Individual and environmental risk factors associated with fecal glucocorticoid metabolite concentrations in zoo-housed Asian and African elephants

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Abstract

A recent large-scale welfare study in North America involving 106 Asian (Elephas maximus) and 131 African (Loxodonta africana) elephants at 64 accredited facilities identified links (i.e., risk factors) between zoo environmental factors and a number of welfare outcomes (stereotypic behavior, ovarian acyclicity, hyperprolactinemia, walking and recumbence, body condition, health status, serum cortisol). For this population of elephants, we used the same epidemiological methods to examine associations between those risk factors and two additional welfare outcomes, mean concentration and individual variability (CV) of fecal glucocorticoid metabolite concentrations (FGM) as indicators of stress. Results indicate that African elephants are more responsive to social stressors than Asians, and that poor joint health is a stress-related welfare problem for Asian, but not African elephants in the North American population. For both species, higher FGM concentrations were associated with zoos located at more northern latitudes, whereas lower FGM concentrations were associated with having free access to indoor/outdoor spaces, and spending more time in managed interactions with staff. Also important for captive management, elephants having diverse enrichment options and belonging to compatible social groups exhibited reduced intra-individual variability in FGM concentrations. Our findings show that aspects of the zoo environment can be potential sources of stress for captive elephants, and that there are management activities that may facilitate coping with zoo conditions. Given species differences in factors that affected FGM, targeted, species-specific management approaches likely are needed to ensure good welfare for all elephants.
Introduction

Modern zoos strive to ensure animals under human care experience a high standard of welfare that meets emotional and physical health needs [1]. Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants in zoos have received considerable scrutiny in the last two decades because of concerns over welfare and management practices [2]. To create sustainable captive populations, it is important that zoo animal programs evaluate the basic husbandry needs of individual animals, as well as the more complex factors that may affect welfare in a captive environment. For example, an earlier study of 112 female zoo-housed elephants in North America found a significant effect of “facility” on longitudinal serum cortisol concentrations, but no significant effect of “species” or “management” (i.e., free contact—elephants and people share the same space; or protected contact—elephants and people are separated by a barrier) [3], suggesting that facility-specific factors exist that may affect stress and welfare status in captive elephants.

A more recent Elephant Welfare Project (EWP) took an epidemiological approach to determine how factors in the zoo environment impact a number of welfare indicators in captive elephants. That study, conducted by a multi-institutional team of researchers, included 237 elephants at 64 Association of Zoos and Aquariums (AZA)-accredited zoos, and found a variety of factors correlated with welfare outcomes. In particular, enrichment (physical items and facility features) and social (herd composition and interactions) factors were important for normal pituitary-ovarian function [4] and reducing stereotypic behaviors [5]; diversity of feeding practices and exercise reduced the likelihood that an elephant would be overweight [6,7]; softer exhibit substrates were good for physical and behavioral health [8,9]; and positive keeper-elephant relationships were mutually beneficial [10]. Overall, environments that provided diversity and choice were of greater importance to elephant welfare—than exhibit size alone [11]. A remaining question is if these factors also affect physiological stress responses in individual elephants.

The most commonly used bio-markers of stress and, by extension welfare, are glucocorticoids (GC) secreted from the adrenal cortex in response to a stressor [12,13]. The primary role of GCs is energy regulation and mobilization [14,15], but at higher concentrations they facilitate physiological changes associated with the stress response [14]. Stimuli both favorable and unfavorable to welfare can increase GC release; however, most studies of captive wildlife focus on how prolonged exposure to psychological or physical stressors increase GCs and may affect well-being, such as causing immunosuppression, decreased wound healing, increased susceptibility to disease, poor reproduction, and development of stereotypic behaviors [16]. Circulating GCs have been measured in elephants [3,17,18,19], although an important consideration is whether the act of collecting blood itself elicits a response [20,21]. For that reason, noninvasive measures of GCs or their metabolites excreted in feces (fecal glucocorticoid metabolites, FGM) have provided a robust tool for assessing welfare in wildlife species [22,23], including elephants [24,25,26,27,28,29,30,31,32].

The biological validity of FGM to monitor adrenal cortex activity has been demonstrated in elephants under a variety of conditions. Normal physiological increases in FGM are observed during parturition [17], in association with musth [29,33] and during the follicular phase of the estrous cycle [19]. Increases also occur in response to stressful conditions, such as negative interactions with humans and episodic loud noises [34], opening of a zoo to the public for the first time [35], work associated with logging [36,37], participating in public festivals and processions [29], being housed in small enclosures [38], construction [39], and in association with transportation and relocation [34,40,41]. More recently, Edwards et al. [42] found positive correlations between the number of clinical cases in the EWP study and the coefficient of
variation (CV) for both serum cortisol and FGM, suggesting that within-individual variation in FGMs also may be an important welfare indicator. Thus, non-invasive glucocorticoid monitoring can be a powerful tool for assessing stress responses and welfare status, especially when combined with evaluations of health or behavior.

The goal of this study was to determine how previously identified risk factors associated with physical [7,9], behavioral [6,8,43], and physiological [4, 18] outcomes measured in the EWP to date affect FGM concentrations using the same epidemiological approach. We hypothesized that risk factors for ovarian acyclicity, hyperprolactinemia, obesity, stereotypy, poor foot and joint health, lower rates of physical activity or recumbence, and higher serum cortisol responsiveness are associated with higher FGM mean concentrations and variability. The ultimate goal is to better understand relationships between FGM and welfare outcomes, and how they are influenced by extrinsic forces—important information needed to optimize management of elephants in zoo settings.

Materials and methods

Ethics statement

This research was approved by the Animal Care and Use Committee of the Smithsonian National Zoo (NZP-ACUC #11/10).

Study population and sample collection

The study consisted of 237 captive elephants, 106 Asian (85 females; 21 males) and 131 African (104 females; 27 males), housed at 64 American Zoo and Aquarium (AZA) accredited facilities throughout North America that participated in the EWP. Fresh fecal samples were collected by keepers at a frequency of every other week for 12 months. Samples were collected by keepers fresh from the ground in the morning within 2 hours of defecation, mixed to obtain homogeneity, and then 5–10 subaliquots (~50–100 g) placed into Whirlpak® plastic bags, and frozen (-20˚C) immediately. All fecal samples were collected at the same time as data for the other EWP studies, which was for 1 year in 2012 [4–9, 18, 42–43].

Fecal extraction and GC metabolite analysis

Fecal samples were lyophilized (Labconco, Kansas City, MO), and 0.1 g (± 0.02) of well-mixed fecal powder was placed into 16 x 125 mm glass tubes (Fisher Scientific; Pittsburgh, PA). Five ml of 80% methanol was then added and the samples were mixed for 30 minutes on a multi-tube vortexer (Glas-Col; Terre Haute, IN), followed by centrifugation for 20 min at 2500 x g (Sorvall RC 3C Plus; Thermo Fisher Scientific, Waltham, MA). Each supernatant was recovered and the remaining pellet was re-suspended in 5 ml of 80% methanol and extracted again. The two supernatants were combined into a 16 x 125 mm glass tubes and dried under forced air in a fume hood overnight. Extracted samples were reconstituted in 1 ml of 100% methanol, dried again, and then buffer (1 ml, 0.149 M NaCl, 0.1 M NaPO₄; with pH 7.0) added and the tubes sonicated (Part# 08895–60; Cole-Parmer, Vernon Hills, IL) for 30 seconds to dissolve particulates. Finally, all samples were diluted (1:8) in assay buffer (Cat. No. X065, Arbor Assays, Arbor, MI, USA) and stored at –20˚C until enzyme immunoassay (EIA) analysis.

