Non-invasive monitoring of a stress biomarker in captive and free-roaming tigers in South Africa

by

Emma Maeve Jepsen

Submitted in partial fulfilment of the requirements for the degree Magister Scientiae in Zoology

in the

Department of Zoology and Entomology

Faculty of Natural and Agricultural Sciences

UNIVERSITY OF PRETORIA

July 2021

© University of Pretoria

SUMMARY

Non-invasive monitoring of a stress biomarker in captive and free-roaming tigers in South Africa

by

Emma Maeve Jepsen

Supervisor:	Prof A. Ganswindt
Co-supervisor:	Dr Juan Scheun
Department:	Zoology and Entomology
University:	University of Pretoria
Degree:	Magister Scientiae in Zoology
Keywords:	Adrenocortical stress response; Animal welfare; Conservation physiology; Cortisol; Ex-situ conservation; Faecal glucocorticoid metabolites; Non-invasive hormone monitoring; Stress

The tiger (*Panthera tigris*) is among the most endangered apex predators in the world and is believed to be the second-most trafficked animal on Earth, after pangolins. In the last century their habitats have been reduced by 95% and their wild populations decimated. They continue to face threats such as habitat loss, persecution, and poaching and unless all of these, and more, are addressed, the future of wild tigers is not guaranteed. However, captive tiger populations seem to do relatively well, outnumbering their wild counterparts hugely. There are many different conservation efforts currently being undertaken, both in the wild and captivity, but all factors, both biological and anthropogenic, that affect their welfare require investigation. The data chapter of this dissertation focused on examining factors that impact the well-being of captive tigers in South Africa in regards to their adrenocortical stress response and how this

© University of Pretoria

compared to both captive and wild Indian tigers. Faecal glucocorticoid metabolite (fGCM) concentrations were measured at eight different study locations; five in South Africa and three in India. In South Africa, three sites were zoos, one was a pet tiger held in a backyard enclosure and one site was a reserve were the tigers were “re-wilded” and allowed to live with little interference from humans. Location, season, sex, and level of exposure to humans was investigated and then these results were compared to those of tigers living in their native country of India; both in the wild and captivity. The data showed no difference in fGCM concentrations between Indian and South African tigers, and while season and sex does not seem to play a role in altering fGCM concentrations, certain locations and higher levels of public exposure did lead to significantly higher fGCM concentrations.

This data are the first to examine the adrenocortical stress response of captive and re-wilded tigers in South Africa with a direct comparison to wild and captive individuals in India. The feasibility of using fGCMs as a non-invasive method to monitor adrenocortical activity is demonstrated, as well as the value of understanding possible stressors that may impact the well-being of tigers in a captive, semi-wild, or wild setting.

© University of Pretoria

RESEARCH OUTPUTS

Journal articles

Jepsen, E.M., Scheun, J., Dehnhard, M., Kumar, V., Umapathy, G., Ganswindt, A. 2021. Non-invasive monitoring of glucocorticoid metabolite concentrations in native Indian, as well as captive and re-wilded tigers in South Africa. *General and Comparative Endocrinology.* In press.

Conference presentations

International Conference on Advancements in Veterinary Sciences for Wildlife Conservation & 13th Annual Meeting of the Association of Indian Zoo and Wildlife Veterinarians, 13-15 November 2019, Laboratory for the Conservation of Endangered Species (LaCONES), CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India

South African National Biodiversity Institute National Zoological Gardens, Annual General Meeting, 20-22 November, 2019, Pretoria, South Africa.

University of Pretoria, Department of Zoology and Entomology Annual General Meeting, 3-4 December, 2020. Pretoria, South Africa.

© University of Pretoria

LIST OF ABBREVIATIONS

USA	United States of America
IUCN	International Union for Conservation of Nature
DNA	Deoxyribonucleic acid
TCM	Traditional Chinese medicine
AD	*Anno Domini*
CITES	Convention on International Trade in Endangered Species
EIA	Environmental Investigation Agency
ECG	Electrocardiogram
HTC	Human-tiger conflict
TRAFFIC	Trade Records Analysis of Flora and Fauna in Commerce
IVF	*in vitro* fertilization
AI	Artificial insemination
ART	Assisted reproductive technology
WWF	World Wildlife Fund
SA	Sympatho-adrenal
HPA	Hypothalamic-pituitary-adrenal
CA	Catecholamines
CRH	Corticotropin-releasing hormones
ACTH	Adrenocorticotropin hormone
GC	Glucocorticoid
fGCM	Faecal glucocorticoid metabolite
NZG	National Zoological Gardens
LP	Lory Pak Zoo
PW	Predator World
TC	Tiger Canyon
NZP	Nehru Zoological Park

© University of Pretoria

KTR	Kanha Tiger Reserve
BTR	Bandhavgarh Tiger Reserve
SANBI	South African National Biodiversity Institute
EIA	Enzyme-immunoassay

© University of Pretoria

DECLARATION

I, Emma Maeve Jepsen, declare that the dissertation, which I hereby submit for the degree Magister Scientiae in Zoology at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

I, Emma Maeve Jepsen, whose name appears on the title page of this dissertation, has obtained, for the research described in this work, the applicable research ethics approval from the animal ethics committee of the University of Pretoria (NAS079/2019), as well as the South African National Biodiversity Institute NZG Research Ethics and Scientific Committee (P19/11).

I, Emma Maeve Jepsen declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

DISCLAIMER

This dissertation consists of a series of chapters that have been prepared as stand-alone manuscripts for the subsequent submission for publication processes. Consequently, unavoidable overlaps and/or repetitions may occur between chapters.

Emma Jepsen

Emma Maeve Jepsen

July 2021

© University of Pretoria

ACKNOWLEDGEMENTS

Where do I begin? So many people have gone above and beyond to help me and to each and every one of you I'm grateful. That's my general disclaimer because far too many individuals have helped me than I can possibly name here.

Now that that's out of the way: to my supervisor Prof André Ganswindt I thank you for receiving all my mails at 17:30 on a Friday afternoon (or 22:00 on Sunday) and still promptly replying, for creating a healthy, fun work environment, and for sending me to India to just miss seeing tigers not once, twice, but three times in a day. Although I'm still a bit sad I couldn't name my thesis SHIT (Stress Hormones in Tigers). To my co-supervisor, Dr. Juan Scheun I appreciate you being the first soundboard for all my drafts, I know not all of them were as decent as you say, and for getting frustrated with the slow sample collection alongside me. To Vinod Kumar and Dr. Govindhaswamy Umapathy for all their hard work collecting samples and running our protocol in their labs as well as being our tour guides around India: you are some of the most generous and hospitable hosts I've ever had the pleasure of meeting. And of course, to Nicole Hagenah, Stefanie Ganswindt, and Chantel Henning of the Endocrine Research Laboratory, who not only were instrumental in running my samples during COVID-19, but also in developing the step-by-step protocol and without whom I would have been helpless.

Who's next on the list? Everyone involved in sample collection and allowing me to work at their sites. Clinton, Clive, Kara, Sophy I am indebted to you for allowing us to use your premises, for helping to collect samples on my behalf. Rodney I am forever grateful for having gotten to experience Tiger Canyon and for your determination in allowing us to conduct research there. And of course, Ben, Chantel and Sam – you made the experience at Tiger Canyon unforgettable and so much more than just a poop collecting trip. Sam keen for any more card games?

I have not yet mentioned my research group – poop groupers you are all all-stars. I appreciate all the feedback on ideas, proposals, presentations, and drafts. I couldn't have gotten more lucky than I did with all of you. Special shout out to Arantxa for being my go-to person for

© University of Pretoria

proof-reading all my papers (shameless plug: she's researching pangolins – go read her stuff!).

And of course where would I be without friends and family? Brett, Graeme, and Tryan, you were there (literally, in the same house), for all of it. I love you all for letting me force you into listening to my presentations and dealing with my stress and still wanting to play games or participate in family trash time. You have become my brothers and I'll always put up with your nonsense if you put up with mine. Bee, you have been there since literally day one of undergrad. We've grown so much together, we survived (barely and I still don't know how) honours together and I'm so so so proud of both of us for living out our dreams. To Kyle, you've now seen this journey through to its end and you've been there for all the breakdowns and celebrations and stuck with me through all of it. You road-tripped at crazy hours of the morning with me and stayed up late to see aardvarks and watch me freak out as I saw my first aardwolf, and you will always have the top pooper-scooper prize. I love you more than I can put to words. Finally, to my parents and brother, you have supported me in my endeavours not just financially, but emotionally, and your support and belief in me has never wavered. I would not be here without you but it's so much more than that. I owe you everything and I don't think I can ever repay that, but I will try. But here's something you can at least hang on your fridge! And that about wraps that up ladies and gentlemen – Emma out.

© University of Pretoria

TABLE OF CONTENTS

© University of Pretoria

© University of Pretoria

CHAPTER 1 GENERAL INTRODUCTON

© University of Pretoria

1.1 Apex predators

Apex predators play important roles in ecosystem functioning, control of disease transmission, bio-indicators of climate change, as well as the control of invasive species (Wilmers et al., 2006; Pongsiri et al., 2009; Wallach et al., 2010; Ritchie et al., 2012). The loss of these top predators can unbalance an ecosystem and cause negative cascades (Ripple et al., 2016). For example, the removal of wolves from the Yellowstone National Park in the USA resulted in the over-abundance of the elk, leading to the decimation of the surrounding vegetation and reduction in plant cover (Ripple and Beschta, 2003). This same scenario has been mirrored countless times all over the world (Sahasrabudhe and Motter, 2011). Despite the important role apex predators play on ecosystem functioning, their persecution and removal from pristine areas continue unabated; as such, apex predators, especially large carnivores, are among the most threatened species in the world (Woodroffe, 2000). Today, due to factors such as urbanisation, exploitation of natural resources, and expansion of agricultural areas, many of the world's predator populations inhabit heavily degraded habitats (Bender et al., 1998; McKinney, 2002). The nutritional demands and home range requirements inherent in large bodied carnivores, a large, undisturbed habitat, is needed (Kelt and Van Vuren, 2001). Tigers are apex predators and are among those large carnivores that have undergone severe range contractions and are at risk of extinction (Goodrich et al., 2015; Wolf and Ripple, 2017).

1.2 Tigers (*Panthera tigris*)

1.2.1 Tigers in the wild

Originating in eastern Asia nearly six million years ago, the Felidae family includes some of the most iconic 'big cats', of which tigers (*Panthera tigris*) are the largest. Other members of the genus are: leopards (*P. pardus*), snow leopards (*P. uncia*), lions (*P. leo*), and jaguars (*P. onca*) (Hemmer, 1981; Collier and O'Brien, 1985; Wayne et al., 1989; Johnson et al., 2006; Lei et al., 2011; Cho et al., 2013). Tigers are classified by the International Union for

© University of Pretoria

Conservation of Nature (IUCN) Red List as endangered with a decreasing population trend (Goodrich et al., 2015). However, this is a broad classification for tigers as a whole and individual subspecies do not have classifications (Goodrich et al., 2015).

1.2.2 Subspecies of tigers

There is a debate on the number of subspecies, with numbers ranging from two to nine (Kitpipit et al., 2012). The putative nine subspecies are: Amur (*P. t. altaica*), South China (*P. t. amoyensis*), Bengal (*P. t. tigris*), Indochinese (*P. t. corbetti*), Sumatran (*P. t. sumatrae*), Caspian (*P. t. virgata*), Javan (*P. t. sondaica*), Bali (*P. t. balica*), and the Malayan tiger (*P. t. jacksoni*). Three of the mentioned subspecies are extinct (*P. t. virgata, P. t. sondaica, P. t. balica*), with *P. t. amoyensis* found only in captivity (Figure 1-1) (Kitpipit et al., 2012).

© University of Pretoria

Amur (P. t. altaica)
Image Source: World Association of Zoos and Aquariums

Bengal (P. t. tigris)
Image Source: Tigers-World

Indochinese (P. t. corbetti)
Image Source: A. Savin

Malayan (P. t. jacksoni)
Image Source: Audubon Nature Institute

Sumatran (P. t. sumatrae)
Image Source: WorldAtlas

South China (P. t. amoyensis)
Image Source: The Tiger Domain

Bali (P. t. balica)
Image Source: YouTube

Caspian (P. t. virgata)
Image Source: Kids Animals Facts

Javan (P. t. sondaica)
Image Source: Busy.org

Figure 1-1: Depiction of the nine different putative subspecies (Kitpipit et al., 2012). Those outlined in green are still found in the wild, in yellow are extinct in the wild, and in red are extinct.

This ongoing debate about the exact number of subspecies seems to harm the conservation effort for tigers as a whole, as there are no clearly defined management units, which can impede translocations or reintroductions (Tilson and Nyhus, 2010; Wilting et al., 2015). For instance, with the South China tiger which exists only in captivity, if this subspecies is to remain un-hybridized, the reintroduction would have to be planned extremely carefully. Both morphological traits (e.g. skull and pelage measurements) and genetics have been used to try to distinguish the subspecies with varied results. As most of the subspecies are

© University of Pretoria

morphologically similar, it seems not a viable method to differentiate between the clades which led to the argument for only recognizing two subspecies, *P.t. tigris* and *P.t. sondaica* in mainland Asia and the Sunda Islands, respectively (Kitchener and Yamaguchi, 2010; Mazák, 2010; Wilting et al., 2015; Cooper et al., 2016; Kitchener et al., 2017). Liu et al. (2018) recently conducted whole genome sequencing on 32 specimens and found there to be three extinct and six extant subspecies with low gene flow between them. These findings are in agreement with the majority of other studies using genetic analyses (Mázak, 1981; Luo et al., 2004; Driscoll et al., 2009; Goodrich et al., 2015; Xue et al., 2015), although not all of them (Driscoll et al., 2009; Kitpipit et al., 2012, Xue et al., 2015). The lack of consensus between the genetic and morphological studies is not uncommon and is evidence for the greater clarity that DNA introduces (Springer et al., 2007). However, differences between clades may be exaggerated simply due to uneven sampling over a cline (Kitchener and Dugmore, 2000; Kitchener and Yamaguchi, 2010). This was shown with Bengal tigers where differences found among the subspecies disappeared when a larger sample pool was analysed (Sharma et al., 2009; Mondol et al., 2013). Liu et al. (2018) offers a clear description of when and how different clades diverged, often due to temperature changes or other climatic events such as volcanic eruptions. Interestingly, the author could not determine the origin of the South China tiger, hypothesizing that they might even have originated with the captive breeding of tigers from different subspecies. Although there is no definitive consensus on tiger subspecies, the species as a whole is under threat largely due to human practices.

1.2.3 Tiger distribution

Tigers were historically distributed from Bali to the Caspian Sea, but are now confined to less than 5% of their historic range due to habitat loss, persecution, and inadequate management and enforcement, leading to increased poaching (Seidensticker, 1999; Dinerstein et al., 2007; Wolf and Ripple, 2017) (Figure 1-2). Furthermore, over the last century, their numbers have declined from over 100,000 individuals in the wild to below an estimated 4000 animals for all

© University of Pretoria

six extant subspecies, of which around one-third are breeding females (Damania et al., 2008; Luo et al., 2008).

