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Development of an evidence-based welfare approach for cheetahs (*Acinonyx jubatus*) under human care

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Abstract

*Societal concern for animals under human care has influenced our approaches to advance animal welfare in a variety of contexts. The Animal Programs Department at the Columbus Zoo and Aquarium sought partnership with the Center for Human-Animal Interactions Research & Education (CHAIRE) at The Ohio State University to develop a holistic welfare approach for the animals within their department using a focal species, the cheetah (*Acinonyx jubatus*). A one-year project using the Five Domains Animal Welfare Model collected data over six 60-day periods to evaluate long-term cortisol production and behavioural observations of cheetahs under changing environmental factors. Species and individual histories were incorporated with behavioural observations and hair cortisol production, giving a holistic view of welfare. Cortisol and behavioural data were analysed using linear models to compare cheetahs at population and individual levels. Participation in a cheetah run activity, housing occupancy, and 60-day period were found to influence all behaviours within the population and stereotypic behaviour also differed within individual cheetahs. No differences in hair cortisol concentrations were found for the group, but further analysis revealed differences within individuals throughout the study. No correlation of stereotypic behaviour and cortisol levels were found. This study created a welfare assessment protocol that can be used within zoological institutes and was the first to measure cortisol concentrations in hair in cheetahs.*

Keywords: *animal welfare, behaviour, cheetah, hair cortisol, human care, zoo*

Introduction

Animal welfare science was established as a scientific field just over 50 years ago. It has evolved into a multi-disciplinary field of study and continues to be influenced by societal concern for animals under human care (Fraser 1999, 2008; Fisher & Mellor 2008). Over time, various frameworks have been developed to measure animal welfare. The Five Domains Animal Welfare Model, first developed in 1994 and most recently updated by Mellor and colleagues in 2020, being one framework that includes the domains of nutrition, physical environment, health, behavioural interactions, and mental state, offering an overall assessment of an individual's welfare state. Absence of negative experiences and opportunities for positive experiences within each domain are integrated to measure welfare over a period of time (Mellor *et al* 2020). This model has aided in the transformation of the zoological industry, including the development of welfare monitoring tools, welfare risk assessments, and welfare research centres at zoos (Appleby *et al* 2018; Brando & Buchanan-Smith 2018; Sherwen *et al* 2018; Rose & Riley 2019). One example being the development of the World Association of Zoos and Aquariums' (WAZA) Animal Welfare Strategy in 2015 which recommends use of the Five Domains Model for development of welfare assessments (Mellor *et al* 2015). This welfare strategy has also been adopted by the European Association of Zoos and Aquaria (EAZA) which provides an Animal Welfare Assessments Library that is publicly accessible online (EAZA 2020a). In addition, beginning in 2017, the Association of Zoos and Aquariums (AZA), primarily in the United States, now requires all members to have a formal animal welfare approach (AZA 2020). In response to this new accreditation standard, the Columbus Zoo and Aquarium's (CZA) Animal Programs Department and the Center for Human-Animal Interactions Research & Education (CHAIRE) at The Ohio State University partnered to develop a holistic welfare approach using a focal species, the cheetah (*Acinonyx jubatus*).

The first record of a captive cheetah in a zoological institution was in 1829 in London (Marker *et al* 2018).

Populations of free-ranging cheetahs have decreased substantially within the last 50 years. IUCN lists the species as threatened, populations decreasing, and less than 10,000 individuals in the wild, (Durant *et al* 2015) with an additional 1,500 in zoos worldwide (Marker *et al* 2018). Beginning in the 1980s, studbooks were developed by collaboration between regional member institutions of organisations such as AZA and EAZA, as well as the creation of the international cheetah studbook leading to record-keeping and the establishment of breeding programmes to promote species diversity of cheetahs under human care (Sengenberger *et al* 2018). Established breeding programmes within zoos have not only helped promote genetic diversity within cheetah populations but have led to many advances in knowledge of the physiology, nutrition, endocrinology, genetics, and disease susceptibility of the species (Koester *et al* 2015).

Management of cheetahs under human care differs regionally as well as globally. For example, in the United States, use of cheetahs as an ambassador animal, often including travelling for educational purposes and/or training to perform behaviours for demonstrations, is recognised as an acceptable role to increase public awareness of the species (Ziegler-Meeks 2009; AZA 2021). In European zoos, handling of cheetahs occurs through protected contact, maintaining separation between staff members and animals through a barrier and direct contact with visitors is not viewed as acceptable (Sengenberger *et al* 2018; EAZA 2020b). Cheetahs may also be housed at breeding facilities, separate from traditional zoo settings, which may or may not be publicly accessible (Ziegler-Meeks 2009). Knowledge gained about differing husbandry practices of cheetahs through scientific research on both wild and captive populations contributed to the development of this study's longitudinal welfare approach using the Five Domains Animal Welfare Model.

The first three domains (nutrition, physical environment, and health) of the Five Domains Model are resource-based measurements and were incorporated into welfare determinations by use of knowledge within literature and individual animal's history (Mellor *et al* 2020). For example, cheetahs are obligate carnivores that typically consume medium-sized ungulates; thus, under human care, cheetahs are regularly fed commercially available diets and water *ad libitum*, which meet the daily nutritional requirements (Dierenfeld 1993; Vester *et al* 2009; Marker *et al* 2018; Sengenberger *et al* 2018). A domain two (physical environment) example is that in the wild cheetahs often inhabit a wide range of areas including grassland and open savannahs and have also been found in high grass and bush areas. They utilise raised areas for marking their territory and bush to remain undetected while stalking their prey. Therefore, adequate platforms and raised areas as well as hay or wood-shavings for bedding in indoor areas are recommended in human care settings. Appropriate ventilation within these areas as well as proper flooring allow for disinfection and prevention of accumulation of harmful substances, such as ammonia in urine (Ziegler-Meeks 2009; Marker *et al* 2018; Sengerberger *et al* 2018).

