

CHARACTERISATION OF POSITIVE WELFARE INDICES IN CAPTIVE AFRICAN LIONS (*Panthera leo*)

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Summary

Providing animals with optimal welfare is a key aim of zoos and aquariums. Zoo and regulatory communities have traditionally approached captive welfare through “input measures” - imposing minimum standards on their housing and management in terms of enclosure size and design. While these “input” measures are important and help to promote adequate welfare for animals, they fail to capture the experience of the animal, which is a critical component of welfare. This project is a first step towards developing and applying “output measures” - positive animal-based metrics - that can be used as a benchmark to assess the welfare of individual animals and promote optimal welfare. The objective of this study was to provide a thorough account of captive lion behaviour in modern facilities and compare this behaviour to that of wild conspecifics to explore similarities for welfare assessment purposes. Nine adult African lions (*Panthera leo*; n=5 females; n=4 males) from three captive facilities were included in the study. Two lions at one facility were used for trialling hair collection over 10 days. Seven lions were used for behavioural data and faecal sample collection daily for a period of 10 days. Behavioural data for each lion was collected through daily instantaneous scan samples every 30 minutes for six hours. Additionally two 30 minute continuous focal follows (a.m. and p.m.) were conducted. A comprehensive behavioural ethogram was generated for captive lions, which identified 28 distinct behaviours. Additionally the activity budget demonstrated lions were inactive for 86% of the observed time. There was not a significant correlation of activity when comparing studied lions to data on wild lions from literature, nor was there a significant time of day effect on activity levels ($p=0.07$). There was a slight trend of more activity during the afternoon, however this was likely shaped through animal management as this was when lions were fed and moved between enclosures. Corticosterone levels were examined as an indicator of stress through trialling the use of hair as a long-term indicator of stress and by assessing faecal glucocorticoid metabolites (FGM's) to develop a comprehensive account of corticosterone levels in captive African lions. The relationship between behavioural and hormonal data was investigated. Additionally, this relationship was tested to validate the accuracy of behavioural and corticosterone analysis as welfare indicators. The use of hair to measure glucocorticoid metabolites was unsuccessful as we were unable to obtain sufficient amounts of hair for

accurate analysis. Overall the lions exhibited variation in daily FGM levels with few peaks, demonstrating that they were not chronically stressed and able to respond normally to stressors. Mean baseline FGM levels in dry faeces was 128.96 ± 10.47 ng/g, however there was a significant difference in mean FGM levels between male and female lions ($p < 0.01$). The mean FGM concentrations of male lions was 177.12 ± 18.5 ng/g dry faeces, while female lions mean concentrations was 97.25 ± 9.71 ng/g dry faeces. This demonstrates a need to consider male and female lions separately when examining stress levels. There was also a significant difference in FGM levels between lions from different facilities ($p = 0.02$). The mean concentrations of FGM levels from the lions at the first facility was 156.17 ± 11.92 ng/g dry faeces, while at the second facility it was 108.73 ± 15.28 ng/g dry faeces. We were not able to determine what factors may have impacted this difference. There was no significant relationship between FGM's and aggression ($p = 0.80$), or between FGM's and activity ($p = 0.27$). This shows that relationships between behaviour and hormones are not always present and prior validations are required to identify certain behaviours as indicators of stress or poor welfare. These results provide an account of captive lion behaviour and stress hormones, in addition to trialling multiple measurements of welfare to provide accurate and robust assessments for captive animals. This study is the first step in linking excreted hormones and observed behaviour for captive lions. The results of this study provide an opportunity for all captive animal institutions, irrespective of participation in this study, to evaluate which specific aspects of their lion husbandry practices already promote optimal lion welfare and which (if any) aspects should be the focus of efforts to improve lion welfare. Regular testing of corticosterone levels as an indicator of stress, particularly surrounding potentially stressful situations (such as animal transport) would provide insight in to individual animal welfare. It would also provide more data to determine a standardised benchmark for future welfare studies, and by also measuring behaviour surrounding periods of high stress levels it may be possible to identify whether certain behaviours are indicative of poor welfare for individual animals.

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Chapter 1

Review of the Literature

1.1 GENERAL INTRODUCTION

Animals have been housed in captivity for centuries, with the first recognised formal zoological parks dating back to the 18th century (Brown, 2014). Since this time, the stated purpose behind keeping animals has shifted from entertainment, to research, conservation and education. Providing animals with housing that attempts to mimic wild conditions and provide stimuli that afford animals the opportunity to display behaviours as they would in the wild has been of increasing importance and focus for captive facilities (Maple and Perdue, 2013). When housing animals in a captive environment, such as in zoos, there is a moral and ethical responsibility to provide animals with an environment conducive to optimal welfare. In terms of available resources, captivity can provide better conditions than might be available in the wild, allowing for greater longevity and improved health for many captive specimens. Despite this some species appear to display poorer welfare in captivity than in the wild. For example, tigers and cheetah in captivity have a higher occurrence of gastroenteritis than those in the wild (Cociu et al., 1974; Terio, Munson and Solnick, 2000), and failure to reproduce in captivity can be due to chronic stress from suboptimal housing and husbandry (Lindburg and Fitch-Snyder, 1994; Mellen, 1991). Some species that have poor welfare in captivity have closely related species that have relatively good captive welfare, and as such there is much unexplained variation in captive welfare (Mason, 2010).

The welfare of an animal in captivity is dependent to a large extent on the housing and husbandry provided by zoo professionals as these shape the environment in which the animal exists, influencing physiological and behavioural responses (Morgan and Tromborg, 2007; Wurbel, 2001). To provide optimal welfare, the needs of animals must be met which can be simple and physical (access to water and shelter), or they can be complex (sufficient sensory input or contact with conspecifics; Broom, 1991).

The “Five Freedoms” of animal welfare was initially formulated by the UK Farm Animal Welfare Council in 1979 as a framework for the ideal states of welfare to be provided for animals in captivity.

The freedoms are as follows;

1. Freedom from thirst, hunger and malnutrition.
2. Freedom from discomfort and exposure.
3. Freedom from pain, injury and disease.
4. Freedom from fear and distress.
5. Freedom to express normal behaviour.

These freedoms are still currently often referred to by animal welfare NGO's (RSPCA Australia, 2009) and have had major impact on welfare legislation (UK Animal Welfare Act 2006). Whilst they were initially targeting the domestic livestock industry, zoos have also adopted these principles in their approach to animal welfare (see; Taronga Conservation Society Animal Welfare Charter; Zoos Victoria Animal Welfare Code).

More recently, the concept of the “Five Freedoms” has been evolved by David Mellor to a new model, the “Five Domains Model” (Mellor, 2016). Mellor outlines that the Five Freedoms do not identify that some negative experiences (e.g. hunger, thirst, sickness or discomfort) can never be eliminated entirely and only temporarily removed, and that minimising these negative experiences does not therefore guarantee a positive experience. The Five Freedoms model focuses largely on the physical and functional aspects of welfare, but fails to incorporate the “affective state” of an animal. The affective state of an animal places more focus on the experiences of animals, which are shaped by the functional domains; nutrition, environment, health and behaviour (Mellor, 2016). As such, Mellor outlines an approach that instead places focus on quality of life, whereby in place of only working to minimise negative experiences it also aims to provide opportunity for positive experiences where animals are able to engage in behaviours they find rewarding (Mellor, 2016).

While the concepts of the “Five Freedoms” and “The Five Domains” are integral to the provision of optimal welfare, they focus only on “input variables”. Using “input” variables alone assumes that if a captive animal is afforded satisfactory resources for its care, that it then must have good welfare.

While the provision of adequate resources surely does have an impact on an animal's welfare, as an assessment tool this technique fails to capture the experience of the animal, and as such does not provide accurate assessment of welfare (Yeates and Main, 2008). Therefore, a method of assessment that uses "output" variables - indicators of an animal's experience - must be developed in order to provide accurate assessment of welfare in captive animals.

1.1.1 Exhibited animals legislation and regulation

In Australia, state governments regulate and develop legislation pertaining to captive animal welfare. In New South Wales, the *Exhibited Animals Protection Act 1986 No 123* (NSW) and the *Exhibited Animals Protection Regulation 2010* (NSW) are the relevant legislative documents governing institutions that display captive animals to the public. Within these, general standards are prescribed that outline institutional requirements. The requirements addressed are psychological and physical animal welfare, educational value of exhibits, public safety, legal effect and guidelines for new or existing displays (*General standards for exhibiting animals in NSW, 2015*). Non-government associations also set levels of professional standards of practice that zoos are encouraged to meet In Australasia; the peak body representing zoos and aquariums is the Zoo and Aquarium Association (ZAA), which offers accreditation and membership for facilities that adhere to their individual policies, as assessed through frequent reviews (*ZAA Membership Policy, 2012; ZAA Animal Welfare Position Statement*). These policies further assist in providing outlines of standards and requirements for animals in captivity, but can also be quite subjective. Although these standards are comprehensive for general requirements across similar taxa - for example, the Standards for Exhibiting Carnivores in New South Wales outlined within the Exhibited Animals Protection Act (1986) outlines housing and management requirements for all carnivores - due to the vast numbers of species housed within captive facilities, much of the legislation is open to individual interpretation. Clause 36, Part 8 of the *General Standards for Exhibiting Animals in NSW 2015* is typically vague in stating, "Each animal must be offered a variety of wholesome and palatable food and water in quantities that are sufficient to provide for its good health and normal growth". This clause does not outline what foods fit those

requirements for each species nor does it outline a method of assessment to determine if certain foods provide good health. Additionally these standards provide little guidance for comprehensive welfare assessment, stating only five non species-specific indicators of psychological stress. The indicators outlined are self-mutilation, excessive grooming, excessive stereotypical behaviour, changes in eating/defecation/urination and abnormal aggression (*ZAA Animal Welfare Position Statement*). Legislation and regulatory bodies are integral to ensuring animals are provided with optimal welfare. However, a non-subjective framework that can be used to assess welfare of animals after the provision of outlined standards of care is needed to determine effectiveness for animal welfare.

1.2 WELFARE MEASUREMENT

Animal welfare can be difficult to assess and quantify. Capturing the experience of an animal is a critical component of animal welfare assessment (Yeates and Main, 2008), but difficult to do objectively and without anthropomorphising the subject. There are three main definitions of welfare covering physical, mental and natural living aspects of an animal's existence in a captive environment (Hewson, 2003). The first is concerned with the biological functioning of an animal, referring to the provision of its functional needs for survival (for example food, water and shelter) and the absence of disease and injury (Fraser et al., 1997). Mental aspects of welfare are concerned with the 'feelings' that animals may experience, suggesting that good welfare is defined by positive emotions such as pleasure and the absence of negative states such as anxiety (Fraser et al., 1997). Lastly, the concept of natural living as a measure of welfare refers to animals being afforded the opportunity to live as they would in the wild; being exposed to similar environmental factors and providing more opportunity for behaviours that are expressed by wild counterparts (Broom, 1988; Fraser et al., 1997). Each of these concepts is measured differently when used to assess welfare. Natural living can be measured through comparing the activity and behavioural repertoire of captive and wild conspecifics. Physical welfare can be assessed through the presence/absence of injury and disease. The assessment of mental wellbeing is more difficult to study as it refers largely to the experience and emotional state of an animal, which is often inferred through behavioural studies (Fraser et al., 1997). These three concepts are interrelated and are all important for overall well being (Fraser et al., 1997), and

assessment of animal welfare should incorporate elements from all three in order to be comprehensive and accurate.

1.3 BEHAVIOURAL ASSESSMENT OF WELFARE

Most welfare studies rely on behavioural observations. Behaviour is fluid and variable across individuals and species (Dingemanse and Réale, 2005; Sih, Bell and Johnson, 2004) so there is a need for a non-subjective, species-specific framework for behavioural assessments of welfare. A common behaviour for one individual animal or species may not be common for another, and assessments rely on the assessor's subjective opinion on what behaviours are indicative of a good or a bad welfare state. There is a broad range of behavioural and physiological indicators of poor welfare, but there are currently few behavioural and physiological measures that are used as indicators of good welfare (Basset and Buchanan-Smith, 2007). As such there is a need to determine which behaviours are indicative of good welfare.

In contrast, certain behaviours are commonly used as indicators of poor welfare and are often used for welfare assessments. Previous studies have used aggressive and aversive behaviours in animal-human interactions as an indicator of welfare in working animals (Pritchard et al., 2005). Stereotypies - repetitive behavioural patterns, such as fixed pattern pacing in big cats (Mason and Rushen, 2005) - are often used as an indicator of poor welfare. Stereotypic behaviour is considered to be a method of coping with an environment in which the animal is frustrated, threatened or lacking in stimulation, and where the animal is able to exert no control over that environment (Broom, 1991). However, using stereotypies as a definitive method of welfare assessment is flawed, as the presence of a stereotypical behaviour could be due to factors other than an animal's welfare (Mason and Latham, 2004). Mason and Latham (2004) suggest four processes that account for complex links between stereotypy and welfare where stereotypies cannot be linked with poor welfare: 1) Beneficial consequences from a behaviour ("do-it-yourself enrichment") where the positive effects from a behaviour encourage repetition; 2) Mantra effect- where calming results from repetition (cf. rhythmic movements such as rocking soothes humans); 3) Stereotypies that have become centrally controlled

(habit-like) that become dissociated from welfare. For example, nail biting in humans is a repetitive, habitual behaviour, but it is not necessarily indicative of poor welfare; 4) Preservative behaviour: stereotypes that can arise from autistic-like changes or central nervous system dysfunction. In humans this type of behaviour is associated with disorders such as autism and Asperger's syndrome, and can result in repetitive behaviour. This repetitive behaviour has been reported to be a source of pleasure in humans (Handley, 2001; Loftin, 2003) and observed in other non-human primates (Sanchez et al. 2001). They are unreliable indicators of current state as they can persist regardless of welfare state.

Bashaw et al. (2003) used increased activity as an indicator of good welfare under the conditions that it was not stereotypic activity and that the increase caused activity levels to be more similar to that of wild conspecifics for African lions and Sumatran tigers. Other behavioural indices of good welfare used were species-specific appetitive behaviours and lack of stereotypes (Bashaw et al. 2003). Increased activity has been identified as a possible indicator of positive welfare, however, this is controversial as the evidence supporting this hypothesis is not conclusive and so it requires validation prior to its use (Clubb and Mason, 2003). There are many instances where behavioural indicators can be associated with welfare, such as self-injuring behaviours demonstrated in clouded leopards (Wielebnowski et al., 2002). Therefore, they should still be considered when assessing the welfare state of an animal. However, caution should be exerted when drawing conclusions about the welfare of an animal from behavioural observations alone (Mason and Latham, 2004). Likewise, simply because an animal is not displaying stereotypical behaviours does not necessarily indicate positive welfare (Mason and Latham, 2004; Melfi, 2009). It can be challenging to identify indicators of positive welfare, but it is possible that studying wild animal preferences could provide good indications of what animals 'want', as wild animals have limited artificial influences and greater options available to them than captive animals.

1.3.1 Natural living

“Natural living” has been identified as an important component of welfare and it has been suggested that it may be developed into a tool for assessing the welfare of captive animals (Veasey, Waran and Young, 1996). Using this model, it would be expected that in a captive situation, well-faring animals would display similar preferences to wild animals when offered similar options. For example, wild meerkats exist in cooperative social groups (Clutton-Brock et al. 2002), and so it is expected that captive meerkats would also exhibit social living behaviour. A meerkat that is exhibiting dispersal behaviour as it might in the wild could therefore consistently be alienated from a social group, as it is a captive environment which prohibits its ability to disperse. This may result in poor social health and therefore compromised welfare. Similarities in behaviour between wild and captive environments, in addition to other well-being factors, such as physical health, could assist with determining if an animal has good welfare (Dawkins, 2004). There have been relatively few published papers that specifically outline and trial the concept of natural living as an assessment tool (Lund, 2006; ; Veasey, Waran and Young, 1996; Wagenaar and Langhout, 2007). If the concept of natural living is used to assess welfare, then exposure to factors that could be considered negative may be of particular importance to provide animals with experiences and opportunities to exhibit behaviour as they would in the wild.

In captivity, animals are exposed to different types and amounts of stimuli than their wild conspecifics. As such, it seems logical that the conditions under which an animal is housed and managed would have an impact on its behaviour. Zoo environments likely impact on daily animal activity as husbandry practices, such as feeding, may occur at different times to when wild conspecifics might ordinarily hunt or forage for food. For some species, their natural environment can provide an indication as to why the species may have poor captive welfare. For example, natural home range sizes have been used as a predictor for the amount of pacing in captivity; animals with normally large home ranges demonstrate higher levels of pacing behaviour when held in captivity (Clubb and Mason, 2003).

Modern captive animal management practices place large focus on enrichment. Enrichment in zoos is aimed at providing stimuli for captive animals to be able to express behaviours that they would in the wild, for example hiding food to elicit foraging behaviour. The provision of enrichment has been demonstrated to significantly decrease stereotypic behaviour and increase activity for captive cougars (Ruskell et al. 2015). For ferrets, enrichment was able to significantly increase play behaviour and

decrease aggressive interactions (Talbot, Freire and Wassens, 2014). It is also important to consider that wild animals are often exposed to factors that can be considered as “negative”. For example, a wild animal must compete with other animals for food, territory and mates. They may not have access to regular supply of food and can starve, be exposed to extremes of temperature, and to disease and injury. Wild animals clearly cope behaviourally with these challenges, but they could have a negative impact on animal welfare when considering this from a biological function and experience aspect (Dawkins, 1980). While starvation and exposure to injury or disease may be a factor for “natural living” they obviously negatively impact an animal’s physical welfare. However, less extreme stimuli may be an adequate substitute in captivity. For example, playbacks of conspecific vocalisations may mimic territorial threats and thereby provide the animals with the opportunity to display natural territorial behaviours as they would in the wild, but without the risk of death or injury.