Concentrations of FGM were determined using a double-antibody enzyme EIA with a polyclonal rabbit anti-corticosterone antibody (CJM006) validated for elephants [32]. Standards (3.9–1000 pg/well; Sigma Diagnostics, St. Louis, MO), samples, and controls were added in duplicate (50 µl per well) to pre-coated goat anti-rabbit IgG, 96-well plates at room temperature. Corticosterone-horseradish peroxidase (25 µl, 1:20,000 dilution) was immediately added.
to all wells, followed by 25 μl anti-corticosterone antibody (1:60,000) that was added to all but non-specific binding wells. The plates were covered with microplate sealers and incubated at room temperature on an agitator (Model E6121; Eberbach Corp., Ann Arbor, MA) for 1 hour. All plates were then washed four times (1:20 dilution, 20X Wash Buffer Cat. No. X007; Arbor Assays), blotted dry, and 100 μl of TMB (3, 3’; 5, 5’-tetramethylbenzidine) (Moss Inc., Pasadena, MD) was added. Plates were incubated for 30–45 min at room temperature without shaking, and the reaction stopped by adding 50 μL of a 1 N HCl solution. Optical density was read in a plate reader at 450 nm (OPsys MR; Dynex Technologies, Chantilly, VA). The inter-assay coefficient of variation (CV %) for the high control was 8.1%, and the low control CV% was 15.1% (n = 200 plates); intra-assay CV was <10% as all samples with duplicate CVs over 10% were reanalyzed. Assay sensitivity (based on 90% binding) was 0.14 ng/ml.

Statistical analysis

Independent variables used for these analyses were chosen based on their significance as risk factors in already-published multi-variable models for other welfare indicators of the EWP: reproductive dysfunction as indicated by ovarian acyclicity and hyperprolactinemia [4], stereotypy [43], body condition [7], foot and joint health [9], walking distance and recumbency [6,8], and serum cortisol [18]. Full details regarding data collection and variable creation are provided in several EWP publications [5,11,44]. Table 1 summarizes the independent variables identified as significant “risk factors” for each welfare indicator and descriptions of each independent variable. Elephant-specific independent variables were: Age, Sex, Percent Time in Mixed-Sex Herds, Social Group Contact, Walking Hours Per Week, Percent Time with Juveniles, Percent Time Housed Separately, Transfers, Percent Time In/Out Choice, Social Experience, Recumbence Rate, Percent Time on Hard Substrate, Percent Time on Soft Substrate, Space Experience Outdoors at Night, Space Experience with In/Out Choice, Joint Health, Space Experience Total at Night, Mean Daily Walking Distance, Mean Serum Cortisol, Elephant Positive Behaviors, and Elephant Interacts with Public. Measured on a zoo-level were Season, Enrichment Diversity, Alternative Feeding Methods, Feeding Diversity, Percent Time Managed, Keeper Positive Opinions of Elephants, Keeper as Herdmate and Latitude of Zoo.

Generalized Linear Mixed Models (GLMM) were used to determine Species and Season effects on mean FGMs, and Species and Sex effects on mean and CV of FGMs. Zoo was treated as a random effect to account for clustering of elephants by facility.

Mean FGM concentrations for elephants of each species, and CV of FGMs for both species combined, were fitted in regression models using Generalized Estimating Equations (GEE), which allow for the individual elephant to be used as the unit of analysis, accounts for clustering of individuals within zoos, and focuses on population-averaged effects [45]. GEE also allows for weaker distributional assumptions than mixed models, and was the technique used in previous EWP reports [4–9, 18, 42–43]. The model included repeated measures of FGMs by Season. Zoos were treated as random effects and an independent correlation structure was specified. We built multi-variable regression models by first assessing individual predictors at the univariate level and then at the bivariate level with each demographic variable (Species, Age, Sex) as potential confounding variables. Confounding variables (those that altered the beta values of input variables by more than 10% during bivariate analysis) were included in all models as necessary. Any variables that predicted FGM mean or CV (P < 0.15) following the univariate and bivariate assessments were retained for evaluation in the hierarchical model building process. The model building process proceeded using the forward selection approach [46]. Models reaching the multi-collinearity criteria, as defined by a variance inflation factor of greater than 10 and a condition index of greater than 30, were not considered for further
Table 1. Significant independent variables that were identified as risk factors for welfare outcomes for either or both species in published multi-variable models from the Elephant Welfare Project.

| Welfare Indicators | Independent Variables | Definition of independent variable |
|--------------------|-----------------------|-------------------------------------|
| Ovarian acyclicity | Percent Time in Mixed Sex Herds (unpub.) | Sum of monthly percent time spent in social groups where both males and females are present |
| Age                | Age of elephant in years in 2012 |
| Enrichment Diversity | Shannon diversity index score of enrichment activities types and frequencies conducted at zoo |
| Hyperprolactinemia | Alternate Feeding Methods | The proportion of all feedings where food was presented in a foraging device, hidden, or hung above the exhibit |
| Social Group Contact | | Maximum number of unique social groups focal animal is part of |
| Body Condition | Walking, Hours/Week | Number of reported hours spent walking elephants each week, ranging from 1 (< 1 hour per week) to 7 (14 or more hours per week) |
| Feeding Diversity | | Shannon diversity index score of feeding types and frequencies conducted at zoo |
| Sex (ref: male) | Male or female |
| Daytime Stereotypy | Percent Time Managed | Sum of percent time spent in activities managed by caretaking staff |
| Percent Time with Juveniles | Sum of monthly percent time spent in social groups where an elephant 7 years or younger was present |
| Percent Time House separate | Sum of monthly percent time spent housed in a social group of one |
| Transfers | Total number of inter-zoo transfers an elephant has experienced |
| Nighttime Stereotypy | Percent Time In/Out Choice | Sum of monthly percent time spent in environments where there is a choice of indoors or outdoors |
| Social Experience | The average weighted (by percent time) size of all social groups in which an elephant spent time |
| Recumbence | Recumbence Rate | Hours recumbent per day, averaged over all days of data collection |
| Percent Time on Hard Substrate | Sum of monthly percent time spent in environment with 100% concrete or stone aggregate substrate |
| Percent Time Soft Substrate | Sum of monthly percent time spent in environment with 100% grass, sand, or rubber substrate |
| Space Experience Outdoor Night (per 500 ft²) | The average weighted (by percent time) size of all environments in which an elephant spent time in outdoor environments only |
| Percent Time Housed Separately | Sum of monthly percent time spent housed in a social group of one |
| Musculoskeletal Score | Space Experience In/Out Choice (per 500 ft²) | The average weighted (by percent time) size of all environments in which an elephant spent time where there is a choice of indoors or outdoors |
| Joint Abnormalities (ref: absence) | Presence or absence of gait change, limb deformity, joint heat or swelling noted from musculoskeletal exam |
| Foot Health | Percent Time In/Out Choice | Sum of percent time spent housed in a social group of one |
| Space Experience Total Night (per 500 ft²) | The average weighted (by percent time) size of all environments in which an elephant spent time at night |
| Walking Distance | Mean Daily Walking Distance | Mean outdoor daily walking distance measured by anklets equipped with GPS data loggers |
| Social Group Contact | Maximum number of unique social groups focal animal is part of |
| Feeding Predictability (ref: unpredictable) | The predictability of feeding times; categorical where 1 is predictable, 2 is semi-predictable, and 3 is unpredictable |
| Space Experience Total Night (per 500 ft²) | The average weighted (by percent time) size of all environments in which an elephant spent time in outdoor environments only |
| Serum Cortisol | Mean Serum Cortisol | Mean of 24 blood samples taken bi-weekly for 1 year |
| Keeper Attitude: Positive Opinions of Elephants | Composite scores (averaged by zoo) of keepers’ opinions of elephants: elephants are playful, like to be trained, like change, are trusting, affectionate, and bond to keepers |
| Keeper Attitude: Keeper as Herdmate | Composite scores (averaged by zoo) of keepers’ perceptions that they are accepted by elephants as part of the herd, elephants are interested in the keepers, keepers connect verbally with elephants, keepers have bonds with elephants |
| Latitude of Zoo | Angular distance of a zoo’s location north of the equator |
| Elephant Positive Behaviors | Composite scores (from keeper ratings) for affiliative/friendly behaviors, food sharing, solo play, wallowing |
| Elephant Interacts with Public | Composite scores (from keeper ratings) for elephant watches and initiates interactions with zoo visitors |

1Identified in published studies of the EWP:  
2Brown et al. [4];  
3Morfeld et al. [7];  
4Greco et al. [43];  
5Holgate et al. [8];  
6Miller et al. [9];  
7Holgate et al. [6];  
8Carlstead et al. [18].

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analysis [46]. The forward selection of variables was continued until the addition of variables no longer resulted in significant models. Interactions were assessed during the final model building stage and the final model was selected based on quasi-likelihood under the independence model criterion (QIC) values [47] and parameter estimates of explanatory variables. With the exception of the univariate stage of the model building process where \( P < 0.15 \) was considered significant for continued analyses, \( P < 0.05 \) was considered statistically significant in the remainder of the model building stages. For other analyses, unless otherwise indicated, differences were considered significant at \( P < 0.05 \). All analyses were conducted using IBM SPSS Statistics Version 25, IBM Corp., Armonk, NY, USA.

