# Can stress and anxiety be assessed in African elephants (Loxodonta africana) using self-directed behaviour?

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## Highlights

- Semi-captive African elephants were shown to use self-directed behaviour (SDB).
- SDB rates were higher when elephants engaged in walk & ride tourist interactions.
- SDBs were not linked to faecal glucocorticoid metabolite concentrations.
- SDBs may be used as an indicator of anxiety or lower-level stress in elephants.
- SDBs could be used as part of a welfare assessment or to ensure optimum husbandry.

## Abstract

Captive African elephants used in the tourism industry face numerous welfare issues which are often stress related and linked to high numbers of tourists or human-elephant interactions. Elephant welfare is commonly assessed by quantifying faecal glucocorticoid metabolite (fGCM) concentrations, and monitoring stereotypic behaviour, which are either costly or problematic in identifying underlying causes. Self-directed behaviours (SDBs), a form of displacement activity, have been substantially linked with stress and anxiety in primates, and could be a potential but not yet utilised behavioural marker in elephants. We thus explored the usage of several trunk, tail, and body related SDBs in a group of 7 semi-captive African elephants maintained at the Knysna Elephant Park, a tourist destination offering a variety of close contact experiences. Using continuous behavioural sampling (focal following), SDBs were recorded as they occurred, along with the numbers of nearby tourists (<5 m to focal; with 3 levels: None, Low 1–5, and High >5), and the nature of the tourist interaction (5 levels: None, Feed, Touch, Walk, and Ride). We then compared SDB rates with fGCM concentrations ($n$ = 115) determined following observations (1 day and 2 days after behavioural sampling). Data were analysed using Generalized Linear Mixed effects Models. SDB rates (p/min) significantly increased during elephant Walks ($p < 0.001$) and Rides ($p < 0.001$), indicating a correlation with their usage during potentially stressful scenarios. The Touch interaction significantly decreased SDBs ($p < 0.05$), whilst the Feed interaction had a non-significant effect ($p > 0.05$), which could indicate the element of control in terms of perceived stress is an important component in welfare, as elephants were free to move away during these interactions. Interestingly, SDBs significantly decreased when tourist numbers were High ($p$ = < 0.05), potentially due to greater quantities of high value food. Additionally, no correlation was found between SDBs and fGCM concentrations determined on day one ($p > 0.05$), and day two ($p > 0.05$). Hence, SDBs may be correlated more with anxiety or lower-level stress, which is not significant enough to activate GC production, and may therefore act as a coping strategy utilised to maintain physiological homeostasis during anxiety-inducing situations. Additional research would benefit from coupling SDB observations with different forms of physiological assessments to better understand the internal motivations, and formally establish SDBs as a reliable, cost-effective, and non-invasive welfare index to identify stressed individuals in real time and ensure optimum husbandry.

**Keywords**

Captive elephant; Welfare assessment; Non-invasive;  - Glucocorticoid metabolites; Displacement activity; Wildlife tourism

**Abbreviations**

SDB: Self-directed behaviour
fGCM: Faecal glucocorticoid metabolite

## 1. Introduction

Captive elephants face greater welfare challenges than other species due to their considerable size, complex social behaviour, and high level of intelligence, which renders meeting their needs whilst maintaining optimum welfare taxing (Veasey, 2006). This is confounded further by their use in the tourism industry, whereby high levels of visitors, human-elephant interactions, and participation in walks and elephant-back rides can provoke a stress response and negatively impact welfare (Bryant and Wielebnowski, 2018, Lama, 2017, Millspaugh et al., 2007, Szott et al., 2019). Assessments of captive elephant wellbeing are therefore crucial to ensuring optimum husbandry. The most commonly used validated methods for evaluating welfare, particularly in terms of stress, are measuring glucocorticoid (GC) output, and monitoring stereotypic activity (Clubb and Mason, 2002, Mason and Veasey, 2010). But these approaches involve costly laboratory testing, in case of quantifying GCs or their metabolites; or in the latter instance, occur once the animal has already developed stereotypies following severe or consistent pressures, a learnt behaviour that can remain even after the original stressors are resolved (Harris et al., 2008). There are, however, forms of established behavioural indices in other species that have seldom been used for evaluating welfare in elephants. Displacement activities, which are behaviours that arise out of motivational conflict and appear irrelevant to the context (Tinbergen, 1952), are regarded as reliable and non-invasive measures of stress and anxiety in non-human primates (Maestripieri et al., 1992), and are a potential but not yet utilised index of welfare in captive elephants (Mason and Veasey, 2010).

Self-directed behaviours (SDBs) are the most common form of displacement activity in primates, and are related to body care maintenance such as self-scratching, auto-grooming, and self-touching (Castles et al., 1999, Daniel et al., 2008, Maestripieri et al., 1992). Numerous studies have demonstrated increased SDBs above baseline levels during stressful situations in primates; for example, in hamadryas baboons (*Papio hamadryas*) with elevated psycho-social stress (Plowman et al., 2005) or in vervet monkeys (*Chlorocebus pygerythrus*) following an agonistic attack (Daniel et al., 2008). In non-social contexts, studies have found chimpanzees (*Pan troglodytes*, Leavens et al., 2001) and mandrills (*Mandrillus sphinx,* Leeds and Lukas, 2018) to show significantly more SDBs during cognitive stress exercises, and captive stump-tailed macaques (*Macaca arctoides*) to display higher rates of SDBs in situations of temporal predictability loss (Waitt and Buchanan-Smith, 2001). Elephants also exhibit displacement activity and auto-related behaviours, but this index has not been fully explored. Several authors discussed trunk manipulation/ self-touching behaviours in free-ranging African elephants (*Loxodonta africana*), and attributed this to states of internal conflict (Douglas-Hamilton, 1975) or situations of apprehension and/or distress (Mason and Veasey, 2010, Douglas-Hamilton et al., 2006, Goldenberg and Wittemyer, 2020). However, the connection between SDBs and potential implications of the elephant's mental state in terms of welfare has not been made to date. In a preliminary study, Jim (2015) observed numerous trunk-related self-touching behaviours in 7 semi-captive African elephants that were at significantly higher rates during walk and elephant-back ride tourist interactions. Since captive working elephants are known to become agitated following tourist interactions and activities (Lama, 2017, Millspaugh et al., 2007), this provided the first evidence that SDBs could be utilised for assessing African elephants during potentially stressful scenarios.

