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Research article

Evaluating physiological and behavioural responses to social changes and construction in two zoo-housed female giraffes

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Abstract

Exposure to external repeated or long-term stressors can alter animal behaviour and physiology. At zoos, construction of new buildings and habitats is one potential unavoidable long-term stressor. During the construction of a new exhibit near the giraffe enclosure at Lincoln Park Zoo (Chicago, IL), the Zoo's two female giraffes, *Giraffa camelopardalis rothschildi* and *Giraffa reticulata*, were monitoed for changes in behavior and faecal glucocorticoid metabolite (FGM) levels during five phases of construction and enclosure access. The FGM analysis was validated by analysing illness and eventual loss of a companion—when one of the giraffes became ill and was euthanised during the study period. In the four months prior to her death, this giraffe exhibited elevated cortisol and corticosterone metabolites; er companion exhibited elevated FGM in the months following her death. Regarding the effects of construction on faecal cortisol metabolite production and behaviour, both giraffes exhibited higher FCMs during the initial demolition phase, but only one individual exhibited elevated FCMs during the prolonged, active construction that followed. This individual also exhibited decreased inactivity and increased locomotion, as well as an increase in the frequencies of abnormal oral and locomotor stereotypies. Such stereotypies included pacing, licking/gnawing of non-food objects, and tongue play during active construction compared to subsequent time periods. IIn addition to such inter-specific and inter-individual variation, both construction and loss of companion were significant sources of stress for the giraffes. Future construction and other stressful long-term events should be paired with careful behavioural and faecal hormone metabolite monitoring, alongside monitoring for variation between individuals, to better inform management decisions regarding zoo animal care.

Introduction

Zoological institutions must make management decisions that promote long-term animal welfare (defined as an animal's collective physical, mental and emotional states over a period of time, and measured on a continuum from good to poor; Association of Zoos and Aquariums Welfare Committee). Species housed in zoos and wildlife institutions often face unique external stressors. Though they do not experience stress that results from predation or inability to find food, factors such as proximity to visitors, abnormal social groups, construction, noise and light conditions, and reduced habitat size can trigger both short- and long-term stress responses (Hosey 2005; Morgan and Tromborg 2007; Mason 2010; Kalioujny et al. 2013).

The stress response to external stimuli is an essential physiological feature. Upon encountering a stressor, the hypothalamic-pituitary-adrenal (HPA) axis activates, and the release of corticotropin-releasing hormone by the hypothalamus stimulates secretion of the adrenocorticotropic

hormone (ACTH) by the pituitary gland. ACTH, in turn, stimulates secretion of glucocorticoids, such as cortisol and corticosterone, by the adrenal cortex (Sapolsky et al. 2011).In the short term, glucocorticoids mobilise energy and change behaviour to improve fitness.The release of glucocorticoids can occur in response to positive external stimuli, including courtship, social hierarchy and even during enrichment and play for animals in zoos (Mostl and Palme 2002). However, stress operates on a continuum, and in increasingly stressful situations, the adrenal cortex secretes higher amounts of glucocorticoids into the blood, which are excreted via urine and faeces following metabolism (Mostl and Palme 2002). Prolonged or repeated stress over a long time has been shown to significantly decrease immune function, reproductive ability and survival (Pride 2005; Sheriff et al. 2011; Tort 2011). Thus, quantifying stress through faecal glucocorticoid metabolite (FGM) analysis has been used widely for species physiology monitoring (Keay et al. 2006; Schwarzenberger 2007; Ganswindt et al. 2012; Kersey and Dehnhard 2014).

To ensure that hormone assays are providing accurate results and that the results make biological sense, it is important to validate FGM analysis, both biochemically and physiologically (Palme 2019). Biochemical analysis occurs in the laboratory using parallelism and percent recovery tests; physiological validation includes ACTH challenges—where individuals are injected with ACTH to prompt a release of glucocorticoids by the adrenal glands (Santymire et al. 2012; Crill et al. 2019). As ACTH challenges are more invasive, researchers prefer biological validations for stress physiology (Cook 2012). Biological validations can include reproductive events (such as pregnancy, mating, or giving birth), stressful events (such as transportation and veterinary procedures), and social interactions, such as fighting, introduction to a new social group, or losing a conspecific (Palme 2019).

FGM monitoring should also take place before, during and after known stressors, to ensure that fluctuations in FGMs accurately reflect the valence of a stress response, rather than a result of HPA axis dysregulation, which causes artificially blunted HPA axis responses following chronic stress (Karin et al. 2020). Careful and continued monitoring over many months can help establish a baseline for the individual's typical physiology that will contextualise future stress responses and reduce the likelihood that decreases in FGM levels are incorrectly interpreted to be indicative of reduced stress. Finally, pairing FGM monitoring with behavioural observations helps further ensure that up- or down-trends in glucocorticoid production match the patterns of short- and long-term stress experienced by the animal. During stressful events, individuals may suppress normal behaviours, such as feeding and foraging, or increase others, such as lethargy (Jakob-Hoff et al. 2019). Increases in the frequency of abnormal behavioural stereotypies, agonistic behaviour and vigilance have also been observed (Mason and Latham 2004; Shepherdson et al. 2013); in larger animals (including ungulates and large carnivores), these include interspecies aggression and oral and locomotor stereotypies, such as excessive grooming, pacing, trunk curling and foot lifting (Loeding et al. 2011; Chosy et al. 2014; Jakob-Hoff et al. 2019). In giraffes, specifically, Bashaw et al. (2001) observed licking of non-food objects and pacing in captive individuals across zoological institutions. When Lincoln Park Zoo (Zoo; Chicago, IL, USA) initiated construction of a new exhibit in October of 2014, animal care staff began monitoring its effects on the Zoo's two female giraffes *Giraffa camelopardalis rothschildi* and *Giraffa reticulata,* which were housed nearby. This study used FGM analysis and behavioural monitoring to investigate whether prolonged construction activity and decreased access to the full enclosure triggered changes in the stress physiology and behavioural health of the two individuals. When one of the giraffes became ill and was euthanised during this study period, the effects of illness and eventual loss of a companion were also analysed. The objectives were to: 1) use the effects of illness and the loss of a companion as a biological validation for FGMs and behaviour; 2) determine the appropriate glucocorticoid to monitor stress physiology using the results of these biological events; and 3) evaluate the effects of different stages of construction and access to the enclosure on long-term stress physiology. It was likely that illness and the eventual loss of a companion would elicit physiological and behavioural stress responses in the two individuals. Additionally, it was likely that the giraffes would also exhibit elevated FGM concentrations from baseline, coupled with increased frequencies of oral and locomotor stereotypic behaviour, following periods of demolition or active construction and/or modified access to the full enclosure. This prediction is based on previous studies, showing that individuals across different species exhibit heightened FGMs and changes in behaviour in response to construction noise, enclosure modifications and social changes. These studies include felids (Sulser et al. 2008; Chosy et al. 2014), giant pandas (Powell et al. 2006), macaques (Westlund et al. 2012), and elephants, giraffes and emus (Jakob-Hoff et al. 2019).