Body condition scoring has been previously established for cheetahs within the literature. Ranging from 1–5, a score of three indicates an 'ideal' cheetah which is the appropriate size and weight as well as being correctly proportioned (Carlstead *et al* 2013) and possessing the right amount of fat deposits. Zoological institutions, including CZA, use measurements such as body condition score for evaluation of domain three, health. Typically, these health measurements are recorded in a database, such as ZIMS Species 360, which is also used to track key life events, medical records, and animals' genetic background (Barber 2009).

The fourth and fifth domains of the Five Domains Model, behavioural interactions and mental state, relate to how animals are coping within their environment. Environmental enrichment is widely used by zoological institutions to promote species-typical behaviours and mental stimulation for positive welfare states in animals (Skibiel *et al* 2007; Mellor *et al* 2020). Natural hunting behaviour for cheetahs is to stalk their prey until within a close distance and then to explode with speed for a relatively short duration (Marker *et al* 2018). A specialised lure system often called the 'cheetah run' has been adopted at various institutions worldwide to simulate the instinctual chase for the cheetah and serve as an enriching activity. Animals are trained to chase the lure and receive a reward after completion (Ziegler-Meeks 2009; Quirke *et al* 2013; Sengerberger *et al* 2018). This activity allows cheetahs to fulfill their motivation to perform this natural behaviour, as well as creating an educational opportunity for guests.

Positive experiences in the fourth domain, behavioural interactions, include free movement and choice and control within their environment as well as the opportunity for bonding with conspecifics (Mellor *et al* 2020). For example, females are typically solitary in the wild except for the period in which they are raising cubs, although they are often housed in pairs or groups under human care (Wielebnowski *et al* 2002). Males in the wild may form small coalitions which has been demonstrated to also occur when under human care (Chadwick *et al* 2013). Although these housing occupancies are not their natural social groupings, previous studies of housing differences of male and female cheetahs in human care found no difference in faecal cortisol levels for those housed singly compared to in a group (Koester *et al* 2015, 2017).

Stereotypic behaviour has often been a behavioural focus of animal welfare research (Quirke *et al* 2012; Shepherdson *et al* 2013; Watters 2014; Greco *et al* 2017). It is described as repetitive behaviour with no goal or function and may appear when an animal is unable to perform natural behaviours that they are motivated to do (Spruijt *et al* 2001). Many studies have been undertaken with the objective of reducing stereotypic behaviours in cheetahs and have found that provision of randomised enrichment, variation in feeding schedules, and activities such as the cheetah run reduce stereotypies (Quirke & O'Riordan 2011; Quirke *et al* 2012, 2013). Other studies found frequent movement of cheetahs between enclosures, enclosure size, and raised areas to increase exploratory

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behaviour, decrease stereotypic behaviour, while the ability to see conspecifics had the opposite effect (Quirke *et al* 2012; Quirke & O'Riordan 2015).

An animal's mental or affective state, the fifth domain, is described as the individual's experience in relation to the other four domains of animal welfare. Affects can be positive or negative in response to resources provided and environmental conditions experienced by the animal. Positive examples include experiencing drinking and taste pleasures (nutrition domain), thermal and visual comfort (physical environment domain), comfort of good health and function (health domain) and experiencing interest and engagement (behavioural interactions domain). Examples of negative experiences include nausea, overheating, pain, and boredom (Mellor *et al* 2020).

Incorporation of a biomarker with behavioural observations to assess animal welfare provides an explanation of the animal's outward expression, behaviour, and inward expression, cortisol, of how they are coping within their environment. Researchers have utilised faecal cortisol as a physiological measurement to measure stress levels in cheetahs (Jurke *et al* 1997; Wielebnowski *et al* 2002; Ludwig *et al* 2013; Koester *et al* 2015, 2017). However, a limitation of the use of faecal cortisol as the biomarker for a welfare assessment is that faecal cortisol represents an acute measurement and activation may be indicative of eustress, resulting from excitement or playfulness, or distress, resulting from negative affects, such as fear (Kupriyanov & Zhdanov 2014; Binding *et al* 2020).

Hair cortisol reflects chronic stress levels and is thus not affected by circadian rhythms or acute stressors which differs from other measurements such as salivary, urine, or faeces (Russell *et al* 2012). Hair cortisol levels have also been utilised in other species such as humans, domestic felines (*Felis catus*) and dogs (*Canis lupus familiaris*), Asiatic black bears (*Ursus thibetanus*), and rhesus monkeys (*Macaca mulatta*) (Davenport *et al* 2006; Sauvé *et al* 2007; Accorsi *et al* 2008; Malcolm *et al* 2013). It is a less-invasive measurement that is less labour-intensive compared to other methods due to its ability to reflect weeks–months of cortisol production (Isaac *et al* 2017).

Based on new zoo accreditation requirements and advances in animal welfare science, the objective of this study was to evaluate a longitudinal welfare approach by combining long-term cortisol production and behavioural observations of cheetahs in response to changing environments. Specifically, this study investigated which changing environmental factors, impacting enrichment capacities, affected an individual's welfare over a one-year period and if differences in housing occupancies influenced cheetah welfare. The hypotheses were as follows: i) participation in the cheetah run activity will result in decreased cortisol levels and stereotypical behaviour; ii) times when cheetahs' exhibit is open to the public and animals are consistently rotated into differing environments will lead to decreased hair cortisol and stereotypic behaviours; and iii) housing occupancy will have no effect on hair cortisol or stereotypic behaviour.