1.4 STRESS IN ANIMALS

Stressors trigger a physiological response that involves activation of the endocrine, nervous and immune systems (stress response; Chrousos and Gold, 1992). Stressors are any factor in an animal’s perceived environment that elicits a physiological stress response. It is important to note that the stress response is not just elicited under negative circumstances. A range of events, including exciting events such as eating and mating, can trigger this response (Toates, 1995). Responding to stressors is common in the lives of all animals and allows survival through adaptation to changes in the environment (Moberg and Mench, 2000). Allostasis is the response to daily events and stressors that allows the maintenance of homeostasis (Sterling and Eyer, 1988). As such, stress is not always a bad thing; it only becomes a problem when the stress response threatens an individual’s well being, exerting deleterious effects on the individual’s biological state (Moberg and Mench, 2000). Hence, measuring physiological stress in captive animals can be useful for providing information on their welfare.

1.4.1 Stress response pathways

Structures involved in the stress response include the brain stem, noradrenergic neurons, sympathetic adrenomedullary circuits and parasympathetic systems (Smith and Vale, 2006). However, the main effectors of the stress response are structures in the hypothalamus-pituitary-adrenal (HPA) axis (Figure 1; Smith and Vale 2006). In response to a stressor, epinephrine and norepinephrine are secreted instantaneously from the adrenal glands, while the pathway regulating the synthesis and release of glucocorticoids is slower and longer acting (Palme, 2005). The hypothalamus releases corticotrophin-releasing hormone (CRH) and vasopressin (AVP), which control the activity of the HPA axis, thereby co-ordinating behavioural and metabolic responses to a stressor (Kloet, Joels and Holsboer, 2005). In response to a stressor, corticotrophin-releasing factor (CRF) is synthesized and secreted by hypophysiotropic neurons in the paraventricular nucleus of the hypothalamus (Rivier and Vale, 1983). CRF accesses the anterior pituitary gland through hypophysial portal vessels and binds to receptors on pituitary corticotopes inducing the release of adrenocorticotrophic hormone (ACTH; Smith and Vale, 2006). ACTH stimulates glucocorticoid (GC) synthesis and secretion in the adrenal cortex. HPA axis activation is regulated through negative feedback systems as GC's suppress the secretion of CRF and ACTH at the hypothalamus and pituitary when elevated (Smith and Vale, 2006).

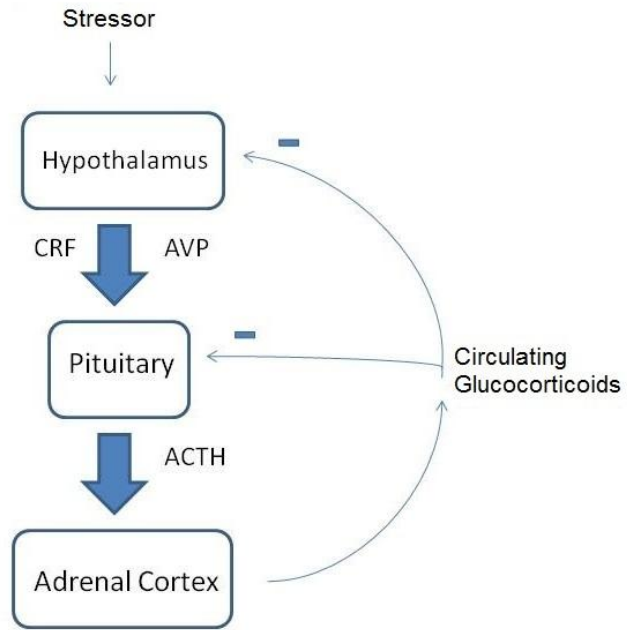


Figure 1. The physiological response to a stressor.

During a stress response, there are peaks in blood concentrations of adrenal GC's which later decline to pre-stress levels once the stressor has been effectively dealt with (Kloet, Joels and Holsboer, 2005). Glucocorticoids are metabolized by the liver to be excreted into urine, and by microbial flora in the gut to be excreted in faeces (Taylor, 1971). There is significant variation between species in the amount of GC metabolites excreted in faeces and urine and the main route of elimination (Palme, 2005). Stressors cause increases in blood glucocorticoid concentrations within minutes (Willemsse et al. 1993), but are excreted over longer time periods. Urine is typically in the body for several hours, dependent on hydration. In mice, intraperitoneal injection of tritiated corticosterone was detected in excreted urine within two hours (Touma, et al. 2003). In general, faecal GCs represent an accumulation of excreted metabolites over a time scale of 12 - 24 hours, providing a daily average of HPA activation (Möstl and Palme, 2002). Hair analyses can provide important information about chronic stressors (Accorsi et al., 2008) and GC's from hair analyses is not impacted by hourly or daily variations in glucocorticoid levels (Koren et al., 2002). Due to differences in growth rate and gut passage time each sample type represents a different time period which can be measured and provide good indication of HPA axis activity.

Glucocorticoids cortisol and corticosterone are generally used for measures of stress in mammals (Figure 2.). The activity of the HPA axis is shown by the concentrations of corticosteroid hormones in the blood, reaching all organs through circulation coordinating brain and body functions for coping with stress, recovery and adaption (Kloet, Joels and Holsboer, 2005). Baseline corticosterone values essentially represent the mean amount of corticosterone present without the impact of a stressor, and are often used as a measure of the "normal" state (; Brown et al., 2004; Chosy, Wilson and Santymire, 2014).

behavioural and physiological stress response demonstrates the need for a robust framework to be developed to accurately use these indices to assess welfare.

1.4.3 Glucocorticoid metabolites as welfare indicators

Corticosteroid or cortisol measurement can provide a physiological indicator of stress (Carlstead, et al. 1992, Konrad and Bagshaw, 1970). Faecal samples provide a non-invasive way of determining adrenal activity. Being steroid hormones and therefore lipid soluble, GC's easily diffuse across the blood-brain barrier and excreted concentrations of GC's reflect those in the central nervous system. The activation of the HPA axis and the release of GC's are processes widely conserved across species (Möstl and Palme, 2002), but the quantity of GC's produced varies across species and is determined by each individual's perception of a stressor.

Additionally, there are differences within species according to sex on GC production. Fanson (2012) suggests GC production may be correlated with ovarian/luteal activity as variation in adrenal activity has been linked with reproductive status and oestrus in baboons (Weingrill et al., 2004), ring-tailed lemurs (Cavigelli, 1999) and red squirrels (Dantzer et al., 2010). However, these differences are not significant in all species; a study by Carbajal et al. (2014) on broiler chickens found no significant relationship between GC's and sex. Where there are differences between sexes, they are not consistent across species. For some species, females have higher levels of GC production (lynx [Fanson et al. 2012]; tigers [Narayan et al. 2013]; clouded leopards [Wielebnowski et al. 2002]). In others, males have higher levels of GC production (jaguars; Conforti et al. 2012). Glucocorticoids are a measure of animal welfare that is broadly applicable, but there is a need for validation for each individual species and each sex (Möstl and Palme, 2002). To use corticosterone or cortisol measurement to determine welfare, ideally a baseline level should be identified for each species, for separate sexes, and possibly even each individual within species. The mean corticosterone or cortisol level for each species should be calculated using a large sample size to determine the baseline. Baselines have been identified for a range of domestic animals (Carbajal et al., 2014; Graham and Brown, 1996; Prola et al. 2013; Rettenbacher et al., 2004), however few baselines are well

established for zoo housed animals and are often limited by sample size (tigers [Narayan et al., 2013]; spider monkeys [Rangel-Negrin et al., 1992]; cheetah [Wells et al., 2004]; clouded leopards [Wielebnowski et al., 2002]). Until baselines have been established for a large number of species using sufficient sample size, it is difficult to determine if a studied animal's corticosterone levels are indicative of good welfare.

1.4.4 Glucocorticoid measurement in various sample types

Glucocorticoids and their metabolites are commonly analysed using radioimmunoassay and enzyme-immunoassays, which are both competitive binding assays to measure GC levels (Sheriff et al., 2011). Radioimmunoassays generate radioactive signals using radioactive isotopes such as tritium or iodine, requiring gamma counters or scintillations (Sheriff et al., 2011). Enzyme immunoassays generate colourimetric or fluorometric signals and utilise a microplate reader (Sheriff et al., 2011). Glucocorticoids can be measured in faeces, urine, hair, blood and saliva. Each medium provides information about different time periods of HPA activity.

Blood samples provide instant information of HPA activity in response to specific stressors. However, blood sampling can be stressful for animals and may require either chemical or physical restraint. This may induce adrenal activity, which would confound results when testing for response to a specific factor if GC's produced from capture stress enters blood before a sample is able to be collected. This could be avoided by training an animal to allow and be comfortable with blood sampling to prevent inducing stress, but this approach is resource intensive. Using urine can also be difficult; urine collection is resource intensive and not always practical, particularly for dangerous animals. Faecal GC's are advantageous as they are easily collected, non-invasive and provide a daily average of HPA activation that can be linked to behavioural observations and any environmental variables that change from day to day (Möstl and Palme, 2002). Circulating cortisol fluctuates diurnally for most mammals, and as faecal monitoring represents an average value for the past day it is useful for determining adrenal activity over time (Graham and Brown, 1996). Unlike urine, faecal and blood analyses, hair samples can provide long-term information on an animal's glucocorticoid production, but not acute

stress (Bechshoft et al., 2011). Hair analyses can provide important information about chronic stressors however it is a relatively new technique and requires further validation for more species (Accorsi et al., 2008). For captive, non-dangerous animals hair is quite easily collected, and any potential acute stress caused during the process will not be reflected in the data as there would not be enough time for GC's to be incorporated in the hair (Sheriff et al., 2011). For dangerous animals hair collection could be through training an animal to allow shaving through protective fencing or alternatively during an anaesthetic procedure. While hair has the potential to be very useful in measuring chronic stress over long periods of time, collection of sufficient samples could be resource intensive for dangerous or fight or flight driven animals.

1.5 COMBINING MULTIPLE METHODS OF WELFARE ASSESSMENT

Using multiple indices to generate a more accurate assessment of an animal's welfare provides more robust results and validates the accuracy of metrics used. Links between hormones and behaviour is well researched. For example, reproductive and parenting behaviour has been shown to have significant correlations with corticosterone or cortisol levels for a range of species (meerkats [Carlson et al., 2006]; macaroni penguins [Crossin et al., 2012]; eastern bluebirds [Davis and Guinan, 2014]; african striped mice [Raynaud, Schradin and Fusani, 2015]). Most studies applying both behavioural and hormonal metrics for use in welfare assessment have focused on domestic animals. Only a limited number of studies have used this method for zoo housed animals. Pritchard et al. (2005) used a combination of physical health and behaviour to determine welfare, focusing on lameness, body condition and the presence of aggressive and aversive behaviour during interactions with handlers, as well as determining that a lack of responsiveness was due to apathy or severe depression.

Correlation analysis between the two welfare measures showed significant correlation between behaviour and some physical aspects of health (body condition, lesions and abnormal gaits) validating the results of both these indices as a measure of welfare (Pritchard et al., 2005). Blanchard et al. (1998) exposed rats to cats, measuring behaviour and physiological responses to stress and found that there were significant relationships between some behaviours (crouching, grooming, rearing) and corticosterone levels after exposure to cats. Carlstead, Brown and Strawn (1993) found

increases in cortisol were significantly correlated with time spent hiding when cats were exposed to stressors. A non-significant trend also suggested stressed cats spent more time alert and awake and were also more active (Carlstead, Brown and Strawn, 1993). Clouded leopards have also been shown to have significant correlation between elevated faecal GCs and behaviours such as pacing, self-injuring, sleeping, hiding and fearful/tense behaviour (Wielebnowski et al., 2002). Ideally for all welfare studies, multiple indices should be used to strengthen results. If studies consistently identify significant correlates between a behaviour and a second welfare measure, one measure of welfare may be sufficient. For example, if certain behaviours are shown to be strongly correlated with elevated stress hormones, future observations of that behaviour can be reliably used as a measure of stress for animals under similar circumstances.

1.6 AFRICAN LIONS AS A FOCUS SPECIES

This study uses African lions as a focal species as they are a popular species commonly housed in captive facilities, with many different enclosure types and social groupings. Wild lion behaviour has been extensively researched (e.g. Bauer, De Longh and Di Silvestre 2003; Benhamau, et al., 2014; Dolreny, 2013; Funston et al 1998; Grinnell, Packer and Pusey, 1995; Grinnel and McComb, 2001; Loarie, Trampling and Asner, 2013; Matoba, Kutsukake and Hasegawa, 2013; McComb, Packer and Pusey, 1994; Mosser and Packer, 2009; Scheel and Packer, 1991; Van Der Waal, Mosser and Packer, 2009). There are fewer studies that examine captive lion behaviour, and these studies focus only on specific factors influencing behaviour. For example, Bashaw et al. (2007) demonstrated significant effects of time of day on resting behaviour. Powell (1995) studied the effect of the provision of enrichment on lion behaviour, finding enrichment increased behaviours relating to environmental exploration such as licking and object manipulation. Enclosure size and complexity have been demonstrated to exert an influence on captive lion behaviour, particularly on aggressive behaviours, resting and pacing (Bashaw et al., 2007; Clarey and Farnsworth, 1983). Only one previous study has thoroughly categorised the behavioural repertoire of captive lions (Clarey and Farnsworth, 1983). This study did not identify the potential occurrence of stereotypic behaviours other than pacing, nor did it

examine lion behaviour without manipulating variables (i.e. moving them to different enclosure). Additionally, management of zoo-housed lions is likely to have changed since this study was conducted and, as such, these results may be outdated. However, the results suggest that when assessing welfare in captivity, social behaviours and stereotypic behaviour can potentially provide an indication of welfare. There are no previously published papers that compare any aspect of captive lion behaviour to wild lion behaviour. Our study will provide a thorough account of the behavioural repertoire of captive lions, in addition to being the first to compare an aspect of wild and captive lion behaviour (daily activity levels). It is also the first to introduce the concept of natural living as a welfare assessment tool for lions.

There are currently few published studies that investigate the stress response of lions. Serum cortisol has previously been measured in wild lions by Brown et al. (1993). Santymire et al. (2012) validated the use of faecal samples for corticosterone measurement, however this study was based on a single lioness. Putman et al. (2015) focused on reproductive hormones and only examined corticosterone in relation to these. Reported corticosterone baselines for lions varied largely between each study (130 ± 20 ng/g dry faeces [Putman, 2015]; 407.9 ± 103.7 ng/g dry faeces [Santymire, 2012]). This study will measure corticosterone levels in captive lion faeces. The results are intended to contribute to determining a normal baseline for captive lions and to validate behavioural data for future reference and welfare assessment.

1.7 CONCLUDING REMARKS AND STUDY OBJECTIVES

Identification and quantification of links between an animal's observable behaviour and its internal physiological state will support the development of a non-invasive, fact-based assessment tool that could be applied across animal industries to better measure the welfare state of individual animals. Using a combined approach (behavioural and physiological assessment) allows for a more complete representation of an animal's welfare (Basset and Buchanan-Smith, 2007). This study will investigate the use of corticosterone through faecal glucocorticoid metabolite levels for welfare assessment, as well as trial the use of hair samples for African lions. A baseline will be developed for lion faecal GC

metabolite levels and a thorough account of captive lion behaviour in modern facilities will be recorded which will also be used for comparisons with wild lion behaviour. The relationship between behavioural and hormonal data will be investigated. This study aims to provide data which can be used as a benchmark for future assessments of captive lion welfare. The following hypotheses will be tested:

- A. Captive lions will display a diverse range of behaviours with little stereotypic behaviour;
- B. There will be similarities in activity levels and behaviour between the captive lions studied and wild lion behaviour (from previously published literature);
- C. There will be an effect of time of day on patterns of lion behaviour;
- D. There will be correlations between behavioural aggression and faecal glucocorticoid metabolite levels; and between daily activity and faecal glucocorticoid metabolite levels.

Chapter 2

Behavioural Characterisation of Captive African Lions

2.1 INTRODUCTION

African lions are a charismatic species of large felid that are commonly housed in captive facilities. There is a need to develop a non-subjective framework for behaviour-based welfare indicators for captive lions to be used as a benchmark across institutions and individuals. Comparing the behaviour of captive individuals to wild conspecifics may be used to indicate captive animal welfare (Chamove, 1989; Lindburg, 1988). Previous behavioural studies on wild lions have focussed variously on dispersal behaviour (Benhamou et al, 2014; Van Der Waal, Mosser and Packer, 2009), hunting behaviour (Funston et al 1998; Loarie, Trampling and Asner, 2013; Scheel and Packer, 1991), social behaviour (Bauer, De Longh and Di Silvestre 2003; Dolreny, 2013; Grinnell, Packer and Pusey, 1995; Grinnel and McComb, 2001; Matoba, Kutsukake and Hasegawa, 2013; McComb, Packer and Pusey, 1994; Mosser and Packer, 2009) and population dynamics (Benhamou et al, 2014). Studies examining behaviour of captive lions often focus on the effect of environmental enrichment (the provision of environmental stimuli that aims to enhance quality of life and improve psychological and physiological well-being; Sherperdson, 1988), particularly on activity levels and proportions of behaviour displayed (Bashaw et al., 2003; Powell, 1995). Other studies have examined lion behaviour in different enclosures and at different times of day (Bashaw et al 2007; Clarey and Farnsworth, 1983). Until now, there has been no comparison of wild and captive behaviours in lions.