Behavioural assessments of stress may be subjective (Pretorius, 2004, Weary et al., 2006) particularly when exploring novel approaches, and therefore, the addition of physiological parameters recorded simultaneously to behaviour can produce more robust conclusions. The most frequently used bio-markers for physiological stress are glucocorticoids (e.g. Brown et al., 2019). When an animal perceives a meaningful stressor, the activated hypothalamic-pituitary-adrenal (HPA) axis releases glucocorticoids which, alongside catecholamines, are an essential aspect of the physiological response to stress, and of maintaining homeostasis (Burchfield, 1979, Ganswindt et al., 2010, Möstl and Palme, 2002, Touma and Palme, 2005). Hence, quantification of glucocorticoids or their metabolites has been established to assess welfare in captive elephant populations utilising blood (Palme et al., 2000), saliva (Dathe et al., 1992), faeces (Chichilichi et al., 2018), and urine (Brown et al., 1995). More recently, non-invasive approaches such as faecal steroid quantification have been favoured, to avoid the stressful and confounding act of capture and restrain for sampling (Bertoli et al., 2019, Touma and Palme, 2005). Moreover, faecal glucocorticoid metabolites (fGCM) are less affected by the circadian rhythm since they represent the accumulation rate of hormones throughout a set time period, and are less influenced by episodic variations or the pulsality of hormone secretion (Viljoen et al., 2008; Touma and Palme, 2005). The validity of fGCM quantification to monitor stress in elephants has been demonstrated in both wild (e.g. Ganswindt et al., 2010) and captive populations (e.g. Bryant and Wielebnowski, 2018).

The aim of this study was to investigate the usage of SDBs in 7 semi-captive African elephants maintained at the Knysna Elephant Park during potentially stressful situations associated with several human-elephant interactions, or high tourist volume. Various trunk and tail related self-directed behaviours were recorded during these circumstances, and correlated with fGCM concentrations. We hypothesized that SDB rates would be elevated during scenarios with high levels of tourist numbers, and high intensity human-elephant interactions (i.e., walks and elephant-back rides), and that SDBs would also correlate with elevated fGCM concentrations. Ultimately, we intended to establish the behaviour set as a novel, non-invasive, and reliable measure of welfare in African elephants.

## 2. Material and methods

### 2.1. Ethical statement

This study was approved by the University of Exeter ethical review process, and ethical clearance was granted prior to data collection (Reference number: 2016/1168). The authors have read the policy relating to animal ethics and confirm that our study complies. All aspects of this study were non-invasive/ observational; the study animals were not manipulated in any way.

### 2.2. Study area and animals

The study took place at the Knysna Elephant Park, a private reserve located in the Western Cape of South Africa (34°02'16.7"South, 23°16'04.6"East). The Park comprises 60 ha and consists predominantly of grass and indigenous shrubland, with areas of forest, deep tree-lined valleys, and two large reservoirs of water. Observations were conducted over a 10-week period during South African Autumn/ Winter months (May-August 2016) with weather fluctuating between heavy rain and dry, hot days, and a mean daily temperature ranging from 12 °C to 16 °C. The reserve is home to 7 semi-captive African elephants, who are intensely managed. At the time of the study, the elephants' ages ranged between 8 and 26 years, including two sexually immature bulls (Table 1). All but two, a mother-daughter pair (Nandi and Thandi), are unrelated. The African Elephant Research Unit (AERU) is based on site, and since research is conducted almost daily, elephants are habituated to researchers in the field. The Park also has daily tourist visits consisting of feeding experiences every 30 mins, and up to twice-daily walks and rides at the start and end of each day. The elephants roamed the park freely in between these different experiences. When required for work, elephant handlers use voice commands to direct the elephants to their positions; a process they have been trained to do using positive reinforcement methods.

**Table 1.** Elephant ID, sex, age class (Juvenile 3–8 yrs; Young adult 9–18 yrs; Adult 19–35 yrs), origin, total number of fGCM samples per individual, and total number of SDBs recorded per individual during the study period May-August 2016, of the Knysna Elephant Park study population (*n* = 7).

| Elephant ID | Sex | Age class | Origin | fGCM samples | Total SDBs |
|---|---|---|---|---|---|
| **SY** | Female | Adult | Kruger National Park | 19 | 2896 |
| **NI** | Female | Adult | Thabazimbi Game Reserve | 19 | 1129 |
| **TI** | Female | Young adult | *Born at Knysna Elephant Park* | 19 | 1746 |
| **KA** | Female | Young adult | Kruger National Park | 20 | 1496 |
| **TO** | Female | Juvenile | Madikwe National Park | 20 | 927 |
| **MU** | Male | Juvenile | Madikwe National Park | 10 | 2103 |
| **SU** | Male | Juvenile | *Born at Knysna Elephant Park* | 9 | 1381 |

### 2.3. Behavioural sampling

Observations were pseudo-randomly scheduled to ensure equally distributed data collection across all days, and occurred between 8.00 am and 5.30 pm. The ethogram for SDBs (Table 2) was developed by AERU, and primarily consists of trunk-related behaviours, with the inclusion of body behaviours to account for actions which may occur when the trunk is occupied. Observers (*n* = 5) were trained to identify behaviours to a high degree of inter-observer reliability (≥ 80%). Inter-observer reliability was evaluated following two examination observation sessions, which were conducted after training had been completed. Simultaneously collected data by both the trainee and the

**Table 2.** Ethogram of elephant self-directed behaviours (SDB) considered in this study. The ethogram includes the name, abbreviation, and description of each individual behaviour. Developed by AERU.