Table 1 **Study population indoor location, sex, age, participation in enrichment activity and housing occupancy.**

Cheetah	Location	Sex	Age	Run	Housing
1	1	Male (intact)	8	No	Paired
2	1	Female	8	Yes	Paired
3	2	Female	6	Yes	Paired
4	2	Female	6	No	Paired
5	3 and 10	Male	4	No	Single
6	4	Female	4	Yes	Paired
7	4	Male	4	No	Paired
8	5	Male	3	Yes	Single
9	6	Female	2	Yes	Triple
10	6	Female	2	Yes	Triple
11	6	Female	2	Yes	Triple

Table 2 **Environmental changes during study that may influence enrichment capacity in each period.**

Period	Exhibit	Cheetah run
1 (Oct–Nov)	Open	Yes
2 (Dec–Jan)	Closed	No
3 (Feb–Mar)	Closed	No
4 (Apr–May)	Mixed	No
5 (Jun–Jul)	Open	Yes
6 (Aug–Sept)	Open	Yes

Materials and methods

The study population included eleven cheetahs (four males, seven females; mean [± SD] age: 4.45 [± 2.25] years) within the Animal Programs Department at CZA located in Columbus, OH. Three of the males were castrated and one was intact. All cheetahs were born at separate facilities and transferred to CZA to be hand-raised at a young age (32.27 [± 21.72] days). Staff members and cheetahs interacted regularly through training programmes, feeding, and shifting into new areas and were managed in free contact, meaning staff members entered environments with cheetahs without a protected barrier. All animals were also leash-trained although they were not regularly walked on leashes throughout the duration of the study. Cheetahs were housed alone, in pairs, or in a group of three (Table 1).

Data collection took place for one year, separated into six 60-day periods (Table 2). Separation of periods allowed for alignment of behavioural observations, which occurred during each 60-day period, and hair growth by each hair sample being collected at the beginning of all periods. Environmental changes occurred across all six periods (Table 2).

Table 3 Cheetah ethogram demonstrating grouping of behaviours for the purposes of this study.

Category	Behaviour	Code	Description
Locomotion			
Active motion	Walking	WA	Forward locomotion at a slow gait
	Running	RU	Forward locomotion at a rapid gait
	Patrolling	PA	Alert, walking around in a calm, deliberate manner
Inactive motion	Standing	ST	Upright position, all four paws on the ground, legs extended
	Lying	LY	Body on the ground in a horizontal position
	Sitting	SI	Upright position, hind legs flexed resting on the ground, front legs extended and straight
Stereotypic	Self-mutilation	SM	Self-injurious behaviour, including such that causes loss of hair and irritation to the skin
	Head-rolling	HR	Head is tossed in circular motion repeatedly
	Pacing	PC	Repetitive locomotion in a fixed pattern, no apparent goal or function
Maintenance	Drinking	DR	Consumption of liquid
	Grooming	GR	Cleaning of self by licking, scratching, biting, or chewing fur on own body
	Feeding	FE	Consumption of solid
	Urinating	UR	Expelling urine
	Defaecating	DE	Expelling faeces
Staff interaction	Interaction with personnel	IP	Training, feeding, or tactile contact with staff, inside or outside holding area
Out of view		OV	Not able to view individual

Environments in which the cheetahs were observed included their inside holding area off view to the public, an exhibit yard on view to the public, and a second yard off view to the public. Inside holding locations remained the same for all cheetahs throughout the study except for Cheetah Five which changed locations at the beginning of period five (Table 1). Seven individuals within the population also participated in a cheetah run activity which occurred during periods one, five, and six (Table 2). Cheetahs that participated in the cheetah run were trained to chase a lure (a yarn ball) around a yard and received a reward of meat after completion. Cheetahs were not permitted outside if outside temperatures did not exceed 32°F which occurred 43 times during the duration of the study.

The region of the zoo in which the cheetahs were located was not open all year round indicating periods when the exhibit was closed (Table 2). During periods when the exhibit was closed, cheetahs were not on view to the public or consistently rotated into yards due to temperature. During period four, the region was open to the public on days of warmer weather, but not completely open for the season until period five, which was therefore recorded as mixed (Table 2). Cheetah Five was not on view to the public during periods five and six and Cheetah Seven was not on view to the public during period six although the region was open.

Information relating to domains 1–3, nutrition, physical environment, and health, were verified by reviewing individual cheetahs' histories acquired by use of ZIMS Species 360 as well as interviews conducted with caregivers. Medical records were obtained from veterinary staff and all animals received routine care. Cheetahs were weighed monthly, and diets rationed to meet target weight and appropriate body condition score, as designated by caregivers and veterinary staff. Diet included Nebraska horse meat and time of feeding varied each day depending on training with caregivers or participation in the cheetah run activity. Environmental conditions met AZA, EAZA, and Cheetah SSP recommendations for captive cheetahs, such as including raised areas and appropriate bedding (see Ziegler-Meeks 2009; Marker *et al* 2018; Sengerberger *et al* 2018).

Behaviour

Behavioural observations were recorded using a scan-sampling technique (one scan per cheetah per 5 min for 2 h). A total of 150 observations took place per period and collection times ranged from 0900 to 1700h. Observations of all cheetahs occurred at the same time. Behavioural observations were conducted by the same individual throughout the study. A modified ethogram (Table 3) was used to catalogue behaviours as active, inactive, maintenance, stereotypic, staff interaction, and out of view (Stanton *et al* 2015).

