pre	During	Post	Pre	During	Post
Q6	20.00 ± 2.31	N/A	16.00 ± 2.00	39.33 ± 1.20	N/A	32.00 ± 4.00	31.36 ± 0.21	36.40	33.23 ± 0.45
Q34	20.67 ± 3.33	N/A	17.33 ± 1.33	37.00 ± 1.53**	N/A	60.00 ± 0.00	31.22 ± 0.20	35.40	32.80 ± 0.23
Q13	15.5 ± 1.50	N/A	18.00 ± 0.00	30.00 ± 2.65**	N/A	48.00 ± 0.00	31.62 ± 0.25	36.00	29.83 ± 0.32
Q17	20.67 ± 3.71	N/A	30.00 ± 0.00	36.33 ± 1.20*	N/A	45.33 ± 3.53	31.96 ± 0.22	35.40	36.17 ± 0.19
Q19	16.75 ± 0.75	N/A	22.67 ± 1.76	31.33 ± 1.76*	N/A	52.00 ± 4.00	31.31 ± 0.13	34.60	31.40 ± 0.36
Q25	19.33 ± 2.40	N/A	22.00 ± 2.00	36.67 ± 1.76	N/A	44.00 ± 4.00	31.47 ± 0.10	35.60	32.30 ± 0.26
Q28	20.67 ± 1.76	N/A	24.00 ± 0.00	39.27 ± 1.45	N/A	34.67 ± 2.67	31.53 ± 0.06	35.20	31.17 ± 0.26
Q0	19.00 ± 1.73	N/A	26.00 ± 2.00	39.00 ± 0.58	N/A	36.00 ± 0.00	30.02 ± 0.16	35.60	32.80 ± 0.17
Average	19.2 ± 1.35	N/A	22.16 ± 1.27	36.2 ± 1.26	N/A	44.1 ± 2.13	31.3 ± 0.16×	35.53	32.5 ± 0.28

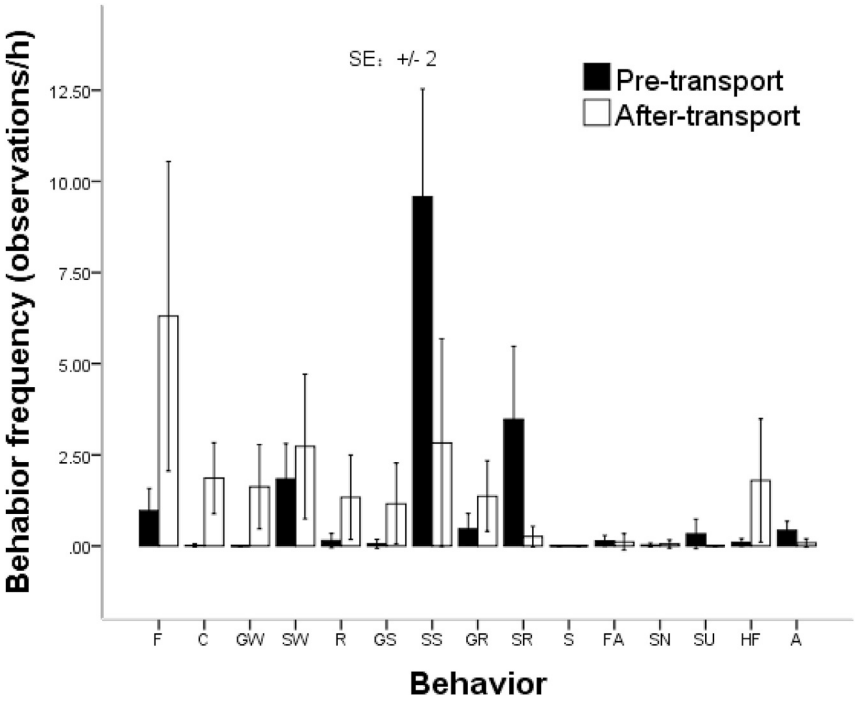

Fig. 2. Mean behavior frequencies across all individuals pre- and post-transport (Mann-Whitney *U* test: **: $p < 0.05$; *: $p < 0.01$). F: Feeding; C: Chewing; GW: Group Walking; SW: Single Walking; R: Running; GS: Group Standing; SS: Single Standing; GR: Group Resting; SR: Single Resting; S: Staring; FA: Feinted attack; SN: Snarling; SU: Submissive; HF: Horn fencing; A: Attack.