Methods

Animals and housing conditions

Lincoln Park Zoo housed two species of giraffe in the Regenstein African Journey (RAJ) building. The two females, giraffe #1729 (*G. c. rothschildi;* deceased at age 28) and #2476 (*G. reticulata;* age 18 at conclusion of study), were born in North American zoological parks and were on loan to the Lincoln Park Zoo since May of 2003.

The giraffe exhibit consisted of two connected enclosures, with access to the inside, outside and holding spaces (Figure 1). Depending on weather conditions, the giraffes either had inside access only, outside access only, or could move between inside and outside enclosures. The RAJ building was carefully climate-controlled and also housed nearby other African species, including aardvarks *Orycteropus afer*, meerkats *Suricata suricatta*, pygmy hippopotamus *Choeropsis liberiensis*, African wild dogs *Lycaon pictus* and several species of birds.

Timeline of events

The timeline of events in this study is summarised in Figure 2. Construction of a new exhibit adjacent to the giraffe outside enclosure began in October of 2014. Demolition of the existing structure lasted until December 2014; during this period, noise and vibration levels were at a maximum near the enclosure. After a period of inactivity, active construction began in April 2015 and ceased in November 2015. On days with heavy construction, giraffes were restricted from accessing the outside enclosure. Work continued on the outdoor giraffe enclosure through October 2016, after which giraffes were given access to a new, larger outdoor space. In mid-March 2017, giraffe #1729 began exhibiting symptoms of illness, and was eventually euthanised on 11 July 2017. Throughout July and August 2017, plains zebra *Equus quagga* were introduced and then co-housed with the remaining giraffe in the full outdoor enclosure.

Faecal sample collection and hormone extraction

Faecal sample collection began on 7 October 2014 and continued until 30 June 2017 for giraffe #1729, and until 20 October 2017 for giraffe #2476. Fresh faecal samples were collected two to three times per week and immediately placed into a labelled, sealed plastic bag and stored in a freezer at −20ºC.

Faecal hormone metabolites were extracted using the protocol described by Loeding et al. (2011). Briefly, 5.0 mL of 90% ethanol (100% ethanol:distilled water) was added to 0.5±0.02 g samples of frozen-thawed faeces and vortexed (Daigger, Vernon Hills, IL).

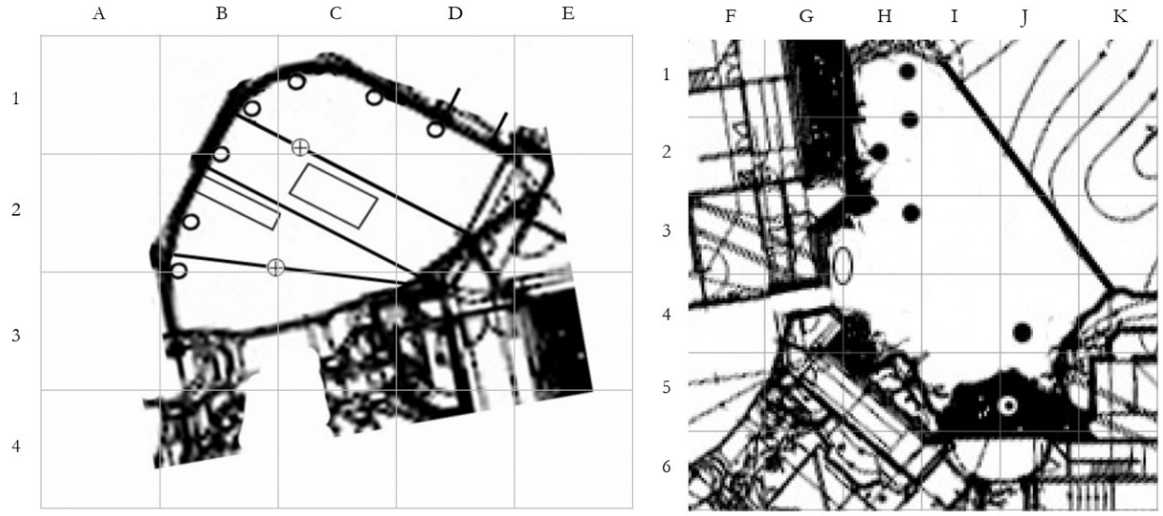

Figure 1. Inside enclosure (left) and outside enclosure (right) of giraffe exhibit in with grid overlay. Open circles represent wooden posts; circles filled with an X are hanging baskets with foodstuff. The large rectangle in C2 is a skylight. Large black dots represent locations of large trees within the enclosure. The viewing area for visitors is south of the enclosure in squares B3 and C3 and represented by the semicircle in squares I6 and J6. Construction occurred in squares marked with wavy solid lines, I1, J1-2 and K1-3.

Samples were shaken on a mixer (Glas-Col, Terre Haute, IN) for 30 min, and then centrifuged at 1500 rpm for 20 min. The supernatant was poured into a second set of tubes. The faecal pellets were then re-suspended in 5 mL of 90% ethanol, vortexed for 30 sec, and centrifuged (1500 rpm; 15 min). The supernatant was combined with the first set before air-drying both sets of supernatant in a warm water bath (60ºC). The extract was reconstituted in 1 mL of phosphate buffered saline (0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, NaCl), then vortexed and sonicated (Fisher Scientific, Waltham, MA) for 20 min. After sonication, 500 µL of the extract was diluted 1:2 with 500 µL of dilution buffer; these 1:2 dilutions were used to create the subsequent dilutions for hormonal analysis.