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Cortisol

Hair samples were collected from each cheetah every 60 days to measure long-term cortisol production. Each cheetah served as their own control by using an individual's first sample as their baseline. Additional sample concentrations were subtracted from the baseline to analyse changes in cortisol due to changing environmental factors. Positive reinforcement methods enabled cheetahs to be trained to lie on their side for hair collection by zoo staff, using battery-operated trimmers (Wahl Clipper Corporation, Sterling, Illinois, USA). Hair samples were collected from the back of the hip and the same location was used each collection time to control data period and mirror hair growth with behavioural observations.

Hair samples were processed, and cortisol extracted by the College of Nursing Stress Science Lab within The Ohio State University as previously described by Meyer *et al* (2014) with slight modifications (Ford *et al* 2016). Briefly, samples were washed using high-performance liquid chromatography (HPLC)-grade isopropanol before being allowed to dry overnight. Samples were then ground to a powder and 1.1 ml of HPLC-grade methanol mixed for 18–24 h. Next, tubes were centrifuged at 5,000 g for 5 min and methanol evaporated from the sample via stream air or nitrogen gas for 6–8 h. Samples were assayed via a commercial ELISA kit (Salimetrics, State College, PA, USA). Inter- and intra-assay coefficients of variation (CV) were 5.4 and 7.4% cumulatively, respectively. Each hair sample averaged 50 mg and no samples under 10 mg were used to avoid false results (Meyer *et al* 2014).

Statistical analysis

All data were analysed using SAS v 9.4 Statistical Software (SAS Institute, Cary, NC, USA). Data were analysed at the population level, to evaluate group differences, and at the individual level, to evaluate differences within individual cheetahs. Data normality was verified through normal quantile-quantile (Q-Q) and normal probability plots (P-P). Multicollinearity of factors was tested through the variance inflation factor and tolerance. Statistically significant values were those at $P < 0.05$.

Group behavioural data were analysed via PROC GLIMMIX, using multinomial logistic regression. Odds ratios were computed comparing the behavioural categories of active, maintenance, stereotypic, staff interaction, and out of view to a baseline category of inactive behaviour. Inactive behaviour was chosen as the baseline category due to frequency of performance (66%). Odds ratios gave a simultaneous representation of the likelihood of performance of behaviours within covariate categories. Fixed effects included in the analysis were participation in the cheetah run, housing occupancy, period, and exhibit being open or closed and thus cheetahs being on vs off view to the public. Reference categories for fixed effects included completed cheetah run, triple housing, and period six. Cheetah was the experimental unit, or random effect, which was blocked by location of their inside holding area.

Individual differences of performance of stereotypic behaviour were analysed via PROC MIXED, using a mixed linear model. Least square means were computed for averages of performance of stereotypic behaviour within individuals across all periods of the study. Cheetah, as well as interactions of cheetah and run and cheetah and exhibit, were the fixed effects in this model. Housing was not included in analyses since it did not vary within individuals throughout the study, meaning all cheetahs remained within the same housing occupancy during data collection. For analysis of individuals, period was used as the random effect to determine changes within individuals over all periods of the study which was also blocked by location.

Group changes in cortisol concentrations were analysed via PROC MIXED, using a mixed linear model. Fixed effects included cheetah run, exhibit, housing, period, age, and sex. Differences of cortisol due to age and sex were additionally tested due to variations within the literature (for a review, see Stalder & Kirschbaum 2012). Least square means were computed to determine averages within each covariate category across the population. Cheetah was again the random effect and blocked by location.

Individual changes in cortisol concentrations were analysed using PROC MIXED. A mixed linear model was used for analysis and least square means computed for averages within individuals throughout all periods of study. Period was used as the random effect and was blocked by location. Fixed effects included cheetah as well as interactions of cheetah and run and cheetah and exhibit.

Descriptive statistics were applied for averages of prevalence of stereotypic behaviours and cortisol concentrations in each period to establish baseline measurements of behaviour and hair sampling in cheetahs. Lastly, correlations of behaviours, cortisol, and factors were analysed via PROC CORR using Pearson correlation coefficients.

Results

Group behaviour

Performance of a Type III Test revealed influence of participation in the cheetah run activity ($P < 0.01$), housing ($P = 0.01$), and period ($P < 0.01$) on all behavioural categories of the cheetah population. The exhibit being open or closed had no effect ($P = 0.49$) on behaviour. Odds ratios of behaviour were considered significant if $P < 0.05$ as well as if 95% confidence intervals did not contain one within their range. Estimates < 1 indicated that cheetahs were less likely to perform the behaviour within the covariate category while estimates > 1 indicated greater likelihood. Results indicated a reduced likelihood of maintenance (OR: 0.27, CI: 0.13–0.56; $P < 0.01$) and stereotypic (OR: 0.62, CI: 0.44–0.89; $P = 0.01$) behaviours as well as staff interactions (OR: 0.52, CI: 0.32–0.84; $P = 0.01$) of cheetahs that did not participate in the cheetah run activity. Cheetahs that were housed in pairs were almost four times as likely (OR: 3.80, CI: 1.41–10.24; $P = 0.01$) and by themselves almost five times as likely (OR: 4.64, CI: 1.40–15.34; $P = 0.01$) to

Table 4 Status of exhibit influence on prevalence of stereotypic behaviours of individual cheetahs.