**Results**

The elephant study population ranged in age from 0 to 64 years (mean age: Asian, 34.3 ± 1.5; African, 27.7 ± 1.1 years). Table 2 presents seasonal mean FGM concentrations for each species. Overall FGM concentrations were higher in Asian (124.4 ± 4.9 ng/g) than African (97.7 ± 3.0 ng/g) elephants. There was a significant main effect of species (\( F = 27.86, \) df1,2 = 1,927, \( P = 0.000 \)), but not season (\( F = 1.30, \) df1,2 = 3,927, \( P = 0.0001 \)). In all seasons, Asian elephants had higher mean concentrations than Africans.

Mean and average variability (CV) of FGMs was calculated for the entire year and is given for each species and sex separately in Table 3. GLMM analysis found significant differences in mean FGM for **Species** (\( F = 8.496, \) df1,2 = 1,236, \( P = 0.004 \)), but not for **Sex** (\( F = 0.124, \) df1,2 = 1,236, \( P = 0.726 \), Table 3). For FGM CV, which is a normalized calculation, there were no significant effects of **Species** (\( F = 0.004, \) df1,2 = 1,236, \( P = 0.950 \)) or **Sex** (\( F = 0.891, \) df1,2 = 1,236, \( P = 0.346 \)). Therefore, mean FGMs were analyzed separately for each species, whereas FGM CVs were analyzed for both species combined.

Descriptive statistics for independent variables are presented for each species in Table 4. For Asian and African elephants separately, univariate linear regressions of independent variables with mean FGM concentrations are shown in Table 5. For Asians, significant negative associations (i.e., lower FGMs) were observed for **Enrichment Diversity**, **Walking (hr/week)**, **Percent Time Managed by Staff**, **Experience Outdoors at Night**, **Space Experience with In/Out Choice**, **Total Space Experienced at Night**, **Mean Daily Walking Distance** and **Latitude of Zoo**. Positive associations (i.e., higher FGMs) were associated with **Percent Time Housed Separately**, **Recumbent Rate**, **Joint Abnormalities**, **Serum Cortisol** and **Keeper as Herdmate**. For Africans, significant negative regressions with mean FGMs were with **Percent Time Managed** (as with Asians), and **Percent Time with In/Out Choice**, and additionally with **Keeper as Herdmate**. Positive associations were with **Percent Time in Mixed Sex Herds**, **Social Experience**, **Social Group Contact**, **Feeding Predictability**, **Latitude of Zoo**, **Mean Daily Walking Distance**, and all three **Space Experience** variables. Therefore, African FGMs were positively associated with

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**Table 2.** Mean (± SEM) and minimum-maximum seasonal fecal glucocorticoid metabolite (FGM) concentrations in Asian (n = 106) and African (n = 131) elephants in North American zoos that participated in the Elephant Welfare Project.

| Season          | Asian Elephants |                  |                  | African Elephants |                  |                  |
|-----------------|-----------------|------------------|------------------|-------------------|------------------|------------------|
|                 | FGM Mean (ng/g) | Min              | Max              | FGM Mean (ng/g)   | Min              | Max              |
| Winter (Jan-Mar)| 146.91 ± 5.01*  | 43.41            | 317.67           | 108.48 ± 3.03*    | 31.83            | 222.49           |
| Spring (Apr-Jun)| 156.83 ± 5.04*  | 57.78            | 286.74           | 107.22 ± 3.01*    | 37.56            | 266.17           |
| Summer (Jul-Sep)| 146.29 ± 4.27*  | 49.74            | 324.18           | 105.04 ± 2.94*    | 28.81            | 229.71           |
| Fall (Oct-Dec)  | 147.78 ± 5.13*  | 37.82            | 310.56           | 110.01 ± 3.08*    | 26.78            | 292.43           |

* Seasonal differences between species are significant (\( P < 0.05 \)).

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Table 3. Mean (± SEM) fecal glucocorticoid metabolite (FGM) concentrations and coefficient of variation (CV) for male and female Asian and African elephants in North American zoos that participated in the Elephant Welfare Project.

|                      | Asian Elephants |                          | African Elephants |                          |
|----------------------|----------------|--------------------------|------------------|--------------------------|
|                      | Male = 21      | Female = 85              | Male = 27        | Female = 104             |
| Mean FGM (ng/ml)     | 121.55 ± 8.69a| 125.47 ± 4.87a          | 99.61 ± 5.70b    | 97.72 ± 3.14d            |
| Mean FGM CV          | 31.53 ± 1.49a  | 32.44 ± 1.28a            | 35.22 ± 2.55a    | 33.17 ± 1.18b            |

Sex differences within species are significant (P < 0.05).

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Table 4. Descriptive statistics (mean, SEM, minimum, maximum) for independent variables of Asian and African elephants in North American zoos that participated in the Elephant Welfare Project.

|                      | Asian Elephants |                          | African Elephants |                          |
|----------------------|----------------|--------------------------|------------------|--------------------------|
|                      | N   | Mean | SEM | Min | Max | N   | Mean | SEM | Min | Max |
| Fecal Glucocorticoid Metabolites (ng/g)—Mean | 106 | 124.69 | 4.26 | 59.69 | 282.88 | 131 | 98.11 | 2.75 | 40.56 | 211.34 |
| Fecal Glucocorticoid Metabolites (ng/g)—CV | 106 | 32.26 | 1.07 | 9.78 | 71.24 | 131 | 33.59 | 1.07 | 15.20 | 92.59 |
| Percent Time in Mixed Sex Herds | 106 | 12.46 | 2.96 | 0.00 | 100.00 | 131 | 23.31 | 3.20 | 0.00 | 100.00 |
| Enrichment Diversity | 93  | 2.91  | 0.015 | 2.54 | 3.16 | 129 | 2.83  | 0.014 | 2.54 | 3.26 |
| Alternate Feeding Methods | 100 | 0.49  | 0.022 | 0.08 | 0.92 | 131 | 0.38  | 0.019 | 0.08 | 0.91 |
| Social Group Contact | 106 | 2.70  | 0.200 | 1.00 | 11.00 | 131 | 4.94  | 0.618 | 1.00 | 30.00 |
| Walking, Hours/Week | 88  | 2.58  | 0.186 | 1.00 | 7.00 | 129 | 1.92  | 0.130 | 1.00 | 7.00 |
| Sex (ref: male) | 106 | 0.80  | 0.039 | 0.00 | 1.00 | 131 | 0.79  | 0.035 | 0.00 | 1.00 |
| Percent Time Managed | 89  | 55.42 | 2.035 | 20.00 | 91.00 | 129 | 49.34 | 1.640 | 13.00 | 100.00 |
| Percent Time with Juveniles | 106 | 18.63 | 3.413 | 0.00 | 100.00 | 131 | 22.78 | 3.310 | 0.00 | 100.00 |
| Percent Time Housed Separately | 106 | 32.96 | 3.817 | 0.00 | 100.00 | 131 | 21.15 | 2.590 | 0.00 | 100.00 |
| Transfers | 106 | 2.69  | 0.204 | 0.00 | 10.00 | 131 | 2.68  | 0.162 | 0.00 | 10.00 |
| Percent Time In/Out Choice | 106 | 15.74 | 2.157 | 0.00 | 77.67 | 131 | 17.30 | 1.820 | 0.00 | 89.82 |
| Social Experience | 106 | 2.17  | 0.106 | 1.00 | 4.93 | 131 | 3.14  | 0.218 | 1.00 | 11.22 |
| Recumbence Rate | 25  | 8.02  | 0.752 | 0.00 | 19.72 | 38  | 5.34  | 0.452 | 0.05 | 9.17 |
| Percent Time on Hard Substrate | 106 | 9.69  | 1.260 | 0.00 | 51.90 | 131 | 13.13 | 1.080 | 0.00 | 50.00 |
| Percent Time Soft Substrate | 106 | 10.82 | 1.228 | 0.00 | 55.90 | 131 | 10.61 | 1.260 | 0.00 | 58.30 |
| Space Experience Outdoor Night (per 500 ft²) | 106 | 34.60 | 3.903 | 0.00 | 187.39 | 131 | 70.75 | 8.910 | 0.00 | 574.28 |
| Space Experience In/Out Choice (per 500 ft²) | 106 | 19.36 | 2.177 | 0.00 | 92.13 | 131 | 38.35 | 5.560 | 0.00 | 312.74 |
| Joint Abnormalities (ref: absence) | 98 | 0.33  | 0.048 | 0.00 | 1.00 | 94 | 0.23  | 0.044 | 0.00 | 1.00 |
| Space Experience Total Night (per 500 ft²) | 106 | 26.74 | 2.760 | 1.09 | 147.05 | 131 | 56.25 | 6.920 | 0.88 | 419.14 |
| Age of Elephant | 106 | 34.84 | 1.459 | 1.00 | 64.00 | 131 | 27.85 | 1.060 | 0.00 | 52.00 |
| Mean Daily Walking Distance | 26 | 5.31  | 0.629 | 1.21 | 17.26 | 34 | 5.42  | 0.260 | 2.19 | 9.71 |
| Feeding Predictability (ref: unpredictable) | 95 | 2.16  | 0.066 | 1.00 | 3.00 | 329 | 1.93  | 0.050 | 1.00 | 3.00 |
| Mean Serum Cortisol | 98 | 17.83 | 0.748 | 5.96 | 40.02 | 115 | 17.95 | 0.583 | 5.87 | 37.26 |
| Keeper Attitude: Positive Opinions of Elephants | 84 | 3.68  | 0.053 | 1.59 | 4.40 | 106 | 3.65  | 0.050 | 2.77 | 5.37 |
| Keeper Attitude: Keeper as Herdmate | 84 | 3.02  | 0.073 | 2.00 | 4.48 | 106 | 2.65  | 0.054 | 1.41 | 4.03 |
| Latitude of Zoo | 103 | 35.81 | 0.567 | 21.00 | 47.00 | 131 | 35.60 | 0.414 | 26.00 | 47.00 |
| Elephant Positive Behaviors | 67 | 4.45  | 0.128 | 1.53 | 6.31 | 93 | 4.67  | 0.080 | 2.21 | 6.42 |
| Elephant Interacts with Public | 67 | 2.48  | 0.107 | 0.98 | 5.68 | 93 | 2.40  | 0.082 | 0.83 | 5.16 |