| Behaviours | Abbreviation | Definition |
|---|---|---|
| **Trunk Behaviours** | | |
| **Trunk swing** | T-swing | Swing trunk side to side or forward to back at least twice |
| **Trunk twist** | T-twist | Trunk twists and then untwists at a moderate pace |
| **Trunk curl** | T-curl | Trunk is curled at the end with the tip sticking out to the side and held for at least 2 s |
| **Trunk suck** | T-suck | Trunk tip placed in own mouth and lips closed around it for at least 2 s |
| **Trunk out curl** | T-oc | Trunk tip is pointing outwards but the lower trunk is curled behind the upper trunk |
| **Trunk to head** | T-head | Trunk tip touches or rests on own head |
| **Trunk to mouth** | T-mouth | Trunk tip touches own mouth (not flehmen to place food or an object in the mouth) |
| **Trunk to ear** | T-ear | Trunk tip touches or scratches own ear |
| **Trunk to eye** | T-eye | Trunk tip touches or rubs own eye |
| **Trunk to temporal** | T-temp | Trunk tip touches or hovers within 10 cm of own temporal gland (TG) or TG secretion |
| **Trunk to trunk** | T-trunk | Trunk tip touches a higher part of own trunk, position held for at least 2 s |
| **Trunk to tusk** | T-tusk | Trunk tip touches own tusk |
| **Trunk to body** | T-body | Trunk tip touches own torso or back |
| **Trunk to leg** | T-leg | Trunk tip touches own leg above the ankle |
| **Trunk to foot** | T-foot | Trunk tip touches own foot below the ankle |
| **Body behaviours** | | |
| **Head shake** | HS | Shake or toss head with a fast-rotating motion |
| **Tail swish** | TS | Fast directed movement of the tail to own body (record only once until the tail has been still > 5 s) |
| **Leg swing** | LS | Lift either fore foot and move it back and forth at least twice, without hitting the ground |

trainer had to be similar by ≥ 80% on both examination occasions to be regarded as acceptable. For a period of 30 mins, one to three times a day, focal animals were surveyed using behavioural sampling continuous recording (1 tally on the data recording sheet = expression of 1 behaviour). During this period, all instances of SDBs displayed by the focal individual were documented. In addition, the number of tourists (within a 5 meter proximity) and nature of the tourist interaction in which the focal elephant was engaged (None, Feed, Touch, Walk, or Ride) were noted. Tourist interaction types were defined as follows: “None” - no tourist interaction was occurring and no tourists within 5 meters; “Feed” – elephants voluntarily stood behind a designated feeding barrier whilst tourists fed fruit buckets to them; “Touch” – following feeding, groups of tourists were brought to within 5 meters of focal elephant and could touch them (the elephant was free to move away); “Walk” – elephants were guided to walk along a predestined path for 20–30 mins, with tourists walking alongside them (individuals were obligated to participate); and “Ride” – elephants were guided along the same predestined path but with a guide and tourist sitting on a rug on their back (all elephants were obligated to participate except for Nandi and Thandi). Tourist numbers were considered ‘Low’ if there were between 1 and 5 people within 5 meters of the focal elephant, and ‘High’ if 6 or more people were present within 5 meters of the focal elephant, based on ticket sales and seasonal trends. A voice recorder was used for efficiency and safety during walks and rides and later transcribed. A total of 135 h of observations were conducted during 30 days across the 10 week study period, with 108 h collected during field shifts, and 27 h of walks and rides. From these observations, a total of 11,725 SDBs were recorded (see Table 1 for total SDBs per individual). The elephants spent the majority of time observed, 85 of the 135 h (62.9%), not involved in any tourist interaction (None).

### 2.4. Faecal sampling and steroid extraction

A total of 115 faecal samples were collected from the 7 focal elephants (Table 1). Samples were collected either the day after SDB sampling ($n$ = 63) or two days after ($n$ = 52), to reflect the gastrointestinal passage of time in African elephants, with fGCM concentrations typically peaking 24–36 h after ACTH stimulation, but still being evident for up to 60 h (Ganswindt et al., 2003). All faeces were collected between 7.00 am and 12.30 pm on the respective day. Samples were obtained within 30 mins post-defecation using latex gloves, and were retrieved from the centre of the dung to avoid cross-contamination with urine or faeces from other individuals in the area (Ganswindt et al., 2003). Samples were put on ice immediately, frozen at − 20 °C within 10 mins – 2 h of collection, and kept frozen until further processing at the Endocrine Research Laboratory, University of Pretoria, South Africa.

Frozen samples were lyophilized for 24 h, pulverized, and sifted to remove solid matter (Fieß et al., 1999). Between 0.050–0.055 g of faecal powder was then mixed with 3 ml 80% ethanol, and the suspension vortexed for 10 mins and centrifuged for 15 mins at 2600 $g$. The supernatants (1 ml) were transferred into microtubes and dried down in a freeze dryer (Modulyo, Pirani 501, Edwards, UK) for 16 h at 45–50 °C.

### 2.5. fGCM quantification

Immunoreactive fGCM concentrations were measured using an enzyme immunoassay (EIA) detecting fGCMs with a 5$\beta$-3$\alpha$-ol-11-one structure following the protocol of Ganswindt et al. (2003). Detailed assay characteristics, including full descriptions of the assay components and cross-reactivities, have been provided by Möstl and Palme (2002), and the EIA has been shown to reliably measure alterations in fGCM concentrations in the species (Ganswindt et al., 2003, Viljoen et al., 2008). The sensitivity of the EIA was 1.2 ng/g faecal DW (11oxoaetiocholanolone I EIA). Serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curves, with relative variation of the slope of the trend lines < 6%. Intra-assay coefficients of variation (CV) of quality controls were 3.3% and 5.6%, and Inter-assay CV were 6.7% and 8.0%. All analyses have been conducted at the Endocrine Research Laboratory, University of Pretoria, South Africa.

### 2.6. Statistical analysis

The predictor variables considered against SDBs were tourist interaction type, numbers of tourists, age class, and sex; all variables were considered categorical and had multiple levels. Tourist interaction type had 5 levels to define each of the possible interactions experienced by the elephants (None, Feed, Touch, Walk, and Ride). The number of tourists was divided into categories of None, Low (1−5), and High (>5). This was defined as the number of tourists present within a 5 meter radius per elephant when tourist levels were Low compared to High (as considered by Knysna Elephant Park based on daily ticket and feeding bucket sales). The ages of the individuals were divided into age class categories; based on the classification of Wittemyer et al. (2021), age classes considered were Juvenile (3–8 yrs), Young adult (9–18 yrs), and Adult (19–35 yrs). In order to reflect the tourist interaction type and number of tourists occurring at the same time as SDBs, data were split into 'bouts', whereby every time one of the two tourism factors changed, each period was considered as a separate data point. SDBs were grouped together to create the total number of SDBs per bout, and the time (Mins) spent in each of these periods was recorded. Total SDBs (SDB) per bout was the response variable, and elephant identity was included as a random effect to account for repeated sampling on the same individuals.

