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Relationship between behavioural diversity and faecal glucocorticoid metabolites: a case study with cheetahs (Acinonyx jubatus)

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Abstract

*The ability to monitor the welfare of animal collections in zoological institutions is critical to the mission of these facilities. Historically, zoos have utilised negative indicators of welfare, such as stereotypic behaviour to examine and monitor collection animals. However, absence of stereotypic behaviour or negative indicators of welfare does not indicate that an animal is thriving. The goal of the current study was to continue efforts to validate behavioural diversity as an indicator of welfare using cheetah (*Acinonyx jubatus*) as a model species. Behavioural and faecal glucocorticoid metabolite data were collected on 18 cheetah at the San Diego Zoo Safari Park over a period of three months to explore the relationship between behavioural diversity and adrenal hormones related to the stress response. Results suggest that behavioural diversity can be utilised as an indicator of animal welfare to monitor animal collections within zoological facilities. However, additional research with other species should be conducted to better understand behavioural diversity as a positive indicator of animal welfare. We hope this manuscript will increase discussion surrounding behavioural diversity as well as increase efforts to validate it as an indicator of welfare.*

Keywords: Acinonyx jubatus*, adrenal activity, animal welfare, behavioural diversity, cheetah, faecal glucocorticoid metabolites*

Introduction

Historically, people have perceived zoological institutions as having poor levels of welfare for some of the animals under their care (Reade & Waran 1996). Most issues cited include species engaging in stereotypic or abnormal behaviours that are most easily identified as maladaptive (Clubb & Mason 2003). Great effort has been devoted to improving the welfare of animals in zoos as demonstrated by the large proportion of peer-reviewed manuscripts on the welfare of wildlife species compared to other industries between 1993 and 2012 (Walker *et al* 2014). However, continuous improvement means always looking for new ways to ensure each individual animal within a zoo collection is thriving.

Until recently, the study of animal welfare typically focused on negative indicators of welfare (Whitham & Wielebnowski 2013). One of the most commonly used indicators of animal welfare within zoological institutions was the presence or absence of pacing (Mason & Latham 2004). This was likely due to ease of study and also to prevalence among carnivores (Clubb & Mason 2003). However, zoological institutions strive not only to ensure adequate welfare but to make sure each individual animal is thriving. As a result of this, other measures of welfare are needed as absence of abnormal or stereotypic behaviour does not equate to high levels of animal welfare (Mason & Latham 2004).

Historically, behavioural diversity has been thought of as a potential positive indicator of animal welfare (eg Swaisgood & Shepherdson 2005; Miller *et al* 2011). Behavioural diversity can be defined as a measure of behavioural richness (number of observed behaviours) and frequency (frequency of observed behaviours). The underlying theory is that if zoological institutions are meeting the behavioural needs of animals then high levels of behavioural diversity would be observed in the collection. In this case, animals would be engaged in behaviours that they are motivated to perform. Alternatively, animals that have low levels of behavioural diversity are likely stereotyping or completely lethargic, neither of which would suggest a positive state of welfare (Grandin 1980; Mason & Latham 2004). Yeates and Main (2008) suggest that positive welfare can be best assessed by behavioural responses to resources that are valued by an animal. This further suggests that having an animal in the correct environment would lead to higher behavioural diversity. In addition, a number of studies conducted within zoological institutions have shown an increase in behavioural diversity in situations thought to be stimulating or positive in nature (eg Swaisgood & Shepherdson 2005; Miller *et al* 2011). The goal of the current study was to examine the relationship between faecal glucocorticoid metabolites and behavioural diversity

Table 1 Subjects for the current study.

ID	Gender	Birth date
C1	Female	15/6/2007
C2	Female	17/6/2009
C3	Female	9/8/2005
C4	Male	26/4/2000
C5	Female	9/8/2005
C6	Female	9/8/2005
C7	Male	28/5/2011
C8	Male	26/7/2001
C9	Male	26/7/2001
C10	Male	28/5/2011
C11	Male	7/12/2003
C12	Female	8/11/2008
C13	Male	7/12/2003
C14	Female	27/6/2004
C15	Female	8/11/2008
C16	Male	7/12/2003
C17	Female	11/10/2003
C18	Female	11/10/2003

to continue efforts to validate a potential positive indicator of animal welfare. The ability to monitor a collection of animals and have quantitative information available will provide animal management staff with information critical to making informed decisions and prioritising efforts.