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Factors affecting fecal glucocorticoid concentrations in zoo elephants

Table 5. Univariate linear regressions of 12-month mean fecal glucocorticoid metabolite concentrations in Asian and African elephants in North American zoos and previously published risk factors (independent variables) from the Elephant Welfare Project. Variables at P<0.15 were considered significant for inclusion in the multi-variable analyses, and are bolded.

| Independent Variable | Asian Elephants | | | | African Elephants | | |
|----------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                      | N | Estimate | SEM | P value | N | Estimate | SEM | P value |
| Percent Time in Mixed Sex Herds (unpub.) | 106 | -0.065 | 0.140 | 0.646 | 131 | 0.211 | 0.073 | 0.005 |
| Enrichment Diversity | 93 | -58.746 | 31.058 | 0.062 | 129 | 14.139 | 16.989 | 0.407 |
| Alternate Feeding Methods | 100 | 16.049 | 20.348 | 0.432 | 131 | 13.994 | 12.529 | 0.266 |
| Social Group Contact | 106 | -0.312 | 2.088 | 0.882 | 131 | 0.944 | 0.383 | 0.015 |
| Walking, Hours/Week | 88 | -4.796 | 2.673 | 0.076 | 129 | -2.274 | 1.864 | 0.225 |
| Feeding Diversity | 95 | -10.397 | 14.750 | 0.483 | 129 | 8.369 | 13.265 | 0.529 |
| Sex (ref: male) | 106 | 3.971 | 10.721 | 0.712 | 133 | -1.543 | 6.804 | 0.821 |
| Percent Time Managed | 89 | -0.545 | 0.253 | 0.034 | 128 | -0.284 | 0.149 | 0.060 |
| Percent Time with Juveniles | 106 | -0.043 | 0.122 | 0.726 | 131 | 0.079 | 0.073 | 0.283 |
| Percent Time Housed Separately | 106 | 0.174 | 0.108 | 0.109 | 131 | 0.023 | 0.093 | 0.804 |
| Transfers | 106 | -0.964 | 2.040 | 0.637 | 131 | -0.852 | 1.479 | 0.566 |
| Social Experience | 106 | -5.197 | 3.918 | 0.188 | 131 | 2.342 | 1.089 | 0.033 |
| Recumbence Rate | 25 | 4.949 | 2.200 | 0.034 | 38 | 0.908 | 1.639 | 0.583 |
| Percent Time on Hard Substrate | 106 | 0.725 | 0.323 | 0.27 | 131 | 0.132 | 0.223 | 0.556 |
| Percent Time Soft Substrate | 106 | -0.115 | 0.340 | 0.735 | 131 | 0.229 | 0.190 | 0.229 |
| Space Experience Outdoor Night (per 500 ft²) | 106 | -0.187 | 0.105 | 0.080 | 131 | 0.073 | 0.026 | 0.006 |
| Space Experience In/Out Choice (per 500 ft²) | 106 | -0.333 | 0.189 | 0.081 | 131 | 0.110 | 0.042 | 0.010 |
| Joint Abnormalities (ref: absence) | 95 | 20.198 | 7.470 | 0.008 | 96 | 0.298 | 7.660 | 0.969 |
| Space Experience Total Night (per 500 ft²) | 106 | -0.282 | 0.149 | 0.060 | 131 | 0.111 | 0.033 | 0.001 |
| Age of Elephant | 106 | 0.261 | 0.285 | 0.361 | 133 | -0.278 | 0.227 | 0.222 |
| Mean Daily Walking Distance | 26 | -5.144 | 2.380 | 0.041 | 34 | 6.428 | 3.264 | 0.058 |
| Feeding Predictability (ref: unpredictable) | 95 | 0.642 | 7.087 | 0.928 | 129 | 6.221 | 4.167 | 0.138 |
| Mean Serum Cortisol | 98 | 1.208 | 0.591 | 0.024 | 117 | 0.196 | 0.475 | 0.680 |
| Keeper Attitude: Positive Opinions of Elephants | 84 | 6.814 | 10.654 | 0.524 | 108 | -3.814 | 4.838 | 0.432 |
| Keeper Attitude: Keeper as Herdmate | 84 | 16.663 | 7.625 | 0.032 | 108 | -10.227 | 4.683 | 0.031 |
| Latitude of Zoo | 106 | -1.153 | 0.665 | 0.086 | 133 | 1.659 | 0.563 | 0.004 |
| Elephant Positive Behaviors | 67 | -5.672 | 4.667 | 0.229 | 93 | -0.505 | 3.728 | 0.893 |
| Elephant Interacts with Public | 67 | -0.212 | 5.644 | 0.970 | 93 | 0.503 | 3.639 | 0.890 |

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three social variables and only one individual variable (Mean Daily Walking Distance), whereas FGMs in Asians were positively associated with only one social variable (Percent Time Housed Separately) and four individual variables. Lastly, there was no age effect on FGM for either species.

Multivariable analyses required the exclusion of Mean Daily Walking Distance and Recumbent Rate because these variables were measured in only a sub-set of the elephants. Also, Social Experience was highly correlated (r = 0.899) with Social Group Contact and so was not included in the multivariable model building process due to collinearity problems. The final models are given in Table 6 for Asian and Table 7 for African elephants.

