PLOS ONE

Individual and environmental risk factors associated with fecal glucocorticoid metabolite concentrations in zoo-housed Asian and African elephants
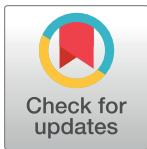
Janine L. Brown[1◉]*, **Kathy Carlstead**[1◉], **Jessica D. Bray**[1,2◉], **David Dickey**[3‡], **Charlotte Farin**[2‡], **Kimberly Ange-van Heugten**[2‡]

1 Center for Species Survival, Smithsonian Conservation Biology Institute, Front Royal, Virginia, United States of America, **2** Department of Animal Science, North Carolina State University, Raleigh, North Carolina, United States of America, **3** Department of Statistics, North Carolina State University, Raleigh, North Carolina, United States of America

◉ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* brownjan@si.edu

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 NaPO$_4$; 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[1]	Definition of independent variable
Ovarian acyclicity[2]	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[2]	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[3]	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[4]	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 Housed Separately	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[4]	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[5]	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 ft^2)	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
Muscoskeletal Score[6]	Space Experience In/Out Choice (per 500ft^2)	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 muscoskeletal exam
Foot Health[6]	Percent Time In/Out Choice	Sum of monthly percent time spent housed in a social group of one
	Space Experience Total Night (per 500 ft^2)	The average weighted (by percent time) size of all environments in which an elephant spent time at night
Walking Distance[7]	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 ft^2)	The average weighted (by percent time) size of all environments in which an elephant spent time in outdoor environments only
Serum Cortisol[8]	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

[1]Identified in published studies of the EWP:

[2]Brown et al. [4];

[3]Morfeld et al. [7];

[4]Greco et al. [43];

[5]Holgate et al. [8];

[6]Miller et al. [9];

[7]Holgate et al. [6];

[8]Carlstead et al. [18].

https://doi.org/10.1371/journal.pone.0217326.t001

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

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[a]	43.41	317.67	108.48 ± 3.03[b]	31.83	222.49
Spring (Apr-Jun)	156.83 ± 5.04[a]	57.78	286.74	107.22 ± 3.01[b]	37.56	266.17
Summer (Jul-Sep)	146.29 ± 4.27[a]	49.74	324.18	105.04 ± 2.94[b]	28.81	229.71
Fall (Oct-Dec)	147.78 ± 5.13[a]	37.82	310.56	110.01 ± 3.08[b]	26.78	292.43

[a,b]Seasonal differences between species are significant (P < 0.05).

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 ± 8.69[a]	125.47 ± 4.87[a]	99.61 ± 5.70[b]	97.72 ± 3.14[b]
Mean FGM CV	31.53 ± 1.49[a]	32.44 ± 1.28[a]	35.22 ± 2.55[a]	33.17 ± 1.18[a]

[a,b]Sex differences within species are significant ($P < 0.05$).

https://doi.org/10.1371/journal.pone.0217326.t003

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	1.070	15.20	92.59
Percent Time in Mixed Sex Herds	106	12.46	2.969	0.00	100.00	131	23.31	3.200	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
Feeding Diversity	95	1.37	0.032	0.31	1.78	129	1.38	0.018	0.98	1.79
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	129	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	51.80	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 ft^2)	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 ft^2)	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 ft^2)	106	27.64	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	129	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

https://doi.org/10.1371/journal.pone.0217326.t004

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
Percent Time In/Out Choice	106	-0.074	0.188	0.695	**131**	**-0.285**	**0.166**	**0.088**
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	0.027	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 ft^2)	**106**	**-0.187**	**0.105**	**0.080**	**131**	**0.073**	**0.026**	**0.006**
Space Experience In/Out Choice (per 500 ft^2)	**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 ft^2)	**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

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 Project[1]. 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 ft^2)	**-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

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

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 Project[1]. Significant variables are bolded.