Materials and methods

This study took place between March 1st and May 31st 2014. The subjects included 18 cheetah (*Acinonyx jubatus*) at the San Diego Zoo Safari Park in Escondido, CA, USA (Table 1). Cheetahs were exhibited in one of two areas, in the park where visitors could view the animals (n = 2) or in a breeding facility closed from the visiting public. All exhibits contained natural vegetation, shade trees and bushes as well as a shelter. In the breeding facility, cheetahs were exhibited adjacent to each other and either solitary (females) or in small groups of two to three individuals (females and males). In the park, the two individuals were exhibited in the same exhibit. For a more complete description of the cheetah exhibits, please refer to Augustus *et al* (2006). Behavioural observations were conducted in person five days a week, twice a day with each observation lasting ten minutes for three months. The ethogram for the current study can be seen in Table 2. Behavioural data were collected using all occurrence sampling for behavioural events (Altmann1974). The focal animal was randomised for order of observation. There were a total of four observers with inter-rater reliability $r > 0.80$. For the current study, behavioural diversity can be defined as the number and frequency of different species-appropriate behaviours exhibited by an individual animal. Behavioural diversity was calculated by using a Shannon diversity index (Shannon & Weaver 1949) to quantify the diversity of behavioural events during each observation. The index was calculated using the following equation where p is the proportion of one particular behaviour observed divided by the total number of behaviours observed, ln is the natural log, and $\sum$ is the sum of all calculations across behaviour(s).

$$(H) = - \sum_{i=1}^{s} p_i \ln p_i$$

The events chosen were all species-appropriate behaviours (eg grooming, playing with object, etc) and did not include abnormal or stereotypic behaviour. The Shannon diversity index was used due to its ability to pick up subtle changes in diversity when one factor (behaviour) is dominant (DeJong 1975). Behavioural diversity scores were averaged across the entire three-month period to create a score for each individual cheetah.

Faecal samples were collected daily, at approximately the same time, and non-toxic glitter was fed to paired animals for individual identification. Faecal samples were stored at –20°C until analysis. Samples were lyophilised for 96 h using a freeze dryer (Flexi-Dry, FTS Systems, Inc Stone Ridge, NY, USA) prior to hormone extraction. Once dried, samples were pulverised and sifted to remove debris. A 0.2 g aliquot of homogenised faecal sample was weighed into a 16 × 100 mm glass tube for hormone extraction. Glucocorticoid metabolites were extracted by adding 5 ml of a modified phosphate-saline buffer containing 50% methanol, 0.1% bovine serum albumin, and 0.05% Tween 20 (polyoxyethylene sorbitan monolaurate, a surfactant) and rocking overnight for 16 h at 400 rpm. Samples were allowed to settle for 1 h after rocking then centrifuged for 1 h at 4,000 rpm. A portion of the supernatant was transferred to a new tube and frozen at –20°C until the assay was pre-formed.

Faecal samples were diluted 2-fold from 1:2 to 1:256 in phosphate buffer saline (PBS) and run in the glucocorticoid assay in order to determine parallelism against the standard curve. The displacement curve of faecal glucocorticoid metabolites was parallel when compared to the standard curve ($r = 0.995$; $P < 0.01$) and extraction of exogenous corticosterone in samples was 102.8%. In order to clarify if there was possible interference with any compounds with the assay, we spiked a range of known amounts of exogenous corticosterone hormone (n = 8) with the sample matrix and the accuracy was determined to be 92.3 (± 11.1)%. Inter-assay coefficient of variation was 6.9% based on duplicates of high-binding corticosterone controls and 7.2% based on duplicates of low-binding corticosterone controls. Intra-assay variation was determined to be 6.60 and 5.3% (n = 20) and assay sensitivity calculated at 23.587 pg per tube which reflects %B/Bo at 90% of our

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Table 2 Ethogram used for the current study.