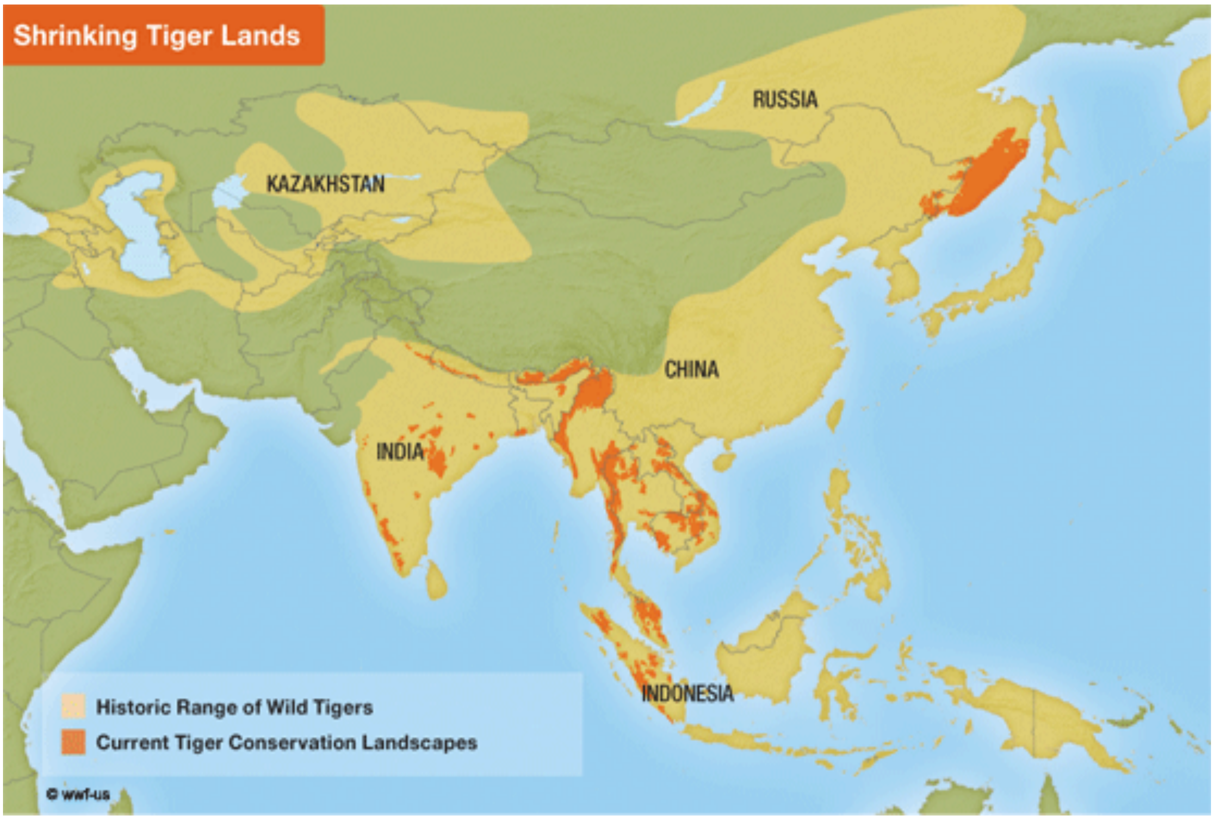


Figure 1-2: Former and current distribution of wild *Panthera tigris* in its natural habitat. Image from: https://suzanneyork.wordpress.com/.

1.2.4 Tiger biology

Tigers are sexually dimorphic with males considerably larger than their female counterparts (Mazák, 1981). However, body size also varies distinctively throughout their range with larger animals found farther north (ranging to below 50° latitude in the Amur basin, Miquelle et al., 1999), and smaller individuals on the islands in the southernmost part of their range (around -10° latitude, Sanderson et al., 2010) (Hooijer and Granger, 1947; Mazák, 1981; Mazák, 1996). The average weight of tigers, when all recognized subspecies are included, is 160 kg for males and 115 kg for females (Kitchener and Yamaguchi, 2010), but males in the wild can reach a mass of 300 kg (Mazák, 1981).

© University of Pretoria

Within a free-ranging environment, tigers are known to reach ten years of age on average (Smith and McDougal, 1991). In contrast to this, captive individuals, in the absence of competition, predation and disease, can exceed twenty years of age (Williamson et al, 2008; Tidière et al., 2016). Sexual maturity is reached at three to four years (females) or four to five years (males) of age (Smith and McDougal, 1991; Kalman, 2004; Vlaming, 2013; Singh et al., 2014). A total gestation period of 100 days, with an interbirth interval of two years, has been observed in the species (Kalman, 2004; Chapron et al., 2008). Although cubs are weaned at three to six months old, they remain with their mother until they are 12 to 18 months of age (Kalman, 2004).

Tigers are solitary and territorial (Goodrich et al., 2010) with a home range size dependent on prey abundance, water availability, and anthropogenic disturbance (Chundawat et al., 1999; Miquelle et al., 2010). Previous studies have found male homes ranges to be around 3.5 – 3.8 times larger than that of females and overlap with home ranges of multiple females. Female home ranges vary from 84.2 ± 40.8 km^2 to 390 ± 136 km^2 and those of males range from 294.1 ± 100.3 km^2 to 1385 ± 539 km^2 (Goodrich et al., 2010; Simcharoen et al., 2014). Wild tigers eat a variety of prey species with seasonal variation in diet, preferring to eat deer, wild pigs and smaller prey items (Chundawat et al., 1999; Sankar et al., 2010). The average weight of their prey is location-dependent and can vary from around 60 kg to 114 kg (Biswas and Sankar, 2002; Bagchi et al., 2003).

Tigers are unmistakable with their bold black and orange coats. Their “orange” fur colour can vary from white to yellow to a deep red (Pocock, 1929; Brongersma, 1935; Mazák, 1981; Mazák, 1996) (Figure 1-3). Ortolani and Caro (1996) found that this colour variation seems to follow Gloger’s rule, which states that the darker animals are found in moister, tropical forests and the lighter coloured individuals are found in grasslands and temperate forests. White tigers are rare variants of a Bengal tiger caused by a single amino acid change and are not albinos (Xu et al., 2013). Most of the white tigers taken from the wild were adults, indicating that the mutation causing this rare colouration does not dramatically affect their fitness (Xu et

© University of Pretoria

al., 2013). The expected extinction of white tigers in the wild is likely due to trophy hunting caused by their rarity as well as habitat loss and degradation (Chundawat et al., 2012). Today, captive white tigers are often bred together continuously which can lead to inbreeding depression and problems such as early death, stillbirth, and deformities (Figure 1-4) (Thornton et al., 1967; Robinson, 1969; Thornton, 1978; Robinson, 1990). These deformities include bull-dog face, where the tigers have a facial deformity due to improper bone development, visual impairment, and strabismus which is crossed eyes (Warrick, 2010; Xu et al., 2013).

Figure 1-1: These tigers are the rich orange often associated with the species (Ranthambore National Park, India). Photo by: Michael Vickers.

© University of Pretoria

Figure 1-4: A) A healthy white tiger, B) A tiger with "bulldog face" and C) strabismus, both often caused by inbreeding. Photo sources: A) Flickr, B) The Sun, C) Australian Veterinary Journal.

1.3 Tigers under threat

1.3.1 Tiger symbolism

Tigers are iconic and charismatic animals that in many countries are religious and cultural symbols; they are found in Buddhist, Hindu, and Islamic symbolism as well as ancient Chinese traditions (Seidensticker, 1996; Damania et al., 2008). Traditionally, tiger skins were worn in Tibet, a practise which is still seen today; tigers are sacred there and believed to have healing powers, and rugs made of their skin were used to repel unwanted pests (Thapar, 1996; Nowell and Ling, 2007). In China, tigers can be found in common myths, legends and folklore (Bascom, 1965; Ting, 1978). Viewed as a ferocious beast, they are often taken as an evil omen or a tiger's action may be the will of the gods (Hammond, 1991). In Confucianism, a tiger is representative of an oppressive government, while in Tao beliefs, tigers could transform into humans, and in other beliefs they are divine agents (Hammond, 1991). Also in

© University of Pretoria

China, a likeness of the animal was used as a protectant against evil spirits and disease (Thapar, 1996) and fevers could be lowered by reading prose of tigers (Thapar, 1996). In Hinduism, tigers represent the goddess Durga, and they are a general symbol of royalty in India (Brittlebank, 1995).

1.3.2 Traditional Chinese Medicine (TCM)

The use of tigers in TCM, which dates back to around 500 AD when tiger fat was used to stop nausea and treat dog bites, is believed to be the largest threat to their survival in the wild (Mainka and Mills, 1995) (Figure 1-5A, 1-5B). Now, almost every part of the tiger is utilized in TCM for a variety of ailments; the testes for glandular swelling, blood for a stronger will, eyes for epilepsy and malaria, bile for convulsions, flesh to stop stomach and spleen problems, bones for joint sprains, tiger grease for stomach pain, whiskers and claws for toothaches, and the list goes on and on (Jackson and Kemf, 1994; Mills and Jackson, 1996; Mainka and Mills, 1995; Laidler and Laidler, 1996; Thapar, 1996; Still, 2003). As all members of the Felidae are known to copulate numerous times a day during their breeding season, they are often regarded as having a large sexual prowess and the penis is viewed as a strong aphrodisiac (Thapar, 1996). Today, tiger bone, specifically the humerus, is the most used part of the animal and is said to treat rheumatism (Mainka and Mills, 1995). The use of tiger parts to treat ailments is not restricted to TCM; in India, tiger fat is used for leprosy and rheumatism, while in Vietnam, bones are used for rheumatism and general illness (Mainka and Mills, 1995).

© University of Pretoria

补骨酒以虎骨加以天然名贵植物和自酿纯正米酒在地窖古法炮制六年而成，酒体色泽红润，酒感醇厚，口味爽净，其味绵长，适当饮用，既强身健体，又延年益寿，是现代生活中必备的送礼时尚佳品。

Figure 1-5: A) A brochure advertises tiger bone wine, and B) pieces of tiger bone on sale at a market in Myanmar. Both of these products, and more, are used for treating a variety of ailments in TCM. Photo source: A) IFAW B) Steve Winter / National Geographic.

1.3.3 Tiger farming

To meet the international demand for tiger parts, several facilities around the globe, with the majority located in Eastern Asia, have started captive breeding/farming practices. There has been an ongoing debate about the practice with proponents for it saying that the ban in tiger trade has not kept the species from crashing and that there will always be a demand for the parts (Kirkpatrick and Emerton, 2009). Furthermore, they say that it provides a livelihood for many millions of people, farming other species has been successful, and flooding the market is believed by some to lead to lowered demand and less poaching (Kirkpatrick and Emerton, 2009). However, in 2007, the Convention on International Trade in Endangered Species (CITES), declared that tigers should not be farmed for their parts (Gratwicke et al., 2008). The

© University of Pretoria

ban on tiger farming was due to two main factors: 1) TCM believes parts from wild tigers are more potent than from farmed tigers, and 2) tiger farms could not alone meet the demand for respective products (Gratwicke et al., 2008). These two reasons indicate that tiger farming is unlikely to lower the price of wild tigers and therefore lower tiger poaching and could even increase demand for parts and provide easy routes to launder illegal products (Kirkpatrick and Emerton, 2009; Abbott and Van Kooten, 2011). Despite the CITES verdict, according to the Environmental Investigation Agency (EIA), there are over 7000 captive tigers in Asia that are illegally traded or harvested for products derived from them.

1.3.4 Human-tiger Conflict

Human-tiger conflict (HTC) occurs when tiger populations overlap with human settlements or activities (Goodrich and Miquelle, 2005) and can be divided into two main types: tiger attacks on humans, and tiger attacks on livestock (Goodrich, 2010). This is an increasing issue, as humans push into the last remaining refuges of tigers, leading to an increase in HTC and a considerable decrease in tiger density and population size (Goodrich, 2010). Tigers, more often than not, do not attack humans for food, but rather as a form of defence, especially when wounded (McDougal, 1987; Gurung et al., 2008). Over the last century, HTC, and the number of human mortalities by tigers, has decreased as tiger populations have dropped and their habitats have undergone considerable contractions (Goodrich, 2010; Nyhus et al., 2010). Nowadays, human mortalities are highest in South Asia, especially rural areas of Bangladesh and India (Karanth and Gopal, 2005; Gurung et al., 2008; Barlow, 2009). Loss of livestock and other domestic animals by tigers is the most common reason for HTC and can be attributed to a lack of their normal prey species due to hunting and habitat degradation/loss (Madhusudan and Karanth, 2002; Wang and MacDonald, 2006; Sangay and Vernes, 2008; Nugraha and Sugardjito, 2009). These conflicts not only lead to increased tiger mortalities but also create a negative perception towards tigers by people living in potential conflict zones (Goodrich, 2010). Unfortunately, if tiger populations increase with successful conservation

© University of Pretoria

initiatives, HTC is expected to become more common (Karanth and Gopal, 2005). Even in well managed areas where tigers are protected, young individuals will disperse to find their own territories or push older/weaker animals farther into human settled areas (Goodrich, 2010). In the past, the method of dealing with HTC was to eliminate the “problem” animals, but that has changed over time, at least in policies, if not in reality, especially rural areas (Treves and Karanth, 2003; Nyhus and Tilson, 2010). Goodrich (2010) divides the approaches to HTC into four main categories: 1) preventative measures, 2) mitigative measures, 3) reactive measures, and 4) integrated programs. Preventative measures include the separation of people and tigers through zoning and relocations, improved livestock management, increased wild prey, educational programmes and reduced human caused injuries to the animals (Goodrich, 2010). Mitigative measures include factors such as compensation, insurance, incentives and programmes in local communities (Goodrich, 2010). Reactive measures include removal of problem animals, monitoring and tracking tiger locations, hazing to frighten tigers, and rehabilitating wounded and sick animals, while integrated programmes would combine any number of the other measures (Rabinowitz, 1986; Dunishenko et al., 1999; Karanth and Madhusudan, 2002; Breitenmoser et al., 2005; Frank et al., 2005; Nyhus et al., 2005; Li et al., 2009; Goodrich, 2010; Smith et al., 2010). Unfortunately, few comprehensive plans to combat HTC have been enacted or followed up on and so there is little evidence showing the success of these measures (Goodrich, 2010).

1.3.5 Tiger Poaching

Poaching is the main threat to the survival of wild tigers and is often conducted by family groups or people with traditional prowess, as well as poaching gangs (Sharma et al., 2014). The practice is ubiquitous, even occurring in seemingly secure reserves (Walston et al., 2010). In the late 1800s, with the increasing availability of guns, tiger poaching increased dramatically (Sharma et al., 2014). Then, in the 1960’s, tiger skin coats came into fashion in the western world, tigers became a tradable commodity, and poaching has not slowed since (Sharma et

© University of Pretoria

al., 2014). Robust statistical data on poaching are hard to get as it is impossible to detect all of the cases; lowered reported poaching rates could be due to a decrease in crime rates or bad policing, which can make it difficult to tell if anti-poaching strategies are working (Sharma et al., 2014). It is estimated by custom officials that the detectability of tiger goods is about 10% of what is actually being poached and traded (Sharma et al., 2014). Tiger poaching has two main parts: the actual killing of the animal and then exporting it to supply the international market, often through major trade hubs (Sharma et al., 2014). The methods used by poachers differ between areas as well as over time (Risdianto et al., 2016). To stop poaching, four kinds of plans can be enacted and they need to bridge difference sectors: protection, outreach, monitoring, and management (Galster et al., 2010). Protection includes rangers, and ranger training courses are needed as better ranger patrols can both deter poachers and find and remove snares (Galster et al., 2010; Linkie et al., 2015; Risdianto et al., 2016). Outreach includes educational programmes and incentivizing the protection of tigers; this can be done locally or on a national or global scale through: (1) major advertising campaigns with well-known celebrities and (2) teaching community members about ways to get alternative income and food supplies (Galster et al., 2010). In connection with protection and outreach, monitoring ensures the presence of experienced rangers that can conduct transects and correctly identify signs of tigers (Galster et al., 2010). Finally, management goes higher up and identifies the need for coordination between all the different segments and support of managers and decision makers (Galster et al., 2010). Penalties imposed on poachers are often insufficient to deter such activities; increasing prison sentences and/or fines may act as a bigger deterrent for criminals (Risdianto et al., 2016).

1.3.6 Tiger diseases

Tigers are susceptible to a number of diseases, both in the wild and in captivity (Quigley et al., 2010; Seimon et al., 2013; Sadler et al., 2016). There have been instances of outbreaks of morbilii viruses, including canine distemper, in Amur tigers (Quigley et al., 2010; Seimon et

© University of Pretoria

al., 2013). Canine distemper is transmitted from a reservoir host, such as a raccoon dog (*Nyctereutes procyonoides*), and can have an impact on the survival of tigers as a species (Seimon et al., 2013), but is entirely preventable with vaccinations (Seimon et al., 2013; Sadler et al., 2016). Degenerative spinal disease also occurs in large felids, including tigers, but as of yet has only been reported in captive individuals (Kolmstetter et al., 2000). As a point of interest, at the time of writing this, tigers in the Bronx zoo in New York City had been diagnosed with COVID-19, most likely contracting the disease from a zookeeper. This was the first recorded instance of a non-domesticated animal contracting the virus and all individuals recovered fully (Daly, National Geographic, 2020).