Few studies provide a detailed list of captive lion behaviours. Clarey and Farnsworth (1983) studied the behavioural repertoire of captive lions in two different zoo exhibits and found differences in lion behaviour between enclosures. In the smaller, less complex exhibit, lions displayed more pacing, more aggression and less interaction with the environment. Aggressive behaviours identified included biting and slapping, pouncing and wrestling, and growling (Clarey and Farnsworth, 1983). Non-aggressive interaction was identified in both exhibits as grooming, sniffing, rubbing, mating, following

or touching and the observed frequency of these interactions were similar between exhibits (Clarey and Farnsworth, 1983). Since this study was conducted, there has been greater focus on housing lions in enclosures that attempt to mimic conditions found in the wild and on stimulating natural behaviours in captivity (Maple and Perdue, 2013). Powell (1995) recorded all behaviours displayed by captive lions and compared whether there were any significant differences in the frequencies of each behaviour displayed before and after enrichment, finding significant increases in behaviours such as licking/gnawing, manipulating objects with paws and sniffing/flehmen when enrichment was offered. Powell (1995) suggested that these behaviours are species typical and providing stimuli that creates opportunities to present these behaviours is beneficial to animal welfare. In captivity, husbandry activity during the daylight hours may also affect captive lion activity.

Schaller (1972) documented an extensive repertoire of wild lion behaviours. These behaviours include: communication behaviours (including roaring, urine-spraying, scuffing, clawing and urine testing); defensive and threat displays; social displays (including rubbing and licking); mating behaviour (including neckbiting, rubbing, slapping); aggressive and threat behaviours (including snarling, slapping, growling, hissing and woofing); and play behaviour (including chasing, wrestling, pawing, stalking and rushing; Schaller, 1972). On average, 60% of wild lion activity is at night with peaks around dawn and dusk (after 1700 and before 0800) and as it gets hotter throughout the day they tend to rest more, averaging approximately 20-21 hours of rest per day (Cozzi et al., 2012; Schaller, 1972). Lion activity is influenced by environmental factors, for example, in areas with sufficient cover, lions will hunt during daylight hours (Schaller, 1972; Sunquist and Sunquist, 2002) while in areas with little cover to stalk prey, lions are largely nocturnal (Schaller, 1972).

Comparing the behaviour of captive lions with their wild counterparts is a potential avenue to examine animal welfare. Indeed “natural living” is a concept of animal welfare that has previously been identified and discussed as an important part of animal welfare (Fraser 2003; Veasey, Waran and Young 1996). Essentially “natural living” refers to an animal being able to perform their full range of behaviours according to how they live in the wild (Hewson, 2003), but to date few studies have used this concept in practice (Wagenaar and Langhout, 2007) and to our knowledge there is no literature

on natural living welfare assessment for zoo housed animals. This chapter will focus on the natural living aspect of animal welfare and utilise this concept in determining welfare for captive lions.

By assessing state and event behaviours, this chapter will provide a comprehensive account of lion behaviour in facilities with modern management that attempts to replicate conditions encountered in the wild.

It is hypothesized that;

1. There will be a diverse range of behaviours displayed by captive lions, with little stereotypic behaviour.
2. There will be similarities in activity levels and behaviour between the captive lions studied and wild lion behaviour from previously published literature (Hayward and Hayward, 2007).
3. There will be an effect of time of day on patterns of lion behaviour, with significantly more activity occurring in the morning compared to the afternoon.

2.2 MATERIALS AND METHODS

2.2.1 Study animals

Seven adult lions were evaluated in this study; three males and four females. The lions were from three groups housed in two Australian institutions, both members of the Zoo and Aquarium Association, and adhering to the same level of professional standards of practice. There were differences between facilities in terms of lion group compositions, sex ratios and exhibit layout. One facility housed two groups, both of one male and one female lion, while the other facility had one group of two females and one male. All lions were aged between eight and 14 and were non-breeding groups. Breeding was prevented through use of contraceptive implant for females that were not yet post reproductive (Deslorelin, LHRH agonist). All facilities had one “on-display” area where members of the public could view them and one “off-display” area for each group of lions. They were confined to their “on-display” areas except for when zookeepers were servicing enclosures; this generally occurred outside of public viewing hours. Exhibit layout differed in terms of areas where the public

could view, furniture within the exhibit and what the lions themselves could see. In one exhibit the lions could be viewed from around the entire area while in the other, lions could be viewed only from the front. One exhibit had more natural vegetation while the other had more artificial climbing structures. One exhibit had no direct view of other animals but was within auditory and olfactory distance, while the other two were in view of other animals.

2.2.2 Characterising captive lion behaviour.

Lions at the first institution were observed continuously for five consecutive days to catalogue and describe the repertoire of behaviours commonly expressed by lions in captive institutions. All behaviours expressed were recorded until no new behaviours were observed. All behaviours were recorded within the first three days, and the last two days of observations were used to verify that no behaviours had been missed. Behaviours observed were described and categorised according to whether the behaviour was a state or event behaviour and compiled into an ethogram. State behaviours were categorised as behaviours that persist over a length of time (e.g. walking), while event behaviours were instantaneous (e.g. jumping); some behaviours could have been either state or event behaviours depending on their duration (e.g. vocalising-- a short vocalisation can be instantaneous while a territorial roar can last minutes; Altmann, 1974). An request was made to access lions for night observation, however, due to safety reasons an observer was not allowed to remain on zoo property outside business hours. Placement of camera traps was tested, , however, these were discovered and destroyed by the lions despite attempts to have the cameras placed out of reach. Due to the layout of the enclosure it was not possible to capture sufficient footage with cameras set up outside the enclosure.

2.2.3 Comparing activity of wild and captive lions.

Behavioural observations were collected over a period of ten consecutive days for each group of lions at the two facilities. A daily activity budget was generated through instantaneous scan sampling, noting at each half hour mark between 09:00 – 16:00 which behavioural state each lion was expressing. Additionally, each lion was observed using continuous sampling for 30 minutes in the morning and again in the afternoon to determine if there was a time-of-day effect on activity. This was

done daily from 10:00-10:30, 10:30-11:00 or 11:00-11:30 in the morning, and 14:30- 15:00, 15:00-15:30 or 15:30 - 16:00 in the afternoon (n=20 per lion). Both state behaviours and event behaviours were recorded. Each lion was rotated between time slots to ensure there was no time-of-day effect skewing individual lion data for the morning or the afternoon. Additionally, one lion per day was observed continuously for a period of 30 minutes from 13:30 – 14:00 as this was often (but not always) prior to zookeepers moving lions off display and then back on to display to facilitate feeding at approximately 14:15. Each lion was observed three times at this time period over the ten day period.

Captive data were compared to results presented by Hayward and Hayward (2007) for wild lion activity in the Addo Elephant National Park in South Africa. Hayward and Hayward (2007) monitored seasonal activity patterns of four adult male lions and two adult female lions for 96 continuous hours each season (summer, spring, autumn and winter) using data from radio collars from December 2003 to Spring 2005. Archived climate data was obtained from the Australian Bureau of Meteorology (BOM; accessed November 2015) and Weatherspark (accessed November 2015) to determine which seasonal climates studied by Hayward and Hayward (2007) were most similar to the seasonal climate in NSW, Australia when data was obtained for our captive lions to minimise influence of climate when comparing lion activity.

2.2.4 Data analysis

Total daily captive lion activity was calculated by using the mean percentage of activity calculated from all half hour instantaneous scan samples from all lions at both facilities. To determine if there was a significant difference between captive lion activity in the morning versus the afternoon, a paired t-test was performed comparing average lion percentage activity from continuous sampling in the morning and the afternoon. The average percentage of stereotypic behaviour displayed was also calculated, as stereotypic behaviour is often used as an indicator of welfare. Mean hourly percentage lion activity was calculated using data from the half hour instantaneous scan samples for all lions from 9:00 – 16:00 and this was then compared to results presented by Hayward and Hayward (2007) showing the average percentage of hourly activity for the same time of day for wild lions in the Addo Elephant National Park in South Africa. Our data was compared only to Hayward and Hayward's

(2007) results presented in a graph (figure 5, page 139) from autumn alone and an average of autumn and winter as these most closely matched environmental conditions to when our data was collected in May/July in NSW, Australia. This was in the interest of minimising meteorological effects such as temperature and rainfall on the assumption that these factors may have influenced activity patterns.. A Shapiro-Wilk test was used to determine normality; no transformation of data sets was performed as all data were normal. A Pearson correlation analysis was performed to determine if there was a significant correlation in hourly percentage activity for wild lions compared to captive lions.

2.3 RESULTS

2.3.1 Characterising captive lion behaviour.

A range of commonly expressed behaviours were recorded and described (Table 1). Mean percentage of time spent inactive throughout the day was 86%, with 13.6% of time spent performing active behaviours. The paired t-test did not show significant differences between proportions of time spent resting in the morning compared to the afternoon, but the trend showed more time spent resting in the morning ($t=-1.67$, $p=0.08$, $df=69$). Pacing behaviour observed was performed for 0.03 % of total daily behaviours displayed. There was not sufficient variation in behaviour displayed to test for differences in behaviour between facilities, groups or individuals.

2.3.2 Comparing activity of wild and captive lions

The Pearson's correlation showed strong correlation in hourly lion activity between captive lions studied and wild lions from the Addo National Park in autumn ($r= 0.84$, $n= 8$, $d.f. = 1$, $r^2=0.70$, $p= 0.0513$). There was also a strong correlation in hourly lion activity when using an average of wild lion activity in autumn and winter for comparison with the captive lions studied ($r= 0.74$, $n= 8$, $d.f. =1$, $r^2=0.55$, $p= 0.29$; Figure 3).

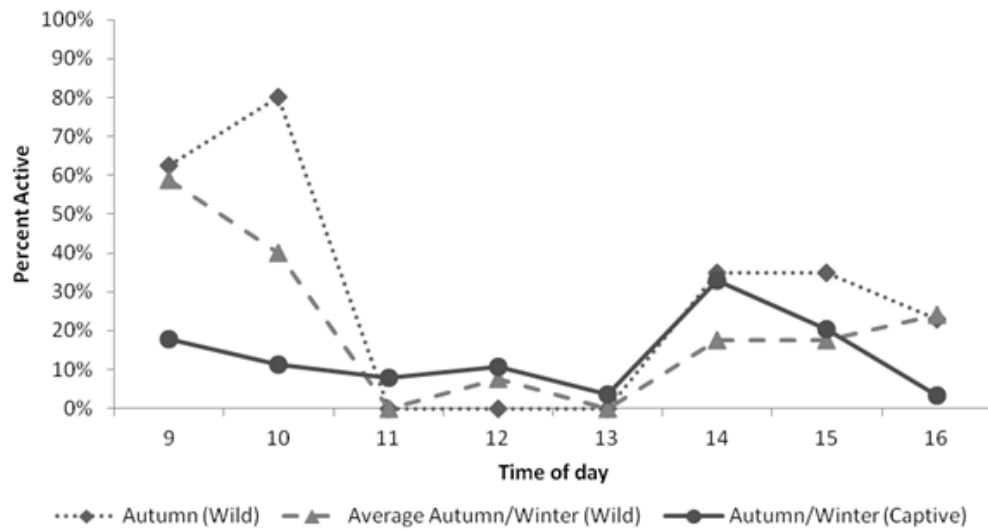


Figure 3. Hourly percentage activity from 0900 to 1600 in autumn, winter and average of autumn and winter for the wild lions in the Addo national park from results from Hayward and Hayward (2007), compared to results from this study conducted over the end of autumn and start of winter for the same time period.

Table 1. Ethogram of common behaviours exhibited by a group of three captive adult lions (one male and two female) over a period of five consecutive days.

Category	Behaviour- Event (E) State (S) or Both (ES)	Description	
Locomotion	Walking	S	Moving from one point to another
	Climbing	E	Scaling tree or other object
	Running	ES	Moving from one point to another at speed
	Jumping	E	Leaping off ground vertically to reach object or land on top of object
	Pacing	S	Moving in fixed manner following the same route repeatedly
Resting	Sitting	S	Back legs folded and hindquarters on ground with front legs straight and forequarters off ground
	Standing	S	Body off ground and weight on all four limbs
	Laying Down	S	Body ground, either on lateral side or on chest/stomach with legs either outstretched or tucked below
	Alert Rest	S	Laying or sitting with head raised and eyes open
	Non-Alert Rest	S	Laying with head on ground and eyes open
	Sleeping	S	Laying with head on ground and eyes closed
Territorial	Scent Marking	E	Spraying urine (typically males) and/or dragging paws on objects (males and females)
	Roaring Vocalisation	S	Deep repetitive grunt like vocalisations typically lasting approximately a minute
Social Interaction	Play- Stalk/Chase	ES	Intently watching other lion from a distance or partially hidden, then rushing out at lion
	Play- Mock Fight	ES	Swiping and batting using paws, jumping on other lions without causing an injury
	Aggressive Display	E	Lunging, baring teeth, swiping at other lion without making physical contact
	Aggressive Action	E	Lunging at, biting or swiping at other lion and making contact
	Social Grooming	ES	Licking other lion
	Social Resting	S	Resting behaviour in physical contact with other lion
	Affection	E	Rubbing head and/or gentle bite on other lion
	Courtship	E	Male: Biting of head/neck, standing over female, rubbing face on females head/neck with teeth bared, sniffing/tasting reproductive organs of female Female: rubbing head on male with teeth bared, laying on stomach submissively.
	Mating	ES	Male: Standing over female, biting head/neck and performing movement for copulation Female: lowered body on ground submissively, moving tail to allow male access
Environmental Interaction	Digging	ES	Using paws to displace earth
	Licking	ES	Moving tongue across object that is not another lion, itself or food item
	Sniffing	ES	Putting nose by object/ground
Other	Eating	S	Consuming food, chewing on branches / leaves/ grass
	Urinating	E	Dispelling of liquid without spraying on object
	Self Grooming	ES	Licking body or licking paws and rubbing on head/face. Scratching self using paw or against object

2.4 DISCUSSION

Although previous studies have examined captive lion behaviour, this is the first to thoroughly describe and characterise the behaviour of lions in a modern facility. Twenty-eight distinct behaviours were recorded and described, covering a range of functions such as social bonding, locomotion, environmental interaction and resting. Lions were inactive for 86% and only active 13.6% of the total observed time, the remaining 0.4% was when lions were not visible to the observer. Although not significantly different, there was a trend of increased activity in the afternoon rather than the morning. This difference was also noticeable when comparing captive lion activity with wild lion activity for both seasons; wild lions were significantly more active in the morning compared to the afternoon (Hayward and Hayward 2007). However, the proportion of time spent active was similar for captive and wild lions at midday and in the afternoon. The overall correlation value was higher between captive lions and wild lions in autumn ($r^2=0.70$, $p= 0.0513$) than for an average of autumn and winter ($r^2=0.55$, $p= 0.29$). When active, the predominant types of active behaviours were exploration and social interaction. This study did aim to further characterise the occurrence of more specific behaviours, but while we did identify many specific behaviours, they were performed quite infrequently. Additionally certain behaviours were performed outside of viewing times/areas and were therefore not able to be captured in the ethogram. An example of this is defecation. While this behaviour was not observed, faecal matter was found within the enclosure and off display holding areas. This made it difficult to do any further analysis outside of descriptions. This issue in the future could be mitigated through observing animals for longer periods of time, and also with a greater sample size of lions.

This study has thoroughly catalogued and described captive lion behaviour in a mixed sex group of adult lions in modern captive facilities. Bashaw et al. (2007) previously described lion behaviour in relation to housing conditions, however they did so very broadly by placing all behaviours into four categories; rest, rest but awake, non-stereotypic activity and pacing. This does not give an indication of the full repertoire of behaviours displayed by lions in captivity, nor does it place any focus on social behaviours which for a social living animal is an important factor in behavioural repertoire analysis. This study examined and described individual behaviours in addition to describing behaviours more broadly using activity levels to generate a more comprehensive ethogram of captive lion behaviour.