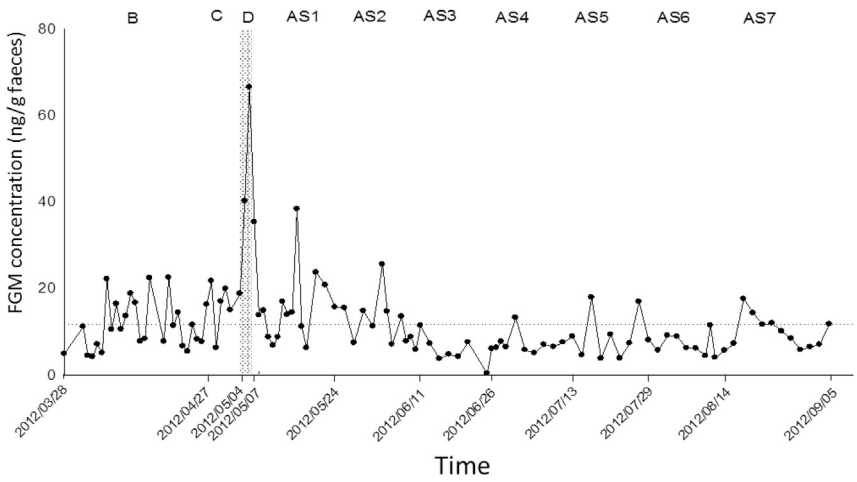

Fig. 3. Development of the overall FGM concentration in white rhinoceros during different phases of the translocation process (B, C, D, AS1, 2, AS3-7). The dotted line shows the mean FGM baseline concentration at Sanya breeding center.

Table 3
FGM concentrations before (B, C), during (D), after transport (AS1, 2) and after normalization (AS3-7).

Phases	Number of samples	Mean FGM concentration (ng/g fresh feces±SE)
Pre-transport (B, Sanya baseline)	170	14.81 ± 1.37
Pre-transport (B, Shilin baseline)	90	7.39 ± 2.48
Capture (C)	45	18.96 ± 2.82
Transport (D)	18	37.16 ± 10.66
Recovery (AS1, AS2)	122	17.27 ± 1.44
Normalized (AS3, AS4, AS5, AS6, AS7)	341	9.34 ± 0.45

Mann-Whitney U tests: B_{Sanya} *vs* C: $p > 0.05$; B_{Sanya} *vs* D: $p < 0.01$; B_{Shilin} *vs* D: $p < 0.01$; B_{Shilin} *vs* AS1-2: $p < 0.05$; B_{Shilin} *vs* AS3-7: $p > 0.05$.