Enzyme immunoassay

Samples were analysed for FGM with both corticosterone and cortisol enzyme immunoassays (EIA). Corticosterone antiserum (CM0006) and horseradish peroxidase (HRP) ligands were used at a 1:15,000 dilution; corticosterone antibody cross-reactivities have been previously published by Santymire and Armstrong (2010). The cortisol antiserum (R4866) and HRP ligands were used at a 1:8,500 and 1:20,000 dilution, respectively; cortisol antibody cross-reactivities have been previously published by Loeding et al. (2011). EIAs were biochemically validated specifically for these two individuals by demonstrating: 1) parallelism between binding inhibition curves of faecal extract dilutions (1:2–1:2,048, r=0.98 for corticosterone; 1:2–1:512, r=0.97 for cortisol) and

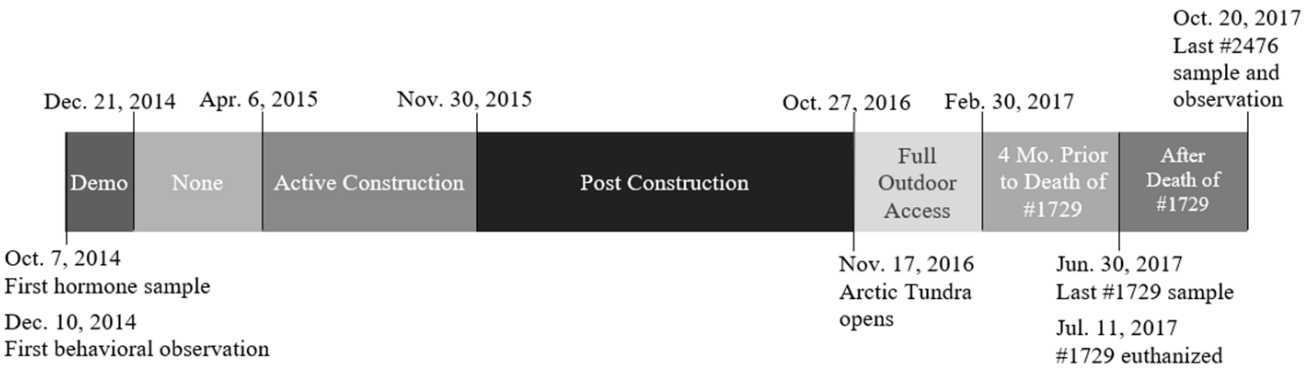

Figure 2. Timeline of construction activity and the collection of faecal samples and behavioural observations for two female zoo-housed giraffes. Faecal samples were collected two to three times per week, and behavioural observations were collected approximately four times a week, over three years of study.

the standard; and 2) significant recovery (>90%) of exogenous corticosterone (3.9–1,000 pg/well) and exogenous cortisol (1.95–1,000 pg/well) added to faecal extracts (y=1.05x–5.47, R^2=0.999 for corticosterone; y=1.01–1.02x, R^2=0.999 for cortisol). Assay sensitivity was 3.9 pg/well (corticosterone) and 1.95 pg/well (cortisol); intra-assay coefficients of variation were <10% and inter-assay coefficients of variation were <15%.

Behavioural data collection

Behavioural observations were recorded by animal care staff and zoo volunteers using the ZooMonitor app (Wark et al. 2019; Lincoln Park Zoo, 2021) on tablet devices. Animal care staff and volunteers passed an inter-observer reliability test with a percent agreement criterion of greater than 85% consistency with a trained observer (Crockett 1996). Observers recorded behaviour and approximate

Table 1. Ethogram used to collect behaviours of two female zoo-housed giraffes using the ZooMonitor application (Ross et al. 2016; Wark et al. 2019). Behaviours are classified within categories.

Behaviour category	Behaviour	Description
Locomotion	Locomotion	Movement of at least one body length from one location to another on the ground by walking or running.
Feed/forage/drink/ruminate	Feed/forage/drink	Using mouth to obtain food items, and chewing and ingesting those food items, or manipulating an object to extract a food item (e.g., includes any food in enclosed containers like boxes, bags, ice blocks, puzzle feeders, etc.), or is ingesting water.
	Ruminating	Regurgitation and chewing cud of previously eaten food. Does not include periods of chewing which might accompany foraging.
	Food play	Repetitive oral manipulation of food.
Social	Affiliative behaviour	Performing any non-sexual affiliative behaviour, including grooming a social partner, playing, or social greetings.
	Agonistic behaviour	Performing any aggressive behaviour, either with or without contact, or any dominance-related behaviour.
Undesirable	Repetitive licking/gnawing	Repeatedly licking or gnawing of a non-food object, including wood or metals posts, rocks or gunite, or concrete floors.
	Pacing	Walking in a repetitive manner along a fixed path without an apparent goal or function, moving along the path at a minimum of three times.
	Head roll	Moving and rolling the head in a fixed stereotypic manner not related to feeding. May be a single bout or repeated movement.
	Tongue play	Tongue repetitively moving in/out (e.g., similar to a firehose flopping around), not for purpose of feeding.
Other solitary	Object manipulation	Engaged with a non-food object in enclosure using mouth or feet.
	Elimination (non-marking)	Excretion of body waste in a non-scent marking manner (i.e., not spraying urine or spreading faeces over a specific target area).
	Focused investigation	Actively sniffing or pawing at a specific area within ½ body's length.
	General exploration	Sniffing the substrate or air in a non-focused manner.
	Object rubbing (non-marking)	Rubbing body against an object.
	Other self-maintenance	Performing any comfort-related behaviour other than wallowing, including self-directed behaviours, stretching, yawning, rolling, or rubbing against objects.
	Vocalisation	Producing sounds through intentional vibration of air for the purpose of communication (may involve vocal cords, stridulation of body parts, vibration of air sacs, or other taxa-specific adaptations).
	Flehmen	Mouth open with a raised upper lip and a grimaced facial expression.
	Solitary play	Locomoting in a non-directed manner or interacting with an object without a clear purpose.
Not visible	Behaviour obscured	Behaviour cannot be determined but the location of the animal is known.
	Animal not visible	Animal is completely not visible, and its location is unknown.

location in the exhibit via instantaneous point sampling every minute for 10 min (Crockett 1996). Observation sessions were conducted separately for inside and outside enclosures (when the giraffes were given outside access). Data collection was conducted from December 2014 to October 2017 between 1000 and 1600 hr (n=681 observations), approximately four times per week.