Data were analysed using Generalized Linear Mixed effects Models (GLMMs). Since data represented counts, a Poisson distribution was fitted. Due to the high variability between the duration (Mins) of each bout, Mins was included as an offset term on the log scale (due to the log link function of the Poisson family distribution), which enabled the regression coefficients to represent the response variable (SDBs) as rates per min. Due to the small sample size, predictor variables were fixed into separate models to enable greater accuracy and reliability. Age class and sex had no significant effect and were subsequently excluded from consideration. Models with a Poisson family distribution and log link function were trialled with the predictor variable (numbers of tourists or tourist interaction type) fixed into the model, Mins as an offset term, and elephant identity as a random factor. None was set as the reference category for tourist interaction type and number of tourists categories to enable conclusions to be drawn from the comparisons with the control (None/ None respectively). Diagnostics tests were carried out using the "DHARMa" package (v 0.4.5; Hartig, 2022), which used a simulation-based approach to assess if the model was correctly specified. Goodness-of-fit tests on the scaled residuals were conducted, and revealed severe overdispersion. Several solutions were trialled, and the best fitting model was selected based on DHARMa test diagnostics, and comparing AIC values, residual standard deviation/ degrees of freedom, and Chi-square overdispersion tests. Data were subsequently analysed using a GLMM ("glmmTMB" package; Brooks et al., 2017) with a Negative Binomial (NB) distribution due to its built in overdispersion parameter. The final models were all checked for over/underdispersion, outliers, heteroscedasticity, uniformity, and zero-inflation using DHARMa by plotting Q-Q probability plots, Residuals vs Predicted plots, and carrying out the DHARMa tests. Model 1 (SDB ~ Tourist interaction type) was still over dispersed despite the change in distribution family; adding age class into the model rectified this.

Post hoc tests using Estimated Marginal Means (EMMs) contrasts were conducted on each of the 2 tourism predictor variables. Because a log link was used with the NB distribution, intervals were back-transformed from the log scale during EMM pairwise contrasts ("emmeans" package; Lenth, 2021). Due to the mixed model composition of the GLMM, the presence of additive random components biases the expected values ("emmeans" package; Lenth, 2021); thus, bias was adjusted for by specifying sigma as the estimate of the Standard Deviation (SD) of the random effect. An offset parameter was specified as 0 to enable predictions to become rates which are relative to the offset specified in the model ("emmeans" package; Lenth, 2021); thus, estimates are presented as rates per unit value of the logged offset. As a result, EMMs are reported on the response scale at a rate of SDB per min. EMM contrasts were plotted using the "ggplot the response" function ("ggplot_the_model" script, Walker, 2021), and bar charts specifying SDB rates per min in each of the different conditions were constructed using ggplots ("ggplot2″ package; Wickham, 2016).

Next, to assess the presence of a correlation between SDBs and fGCM concentrations, SDBs were standardised to rates per elephant per day by dividing the total number of SDBs by the total number of mins of observation, providing us with a mean daily SDB rate for each elephant. These mean SDB values were matched to faecal samples obtained the following day/days (as described above). Since faecal samples were not always obtained, only mean SDB data points which had a coinciding faecal sample were considered, leaving 115 mean SDB data points and 115 matched faecal glucocorticoid metabolite samples to be entered into analyses. Data were split based on day of collection, with 63 samples occurring after 1 day (14–29.5 h after observation; min = 0.08 μg/g, max = 0.77 μg/g, median = 0.42 μg/g), and 52 samples occurring on the second day (38–53.5 h after observation, min = 0.23 μg/g, max = 1.44 μg/g, median = 0.38 μg/g). Due to the variation in collection time on any given day, a GLMM was used to assess whether fGCM concentrations differed pre and post 9.00 am, whilst taking into account the individual elephants' identity. Faecal GCM concentrations were not statistically different with respect to time of collection ($SE$ = 0.045, $t$ = 0.483, $p$ = 0.630), and so all samples were included in the analyses. Raw data were plotted with each distribution option, using the fitdistr() function ("MASS" package; Venables and Ripley, 2002), and both day 1 fGCM values and day 2 fGCM values best fit a Gamma distribution, particularly due to the non-integer, continuous nature of the data. Faecal GCM concentrations were the outcome variable, and two separate models were run for each day; fGCM concentrations 1 day after SDB observations, and fGCM concentrations 2 days after SDB observations. The predictor variable fixed into both models was SDB rate per day (matched with coinciding fGCM concentration), with age class (Juvenile, Young adult, and Adult) and sex included to account for variation in hormone secretion due to these factors. Additionally, elephant identity was included as a random factor to control for repeated measures on the same individuals. Data were analysed using GLMMs ("glmmTMB" package; Brooks et al., 2017) with a Gamma distribution and log link function. The final models were once again checked for over/underdispersion, outliers, heteroscedasticity, uniformity, and zero-inflation using DHARMa by plotting Q-Q probability plots, Residuals vs Predicted plots, and carrying out the DHARMa tests ("DHARMa" package; v 0.4.5; Hartig, 2022). Mean daily SDBs and fGCM concentrations (1 and 2 days after SDB observation) were plotted using ggplots ("ggplot2″ package; Wickham, 2016). All statistical analyses were carried out in RStudio (v 2022.02.3, Build 492) and R (v 4.1.2; R Core Team, 2017).