The behaviours observed covered a greater range of functions than outlined by Bashaw et al. (2007); social behaviours, territorial behaviours, locomotion behaviour, environmental interaction behaviour and resting behaviours. This information is useful for comparisons between wild and captive lion behaviour as it provides a more detailed account of captive lion behavioural repertoire, particularly as the lions in this study were from mixed sex groups of different sizes, allowing for more opportunity to display certain behaviours, particularly social and breeding behaviours. Clarey and Farnsworth (1983) found an effect of environment on social behaviour, with less aggression in larger and more complex environments. They did not identify the potential occurrence of stereotypic behaviours, other than pacing, nor did they examine lion behaviour without manipulating variables (i.e. moving them to different enclosures). However, Clarey and Farnsworth (1983) suggest that when assessing welfare in captivity, social behaviours and stereotypic behaviour can potentially provide an indication of welfare. Clarey and Farnsworth (1983) examined the behavioural repertoire of three captive adult lions in two enclosures of contrasting size, and alluded to the possibility that the lions likely had poorer welfare in the smaller enclosure compared with the larger enclosure. While not tested statistically, Clarey and Farnsworth (1983) found far less repetitive behaviour and social aggression in the larger and more complex exhibit. The lions in this study were housed in modern captive facilities that adhere to standards of housing including in terms of enclosure size, and analysis showed low amounts of stereotypic activity (for 0.03 % of total daily behaviours displayed) and only 9 total observations of aggression between individuals in a total of 10 hours of observation for each lion (70 hours in total). These results provide insight into the effect of environment on behaviour; however, the study does not provide insight to management techniques, provision of enrichment or any other factors that may influence behavioural repertoire - and in recent years animal management within modern zoos has changed considerably. There have been previous studies examining behaviour in response to enrichment (Powell 1995; Van Metter, Harriger and Bolen 2008), generating ethograms and demonstrating significant differences in behaviour in relation to the provision of enrichment, but both these studies have only examined a small number lions at one facility. Future studies should investigate how management practices influence lion behaviour with more lions in different social groups and facilities.

This study found that there was no significant difference in activity levels according to time of day observed, although the general trend suggested a higher degree of activity in the afternoon compared to the morning ($t=-1.77$, $p=0.07$, $df=132$). Bashaw et al. (2007) found that there was significantly more time spent resting in the midday and afternoon for captive lions. The trend of higher amount of activity observed in the afternoon in our study was likely due to the management practices of the facilities, as it was in the afternoon that the lions in our study were shifted between enclosures and fed. It is possible that without management influence, the time spent resting in the afternoon would have been significantly more than in the morning. Lions were observed to be resting in the afternoon until the presence of zookeepers was noticed. As feeding and shifting times were the same each day they were conditioned to associate keeper presence with routine shifting and feeding, eliciting expectant activity. After shifting and feeding, lions resumed resting behaviours. It was at this time of the day pacing was observed was and not at any other time throughout the day, suggesting this stereotypical behaviour was due to anticipation of some kind of management activity which generally incorporated food. Lions were not able to be monitored after hours; however, the influence of management on lion behaviour should be further investigated to also incorporate lion activity levels throughout the full 24 hours of the day, as 60% of wild lion activity occurs overnight (Cozzi et al 2012). The effect of management on total activity budget of lions in captivity would provide further insight as to how captive lion behaviour is shaped and the implications of this when using wild lion activity as a comparison tool to monitor captive lion welfare.

This study is the first to utilise the concept of “natural living” as a comparison tool for captive and wild lion behaviours, with a particular focus on levels of activity. Natural living has been identified as an important factor in animal welfare, but in practice is rarely used in studies of animal welfare (Fraser 2003; Veasey, Waran and Young 1996; Wagenaar and Langhout 2007). Moderate correlations between activity levels of captive and wild lions were found, using wild lion activity data presented in Hayward and Hayward (2007). Wild lions are typically active at night with peak activity around dawn and dusk when most hunting occurs (Cozzi et al 2012, Schaller 1972). A controlled captive environment means that there are certain behaviours wild lions are required to perform that captive lions do not. For example, lions in captivity are provided with food and do not have the opportunity to hunt, as such, it is logical to assume that they would also generally be less active at peak hunting

times for wild lions if they were being fed at different times of the day. Wild lions typically rest 20 – 21 hours per day, or for 85% of a total 24 hours period (Schaller, 1972) and the mean resting time of the lions in this study was 86% of total observed time. Veasey, Waran and Young (1996) suggests that a captive animal performing behaviours similar to its wild conspecifics is more likely to have a similar quality of life compared to wild counterparts than one that is not, but the absence of some wild behaviours does not necessarily indicate reduced quality of life. Some behaviour expressed in wild animals could be due to various stressors that captive lions would not be exposed to, such as competition from extra-pride individuals and other predators, but the absence of these behaviours in captive lions would not indicate poor welfare. It should also be noted that there is much more variation in social group compositions and sizes in the wild, and this variation likely also provides opportunity for different and broader ranges of behaviour than a stable, captive group. An environment that provides opportunity to express natural behaviours may enhance welfare; however other measures of welfare are ideally required to make a comprehensive assessment (Veasey, Waran and Young, 1996). Further investigation should focus on comparing wild and captive lion behaviour at night.

This study has provided valuable insight in to the behaviour and activity of captive lions in a modern facility and has been the first to incorporate the concept of natural living for zoo housed animals. As hypothesized, little stereotypic behaviour was displayed and there was a strong correlation in activity levels/patterns between wild and captive lions. Twenty-eight distinct behaviours were observed whilst generating the ethogram. When examining activity levels and behavioural diversity during fixed observation periods there was generally little variation in behaviour displayed. This may have been limited windows of observation, in particular the inability to observe lions at night. We were therefore unable to determine exactly how diverse their behavioural range was. As hypothesized, there was a strong correlation hourly lion activity between captive lions studied and wild lions from the Addo National Park. However, there was no significant impact of time on day on behaviour which was. The trend did indicate higher amounts of activity in the morning, as predicted in the hypothesis. These results have established a benchmark for captive lion activity and behavioural repertoire.

CHAPTER 3

Faecal glucocorticoid metabolite profiling of captive African lions

3.1 INTRODUCTION

Endocrine analysis is increasingly being used to measure stress in order to assist in welfare assessment of captive animals (Carbajal et al., 2014; Owen and Lane, 2006; Palme et al., 2000; Prola et al., 2013; Wasser et al., 2000). Accurate assessment of stress levels in captive animals will provide an important opportunity to develop best practice husbandry procedures. A stressor will activate the hypothalamic-pituitary-adrenal (HPA) axis, causing the hypothalamus to secrete corticotrophin releasing factor (CRF) which triggers the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland (Sapolsky, Krey and McEwen, 1986). ACTH stimulates the zona fasciculata of the adrenal glands to produce glucocorticoids (GCs; Sapolsky, Krey and McEwen, 1986). GCs dampen the effect of adrenocorticotrophic hormone (ACTH) suppressing further GC release from the adrenal cortex as part of a negative feedback system (Endroczi, 1963, Sapolsky, 2000). During a typical stress response in mammals, the adrenal gland will activate the release of epinephrine and norepinephrine which facilitate immediate physical reactions to cope with stress. Blood concentrations of adrenal GCs generally peak within an hour and later decline to pre-stress levels once the stressor has been effectively managed (Kloet et al., 2005).

Chronic stress occurs when an animal continues to perceive a stressor for a prolonged period of time. This results in an increased output of arginine vasopressin (AVP), which in turn increases CRH action to release ACTH, resulting in maintained elevation of glucocorticoid levels. Chronic stress causes dysregulation of the normal negative feedback mechanisms and has a deleterious effect (Moisan, 2012), reducing an individual's ability to cope with an acute stress event due to allostatic overload of the HPA axis (McEwen, 2004). Chronic stress has negative implications for welfare and can cause a range of issues, such stereotypic behaviour, excessive sleeping, hiding, decreased exploratory behaviour and self-mutilation, all which have been linked with physiological measures of stress in

species including domestic cats and clouded leopards (Carlstead, Brown and Strawn, 1993; Konrad and Bagshaw, 1970; Moberg, 2000; Wielebnowski et al., 2002). Chronic stress can also cause physiological consequences such as immunosuppression and inhibited reproduction (Birkett and Newton-Fisher, 2011; Carlstead and Brown, 2005).

Glucocorticoid metabolites can be measured to provide information about HPA axis activity (Bortolotti et al. 2008). Glucocorticoids and their metabolites can be measured in a number of sample types and different samples can all be successfully used but present information on different timescales (Brown et al. 1993; ; Brown et al. 1994; Möstl and Palme, 2002; Schwarzenberger et al. 1996). Serum cortisol concentrations have been shown to peak in lions (n=13) 45 to 60 minutes after ACTH injection and return to baseline levels after approximately 200 minutes post-injection (Brown et al. 1993).

Metabolites can be measured in faecal extracts between approximately 12 hours and 2 days, depending on species and intestinal passage time (Möstl and Palme, 2002; Schwarzenberger et al. 1996). Faecal glucocorticoid metabolites (FGM's) have been shown to peak 24 hours after ACTH injection in lions and return to baseline levels approximately 60 hours post-injection (Santymire et al. 2012). Glucocorticoids can also be detected in hair and urine in some species (Galuppi et al. 2013; Kolevská and Svoboda 2000; Macbeth et al. 2010; Stalder and Kirschbaum 2013; Touma et al. 2003; Turpeinen and Hämäläinen, 2013), but this has not yet been tested in lions. The time course of urine excretion varies according to species and hydration, but studies have shown that infused cortisol can be seen in urine close to time of infusion (Möstl and Palme, 2002). Hair has been shown to contain cortisol correlated with corresponding faecal samples from the same time period, reflecting average cortisol levels during the time of hair growth (Accorsi et al. 2008). Glucocorticoids in hair has been shown to be unaffected by ACTH injection in reindeer (Ashley et al., 2011), while other studies found ACTH injection led to significantly higher hair glucocorticoid concentrations (cattle [Del Rosario et al., 2011]; lynx [Terwissen, Mastro Monaco and Murray, 2013]). An ACTH challenge is required for validating use of hair glucocorticoid measurement for lions. This study will be the first to trial measuring glucocorticoids in hair for African Lions.

Institutions that house captive animals are responsible for providing animals with optimal welfare and understanding sources of stress in captivity can provide information to inform animal management with a view on providing optimal conditions. Captive environments are generally stable and predictable; with a reliable food source, standard day-to-day management and a familiar environment. Predictability has been shown to reduce stress as it can allow individuals to relax (Weinberg and Levine 1980), although high environmental predictability can cause boredom (Basset and Buchanan-Smith 2007). It is important to note that it is not always a negative circumstance that elicits a stress response; GC levels can rise due to positively perceived events such as eating (Toates 1995). Captive animals are invariably kept in an environment that is smaller and less complex than their natural habitat (Basset and Buchanan-Smith, 1997). Individuals are likely to be exposed to less variation and therefore fewer events that could elicit an acute stress response. Chronic stress has previously been demonstrated in captive animals and has been suggested to be a result of restricted access to food and also due to environmental sources of stress including lack of space and loud sounds (Hughes and Duncan 1988; Morgan and Tromborg 2007). However, increasing focus on captive animal welfare in recent years has put measures in place to prevent chronic stress, such as legislation relating to standards of management and care and enrichment regimes, namely, the *Exhibited Animals Protection Act 1986*, and the *Exhibited Animals Protection Regulation 2010* (NSW). This legislation outlines minimum requirements for various environmental conditions for captive animals with a view to providing a standardised platform for captive animal management in NSW, although these are general across taxa and therefore subjective and open to interpretation.

Previous studies have shown that there are often differences in glucocorticoid metabolites between male and female animals. Some studies suggest that sex differences are due to reproductive status (Cavigelli, 1999; Fanson et al., 2012; Weingrill et al., 2004) while other studies show that social structure can influence GC levels (Louch and Higginbotham 1967; Schuhr, 1987). Significant differences in faecal glucocorticoid metabolites (FGMs) according to sex have been documented in various felid species for basal levels (jaguars [Conforti et al 2012]; lynx [Fanson et al 2002]; tigers [Narayan et al. 2013]). In lynx, clouded leopards and tigers, females had higher mean FGM's than males (lynx [Fanson et al. 2012]; tigers [Narayan et al. 2013]; clouded leopards [Wielebnowski et al. 2002]) while in jaguars, males had higher levels of FGMs (Conforti et al. 2012). It is hypothesized that

female lions will have higher baseline FGM levels than males as this is the trend for previous studies on felids that have tested sex differences. It is important to determine if these sex differences in FGM levels are present for lions in order to accurately characterise and profile captive lion FGM levels.

This study will determine faecal glucocorticoid metabolite (FGM) levels of captive African lions using hair and faecal samples.

It is hypothesised that;

1. Hair and faeces can be used to non-invasively measure faecal glucocorticoid metabolite levels in lions;
2. Lions in the study will have similar faecal glucocorticoid metabolite profiles to other captive conspecifics; with comparable peak concentrations and variability in faecal glucocorticoid metabolite levels;
3. Faecal glucocorticoid metabolite levels in lions will be comparable across studied captive institutions;
4. Female lions will have higher baseline faecal glucocorticoid metabolite levels than male lions.

3.2 METHODS

3.2.1 Study animals

Nine adult, captive-born lions were evaluated in this study; four males and five females. The lions studied were from three Australian institutions, all of which are members of the Zoo and Aquarium Association. They housed lions under comparable conditions and are regulated by the same state legislation as outlined by the *Exhibited Animals Protection Act 1986*, and the *Exhibited Animals Protection Regulation 2010* (NSW). There were differences between facilities in terms of lion group compositions, sex ratios, and exhibit size/design. All groups of lions were non-breeding and groups were comprised of one male with either one or two females. Females were either post reproductive or on a contraceptive implant (Deslorelin, LHRH agonist). Two lions at Facility 3 were used for collection of hair for corticosterone analyses; these lions were subsequently removed from further study due to

management changes. Seven lions at Facility 1 and 2 were used for the faecal glucocorticoid metabolite (FGM) analyses. Methods were reviewed and given approval (approval number 3a/06/13) by the Animal Ethics Committee at Taronga Conservation Society Australia.

3.2.2 Hair Collection

Non-invasive collection of hair was trialled over a period of seven days using thick-bristled scrub brushes attached to enclosed passages between on- and off- display areas. Brushes were placed to collect hair as lions (n=2) moved between enclosures twice daily and hair was collected from brushes at the end of the five day collection period.

3.2.3 Faecal Collection

Faecal samples were collected daily from each lion (n=7) for a period of 10 (Facility 2) or 15 (Facility 1) days. Occasionally, there was no faecal sample available to collect, particularly after a starve day. The total number of faecal samples collected from lions ranged between n=6 and n=14. All samples were collected during the cooler months of May and June in 2015 and 2014, respectively.

As lions were housed in social groups of 2 or 3, faecal markers were used to distinguish samples from different individuals. To mark samples, 10g of coloured non-toxic glitter (Sulyn Industries, FL, USA), or half a teaspoon of Wilton gel food dye (Wilton Products Inc., IL, USA) was added to an individual's daily ration. Each lion was fed separately. Faecal samples of approximately 100g from identified individuals were collected and placed in separate zip-lock bags within 2 hours of defecation, immediately frozen and stored at -20°C until processing.

3.2.4 Faecal Processing

Frozen samples were thawed and dried overnight in a 60°C oven to eliminate variability due to water content. Dried faeces were pulverized and sieved to remove hair and bones. Cortisol metabolites were extracted from faeces by combining 0.2 +/- 0.05g of dry faecal powder with 5ml of 80% methanol, vortexing each sample for 10 seconds, and gently mixing samples on a rotator (5 RPM)

overnight. Faecal extracts were centrifuged at 635 *xg* for 15 minutes and the supernatant containing the hormone metabolites was decanted into clean glass vials.

3.2.5 Glucocorticoid assay

Faecal glucocorticoid metabolites (FGM's) were quantified using an enzyme-immunoassay (EIA) previously validated for measuring FGM's in lions (Santymire et al., 2012). Briefly, sample hormone competed with a known quantity of horseradish peroxidase-labelled corticosterone (C. Munro, UC Davis) for binding with an anti-corticosterone antibody (CJM006: C. Munro, UC Davis). FGM detection is through an observable colour change, and due to competition with known quantities of the horseradish peroxidase-labelled corticosterone the quantities in the samples can be measured by comparison against a standard curve. The cross-reactives for CJM006 were: 100% for corticosterone, 14.25% for desoxycorticosterone, 0.90% for tetrahydrocorticosterone, 0.23% for cortisol, <0.01% for cortisone, 2.65% for progesterone, 0.64% for testosterone and <0.01% for estradiol 17 β . Serial dilution (1:4) of lion faecal extracts were parallel to corticosterone standards ($r^2=0.94$) and inter- and intra-assay coefficients of variation were 8.1% and 11.9%, respectively. A parallelism was conducted prior to the commencement of this study, indicating that a 1:4 dilution was appropriate for use.

3.2.6 Statistical analysis

Baseline FGM concentration for each lion was calculated through iterations, where peak values of FGM concentrations were identified as values that fell outside 1.5 standard deviations of the mean and subsequently removed until no values exceeded the mean plus 1.5 standard deviations (Brown et al. 2004; Chosy, Wilson and Santymire, 2014). The mean of the remaining values was identified as the baseline FGM concentration for each lion.

A Shapiro-Wilk test was used to test the normality of baseline FGM concentrations. Students T-Test was used for comparisons between facilities and for comparisons between sexes. As t-tests are a

relatively robust test, the slight non-homogeneity of the samples did not significantly skew results. A two-sample f-test was used for all comparisons to determine if the variance was the same for each group being compared. Where the variance was significantly different, an unpaired, two-sample t-test for unequal variance was conducted. Where variance was not significantly different, an unpaired two-sample t-test for equal variance was conducted. Results were presented as mean concentrations \pm standard error. The following groupings were compared:

1. Facility 1 vs. facility 2 FGM mean concentrations (all faecal sample results included).
2. Facility 1 vs. facility 2 FGM baseline concentrations (peak value faecal sample results removed).
3. Male vs. female FGM mean concentrations (all faecal sample results included).
4. Male vs. female FGM concentrations (peak value faecal sample results removed).