Using the ZooMonitor app, observers recorded giraffe location within the habitat, outside temperature, construction activity (ongoing or off), weather conditions, crowd size and whether the giraffes had inside and outside access. For behavioural data, observers recorded behaviour from a general activity ethogram (Table 1). Pacing, repetitive licking/gnawing of non-food objects, tongue play and head roll were defined as abnormal repetitive behaviours (Seeber et al. 2012).

Data analysis

FGM data were analysed using Microsoft Excel (MS Office 365) and Sigma Plot Version 11.0 (SPSS Inc., Chicago, IL). A Kolmogorov-Smirnov test was used for normality assumption testing, and Levene's median test was used for equal variance assumption testing. Nonparametric tests were used for nonnormal data. Values are reported as the mean±standard error (SEM). FGM baseline values for each giraffe were calculated using an iterative process, in which high values that exceed the mean plus 1.5 standard deviations (SD) are excluded before baseline value is determined (Loeding et al. 2011). Elevated FGM values are defined as values greater than the baseline FGM plus 1.5 SD. A Mann-Whitney rank sum test was used to analyse the effect of illness and loss of companion on giraffe #1729 and giraffe #2476, respectively. Friedman repeated measures ranks and one-way analysis of variance (ANOVA) tests were used to determine differences in mean FGM values between the five phases of construction (demotion, none, active, post and full outdoor to illness/full outdoor access) for each individual. Post-hoc comparisons were conducted using the Student-Newman-Keuls test.

Behavioural data were analysed using R statistical software (R Core Team 2018). To limit the number of behavioural comparisons and investigate broad changes in the activity budget of the giraffes, behaviour data were grouped and analysed in the following categories: inactive, locomotion, feed/forage/drink/ruminate (FFDR), social, undesirable (consisting of abnormal repetitive behaviours listed above) and other solitary (Table 1). As visibility of the giraffes changed throughout the season (based on the enclosure access determined by animal care staff); data were summarised for each session as the percentage of scans visible. Sessions with low visibility (<5 scans visible) were excluded from analyses to prevent artificially inflated percentages.

Analysis of loss of companion on giraffe #2476's behaviour was conducted using the Kruskal-Wallis test. Giraffe #1729's behavioural changes during illness were assessed using the Mann-Whitney rank sum test. Kruskal-Wallis and Dunn tests of multiple comparisons were used to assess behavioural changes across the four construction phases. Behavioural observations began after demolition was completed; accordingly, the construction analysis did not include this study phase. Data from the full outdoor phase were also excluded from analysis of construction effects on giraffe #1729, as she began to show potential illness-related behavioural changes.

Results

Biological validation of FGM analysis

The effects of the loss of giraffe #2476's companion on her FGM, and giraffe #1729's time of illness were used to determine the appropriate glucocorticoid (corticosterone or cortisol) for evaluating giraffe stress physiology. Unsurprisingly, giraffe #1729 exhibited a higher (2.3 fold higher; U=63.0, P=0.003) mean faecal cortisol metabolite concentration after contracting illness in the

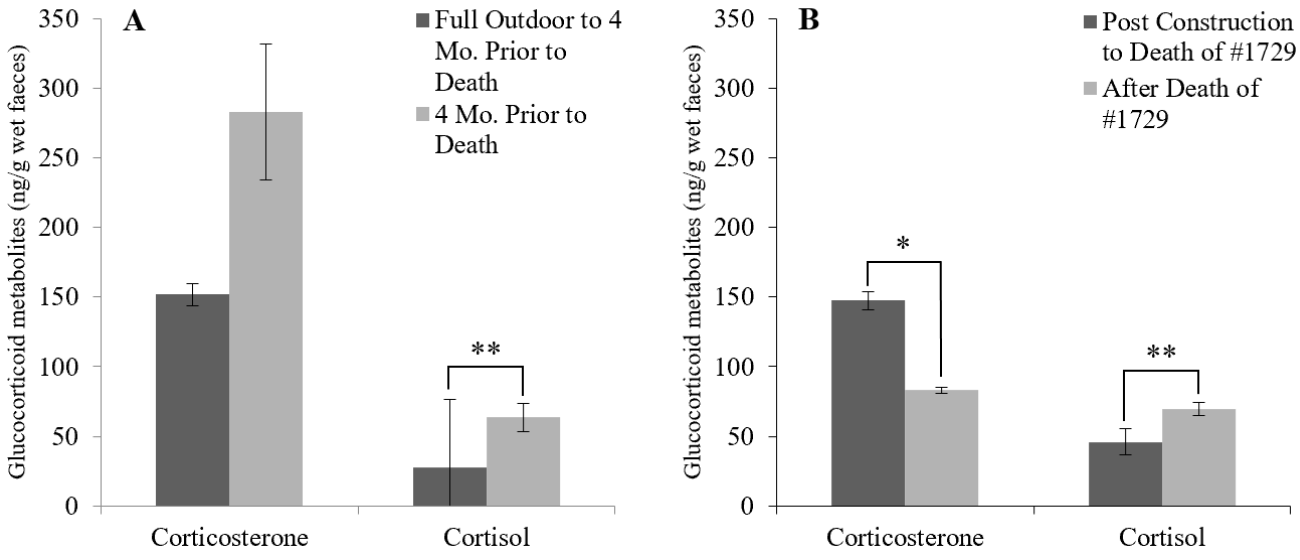

Figure 3. Mean (±SEM) glucocorticoid metabolites (ng/g wet faeces), corticosterone and cortisol, for giraffe #1729 (A) post full outdoor access and 4 months prior to death, and for giraffe #2476 (B) prior to and post giraffe #1729's death. Asterisks denote significance at the P<0.05 level; double asterisks denote significance at the P<0.01 level.