## 3. Results

### 3.1. Effect of tourist interaction type on SDB rates

SDB rates (p/min per bout) significantly increased during the Walk (coef = 0.82, $SE$ = 0.17, $p < 0.001$) and Ride (coef = 0.63, $SE$ = 0.15, $p < 0.001$) tourist interaction types, and significantly decreased during the Touch (coef = −0.23, $SE$ = 0.09, $p$ = 0.012) interaction type (Fig. 1, Table 3). There were no significant changes in behaviour rates during the Feed interaction condition ($p > 0.05$). Age class was included as a covariate in the model and had no significant effect on SDB rates ($p > 0.05$). Estimated Marginal Means (EMM) pairwise contrasts (Fig. 1) revealed significant differences between Touch when compared to None ($t$ (908) = −2.53, $p$ = 0.012); Touch when compared to Feed ($t$ (908) = −2.08, $p$ = 0.038); Walk when compared to None ($t$ (908) = 4.79, $p < 0.001$); Walk when compared to Feed ($t$ (908) = 4.51, $p < 0.001$); Walk when compared to Touch ($t$ (908) = 5.68, $p < 0.001$); Ride when compared to None ($t$ (908) = 4.31, $p < 0.001$); Ride when compared to Feed ($t$ (908) = 4.03, $p < 0.001$); and Ride when compared to Touch ($t$ (908) = 5.41, $p < 0.001$). There was no statistical difference between Feed and None ($t$ (908) = −0.02, $p$ = 0.982); and Ride and Walk ($t$ (908) = −0.84, $p$ = 0.401).

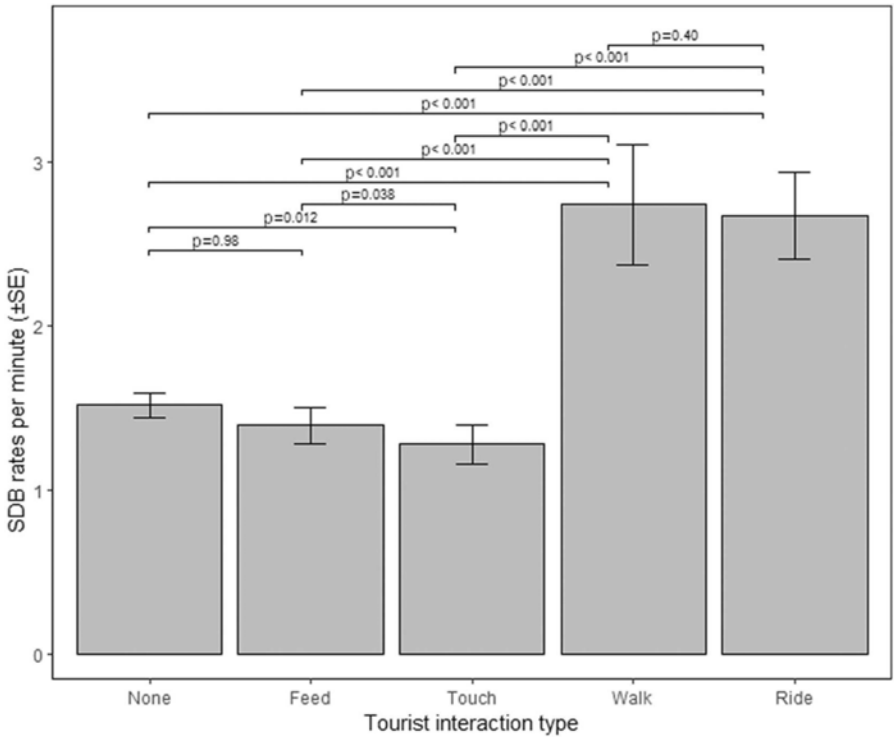


**Fig. 1.** Mean SDB rates scaled to SDBs per min per bout for the African elephants (*n* = 7) housed at Knysna Elephant Park during each of the 5 different tourist interaction types (None, Feed, Touch, Walk, and Ride). Error bars represent Standard Error of the scaled data.

**Table 3.** Coefficient estimates and associated *SE*s and *p*-values from the Generalized Linear Mixed effects Model (GLMM) analysis, with Estimated Marginal Means (EMM) post hoc tests (reported on the response scale, showing estimated negative binomial rates with offset = 0, corrected for mixed model bias by specifying sigma as the standard deviation estimate of the random effect) and corresponding confidence limits, of the self-directed behaviour (SDB) rates in the African elephant study population (*n* = 7) at Knysna Elephant Park between May – August 2016. Coefficients indicate the influence of each variable (tourist interaction type or tourist numbers categories) on the frequency of elephant SDB rates (1 unit increase in coefficient estimate indicative of an increase/ decrease of SDBs per min spent in each condition). None was set as the reference category for tourist interaction type and tourist numbers category.

| Fixed factor | Coefficient estimate | *SE* | EMM (response) | Lower CL | Upper CL | *p* |
|---|---|---|---|---|---|---|
| **Model 1 - SDBs ~ Tourist interaction type + Age + (1\|Elephant) + offset(log(Mins))** | | | | | | |
| **None (ref)** | | | 13.0 | 9.20 | 18.3 | |
| **Feed** | -0.00 | 0.09 | 13.0 | 9.01 | 18.7 | 0.982 |
| **Touch** | -0.23 | 0.09 | 10.3 | 7.13 | 14.9 | 0.012 * |
| **Walk** | 0.82 | 0.17 | 29.4 | 18.49 | 46.8 | < 0.001 *** |
| **Ride** | 0.63 | 0.15 | 24.5 | 15.91 | 37.7 | < 0.001 *** |
| **Model 2 - SDBs ~ Tourist numbers category + (1\|Elephant) + offset(log(Mins))** | | | | | | |
| **None (ref)** | | | 13.6 | 10.34 | 17.9 | |
| **Low** | 0.04 | 0.08 | 14.2 | 10.61 | 19.0 | 0.594 |
| **High** | -0.28 | 0.09 | 10.2 | 7.56 | 13.9 | 0.002 ** |

### 3.2. Effect of number of tourists on SDB rates

High numbers of tourists appeared to significantly decrease SDB rates (coef = −0.28, *SE* = 0.09, $p = 0.002$, Fig. 2, Table 3), whilst the Low tourist number category had no significant effect on SDB rates ($p > 0.05$). EMM pairwise contrasts (Fig. 2) revealed a significant difference when there were High tourist numbers compared to no tourists (None; $t$ (912) = −3.06, $p = 0.002$), and when there were High tourist numbers compared to Low ($t$ (912) = −3.10, $p = 0.002$). EMM pairwise contrasts also showed no statistical difference between Low when compared to None ($t$ (912) = 0.53, $p = 0.595$).