	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 ft^2)	**0.06**	**0.02**	**0.004**
Percent Time In/Out choice	**-0.20**	**0.09**	**0.032**

[1]*Age* 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*.

when all other independent variables are held constant, the absence of *Joint Abnormalities* decreases FGM by 21.14 ng/g, and for every 5000 ft^2 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 ft^2 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*

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 multi-variable 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 ft^2)	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 ft^2)	**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

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[1]. Significant variables are **bolded**.

Independent variable	Beta	SEM	P value
Species[1] (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**

[1]Species is a confounder of *Social Group Contact* and *Enrichment Diversity*.

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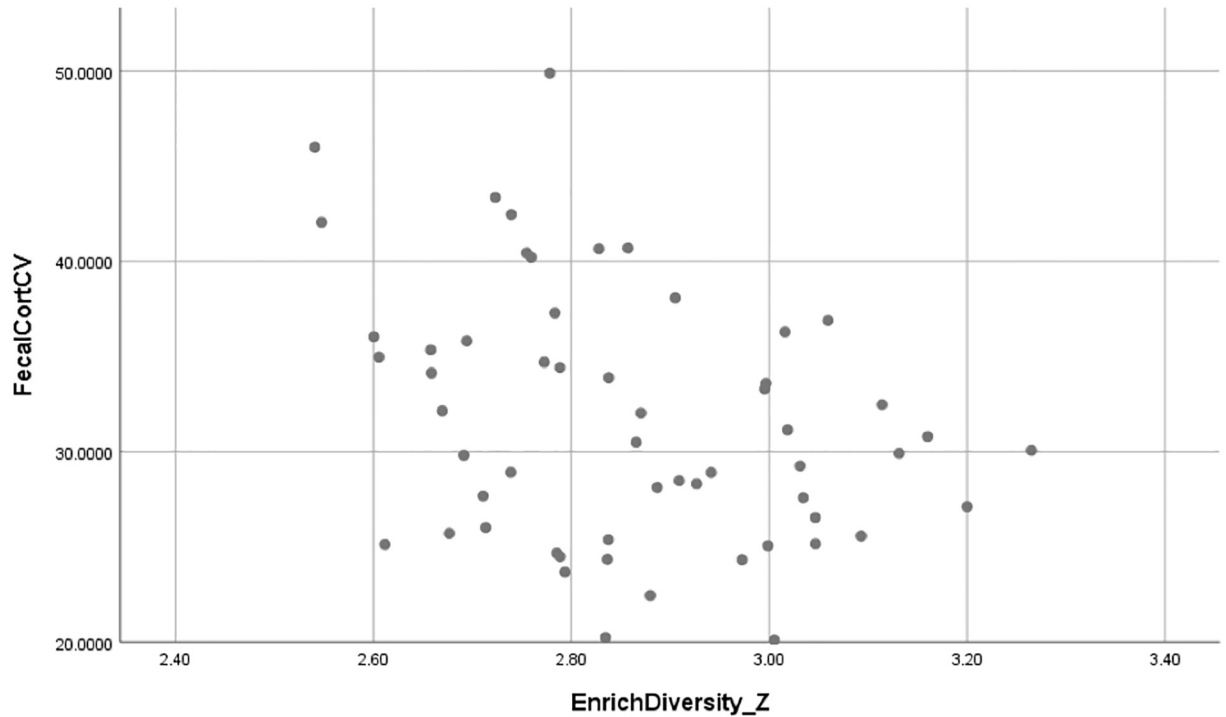
Fig 1. Correlation between zoos' enrichment diversity scores and mean coefficient of variation (CV) of fecal glucocorticoid metabolite concentrations at zoos (r = -0.339, n = 57, *P* = 0.010).

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 [52]. 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*

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 day time, 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. Cochrem [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 hypothalamo-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.