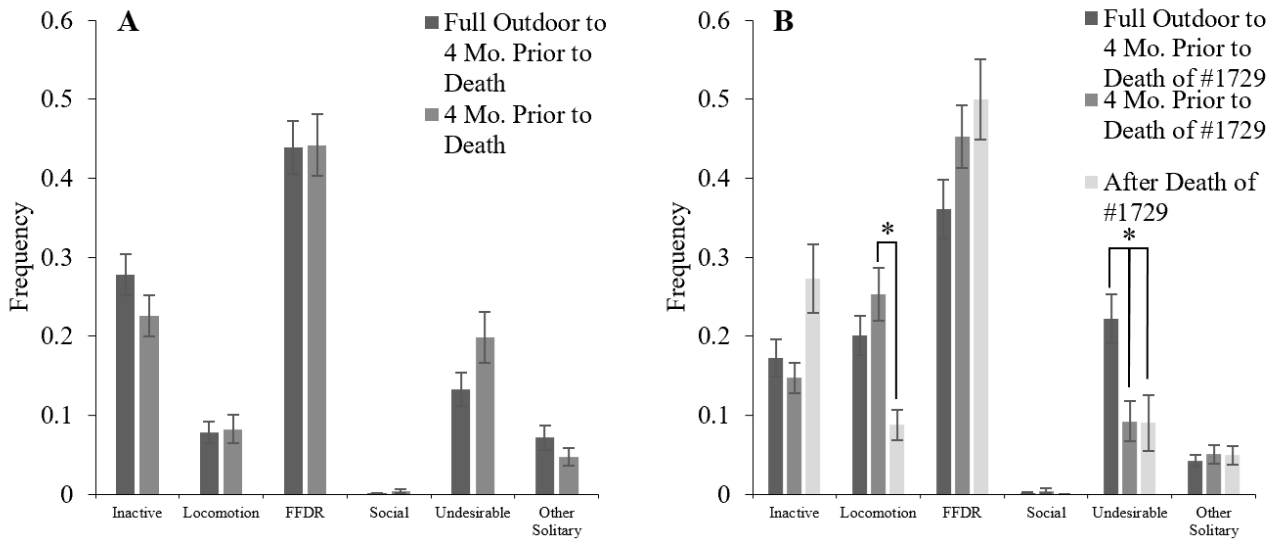

Figure 4. Mean (±SEM) frequencies of behavioural categories for giraffe #1729 (A) post full outdoor access and 4 months prior to death; and giraffe #2476 (B) preceding giraffe #1729's illness, during her illness and after her death. FFDR denotes the feed/forage/drink/ruminate behavioural category. Asterisks denote significance at the P<0.05 level within behavioural category

4 months prior to her death than during the post-construction, full outdoor access phase (Figure 3). Following the loss of her companion, giraffe #2476's faecal cortisol metabolites increased (1.5-fold increase; U=410.0, P<0.001) while corticosterone metabolites declined (1.8-fold decrease; U=420.0, P<0.001) compared to the 4 months prior (Figure 3). Because cortisol metabolites increased for both individuals during what were considered 'stressful' events, faecal cortisol metabolites were used as the primary marker for analysing stress response to construction in both individuals (though the decrease in faecal corticosterone metabolites may also point to possible HPA axis dysregulation; see Discussion). Giraffe #1729's faecal cortisol metabolite values were as follows: mean, 40.88±1.41; baseline, 22.82 ng/g wet faeces; range, 15.53 to 128.96 ng/g wet faeces. Giraffe #2476's faecal cortisol metabolite values were as follows: mean, 41.12±1.40; baseline, 19.01 ng/g wet faeces; range, 15.53 to 128.96 ng/g wet faeces (Figure 5).

Giraffe #2476 exhibited behavioural changes following giraffe #1729's death. Specifically, giraffe #2476 exhibited decreased locomotion compared to previous time periods (Figure 4) (χ^2=10.18, df=2; P=0.006, 4 months prior to death of #1729 vs after death of #1729: Z=3.15, P=0.005; after death of #1729 vs full outdoor to 4 months prior to death of #1729: Z=−2.45, P=0.029). Contrary to expectations, compared to the preceding full outdoor phase, giraffe #2476 exhibited decreased undesirable behaviours during the 4 months prior to and after giraffe #1729's death (Figure 4) (χ^2=14.34, df=2, P<0.001; 4 months prior to death of #1729 vs full outdoor to 4 months prior to death of #1729: Z=−3.36, P=0.0023; after death of #1729 vs full outdoor to 4 months prior to death of #1729: Z=−2.85, P=0.0088). The frequency of giraffe #2476's behaviours in the remaining behavioural categories (inactive, FFDR, social and other solitary) was similar (P>0.05) across these three phases (overall means: inactive, 0.198±0.029; FFDR, 0.438±0.043; social, 0.002±0.001; other solitary, 0.047±0.010). Meanwhile, giraffe #1729's behavioural patterns (P>0.05) remained consistent between the full outdoor phase and the 4 months prior to her death (overall mean: inactive, 0.252±0.026; locomotion, 0.080±0.016; FFDR, 0.441±0.036; social, 0.002±0.002; undesirable, 0.166±0.027; other solitary, 0.059±0.013).

Effect of construction and enclosure access on faecal cortisol metabolites and behaviour

As expected, giraffe #1729's faecal cortisol metabolites were highest ($F_{4,72}$=24.549, P<0.001) during demolition and active construction, compared to no construction, post-construction and in the months prior to her illness (Figure 6). Giraffe #2476's faecal cortisol metabolites were similarly elevated during demolition, but actually lowest ($F_{4,85}$=27.342, P<0.001) during active construction compared to all other phases (Figure 6). The switch to a larger outdoor enclosure, post-construction, had no effect (P>0.05) on faecal cortisol metabolites for either individual.

Consistent with the hypothesis, behavioural changes across construction phases were observed in both individuals (Figure 7). Giraffe #2476 exhibited a decrease in frequency in other solitary behaviours (χ^2=13.2, df=3, P=0.004) during active construction and the full outdoor phase, compared to previous active (Z=−3.22, P=0.008) and post (Z=2.90, P=0.019) construction phases. Giraffe #1729 exhibited broader changes in response to construction across three behavioural categories: undesirable (χ^2=13.10, df=2, P=0.001), locomotion (χ^2=9.52, df=2, P=0.009), and inactive (χ^2=15.49, df=2, P<0.001). During active construction, giraffe #1729's undesirable (Z=3.57, P=0.001) and locomotor (Z=3.071, P=0.0064) behaviours increased, and inactive behaviours decreased (Z=−3.93, P<0.001).