3.3 RESULTS

3.3.1 Hair Corticosterone

Collection of hair using brushes was unsuccessful. Both lions avoided touching the brush and as a result, insufficient hair was collected for analyses.

3.3.2 Faecal Corticosterone

All lions at facility 1 exhibited peaks in faecal glucocorticoid metabolites (FGM), while the lions at facility 2 did not (Table 2). Figure 4 provides a visual representation of the FGM profiles of the lions who displayed peak values. Samples containing peak FGM concentrations occurred on different days with the exception of Lion 1 and 3 who both exhibited a peak value in FGM concentrations on the 20th of June 2014 (Figure 4). Average FGM concentrations across all lions was 128.96 ± 10.47 ng/g dry faeces; ranging from 20.6 to 516.50 ng/g dry faeces. Average baseline concentrations across all lions was 115.46 ± 8.53 ng/g dry faeces. The mean FGM value for all female lions was 97.25 ± 9.71 ng/g dry faeces and for males 177.12 ± 18.51 ng/g dry faeces.

Table 2. Faecal glucocorticoid metabolite (FGM) concentrations in lions

Lion	Sex	Facility	<i>n</i>	Mean \pm SE (ng/g)	Baseline \pm SE ng/g	No. Peaks	Highest Peak Value (ng/g)
1	Male	1	13	178.21 \pm 33.58	132.72 \pm 10.60	2	516.50
2	Female	1	12	74.34 \pm 13.25	56.71 \pm 6.50	2	181.41
3	Female	1	14	73.70 \pm 17.09	50.24 \pm 7.25	2	216.66
4	Female	2	7	123.21 \pm 20.36	123.21 \pm 20.36	0	NA
5	Male	2	6	148.47 \pm 15.89	148.47 \pm 15.89	0	NA
6	Female	2	8	150.10 \pm 18.81	150.10 \pm 18.81	0	NA
7	Male	2	8	196.84 \pm 29.59	196.84 \pm 29.59	0	NA

Number of faecal samples = *n*. Mean values are average FGM concentrations prior to the removal of peak values (Mean \pm SE). Peak values were values greater than 1.5 standard deviations of the mean. Baseline values are mean FGM concentrations with peak values removed (Baseline \pm SE)

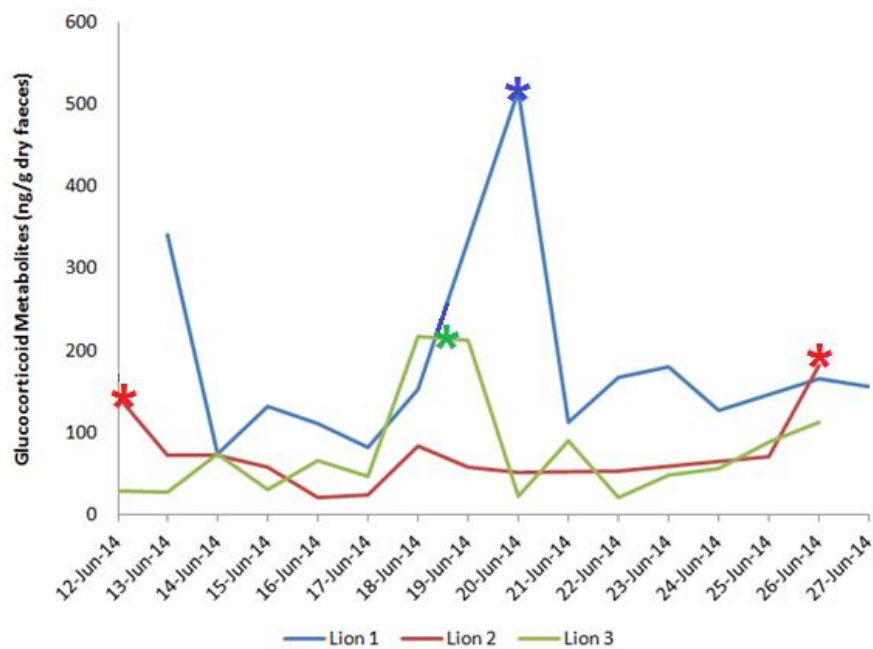


Figure 4. Faecal glucocorticoid metabolite (FGM) (ng/g dry faeces) profiles for individual lions 1, 2 and 3. Lion 1 is a male, lions 2 and 3 are female. * Denotes peak values of FGM levels.

3.3.3 Faecal glucocorticoid metabolite (FGM) concentrations between facilities

The group mean FGM concentration of the lions were significantly different between facility 1 (156.17 ± 11.92 ng/g dry faeces; $p= 0.017$) and facility 2 (108.73 ± 15.28 ng/g dry faeces; Figure 5), as was the group mean baseline FGM concentrations between facility 1 (79.69 ± 8.13 ng/g dry faeces) and facility 2 (156.17 ± 11.92 ng/g dry faeces; $p < 0.001$).

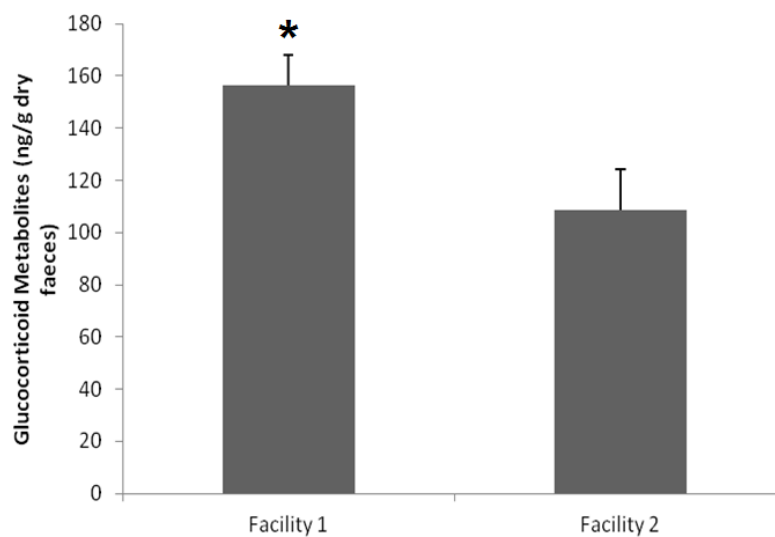


Figure 5. Group mean faecal glucocorticoid metabolite (FGM) concentrations (ng/g dry faeces) from lions (n=7) at two facilities. * The difference in FGM concentrations between facilities was statistically significant ($p=0.017$).

3.3.4. Faecal glucocorticoid metabolite (FGM) concentrations between male and female lions

The mean FGM concentrations of male lions (177.12 ± 18.5 ng/g dry faeces) compared with female lions (97.25 ± 9.71 ng/g dry faeces) were significantly different ($p=0.004$; Figure 6), as was the mean baseline FGM concentrations of male lions (157.01 ± 12.15 ng/g dry faeces) compared with female lions (87.39 ± 9.27 ng/g dry faeces; $p=0.0003$).

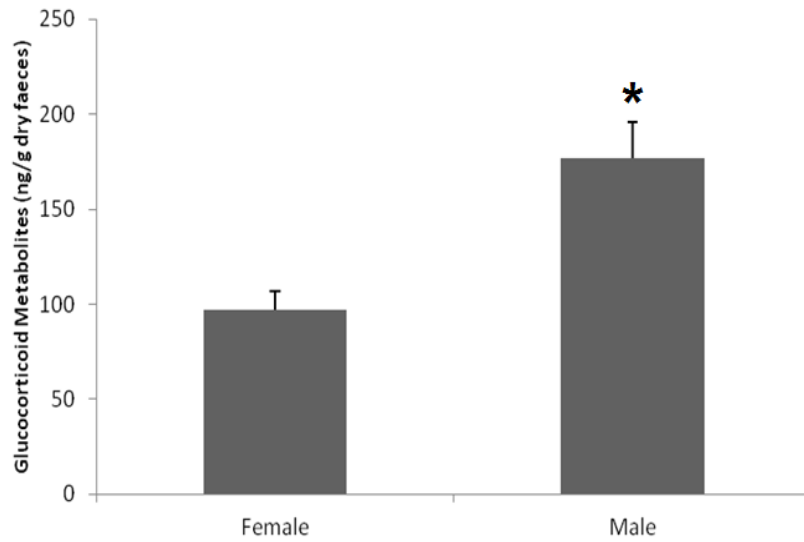


Figure 6. Mean faecal glucocorticoid metabolite (FGM) concentrations (ng/g dry faeces) of male lions (n=3) compared with female lions (n=4). Samples taken in an 11 day period. *The difference between male and female FGM mean concentrations was statistically significant ($p=0.004$).

3.4 DISCUSSION

3.4.1 Hair samples for corticosterone measurement

This study is the first known attempt to trial the non-invasive collection and use of hair samples for corticosterone concentration measurement in lions. Analysis of hair samples has potential to provide long-term information on an animal's glucocorticoid production. This gives data indicative of hypothalamic-pituitary-adrenocortical system activity over the period of hair growth (Bechshøft et al 2012) providing information of the effect of chronic stressors. Hair collection was trialled using non-invasive methods in order to prevent distress to lions and to avoid having to use chemical restraint. Ideally, collection of hair samples should be a non-invasive procedure to prevent eliciting a stress response which could confound data and also for ethical reasons. Collecting sufficient amounts of hair can present logistical difficulties; animals need to be housed individually to determine hair identity and approximately two grams is required. Non-invasive hair collection was trialled by attaching brushes to passageway entrances at a low level to brush against lions as they passed through, however the lions ducked under the brushes, thus insufficient amounts of hair were collected for analysis. With greater resources and time, animals could be trained to allow shaving. Hair should be clipped prior to the start of an observation period, with the regrown hair clipped again at the end to obtain a real time record of glucocorticoid levels for accurate determination of the relevant time period (Sheriff et al 2011). In our study, hair could not safely be clipped without performing an anaesthetic procedure on lions, which was not ethically justifiable for only hair collection. In a zoo environment, a routine anaesthetic procedure for health check purposes could easily obtain sufficient amounts of hair for analyses to provide information on mean corticosterone levels, therefore requiring no extra resources for sample collection. Hair collection during every anaesthetic procedure throughout a lion's life can provide an indication of the effect of stressors over a time period of years, and potentially be linked to any substantial changes in an animal's environment, social status, reproductive status or health. Future studies should focus on validating the use of hair for corticosterone analysis in felids by attempting different methods of hair collection, such as training animals to allow shaving. Using hair is advantageous as it provides information on chronic stress over long periods of time.

3.4.2 Faecal samples for corticosterone measurement

Our study found that in a captive environment faecal samples were an ethical, effective and practical sample type to measure corticosterone non-invasively. Samples could be easily collected in line with normal working procedures, were easily identified using faecal markers such as non-toxic dye or glitter and required no unusual or stressful interaction between lions and people to collect.

Faecal samples provide information of a time period reflecting intestinal passage time as glucocorticoids are incorporated into faeces in the gastro-intestinal tract, although this is variable according to species (Möstl and Palme 2002). While there is currently no literature on total transit time (time for food to pass from mouth to anus) or on gastric emptying time (time for food/waste to move through the intestine) for lions an ACTH injection challenge on a female lion showed that faecal GC's peaked approximately 24 hours post injection (Santymire et al. 2012). Therefore any measures of FGMs generally represent an average of the previous 24 hours. Diet likely also influences transit time as digestion has been shown to differ in large felids in terms of faecal output and composition according to type of meat fed (Vester et al. 2010) and different types of food affect gastric emptying time in other species, such as dogs (Lewis et al. 1994). In this study, the lions were fed similar food types (beef and chicken), but did have some variation in feeding schedules and amounts. Following a starve day the lions sometimes did not defecate for up to 48 hours after the previous defecation. In a captive environment management can be altered to feed every day and give a greater chance of daily defecation throughout the study period. Further investigation in to the impact of diet may have on FGM measurement is needed. Faecal samples alone cannot identify specific causes of stress events although tracking changes in management and observing behaviour could provide a reasonable idea of what caused any stress responses seen in faecal samples. Faecal samples could perhaps be more readily used to identify chronic stress and can potentially be used to identify a period of acute stress, but not to conclusively determine causation of acute stress.

3.4.3 Lion Faecal Glucocorticoid Metabolite Profiling

The current results show significant differences in combined mean faecal glucocorticoid metabolite (FGM) levels between lions from different institutions. The data collected represents an important addition to the information about captive lion faecal corticosterone, as few published studies have examined stress hormone metabolites in lions. Only one previous study examines wild lion blood serum cortisol and only two examine FGM's in female captive lions (Brown et al. 1993; Putman et al. 2015; Santymire et al. 2012). Putman et al. (2015) studied only female lions and measured FGM levels in relation to reproductive parameters and contraception, finding that the mean baseline of adult female lion FGM levels was 130 ± 20 ng/g dry faeces. Santymire et al. (2012) validated the use of corticosterone EIA through an ACTH challenge and found that the pre-ACTH mean FGM concentration was 407.9 ± 103.7 ng/g dry faeces (Santymire, 2012; Santymire pers comms.). However only one individual with a total of five faecal samples was included, of which only two were collected prior to the ACTH injection. The technique used by both Putman et al. (2015) and Santymire et al. (2012) to process samples varied slightly from the methods performed here, as samples were dried in a lyophilizer, 90% ethanol was used for the extraction and boiled to agitate samples. Putman et al. (2015) also reconstituted extracts with 1 ml of 100% methanol, dried under forced air and reconstituted with 1ml of phosphate buffer. Our simplified method used a conventional oven for drying, 80% methanol and agitated through vortexing and rotating. Methanol has been shown to extract significantly more glucocorticoids than ethanol and 80% methanol diluted with water has been shown to extract significantly more glucocorticoids than when methanol is diluted with acetone when tested using Gelada faeces (Pappano, Roberts, and Beehner, 2010). The results from the present study demonstrate that the mean basal level of FGM's in lions is 128.96 ± 10.47 ng/g dry faeces based on a total of 68 samples from seven individuals. Daily variation in FGM levels was seen for all studied lions with peaks present in three out of seven lions. The differences in faecal processing between this study and previous investigations by Putman et al. (2015) and Santymire et al. (2012) may have impacted results and further testing is needed to determine whether the processing method significantly affects results.

Positive events can also elicit a rise in GCs and a healthy negative feedback system allows levels to return to baseline (Endroczi, Schreiber and Lissak 1963; Toates, 1995). Therefore, variability in day-to-day corticosterone levels is indicative of healthy functioning of the HPA axis and the ability to respond normally to environmental variation and stressors. During the current study, events that were likely perceived as positive were observed (e.g. playing and eating), as well as some minor aggression (e.g. teeth bearing and swiping lasting only a few seconds) which may have provided negative stimuli. Day-to-day variability in corticosterone levels was seen, with peak values declining to non-peak values within 24 hours. This suggests that the studied animals have good welfare from a physiological perspective. To determine the accuracy of our findings for mean FGM concentrations, repetition with more lions in a range of captive institutions, as well as in the wild is required. To date, only Brown et al. (1993) has examined stress in wild lions using serum cortisol, so comparison between wild and captive lions requires either serum cortisol to be tested in a captive environment, or FGMs to be tested in the wild. In other species, comparative studies of FGMs in wild versus captive animals have shown that captive animals have higher mean FGM levels than wild counterparts (starlings [Cyr and Romero 2008]; lynx [Fanson et al. 2012]; spider monkeys [Rangel-Negrin; 2009]; cheetah [Wells et al 2004]). This study has demonstrated significant differences in average FGMs between facilities. A study examining serum cortisol concentrations in wild lions from two different locations showed similar baseline concentrations (Brown et al. 1993). Further testing is needed to determine if differences between facilities in FGM concentrations is indicative of specific factors in a captive environment.

There were significant differences between facilities in mean and baseline faecal glucocorticoid metabolite (FGM) concentrations, as well as significant differences in mean and baseline FGMs according to sex. With further testing on a greater sample size these results could be further validated. Conforti et al. (2012) measured FGM's in jaguars from eight different zoos and compared wild-born to captive-born jaguars, finding significant differences between jaguars with different birth origins. Captive born male jaguars had higher FGM levels than wild males, but captive females had lower FGM levels. A study examining cheetah FGM levels pre- and post-movement showed that movement between facilities resulted in a prolonged stress response and by comparison between facility types, determined that environment type influenced the length and magnitude of this response

(Wells et al. 2004). In the current study, there were significant differences in mean baseline FGM's between lions from different facilities. While neither facility had lions that exhibited overt/physical signs of chronic stress, these results suggest an effect of facility type on corticosterone levels. Without further determining the effect of specific facility aspects on corticosterone levels, it is difficult to determine what the exact causation is. Future studies should collect information on a range of environmental aspects in addition to corticosterone to determine more specifically what aspects of an animal's environment has the greatest effect on corticosterone levels.