1.4 Conservation measures

1.4.1 Habitat protection

There are a number of practices being implemented both in tigers' native habitats as well as elsewhere in captive or *ex situ* locations. The most local of these is basic habitat protection. There are many reserves for tigers throughout their current range, but these introduce new challenges. Often, these reserves are isolated from one another, inhibiting animal movements and risking local extinctions (Gopal et al., 2010). Additionally, in India and other locations, there are often human settlements in the reserves that encroach on tiger habitat, resulting in increased poaching of both animals and other resources such as timber, and create instances of HTC (Tilson et al., 2004). Despite HTC, villages often stand to benefit from being located in the reserve due to the increased sources of jobs and revenue and may also help to increase the education of local people around the animals and give them a motivation to protect them (Sekhar, 2003). In some parks there have been initiatives that have undertaken relocations of villages (both voluntary and forced) out of the protected areas such as in Sariska, Nagarahole, and Mudumalai Tiger Reserves, among others (Bhattacharjee et al., 2015). In many villages, authorities have informed them of the event for years, even decades before it may come to

© University of Pretoria

fruition (Ghate, 2005; Desai and Bhargav, 2010). It has been shown that these relocations lead to reduced human disturbance. Moef (2008) reported that in Corbett Tiger Reserve, post-relocation of 411 families, the tiger population increased by 52% and 273 hectares were restored to prime tiger habitat (Lasgorceix and Kothari, 2009). Moreover, other species, both predators and prey, (*e.g.* chital, sambar, leopard) have shown similar changes in population densities post-relocation (Panwar, 1978; Karanth, 2006). Similarly, a decrease in reported fires and HTC incidences have also been observed following relocation events (Lasgorceix and Kothari, 2009). In terms of the effects of relocation on socio-economic factors, it can be very difficult for the communities moved, due to economic and cultural reasons (Lasgorceix and Kothari, 2009). Many of these villages are very remote and have strong cultural traditions which then have to be merged with the traditions of the new areas they are moved to, which are often larger and more centred around money and less about community (Lasgorceix and Kothari, 2009). Furthermore, as prime land is often already taken, available space becomes an issue and the quality of the land may not be equal to formally settled regions, making their old ways of subsistence farming or forestry more difficult (Dasgupta, 2003; Wani and Kothari, 2006; Lasgorceix and Kothari, 2009). A good practice used in some reserves, such as Tadoba Tiger Reserve, is that the visitors are not allowed to drive themselves. Drivers and guides are normally people from local villages who know the area and the animals well and they do not make use of radios so when a tiger is spotted they cannot call to other vehicles, which could potentially overwhelm and stress the individual (Emma Jepsen, personal observation).

1.4.2 Captive breeding programs

Captive breeding is another conservation measure which can be implemented. Here, animals are bred in captivity with the aim of reintroduction into the wild. There are, as always, two sides to this. Some naysayers argue that this method can hinder the conservation of different subspecies as there may be interbreeding between them and they would no longer be distinguishable (Kitchener and Dugmore, 2000). However, breeding programmes can be used

© University of Pretoria

to conserve subspecies, as is being done at Laohu Valley in the Karoo of South Africa, where they have some of the few remaining South China tigers left, and are currently breeding them with the hope of future release into the wild. Other similar programmes are underway worldwide to conserve Sumatran tigers (the World Association of Zoos and Aquariums, WAZA as well as the Association of Zoos and Aquarums, AZA), Amur tigers (AZA, EAZA Ex-Situ Programme (EEP), and Malayan tigers (AZA). These programmes follow specific regulations with the goal of long-term preservation of genetic diversity in order to preserve individual subspecies. Captive breeding programs, if done correctly, could help with the conservation of the species by bolstering wild populations through reintroductions and improving the gene pool though the addition of healthy, genetically diverse individuals (Carillo et al., 2015), but they need to follow strict guidelines and be closely monitored. Unfortunately, many captive breeding programmes are presumably just fronts to farm tigers for their parts, or use the animals for trophy hunting and have no conservation value (TRAFFIC/Wildcru report, *Bones of Contention*). However, as the number of wild tigers are rapidly decreasing, the main focus should be on the conservation at species- rather than subspecies level (Wilting et al., 2015).

Closely tied with captive breeding programs is the use of *in vitro* fertilization (IVF) and artificial insemination (AI) to aid in the birth rates of cubs or to increase genetic diversity between geographically distant locations (Wildt and Roth, 1997). Tiger cubs have been successfully born using invasive assisted reproductive technology (ART) techniques such as IVF laparoscopic methods since the 1990s (Donoghue et al., 1990; Donoghue et al., 1996). Recently, the first lion cubs were born using a non-surgical AI technique; a technique that is better for the welfare of the animal, and this can potentially be a model going forward for use in tigers (Callealta et al., 2019). When done ethically and following proper guidelines, ART is potentially very useful for conservation efforts. However, a governing body of sorts should be monitoring this as, in the wrong hands, it could potentially be used in tiger farming and other unethical practices.

1.4.3 Reintroductions

© University of Pretoria

Furthermore, hand in hand with captive breeding programs are reintroductions and translocations. Reintroductions often take place in areas where tigers where previously extirpated in order to try and establish a new population there. Of course, this strategy only works if the original reason for the loss of tigers in the area has been eliminated. Reintroductions have been done in India with varying levels of success, which is often measured as both survival and reproductive rates (Bhattacharjee et al., 2015). Sarkar et al. (2016) showed that in Panna Tiger Reserve, reintroduced tigers behaved exactly the same way as tigers did prior to their extirpation. These tigers were a mix of wild-caught and bred in captivity, showing that captive breeding efforts can lead to successful reintroductions (Sarkar et al., 2016). With the South China tiger, which has been declared extinct in the wild, a reintroduction strategy is the end goal. However, several obstacles may hinder such progress, including habitat quality, prey availability, connectivity between parks, better enforcement and protection and education of local people (Qin et al., 2015). A study on the South China tiger found that presence of the mother in early developmental phases is not necessary for learning to hunt, but it does contribute to a higher kill rate (Fàbregas et al., 2015). Additionally, Ning et al. (2020) suggested that for animals destined for reintroduced, adapting the gut microbiota to match that of wild tigers would further the reintroduction success as the body will be better prepared for a wild diet. All of these factors and more prove that the reintroduction of tigers is a very complicated process but can be vital to the survival of the species.

1.4.4 Translocations

There are a number of reasons why translocations may be used in conservation. They can mitigate HTC, introduce new genes to an area to avoid inbreeding, or restore populations (Massei et al., 2010; Germano et al., 2015). However, there are some potential consequences for animals during translocation: increased stress levels which may result in lowered immune function, malnutrition, spread of disease and lower fitness and survival have been reported (Cunningham, 1996; Massei et al., 2010). Furthermore, in areas where there are resident

© University of Pretoria

tigers, it can make it difficult for translocated individuals to establish territories; either the resident of translocated individual may have to be displaced (Priatna et al., 2012). Ideally, resident animals at release sites should be accustomed to the arrival of presumably young individuals looking for territories (Goodrich, 2010). There have been successful translocations of tigers, most often to avoid HTC (Miller et al., 2011; Mardiastuti, 2018). However, there have been instances where translocated tigers have been poached soon after introduction (Goodrich and Miquelle, 2005). To mitigate the poaching threat, tigers could be collared and closely monitored to ensure individual safety (Miller et al., 2011). Furthermore, a program to educate local communities within the translocation region should also be put in place (Mardiastuti, 2018). When translocation is being considered, all possible outcomes, both for the animals and human settlements should be weighed before a decision is made.

1.5 Captive tigers

1.5.1 Tigers in captivity

Captive tigers, which in this case are tigers kept in comparatively small enclosures (when compared to wild tigers) where they do not have free access to food/water without human caretakers, number between 15,000 to 20,000 individuals worldwide, and are five to seven times more abundant than their wild counterparts (Luo et al., 2008). According to the World Wildlife Fund (WWF), there are at least 5000 captive tigers in the USA alone. Captive tigers are confined to enclosures which are more often than not considerably smaller than the natural home range size of their free-ranging counterparts which measure between 7-1000 km^2 (Breton and Barrot, 2014). The upper size limit of enclosure size of captive tigers is not known, but in South Africa, some tigers have been found in cages barely larger than their bodies (TRAFFIC/Wildcru report, *Bones of Contention*). Captive tigers often face conditions vastly different than those that would be encountered naturally and are usually fed, similarly to most large captive felids, a diet mainly consisting of raw beef, horse, or some other similar meat; it

© University of Pretoria

is currently unknown whether this diet is sufficiently suitable for tigers (Vester et al., 2008). Tigers in zoos have been shown to use only a third of their enclosure, often pacing along fence lines, while resting for up to 75% of the day (Lyons, 1997; Pitsko, 2003) as compared to wild tigers which Seidensticker (1976) found to be active for 75% of the day. There are many aspects of captivity that can influence the welfare of the animals: larger enclosures, more caring and consistent caretakers, and innovative and interactive habitat features and feeding techniques, among other things, can all help improve the well-being of animals in captivity.

1.5.2 Stereotypical behaviours

Carlstead (1996) defined stereotypic behaviour as an unchanging movement pattern that serves no obvious purpose. These behaviours can be caused by a number of factors including fear, boredom, frustration, unattainable goals (i.e. not being able to feed normally or reach another individual), genetics, early developmental experiences (i.e. maternal deprivation), physical restraints, animal density, housing quality, low levels of stimulation, and visitor presence (Schaller, 1967; Pitsko, 2003; Clubb and Vickery, 2006; Bashaw, 2007; Moreira et al., 2007; Latham and Mason, 2008; Jones et al., 2010). Rose et al. (2017) suggests that stereotypic behaviours may be a coping mechanism, suggesting that some animals are far less suited to captivity than others. Carnivores are reported to be more susceptible to stereotyped behaviours than other animals (Boorer, 1972, Berkson, 1983, Kolter, 1995), perhaps due to their natural activity levels and often wide-ranging habits (Meyer-Holzapfel, 1968; Forthman-Qiuck, 1984; Kreger et al., 1998; Shepherdson, 1998). Pacing was one of the first stereotyped behaviours ever noted for captive predators (Clubb and Vickery, 2006). Pacing, and other unnatural behaviours, are considered an indication of stress in animals and may lead to injuries such as cuts and abscesses (Morris, 1964; Meyer-Holzapfel, 1968; Mason, 1991; Carlstead, 1996). Individuals exhibiting stereotypical behaviours are less likely to be used for public education programmes, and are often less desirable candidates for reintroduction events (Ormrod, 1987; Vickery and Mason, 2003b). This is due to the

© University of Pretoria

persistence of abnormal behaviour which could lead to lower survival success in the wild as they are not as adaptable as they may need to be (Vickery and Mason, 2003b). It is important to understand the cause of the behaviours in each individual to be able to best improve the welfare of that individual. If the stereotypic behaviours are caused by genetics or early social and developmental experiences, they may be more difficult to remedy than behaviours caused by feeding times, or lack of habitat diversity. With behaviours caused by boredom or lack of stimulation, Pitsko (2003) showed that enclosures mimicking the natural environment and containing various enrichment practices resulted in an increase in natural behaviours. In a study conducted by Vaz et al. (2017), leopards that had access to trees, pools, or dens, as well as zoo-born individuals spent significantly less time performing stereotyped behaviours. Additionally, to reduce stereotypic behaviour, synchronising the activity times of the animals with when they are fed and interacted with, as well as increasing the feeding frequency, can lower the amount of time spent pacing by up to half and increase exploratory behaviours (Erwin and Deni, 1979; Stevenson, 1983; Carlstead, 1998; Altman et al., 2005). Vlaming (2013) found that tigers paced significantly more with every passing day since the last feed, and that older individuals’ pace more as pacing behaviour relates to territoriality (Clubb and Vickery, 2006).

It was also found that in captive tigers, their skulls develop external occipital protuberances, thought to be caused by reduced jaw activity (not having to catch their own food) combined with increased lateral head rotation (due to increased grooming and pacing activity) (Duckler, 1998). Excessive grooming is, in many species, an indicator that animals are stressed (Gispen and Isaacson, 1981; Willemse and Spruijt, 1995). Bashaw et al. (2003) found that enriching tigers’ diet with live fish and horse bones reduced the amount of time spent displaying stereotypic behaviour.

1.6 Tigers in South Africa

South Africa has at least 280 tigers held in captivity in at least 44 facilities, as reported in 2015 by the EIA, a TRAFFIC/Wildcru report, *Bones of Contention*. However, an investigation by

© University of Pretoria

Ban Animal Trading and the *EMS Foundation,* which are two South African CITES Observer organisations, reported a minimum of 64 facilities found in eight of the nine provinces (excluding the Northern Cape). None of these facilities are registered CITES breeding facilities even though tigers are Appendix 1 animals and thus all breeding facilities have to be registered and trade is “permitted only in exceptional circumstances” (www.cites.org). There are also major problems with inbreeding as well as cross-breeding with other subspecies of tiger as well as lions, due to the lack of regulation. CITES has reported that in the last five years South Africa has exported over 200 live captive-bred tigers to Asia, mostly Vietnam and Thailand. Along with these live tigers, countless tiger trophies, including bones, claws and skulls, have been exported. This has been shown to occur through laundering the tiger bones as lion bones which is possible as lions are listed under Appendix II which are animals where “trade is controlled in order to avoid utilization incompatible with their survival” (www.cites.org).

Tigers in South Africa are kept in a variety of different facilities ranging from enclosures as small as 36 m^2 to ‘free-roaming’ tigers that are essentially allowed to live freely on a large tract of land and hunt and fend for themselves. Free-roaming tigers located in South Africa offer an ideal opportunity to investigate the effect novel environments and diets may have on the physiology, adrenocortical activity, and health of these individuals. This information can aid in improving welfare of captive tigers, and give important insights into tiger conservation.

1.7 Stress

1.7.1 Defining stress

The original definition of stress, proposed by Selye (1936), is “the nonspecific response of the body to any demand”; however, there seem to be no agreed upon definitions today and the definitions differ in relation to the field of study. For this study, a suitable way to describe it is as any disruption to the body’s homeostasis and the physiological responses to these disturbances (Buchanan, 2000; Atkinson et al., 2015). In this regard, a three stage model of

© University of Pretoria

stress was suggested by Moberg and Mench (2000): 1) recognition of the stressor, 2) biological defence, whereby the body increases heart rate, stops digestion and other non-essential processes such as reproduction, and releases stored energy, all to help the animal survive the potential stressor, and 3) the ramifications of the stress response. Stress is important to understand and be able to measure, because it can affect an individuals' immune system (Khansari et al., 1990), reproduction (Pottinger, 1999), as well as behaviour and cognition (Lupien et al. 2009).

1.7.2 Physiological stress response

The physiological stress response is mediated by two main pathways: the sympatho-adrenal (SA) axis, which is activated within a fraction of a second, and the hypothalamic-pituitary-adrenal (HPA) axis, which takes longer for hormonal expression to appear (Palme et al., 2005). The adrenal glands are instrumental in both (Palme et al., 2005). With activation of the sympathetic nervous system, catecholamines (CAs), such as epinephrine, and norepinephrine, are released from the adrenal medulla and lead to the fight-or-flight response (Palme et al., 2005; Arun, 2004).