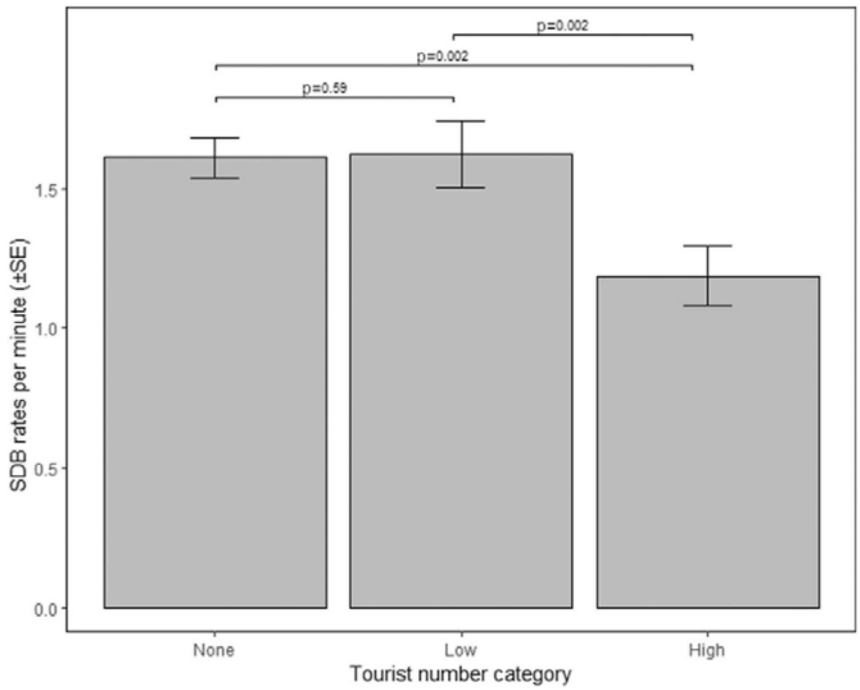


**Fig. 2.** Mean SDB rates scaled to SDBs per min per bout for the African elephants ($n = 7$) housed at Knysna Elephant Park during each of the 3 different tourist number categories (None = 0, Low = 1–5, and High = 6 +, in < 5 meters to focal elephant). Error bars represent Standard Error of the scaled data.

### 3.3. Correlation of SDB rates with fGCM concentrations

SDB rates were not significantly correlated with fGCM concentrations determined on day one (coef = 0.02, $SE = 0.04$, $p = 0.576$), and day two (coef = 0.03, $SE = 0.06$, $p = 0.649$) post behavioural observations (Fig. 3, Table 4). Age class and sex were also found to have no significant relationship with fGCM concentrations (all $p > 0.05$).

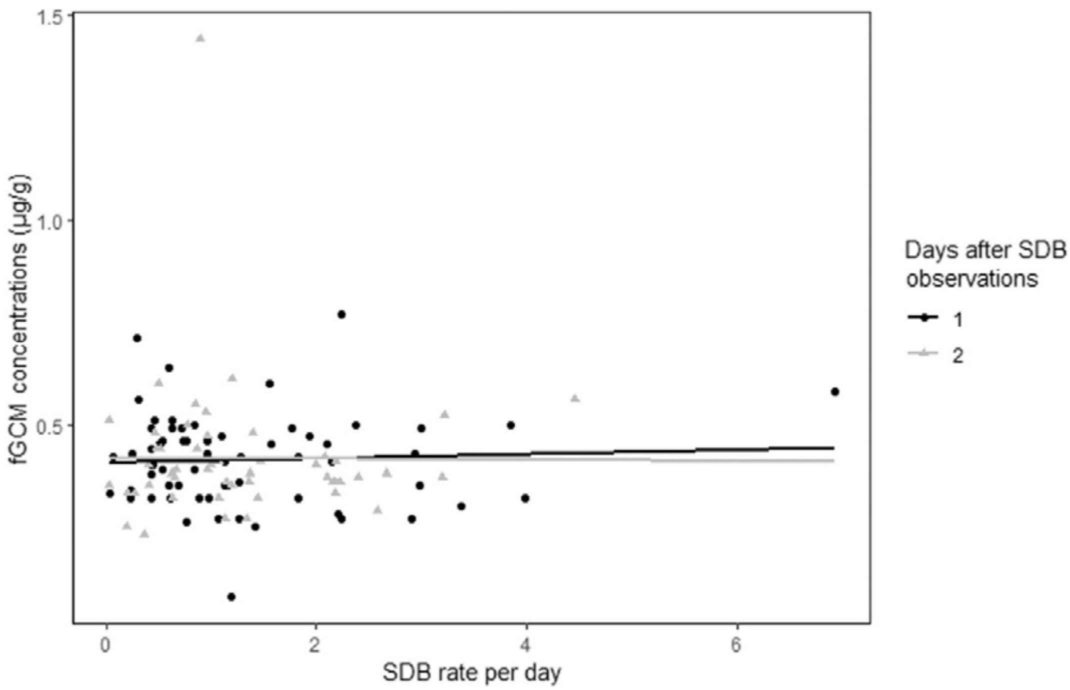


**Fig. 3.** Faecal glucocorticoid metabolite (fGCM) concentrations (µg/g DW) collected 1 day (black/ solid) and 2 days (grey/ dashed) after behavioural observations, against self-directed behaviour (SDB) rates standardised to rates per day for the African elephants ($n = 7$) housed at Knysna Elephant Park. Trend lines represent a linear model.