Previous studies in felids have shown significant differences in FGM levels between males and females; however, the trend is not consistent among species. In various carnivores, females had higher mean FGMs than males (lynx [Fanson et al. 2012]; clouded leopards [Narayan et al. 2013]; tigers [Wielebnowski et al. 2002]) whilst in jaguars, males had higher levels of FGMs (Conforti et al. 2012). The male lions in the current study had higher mean FGM levels than female lions. As the differences are not consistent in terms of which sex generally secretes greater amounts of FGMs, it is not clear as to what the specific cause is for each different species. Some studies have suggested that reproductive status influences FGM levels. Pregnant females have significantly higher FGM's than non-pregnant females in some species including lynx, baboons, and ring-tailed lemurs (ring-tailed lemurs [Cavigelli 1999]; lynx [Fanson et al. 2012]; baboons [Weingrill et al. 2004,]). Fanson et al. (2012) demonstrated that spayed female lynx have FGM concentrations similar to males. Here, female lions had significantly lower FGM concentrations than the males. The females were all in regular contact with males, but were post-reproductive or had a contraceptive implant (Deslorelin, LHRH agonist) and so were non-breeding females. It is possible that social structure can play a role in FGM levels, as studies on mice have shown that dominant animals had significantly lower plasma corticosterone levels than subdominant animals (Louch and Higgenbotham 1967, Schuhr 1987). As lions live in social groups, there may be an effect of social structure on FGM levels on individual lions. In our study it was difficult to determine which lions were dominant over others, since no dominance behaviours were observed. The route of excretion of glucocorticoid metabolites has also been demonstrated to differ between males and females in mice, with males excreting higher amounts of radiolabelled metabolite in urine than females (Touma et al. 2003). Excretion time has not yet been tested for lions, and further investigation should determine if faecal excretion is significantly different

between male and female lions. Goymann (2012) suggests that validations of hormone metabolite measurement should be performed separately for each sex and these results support this statement. Further studies focusing on differences in hormone metabolism in felids is needed to determine the extent of these differences and how to integrate the differences between male and female animals in future hormone studies.

This study has been the first to profile faecal glucocorticoid metabolite (FGM) concentrations of multiple lions, of both sexes, across different institutions. As hypothesized faeces proved to be a good non-invasive measurement of FGM levels in lions, however the use of hair requires further investigation. We hypothesized there would be similarities in FGM profiles between the lions we studied and lions studied in previous literature. We found significant differences in baseline FGM levels in our lions compared to other captive lions from previous studies, as well as their peak values; this is likely a result of differences in faecal processing techniques between laboratories.

Unexpectedly, there was also a significant difference in mean FGM levels of the lions between facilities and further investigation would determine what specific institutional factors influence this which disproved our hypothesis. Contrary to our prediction, all male lions had significantly higher baseline FGM levels. Future studies should focus on the mechanism behind this particularly as this sex difference is inconsistent between species. The results give an indication of the variability and mean corticosterone levels of captive lions in modern facilities where welfare is a priority, showing clear differences between sexes and outlining the effect of environment. These results will provide a platform for comparison for future studies and welfare assessments that examine FGM levels in lions.

CHAPTER 4

Combining multiple Methods of Welfare Assessment for Captive African Lions

4.1 INTRODUCTION

Welfare evaluation of captive animals that utilises “output” variables often only use a single type of metric to determine whether an animal has good or bad welfare (Bashaw et al. 2003; Mason and Rushen, 2005; Pritchard et al., 2005; Wielebnowski et al., 2002). Welfare from a physical aspect is relatively easy to determine as it uses presence/absence of disease or injury and non-subjective body scoring to determine condition. However, evaluating mental aspects of animal welfare can be difficult to quantify as it often requires subjective evaluations of behaviour, drawing conclusions based on displayed behaviours.

Certain behaviours such as stereotypies or aggression are often considered indicative of negative welfare (Broom, 1991; Pritchard et al., 2005), however the presence of these behaviours does not conclusively provide evidence of poor welfare (Mason and Latham, 2004), nor does the absence of these behaviours prove that an animal’s welfare is good (Melfi, 2009). As such, validation is required when using behaviour to determine welfare and a secondary measure of welfare using a different metric can provide insight as to the validity and accuracy of behaviour-based welfare assessment. Using multiple metrics for welfare assessment is ideal; however, it does require additional resources and funding which may be limited in captive facilities in particular.

It is possible that certain behaviours are consistently linked with poor welfare. Previous studies have demonstrated significant correlations between certain behaviours and stress levels; in cats, hiding behaviour was significantly correlated with increased cortisol levels (Carlstead, Brown and Strawn, 1993). Clouded leopards have shown significant correlations between FGM levels and pacing and self-injuring, as well as significant correlations between activity and FGM levels (Wielebnowski et al. 2002). However, as particular behaviours may be species-specific, separate pilot studies should be

performed for each species to determine whether correlations exist between specific behaviours and a secondary measure of welfare (e.g. GC levels as a measure of stress). If correlations exist between GC levels and specific behaviours for lions, the prevalence of that behaviour could be reliably used in future welfare assessments.

This study aims to determine if there are any links between FGM levels and various aspects of behaviour including activity, aggression and presence of stereotypies in lions. The presence of significant links between any behaviours and stress levels can be used to develop a rigorous framework using behaviour as an assessment tool that can be applied to all captive facilities for lions. To this end, we hypothesize that;

1. There will be a significant positive correlation between amount of aggression observed and FGM levels;
2. There will be a significant negative correlation between daily levels of all activity and FGM levels.

4.2 METHODS

Behavioural data (chapter two) were compared with endocrine data (chapter three). Behavioural observations were paired with endocrine data from the corresponding faecal sample of each lion. Faecal samples were matched with behavioural data collected the previous day, as faecal samples reflect a pooled average faecal glucocorticoid metabolite levels for the previous 12-24 hours in felids (Brown et al. 1994).

To determine if there was a significant relationship between aggression and faecal glucocorticoid metabolite (FGM) levels for lions, total counts of observed aggression displayed from two 30 minute continuous sampling periods each day (a.m. and p.m.) were compared with corresponding faecal samples. This was tested using spearman's rank correlation test. FGM levels were also compared with activity levels for each individual lion from the previous day using spearman's rank correlation test. Activity levels were measured through daily time budgets generated from half hour scan samples as outlined in chapter two, and were presented as proportion of time spent active.

4.3 RESULTS

There was no significant relationship between FGM levels and aggressive displays ($\rho = -0.0348$, $p = 0.80$; d.f.= 6 Figure 7). The relationship between activity levels and FGM's was also not significant ($\rho = 0.153$, $p = 0.27$, d.f.=6; Figure 8). There was insufficient individual behavioural variation and counts of aggression to determine a whether there was a significant relationship between individual FGM levels and aggression or activity.

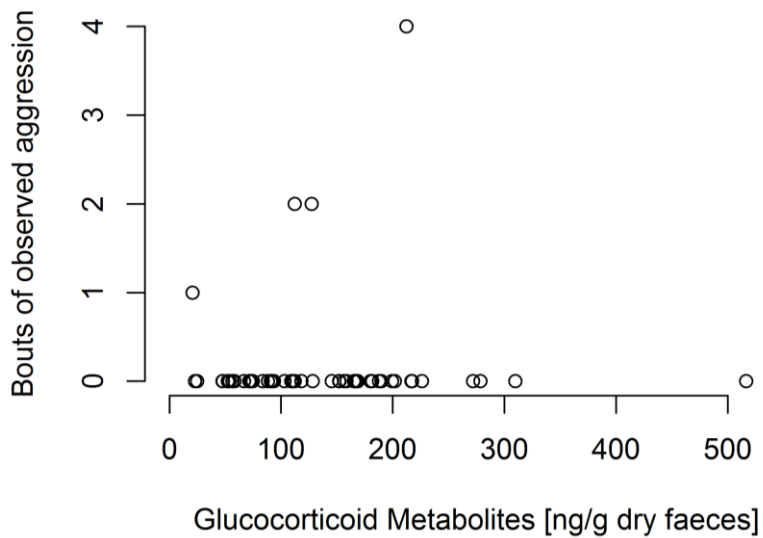


Figure 7. Daily total counts of aggression compared to faecal glucocorticoid metabolite concentrations from faeces from n=7 African lions over a period of 10 days, using two 30 minute continuous sampling periods (a.m. and p.m.) per day.

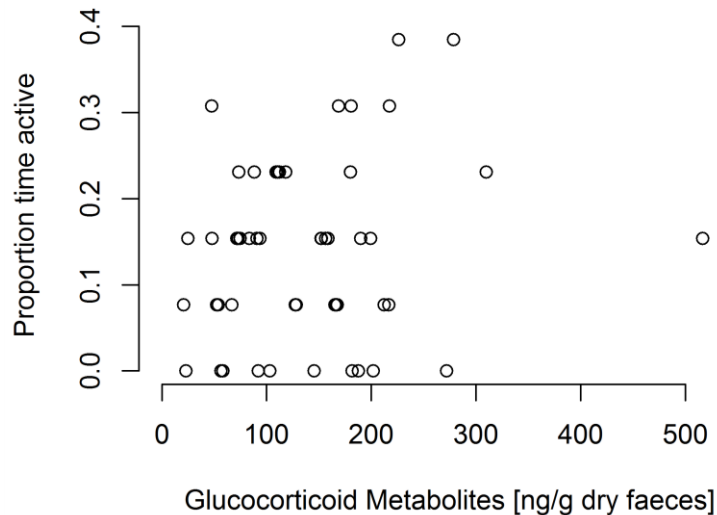


Figure 8. Daily proportion of time spent active compared to daily glucocorticoid metabolite concentrations from faeces from n=7 African lions over a period of 10 days, using instantaneous scan samples every half hour for six hours per day.

4.4 DISCUSSION

The use of multiple indices has the potential to strengthen and validate results of welfare assessment in animals. This study demonstrated a lack of significant relationship between corticosterone and aggression, or corticosterone and activity. This shows that in this instance, making predictions from either behaviour or corticosterone alone to determine the welfare of the lions studied may have resulted in varying outcomes.

Contrary to the non-significant results in this study, previous studies have identified significant relationships between certain behaviours and corticosterone or cortisol levels for a range of species (meerkats [Carlson et al. 2001]; macaroni penguins [Crossin et al. 2012]; eastern bluebirds [Davis and Guinan, 2014]; mules and horses [Pritchard, 2005]; african striped mice [Raynaud, Schradin and Fusani, 2015]). However, Kuhar, Bettinger and Luadenslager (2005) reported no significant relationship between behaviour and cortisol levels in gorillas and these results, in combination with our own, show that links between behaviour and hormones can be, but aren't always, present. It is possible that the relationship between behaviour and hormones may only be observable in circumstances where a major stress response is elicited in individuals. Future studies of captive

animal welfare would be strengthened by determining the presence of these links prior to making an assessment.

It is quite possible that these links do exist for lions; however, as this study examined lions without influencing or changing their day-to-day management there may simply have been an absence of sufficient stressors to be captured in this study. Previous studies that have identified these relationships have manipulated the study to introduce a certain stressor to elicit a response. For example, Blanchard et al. (1998) exposed rats to a predator-based stressor (cats) and subsequently found correlations between corticosterone and crouching, grooming and rearing. Carlstead, Brown and Strawn (1993) altered daily management of captive cats to induce stress and also found significant results between cortisol and hiding behaviour. Wielebnowski et al. (2002) did not manipulate the environment to induce stress, but behavioural data was collected through questionnaires completed by zookeepers and showed quite a high proportion of self-injuring and stereotypic behaviour. This study specifically attempted to determine the presence of a relationship without introducing a stressor. Behavioural results from chapter two demonstrated only a very small proportion of pacing behaviour (0.03% of total daily behaviour) and only a total of nine counts of aggression from all lions across the entire ten day period. Only three lions demonstrated peaks in FGM concentrations and each only demonstrated two peaks across the entire observation period. Had variables been manipulated to induce stress, the presence of a significant relationship may have been detected that was otherwise masked in the dataset by a lack of variation, or indeed a lack of stress. Additionally, limitations on the observation period prevented behaviour data collection after zoo closing hours due to safety regulations and husbandry practices. Wild lions are active largely at night and rest throughout the day (Cozzi et al., 2012; Schaller, 1972); had behavioural observations been collected throughout this nocturnal period it may have impacted results. Attempts were made to capture behaviour overnight through installing camera traps. Unfortunately, these cameras were discovered and destroyed by lions prior to being able to retrieve footage. As the faecal samples represent a pooled average of FGM's from the previous 12 – 24 hours (Möstl and Palme, 2002) they therefore provided information from the evening outside of behavioural observation hours, and possible behavioural indicators of stress that were displayed at this time (including perhaps

aggression and activity) were not recorded in diurnal observations. Given there were few peaks in FGM levels from only three of seven lions, had we been able to capture behaviour over the entire 24 hour period it is unlikely to have impacted correlation results between FGM's and activity or aggression. The current behavioural observation component was limited to zoo opening hours, but it is also worth noting that future assessments of zoo animal welfare and behavioural observation will likely be tested during opening hours due to similar limitations. Therefore, our results are still reflective of future welfare assessments and valuable for future reference. However, use of cameras that are able to record overnight that are confirmed to be inaccessible to lions would certainly strengthen results. When conducting similar assessments on other species, the time of observation should be based on the time of activity that wild counterparts display in order to have greater success at characterising their behaviour.

Validating various measurements of welfare is integral to providing robust results and an accurate tool for welfare monitoring. ACTH challenges are used to validate measurement of corticosterone in faeces (Brown et al. 1991; Santymire et al., 2012). Introducing animals to a stressor and then measuring behaviour can determine what behaviours are associated with stress, and therefore validate future measurement of welfare through behavioural observations. Neither the presence nor absence of stereotypic behaviours is sufficient to determine welfare (Mason and Latham, 2004; Melfi, 2009). This study disproved the hypothesis that there would be a significant positive correlation between aggression and FGM levels, as well as the hypothesis that there would be a significant negative correlation between activity and FGM levels. Future welfare studies should continue to combine multiple indices of welfare, certainly until links between specific behaviours and secondary measures of welfare are consistently demonstrated. Validation studies such as these provide the foundations of a non-subjective, fact-based welfare assessment tool for future use. They must be carefully designed and ensure that the entire period is reflected in their corticosterone, particularly during the validation phase. Future studies should aim to collect behaviour data over a full 24 hour period, and investigate digestion times. This would allow for more specific correlations to be made between behaviour and corresponding FGM levels.

CHAPTER 5

General Discussion

This study has thoroughly characterised, described and compared captive lion behaviour and stress levels in a welfare context, using a combination of different assessment tools. Currently, most welfare assessment and legislation relating to captive animals relies very heavily on measuring the “input” variables of a captive animal’s environment. This is of course important, as the needs of animals must be met in order to provide an environment conducive to positive welfare, but it does not provide information about the experience of an animal (Yeates and Main, 2008). Here, multiple methods of welfare assessment were used, focussing only on these “output” measures in order to capture this critical component of animal welfare assessment.

Overall, the results of this study indicate that the lions likely had positive welfare. Through observations, this study identified 28 distinct behaviours of captive adult lions housed in mixed sex groups in modern captive facilities; previous studies in modern facilities have only broadly described four different categories of behaviours (Bashaw et al. 2007). Identifying the full repertoire of captive lion behaviour is essential for welfare studies as behavioural diversity is often used as a measure of welfare (Hirt and Wechsler, 1994; Rabin, 2003). Behaviourally, the lions in this study displayed very low proportions of stereotypic activity and aggression, both of which are commonly used as indicators of poor welfare. Future research should build on these results by studying behavioural repertoire in different groupings of lions with variability in group size, age, sex ratio and reproductive status. This was an intended avenue of research in this study with many facilities approached to request participation and the sample size was determined by participation of these facilities. A fourth bachelor group of three lions at another facility were initially included in the study; however, inconsistent sample collection meant the data could not be used. Additionally, the current study was limited as the lions were all non-breeding adults. Studying lions in a breeding situation, as well as inclusion of cubs, would provide the opportunity to capture breeding behaviour, parenting behaviour and the presumed changes in social repertoire associated with this group makeup.

This study is the first to compare captive lion behaviour to wild conspecifics to explore the concept of natural living; an important factor of welfare (Veasey, Waran and Young 1996). Environments that encourage expression of natural behaviours are likely to enhance welfare (Veasey, Waran and Young, 1996) and there were similarities in the activity budget of the captive lions studied and wild lions studied by Hayward and Hayward (2007). This provides good evidence of positive welfare in the lions studied according to the concept of natural living as a welfare indicator.

From a physiological standpoint, the corticosterone levels were also indicative of good welfare. As previously mentioned, stressors can also be indicative of factors that are not considered negative (Möstl and Palme, 2002) and the ability to respond effectively to stressors is essential for the maintenance of homeostasis (Sterling and Eyer, 1988). Stress only threatens the welfare of an animal when it cannot be managed and where it is sustained over a long period, thereby exerting a deleterious effect on the individual's biological state (Moberg and Mench, 200). All of the studied lions exhibited variable corticosterone levels with only three lions displaying no more than two peaks each, none of which were sustained for more than a day. The short duration of peak corticosterone levels indicated success in responding appropriately to a stressor, which would be expected from an animal with positive welfare.