The initial, best multi-variable model for Asian elephant FGMs showed trending effects for Season: Spring and Latitude of Zoo (P = 0.076 and 0.051, respectively), so Season’ Latitude of Zoo was added as an interaction term in the model. The rationale for this was that the degree of climatological change between seasons is a function of how far north the zoo lies. With the interaction term added to the model, Latitude of Zoo was no longer significant as a main effect.
Table 6. Multi-variable model of seasonal fecal glucocorticoid metabolite concentrations for Asian elephants (n = 106) in North American zoos that participated in the Elephant Welfare Project1. Significant variables are bolded.

| Variable                                      | Beta Estimate | SEM  | P value |
|-----------------------------------------------|---------------|------|---------|
| Intercept                                     | 118.69        | 23.60| 0.001   |
| Season: Winter (Jan-Mar)                      | -2.43         | 24.92| 0.922   |
| Season: Spring (Apr-Jun)                      | -42.59        | 24.01| 0.076   |
| Season: Summer (Jul-Sep)                      | -10.91        | 21.21| 0.606   |
| Season: Fall (Oct-Dec) (ref)                  | 0             |      |         |
| Sex: Female                                   | -3.15         | 6.83 | 0.644   |
| Sex: Male (ref)                               | 0             |      |         |
| Age of Elephant                               | 0.34          | 0.22 | 0.128   |
| Joint Health: No Abnormalities                | -21.14        | 8.58 | 0.014   |
| Joint Health: Abnormalities (ref)             | 0             |      |         |
| Space Experience In/Out Choice (per 500 ft²)  | -0.41         | 0.13 | 0.003   |
| Season: Winter\ Latitude of Zoo               | 0.61          | 0.66 | 0.350   |
| Season: Spring\ Latitude of Zoo               | 1.81          | 0.77 | 0.019   |
| Season: Summer\ Latitude of Zoo               | 0.66          | 0.62 | 0.288   |
| Season: Fall\ Latitude of Zoo (ref)           | 0.39          | 0.55 | 0.473   |

1Age is a confounder for Sex and Latitude of Zoo.

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and was dropped from the model (Table 6). The interaction factor was a significant risk factor for higher FGM only in the spring season at higher latitudes. When all other independent variables are held constant, an increase of one degree in Latitude of Zoo corresponds to a 1.81 ng/g increase in FGM during April—June. For Asian elephants, risk factors for higher FGMs were Joint Abnormalities and limited Space Experience with In/Out Choice. Our analysis found that,

Table 7. Multi-variable model of seasonal fecal glucocorticoid metabolite concentrations for African elephants (n = 131) in North American zoos that participated in the Elephant Welfare Project1. Significant variables are bolded.

| Variable                                      | Beta Estimate | SEM  | P value |
|-----------------------------------------------|---------------|------|---------|
| Intercept                                     | 16.67         | 26.24| 0.525   |
| Season: Winter (Jan-Mar)                      | -3.79         | 2.94 | 0.197   |
| Season: Spring (Apr-Jun)                      | -1.10         | 3.03 | 0.716   |
| Season: Summer (Jul-Sep)                      | -1.71         | 2.80 | 0.541   |
| Season: Fall (Oct-Dec) (ref)                  | 0             |      |         |
| Sex: Female                                   | -5.53         | 6.69 | 0.409   |
| Sex: Male (ref)                               | 0             |      |         |
| Age                                           | -0.10         | 0.28 | 0.719   |
| Percent Time Managed                          | -0.27         | 0.13 | 0.045   |
| Latitude of Zoo                               | 2.62          | 0.58 | 0.001   |
| Percent Time in Mixed-Sex Herds               | 0.19          | 0.09 | 0.039   |
| Space Experience Outside at Night (per 500 ft²)| 0.06          | 0.02 | 0.004   |
| Percent Time In/Out choice                   | -0.20         | 0.09 | 0.032   |

1Age of elephant is a confounder of Percent Time Managed and Latitude of Zoo. Latitude of Zoo was a confounder of Percent Time in Mixed-Sex Herds and Space Experience Outside at Night.

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when all other independent variables are held constant, the absence of Joint Abnormalities decreases FGM by 21.14 ng/g, and for every 5000 ft² increase in Space Experience with In/Out Choice there is a 4.1 ng/g decrease in FGM.

The multivariable model for African elephant FGMs also demonstrated effects of Latitude of Zoo on FGM, but no seasonal effects (Table 6). As latitude increases by one degree, FGMs increase by 2.67 ng/g. There were four additional risk factors in the multivariable model: Percent Time In/Out Choice, and Percent Time Managed by staff. For every 10% increase in Percent Time In/Out Choice there is a 2.00 ng/g decrease in FGM. Similarly, a 10% increase Percent Time Managed decreases FGMs by 2.70 ng/g. By contrast, Percent Time in Mixed-Sex Groups and Space Experience Outdoors at Night increase FGMs: a 10% increase in time produces a 1.90 ng/g increase, and a 5000 ft² increase in space experience produces a 0.60 ng/g in FGMs.

Table 8 presents univariate regressions of the independent variables and FGM CV. Associated with lower FGM variability were Enrichment Diversity, Social Group Contact and Social Experience. Variables at P < 0.15 were considered significant for inclusion in the multivariable analyses, and are bolded.

Table 8. Univariate linear regressions between CV of fecal glucocorticoid metabolite concentrations and previously published risk factors (independent variables) for Asian and African elephants in North American zoos that participated in the Elephant Welfare Project. Variables at P < 0.15 were considered significant for inclusion in the multivariable analyses, and are bolded.

| Independent variable                              | N   | Beta  | SE   | P value |
|---------------------------------------------------|-----|-------|------|---------|
| Percent Time in Mixed Sex Herds (unpublished)     | 237 | -0.015| 0.022| 0.507   |
| Enrichment Diversity                              | 222 | -14.524| 4.566| 0.002   |
| Alternate Feeding Methods                         | 231 | -2.216| 3.421| 0.518   |
| Social Group Contact                              | 237 | -0.451| 0.135| 0.001   |
| Walking (14 or more hours per week)               | 217 | -0.342| 0.470| 0.468   |
| Feeding Diversity                                 | 224 | -1.395| 3.008| 0.643   |
| Sex (ref: male)                                   | 237 | -0.790| 1.894| 0.677   |
| Percent Time Managed                              | 218 | 0.022 | 0.040| 0.580   |
| Percent Time with Juveniles                       | 237 | -0.042| 0.021| 0.044   |
| Percent Time Housed Separately                    | 237 | -0.004| 0.022| 0.858   |
| Transfers                                         | 237 | 0.411 | 0.363| 0.260   |
| Percent Time In/Out Choice                        | 237 | 0.102 | 0.035| 0.004   |
| Social Experience                                 | 237 | -0.830| 0.370| 0.026   |
| Recumbence Rate                                   | 63  | 0.229 | 0.465| 0.625   |
| > 0 Percent Time on Hard Substrate                | 237 | -0.012| 0.060| 0.838   |
| > 0 Percent Time Soft Substrate                   | 237 | 0.039 | 0.056| 0.486   |
| Space Experience Outdoors Night                   | 237 | -0.016| 0.009| 0.076   |
| Space Experience In/Out Choice (per 500 ft²)      | 237 | -0.016| 0.015| 0.304   |
| Joint Health: Absence or presence of joint abnormalities | 194 | 0.952 | 1.940| 0.624   |
| Space Experience Total Night (per 500 ft²)        | 237 | -0.020| 0.012| 0.099   |
| Age of Elephant                                   | 237 | 0.039 | 0.055| 0.477   |
| Mean Daily Walking Distance                       | 60  | -1.832| 0.640| 0.041   |
| Feeding Predictability (ref: Unpredictable)       | 224 | -2.564| 1.145| 0.026   |
| Mean Serum Cortisol                               | 215 | -0.023| 0.116| 0.844   |
| Keeper Attitude: Positive Opinions of Elephants   | 192 | -1.561| 1.641| 0.343   |
| Keeper Attitude: Keeper as Herdmate               | 192 | 1.373 | 1.356| 0.312   |
| Latitude of Zoo                                   | 237 | -0.358| 0.146| 0.015   |
| Elephant Positive Behaviors                       | 160 | 1.363 | 1.010| 0.179   |
| Elephant Interacts with Public                    | 160 | -0.822| 1.106| 0.458   |
| Species (ref = 2, Asian)                          | 237 | -1.282| 1.52 | 0.402   |

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Experience, Percent Time with Juveniles, both Space Experience at Night variables, Mean Daily Walking Distance, Feeding Predictability and Latitude of Zoo. The variable associated with increased variability was Percent Time with In/Out Choice.

The multivariable model for FGM CV (Table 9) indicates that Percent Time In/Out Choice increases FGM variability: when other variables are held constant, for each 10% increase in time there is a 0.9% increase in CV of FGM. Enrichment Diversity and Social Group Contact both decreased variability. Each 1.0 increase in the Shannon Diversity Index of enrichment is associated with a 13.4% decrease in the CV of FGMs, and each additional Social Group Contact results in a 0.5% decrease. Species confounds Enrichment Diversity and Social Group Contact due to Asian elephants receiving, on average, slightly more enrichment than Africans (see Table 4), and Africans having contact with more social groups than Asians (Table 4), primarily because Africans are kept more often in larger groups.