Cheetah	Exhibit open	Exhibit closed	Exhibit mixed
1	9.67 (± 3.79) **(0.02)**	33.50 (± 4.65) **(< 0.01)**	30.00 (± 6.57) **(< 0.01)**
2	6.67 (± 3.79) (0.10)	15.50 (± 4.65) **(< 0.01)**	13.00 (± 6.57) (0.07)
3	8.67 (± 3.79) **(0.04)**	20.50 (± 4.65) **(< 0.01)**	24.00 (± 6.57) **(< 0.01)**
4	4.33 (± 3.79) (0.27)	12.50 (± 4.65) **(0.02)**	7.00 (± 6.57) (0.30)
5	20.00 (± 6.57) **(0.01)**	9.50 (± 3.29) **(0.01)**	16.00 (± 6.57) **(0.03)**
6	6.14 (± 3.67) (0.12)	1.29 (± 4.41) (0.77)	< 0.01 (± 6.57) (1.00)
7	10.05 (± 6.57) **(0.04)**	7.64 (± 3.67) (0.06)	10.00 (± 6.57) (0.15)
8	12.33 (± 3.79) **(0.01)**	7.00 (± 4.65) (0.15)	8.00 (± 6.57) (0.24)
9	3.33 (± 3.79) (0.39)	3.50 (± 4.65) (0.46)	13.00 (± 6.57) (0.07)
10	7.00 (± 3.79) (0.09)	5.50 (± 4.65) (0.26)	6.00 (± 6.57) (0.38)
11	1.67 (± 3.79) (0.67)	1.50 (± 4.65) (0.75)	< 0.01 (± 6.57) (1.00)

Effect of interaction of cheetah and exhibit status on average performance of stereotypic behaviour (P = 0.04).
Bold text indicates P < 0.05.

perform stereotypic behaviours when compared to the reference category of cheetahs that were housed within a group of three. Periods one (OR: 3.68, CI: 2.60–5.21; P < 0.01), two (OR: 6.33, CI: 2.09–19.20; P < 0.01) and three (OR: 5.86, CI: 1.93–17.78; P < 0.01) included an increased likelihood of performance of stereotypical behaviours when compared to the reference category period six. Increased likelihood of staff interaction (OR: 1.92, CI: 1.32–2.80; P < 0.01) also occurred in period one as well as increased active (OR: 0.66, CI: 0.49–0.90; P = 0.01) and maintenance (OR: 2.33, CI: 1.21–4.47; P = 0.01) behaviours in period five compared to six.

Individual behaviour

A Type III Test of stereotypic behaviour at the individual level revealed differences within individual cheetahs (P < 0.01), the interaction of cheetah and exhibit (P = 0.04) and cheetah and run (P = 0.02). Results of least square means indicated increases of stereotypic behaviour within all cheetahs except for Cheetahs Six, Nine, and Eleven across all periods. Significance of interactions of cheetah and exhibit (Table 4) and cheetah and cheetah run (Figure 1) varied with individuals. For example, Cheetah One showed increased stereotypic behaviour regardless of changes in exhibit status: open (P = 0.02); closed (P < 0.01); and mixed (P < 0.01) although Cheetah Two did not show significance when the exhibit was open (P = 0.10) or mixed (P = 0.07) but did during closed (P < 0.01). Cheetah Eight showed increased stereotypic behaviour during periods when the cheetah run was occurring (P = 0.01) and no significance during times when the activity did not occur (P = 0.11). It is important to note that not all cheetahs participated in the cheetah run and therefore estimates for those individuals were not included in Figure 1.

Group cortisol

Hair growth for cheetahs ranged between 0.50–2.50 cm over 60 days with an average growth rate of 1.36 (± 0.15) cm. No seasonal variation in cortisol levels were found which was in accordance with previous research in other feline species (Leyva *et al* 1984; Newell-Fugate *et al* 2007). Results from the Type III Test revealed no difference of exhibit (P = 0.77), housing (P = 0.50), cheetah run activity (P = 0.64), and period (P = 0.37) on changes of group cortisol levels. Effects of age (P = 0.76) and sex (P = 0.36) were also tested which also showed no influence on cortisol levels.