**Table 4.** Coefficient estimates and associated *SE*s and *p*-values from the Generalized Linear Mixed effects Model (GLMM) analysis, of the fGCM concentrations (µg/g DW) in the African elephants ($n = 7$) housed at Knysna Elephant Park between May – August 2016. Coefficients indicate the influence of SDB rates per min per day on the fGCM concentrations collected 1 day and 2 days after self-directed behavioural observations (1 unit increase in coefficient estimate indicative of an increase/ decrease in fGCM concentrations per SDB performed). Age class and sex were included in the model to account for hormonal variation based on these factors.

| **Fixed factor** | **Coefficient estimate** | ***SE*** | ***p*** |
|---|---|---|---|
| **Self-directed behaviour against faecal glucocorticoid metabolite concentrations sampled 1 day after behavioural observations** | | | |
| **SDB rate p/m p/d** | 0.02 | 0.04 | 0.576 |
| ***Age class*** | | | |
| **Juvenile** | 0.16 | 0.13 | 0.213 |
| **Young adult** | 0.04 | 0.10 | 0.679 |
| ***Sex*** | | | |
| **Male** | 0.00 | 0.12 | 0.970 |
| **Self-directed behaviour against faecal glucocorticoid metabolite concentrations sampled 2 days after behavioural observations** | | | |
| **SDB rate p/m p/d** | 0.03 | 0.06 | 0.649 |
| ***Age class*** | | | |
| **Juvenile** | 0.19 | 0.16 | 0.282 |
| **Young adult** | 0.20 | 0.13 | 0.129 |
| ***Sex*** | | | |
| **Male** | -0.19 | 0.16 | 0.261 |

**4. Discussion**

We quantified SDB occurrence in a group of 7 semi-captive African elephants participating in tourism activities, and assessed variation in the frequency of these behaviours with respect to tourist interaction types, numbers of tourists, and fGCM concentrations, whilst controlling for sex and age class, and including elephant identity. We observed a significant increase in SDB rates during Walks and Rides, and a significant decrease in SDB rates during the Touch tourist interaction type. High numbers of tourists within 5 meters of the focal elephant significantly decreased SDB rates, whilst Low numbers of tourists had no effect. Furthermore, we found no correlation between SDB occurrence and fGCM concentrations.

The finding of the significant increase of SDB usage during Walks and Rides is in line with the conclusions of Jim (2015), who conducted a similar study on self-directed behaviours in the same population and also demonstrated SDBs above baseline levels during these tourist interactions. Our findings also fit with previous literature demonstrating behavioural and physiological stress responses to walk and ride interactions in this species (e.g. Lama, 2017; Millspaugh et al., 2007), which provides evidence for SDBs to be used in stressful scenarios. Interestingly, SDB rates decreased during the Touch interaction, and were not significantly affected by the Feed interaction, which is in contrast with previous studies demonstrating elevated stress levels in elephants following close-contact tourist interactions (e.g. Millspaugh et al., 2007). Our finding of an SDB decrease when tourist numbers were High also contradicts previous literature evidencing captive elephants to perceive this occurrence as stressful (Byrant & Wielebnowski, 2018). This could possibly be explained by the increase in the number of high value food resources (fruit buckets) each elephant received during this period, which could be positively reinforcing. Similar results have been seen in captive chimpanzees (*Pan troglodytes*), whereby individuals were motivated to interact with humans to obtain food (Cook and Hosey, 1995), demonstrating the possibility for captive individuals to view visitors more positively, and not necessarily as a source of stress. This might also explain the lack of correlation with SDBs and the Feed tourist interaction, since this occurrence involves large quantities of high value food. In accordance with this, captive giraffes (*Giraffa camelopardalis*) have been shown to exhibit a reduction in oral stereotypies during tourist feeding programs (Orban et al., 2016), which the authors speculate could indicate an environmental enrichment element through the alleviation of unsatisfied foraging motivations. It could in our case, however, also be due to an increase in trunk usage during periods of eating, and thus simply means less free time for the trunk to display SDBs. The same may be true of the significant SDB reduction in the Touch condition, if elephant handlers preferentially chose elephants that were already grazing for this type of interaction. Future research would benefit from assessing the physiological response with respect to these tourism factors, as well as the other variables at play during tourist interactions.

Additionally, the element of control may play a pivotal role. Koolhaas and colleagues (2011) state that if an individual perceives control over a situation, the stress experienced is negated. Thus, it might be possible that the elephants in this study have become habituated to some aspects of the obligatory participation during Feed and Touch interactions, and when tourist numbers within a close proximity are High, but that ultimately, they are able to move away if they choose to. This would also correspond with the findings during the Ride and Walk conditions, which are associated with a prolonged inability to control their own actions, and thus creating the uncontrollability element of stress. Potentially, these findings could indicate that welfare can be optimised by giving animals more choice over their interactions with tourists, and the freedom to move as they please. In support of this, Dorning et al. (2016) reported that captive animals, during an extended period of uncontrollability attempt to achieve control through maladaptive behaviour such as stereotypies.

Interestingly, SDB rates were not correlated with fGCM, and so we offer some potential explanations. Firstly, the primary role of glucocorticoids is to mobilize energy and suppress non-essential biological functions to overcome immediate threats (Sapolsky, 2002). Therefore, since the elephants at Knysna Elephant Park regularly experience tourist interactions, they are potentially habituated, and as such these interactions may not be meaningful enough to activate the HPA axis and increase glucocorticoid output. In support of this, a semi-captive elephant population engaging in elephant-back safaris did not have statistically higher concentrations of fGCM compared with a wild population in the same ecological environment (Grotto et al., 2020), thereby demonstrating that elephants frequently exposed to tourists do not show an increase in GC output. Secondly, the fact that no correlation was found between SDBs and fGCM could indicate that SDBs play a different role altogether. Hinde (1970) refers to displacement behaviours as actions which the animal displays during periods of anxiety or frustration to restore physiological homeostasis. Thus, SDBs may be more related to anxiety than stress (Maestripieri et al., 1992), especially since this behavioural index has long been considered an indicator of stress and anxiety in primates, but these two physiological occurrences are not interchangeable. In support of this, marmosets (*Callithrix penicillata*) administered with anxiolytic drugs minimised SDB usage following exposure to a mock predator (Barros et al., 2000). Additionally, Higham et al. (2009) found no significant correlation with SDBs and fGCM in female Olive baboons (*Papio anubis*), which the authors postulate could indicate SDBs are an indicator of short-term lower-level stress not associated with prolonged elevated levels of GC secretion. Ellis et al. (2011) reached the same conclusion by examining the correlation between SDBs and fGCM in male wild olive baboons. Thus, since fGCM levels are indicative of the accumulation of hormones (Touma and Palme, 2005), they may be too general to reflect fleeting moments of lower-level stress.