The HPA axis begins with the perception of a stressor, which leads to the release of corticotropin-releasing hormones (CRH) by the hypothalamus. CRH in turn acts on the anterior pituitary gland to release adrenocorticotropin hormone (ACTH), which goes through the blood to the adrenal gland and signals the release of glucocorticoids (GCs) (Dedovic et al., 2009) (Figure 1-6). This pathway is under negative feedback control; with a stress response the release of GCs halts further production and release of elevated GCs (Sheriff et al., 2011). However, with long-term exposure to a stressor, the negative feedback response may be reduced or disrupted (Mizoguchi et al., 2001; Mizoguchi et al., 2003). The release of GCs leads to the reallocation of resources and processes including gluconeogenesis, fat breakdown to release glucose, and reproductive suppression, all to better allow the animal to survive the immediate threat (Whirledge and Cidlowski, 2010). There are two major GCs:

© University of Pretoria

cortisol and corticosterone; cortisol is the primary GC hormone produced in most mammals, including tigers, while corticosterone is the most abundant GC hormone in birds, reptiles, amphibians and most rodents (Boonstra, 2004).

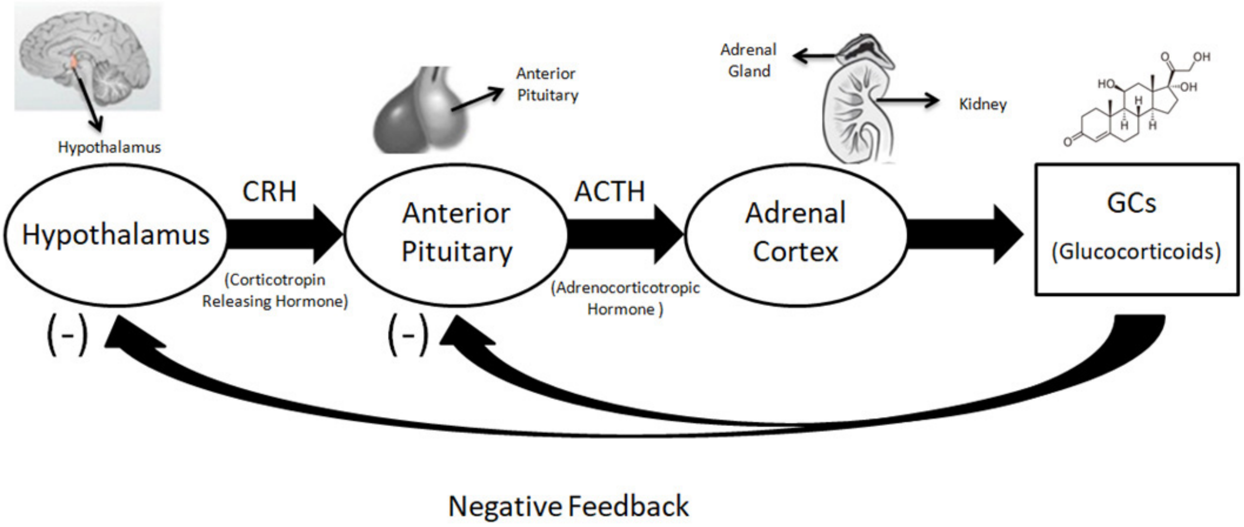


Figure 1-6: Schematic diagram of the HPA Axis with feedback loops.Source: Bruce Crossey, MSc thesis.

1.7.3 Short- and long-term stress

The duration of the perception of a stressor can be categorized into two main groups: short-term and long-term. Short-term stress is short-lived and the hormone release can be beneficial by mobilising energy reserves, whereas long-term stress is long lasting and consequentially can negatively impact the well-being of animals through chronically elevated GCs, leading to effects such as lowered immune responses and increased blood pressure, and can lead to long-term damage or even death (Dhabar, 2009). However, frequent exposure to short-term stressors can also lead to a long-term stress response by the HPA axis (Busch et al., 2008). Wild animals face both natural and anthropogenic stressors such as predators, finding food, social hierarchy, weather events, urbanization, and habitat degradation, but not all stressors

© University of Pretoria

effect animals in the same way or even affect them at all (Reeder and Kramer, 2005; Norris and Carr, 2013). However, most often you will not find chronically stressed animals in the wild unless they are sick, have long-term environmental stressors, anthropogenic stress, or high levels of social stress (Sapolsky, 1990; Terio et al., 2004). Captive animals are often healthier and longer lived than wild species, but the actual effect of captivity on the stress response of an individual can vary and can differ from setting to setting as well as species to species (Mason, 2010). Finally, in addition to short- and long-term stressors, there are cases of adaptive stress responses where animals maximise reproductive output, often in a single season, leading to reduced survival (Delehanty and Boonstra, 2011). This is a phenomenon most often observed in animals that have a single breeding season in their life (Boonstra and Boag, 1992), an exception being the arctic ground squirrels (*Spermophilus parryi plesius*) which are not dasyurids, but do have high male mortality after the breeding season (Boonstra et al., 2001).

1.7.4 Monitoring stress

Stress can be monitored either through behavioural patterns, heartrate, or by quantifying hormone concentrations (Keeling and Jensen, 2002; Möstl and Palme, 2002; Von Borell et al., 2007). Behaviours indicative of stress are any abnormal or stereotyped behaviours that would not be seen under usual circumstances (Keeling and Jensen, 2002). These are often present in captive animals that have undergone long-term exposure to stressors or lack of stimulation, and can present in such ways as pacing or excessive grooming (Carlstead, 1996). However, stereotyped behaviour does not necessarily mean that the HPA axis is activated (Burgener el al., 2008; Fureix et al., 2013). Activity budgets are a way to monitor behaviour and the time spent performing different behaviours throughout the day (e.g., resting, eating etc. which can be measured with some accelerometers), and altered activity budgets have been used as indicators of stress in Back Grouse (*Lyrurus tetrix*; Arlettaz et al., 2015) and cows (*Bos taurus*; Cook et al., 2007). Additionally, behavioural diversity indices, which

© University of Pretoria

measure the variety of behaviours performed, and usually equate fewer behaviours to poorer welfare, have also been used (Miller et al., 20). Moreover, vocalisations have been used in domestic cats (*Felis silvestris catus*; Urrutia et al., 2019) as well as pigs (*Sus scrofa domesticus*; Von Borell et al., 2009) as indicators of stress, and more recently, facial expressions have been investigated for what they may reveal about animal stress and welfare (Descovich et al., 2017). Measuring heart rate variability (HRV) is a non-invasive method that can relay information about changes in the sympatho-adrenal axis in relation to diseases, or environmental or physiological stressors (Von Borell et al., 2007). This is usually done using inter-beats intervals and can give only broad insights into disruptions in the SA axis (Singer, 1995; Von Borell et al., 2007). It is measured using electrocardiograms (ECGs), Polar recorders, portable HR devices, or measuring the arterial pulse (Kovács et al., 2014). Interestingly, Martinez et al. (2006) suggested the use of an ingestible pill that has a microphone that can capture heart rate. Heart rate variability requires standardization as many factors can impact heart-rate variability, and baseline measurements should be taken when the animals are resting (Langbein et al., 2004; Hagen et al., 2005). There is also some evidence that using infra-red thermography to monitor skin temperatures can indicate compromised welfare, potentially indicative of stress, but this needs to be investigated further (Whitham and Miller, 2016).

Stress-related physiological biomarkers, like GCs, can be quantified directly through the analysis of plasma or serum samples collected invasively from the animals of interest or determined non-invasively through the utilization of alternative matrices (Ganswindt et al., 2012). Invasive techniques, such as drawing blood, although with some advantages such as easier sample preparation and real-time hormone levels, have drawbacks that have led to a burgeoning interest in using non-invasive methods (Hodges et al., 2010). Among these drawbacks is that the sampling procedure, the involvement of capture, restraint, and handling of animals, can all lead to immediately heightened levels of GCs and thus results that do not accurately reflect the hormone concentrations from the time period of interest (Hodges et al., 2010).

© University of Pretoria

1.7.5 Non-invasive hormone monitoring

Non-invasive techniques for the quantification of GCs and their metabolites include using faecal matter, urine, saliva, or feathers (Sheriff et al., 2011; Ganswindt et al., 2012), and can provide information about hormone levels over a length of time (Berk et al., 2016). In faeces and urine, GC metabolites are measured, as GCs are not always present in the matrix (Hodges et al., 2010, Lattin et al., 2011). With faecal samples, conjugated or unconjugated steroids are excreted after passing from the blood and bile to the gastrointestinal tract (Hodges et al., 2010). Compared to invasive techniques, advantages of faecal sampling include the ease of collection and the ability to collect multiple samples from one individual without impacting their behaviour (Touma and Palme, 2005). Moreover, as these samples are representative of a period of time, rather than an instant as with plasma samples, they are less likely to be influenced by stochastic events (Palme, 2012). This is often advantageous for longer term sampling of a chronically active HPA (Stubsjøen et al., 2015). With faecal samples, only a small amount of matter needs be collected, whereby the sample is frozen to inhibit changes in the faecal glucocorticoid metabolite (fGCM) composition due to microbial and environmental degradation (Hodges et al., 2010). However, knowledge of the physiology of the study species is important as there is interspecific variation in terms of the quantity of metabolites excreted (Palme et al., 2005; Hodges et al., 2010). There are also disadvantages to this method, including the time-consuming preparation of the sample (Hodges et al., 2010) and how the sample can degrade if not stored properly (Majelantle et al., 2020). Additionally, in the wild when studying herd animals, it can be hard to identify which individual provided the sample (Ganswindt et al., 2012) and this needs to be accounted for as well as the lag time that occurs due to animals' digestive times and activity patterns (Touma and Palme, 2005). In addition, there are many factors that can influence the metabolism and excretion of fGCMS (Palme, 2012). These can be sexual, as males and females often vary in their hormone metabolism, dietary, as different food items can change hormone metabolism, temperature related, with different temperatures effecting the metabolic rate of animals, or changes in the

© University of Pretoria

bacterial composition in the gut as this is what is largely responsible for hormone metabolism (Goymann, 2012). All of these factors may influence results, and thus, conclusions that can be drawn, and as such, need to be thoroughly investigated and considered for each individual study (Goymann, 2012).

1.7.6 Assay Validation

When using a particular non-invasive technique for a species for the first time, the respective approach has to be carefully validated to examine the applicability for the species-specific hormone matrix of interest to ensure a reliable quantification of respective GC metabolites (Touma and Palme, 2005; Sheriff et al., 2011). Assay validations usually include analytical approaches such as sensitivity, precision, accuracy, and specificity, as well as physiological or biological approaches (Touma and Palme, 2005). Physiological validations cause controlled changes in circulating GCs, most often involving injecting the animal with a synthetic hormone, by pharmacologically activating part of the HPA axis (Palme, 2019). A benefit of this method is that these validations directly activate the adrenal glands, and thus the entirety of the HPA axis is not stimulated (Palme, 2019). Biological validations are when the animal undergoes an event perceived to be stressful (*e.g.* translocation) (Touma and Palme, 2005). Unlike with physiological validations which require authorization to perform, biological validations do not necessarily require prior permission and are, in this regard, often easier to conduct on endangered species (Palme, 2019). Additionally, if no activation of the HPA axis is found to have occurred, it is not conclusive, as the event in question may simply not have been a large enough stressor to have caused a response (Palme, 2019).

1.7.7 Stress monitoring in Tigers

Non-invasive hormone monitoring has been used for assessing the stress responses of tigers under various environmental conditions. Naidenko et al. (2011) showed that wild Siberian tigers have higher fGCM concentrations and a more distinct seasonal pattern than captive

© University of Pretoria

individuals, presumably due to unfavourable winter conditions. A study on captive Bengal and Sumatran tigers found that females have comparatively higher GC/fGCM concentrations than their male counterparts, with no significant difference between GC/fGCM concentrations of the two proposed sub-species (Narayan et al., 2013). Bhattacharjee et al. (2015) looked at the effect that anthropogenic activity had on the fGCM concentrations of reintroduced tigers in India and found that more humans and livestock, as well as closer proximity to roads, led to increased levels of fGCM concentrations. They also found in this study that females had comparatively higher fGCM concentrations than males and concluded that the high stress-related hormone levels were leading to lower reproductive success (Bhattacharjee et al., 2015). A recent study also looked at fGCM concentrations in reintroduced tigers, comparing two reserves where the population growth differed considerably and found that the levels of anthropogenic disturbance as well as habitat complexity, including but not limited to terrain, water and prey availability were major determinants in how affected the tigers were (Malviya et al., 2018). Interestingly, a study by Sajjad et al. (2011) found no differences in plasma GC levels in tigers housed in zoos compared to individuals in a wildlife park. However, blood may have been a suboptimal matrix due to elevated GC concentrations caused by capture and restraint. As mentioned earlier, limited space and more unnatural and un-enriched environments lead to higher GC concentrations in tigers (Breton and Barrot, 2014), but there are other contributing factors as well; e.g. transportation leads to elevated GC levels of up to ~500% above baseline (Dembiec et al., 2004) and time of collection can impact GC concentrations due to the impact circadian rhythms have on the HPA axis (Kalsbeek et al., 2012).

1.8 Project rationale

Tigers are endangered and, with only a few thousand left in the wild, their ability to persist in their natural environment for generations to come is not guaranteed (Damania et al., 2008; Luo et al., 2008; Watson et al., 2010). As there are many challenges with the conservation

© University of Pretoria

methods currently applied and in the majority of their remaining habitat, where numbers are still decreasing, it would be advisable to look at alternative conservation and welfare measures to improve the situation for the species as a whole. By examining how different stress-associated variables, both environmental and anthropogenic in origin, affect tiger welfare, we can work towards improved conditions for these cats everywhere. Specifically, with the unique situation in South Africa of having free-roaming tigers, there is the opportunity to learn about the welfare of tigers living in an ex-situ environment and how this can be used to further the conservation efforts of this species.

1.9 Project aim and research questions

1.9.1 Aim

The aim of this study was to quantify fGCMs as a measure of physiological stress in relation to environmental and anthropogenic variables, in tigers living in three different environments: naturally occurring habitats, free-roaming in an exotic habitat, and in captivity. For the purposes of this study, the tigers in their native, naturally occurring habitats are referred to as "wild". "Free-roaming" which is used interchangeably with "re-wilded" refers to tigers in exotic habitats that are allowed to move more or less freely across large areas of land and hunt for themselves. Finally, "captive" in the context of this thesis, refers to animals kept in zoos/small indoor/outdoor enclosures that have are regularly fed by humans and have comparatively limited space.

1.9.2 Specific objectives

- Examining the impact of local environmental changes and sex on fGCM concentrations in free-roaming tigers in South Africa.
- Examining the impact of anthropogenic variables (*e.g.* proximity to visitors) on fGCM concentrations in free-roaming tigers in South Africa.

© University of Pretoria

- Compare baseline fGCM concentrations of captive tigers with those from free-roaming tigers in South Africa and India.

© University of Pretoria

CHAPTER 2 DATA CHAPTER

Non-invasive monitoring of glucocorticoid metabolite concentrations in native Indian, we as well as captive and re-wilded tigers in South Africa*

***Published in *General and Comparative Endocrinology*, Volume 308.**

© University of Pretoria

2.1 Abstract

Over the last century, wild tiger (*Panthera tigris*) numbers have declined from over 100,000 individuals to fewer than 4,000, with animals now confined to less than 5% of their historic range due to habitat loss, persecution, inadequate management, and poaching. In contrast, 15,000 - 20,000 tigers are estimated to be housed in captivity, experiencing conditions vastly different than their wild counterparts. A total of 280 tigers are currently held at 44 different facilities within South Africa, including zoos, semi-captive re-wilded populations, and pets; these animals provide a unique opportunity to measure the impact of extrinsic factors, found in exotic habitats, on the adrenocortical activity of tigers. By monitoring and comparing stress-related faecal glucocorticoid metabolite (fGCM) concentrations of tigers housed at different locations, and free ranging tigers in natural tiger reserves, this project aimed to get a better understanding of the impact of extrinsic factors on adrenocortical function as a measure of stress. The results of this study showed no significant difference in fGCM concentrations between captive, re-wilded, and free-ranging tigers with the exception of one site. Furthermore, factors such as sex and season were not significant drivers of fGCM concentrations. One study group had elevated fGCM concentrations, showing population variation in the stress response. This indicates that populations are able to cope with exotic environments, however, as population-specific differences in the stress response exist, we suggest management protocols be created for each population. This study offered the unique opportunity to see how well tigers are faring outside of their native range and if having re-wilded tigers in exotic locations is a potential welfare-acceptable management option for tiger conservation globally.