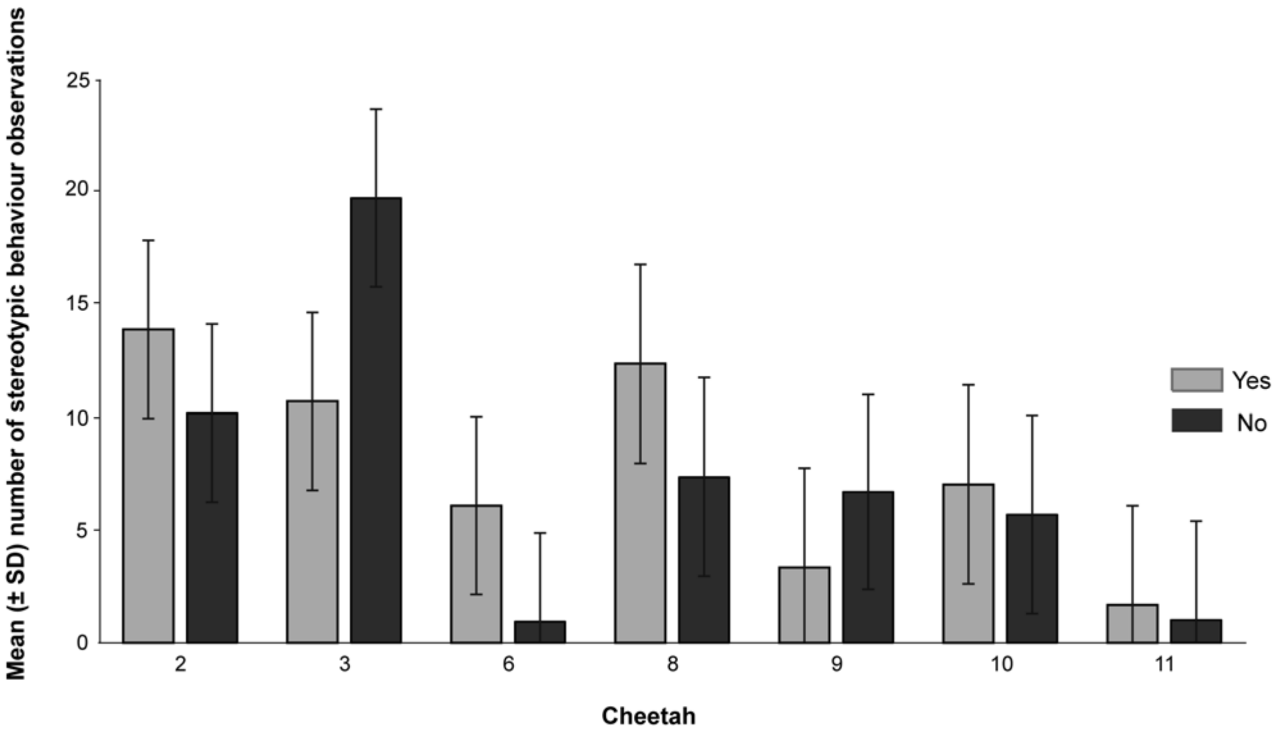
Individual cortisol

A Type III Test for differences of changes in cortisol at the individual level revealed differences within individual cheetahs (P = 0.01), a trend of interaction between cheetah and run (P = 0.05), and no effect of interaction between cheetah and exhibit (P = 0.17) across all periods. Least square means revealed increased cortisol levels of Cheetahs One, Seven, and Ten. Increased cortisol levels of Cheetah Ten were found during periods when the cheetah run activity was not occurring (P = 0.03). Results of the influence of the cheetah run on individual cortisol concentrations are shown in Figure 2. Negative values for some cheetahs occurred due to the first collection being used as each individual's baseline value.

Correlation

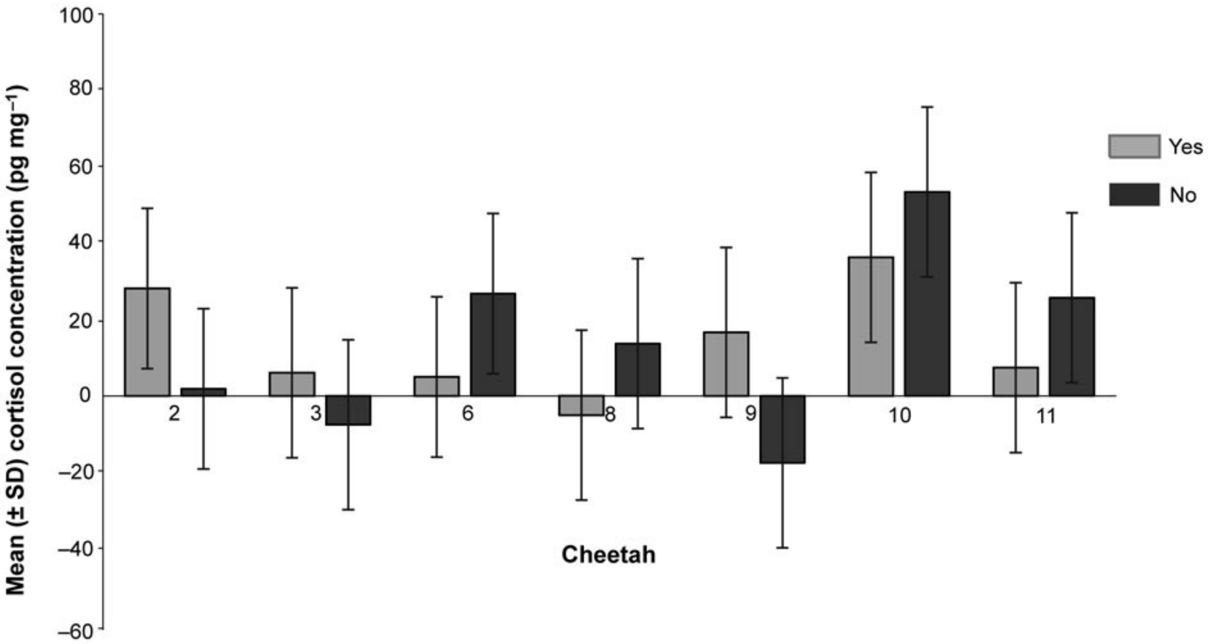
Lastly, no correlation of stereotypic behaviour (R^2 = 0.06; P = 0.65), active behaviour (R^2 = –0.05; P = 0.60), or staff interactions (R^2 = –0.01; P = 0.93) with cortisol were found, although a slight correlation existed between maintenance behaviour (R^2 = 0.25; P = 0.05) and cortisol. Correlations of