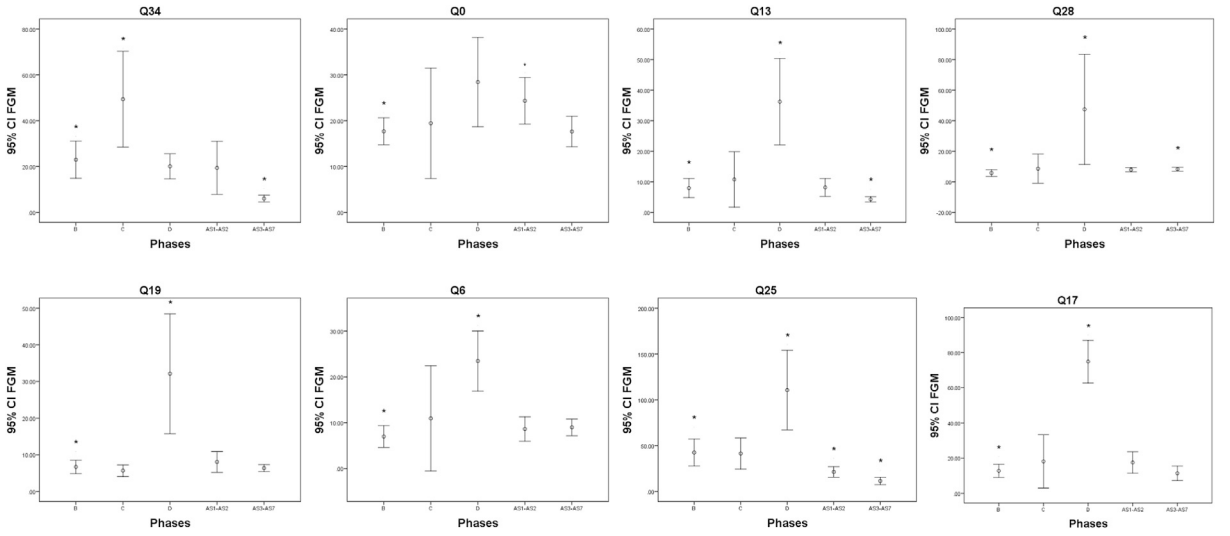

Fig. 4. The difference of individual FGM concentrations of eight white rhinoceros during different phases compared to their own baseline values in Sanya. Note: '*' means significant difference.

(Fig. 4); Q0 restore to the FGM baseline concentration in the phase (AS3-7) (Mann-Whitney *U* test: $N = 8$, $p > 0.05$); only the individual Q25 hormone levels is changing all the time, there is no back to the baseline after transportation (Mann-Whitney *U* test: $N = 8$, $p < 0.05$) (Fig. 4).

4. Discussion

Generally, the stress status of an animal can be assessed by behavioral observations, the vital signs or its endocrinology (Estes, 1991; Hopster and Blokhuis, 1994; Brown et al., 2001; Carlstead and Brown, 2005, Von Borell et al., 2007). Moreover, the respiratory and heart rate are also commonly used to estimate the nervous system's response to physiological stress (Hopster and Blokhuis, 1994; Von Borell et al., 2007). The behaviors of white rhinoceros can be divided into communication behavior, parent-offspring behavior, antagonistic behavior, reproductive behavior and defensive behavior (Estes, 1991), whereby the latter three are known to be linked to physiological stress. We therefore, assumed that increased frequencies of antagonistic and defensive behavior are the consequence of physiological stress and can be used to indirectly assess the stress status of individual white rhinoceros during and after transport.

As predicted, increased short-term stress levels had a strong impact on the vital signs of white rhinoceros after transport. Respiratory and heart rates, but also temperatures were significantly increased during and after transport (Table 2). Increased stress levels also influenced the behavior of white rhinoceros after transport. Behavioral observation revealed that the frequency of Horn Fencing (HF) and group behavior increased significantly during the first weeks after transport (Fig. 2), indicating that translocated white rhinoceros were eager to join the new community, but accompanied by intense physiological stress. High FGM concentrations co-occurred with increased frequencies of Horn Fencing, suggesting that this behavioral change was the result of physiological stress. Behavioral observations also showed that females increased the frequencies of group activities after transport (Fig. 2), suggesting that females were more adaptive to new environments and new communities than males. This result was expected since it corresponds to observation reported from white rhinoceros in their natural habitat (Estes, 1991).

High FGM concentrations are reported to reduce the food intake of animals (Makino et al., 1998). FGM tests showed that Q19, Q6, and Q17 had less physiological stress than other individuals post-transport, while their food consumption increased after transport (Figs. 2 and 4). The overall increasing frequency of Feeding (F) after transport indicated great energy consumption during transport. However, the increasing frequency of Feeding varied among individuals. Q0, Q6 and Q28 showed a higher Feeding frequency than others, which might be due to the lower FGM levels. Alongside increased FGM concentrations, increased respiratory rate and heart rate may have also caused the higher energy consumption and thus the increased food intake. This finding implies that supplementary food should be provided to white rhinoceros on transport.

Individuals with increased physiological stress before transport could easily turn to distress during the first week after transport (Smith et al., 1997; Linklater et al., 2010). For example, capture caused the FGM concentration of individual Q34 to soar, but rapidly decreased again during transport (Fig. 4), probably due to the intrinsic physiological control of the animal. After transport, the FGM concentration, but also the heart rate, increased slightly again but normalized over a period of three months after transport (AS3-7; Fig. 4). The above observation strongly indicates that Q34 experienced severe distress, especially during the capture. Individuals with previous transport experience, such as Q0, did not have physiological stress during the entire translocation process since heart rate and FGM level were both relatively stable during and after transport.