Whilst SDBs are an accepted method of welfare assessment in primates, previous research on this taxon has failed to conclusively link them with fGCM. This may provide evidence that the role of SDBs could be better explained as a form of self-pacification. In other words, they may have developed as an adaptive stress appeasement strategy to cope with anxiety, a short-term response, and prevent the body from entering a state of longer-term, chronic stress which would otherwise be detrimental to the animal’s fitness (Sapolsky, 2002). SDBs as a function of stress appeasement could also explain the significant increases during Walks and Rides. These two more restrictive tourist activities occurred only twice daily. Therefore, it could be possible that elephants utilise SDBs when they feel anxious, and this mediates the overall stress experienced, resulting in no significant change in fGCM. Some evidence for this comes from studies on stereotypic behaviour - a repetitive, functionless set of activities that develop due to chronic stress, past trauma, or poor welfare (Dorning et al., 2016; Mason and Veasey, 2010). There is indication that their use acts as a coping mechanism, and results in lower levels of cortisol (for a review, see Mason, 1991). For example, Bansiddhi et al. (2019) found that Asian elephants (*Elephas maximus*) in Thai riding camps who exhibited stereotypies had lower levels of fGCM than elephants who showed none. This also agrees with the conclusions of Dorning et al. (2016) regarding maladaptive behaviour as a way to regain control. Thus, this may potentially also be the case for

SDBs, but it is important to note the theoretical nature of this explanation. Ultimately, whilst these self-directed behaviours displayed by elephants differ to those of primates, the similarities of situational utilisation and emotional motivation are noteworthy.

It is important to consider that animals experiencing moments of stress is not inherently bad. However, it could lead to poor welfare, since frequent bouts of anxiety or chronic stress can result in distress, a highly undesirable state in captive animals, and a violation of the five freedoms (Clubb and Mason, 2002, FAWC, 2010, Wielebnowski, 2003). The five freedoms are the most basic animal welfare requirements and explain that animals must be free from experiences such as hunger, pain, illness, and distress (FAWC, 2010), therefore a violation is unacceptable. Displacement activity, if repeated often enough due to consistent environmental or social stressors, is believed to precede, and ultimately lead to the development of stereotypic behaviour (Clubb and Mason, 2002, Dorning et al., 2016, Mason and Veasey, 2010). Therefore, the implementation of SDB assessments for elephant welfare would be advantageous to address problems before abnormal behaviours develop. Further, unlike stereotypies, SDBs are in response to an immediate feeling, and so they would grant the observer an instantaneous insight into the animal's state of mind. Thus, they would also allow for swift identification and rectification of stressors before they evolve and become detrimental to the animal. This is not always possible with the more permanent stereotypic behaviour due to their persistence after problem resolution and subsequent difficulty to causally identify (Harris et al., 2008). Furthermore, since SDBs are not unique to captive elephants (Douglas-Hamilton, 1975, Mason and Veasey, 2010), they could have applications for wild elephant conservation and human-elephant conflict mitigation strategies. Working with elephants in free contact situations can be extremely dangerous (Dunham et al., 2010, Gore et al., 2006), and so the ability to identify agitation prone, emotionally aroused elephants or astute potential warning signals before they escalate would be advantageous in numerous situations. This is particularly important since increased deliberate aggression towards humans or engagement in human-elephant conflict occurs at higher rates when the animal is chronically stressed (Gore et al., 2006, Jachowski et al., 2012, Mumby and Plotnik, 2018). Additionally, elephant handlers in captive/ semi-captive settings would benefit from this skillset, which may contribute to improving elephant handler safety by allowing individuals to recognise more subtle clues earlier on.

Finally, there are several limitations of this study to address. Firstly, there was little variation within the fGCM concentrations, which could explain the lack of significant results. In faeces, glucocorticoid metabolite excretion lags are broad (Ganswindt et al., 2003, Wasser et al., 2000), which can make pinpointing a meaningful sample difficult, and brings into question the reliability of a single daily sample to provide enough meaningful data. In the future, additional faecal samples throughout the day would provide a much-needed broadening of the data set, and in turn a more complete, robust picture. Secondly, whilst effort was made to control for age and sex, it was not possible to obtain information regarding oestrus cycles, which is known to impact glucocorticoid secretion (Touma and Palme, 2005). It is also imperative to consider the volatile aspect of quantifying fGCM concentrations, and thus the difficulties in interpreting this type of physiological variable. Faecal GCM can be affected by a number of intrinsic factors, such as diet, parasite load, social factors, diseases (Goymann, 2005, Seltmann et al., 2020, Seltmann et al., 2022), and variation in the distribution of glucocorticoid metabolites throughout the faeces (Millspaugh and Washburn, 2003). Considerations must then be in place regarding these confounding variables in the future. Samples in the current study were taken from the centre of the dung in a single section, and thus were not necessarily representative of the true hormone levels which may be distributed unevenly throughout the faeces. The method by Parnell and colleagues (2015) describes cross segment sampling and mixing prior to collection, which was demonstrated to improve the consistency of hormone distribution in big cat scat, and may be a fruitful future endeavour to improve reliability. Lastly, the current study sample size consisted of only 7 individuals, and this must also be taken into account when considering the validity of the results. Clear patterns emerged in this population, but caution must be taken regarding any firm conclusions; the small sample size is not necessarily indicative of trends at the species level. Therefore, more work must be done on additional individuals to expand the evidence, and improve the validity and reliability regarding the occurrence and motivations of self-directed behaviour in African elephants.

## 5. Conclusion

This study provides evidence of SDB usage in African elephants during anxiety-inducing scenarios, and is a promising start to validating the behaviour set as a welfare tool. It warrants further research since the implementation of such a strategy would be beneficial for instantaneously monitoring anxiety and lower-level stress in captive elephant populations worldwide. Subsequently, it would provide the opportunity to identify and rectify issues before they manifest as more permanent behavioural and physiological stress responses. Preliminary results during the period of this study have already been instrumental in discontinuing the elephant-back rides at the Knysna Elephant Park. Establishing SDBs could also eliminate the need for costly and time-consuming laboratory testing of physiological parameters by providing a reliable behavioural alternative. Further studies are however needed to correlate SDBs with physiological changes in order to conclusively explain the underlying motivation. Furthermore, since self-directed behaviour is evident in both captive and wild populations, the potential application to wild elephant conservation and human-elephant conflict mitigation strategies is compelling.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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