Acknowledgments

Dickerson Park Zoo, Disney's Animal Kingdom, El Paso Zoo, Fresno Chaffee Zoo, Greenville Zoo, Honolulu Zoo, Houston Zoological Gardens, Indianapolis Zoological Society, Inc., Jacksonville Zoological Gardens, Knoxville Zoological Gardens, Lee Richardson Zoo, Little Rock Zoological Garden, Los Angeles Zoo and Botanical Gardens, Louisville Zoological Garden, Lowry Park Zoological Garden, Maryland Zoo, Memphis Zoological Garden and Aquarium, Metropolitan Toronto Zoo, Milwaukee County Zoological Gardens, Montgomery Zoo, Nashville Zoo, National Zoo, Niabi Zoo, North Carolina Zoological Park, Oakland Zoo, Oklahoma City Zoological Park, Oregon Zoo, Parque Zoologico de Leon, Phoenix Zoo, Point Defiance Zoo and Aquarium, Reid Park Zoo, Riverbanks Zoological Park, Roger Williams Park Zoo, Rosamond Gifford Zoo at Burnet Park, San Antonio Zoological Gardens & Aquarium, San Diego Safari Park, San Diego Zoo, Santa Barbara Zoological Gardens, Sedgwick County Zoo, Seneca Park Zoo, Saint Louis Zoo, The Kansas City Zoo, Toledo Zoo, Topeka Zoological Park, Tulsa Zoological Park, Utah's Hogle Zoo, Virginia Zoological Park, Wildlife Conservation Society—Bronx Zoo, Wildlife Safari, Woodland Park Zoo, Zoo Atlanta, Zoo de Granby, Zoo Miami.

Author Contributions

Conceptualization: Janine L. Brown, Kathy Carlstead.

Data curation: Charlotte Farin.

Formal analysis: Kathy Carlstead, Jessica D. Bray, David Dickey, Charlotte Farin, Kimberly Ange-van Heugten.

Funding acquisition: Janine L. Brown, Kathy Carlstead.

Investigation: Jessica D. Bray.

Methodology: Janine L. Brown, Kathy Carlstead, Jessica D. Bray, David Dickey, Charlotte Farin, Kimberly Ange-van Heugten.

Project administration: Janine L. Brown.

Resources: Janine L. Brown.

Software: David Dickey, Charlotte Farin.

Supervision: Janine L. Brown, David Dickey, Kimberly Ange-van Heugten.

Validation: Janine L. Brown.

Visualization: Janine L. Brown.

Writing – original draft: Janine L. Brown, Jessica D. Bray.

Writing – review & editing: Janine L. Brown, Kathy Carlstead, Kimberly Ange-van Heugten.

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(2):256–73. 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

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: 27414654

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):e0153301. https://doi.org/10.1371/journal.pone.0153301 PMID: 27414809

9. Miller MA, Hogan JN, Meehan CL. Housing and demographic risk factors impacting foot and musculo-skeletal 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: 27415763

10. Carlstead K, Mench JA, Meehan C, Brown JL. An epidemiological approach to welfare research in zoos: the Elephant Welfare Project. J Appl Anim Welf 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: 27414034

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 responseof female rhesus monkeys to venipuncture in homecage 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, Münscher, 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, Shivaji S, Umapathy G. Non-invasive assessment of reproductive status and stress in captive Asian elephants in three south Indian zoos. Gen and Comp Endocrinol 2014; 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 fecal 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. Mumby 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. Mumby HS, Mar KU, Thitaram C, Courtiol A, Towiboon P, Min-Oo Zaw, et al. 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 Afri Vet Assoc. 2000; 71:192–6.

39. Boyle SA, Roberts B, Pope BM, Blake MR, Leavelle SE, Marshall JJ, et al. Assessment of flooring renovations 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 serum, urine, 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: 31170219

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/10.1002/zoo.21289 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. Casares 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, Punyapornwithaya 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, Wittemyer G. A comparison of social organization in Asian elephants and African savannah elephants. Int J Primatol. 2012; 33(5):1125–41.