Figure 1



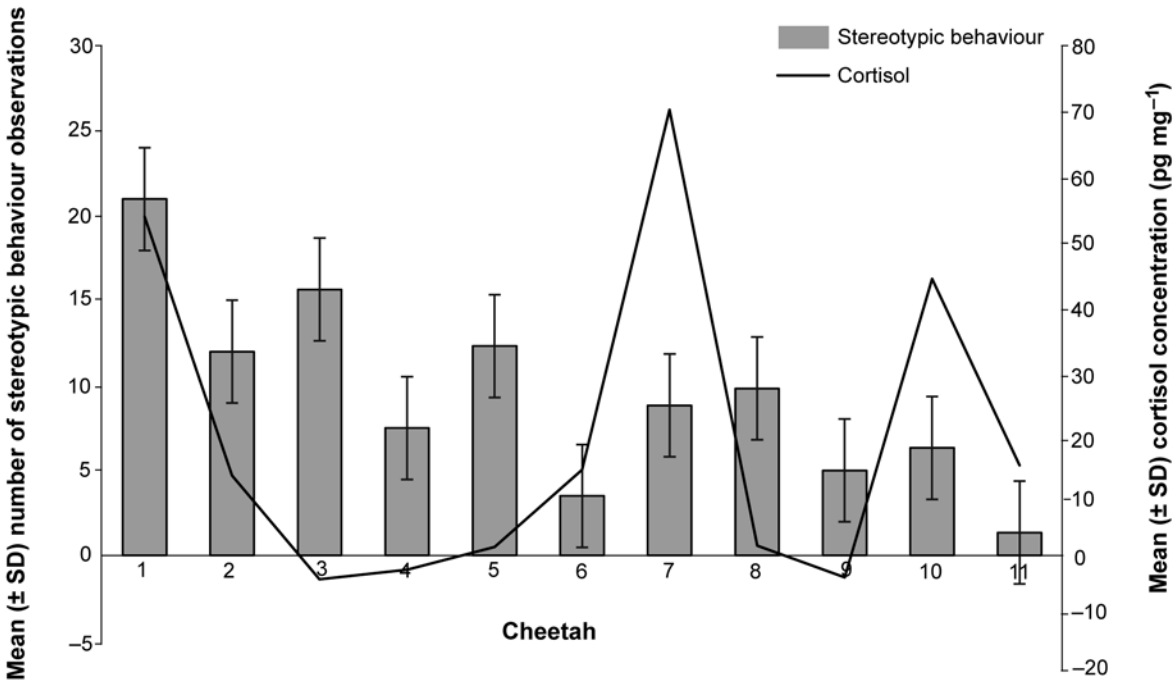
Influence of cheetah run occurrence on prevalence of individual stereotypic behaviours. Effect of interaction of cheetah and run on average stereotypical behaviour (*P* = 0.02).

Figure 2



Influence of cheetah run occurrence on individual hair cortisol concentrations. Effect of interaction of cheetah run on average cortisol concentrations (*P* = 0.05). Hair samples collected every 60 days and compared to baseline.

Figure 3



Individual averages of cortisol concentrations (P = 0.01) and stereotypic behaviour (P < 0.01) across all periods of study. Behavioural observations recorded using scan sampling and hair samples collected every 60 days and compared to baseline.

Table 5 Mean (± SD) cortisol concentrations, change from cheetah baseline measurement, and prevalence of stereotypic behaviours in each period.

Period	Cortisol concentration (pg mg^{-1})	Cortisol change from baseline (pg mg^{-1})	Prevalence of stereotypic behaviour (150 total behaviour observations each period)
1	33.00 (± 12.75)	9.40 (± 17.54)	11.45 (± 7.53)
2	38.79 (± 25.39)	12.37 (± 31.90)	11.91 (± 13.67)
3	63.24 (± 39.04)	34.98 (± 38.47)	11.55 (± 7.09)
4	50.00 (± 38.42)	23.57 (± 37.40)	11.55 (± 9.23)
5	40.74 (± 48.07)	15.59 (± 52.01)	5.27 (± 5.46)
6	62.14 (± 59.46)	36.99 (± 53.48)	4.36 (± 3.53)

factors included exhibit status and cheetah run (R^2 = 0.66; P < 0.01), although all factors produced low variance inflation factors (exhibit [VIF = 2.35], cheetah run [VIF = 2.69], housing [VIF = 2.06], and period [VIF = 1.29]) indicative of no multicollinearity of factors. Figure 3 illustrates individual averages of stereotypic behaviour and changes in cortisol levels across periods. Table 5 includes group averages of prevalence of stereotypic behaviours, cortisol concentrations, and average change in cortisol compared to the baseline in each period.

Discussion

Animal welfare has been described as "a state that is subjectively experienced by an animal" (Mellor 2016). Information within the first three domains of the Five Domains Model (nutrition, physical environment, and health) were important to incorporate into the overall welfare assessment of the cheetahs to verify that the animals' basic needs for survival were met, although meeting the recommended husbandry requirements for animals in any setting does not guarantee a positive welfare

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state (Mellor *et al* 2020). Positive effects can occur by animals' survival-critical factors being met, such as pleasure of consumption of a species-appropriate diet and absence of illness, injury, and disease due to regular veterinary care (Mellor & Beausoleil 2015). Therefore, incorporation of these resource-based domains was important for interpretation of an individual's subjective experience, reflected by behavioural outputs and cortisol concentrations, to determine welfare states.

The method of data analysis was chosen to examine the welfare of the cheetahs as a group, a sample to be compared to other populations of cheetahs under human care, and differences within individuals, to focus on the CZA population, over the periods of the study. Beginning first with group behaviour, by use of odds ratios, results revealed that cheetahs that participated in the cheetah run activity were more likely to demonstrate stereotypic behaviour compared to those that did not participate and therefore rejection of a portion of the first hypothesis. Anticipatory behaviour, often pacing, is described as a goal-directed behaviour in which individuals perform in response to recollection of a previous reward (Spruijt *et al* 2001). Since individuals received a reward of meat after completion of the cheetah run activity, one possible explanation is that the stereotypic behaviour that occurred was actually anticipation of participation in the activity, indicative of a positive affective state by release of dopamine upon occurrence (Watters 2014). This aligns with the fact that there were no differences in cortisol levels due to the cheetah run activity for the population, indicating that the absence of the run during periods of the year (Table 2) did not negatively influence cheetah welfare. One exception was the findings for Cheetah Ten, for which cortisol concentrations increased during periods when the cheetah run was not occurring. This resulted in additional management consideration for that individual during those periods to provide opportunities for a positive welfare state. Rejection of the first hypothesis also revealed that although four cheetahs did not participate in the cheetah run activity during the duration of the study, welfare of these individuals was not compromised due to absence of the activity.