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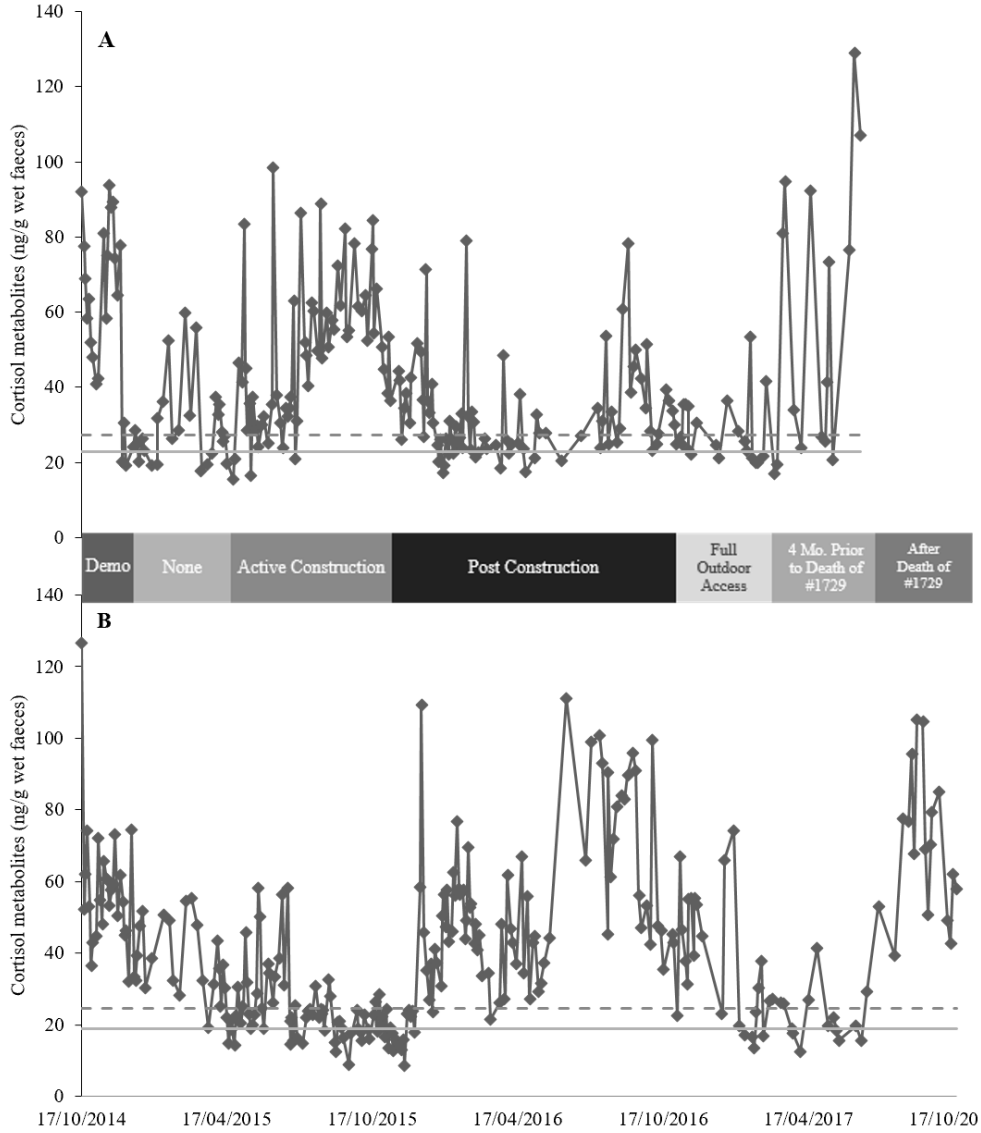

Figure 5. Faecal cortisol metabolite values (ng/g wet faeces) for giraffe #1729 (A) and giraffe #2476 (B) from October 2014 to October 2017. Solid lines reflect mean baseline values; dashed lines reflect elevated values, which are the mean baseline plus 1.5 SD.

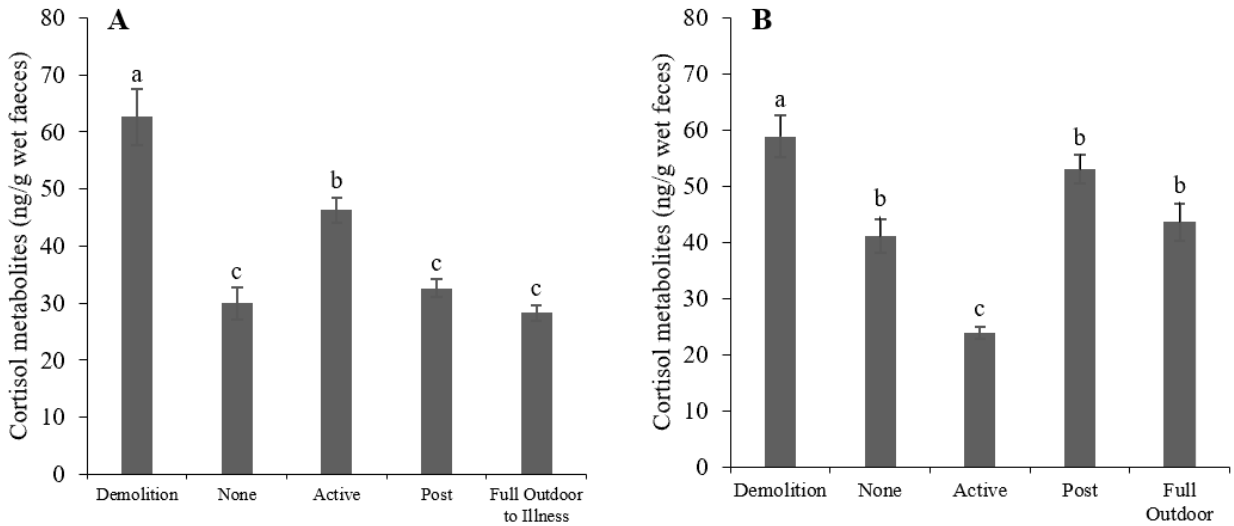

Figure 6. Mean (±SEM) cortisol metabolites (ng/g wet faeces) for giraffe #1729 (A) and giraffe #2476 (B) during phases of construction. Superscripts indicate differences among construction phases at the P<0.001 level for that individual.