In several individuals (Q28, Q19, Q17; Fig. 4), the FGM level declined after transport, even below the baseline concentration. Linklater et al. (2010) attribute this phenomenon to an active FGM suppression.

The FGM baseline concentration established in the Sanya breeding center was significantly higher than that measured in white rhinoceros from the Shilin breeding center. When comparing the climate and the captive environments of the two breeding centers, it became apparent that the climate and altitude of Shilin breeding center were closer to that experienced by white rhinoceros in their natural habitats. It was therefore assumed that the FMG concentration declined after transport due to an improvement of environmental and climatic conditions, rather than physiological stress suppression as described by Linklater et al. (2010) for white rhinoceros or by Viljoen et al. (2008) for African elephants (*Loxodonta africana*). Turner's et al. (2002) study on black rhinos (*Diceros bicornis*) concluded that they need six weeks to recover from transport stress. The authors used declining corticoid levels as evidence for acclimatization to the new environment but without establishing a comparative pre-capture corticoid concentration. Turner et al. (2002) conclusion could, therefore, not prove that FGM concentration declined due to an adaption to the new environment.

Linklater et al. (2010) attributed the decline in FGM concentration after transport to distress and the function of intrinsic control through increased adrenaline levels. However, Linklater et al. (2010) did not consider the geographic and climatic differences between locations. In this study, FGM concentrations showed a remarkable regularity amongst individuals with declining FGM levels during the first week's post-transport and normalization to pre-transport levels in the months thereafter (Fig. 4). Similar physiological processes were also described from other species, such as the African elephant (Viljoen et al., 2008). In our study, an average of about 32 days was needed for white rhinoceros to recover to FGM baseline concentration after transport stress occurred. However, due to pronounced individual differences, this time varied from 0 to 45 days. For example, in Q19, Q6 and Q17 the post-transport FGM concentration rapidly declined to baseline in phase AS1 (i.e., within 15 days) while in Q0 the FGM concentration remained constant throughout the translocation process. There is showed no significant physiological stress before and during the transport, which was likely due to their previous experiences of transport (Grandin, 1997). Individuals Q19 and Q17 showed a FGM trend similar to the overall FGM development established in this study. However, it needs to be investigated whether the rapid normalization in individuals like Q25 and Q28 is due to the individuals' ability to quickly adapt to the new environment or to a strong intrinsic physiological control (Smith et al., 1997).

The study on individual differences in white rhinoceros during translocation will help to improve the management and welfare of transported rhinoceros. For individuals with an intense intrinsic physiological control, supplementary feeding at quarantine is necessary to help those individuals to acclimatize faster to the new environment after transport. By contrast, individuals with healthy physiological response and/or transport experience could be integrated into the new environment soon after being transported. Moreover, it would be advisable to combine well habituated individuals and those with intense intrinsic physiological control during transport in order to level out exaggerated stress responses and to calm down inexperienced individuals.

5. Conclusions

The method of FGM measurement has provided researchers an accurate pathway to understand the endocrinology of wildlife in captivity (Brown et al., 2001). Periods of stress and tension could be regularly monitored, recognizing changes in time, and therefore providing time to improve the health and welfare of captive wildlife. From the point of view of wildlife management, it is imperative to know the time and intensity tress occurs, but also the means to rapidly decline stress once it occurs. Our study contributed to this task by providing the following recommendations:

(1) The overall FGM development during the translocation process showed a remarkable regularity but also an apparent individual difference.
(2) Individuals stress responses shall be determined by regular FGM measurements in combination with monitoring vital signs and behavior.
(3) Keeping the white rhinos' endocrinology stable prior to transport is the key to avoid distress during transport.
(4) It is necessary to provide a relatively stable environment during the first 30 days post-transport to allow for physiological stress recovery.
(5) Transport experience can reduce translocation stress.

Author contributions

Yang LL, Lu J and Deng HQ conceived and designed the study, Yang LL, Wang WX and Wang Y performed the experiments and collected the data, Wronski T and Huang SL processed the data and wrote the manuscript. All authors reviewed the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2019.e00588.

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