In reference to exhibit, the hypothesis was rejected due to results revealing no effect on group behaviour and cortisol levels. A multi-institutional study of 22 cheetahs across ten institutions also found no differences of faecal cortisol levels in cheetahs that were on view to the public vs cheetahs housed at private breeding centres (Koester *et al* 2017). Although the CZA cheetahs rotate roles throughout the year, the results indicated that animals were able to adapt to changes within their environment, including inconsistencies of rotation into yards, with no influence on their welfare. Another possible explanation for this finding was the extensive relationship the cheetahs share with their caregivers. Throughout the entire duration of the study, animals were actively engaged in training programmes irrespective of whether or not the public were present to view the performance of behaviours. Although management of CZA cheetahs is unique, the extent of the human-animal interactions that occurred can be expected to generate positive affects for both the caregivers and cheetahs involved (Mellor *et al* 2020).

Housing occupancy differences were predicted to have no influence on behaviour and cortisol levels. Odds ratio analysis revealed decreased stereotypic behaviours for the cheetahs that were housed as a group of three and no influence of housing occupancy on cortisol levels, causing rejection of the third hypothesis. These results support findings from a multi-institutional study of 112 cheetahs in 88 different enclosures which found increased stereotypic behaviour of cheetahs housed as a single compared to those housed as a group as well as cheetahs that could see conspecifics in other enclosures (Quirke *et al* 2012). Although CZA cheetahs are housed side-by-side within their inside holding areas, they are unable to see other conspecifics due to solid walls between enclosures. It is important to also note that one limitation of the study population was that there was only one grouping of three cheetahs, while there were three groupings of pairs and two singles (Table 1).

The results of no influence of housing on hair cortisol are in accordance with previous studies finding no difference of housing on faecal cortisol levels in other cheetah populations (Koester *et al* 2015, 2017). Although the study population included housing of cheetahs that differ from their wild counterparts, meaning groupings included multiple females housed together as well as females and males housed together (Table 1), findings indicate no negative effects on their welfare. One may even argue that housing in groups provides opportunities for positive experiences through bonding with conspecifics and engaging in rewarding interactions (Wolfensohn *et al* 2018; Mellor *et al* 2020).

Although various studies within the literature revealed correlations of stereotypic behaviour and cortisol levels, all studies utilised faecal measurement, a reflection of acute stress within their animals (Wielebnowski *et al* 2002; Shepherdson *et al* 2013; Miller *et al* 2016). These studies should not be discounted as they provide important information for improvements in husbandry practices for species within human care, such as increased enrichment items for polar bears (*Ursus maritimus*) (Shepherdson *et al* 2013). The current study's use of hair sampling and behaviour allowed for comparison of potential chronic stress throughout the year and identified differences within individuals in the CZA population. For example, for Cheetahs One and Seven, increased cortisol levels were found throughout the study resulting in recommendations for those individuals. These evidence-based recommendations afford CZA the opportunity to provide optimal welfare for all the cheetahs within their care. The results of this study reflect the ability of the animals to cope appropriately within their changing environments and minimal management recommendations were made to improve the welfare of specific individuals.

Animal welfare implications

As animal welfare science continues to evolve in zoological settings, the use of the Five Domains Model as a framework for assessment of species is recommended. Although many animal welfare studies have been undertaken for an array of species, incorporation of all domains is necessary for a comprehensive representation of welfare (Mellor & Beausoleil 2015). The current study employed a method of measurement which enhances the Five Domains Animal Welfare Model that can be applied to other cheetah populations as well as various other species under human care. Use of this longitudinal comprehensive methodology is recommended for species experiencing a major life change, such as a transfer to another institution or giving birth, to measure how the individual is coping with the change or if the individual is demonstrating signs of chronic stress. Additionally, zoos and aquaria should continue to utilise welfare-monitoring tools developed for evaluation of animal welfare on a daily basis to influence animal-care decisions (see Whitham & Wielebnowski 2013; Orban *et al* 2017; Sherwen *et al* 2018).

The current study was the first to use hair sampling to measure cortisol production in cheetahs. Hair sampling was an appropriate measurement to fill this gap within the literature and to demonstrate its feasibility for welfare assessments of species within human care. Hair sampling to measure long-term cortisol production proved a less-invasive, low-labour-intensive biomarker for determining potential chronic stress levels. Animal training for collection is recommended, and hair collection was carried out relatively easily here due to the rapport between the cheetahs and their caregivers. Although faecal sampling has been utilised in the past, the ability of hair to reflect chronic stress levels meant it was the most appropriate method for this longitudinal welfare study (Wielebnowski *et al* 2002; Russell *et al* 2012; Ludwig *et al* 2013; Koester *et al* 2015, 2017). Use of behavioural observations allowed for investigation of performance of different behaviours within the population as well as investigation of stereotypical behaviour within individuals. Combining both hair sampling and behavioural observations, as well as knowledge of both individual animals and the species as a whole, provided a holistic view of an animal's welfare state. Data analysis at both the population and individual levels were critical to adequately provide recommendations catered to each individual cheetah. Future longitudinal welfare assessments should include both analyses to enable elucidation of potential differences within individuals.

Conclusion

Zoos should incorporate evidence-based management decisions and open communication with the public to maintain their social licence. As societal concern for animals under human care continues to increase, it will be important for the zoological community to demonstrate animal welfare as a priority. As cheetah populations continue to decline and societal concern for animals under human care continues to rise, it is important for zoological institutions to demonstrate objective measurements of their animals and use results for evidenced-based management decisions.

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