2.2 Introduction

Apex predators play important roles in ecosystem functioning (Ritchie et al., 2012) and can help protect against disease transmission (Wilmers et al., 2006), the effects of climate change (Pongsiri et al., 2009), and invasive species (Wallach et al., 2010). The loss of these top

© University of Pretoria

predators can unbalance an ecosystem and cause a trophic cascade (Wallach et al., 2015). Although important in their ecosystems, constant persecution has resulted in large carnivores being among the most threatened species globally (Woodroffe, 2000).

Tigers (*Panthera tigris*) have undergone severe range contractions and are at risk of extinction (Kenney et al., 2014). Due to habitat loss, persecution, poaching, and inadequate management and protection practices, tiger numbers have decreased from 100,000 to just below 4,000, and occupy <5% of their historic range (Dinerstein et al., 2007; Damania et al., 2008; Luo et al., 2008; Kenney et al., 2014; Duangchantrasiri et al., 2015; Wolf and Ripple, 2017).

Tigers under human care number between 15,000 to 20,000 individuals worldwide, and thus are five to seven times more abundant than their wild counterparts (Luo et al., 2008). Captive tigers experience environmental conditions vastly different from those encountered by their free-ranging counterparts (Szokalski et al., 2012). Firstly, compared to the size of their natural home ranges, which range from seven to 1000 km^2, captive tigers are often confined to small enclosures (Breton and Barrot, 2014). Furthermore, many captive tigers face major problems with in- and out-breeding practices (Nyhus et al., 2010). The captive environment can be detrimental and alter behaviour as has been seen in tigers; Pitsko (2003) showed that tigers kept in more stimulating and natural environments display more natural behaviours, including exploring, and less unnatural, stereotyped behaviours, such as pacing. Tigers within novel environments frequently encounter a range of stressors, and thus often display stereotypical behaviours; behavioural alterations that help animals cope with the perceived stressors of their unnatural surroundings and are generally considered an indication of stress in captive animals (Carlstead, 1996; Quirke et al., 2012; Mason et al., 2013).

The original definition of stress, proposed by Selye (1936), is the nonspecific response of the body to any demand; that is, any disruption to the body's homeostasis and the response of the animal. When a noxious stimulus is perceived by an individual, there are both behavioural and physiological responses, such as heightened alertness, the suppression of reproductive

© University of Pretoria

and feeding processes, increased heart rate and blood pressure, and the redirection of energy reserves (Stratakis and Chrousos, 1995; Vingerhoets and Perski, 2000). Physiologically, two main response axes are activated; firstly, the sympatho-adrenal axis which causes the release of catecholamines from the adrenal medulla in the fight-or- flight response (Palme et al., 2005; Arun, 2006) and secondly, the hypothalamic pituitary-adrenal (HPA) axis, which results in the secretion of glucocorticoids (GCs) (Dedovic et al., 2009). Short-term elevation in GC concentrations is often advantageous as it can boost immune responses and mobilize energy reserves (Dhabhar, 2009). However, long- term elevation of GC concentrations can be deleterious as it can negatively affect reproduction, survival, and fitness of an animal (Tilbrook et al., 2000; Glaser and Kiecolt-Glaser, 2005; Dhabhar, 2009). As such, GC concentrations can act as a robust proxy of adrenocortical function and animal health (Khansari et al., 1990; Pottinger, 1999; Lupien et al., 2009).

Glucocorticoids can be quantified via invasive or non-invasive techniques (Ganswindt et al., 2012). Invasive techniques, such as hormone quantification in blood, have advantages such as real-time hormone concentrations. However, they have major drawbacks such as the restraint of animals that can lead to heightened GC concentrations and thus biased results (Hodges et al., 2010). These drawbacks have led to a burgeoning interest in exploring the use of alternative matrices to study adrenocortical function; as a result, non-invasive approaches have become the cornerstone of hormone monitoring in wildlife (Hodges et al., 2010). Non-invasive faecal sampling in particular, has advantages including the ease of collection and the ability to collect multiple samples from one individual without requiring direct human-animal interaction or impacting individual behaviour (Touma and Palme, 2005). Additionally, as faecal samples integrate fGCM concentrations over a period of time, they are less likely to show fluctuations due to stochastic events (Touma and Palme, 2005). However, non-invasive techniques always need to be validated for a new species, either biologically using a presumed stressor such as transportation, or physiologically using a synthetic hormone (Palme, 2019). When the two methods are used in parallel, the most understanding is gained (Touma and Palme, 2005).

© University of Pretoria

Tigers within the captive environment offer a unique source to supplement sparse wild populations and ensure species survival. As such, facilities where tigers are kept should focus on enhancing management practices, specifically, removing unnecessary stressors within their immediate environment. Non-invasive endocrine monitoring in tigers offers a perfect tool to assess adrenocortical activity in the species, as previously shown (Naidenko et al., 2011; Narayan et al., 2013; Bhattacharjee et al., 2015; Malviya et al., 2018; Tyagi et al., 2019). This can benefit tigers by improving their welfare, fitness, survival, and reproduction, leading to the potential to reintroduce more tigers into the wild, a management programme that has already had some success (Bhattacharjee et al., 2015; Sarkar et al., 2016). The aim of this study was to quantify faecal glucocorticoid metabolites (fGCMs) as a measure of physiological stress in relation to season and environmental variables in tigers occurring in three different environments: the wild (within nature reserves in their native range in India), re-wilded (free roaming tigers in a reserve in South Africa that hunt for themselves and have no man-made shelter), and in captivity (tigers kept in relatively limited space in zoo-like indoor-outdoor enclosures).

2.3 Materials and Methods

2.3.1 Study animals and sample collection

Faecal samples were collected from tigers in eight locations; five in South Africa (Figure 2-1) and three in India (Figure 2-2). The five locations in South Africa included the National Zoological Gardens (NZG), Lory Park Zoo (LP), Predator World (PW), a pet tiger (hereafter referred to as House), and Tiger Canyon (TC). The three locations in India were Nehru Zoological Park (NZP), Kanha Tiger Reserve (KTR), and Bandhavgarh Tiger Reserve (BTR). Related information on climate (climate-data.org) and tiger location is given in Table 2-1. In South Africa, the specific subspecies are not known, but many are suspected to be hybrids of Bengal (*P.t. tigris*) and Siberian (*P.t. altaica*), while the Indian tigers we collected samples from were all Bengal tigers. Faecal samples for fGCM monitoring were collected seasonally in

© University of Pretoria

South Africa (winter: June – July 2019, and summer: December 2019 – January 2020). Due to logistical challenges, seasonal sampling could not be conducted at the three Indian sites, and samples were collected from the NZP between January and March of 2019, and in September of 2015 for KTR and BTR. No samples were kept in a freezer for longer than eight months prior to drying.

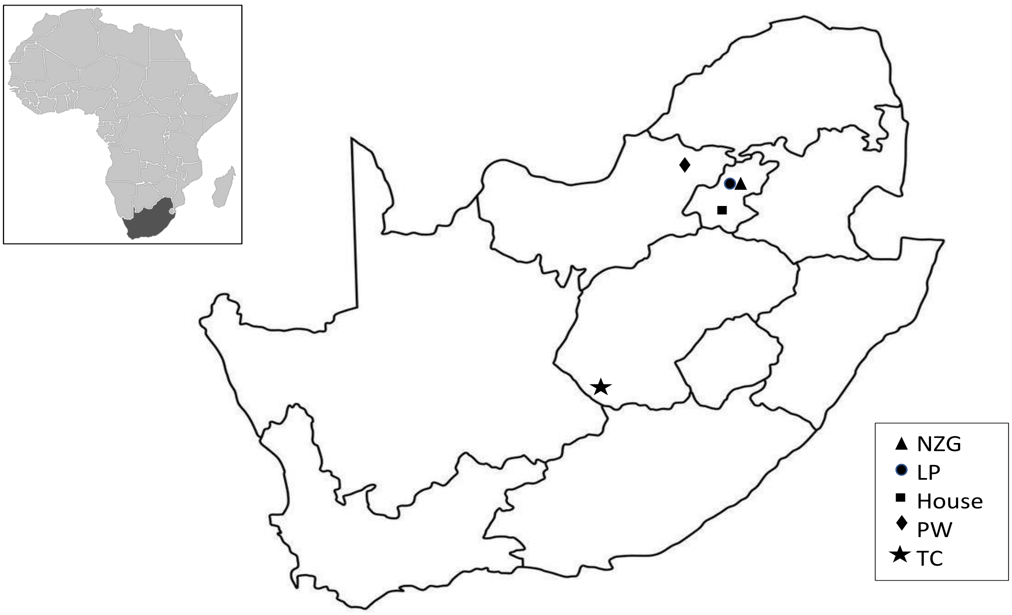


Figure 2-1: A map showing the location of the study sites within South Africa. The insert shows where South Africa is located on the African continent.

© University of Pretoria

Figure 2-2: A map showing the location of the study sites within India. The insert shows where India is located on the Asian continent.

© University of Pretoria

Table 2-1: Climate and sample data for all study sites. Rainfall is the average annual rainfall and temperature range is the average temperature from the coldest month to the average temperature from the warmest month. The coordinate for House is a general coordinate for the city of Johannesburg as exact coordinates are confidential. Visitors is the number of visitors in 2019. The number of samples reflects the number that were collected (W – winder, S – Summer) and analyzed, and individual collection refers to whether there could be individual identification of the faecal samples that were deposited.

	Location							
	NZG	**LP**	**PW**	**House**	**TC**	**NZP**	**KTR**	**BTR**
Environment	Captive	Captive	Captive	Captive	Re-wilded	Captive	Wild	Wild
Province	Gauteng	Gauteng	Northwest	Gauteng	Free State	Telangana	Madhya Pradesh	Madhya Pradesh
Coordinates	25.7361S, 28.1902E	26.0098S, 28.1522E	25.3553S, 27.1636E	26.2041S, 28.0473E	30.2514S, 25.0394E	17.3507N, 78.4513E	22.345N, 80.6115E	23.4158N, 80.5743E
Rainfall (mm)	697	697	663	790	391	766	1277	1277
Temp range (°C)	11.0-22.4	11.0-22.4	11.5-23.7	10.0-19.9	6.4-23.0	21.5-33.3	16.7-34.1	16.7-34.1
Visitors	340386	48251	5876	NA	655	NA	NA	NA
No. tigers	2F 1M	2F 2M	5F 3M	1M	~18 (var.)	11F 11M	60 total	60 total
Samples per season	6 W 2 S	109 W 70 S	21 W 21 S	4 W 2 S	39 W 24 S	39 total	36 total	20 total
Individual collection	Yes	Yes	No	Yes	No	Yes	No	No
Born at the facility	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Public view	Yes	2Yes 2No	Yes	No	Variable	Variable	Variable	Variable

At the NZG, three tigers were kept in two enclosures (a male-female pair and a single female). At night, animals were kept in separate night rooms; all faecal samples excreted in these rooms were collected the following morning. Lory Park has four tigers, kept in two enclosures. A male-female pair is housed in an enclosure open for public viewing; the remaining male-female pair is housed in a separate enclosure which is only accessible by LP staff members. After defecation, the tigers were moved into a holding pen to allow for safe sample collection. Predator World has eight tigers, housed in pairs or groups of three, and samples were collected in a similar fashion to LP. Samples collected from the pet tiger occurred after defaecation when he was moved into his holding pen.

Tigers located at TC were all born on-site and kept in three large fenced off areas averaging 1200 ha where they have to fend for themselves; these animals are defined as “re-wilded”. This study population fluctuated with births, deaths and translocations of young tigers old enough to disperse; a new area was also opened for tiger habitation during the study. Fresh faecal samples were collected opportunistically by driving around the areas, especially on boundary lines. Samples were positively identified as tiger samples using the information provided by Pagett (2007) and due to the fact that they were the only carnivores of that size in their enclosures.

During the study period Nehru Zoological Park had 22 tigers. Samples were collected every morning when the cages were cleaned and the tigers moved into a sectioned-off area. Kanha Tiger Reserve (940 km^2, ~60 tigers) and BTR (1598 km^2, ~60 tigers) are both located in the state of Madhya Pradesh. Fresh samples were collected during forest surveys of roads and trails during non-tourism (September). Sample age at BTR and KTR was morphologically identified by outline shape, moisture content, and smell (Tyagi et al., 2019). Samples were collected evenly across the reserve to eliminate any potential sampling bias. All samples were collected using gloves and placed into small, sealable sample bags and immediately put on ice until they could be frozen at -20°C. Gloves were replaced after every use to avoid cross-contamination of samples.

© University of Pretoria

This study was approved by the animal ethics committee of the University of Pretoria (NAS019/2019) as well as the South African National Biodiversity Institute (SANBI) NZG Research Ethics and Scientific Committee (P19/11). Research permission to collect tiger faecal samples from the Indian tiger reserves were also obtained (from the Principal Chief Conservator of Forests, Madhya Pradesh letter Reference No. 7616, dated 12 October 2014).

2.3.2 Alteration in fGCM concentrations post-defecation

To determine the stability of fGCM concentrations post-defecation, six additionally collected faecal samples from two LP tigers were pooled, well-mixed, subdivided into 21 subsamples, and placed outside in partial sun under a slatted roof. Over six days, the samples were exposed to the elements. The average ambient temperature was 17°C, with no cloud cover or rain during this period. Three subsamples were taken immediately after sub-dividing (0 hours) and three additional subsamples were taken at time intervals of 1 hour, 6 hours, 12 hours, 48 hours, 96 hours, and 144 hours as described by Webster et al. (2018), and immediately frozen at -20°C, a treatment that has been shown to have little effect on fGCM concentrations, even if frozen for years (Hunt and Wasser, 2003).

2.3.3 Steroid extraction and enzyme-immunoassay (EIA) analyses

The extraction process of the South African and Indian samples was identical apart from the initial drying process. Frozen faeces from South African tigers were lyophilized (as described by Heistermann et al., 2006), while respective samples from Indian tigers were dried in a hot air oven at 60°C for two - three days (as described by Naidenko et al., 2019). Subsequently, individual samples were pulverized and sieved through a mesh strainer to remove undigested material such as bones and/or plant material (Fieß et al. 1999). Following this, 0.100 – 0.110 g of dried faecal powder was extracted with 3 ml 80% ethanol. After vortexing for 15 minutes, the suspension was centrifuged for 10 minutes at 1500 g (Ganswindt et al. 2002). The

© University of Pretoria

supernatants were then transferred into microcentrifuge tubes, and stored at −20°C until analysis.

Steroid extracts were analysed using an 11-oxoetiocholanolone II EIA (detecting fGCMs with a 5β-3α-ol-11-one structure) as described by Möstl et al. (2002) following EIA validation (see below). The sensitivity for the assay, calculated at 90% binding, was 0.18 ng/g DW, while the coefficients of variance, determined by repeated measurements of high- and low-value quality controls, were 6.1% and 8.7% (intra-assay) and 13.3% and 15.5% (inter-assay). Serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curve (relative variation of the slope of the trendlines < 3%) (Figure 2-3). The same quality controls, steroid solution utilizing the commercially available 11-oxoetiocholanolone (5β-androstane-3α-ol-11, 17-dione) from Steraloids Inc. (product number: A3460-000), were used on both the South African and Indian sample extracts to ensure comparable results.