Because Enrichment Diversity was calculated on a zoo-level, Fig 1 shows the correlation between a zoo’s enrichment diversity score and the average FGM CV of the elephants at a zoo.

**Discussion**

Epidemiological analyses of the EWP data point to a number of individual, social, housing and management factors that might affect adrenal activity in the zoo-housed elephant population in North America. A higher risk of elevated FGM concentrations was found for Asian elephants with joint abnormalities, and African elephants housed in mixed-sex herds, whereas all elephants housed in northern latitudes had an increased risk of higher FGM in the spring (Asians) or all seasons (Africans). More importantly, the results point to management factors that decrease FGMs in both species: having choice of being indoors and out, and management interactions with staff (Africans). The variability in FGM concentrations (CV) was reduced by enrichment and social groupings, and increased by having a choice of indoor and outdoor spaces. Interestingly, univariate analyses indicated that walking distance and all three space experience variables were negatively correlated to FGM in Asian elephants, but positively associated in African elephants. These patterns suggest there are species differences in how housing space is experienced, which may indicate that species-specific management protocols are needed.

Having the choice to be indoors or out appears to decrease adrenal activity for both species, as indicated by significant negative associations between mean FGM concentrations and the independent variables Space Experience with In/Out Choice (Asians) and Percent Time with In/Out Choice (Africans). Greco et al. [43] also identified Percent Time with In/Out Choice as a factor that reduced the frequency of nighttime stereotypy in the current population. Choice is

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**Table 9. Multi-variable model of CV of fecal glucocorticoid metabolite concentrations for Asian (n = 106) and African (n = 131) elephants in North American zoos that participated in the Elephant Welfare Project.** Significant variables are bolded.

| Independent variable                  | Beta  | SEM   | P value |
|---------------------------------------|-------|-------|---------|
| Species (ref: Asian)                  | 0.925 | 1.3855| 0.504   |
| Sex (ref: female)                     | 0.828 | 1.7213| 0.630   |
| Age                                   | -0.050| 0.0698| 0.477   |
| Percent Time In/Out Choice            | 0.090 | 0.0390| 0.021   |
| Enrichment Diversity                  | -13.430| 4.1904| 0.001   |
| Social Group Contact                  | -0.516| 0.0983| 0.000   |

Species is a confounder of Social Group Contact and Enrichment Diversity.

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generally beneficial to the welfare of captive animals because it increases an animal’s perceived control over its environment [48] and being given a choice of moving between indoor and outdoor areas at will has been associated with reduced stereotypic behaviors in polar bears [49], Asian elephants [50], and giant pandas [51]. For Asian elephants, those with joint problems had higher FGMs than those that did not, presumably due to pain. This could be the result of spending more time on hard surfaces and being older on average than African elephants in this population, because Time on Hard Surfaces and Age are both risk factors for joint problems [9].

Latitude of Zoo was a risk factor for higher FGMs in African elephants, increasing as a zoo’s location was more northwards. For Asians, this effect was only identified in the spring. Carlstead et al. [18] also found that Latitude of Zoo was a predictor of higher serum cortisol in this same population of Asian elephants. There are a variety of elephant management modifications that take place as seasons change, such as elephants spending more time confined inside or outside, with potential changes in social density or social contact that could account for increased social stress [32]. Higher glucocorticoids have been reported during colder seasons among small numbers of zoo-housed Asian [53] and African [54] elephants. In Thailand, mean FGM concentrations were ~28% higher in winter compared to the summer and rainy seasons, and were negatively associated with temperature and rainfall, but not humidity [55]. The need for more energy to maintain optimum body temperature and ensure survival in cooler temperatures could be related to this finding.

There were three other risk factors identified for African FGMs. First, Percent Time Managed by staff reduces FGMs, and also reduces daytime stereotypies for both species [43]. In Asians, there was a significant univariate correlation between FGMs and Percent Time

Fig. 1. Correlation between zoos’ enrichment diversity scores and mean coefficient of variation (CV) of fecal glucocorticoid metabolite concentrations at zoos ($r = -0.339$, $n = 57$, $P = 0.010$).

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Managed, but it did not make it into the multivariable model. Therefore, stress in African elephants, as indicated by higher FGM concentrations and higher rates of stereotypy in the daytime, may be due to insufficient time spent in interactions with staff (i.e. cleaning and grooming, feeding, exercising and training). Positive interactions with keeper staff have been shown to be predictors of lower serum cortisol concentrations for both species [18]. The evidence points strongly to interactions with staff being stress-reducing for elephants. Second, Percent Time in Mixed-Sex Herds was associated with increased FGMs, possibly related to having bulls for breeding, a natural stressor. The third risk factor for African FGMs was Space Experience Outdoors at Night. There is no obvious explanation for why having more outdoor space at night would be associated with increased adrenal activity. Perhaps there are more social interactions occurring under the cover of darkness, without keepers nearby, which for some elephants might be stressful or, alternatively, stimulating. Posta et al. [56] reported that two zoo-housed African elephants spent a greater portion of their time outdoors at night walking, while others report significant social behaviors occurring during the night with free access to indoor and outdoor areas [57,58]. Holdgate et al. [6] also found that a subset of elephants from this population had a greater Mean Walking Distance if they had a greater Space Experience at Night. Therefore, evidence suggests that outdoor space at night facilitates activity of African elephants, and increased activity could account for the slight increase in FGMs identified in the multi-variable model.

In assessments of FGM CVs, three risk factors were identified: Percent Time In/Out Choice, Enrichment Diversity and Social Group Contact. Having more choice of being indoors or outdoors was associated with a decrease in mean FGM in both species. Therefore, while the overall population effect of choice appears to be stress-reducing, it leads to slightly increased variability within individuals. We speculate that this may be due to movements of other elephants in the herd going in and out in an unpredictable manner. A given individual might benefit from having increased choice and control over its own situation, but it has no control over the whereabouts of other elephants, potentially resulting in more variable stress responses. Cochrane [59] points out that CV should be included in studies of GCs because the factors that account for within-individual variation and their adaptive significance, such as personality, coping styles, genetic or maternal influences, are little known for most species. For example, increased variability in FGMs was correlated with abnormal reproductive function, higher rates of fighting, and institutional mortality rates in rhinoceros [60], leading to the conclusion that the variability of FGMs is a valuable measure of stress responsiveness that may have biological costs to the animal. The subject of individual variation in GC responses to stressors has included investigations of differences in coping styles and disease susceptibility [61]. A better understanding of inter- and intra-individual variation in hypothalomo-pituitary-adrenal activity would be beneficial to our use of GCs as a welfare measure as suggested by Edwards et al. [42].

Enrichment Diversity was strongly associated with a reduction in CV of FGMs, but not with mean FGMs, suggesting that having multiple enrichment options functions to moderate adrenal reactivity of individuals. Brown et al. [4] found enrichment diversity to be positively correlated with reproductive health in African females of the EWP, both in terms of reduced acyclicity and normalization of prolactin secretion, and our results support enrichment as an important management factor for zoo elephant welfare. All elephants of the EWP received some form of enrichment at their zoo, and the frequency with which different enrichments were provided was found to impact the variability of FGMs within, but not between individuals. An analogous experiment with mice found that housing in enrichment diverse “calming” environments, consisting of a large cage with a cardboard nest box, paper nesting material, and a tube, exhibited significant and lasting reductions over time in FGM levels compared to
mice housed in less enriched, standard caging [62]. In our study, Enrichment Diversity scores were derived from surveys of zoo managers providing the percentage of days their elephants had access to 30 different types of enrichment items, ranging from exhibit features such as sand or dirt piles, mud wallows, pools, logs, scratching posts and sprinklers, to the provision of manipulatable objects such as balls, tires and hanging objects, to feeding items such as browse and treat boxes/bags, and scents, music and problem-solving tasks [5]. We found the zoo average FGM CVs to be negatively correlated with the frequency of only three of the 30 enrichment types: problem-solving ($r = -0.348$, $n = 57$, $p = 0.007$), hanging objects ($r = -0.261$, $p = 0.048$) and scratching posts ($r = -0.340$, $p = 0.009$); three enrichments that intensely engage elephants. All evidence together strongly suggests that enrichment has a “calming” effect on stress responses of elephants, most likely by providing additional behavioral options and/or cognitive opportunities to cope with their daily lives.