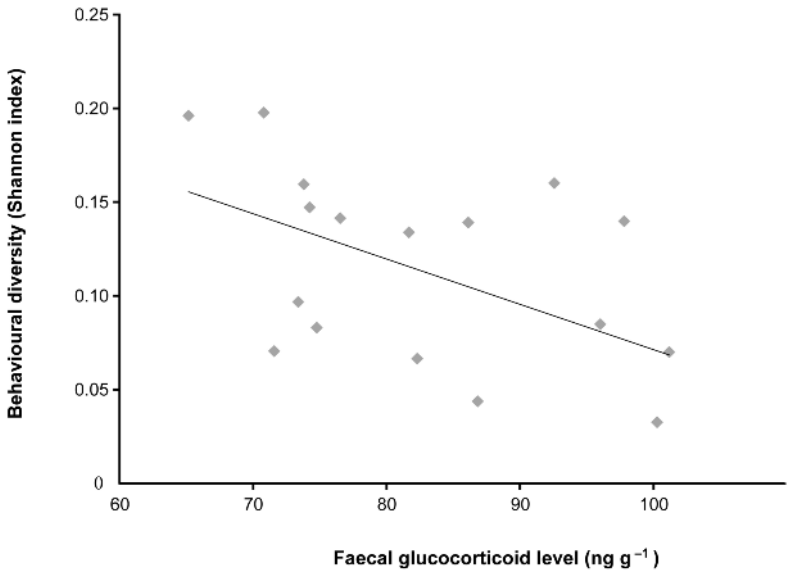
Behavioural events	Definition
Groom other	Licking body of another individual (head, neck, ears, flanks, legs or tail)
Body contact	Positive social interaction resulting in physical contact from focal animal to another conspecific not including contact by head of focal animal
Tail flick	Moving tail vigorously from side-to-side while lying, sitting, standing or walking (with presence of other sex)
Roll	Focal animal rolls on back, rubbing the back on the ground while all paws are in the air, or rolls from one side to the other (each roll is then recorded as one occurrence); member of other sex within two body lengths
Head rub	Focal animal rubs head against other individual in affiliative manner
Chase (A)	Focal animal is following another individual at a steady pace with another form of aggressive interaction happening either immediately before or after the chase
Bite (A)	Focal animal bites another animal with force
Paw swipe	Focal animal swipes at another individual with intent of doing harm
Chase (P)	Focal animal is following another individual at a steady pace without an aggressive interaction happening either immediately before or after the chase
Bite (P)	Focal animal puts mouth around body part of another individual without biting with force
Roll (P)	Focal animal rolls on back, rubbing the back on the ground while all paws are in the air, or rolls from one side to the other (each roll is then recorded as one occurrence) without member of other sex within two body lengths
Play with object	Manipulating, pulling, pushing or chewing an object within the exhibit
Lick object	Animal licks object within the exhibit
Sniff object	Animal sniffs object within exhibit
Scratch	Animal actively uses paws/claws to dig at the ground or other part of the environment
Scent mark	Individual urinates on part of environment (eg tree)
Groom self	Licking, chewing or scratching body or tail
Urinate	Process of urination
Defaecate	Process of defaecation

lowest standard. Results are presented as nanograms per gram (ng g^{-1}) dry faecal weight.

Extracted faecal glucocorticoid metabolites were measured by ^{3}H radioimmunoassay (RIA) using an antibody produced against corticosterone-3-carboxymethyloxime: BSA (ICN Biomedicals, Costa Mesa, CA, USA). The antibody cross-reacts 100% with corticosterone, 2.30% with desoxycorticosterone, 0.47% with testosterone, 0.35% with prednisolone, 0.33% with 17α-hydroxyprogesterone, 0.27% with cortisol, 0.17% with progesterone, 0.14% with 11-desoxycortisol, 0.07% with 20α-dihydroprogesterone, 0.05% with aldosterone, 0.03% with dihydrotestosterone, 0.02% with androstenedione and < 0.01% with 20β-dihydroprogesterone, cortisone, estradiol-17α, dihydroepiandrosterone-sulfate and 17α-hydroxypregnenolone. Tritiated corticosterone (10,000 cpm, PerkinElmer Life Sciences, Boston, MA, USA) was used in the assay to compete with the endogenous glucocorticoid metabolites.

A 20 µL portion of the cheetah extract was assayed with 300 µL of PBS with bovine serum albumin (BSA 0.35%) and combined with 200 µL PBS without BSA. Samples were run in duplicate and allowed to incubate overnight at 4°C. Following incubation, 250 µL of charcoal dextran solution (6.25 g charcoal: 0.625 g dextran in 1 L PBS) was added to end the competitive binding reaction and the assay was incubated for an additional 30 min at 4°C. Samples were centrifuged at 3,400 rpm for 15 minutes at 4°C and the supernatants decanted into scintillation vials. Finally, 3.5 mL of scintillation fluid was added prior to samples being counted in an LS 1801 Beckman liquid scintillation spectrometer (Beckman Instruments, Brea, CA, USA). Faecal glucocorticoid metabolite scores were averaged across the entire three-month period to create a score for each individual cheetah. All statistical tests were run using IBM SPSS Statistics 22. Both variables were examined for normality and the relationship between behavioural diversity and faecal glucocorticoid metabolites was tested using a two-tailed regression with alpha set at $P < 0.05$. All results are presented as means (± SD).