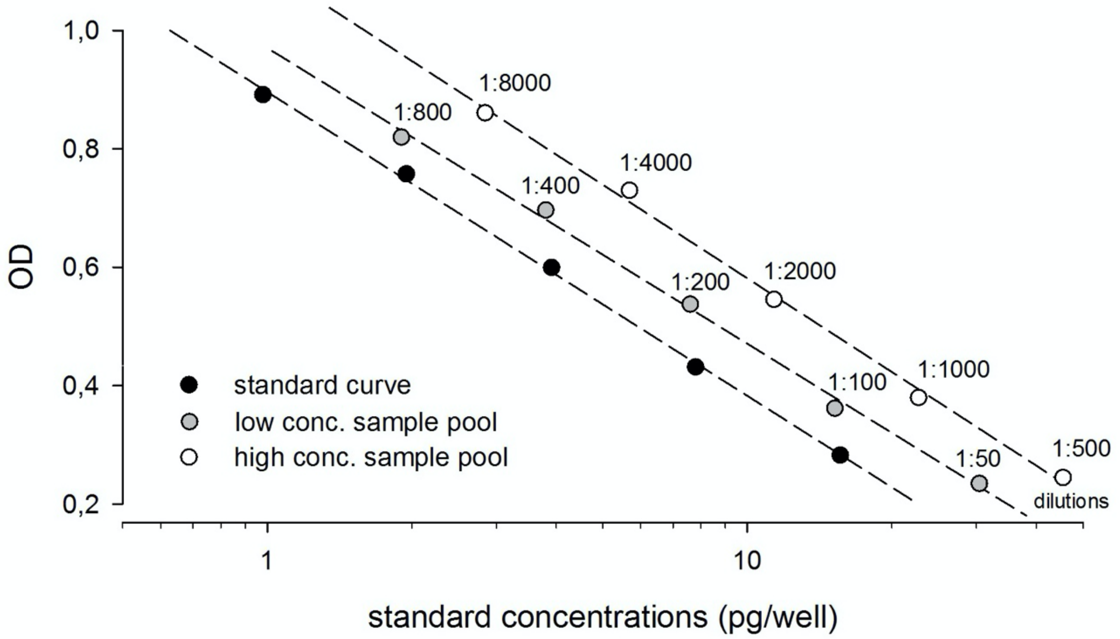


Figure 2-3: Parallelism test for serial dilutions of low and high concentration sample pools for the selected 11-oxoetiocholanolone II enzyme-immunoassay.

© University of Pretoria

All analyses of South African samples were performed at the Endocrine Research Laboratory, University of Pretoria, and all Indian samples were analysed at the Laboratory for the Conservation of Endangered Species, Centre for Cellular and Molecular Biology, as described by Ganswindt et al. (2002).

2.3.4 Biological validation of enzyme-immunoassays (EIA)

To evaluate EIA suitability, faecal extracts from a male and female Bengal tiger at the NZG were utilized. All faecal samples deposited 5 days prior to (total n=10) and 10 days post (total n=31) a translocation event were collected and analysed using five different EIAs: i) cortisol; ii) 11-oxoetiocholanolone I (detecting 11,17 dioxoandrostanes); iii) 11-oxoetiocholanolone II (detecting fGCMs with a 5β-3α-ol-11-one structure); iv) corticosterone; v) (iii) 5α-pregnane-3β,11β,21-triol-20-one (measuring 3β,11β-diol-cortisol metabolites). The respective assay characteristics including antibody cross-reactivities for the additional four tested EIAs are described by Palme and Möstl (1997) for the cortisol, 11-oxoetiocholanolone I, and corticosterone EIAs, and by Touma et al. (2003) for 5α-pregnane-3β,11β,21-triol-20-one EIA. The sensitivities for these four assays were 0.18 ng/g (cortisol and 11-oxoetiocholanolone I), 0.54 ng/g (corticosterone) and 0.72 ng/g (5α-pregnane-3β,11β,21-triol-20-one). The intra-assays coefficients of variance were 9.5% and 11.4% (cortisol), 5.5% and 6.2% (11-oxoetiocholanolone I), 4.5% and 7.9% (corticosterone), and 4.9% and 6.3% (5α-pregnane-3β,11β,21-triol-20-one), while the inter-assay coefficients of variance were 10.8% and 18.4% (cortisol), 13.9% and 17.7% (11-oxoetiocholanolone I), 6.9% and 14.5% (corticosterone), and 12.2% and 16.6% (5α-pregnane-3β,11β,21-triol-20-one).

The study was approved by the NZG Research Ethics and Scientific Committee (P14-09).

2.3.5 Statistics

© University of Pretoria

All analyses were conducted in R (R Core Team, 2017). All data were tested for normality using a Shapiro-Wilk goodness of fit test; only the post-defecation alteration data were parametric.

For the biological validation of the EIAs, median fGCM concentrations prior to the translocation event were used as the baseline for each animal and set as 0%. A repeated measure analysis of variance (ANOVA) was run to test for alterations in fGCM concentrations post-defaecation. The different time points at which the subsamples were taken were considered separate treatments. A general linear model (GLM) was conducted to determine the effect of season on fGCM concentrations; both rainfall and visitor number were found to be dependent on season and thus were not run as separate factors. Kruskal Wallis analyses of variance were run to quantify the effects of location and sex on fGCM concentrations. Additionally, a post-hoc pairwise Wilcoxon rank sum test was conducted to determine which locations had significant differences in fGCM concentrations, as well as to determine whether there was any difference between fGCM concentrations of on-display and off-display tigers at LP. Individual differences in fGCM concentrations were also tested at LP with a Kruskal Wallis analysis of variance followed by a Mann Whitney U post-hoc analysis. Differences in fGCM concentrations between individuals at other locations could not be conducted, as for most sites, only the sex of the tiger that provided the sample was supplied and not the individual identity. A Kruskal-Wallis analysis of variance was used to compare sexes in the different locations, with post-hoc Mann Whitney U tests, as well as to compare fGCM concentrations of pregnant and non-pregnant females at the NZP. All models were run with and without LP as a factor to determine any effects that location may have, and none were found.

2.4 Results

2.4.1 Biological validation of enzyme-immunoassays (EIA)

Although all tested EIAs showed a suitable increase in fGCM concentrations following the translocation event in both the female and male, the 11-oxoetiocholanolone II EIA displayed

© University of Pretoria

the biggest increases for both sexes when considered together (F: 274%, M: 81%) (Table 2-2). Both sexes had a peak increase in fGCM concentration in the first sample excreted post-translocation (F: 3 days, M: 1 day). As such, all further analyses were conducted using this EIA.

Table 2-2: The median baseline and peak fGCM concentrations (µg/g WW) as well as the % increase for the five EIAs tested in the biological validation study. The peak fGCM concentration was found to be in the first sample deposited post-translocation in both the female (3 days post-translocation) and the male (1 day post-translocation).

	Animal 1 - Female			**Animal 2 - Male**		
	Base-line fGCM	**Peak fGCM**	**% increase**	**Base-line fGCM**	**Peak fGCM**	**% increase**
Cortisol	0.02	0.03	50%	0.03	0.13	351%
11-oxoetiocholanolone I	0.78	1.96	151%	0.79	0.64	-19%
11-oxoetiocholanolone II	1.37	5.11	274%	2.07	3.74	81%
5α-pregnane-3β,11β,21-triol-20-one	0.14	0.68	404%	0.29	0.39	36%
Corticosterone	0.18	0.28	52%	0.21	0.64	200%

2.4.2 Changes in fGCM concentrations post-defecation

Median fGCM concentrations fluctuated over time with maximum increases of 32.0% and 30.8% at six and 144 hours compared to fGCM concentrations immediately following defecation. However, respective fGCM concentrations did not differ significantly over time ($F_{6,14} = 0.54$, $P = 0.77$) (Figure 2-4).

© University of Pretoria

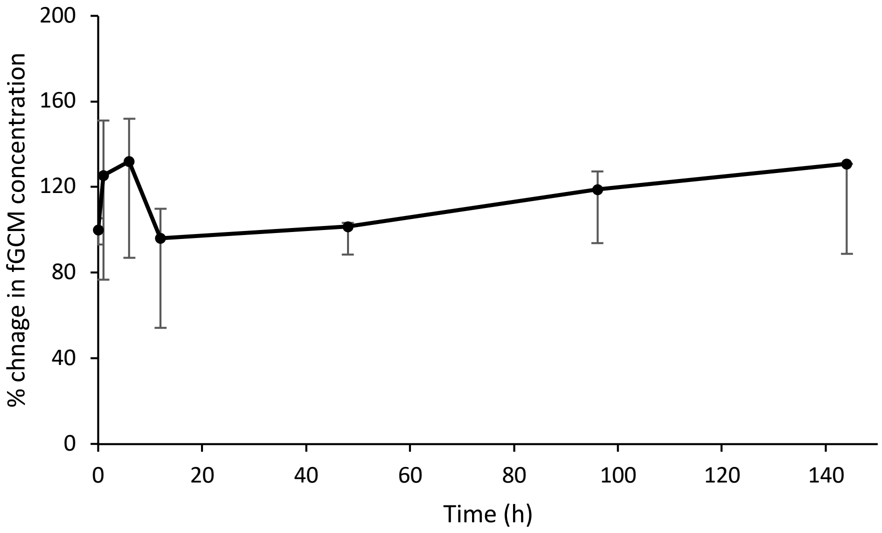


Figure 2-4: Relative change (%) in fGCM concentration in tiger (*Panthera tigris*) faeces over time (0h, 1h, 6h, 12h, 48h, 96h, 144h) post-defecation. The median, 25th, and 75th percentile values from each time point are shown and 100% concentration measured at Hour 0 was used to calculate the percent changes at all other time points.

2.4.3 fGCM concentrations of tigers at different locations

Tiger fGCM concentrations differed significantly between study locations (H_7 = 115.27, $P <$ 0.001), with fGCM concentrations of animals at LP being significantly higher compared to all other sites (H_7 = 115.27, $P < 0.001$). Overall median fGCM concentration of LP animals (16.70 µg/g DW) were up to 22-fold higher than respective values from animals at the other seven locations (0.76 – 3.46 µg/g DW) (Figure 2-5). With the exception of LP, there was no significant difference in fGCM concentrations between the South Africa and Indian study sites ($H=52_{52}$, P = 0.474).

© University of Pretoria

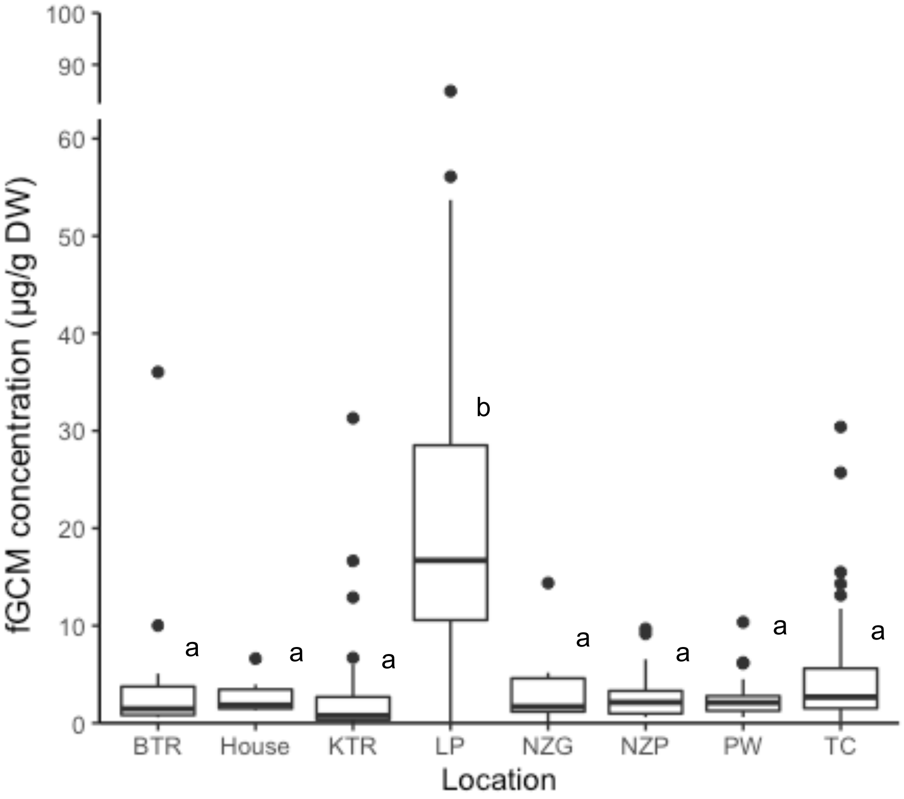


Figure 2-5: Boxplots of grouped fGCM concentrations (µg/g DW) in tigers (*Panthera tigris*) at the eight different study sites. The boxes show the median value and the upper and lower quartile values; the whiskers show the 10th and 90th percentiles of the values, the dots outliers. Different superscripts indicate significant differences between sites. N = 20 (BTR), six (House), 36 (KTR), 65 (LP), eight (NZG), 39 (NZP), 38 (PW), 61 (TC).

2.4.4 fGCM concentrations of tigers on/off display

At LP the two on-display animals (26.65 ± 15.34 µg/g DW) showed significantly higher fGCM concentrations (W = 713, $P < 0.01$) compared to the two animals off-display (17.07 ± 14.85 µg/g DW). When comparing individual fGCM concentrations the two individuals on display (Kimberley: 27.78 ± 14.65 µg/g DW; Ashan: 25.61 ± 16.48 µg/g DW) had significantly higher fGCM concentrations than one of the two off-display animals (Aaron: 12.17 ± 6.66 µg/g DW; $H_2 = 12.008$, $P < 0.01$), but not the other (Kiska: 21.98 ± 18.92 µg/g DW, W = 313, $P = 0.155$).

2.4.5 fGCM concentrations in relation to season, sex, and reproductive state

There was no significant difference in fGCM concentrations between seasons across the five South African study sites (winter: 11.21 ± 14.51 µg/g DW; summer: 8.31 ± 10.18 µg/g DW)

© University of Pretoria

(GLM, t_{177}= 0.218, P = 0.828). The Indian locations could not be included in the seasonal analysis as samples were only collected during one season.

There were no significant intra-site differences in fGCM concentrations between males (10.12 ± 11.73 µg/g DW) and females (15.92 ± 17.09 µg/g DW) (LP: W = 404, P = 0.105; PW: W = 139.5, P = 0.914; NZG: W = 6, P = 1.00; NZP: W = 55, P = 0.845; KTR: W = 105, P = 0.855; BTR: W = 52, P = 0.545) (Figure 2-6). For seven of the eight sites, females showed larger variations in their fGCM concentrations compared to males.

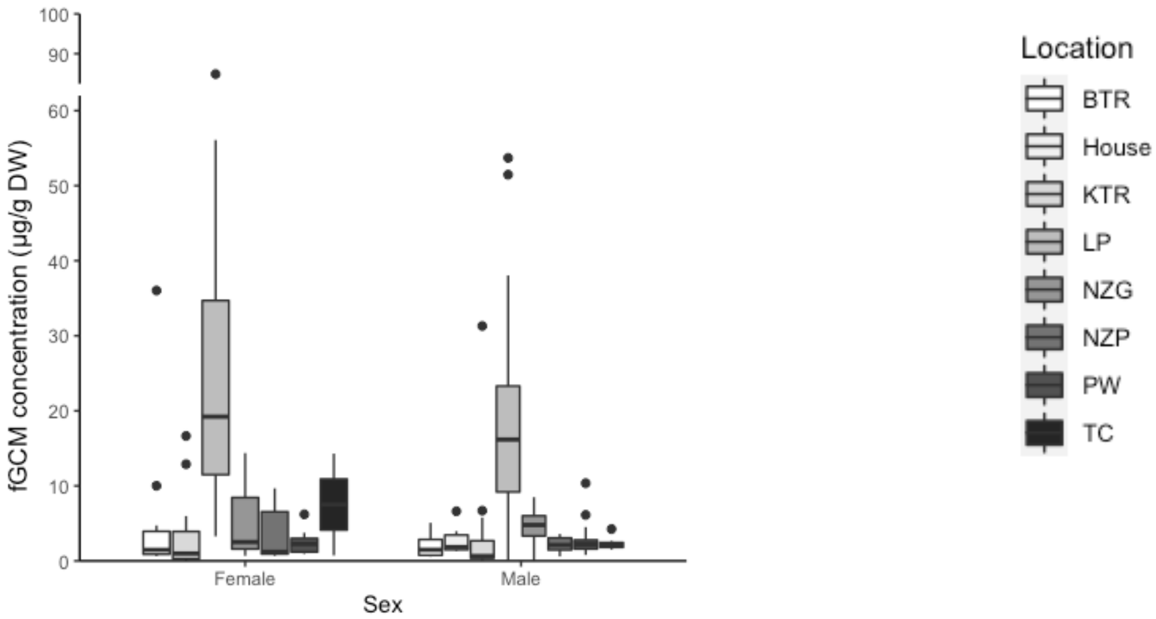


Figure 2-6: Boxplot of fGCM concentration (µg/g DW) of male and female tigers (*Panthera tigris*) across sites. The boxes show the median value and the upper and lower quartile values; the whiskers show the 10th and 90th percentiles of the values. House is only represented on the male side as the single tiger there was male.