Other studies have demonstrated that there are significant differences in faecal glucocorticoid metabolite (FGM) levels between captive animals and wild animals of the same species, with captive animals having higher cortisol levels than wild conspecifics (lynx [Fanson et al. 2012]; spider monkeys [Rangel-Negrin; 2009]; cheetah [Wells et al 2004];). Interestingly, captive spider monkeys only had significantly higher cortisol levels than wild spider monkeys living in protected forest; wild spider monkeys in fragmented forest had similar levels to captive conspecifics (Rangel-Negrin; 2009). It would be interesting to evaluate this for lions to determine if captivity or environment encroachment has a significant impact on baseline FGM levels. This may provide important information about stress and welfare of animals in captivity which could then be used to determine the causation of any possible differences, which may inform management.

Interestingly, this study identified significant differences in mean and baseline FGMs according to sex for captive African lions. This difference between sexes has also been demonstrated for a number of

other felids. In clouded leopards, tigers and lynx, females had higher mean FGM's than males, however male jaguars had higher levels of FGM's than females (Cavigelli 1999; Fanson et al. 2012; Weingrill et al. 2004). It is possible that these differences are due to reproductive status based on findings from similar studies. Pregnancy has been shown to result in significant increase in FGM's when compared to non-pregnant females for a range of species (ring-tailed lemurs [Cavigelli 1999]; lynx [Fanson et al. 2012]; baboons [Weingrill et al. 2004,]); spayed female lynx were shown to have similar FGM levels to male lynx (Fanson et al. 2012). The female lions in this study were either contracepted or post-reproductive and interestingly they had significantly lower FGM levels than the males. Reproductive status may have a large influence over FGM levels for female lions, and those that are reproductively active may have higher mean FGM levels. Should this be the case they will need to be considered separately from reproductively inactive females, as well as males. Further research investigating FGM levels for female lions with differences in reproductive status is needed to determine if these differences are due to reproductive status, or another variable such as social structure. Social structure has been shown to influence the FGM levels of mice, with dominant mice having lower plasma corticosterone levels than subordinate mice (Louch and Higgenbotham 1967; Schuhr 1987). As lions have a complex social system that is unique amongst felids, this may play an important part in HPA activity, and therefore FGM levels. This should be further investigated and the differences between males and females should be integrated into future hormone, and possibly also welfare studies.

By using a combined approach we have attempted to provide a more complete representation of captive lion welfare. As each different approach has displayed results that indicate positive welfare, they have validated one another to provide a stronger assessment. This is particularly important as the results from chapter four have demonstrated that there are not always significant relationships between behaviour and stress levels, even when certain behaviours such as stereotypies are traditionally considered negative (Broom, 1991). This further supports previous literature stating that use of single measures to assess welfare do not necessarily provide conclusive evidence of an animal's welfare state (Clubb and Mason, 2003; Mason and Latham, 2004; Melfi, 2009).

While this study found no relationship between behaviour and stress levels, previous studies have identified these links (Carlson et al. 2001; Crossin et al. 2012; Davis and Guinan, 2014; Raynaud, Schradin and Fusani, 2015). However, only a handful of studies have explored this relationship in a welfare context. In rats, crouching, grooming and rearing was correlated with corticosterone (Blanchard et al., 1998). In felids, hiding behaviour and cortisol levels are correlated in domestic cats (Carlstead, Brown and Strawn, 1993) and clouded leopards that display self-injuring behaviour and pacing show correlations between these behaviours and elevated corticosterone levels (Wielebnowski et al., 2002). Further study into the potential presence of these relationships is certainly needed to determine if they exist, and under which circumstances these links are present and significant. Future investigation should introduce a stressor to an experimental group and compare results with that of a control group. However, real-life welfare assessments require minimal manipulation and changes of the day-to-day life of animals to make an accurate assessment.

To conclude, this study demonstrated strong correlations between wild and captive lion activity, with little stereotypic behaviour displayed in the captive lions studied. The captive lions tended to be more active in the morning and displayed a range of behaviours in total; however there was not much variation in observed day to day behaviour. Faeces was shown to be an accurate non-invasive method of measuring corticosterone levels in lions, however more testing is needed to investigate the potential use of hair. This study displayed significant differences in faecal glucocorticoid metabolites (FGM) levels between facilities and also between males and females. There was no significant relationship displayed between FGM levels and aggression or activity, however throughout this study the lions were not specifically exposed to a stressor. The results of this study provide a framework for the development of a non-invasive, multi-faceted, objective assessment tool that utilises “output” measures of captive lion welfare. As the lions studied here appear to have good welfare, the characterisation of their behaviour and their corticosterone levels can be used in future assessments of captive lion welfare at other facilities. Our results have provided a benchmark for captive lion welfare studies that utilise behaviour and stress.

Reference List

- Accorsi, P.A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., Seren, E. (2008). Cortisol determination in hair and faeces from domestic cats and dogs. *General and Comparative Endocrinology*. **155**: 398-402.
- Altmann, J. (1974). Observational study of behaviour: sampling methods. *Behaviour*. **49**: 227-267.
- Animal Welfare Act 2006 (UK). Retrieved from:
http://www.legislation.gov.uk/ukpga/2006/45/pdfs/ukpga_20060045_en.pdf.
- Ashley, N. T., Barboza, P. S., Macbeth, B. J., Janz, D. M., Cattet, M. R. L., Booth, R. K., & Wasser, S. K. (2011). Glucocorticosteroid concentrations in feces and hair of captive caribou and reindeer following adrenocorticotrophic hormone challenge. *General and comparative endocrinology*. **172**: 382-391.
- Australian Bureau of Meteorology (BOM). (2014). June 2014 Daily Weather Observations, Sydney. <http://www.bom.gov.au/climate/dwo/201406/html/IDCJDW2124.201506.shtml> accessed 5 November 2015
- Australian Bureau of Meteorology (BOM). (2015). May 2015 Daily Weather Observations, Batemans Bay. <http://www.bom.gov.au/climate/dwo/201505/html/IDCJDW2011.201505.shtml> accessed 5 November 2015
- Bashaw, M.J., Bloomsmith, M.A., Marr, M.J., Maple, T.L. (2003). To hunt or not to hunt? A feeding enrichment experiment with captive large felids. *Zoo Biology*. **22**: 189-198.
- Bashaw, M.J., Kelling, A.S., Bloomsmith, M.A., Maple, T.L. (2007). Behaviour of Zoo-house lions and tigers, with a case study of the effect of a visual barrier on pacing. *Journal of Applied Animal Welfare Science*. **10**: 95-109.
- Basset, L, Buchanan-Smith, H.M. (2007). Effect of predictability on the welfare of captive animals. *Applied Animals Behaviour Science*. **102**: 223-245.
- Bauer, H., De longh, H.H., Di Silvestre, I. (2003). Lion (*Panthera leo*) social behaviour in the West and Central African savannah belt. *Mammalian Biology*. **68**: 239-243.
- Bechshøft, T.Ø., Sonne, C., Dietz, R., Born, E.W., Novak, M.A., Henchey, E., Meyer, J.S. (2011). Cortisol levels in hair of East Greenland polar bears. *Science of the Total Environment*. **409**: 831-834.

- Benhamou, S., Valeix, M., Chamaille-Jammes, S., Macdonald, D.W., Loveridge, A.J. (2014). Movement-based analysis of interactions in African Lions. *Animal Behaviour*. **90**: 171-180.
- Birkett, L.P., Newton-Fisher, N.E. (2011). How abnormal is the behaviour of captive, zoo-living chimpanzees? *PLOS One*. **6**: 20101.
- Blanchard, R.J., Nikulin, J.N., Sakai, R.R., McKittrick, C., McEwen, B., Blanchard, D.C. (1998). Behavioural and endocrine change following chronic predatory stress. *Physiology and Behaviour*. **63**: 561-569.
- Bortolotti, G. R., Marchant, T.A., Blas, J., German, T. (2008). Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Functional Ecology*. **22**: 494-420.
- Broom, D.M. (1988). Needs, freedoms and the assessment of welfare. *Applied Animal Behaviour Science*. **19**: 384-386.
- Broom, D.M. (1991). Animal welfare: concepts and measurement. *Journal of Animal Science*. **69**: 4167.
- Brown, J.L., Bush, M., Packer, C., Pusey, A.E., Monfort, S.L., O'Brien, S.J., Janssen, D.L., Wildt, D.E. (1993). Hormonal characteristics of free-ranging female lions (*Panthera leo*) of the Serengeti Plains and Ngorongoro Crater. *Journal of Reproduction and Fertility*. **97**: 107-114.
- Brown, J.L., Wasser, S.K., Wildt, D.E., Graham, L.H. (1994). Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biology of reproduction*. **51**: 776.
- Brown, T. (2014). 'Zoo proliferation'—The first British zoos from 1831-1840. *Der Zoologische Garten*
- Carbajal A., Tallo-Parra O., Sabes-Alsina M., Mular I., Lopez-Bejar M. (2014). Feather corticosterone evaluated by ELISA in broilers: A potential tool to evaluate broiler welfare. *Poultry Science*. **93**: 2884-2886.
- Carlson, A. A., Manser, M. B., Young, A. J., Russell, A. F., Jordan, N. R., McNeilly, A. S., Clutton-Brock, T. (2006). Cortisol levels are positively associated with pup-feeding rates in male meerkats. *Proceedings of the Royal Society B: Biological Sciences*. **273**: 571-577.
- Carlstead, K., Brown, J.L., Monfort, S.L., Killens, R., Wildt, D.E. (1992). Urinary monitoring of adrenal responses to psychological stressors in domestic and nondomestic felids. *Zoo Biology*. **11**: 165-176.
- Carlstead, K., Brown, J.L., Strawn, W. (1993) Behavioural and physiological correlates of stress in laboratory cats. *Applied Animal Behaviour Science*. **38**: 143-158.

- Carlstead, K., Brown, J.L. (2005). Relationships between patterns of fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. *Zoo Biology*. **24**: 215-232.
- Cavigelli, S.A. (1999). Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. *Animal Behaviour*. **57**: 935-944.
- Chamove, A.S., Anderson, J.R. (1989). *Examining environmental enrichment*. In: Segal, E.F. (Ed.) *Housing, Care and Psychological Well-Being of Captive and Laboratory Primates*. New Jersey: Noyes Publications.
- Chandler, M. L., Guilford, G., Lawoko, C. R. (1997). Radiopaque markers to evaluate gastric emptying and small intestinal transit time in healthy cats. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine*. **11**: 361-364.
- Chosy, J., Wilson, M., Santymire, R. (2014). Behavioural and physiological responses in felids to exhibit construction. *Zoo Biology*. **33**: 267–274.
- Chrousos GP., Gold PW (1992). The concepts of stress and stress system disorders. Overview of physical and behavioural homeostasis. *Journal of the American Medical Association*. **267**: 1244–1252.
- Clarey, S. Farnsworth, R.J. (1983). The effect of environmental change on the behaviour of confined African lions (*Panthera leo*). *The Journal of Zoo Animal Medicine*. **14**: 98-102.
- Clubb, R., Mason, G.J. (2003). Animal welfare: captivity effects on wide-ranging carnivores. *Nature*. **425**: 473-474.
- Clubb, R., Mason, G.J. (2007). Natural behavioural biology as a risk factor in carnivore welfare: How analysing species differences could help zoos improve enclosures. *Applied Animal Behavioural Science*. **102**: 303-328.
- Clutton-Brock, T. H., Russell, A. F., Sharpe, L. L., Young, A. J., Balmforth, Z., McIlrath, G. M. (2002). Evolution and development of sex differences in cooperative behaviour in meerkats. *Science*. **297**: 253-256.
- Cociu, M., Wagner, G., Micu, N.E., Mihaescu, G. (1974). Adaptational gastro-enteritis in Siberian tigers: *Panthera tigris altaica*: at Bucharest Zoo. *International Zoo Yearbook*. **14**: 171-174.
- Conforti, V.A., Morato, R.G., Augusto, A.M., de Oliveira e Sousa, Lúcio, de Avila, D.M., Brown, J.L., Reeves, J.J. (2012). Noninvasive monitoring of adrenocortical function in captive jaguars (*Panthera onca*). *Zoo Biology*. **31**: 426-441.

Cozzi, G., Broekhuis, F., McNutt, J.W., Turnbull, L.A., Macdonald, D.W., Schmid, B. (2012), Fear of the dark or dinner by moonlight? Reduced temporal partitioning among Africa's large carnivores. *Ecology*. **93**: 2590-2599.

Crossin, G. T., Trathan, P. N., Phillips, R. A., Gorman, K. B., Dawson, A., Sakamoto, K. Q., Williams, T. D. (2012). Corticosterone predicts foraging behaviour and parental care in macaroni penguins. *The American Naturalist*. **180**: E31-E41.

Cyr, N.E., Michael Romero, L. (2007). Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. *General and Comparative Endocrinology*. **151**: 82-89.

Dantzer, B., McAdam, A.G., Palme, R., Fletcher, Q.E., Boutin, S., Humphries, M.M., Boonstra, R. (2010) Fecal cortisol metabolite levels in free-ranging North American red squirrels: assay validation and the effects of reproductive condition. *General and Comparative Endocrinology*. **167**: 279–286.

Davis, J. E., Guinan, J. A. (2014). Parental behaviour correlates to baseline corticosterone of mates and offspring in nesting eastern bluebirds (*Sialia sialis*). *General and Comparative Endocrinology*. **201**: 1-7.

Dawkins, M.S. (1980). *Animal suffering: the science of animal welfare*. London: Chapman and Hall.

Dawkins, M.S. (2004). Using behaviour to assess animal welfare. *Animal Welfare*. **13**: 3-7

De Boer, S.F., Slangen, J.L., Van der Gugten, J. (1990). Plasma catecholamine and corticosterone levels during active and passive shock-prod avoidance behavior in rats: effects of chlordiazepoxide. *Physiology and Behavior*. **57**: 1089-98.

Del Rosario, G.D.L.V., Valdez, R.A., Lemus-ramirez, V. Vázquez-Chagoyán, J. C., Villa-Godoy, A., Romano, M. C. (2011). Effects of adrenocorticotrophic hormone challenge and age on hair cortisol concentrations in dairy cattle. *Canadian Journal of Veterinary Research*. **75**: 216-221.

Dingemanse, N.J., Réale, D. (2005). Natural selection and animal personality. *Behaviour*. **142**: 1165-1190.

Dolrenry, S. (2013). *African Lion (Panthera Leo) behaviour monitoring and survival in human-dominated landscapes*. PhD. University of Wisconsin-Madison, 2013. Retrieved from:

<http://lionguardians.org/wp-content/uploads/2012/04/Dolrenry-Stephanie-PhD-2013.pdf>

Endroczi, E., Schreiber, G., Lissak, K. (1963). The role of the central nervous activating and inhibitory structures in the control of pituitary-adrenocortical function. Effect of intracerebral

cholinergic and adrenergic stimulation. *Acta Physiologica Academiae Scientiarum Hungaricae*. **24**: 211-221.

Estes, R. (2012). *The behaviour guide to African mammals*. Berkeley: University of California Press.

Exhibited Animals Protection Act 1986 No 123 (NSW). Retrieved from:
<http://www.legislation.nsw.gov.au/#/view/act/1986/123>.

Exhibited Animals Protection Regulation 2010 (NSW). Retrieved from:
<http://www.legislation.nsw.gov.au/regulations/2010-444.pdf>.

Fanson, K.V., Wielebnowski, N.C., Shenk, T.M., Lucas, J.R. (2012). Comparative patterns of adrenal activity in captive and wild Canada lynx (*Lynx canadensis*). *Journal of Comparative Physiology*. **182**: 157-165.

"Five Freedoms". Farm Animal Welfare Council / Farm Animal Welfare Committee. Accessed 17 March 2017 from <http://www.fawc.org.uk/freedoms.htm> the original

Fokkema, D. S., Smit, K., van der Gugten, J., Koolhaas, J. M. (1988). A coherent pattern among social behaviour, blood pressure, corticosterone and catecholamine measures in individual male rats. *Physiology and Behavior*. **42**: 485-489.

Fraser, D, Weary, D.M. Pajor, E.A., Milligan, B.N. (1997). A scientific conception of animal welfare that reflects ethical concerns. *Animal Welfare*. **6**: 187-205.

Fraser, D., Duncan, I.J. H. (1998). 'Pleasures', 'pains' and animal welfare: toward a natural history affect. *Animal welfare*. **4**: 383-396.

Fraser, D. (2003). Assessing animal welfare at the farm and group level: the interplay of science and values. *Animal Welfare*. **12**: 433-443.

Funston P.J., Mills, M.G.L. Biggs, H.C., Richardson, P.R.K. (1998). Hunting by male lions: ecological influences and socioecological implications. *Animal behaviour*. **56**: 1333-1345.

Galuppi, R., Leveque, J.F.C., Beghelli, V., Bonoli, C., Mattioli, M., Ostanello, F., Tampieri, M.P., Accorsi, P.A. (2013). Cortisol levels in cats' hair in presence or absence of *Microsporium canis* infection. *Research in veterinary science*. **95**: 1076.

Goymann, W. (2012). On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. *Methods in Ecology and Evolution*. **3**: 757-765.

Graham, L.H., Brown, J.L. (1996). Cortisol metabolism in the domestic cat and implications for non-invasive monitoring of adrenocortical function in endangered felids. *Zoo Biology*. **15**: 71-82.

Grinnel, J., McComb, K. (2001). Roaring and social communication in African lions: the limitations imposed by listeners. *Animal Behaviour*. **62**: 93-98.

Grinnel, J., Packer, C., Pusey, A.E. (1995). Cooperation in male lions: kinship, reciprocity or mutualism? *Animal Behaviour*. **49**: 95-105.