Figure 1



Relationship between faecal glucocorticoid metabolites and behavioural diversity in cheetahs.

Results

Behavioural diversity ranged between 0.03 and 0.20 (mean = 0.11 [± 0.05]). Faecal glucocorticoid metabolite concentrations ranged between 65.16 and 101.17 ng g^{-1} (mean = 83.62 [± 11.85]). A significant inverse relationship was found between behavioural diversity and faecal glucocorticoid metabolite concentrations ($F_{1,16} = 6.605$; $P < 0.05$; Figure 1). Individuals with higher behavioural diversity were found to have lower levels of faecal glucocorticoid metabolites.

Discussion

Here, we begin to demonstrate the usefulness of behavioural diversity as a behavioural indicator of animal welfare for institutions to monitor welfare. Our results suggest that a relationship exists between faecal glucocorticoid metabolites and behavioural diversity with lower faecal glucocorticoid levels relating to higher behavioural diversity. We anticipate that many zoological institutions, sanctuaries and research laboratories as well as the agriculture and pet industries could benefit by using behavioural diversity as an indicator of welfare to ensure each individual animal under their professional care is thriving.

To the best of our knowledge, this is the first study to demonstrate a relationship between behavioural diversity and a physiological measure of welfare. Our results demonstrate that behavioural diversity can be a way to monitor the welfare of animals within a professionally managed collection. Having high levels of behavioural diversity may be important due to the likelihood that the behavioural needs of animals are being met (Duncan 1998). This suggests that having high levels of behavioural diversity could be a potential indicator of psychological well-being. The Shannon diversity index serves as a method of calculating behavioural diversity that could be useful moving forward. It is important to point out that the index increases with both an improvement of richness (number of behaviours observed) as well as frequency (number of each behavioural occurrence). However, both of these ideas could be important to meeting the needs of animals by providing opportunities (richness) for the animals at a rate (frequency) similar to what would be normal in the wild. Finally, it is also important to note the limitations of faecal glucocorticoid assessments and potential factors outside the stress response that could influence those values (Touma & Palme 2005).

While it is always considered best practice to use multiple indicators of welfare to ensure an individual animal is thriving, behavioural diversity may be a low-cost alternative to processing faecal samples for metabolite concentrations. Exploring behavioural diversity across individuals could help ensure that each individual animal within a collection is thriving and allow institutions to prioritise resources based on individual animals demonstrating low levels of diversity. Moving forward, we anticipate that institutions will start looking at behavioural diversity in addition to or in favour of behaviours such as pacing to ensure high levels of animal welfare. With the link between animal welfare and reproductive success (Broom 1991), this could impact not only the welfare of these animals but also the sustainability of wildlife populations in zoos that are conservation-dependent as well as potential economic benefits for the agricultural and pet industry. Finally, with the mission of most modern zoos and aquaria to inspire visitors to conservation action, ensuring high levels of behavioural diversity with species-appropriate behaviour can assist with that mission (Miller 2012).

Additional research around behavioural diversity could add strength to its use as a measure of animal welfare. A better understanding of how behavioural diversity relates to other measures of animal welfare would also be beneficial. Finally, establishing the minimum levels of behavioural diversity necessary to ensure an individual animal is prospering could be important for animal management when animals are under professional care.

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Animal welfare implications

Results from the current study using cheetahs as a model species suggest that behavioural diversity can be used as a behavioural indicator of welfare potentially representative of an animal's state. It is considered best practice to use multiple measures of welfare to better understand where an animal falls on a continuum from poor to thriving and the current results suggest behavioural diversity may be another beneficial measure indicative of positive welfare. While future efforts are needed to demonstrate this similar relationship across a variety of species, we hope this paper will increase interest in behavioural diversity as an indicator of animal welfare. Through collective efforts within the field of animal welfare, we can determine positive indicators of welfare to ensure animals under our care have the opportunity to thrive.

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