Last, being a member of more social groups (Social Group Contact) also was associated with lower variability in FGMs. Therefore, being a familiar and accepted member of multiple social groups may also stabilize activity of the adrenal cortex in a manner similar to Enrichment Diversity, effectively increasing social enrichment diversity, a clear benefit for elephant welfare.

Conclusions

Results elucidate species differences in FGM concentrations of elephants in relation to a variety of zoo environments. A stress-related welfare problem was identified among Asian elephants with joint health problems. African elephants appear to be more responsive to social stressors than Asians, which fits with their natural history. African elephants form complex, multi-tiered social groups that are important to survival, whereas Asian herds are smaller and bonds are more fluid [63]. One factor that reduced FGMs for both species was more time being managed, suggesting time spent with keepers has a positive effect. More time being managed also was associated with reduced stereotypy [43]. Finally having diverse enrichment options and contact with multiple social groups also appears to be calming for elephants, reducing intra-individual variability in FGMs. Together, all evidence points to the beneficial effects of diverse enrichment opportunities, including cognitive enrichment for zoo-housed elephants. We conclude that there are many avenues for further research on stress in zoo-housed elephants, and monitoring FGMs longitudinally is a proven non-invasive method for determining factors contributing to adrenal function, stress and coping responses in elephants. The species differences in FGM responses to zoo factors suggests that a one-size-fits-all management strategy may not be appropriate, and that more species-specific approaches to husbandry are needed.

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**References**

1. Wolfensohn S, Shotton J, Bowley H, Davies S, Thompson S, Justice WSM. Assessment of welfare in zoo animals: towards optimum quality of life. Animals. 2018; 8(7):110–26.

2. Mason GJ, Veasey JS. What do population-level welfare indices suggest about the well-being of zoo elephants. Zoo Biol. 2010; 29(3):256–73. [https://doi.org/10.1002/zoo.20303 PMID: 20140956](https://doi.org/10.1002/zoo.20303 PMID: 20140956)

3. Proctor CM, Brown JL. Influence of handling method on adrenal activity in zoo African and Asian elephants. J Zoo Aqua Res. 2015; 3: [https://doi.org/10.19227/jzar.v3i1.100](https://doi.org/10.19227/jzar.v3i1.100)

4. Brown JL, Paris S, Prado-Oviedo NA, Meehan CL, Hogan JN, Morfeld KA, et al. Reproductive health assessment of female elephants in North American zoos and association of husbandry practices with
reproductive dysfunction in African elephants (Loxodonta africana). PLoS One. 2016; 11(7):e0145673. https://doi.org/10.1371/journal.pone.0145673 PMID: 27416141

5. Greco BJ, Meehan CL, Miller LJ, Shepherdson DJ, Morfeld KA, Andrews J, et al. Elephant management in North American zoos: environmental enrichment, feeding, exercise, and training. PLoS One. 2016; 11(7):e0152490. https://doi.org/10.1371/journal.pone.0152490 PMID: 27416454

6. Holdgate MR, Meehan CL, Hogan JN, Miller LJ, Soltis J, Andrews J, et al. Walking behavior of zoo elephants: associations between GPS-measured daily walking distances and environmental factors, social factors, and welfare indicators. PLoS One. 2016; 11(7):e0150331. https://doi.org/10.1371/journal.pone.0150331 PMID: 27414411

7. Morfeld KA, Meehan CL, Hogan JN, Brown JL. Assessment of body condition in African (Loxodonta africana) and Asian (Elephas maximus) elephants in North American zoos and management practices associated with high body condition scores. PLoS One. 2016; 11(7):e0155146. https://doi.org/10.1371/journal.pone.0155146 PMID: 27415629

8. Holdgate MR, Meehan CL, Hogan JN, Miller LJ, Rushen J, de Passille AM, et al. Recumbence behavior in zoo elephants: determination of patterns and frequency of recumbent rest and associated environmental and social factors. PLoS One. 2016; 11(7):e0152490. https://doi.org/10.1371/journal.pone.0152490 PMID: 27414654

9. Miller MA, Hogan JN, Meehan CL. Housing and demographic risk factors impacting foot and musculoskeletal health in African elephants (Loxodonta africana) and Asian elephants (Elephas maximus) in North American zoos. PLoS One. 2016; 11(7):e0155223 https://doi.org/10.1371/journal.pone.0155223 PMID: 27414809

10. Carlstead K, Mench JA, Meehan C, Brown JL. An epidemiological approach to welfare research in zoos: the Elephant Welfare Project. J Appl Anim Well Sci. 2013; 16(4):319–37. https://doi.org/10.1080/10888705.2013.827915 PMID: 24079487

11. Meehan CL, Hogan JN, Bonaparte-Saller MK, Mench JA. Housing and social environments of African (Loxodonta africana) and Asian (Elephas maximus) elephants in North American Zoos. PLoS One. 2016; 11(7):e0146703. https://doi.org/10.1371/journal.pone.0146703 PMID: 27414934

12. Möstl E, Palme R. Hormones as indicators of stress. Domest Anim Endocrinol. 2002; 23(1–2):67–74. PMID: 12142227

13. Palme R. Non-invasive measurement of glucocorticoids: Advances and problems. Physiology & Behavior 2019; 199, 229–43.

14. Busch DS, Hayward LS. Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biol Conserv. 2009; 142:2844–53.

15. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev. 2000; 21:55–89. https://doi.org/10.1210/edrv.21.1.0389 PMID: 10696570

16. Moberg GP, Mench JA, editors. The biology of animal stress: basic principles and implications for animal welfare. CABI; 2000.

17. Brown JL, Lehnhardt J. Serum and urinary hormones during pregnancy and the peri- and postpartum period in an Asian elephant (Elephas maximus). Zoo Biol. 1995; 14:555–64.

18. Carlstead K, Paris S, Brown JL. Good keeper-elephant relationships in North American zoos are mutually beneficial to welfare. Appl Anim Behav Sci. 2018; 211:103–11.

19. Fanson KV, Keeley T, Fanson BG. Cyclic changes in cortisol across the estrous cycle in parous and nulliparous Asian elephants. Endocr Connect. 2014; 3(2):57–66. https://doi.org/10.1530/EC-14-0025 PMID: 24623735

20. Reinhardt V, Cowley D, Scheffler J, Vertein R, Wegner F. Cortisol response of female rhesus monkeys to venipuncture in home cage versus veni-puncture in restraint apparatus. J Med Primatol. 1990; 19:601–6. PMID: 2246780

21. Cook CJ, Mellor DJ, Harris PJ, Ingram JR, Matthews LR. Hands-on and hands-off measurement of stress. In Moberg G. P. and Mench J. A. (eds.), The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. Wallingford, Oxon, UK: CABI. Pp. 123–46. 2000.

22. Whitham JC, Wielebnowski N. New directions for zoo animal welfare science. Appl Anim Behav Sci. 2013; 147(3–4):247–60.

23. Wielebnowski N. Stress and distress: evaluating their impact for the well-being of zoo animals. JAVMA 2003; 223(7):973–7. PMID: 14552484

24. Brown JL, Wemmer CM, Lehnhardt L. Urinary cortisol analyses for monitoring adrenal activity in elephants. Zoo Biol. 1995; 14:533–42.

25. Dathe HH, Kuckelkorn B, Minnemann D. Salivary cortisol assessment for stress detection in the Asian elephant (Elephas maximus)—a pilot study. Zoo Biol. 1992; 11:285–89.
26. Foley CAH, Papageorge S, Wasser SK. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Cons Biol. 2001; 15:1134–42.

27. Ganswindt A, Münchscher, Henley M, Palme R, Thompson P, Bertchinger H. Concentrations of faecal glucocorticoid metabolites in physically injured free-ranging African elephants *Loxodonta africana*. Wildl Biol. 2010; 16:323–32.

28. Gobush KS, Mutayoba BM, Wasser SK. Long-term impacts of poaching on relatedness, stress physiology, and reproductive output of adult female African elephants. Cons Biol. 2008; 22(6):1590–9.

29. Kumar V, Reddy VP, Kokkiligadda A, Shivaaj S, Umopathy G. Non-invasive assessment of reproductive status and stress in captive Asian elephants in three south Indian zoos. Gen and Comp Endocrinol 2013; 201:37–44.