Handley, G. (2001). Asperger's syndrome from the horse's mouth. Available at: <http://www.webspawner.com/users/aspberger>.

Hayward, M.W., Hayward, G.J. (2007). Activity patterns of reintroduced lion *Panthera leo* and spotted hyaena *Crocuta crocuta* in the Addo Elephant National Park, South Africa. *African Journal of Ecology*. **45**: 135-141.

Hirt, H., Wechsler, B. (1994). Behavioural diversity as a measure of welfare: A study in pigs. *Applied Animal Behaviour Science*. **40**: 82-83.

Hernot, D.C., Dumon, H.J., Biourge, V.C., Martin, L.J., Nguyen, P.G. (2006). Evaluation of association between body size and large intestinal transit time in healthy dogs. *American Journal of Veterinary Research*. **67**: 342-347.

Hewson, C.J. (2003). What is animal welfare? Common definitions and their practical consequences. *Canadian Veterinary Journal*. **44**: 496-499.

Hughes, B.O., Duncan, I.J.H. (1988) The notion of ethological 'need', models of motivation and animal welfare. *Animal Behaviour*. **36**: 1696-1707.

Jenni-Eiermann, S., Helfenstein, F., Vallat, A., Glauser, G., Jenni, L. (2015). Corticosterone: effects on feather quality and deposition into feathers. *Methods in Ecology and Evolution*. **6**: 237-246.

Kloet, R.D., Joels, M., Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews*. **6**: 463-475.

Kolevská, J., Svoboda, M. (2000). Immunoreactive cortisol measurement in canine urine and its validity in hyperadrenocorticism diagnosis. *Acta Veterinaria Brno*. **69**: 217.

Konrad, K.W., Bagshaw, M. (1970). Effect of novel stimuli on cats reared in a restricted environment. *Journal of Comparative and Physiological Psychology*. **70**: 157-164.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., Blokhuis, H. J. (1999). Coping styles in animals: Current status in behaviour and stress-physiology. *Neuroscience and Biobehavioral Reviews*. **23**: 925-935.

Koren, L., Mokady, O., Karaskov, T., Klein, J., Koren, G., Geffen, E. (2002). A novel method using hair for determining hormonal levels in wildlife. *Animal Behaviour*. **63**: 403-406.

- Korte, S. M., Buwalda, B., Bouws, G. A. H., Koolhaas, J. M., Maes, F. W., Bohus, B. (1992). Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioural passivity and activity in aged and in young rats. *Physiology and Behavior*. **51**: 815-822.
- Kuhar, C.W., Bettinger, T.L., Laudenslager, M.L. (2005). Salivary cortisol and behaviour in an all-male group of western lowland gorillas (*Gorilla g. gorilla*). *Animal Welfare*. **13**: 187-193
- Lewis, L.D., Magerkurth, J.H., Roudebush, P., Morris, M.L., Mitchell, E.E., Teeter, S.M. (1994). Stool characteristics, gastrointestinal transit time and nutrient digestibility in dogs fed different fiber sources. *The Journal of Nutrition*. **124**: 2716-2718.
- Lindburg, D., Fitch-Snyder, H. (1994). The use of behaviour to evaluate reproductive problems in mammals. *Zoo Biology*. **13**: 433-445.
- Loarie, S.R., Tambling, C.J., Asner, G.P. (2013). Lion hunting behaviour and vegetation structure in an African savanna. *Animal Behaviour*. **85**: 899-906.
- Loftin, A. (2003). Survey of Autism. Available at: <http://www.spacehost.us/~aliki/autism/index/html>.
- Louch, C.D., Higginbotham, M. (1967). The relation between social rank and plasma corticosterone levels in mice. *General and Comparative Endocrinology*. **8**: 441-444.
- Lund, V. (2006). Natural living- a precondition for animal welfare in organic farming. *Livestock Science*. **100**: 71-83.
- Macbeth, B.J., Gibeau, M.L., Janz, D.M., Cattet, M.R.L., Stenhouse, G.B. (2010). Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (*Ursus arctos*): considerations with implications for other wildlife. *Canadian Journal of Zoology*. **88**: 935-949.
- Manojlović-Stojanovski, M., Nestorović, N., Milošević, V., (2012). *Prenatal Glucocorticoids: Short-Term Benefits and Long-Term Risks, Glucocorticoids*. In:- New Recognition of Our Familiar Friend, Dr. Xiaoxiao Qian (Ed.). Available from: <http://www.intechopen.com/books/glucocorticoids-new-recognition-of-our-familiar-friend/prenatal-glucocorticoids-short-term-benefits-and-long-term-risks>
- Maple, T.L., Perdue, B.M., (2013). *Zoo Animal Welfare*. Berlin: Springer.
- Mason, G.J., Latham, N.R., (2004). Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? *Animal Welfare*. **13**: 57-69.
- Mason, G.J., Rushen, J. (2006; 2005). *Stereotypic animal behaviour: fundamentals and applications to welfare*. 2nd edition. Cambridge: CABI Publishing.

- Mason, G.J. (2010). Species differences in responses to captivity: stress, welfare, and the comparative method. *Trends in Ecology and Evolution*. **25**: 713-721.
- Matoba, T., Kutsukake, N., Hasegawa, T. (2013). Head rubbing and licking reinforce social bonds in a group of captive African lions, *Panthera leo*. *PloS One*. **8**: 73044.
- McComb, K, Packer, C, Pusey, A., (1994). Roaring and numerical assessment in contests between groups of female lions *Panthera leo*. *Animal behaviour*. **47**: 379-387
- McEwen, B. S. (2004). Protection and Damage from Acute and Chronic Stress: Allostasis and Allostatic Overload and Relevance to the Pathophysiology of Psychiatric Disorders. *Annals of the New York Academy of Sciences*. **1032**: 1-7.
- Melfi, V.A. (2009). There are big gaps in our knowledge, and thus approach, to zoo animal welfare: a case for evidence-based zoo animal management. *Zoo Biology*. **28**: 574.
- Mellen, J. D. (1991). Factors influencing reproductive success in small captive exotic felids (*Felis* spp.): A multiple regression analysis. *Zoo Biology*. **10**: 95-110.
- Mellor, D.J. (2016). Updating animal welfare thinking: moving beyond the “Five Freedoms” towards “A Life Worth Living”. *Animals*. **6**: 21
- Moberg G.P. (2000). Biological response to stress: implications for animal welfare. In Moberg G.P., and Mench J.A. (eds): *The Biology of Animal Stress Basic Principles and Implications for Animal Welfare*. New York: CABI Publishing. pp. 1-2.
- Moisan M.P. (2012). Overview of acute and chronic stress responses. *Medicine Sciences*. **28**: 612.
- Morgan, K.N., Tromborg, C.T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*. **102**: 262-302.
- Mosser, A, Packer, C. (2009). Group territoriality and the benefits of sociality in the African lion *Panthera leo*. *Animal Behaviour*. **78**: 359-370.
- Möstl E, Palme R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*. **23**: 67-74.
- Narayan, E.J., Parnell, T., Clark, G., Martin-Vegue, P., Mucci, A., Hero, J. (2013). Faecal cortisol metabolites in Bengal (*Panthera tigris tigris*) and Sumatran tigers (*Panthera tigris sumatrae*). *General and Comparative Endocrinology*. **194**: 318.
- Owen, D.J., Lane, J.M. (2006) High levels of corticosterone in feather-plucking parrots (*Psittacus erithacus*). *The Veterinary Record*. **158**: 804-805.

- Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M., Möstl, E. (2005). Stress hormones in mammals and birds. Comparative aspects regarding metabolism, excretion, and noninvasive, measurement in fecal samples. *Annals New York Academy of Sciences*. **1040**: 162-171.
- Powell, D.M. (1995). Preliminary evaluation of environmental enrichment techniques for African Lions (*Panthera leo*). *Animal Welfare*. **4**: 361-370.
- Pritchard, J.C., Lindberg, A.C., Main, D.C.J., Whay, H.R. (2005). Assessment of the welfare of working horses, mules and donkeys, using health and behaviour parameters. *Preventive Veterinary Medicine*. **69**: 265-283.
- Prola, L., Cornale, P., Renna, M., Macchi, E., Perona, G., Mimosi, A. (2013). Effect of breed, cage type, and reproductive phase on faecal corticosterone levels in doe rabbits. *Journal of Applied Animal Welfare Science*. **16**:140.
- Putman, S.B., Brown, J.L., Franklin, A.D., Schneider, E.C., Boisseau, N.P., Asa, C.S., Pukazhenth, B.S. (2015). Characterization of Ovarian Steroid Patterns in Female African Lions (*Panthera leo*), and the Effects of Contraception on Reproductive Function. *PloS One*. **10**: 140-373.
- Rabin, L. A. (2003). Maintaining behavioural diversity in captivity for conservation: Natural behaviour management. *Animal Welfare*. **12**: 85-94.
- Rangel-Negrin, A., Alfaro, J.L., Valdez, R.A., Romano, M.C., Serio-Silva, J.C. (2009). Stress in Yucatan spider monkeys: effects of environmental conditions on faecal cortisol levels in wild and captive populations. *Animal Conservation*. **12**: 496-502.
- Raynaud, J., Schradin, C., Fusani, L. (2015). Corticosterone levels correlate with alloparental care in a sex-dependent manner in African striped mice, *Rhabdomys pumilio*. *Ethology*. **121**: 57-67.
- Rettenbacher, S., Möstl, E., Hackl, R., Ghareeb, K., Palme, R. (2004). Measurement of corticosterone metabolites in chicken droppings. *British Poultry Science*. **45**: 704-711.
- Rivier C., Vale W. (1983). Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. *Nature*. **305**: 325–327.
- Sanchez, M., Ladd, C., Plotsky, P. (2001). Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Development and Psychopathology*. **13**: 419-449
- Santymire, R.M., Freeman, E.W., Lonsdorf, E.V., Heintz, M.R., Armstrong, D.M. (2012). Using ACTH challenges to validate techniques for adrenocortical activity analysis in various African wildlife species. *International Journal for Animal and Veterinary Advances*. **4**: 99-108.

Sapolsky, R.M., Krey, L.C., McEwen, B.S., (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrine Reviews*. **7**: 284–301.

Sapolsky, R.M., Romero, L.M., Munck, A.U. (2000). How do glucocorticoids influence stress response? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Reviews*. **21**: 55–89.

Sih, A., Bell, A., Johnson, J.C. (2004). Behavioural syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution*. **19**: 372-378.

Schaller, G.B. (1972). *The Serengeti lion: a study of predator-prey relations*. Chicago: University of Chicago Press.

Scheel, D., Packer, C. (1991). Group hunting behaviour of lions: a search for cooperation. *Animal Behaviour*. **41**: 697-709.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia*. **166**: 869-887.

Smith, S.M., Vale, W.W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*. **8**: 383-395.

Schuur, B (1987). Social structure and plasma corticosterone level in female albino mice. *Physiology and Behavior*. **40**: 689-69.

Schwarzenberger, F., Möstl, E. Palme, R., Bamberg, E. (1996). Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Animal Reproduction Science*. **42**: 515-526.

Sgoifo, A., De Boer, S. F., Haller, J., Koolhaas, J. M. (1996). Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: Relationship with aggression. *Physiology and Behavior*. **60**: 1403-1407.

Shille, V.M., Haggerty, M.A., Shackleton, C., Lasley, B.L. (1990). Metabolites of estradiol in serum, bile, intestine and faeces of the domestic cat (*Felis catus*). *Theriogenology*. **34**: 779-794.

Stalder, T., Kirschbaum, C. (2012). Analysis of cortisol in hair--state of the art and future directions. *Brain, Behavior, and Immunity*. **26**: 1019-1029.

Standards for exhibiting carnivores in New South Wales. *Exhibited Animals Protection Act, 1986*.

Retrieved from: http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0015/121542/carnivore-exhibition-standard.pdf.

- Sterling, P, Eyer J. (1988) *Allostasis, a new paradigm to explain arousal pathology*. In: S. Fisher, J. Reason (Eds.), *Handbook of Life Stress, Cognition and Health*. New York: John Wiley & Sons.
- Sunquist, M.E., Sunquist, F. (2002). *Wild cats of the world*. Chicago: University of Chicago Press.
- Talbot, S., Freire, R., Wassens, S. (2014). Effect of captivity and management on behaviour of the domestic ferret. *Applied Animal Behaviour Science*. **151**: 94-101.
- Taronga Conservation Society Australia Animal Welfare Charter, Accessed 17 March 2017 from <https://taronga.org.au/sites/tarongazoo/files/downloads/awc-web.pdf>
- Taylor, W. (1971). The excretion of steroid hormone metabolites in bile and faeces. *Vitamins and Hormones*. **29**: 201–285.
- Terio, K.A., Munson, L., Solnick, J.V. (2000). Infection with H.pylori-like organisms is associated with gastritis in captive but not wild cheetahs. *Gastroenterology*. **118**: A746-A747.
- Terwissen, C. V., Mastromonaco, G. F., & Murray, D. L. (2013). Influence of adrenocorticotrophin hormone challenge and external factors (age, sex, and body region) on hair cortisol concentration in Canada lynx (*Lynx canadensis*). *General and comparative endocrinology*. **194**: 162-167
- Toates (1995). *Stress: Conceptual and Biological Aspects*. Chichester UK: John Wiley and Sons.
- Touma, C., Sachser, N., Möstl, E., Palme, R. (2003). Effects of sex and time of day on metabolism and excretion of corticosterone in urine and faeces of mice. *General and Comparative Endocrinology*. **130**: 267-278.
- Turpeinen, U., Hämäläinen, E. (2013) Determination of cortisol in serum, saliva and urine. *Best Practice and Research. Clinical Endocrinology and Metabolism*. **27**: 795-801.
- Van der Waal, K. L., Mosser, A., Packer, C., (2009). Optimal group size, dispersal decision and postdispersal relationships in female African lions. *Animal Behaviour*. **77**: 949-954.
- Van Metter, J.E., Harriger, M.D., Bolen, R.H. (2008). Environmental enrichment utilizing stimulus objects for African lions (*Panthera leo leo*) and Sumatran tigers (*Panthera tigris sumatrae*). *BIOS*. **79**: 1-16.
- Veasey, J.S., Waran, N.K., Young, R.J. (1996). On comparing the behaviour of zoo housed animals with wild conspecifics as a welfare indicator, using the giraffe (*Giraffa camelopardalis*) as a model. *Animal Welfare*. **5**: 139-153.
- Vester, B. M., Beloshapka, A. N., Middelbos, I. S., Burke, S. L., Dikeman, C. L., Simmons, L. G., Swanson, K. S. (2010). Evaluation of nutrient digestibility and faecal characteristics of exotic felids

fed horse- or beef-based diets: use of the domestic cat as a model for exotic felids. *Zoo Biology*. **29**: 432–448.

Wagenaar, J.P., Langhout, D.J. (2007). Practical implications of increasing 'natural living' through suckling systems in organic dairy calf rearing; Theme: Values in Organic Agriculture. *NJAS Wageningen Journal of Life Sciences*. **54**: 375-386.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millsbaugh, J.J., Larson, S., Monfor S.L. (2000). A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology*. **120**: 260–275.

WeatherSpark (2016). <https://weatherspark.com/history/29027/2000/Port-Elizabeth-Eastern-Cape-South-Africa>. Accessed 5 November, 2015.

Weinberg J., Levine S. (1980). Psychobiology of Coping in Animals: The Effects of Predictability. *NATO Conference Series*, **12**: 39-59.

Weingrill, T., Gray, D.A., Barrett, L., Henzi, S.P. (2004). Faecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. *Hormones and Behaviour*. **45**: 259-269.

Wells, A., Terio, K.A., Ziccardi, M.H., Munson, L. (2004). The stress response to environmental change in captive cheetahs (*Acinonyx jubatus*). *Journal of Zoo and Wildlife Medicine*. **35**: 8-14.

Wielebnowski, N. C., Fletchall, N., Carlstead, K., Busso, J.M., Brown, J.L. (2002). Non invasive assessment of adrenal activity associated with husbandry and behavioural factors in the North American clouded leopard population. *Zoo Biology*. **21**: 77-98.

Willemse, T., Vroom, M.W., Mol, J.A., Rijnberk, A. (1993). Changes in plasma cortisol, corticotrophin, and alpha-melanocyte-stimulating hormone concentrations in cats before and after physical restraint and intradermal testing. *American Journal of Veterinary Research*. **54**: 69-72.

Wurbel, H. (2001). Ideal homes? Housing effects on rodent brain and behaviour. *Trends in Neuroscience*. **24**: 207-211.

Yeates, J.W., Main, D.C.J. (2008). Assessment of positive welfare: A review. *The Veterinary Journal*. **175**: 287-430.

ZAA Membership Policy, 2012. Accessed 19 May 2016 from http://www.zooaquarium.org.au/wp-content/uploads/2015/08/2_0_a_P_MembershipPolicy.pdf 19/05/2016

ZAA Animal Welfare Position Statement. Accessed 19 May 2016 from

http://www.zooaquarium.org.au/wp-content/uploads/2014/01/ZAA_AnimalWelfare_PS

Zoos Victoria Animal Welfare Code. Accessed 17 March 2017 from
<https://www.zoo.org.au/sites/default/files/ZV-animal-welfare-code.pdf>.