Pregnant females at NZP had significantly higher fGCM concentrations (H_2 = 17.89, P < 0.001) than their non-pregnant counterparts (pregnant: 12.07 ± 8.09 µg/g DW, non-pregnant: 3.90 ± 3.77 µg/g DW, W = 24, P <0.01) as well as males (2.13 ± 1.01 µg/g DW, W = 19, P < 0.001) (Figure 2-7).

© University of Pretoria

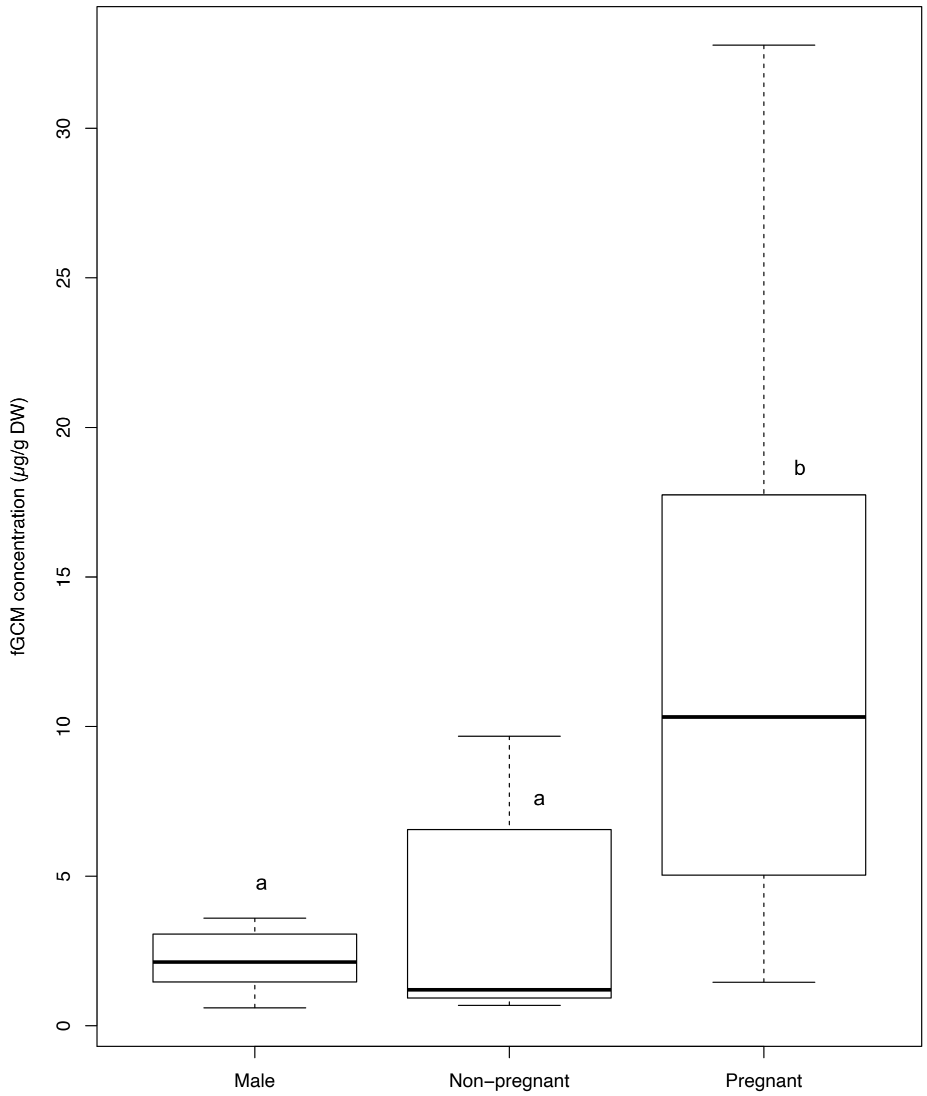


Figure 2-7: Boxplot (median, 25th percentile, 75th percentile) of fGCM concentration (µg/g DW) of males, pregnant, and non-pregnant female tigers (*Panthera tigris*) at NZP. Different superscripts indicate significant differences between groups. N = 13 (Male), 9 (Non-pregnant), 18 (Pregnant).

2.5 Discussion

This study on tigers was the first to implement non-invasive fGCM monitoring in captive, re-wilded, and free-ranging animals in native (India) and exotic environments (South Africa). We successfully established an EIA for measuring fGCM concentrations in faecal samples of tigers and showed that fGCM concentrations can be reliably determined up to 6 days post-defecation. Furthermore, a comparison of fGCM concentrations in captive, re-wilded, and free-

© University of Pretoria

ranging tigers revealed that in and of itself, being in an exotic environment does not increase fGCM concentrations. However, this is site and population specific, with elevated fGCMs found at one location. Additionally, sex and season do not influence fGCM concentrations, but reproductive status of females does.

This study used a biological validation, transportation in this case, to determine the most appropriate EIA for monitoring fGCM concentrations in tigers. This validation was successful for the 11-oxoetiocholanolone II assay detecting fGCMs with a 5β-3α-ol-11-one structure, which has also been used to measure fGCM concentrations in a variety of species including male leopards (*Panthera pardus*), (Webster et al., 2018), Samango monkeys (*Cercopithecus albogularis*) (Scheun et al., 2020), and giraffes (*Giraffa camelopardalis*) (Bashaw et al., 2016). In contrast to previous studies investigating alterations in immunoreactive fGCM concentrations post-defecation in predatory species indicating either a sudden increase (African clawless otter, *Aonyx capensis;* Majelantle et al., 2020; African wild dogs, *Lycon pictus*; Crossey et al., 2018) or decrease (brown hyena, *Hyaena brunnea*; Hulsman, 2010; banded mongoose, *Mungos mungo*; Laver et al., 2012) within 24 h of the defecation event, fGCM concentrations within tigers remained constant up to 144 h post-defecation. This is consistent with similar studies on other members of the *Panthera* genus: both jaguars (*Panthera onca*) and leopards have been shown to have stable fGCM concentrations for up to a week post-defecation (Mesa Cruz, 2014; Webster et al., 2018). Our finding is in direct contradiction to Parnell et al. (2015) who found that fGCM concentrations in their study on tigers differed significantly after 48 h. However, Parnell et al. (2015) used a different EIA, and antibody specificities can contribute to overall different patterns in fGCM concentrations, as seen in Lexen (2008), thus making the results incomparable. Although the prolonged comparability of fGCM concentrations found in this study allows for a more practical solution to sample collection as transects can be walked once a week rather than every day with no concern for alterations in fGCM concentrations (Webster et al., 2018), the degradation results of this study were performed under constant environmental conditions (~17 Celsius, no rainfall). Thus, further research into the rate of fGCM alteration in faeces exposed to varying

© University of Pretoria

environmental conditions in both winter and summer months should be conducted, as fluctuations in temperature, radiation, and UV exposure can all impact the rate of enzymatic bacterial metabolism of hormone metabolites (Lexen et al., 2008, Palme et al., 2013).

Due to the tigers at TC having to hunt for themselves and being more exposed to the environment and therefore experiencing more physical demands, we expected to see a difference in fGCM concentrations between the re-wilded and captive tigers. The lack of significance found in this study aligns with Sajjad et al. (2011), who found no significant difference in plasma cortisol concentrations between zoo tigers and tigers kept in a semi-natural environment where they had ample space but were still fed. While sample sizes were low and more should be collected in future studies, the lack of significance between TC and most of the other study sites may be due to the fact that all of these tigers were born in their respective settings and did not have to adjust to a new environment. It has been shown that in some species, genetic adaption to captive conditions can happen in a single generation, leading to behavioural and physiological adjustments that allow for better fitness (Håkansson and Jensen, 2005; Christie et al., 2012). To get an inclusive picture of the animal's welfare however, additional monitoring techniques to assess the health of captive and free-ranging animals should be implemented, including measuring heart rate and behavioural observations (Keeling and Jensen, 2002), especially stereotyped behaviours (Pitsko, 2003; Clubb and Vickery, 2006). Like almost all reserves, zoos, and parks, TC has anthropogenic stressors from fences and maintenance work being performed. However, the stress from close human interactions was minimal, having low visitor levels year-round. Thus, places like TC offer opportunities for a novel approach of conserving tigers. It allows the animals to live a more natural life in an exotic environment, while being free from many anthropogenic pressures they face in the wild including habitat infringement, poaching, and persecution. Furthermore, it can enhance people's education and understanding of these cats as they can observe them performing behaviours such as hunting which they would not be able to in captivity.

© University of Pretoria

Similar fGCM concentrations observed in this study at the majority of study sites indicate that captivity and management protocols are not main drivers of adrenocortical activity. In fact, these aspects are much less likely to cause increased fGCM concentrations than natural stressors like pregnancy. This contradicts what Naidenko et al. (2011) found in Siberian tigers, with wild tigers having significantly higher fGCM concentrations than captive tigers, which the authors explained by the wild tigers having higher metabolic activity due to their increased activity including hunting and other factors that also influenced their metabolism. In the same study, they found the wild tigers had the highest fGCM concentrations in winter when ambient temperatures decreased (Naidenko et al., 2011). The extreme conditions faced by wild tigers in the study by Naidenko et al. (2011) may explain the reported significant difference between wild and captive individuals compared to the more pleasant conditions experienced by tigers of this study. However, our finding is positive for tigers under human care, indicating that they do not seem to be under long-term stress that could negatively affect their fitness and survival. Nonetheless, as mentioned prior, all aspects of the animal's physiology and behaviour should be monitored to get a more inclusive understanding of their well-being. Additionally, although Narayan et al. (2013) found there to be no difference in fGCM concentrations between captive subspecies, we recommend genetic studies be conducted in South Africa to determine the specific subspecies being sampled to allow for more exhaustive comparisons to tigers elsewhere in the world

An exception to the comparable fGCM values found in free-ranging and captive tigers in this study are the four animals at LP. Although LP tigers were born in captivity, other factors such as enclosure size and quality, as well as proximity to both people and other animals may be the reason for the comparatively higher fGCM concentrations found in those animals. Where LP differed from the other sites was the proximity to visitors; the walkway past the cages was minimum 1.5 m closer here than at any other site. Moreover, the LP tigers on display, were bordered on one side by two lions, and by two jaguars on the other side, both possible competitors. A similar situation was shown to occur in clouded leopards (*Neofelis nebulosa*) when they were situated near potential predators (Wielebnowski et al., 2002), indicating that

© University of Pretoria

felids may be disturbed by the presence of top predators. Future studies should incorporate factors such as enclosure size, the proximity of human visitors and the presence of other predators, such as lions, to their analysis, as all of these have been shown to activate the physiological stress response (De Rouck et al., 2005; Vick et al., 2012; Vaz et al., 2017). Such information might assist in determining why populations such as the LP tigers showed such elevated fGCM concentrations.

The lack of winter/summer variation in fGCM concentrations in our captive study animals reflects what other studies have found on captive Siberian tigers (Byers et al., 1990; Naidenko et al., 2011). This is in contradiction to a study by Ivanov et al. (2017), where captive Siberian tigers had increased fGCM concentrations during lower ambient temperatures and only gradually adapt to temperature changes. In captivity, the general lack of seasonal variation in fGCM concentrations can most likely be explained by the constant availability of resources such as food and housing, which might minimise the impact of changing environmental factors (temperature/rainfall). Furthermore, captive tigers have likely adapted to these conditions and the presence of humans. Although exposed to season variation in temperature and vegetation cover, a constant prey base likely allowed TC tigers to maintain stable fGCM concentrations.

Pregnancy has been shown to cause higher fGCM concentrations in a number of species including Canadian lynx (*Lynx canadensis*) (Fanson et al., 2012), baboons (*Papio ursinus*) (Weingrill et al., 2004), and red squirrels (*Sciurus vulgaris*) (Dantzer et al., 2010). This same result was mirrored in our study, with pregnant females from NZP showing significantly elevated fGCM concentrations between sexes. The lack of significant difference in fGCM concentrations between sexes (when only including non-breeding females) align with results from Vaz et al. (2017), but contradict Narayan et al. (2013) and Parnell et al. (2014) who both found that females had comparatively higher fGCM concentrations than males. This phenomenon might be result of potential sexual differences in hormone metabolism and excretion, as found in domestic cats (*Felis silvestris catus*) and dogs (*Canis familiaris*) (Schatz and Palme, 2001), in combination with differences in the specificity of antibodies used to quantify fGCMs. The higher variation in fGCM concentrations found in females in this study

© University of Pretoria

could be due to the reproductive cycles of females, as cyclic expression of reproductive hormones during the different phases can impact fGCM concentrations (Palme et al., 2005; Kinoshita et al., 2011). The larger variations in the fGCM concentrations of the females are most likely, like Narayan et al. (2013) and Parnell et al. (2014) speculated, indicative of different stages in their reproductive hormone cycle (Kudielka and Kirschbaum, 2005; Palme et al., 2005; Fanson et al., 2012) which can lead to varying metabolic demands (Goymann et al., 1999; Touma et al., 2003; Cavigelli, 1999).

2.6 Conclusion

This study confirmed that fGCM concentrations can be reliably quantified in tigers using an 11-oxoetiocholanolone II EIA detecting fGCMs with a 5β-3α-ol-11-one structure and that tiger faecal samples can potentially be utilized up to a week after defecation, enabling easier sample collection and reliable results. While captive animals seem to have habituated to their surroundings, visitors included, this pattern was not repeated at one captive site, meaning that the physiological response to the same factors (humans/management regime etc.) might differ between populations, or subspecies, and should be kept in mind.

This study adds further support to the importance of tiger conservation, and the need to use a multi-faceted approach to understand tigers welfare and how best to manage and save the species. As current conservation methods have several shortcomings, especially when used in a less than preferred environment, it is imperative that new and existing techniques be included to ensure the robust measurements of animal welfare and population trends. By examining how different stress-associated variables, both environmental and anthropogenic in origin, affect tiger welfare, enhanced management protocols can be developed to ensure successful species conservation.

© University of Pretoria

CHAPTER 3 GENERAL DISCUSSON AND CONCLUSIONS

© University of Pretoria

3.1 General discussion

With a dwindling free-ranging population, captive tigers may be the key to saving this very vulnerable species as the wild individuals are facing an unprecedented number of threats (Goodrich et al., 2015; Wolf and Ripple, 2017). Of course, any intense management intervention such as this requires extensive research and knowledge to ensure the best possible practices are put in place.

Chapter two of this dissertation aimed to explore some of the nuances of captive tiger welfare. This was the first study to look into the welfare of free-ranging and captive tigers kept in a range of conditions in an exotic habitat. Tiger Canyon, with its population of free-ranging re-wilded individuals is unique worldwide with the tigers being free to hunt, form territories, compete with conspecifics and, to some extent, reproduce. The results found that captivity in and of itself does not influence fGCM concentrations above or below those levels found in wild individuals. Other factors taken into account in this study such as sex and season had no significant effect of fGCM concentrations of tigers while one location, level of proximity to humans, and reproductive status did cause significant increases.

3.2 Implications

Tigers in an exotic, free-ranging area did not have elevated adrenocortical activity in comparison to animals in India. This suggests that the welfare of tigers in captivity might not be negatively impacted if they are managed properly. For the majority of captive sites where human traffic was high, we saw no elevation in fGCM concentrations, which could indicate that either tigers are not affected by high levels of human presence or they are habituated against it. Despite this, we saw elevated fGCM concentrations for one captive population exposed to tourism, as well as behind the scenes, indicating location specific stressors, or population differences in the stress response. Thus, one management plan does not fit all locations. Actions should be taken to mitigate possible stressors such as making sure the tigers enclosures sit a distance away from the visitors, that they have spaces where they can retreat to if they so desire, and rules on noise levels and behaviour of visitors. Additionally, the

© University of Pretoria

layout of the enclosures should be well thought out to avoid having possible competitors (i.e. tigers and lions) adjacent to one another. The finding that sex did not have an impact on fGCM concentrations of captive and re-wilded tigers shows that while there may be fluctuations in females' fGCM levels with regards to their reproductive cycles, or sex-specific metabolic differences, they are not large enough to cause significant changes in this study. Similarly, with season, captive held tigers may face such stable conditions that temperature, rainfall, or other seasonal changes do not impact them greatly. Furthermore, in regards to season, the animals at TC may either not face large enough seasonal changes that it greatly impacts their HPA axis, or their prey availability is stable enough year-round that their fGCM concentrations remain stable. Finally, pregnancy, while stress-inducing, is a natural condition and thus heightened fGCM concentrations in pregnant females should not be an immediate reason for concern.