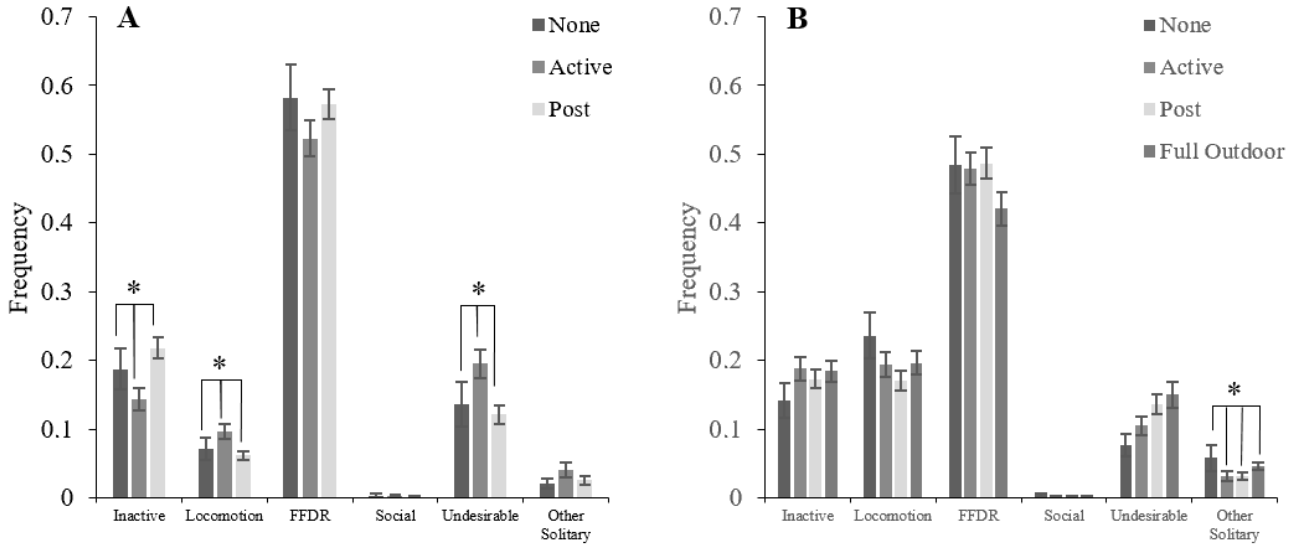

Figure 7. Mean (±SEM) frequencies of behavioural categories across construction phases for #1729 (A) and #2476 (B). FFDR denotes the feed/forage/drink/ruminate behavioural category. Asterisks denote significance at the P<0.05 level within behavioural category.

Discussion

When evaluating wildlife stress physiology using FGMs, it is important to ensure that the results make biological sense. There are several ways to validate the hormonal results— biochemically (in the laboratory), physiologically (using ACTH challenges), and biologically (using known stressful events such as transportation, veterinary procedures and social interactions like the loss of a member of the group) (Palme 2019). Here, the loss of giraffe #2476's companion provided an unplanned opportunity to biologically validate which glucocorticoid (corticosterone or cortisol) is a more effective measure of stress physiology in giraffes. Following the loss of her companion, giraffe #2476's cortisol metabolites increased, but her corticosterone metabolites decreased. Distress caused by a loss of companion for species that form social groups has often been observed (Koyama et al. 2012; Reggente et al. 2016), and for two individuals in a pair bond, a decrease in FGMs following the loss of a companion is unusual.

The predominant glucocorticoid for measuring stress response should make sense biologically, and it is possible that one glucocorticoid may not be as relevant or responsive to external stressors as another (as Murray et al. 2020 found in southern sea otter). It is also possible that giraffe #2476's decline in faecal corticosterone metabolites following her companion's death, as well as the depressed level of faecal cortisol metabolites during active construction, could be indicative of a pathology in which the HPA axis is downregulated following a period of prolonged stress (Karin et al. 2020). Low levels of faecal corticosterone metabolites might accordingly signal HPA axis dysfunction, rather than the lack of responsiveness to a stressor. However, occurrence of HPA axis dysregulation would likely yield a blunted response in both glucocorticoids, corticosterone and cortisol, rather than the opposite trend found here in giraffe #2476.

Though Chinnadurai et al. (2009) previously validated the use of corticosterone as the dominant glucocorticoid in giraffes and other South African herbivores, Bashaw et al. (2016) found that in fact 11-oxoetiocholanolone EIAs best and most consistently identified FGM peaks following an ACTH challenge, and corticosterone was especially inconsistent in detecting FGM elevation from baseline. In this study, it was not possible to conduct 11-oxoetiocholanolone assays, and because greater consistency was observed in cortisol assays under loss of companion and construction conditions, the cortisol analysis was chosen as the predominant glucocorticoid for measuring adrenocortical activity.

Giraffe #1729's endocrinological profile showed significantly elevated concentrations of faecal cortisol metabolites from mid-March 2017 through early July 2017. During this four-month interval, she had been managed for repeated episodes of colic of increasing severity and concurrent weight loss; this concluded in a euthanasia recommendation on 11 July 2017. At necropsy, a marked chronic gastrointestinal issue was identified as the probable cause of the ante-mortem presentation. The attending veterinarian concurred that the cortisol rise was correspondent as an index of physiologic distress and pain as a result of the lesions identified in this individual. But giraffe #1729's behavioural profile did not show a corresponding change in stress behaviour. In the four months prior to her death, the frequency of giraffe #1729's undesirable behaviours did increase, but there were no detectable changes to her activity budget. Further, giraffe #2476 did not exhibit a significant change to the frequency of abnormal repetitive stereotypies following her companion's death. Given giraffe #2476's solitary housing condition for several months after the death of #1729, it would be expected that the loss of her companion would result in an increase in potentially anxiety-related behaviours, like stereotypies—as other studies on loss of companion have shown (Tarou et al. 2000; Koyama et al. 2010)—but that was not observed here. Other studies have described older individuals to be dominant in zoo-housed giraffe herds (Horová et al. 2015) and the death of #1729, as the older animal, may have represented a relaxed social pressure on giraffe #2476. However, a clear dominance hierarchy between the two giraffes in this study was not observed, making this explanation tentative. Giraffe #2476 did exhibit a significant decrease in locomotor behaviours following giraffe #1729's death, attributable perhaps to fewer social interactions that would otherwise drive locomotion (manoeuvring around a companion, for example). The divergence between hormonal and behavioural responses in this

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case highlights the importance of combining physiological and behavioural welfare monitoring, as an individual's response to stress may not be captured well in only one methodology.