30. Schmid J, Heistermann M, Gansloßer U, Hodges JK. Introduction of foreign female Asian elephants (*Elephas maximus*) into an existing group: Behavioural reactions and changes in cortisol levels. Anim Welf. 2001; 10(4):357–72.

31. Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh JJ, Larson S, Monfort SL. A generalized faecal glucocorticoid assay for use in a diverse array of non-domestic mammalian and avian species. Gen Comp Endocrinol. 2000; 120:260–75. https://doi.org/10.1006/gcen.2000.7557 PMID: 11121291

32. Watson R, Munro C, Edwards KL, Norton V, Brown JL, Walker SL. Development of a versatile enzyme immunoassay for non-invasive assessment of glucocorticoid metabolites in a diversity of taxonomic species. Gen Comp Endocrinol. 2013; 186:16–24. https://doi.org/10.1016/j.ygcen.2013.02.001 PMID: 23462197

33. Brown JL, Somerville M, Riddle H, Keele M, Duer C, Freeman EW. Comparative endocrinology of testicular, adrenal and thyroid function in Asian and African elephant bulls. Gen. Comp. Endocrinol. 2007; 151:153–62. https://doi.org/10.1016/j.ygcen.2007.01.006 PMID: 17336304

34. Millspaugh JJ, Burke T, Dyk GV, Slotow R, Washburn BE, Woods RJ. Stress response of working African elephants to transportation and safari adventures. J Wildlife Manage. 2007; 71(4):1257–60.

35. Menargues A, Urios V, Mauri M. Welfare assessment of captive Asian elephants (*Elephas maximus*) and Indian rhinoceros (*Rhinoceros unicornis*) using salivary cortisol measurement. Anim Welf. 2008; 17:305–12.

36. Mumbay HS, Mar KU, Hayward AD, Htut W, Htut-Aung Y, Lummaa V. Elephants born in the high stress season have faster reproductive ageing. Sci Rep-UK. 2015; 5:13946.

37. Mumbay HS, Mar KU, Hayward AD, Htut W, Htut-Aung Y, Lummaa V. Elephant stress and body condition are associated with climate and demography in Asian elephants. Conserv Physiol. 2015; 3:1–14.

38. Stead S, Meltzer D, Palme R. The measurement of glucocorticoid concentrations in the serum and faeces of captive African elephants (*Loxodonta africana*) after ACTH stimulation. J S Afr Vet Assoc. 2000; 71:192–6.

39. Boyle SA, Roberts B, Pope BM, Blake MR, Leavelle SE, Marshall JJ, et al. Assessment of flooring reno-vations on African elephant (*Loxodonta africana*) behavior and glucocorticoid response. PLoS One. 2015; 10(11):e0141009. https://doi.org/10.1371/journal.pone.0141009 PMID: 26535582

40. Fanson KV, Lynch M, Vogelnest L, Miller G, Keeley T. Response to long-distance relocation in Asian elephants (*Elephas maximus*): Monitoring adrenocortical activity via urine, serum, and feces. Eur J Wildlife Res. 2013; 59:655–64.

41. Laws N, Ganswindt A, Heistermann M, Harris M, Harris S, Sherwin C. A case study: Fecal corticosteroid and behavior as indicators of welfare during relocation of an Asian elephant. J Appl Anim Welf Sci. 2007; 10(4):349–58. https://doi.org/10.1080/10888700701555600 PMID: 17970634

42. Edwards KL, Miller MA, Carlstead K, Brown JL. Relationships between housing and management factors and clinical health events in elephants in North American zoos. 2019; PLoS One 2019; 14(6): e0217774. https://doi.org/10.1371/journal.pone.0217774 PMID: 3170219

43. Greco BJ, Meehan CL, Hogan JN, Leighty KA, Mellen J, Mason GJ, et al. The days and nights of zoo elephants: using epidemiology to better understand stereotypic behavior of African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) in North American zoos. PLoS One. 2016; 11(7):e0144276. https://doi.org/10.1371/journal.pone.0144276 PMID: 27416071

44. Meehan CL, Mench JA, Carlstead K, Hogan JN. Determining connections between the daily lives of zoo elephants and their welfare: an epidemiological approach. PLoS One. 201611(7):e0158124.

45. Hardin JW, Hilbe JM. Generalized estimating equations: Introduction. Wiley Encyclopedia of Clinical Trials, John Wiley and Sons, Inc., USA. 2007.

46. Cohen J, Cohen P, West SG, Aiken LS. Applied multiple regression/correlation analysis for the behavioral sciences. New York: Routledge. 2003.

47. Pan W. Akaike’s information criterion in GEE. Biometrics 2001; 57: 120–125. PMID: 11252586
48. Buchanan-Smith HM. Environmental enrichment for primates in laboratories. Adv Sci Res. 2010; 5:41–56.
49. Ross SR. Issues of choice and control in the behaviour of a pair of captive polar bears (Ursus maritimus). Behav Processes, 2006; 73(1):117–20. https://doi.org/10.1016/j.beproc.2006.04.003 PMID: 16687218
50. Powell DM, Vitale C. Behavioral changes in female Asian elephants when given access to an outdoor yard overnight. Zoo Biol. 2016; 35(4):298–303. https://doi.org/10.1016/j.zookeys.2016.04.003 PMID: 27128882
51. Owen MA, Swaisgood RR, Czekala NM, Lindburg DG. Enclosure choice and well-being in giant pandas: is it all about control? Zoo Biol. 2005; 24(5):475–81.
52. Schulte BA, Feldman E, Lambert R, Oliver R, Hess DL. Temporary ovarian inactivity in elephants: Relationship to status and time outside. Physiol Behav. 2000; 71:123–31. https://doi.org/10.1016/s0031-9384(00)00316-4 PMID: 11134694
53. Marcilla AM, Urios V, Limiñana R. Seasonal rhythms of salivary cortisol secretion in captive Asian elephants (Elephas maximus). Gen Comp Endocrinol. 2012; 76(2):259–64.
54. Cazares M, Silván G, Carbonell MD, Gerique C, Martinez-Fernandez L, Cáceres S, et al. Circadian rhythm of salivary cortisol secretion in female zoo-kept African elephants (Loxodonta africana). Zoo Biol. 2016; 35(1):65–9. https://doi.org/10.1002/zoo.21262 PMID: 26748465
55. Norkeaw T, Brown JL, Bansiddhi P, Somgird C, Thitaram C, Punyaporwithaya V, et al. Body condition and adrenal glucocorticoid activity affects metabolic marker and lipid profiles in working female tourist elephants in Thailand. PLoS One. 2018; 13(10):e0204965. https://doi.org/10.1371/journal.pone.0204965 PMID: 30278087
56. Posta B, Huber R, Moore DE III. The effects of housing on zoo elephant behavior: A quantitative case study of diurnal and seasonal variation. Int J Comp Psychol. 2013; 26(1):37–52.
57. Horback KM, Miller LJ, Andrews JR, Kuczaj SA. Diurnal and nocturnal activity budgets of zoo elephants in an outdoor facility. Zoo Biol. 2014; 33(5):403–10. https://doi.org/10.1002/zoo.21160 PMID: 25113850
58. Wilson ML, Bashaw MJ, Fountain K, Kieschnick S, Maple TL. Nocturnal behavior in a group of female African elephants. Zoo Biol. 2006; 25(3):173–86.
59. Cockrem JF. Individual variation in glucocorticoid stress responses in animals. Gen Comp Endocrinol. 2013; 181:45–58. https://doi.org/10.1016/j.ygcen.2012.11.025 PMID: 23298571
60. Carlstead K, Brown JL. Relationship between patterns of fecal corticoid excretion and behavior, reproduction and environmental factors in captive black (Diceros bicornis) and white (Ceratotherium simum) rhinoceros. Zoo Biol. 2005; 24:216–32.
61. Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, et al. Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev. 1999; 23(7):925–35. PMID: 10580307
62. Gurfein BT, Stamm AW, Bacchetti P, Dallman MF, Nadkarni NA, Milush JM, et al. The calm mouse: an animal model of stress reduction. Molecular Med. 2012; 18(4):606–17.
63. De Silva S, Wittemeyer G. A comparison of social organization in Asian elephants and African savannah elephants. Int J Primatol. 2012; 33(5):1125–41.