In response to nearby construction, the giraffes at Lincoln Park Zoo exhibited varying levels of physiological and behavioural responses. Both individuals exhibited significantly elevated faecal cortisol metabolites during demolition when noise and vibration levels outside the enclosure were likely at a maximum. However, while giraffe #1729's faecal cortisol metabolites remained elevated during the active construction that followed, giraffe #2476's mean faecal cortisol metabolite values dropped to its lowest value in that same time period. The giraffes' behavioural responses to construction were similarly varied. Giraffe #1729 spent more time performing repetitive stereotypies during active construction, as well as increased locomotion and decreased inactivity. These changes suggest a negative response to construction activities by giraffe #1729. On the other hand, no significant change was observed in giraffe #2476's activity budget during active construction. The variation in the giraffes' stress responses during active construction may be due to the age difference between the two individuals; giraffe #1729 (at age 27) may have responded more significantly and with greater variability to external stressors than giraffe #2476 (at age 18). As other studies have shown, age can be a source of variability in glucocorticoid metabolism (Anestis et al. 2006). Other factors of inter-individual variability—such as personality, maternal influences, responsiveness to handling method, glucocorticoid plasticity, or the use of stereotypies as coping mechanisms—have also been observed both in the wild (Cockrem 2013; Guindre-Parker et al. 2019) and in zoo-housed species such as elephants (African and Asian; Proctor and Brown 2015) and polar bears *Ursus maritimus* (Shepherdson et al. 2013). The two individuals in this case study also belong to distinct species of giraffe, and genome sequencing studies have suggested greater genetic complexity between giraffe species than previously assumed (Fennessy et al. 2016). In an ACTH challenge comparing two individuals from different species (*G. c. giraffa* and a hybrid species of *G. camelopardalis*), Bashaw et al. (2016) noted significant differences in FGM peaks and peak duration (for cortisol and corticosterone) across species as well. In this same study, researchers measured FGM responses to transportation (a similar stressful event as construction) using 11-oxoetiocholanolone EIAs and found that individuals of species *G. c. reticulata* had exceptionally high FGM concentrations (over a 7-fold increase from baseline) following transport compared to individuals of species *G. c. rothschildi* and *G. c. angolensis* (between a 1-fold and 2-fold increase from baseline). In this study, giraffe #2476 (*G. c. reticulata*) showed a near 6.5-fold increase from baseline in peak cortisol metabolites during demolition, while giraffe #1729 (*G. c. rothschildi*) exhibited closer to a four-fold increase in peak cortisol during that same time. Accordingly, inter-individual and interspecies variation may each play a role in the level of response to external stressors exhibited by the two individuals.

While it is promising that one individual in this study did not exhibit a significant stress response to an expected external stressor, the results suggest that construction activity can be a substantial source of stress for some zoo animals, particularly when layered with the potential effects of inter-individual or inter-species variation. Previous studies have similarly identified construction noise as a physiological and behavioural stressor for captive animals (Powell et al. 2006; Sulser et al. 2008; Chosy et al. 2014; Jakob-Hoff et al. 2019). Moreover, the impact of chronic anthropogenic noise pollution (not only from construction, but also industrial activity, traffic and other ambient noise) on the long-term health of various species in the wild has been well documented (Barber et al. 2010; Kight and Swaddle 2010; Laiolo 2010).

Alongside construction noise, restriction to the inside enclosure may have provided an additional external stressor for the giraffes. Occurrences of oral and locomotor stereotypies (pacing and licking/gnawing) were notably present under periods of active construction that resulted in restrictions to outside access. Once giraffes were given access to the full outdoor enclosure, increases were observed in the time giraffe #2476 spent performing other solitary behaviours, including exploratory behaviours. The novelty of a larger enclosure with new enrichment objects, and the eventual introduction of zebra into the exhibit, may have also increased stimulation and investigatory behaviour. For many species of animals housed ex situ, enclosure conditions and changes in spatial density can have significant impacts on physiological stress and abnormal behaviour (Duncan et al. 2013; Polverino et al. 2015; Miller et al. 2019). Especially for larger animals, including ungulates and large carnivores, restricted access to outdoor enclosures and/or a lack of large, complex spaces can result in marked increases in oral and locomotor stereotypies (Hosey 2005; Shepherdson et al. 2013). Locomotor stereotypies such as pacing, observed in captive mammals, are importantly distinct from normal instances of locomotion. Certainly, many possible motivations may underlie this behaviour, but researchers generally understand these stereotypies to be a welfare concern that requires greater attention (Greening 2019).

Importantly, having only two individuals in the study creates limitations to the scope of the research—the findings cannot be generalised to understand how all giraffes may respond to stressors like illness, loss of companion, or construction. However, the findings do illustrate current methods of welfare monitoring for zoo-housed species can help researchers identify potential physiological and behavioural responses during periods of prolonged stress.

As zoos evolve and continue to improve the habitats for animals in their care, construction and changes in social conditions are inevitable. Larger species, like giraffes, are difficult to transport within or across zoos during construction activity, and additional transport can introduce further stressors for animals. In instances where prolonged exposure to construction noise and associated activity is unavoidable, zoos should proactively identify specific measures that can ameliorate the impact of such stressors. Jakob-Hoff et al. (2019) suggest multiple actions, including timing construction activities, providing enrichment and training as distractions, and implementing sound-absorbent materials. Although future studies are needed to evaluate these mitigation efforts, animal behaviour and hormone monitoring, as performed in this study, represent an important first step in identifying validated measures that can provide important individual insights and guide future efforts.

Conclusion

This study opportunistically performed a biological validation of cortisol and corticosterone EIA in giraffe following the death of a social partner. The study identified greater responsiveness in faecal cortisol metabolites, but notes the possibility of HPA axis dysregulation as a potential cause for a blunted corticosterone response. Using cortisol and additional behavioural data, the study found that construction activities and loss of a social partner represented a significant source of stress for these giraffes, notwithstanding individual and interspecific variation in responsiveness. An approach that combines hormone and behavioural data was especially helpful in contextualising and interpreting the findings, and the study accordingly recommends incorporating both methodologies for future studies of giraffe stress physiology.

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