3.3 Future directions and research needs

Tracking fGCM concentrations of female captive and re-wilded South African tigers alongside their reproductive cycle would lead to a better understanding of how the two are interlinked and whether this may lead to females having a larger variation in their fGCM concentrations. Additionally, the seasonal effects on fGCM concentrations of Indian tigers were not investigated in this study due to a lack of time. However, understanding how wild and captive Indian tigers respond to season alongside their captive South African counterparts should be investigated. Furthermore, the fGCM concentrations of South African tigers should be compared to those of tigers from other locations to be able to identify, as much as possible, the potential difference in fGCM concentrations between putative subspecies. In line with this, management practices, as well as exposure to visitors should be investigated in more detail, the latter through monitoring factors such as noise levels, the number of children visiting, and the average proximity to the tigers for the period of any given sample. This would provide important information for informing management practices. In general, more samples should be collected as a few locations only provided a very limited sample set, and individual

© University of Pretoria

identification should be done at every location, especially TC, as looking at dominance and age class may play a role. Family history of all captive tigers should be included to see if there are any instances of inbreeding which may impact their welfare as well. Finally, as well as monitoring fGCM concentrations, behaviour and general health check-ups would allow for a more well-rounded view into the welfare of the animals.

3.4 Conclusions

The results presented in this dissertation add further support to the importance of conservation for tigers, and the need to use a multi-faceted approach to understand tigers' welfare and how best to manage and save the species. In their native habitat, many management plans are underway to conserve what is left of tigers and their natural habitat. However, in many non-native countries, there are not strict laws governing tigers and no consensus on how best to manage them, leading to vast differences in the conditions they are kept in and management protocols. This, of course, impacts their welfare. Increasing our understanding of environmental factors which can lead to a stress response in tigers can help people the world over to create the best possible situations for tigers, hopefully with the goal of bolstering their wild population numbers and stopping their decline.

© University of Pretoria

CHAPTER 4 REFERENCES

© University of Pretoria

Abbott, B. and Van Kooten, G.C., 2011. Can domestication of wildlife lead to conservation? The economics of tiger farming in China. *Ecological Economics*, *70*(4), pp.721-728.

Altman, J.D., Gross, K.L. and Lowry, S.R., 2005. Nutritional and behavioral effects of gorge and fast feeding in captive lions. *Journal of Applied Animal Welfare Science*, *8*(1), pp.47-57.

Arlettaz, R., Nusslé, S., Baltic, M., Vogel, P., Palme, R., Jenni-Eiermann, S., Patthey, P. and Genoud, M., 2015. Disturbance of wildlife by outdoor winter recreation: allostatic stress response and altered activity–energy budgets. *Ecological Applications*, *25*(5), pp.1197-1212.

Arun, C.P., 2004. Fight or flight, forbearance and fortitude: the spectrum of actions of the catecholamines and their cousins. *Annals of the New York Academy of Sciences*, *1018*(1), pp.137-140.

Atkinson, S., Crocker, D., Houser, D., Mashburn, K., 2015. Stress physiology in marine mammals: how well do they fit the terrestrial model? *Journal of Comparative Physiology B* 185(5), pp.463-486.

Bagchi, S., Goyal, S.P. and Sankar, K., 2003. Prey abundance and prey selection by tigers (*Panthera tigris*) in a semi-arid, dry deciduous forest in western India. *Journal of Zoology*, *260*(3), pp.285-290.

Barlow, A.C., 2009. The Sundarbans tiger: adaptation, population status and conflict management (PhD thesis). University of Minnesota, Saint Paul, USA.

Bascom, W., 1965. The forms of folklore: Prose narratives. *The Journal of American Folklore*, *78*(307), pp.3-20.

© University of Pretoria

Bashaw, M.J., Bloomsmith, M.A., Marr, M.J. and Maple, T.L., 2003. To hunt or not to hunt? A feeding enrichment experiment with captive large felids. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *22*(2), pp.189-198.

Bashaw, M.J., Kelling, A.S., Bloomsmith, M.A. and Maple, T.L., 2007. Environmental effects on the behavior of zoo-housed lions and tigers, with a case study of the effects of a visual barrier on pacing. *Journal of Applied Animal Welfare Science*, *10*(2), pp.95-109.

Bashaw, M.J., Sicks, F., Palme, R., Schwarzenberger, F., Tordiffe, A.S. and Ganswindt, A., 2016. Non-invasive assessment of adrenocortical activity as a measure of stress in giraffe (*Giraffa camelopardalis*). *BMC Veterinary Research*, *12*(1), pp. 235.

Bender, D.J., Contreras, T.A. and Fahrig, L., 1998. Habitat loss and population decline: a meta-analysis of the patch size effect. *Ecology*, *79*(2), pp.517-533.

Berk, S.A., McGettrick, J.R., Hansen, W.K., Breuner, C.W., 2016. Methodological considerations for measuring glucocorticoid metabolites in feathers. *Conservation Physiology* 4(1).

Berkson, G. (1983) Repetitive stereotyped behaviors. *American Journal of Mental Deficiency* 88(3), 239–246.

Bhattacharjee, S., Kumar, V., Chandrasekhar, M., Malviya, M., Ganswindt, A., Ramesh, K., Sankar, K. and Umapathy, G., 2015. Glucocorticoid stress responses of reintroduced tigers in relation to anthropogenic disturbance in Sariska Tiger Reserve in India. *PloS one*, *10*(6), p.e0127626.

© University of Pretoria

Biswas, S. and Sankar, K., 2002. Prey abundance and food habit of tigers (*Panthera tigris tigris*) in Pench National Park, Madhya Pradesh, India. *Journal of Zoology*, *256*(3), pp.411-420.

Boonstra, R., 2004. Coping with changing northern environments: the role of the stress axis in birds and mammals. *Integrative and Comparative Biology* 44(2), 95-108.

Boonstra, R. and Boag, P.T., 1992. Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones. *Journal of Animal Ecology*, pp.339-352.

Boonstra, R., McColl, C.J. and Karels, T.J., 2001. Reproduction at all costs: the adaptive stress response of male arctic ground squirrels. *Ecology*, *82*(7), pp.1930-1946.

Boorer, M.K. (1972) Some aspects of stereotypic patterns of movement exhibited by zoo animals. *International Zoo Yearbook* 12, 164–168.

Breitenmoser, U., Angst, C., Landry, J., Breitenmoser-Würsten, C., W., Linell, J.D.C., Weber, J. 2005. Non-lethal techniques for reducing depredation. In: Woodruffe R, Thirgood S, Rabinowitz A, eds. People and Wildlife: Conflict or Co-Existence? Cambridge University Press, Cambridge, UK, pp. 49–71.

Breton, G. and Barrot, S., 2014. Influence of enclosure size on the distances covered and paced by captive tigers (*Panthera tigris*). *Applied Animal Behaviour Science*, *154*, pp. 66-75.

Brittlebank, K., 1995. Sakti and Barakat: The Power of Tipu's Tiger: An Examination of the Tiger Emblem of Tipu Sultan of Mysore. *Modern Asian Studies*, *29*(2), pp.257-269.

© University of Pretoria

Brongersma, L.D., 1935. Notes on some recent and fossil cats, chiefly from the Malay Archipelago. *Zoologische Mededelingen*, *18*, pp.1-89.

Buchanan, K.L., 2000. Stress and the evolution of condition-dependent signals. *Trends in Ecology & Evolution* 15(4), pp.156-160.

Burgener, N., Gusset, M. and Schmid, H., 2008. Frustrated appetitive foraging behavior, stereotypic pacing, and fecal glucocorticoid levels in snow leopards (*Uncia uncia*) in the Zurich Zoo. *Journal of Applied Animal Welfare Science*, *11*(1), pp.74-83.

Busch, D.S., Sperry, T.S., Peterson, E., Do, C.T., Wingfield, J.C. and Boyd, E.H., 2008. Impacts of frequent, acute pulses of corticosterone on condition and behavior of Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *General and Comparative Endocrinology*, *158*(3), pp.224-233.

Byers, A.P., Hunter, A.G., Seal, U.S., Graham, E.F. and Tilson, R.L., 1990. Effect of season on seminal traits and serum hormone concentrations in captive male Siberian tigers (*Panthera tigris*). *Reproduction*, *90*(1), pp. 119-125.

Callealta, I., Ganswindt, A., Malan, M. and Lueders, I., 2019. Non-surgical artificial insemination using a GnRH analogue for ovulation induction during natural oestrus in African lions (*Panthera leo*). *Theriogenology*, *139*, pp.28-35.

Carillo, L., Johnson, L and Mendelson III, J.R., 2019. Principles of program development and management for amphibian conservation captive breeding programs. *International Zoo News*, *62*(2), pp.96-107.

© University of Pretoria

Carlstead, K., 1996. Effects of captivity on the behavior of wild mammals. University of Chicago Press, USA.

Carlstead, K. 1998. “Determining the causes of stereotypic behaviors in zoo carnivores: Toward appropriate enrichment strategies”. In *Second nature: Environmental enrichment for captive animals*, Edited by: Shepherdson, D. J., Mellen, J. D. and Hutchins, M. pp. 72–183. Washington, DC: Smithsonian Institution Press.

Cavigelli, S.A., 1999. Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. *Animal Behaviour*, *57*(4), pp. 935-944.

Cho, Y.S., Hu, L., Hou, H., Lee, H., Xu, J., Kwon, S., Oh, S., Kim, H.M., Jho, S., Kim, S. and Shin, Y.A., 2013. The tiger genome and comparative analysis with lion and snow leopard genomes. *Nature Communications*, *4*, p.2433.

Christie, M.R., Marine, M.L., French, R.A. and Blouin, M.S., 2012. Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Sciences*, *109*(1), pp. 238-242.

Chundawat, R.S., Gogate, N. and Johnsingh, A.J.T., 1999. Tigers in Panna: preliminary results from an Indian tropical dry forest. *Riding the tiger: tiger conservation in human-dominated landscapes*, pp.123-129.

Chundawat, R.S., Habib, B., Karanth, U., Kawanishi, K., Ahmad Khan, J., Lynam, T., Miquelle, D., Nyhus, P., Sunarto, S., Tilson, R. 2012. *Panthera tigris*. In IUCN (2012) Red List of Threatened Species Version 2012. http://www.iucnredlist.org.

© University of Pretoria

Clubb, R. and Vickery, S., 2006. Locomotory stereotypies in carnivores: does pacing stem from hunting, ranging, or frustrated escape. *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare*, *2*, pp. 58-79.

Collier, G.E. and O'Brien, S.J., 1985. A molecular phylogeny of the Felidae: immunological distance. *Evolution*, *39*(3), pp.473-487.

Cook, N.B., Mentink, R.L., Bennett, T.B. and Burgi, K., 2007. The effect of heat stress and lameness on time budgets of lactating dairy cows. *Journal of Dairy Science*, *90*(4), pp.1674-1682.

Cooper, D.M., Dugmore, A.J., Gittings, B.M., Scharf, A.K., Wilting, A., and Kitchener, A.C. (2016). Predicted Pleistocene–Holocene range shifts of the tiger (*Panthera tigris*). *Diversity and Distributions, 22*, pp.1199–1211.

Crossey, B., Ganswindt, A. and Chimimba, C., 2018. Faecal glucocorticoid metabolite concentrations and their alteration post-defaecation in African wild dogs *Lycaon pictus* from South Africa. *Wildlife Biology*, *2018*(1).

Cunningham, A.A., 1996. Disease risks of wildlife translocations. *Conservation Biology*, *10*(2), pp.349-353.

Daly, N., 2020. *Tiger Tests Positive For Coronavirus At Bronx Zoo, First Known Case In The World*. [online] National Geographic. Available at: <https://www.nationalgeographic.com/animals/2020/04/tiger-coronavirus-covid19-positive-test-bronx-zoo/> [Accessed 6 April 2020].

© University of Pretoria

Damania, R., Seidensticker, J., Whitten, A., Sethi, G., Mackinnon, K., Kiss, A. and Kushlin, A., 2008. A future for wild tigers. Smithsonian's National Zoological Park, Washington DC. USA.

Dantzer, B., McAdam, A.G., Palme, R., Fletcher, Q.E., Boutin, S., Humphries, M.M. and 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*(2), pp. 279-286.

Dasgupta, J., 2003. Transhumance pastoralists to sedentary farmers: A case study of the transformation of the Van Gujjar community of Rajaji National Park and Lansdowne Forest Division in Uttaranchal. *CSD (ibid)*.

De Rouck, M., Kitchener, A.C., Law, G. and Nelissen, M., 2005. A comparative study of the influence of social housing conditions on the behaviour of captive tigers (*Panthera tigris*). *Animal Welfare*, *14*(3), pp. 229.

Dedovic, K., Duchesne, A., Andrews, J., Engert, V. and Pruessner, J.C., 2009. The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. *Neuroimage*, *47*(3), pp. 864-871.

Delehanty, B. and Boonstra, R., 2011. Coping with intense reproductive aggression in male arctic ground squirrels: the stress axis and its signature tell divergent stories. *Physiological and Biochemical Zoology*, *84*(4), pp.417-428.

Dembiec, D.P., Snider, R.J. and Zanella, A.J., 2004. The effects of transport stress on tiger physiology and behavior. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *23*(4), pp.335-346.

© University of Pretoria

Desai, A. and Bhargav, P., 2010. Report on the Progress of Village Relocation: Nagarhole and Mudumalai Tiger Reserves. *New Delhi: National Tiger Conservation Authority*.

Descovich, K.A., Wathan, J., Leach, M.C., Buchanan-Smith, H.M., Flecknell, P., Framingham, D. and Vick, S.J., 2017. Facial expression: An under-utilised tool for the assessment of welfare in mammals. *Altex*.

Dhabhar, F.S., 2009. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation*, *16*(5), pp. 300-317.

Dinerstein, E., Loucks, C., Wikramanayake, E., Ginsberg, J., Sanderson, E., Seidensticker, J., Forrest, J., Bryja, G., Heydlauff, A., Klenzendorf, S. and Leimgruber, P., 2007. The fate of wild tigers. *AIBS Bulletin*, *57*(6), pp. 508-514.

Donoghue, A.M., Johnston, L.A., Seal, U.S., Armstrong, D.L., Tilson, R.L., Wolf, P., Petrini, K., Simmons, L.G., Gross, T. and Wildt, D.E., 1990. In vitro fertilization and embryo development in vitro and in vivo in the tiger (*Panthera tigris*). *Biology of Reproduction*, *43*(5), pp.733-744.

Donoghue, A.M., Byers, A.P., Johnston, L.A., Armstrong, D.L. and Wildt, D.E., 1996. Timing of ovulation after gonadotrophin in duction and its importance to successful intrauterine insemination in the tiger (*Panthera tigris*). *Reproduction*, *107*(1), pp.53-58.

Driscoll, C.A., Yamaguchi, N., Bar-Gal, G.K., Roca, A.L., Luo, S., Macdonald, D.W., and O'Brien, S.J. (2009). Mitochondrial phylogeography illuminates the origin of the extinct Caspian tiger and its relationship to the Amur tiger. *PLoS ONE, 4,* e4125.

© University of Pretoria

Duangchantrasiri, S., Umponjan, M., Simcharoen, S., Pattanavibool, A., Chaiwattana, S., Maneerat, S., Kumar, N.S., Jathanna, D., Srivathsa, A. and Karanth, K.U., 2015. Dynamics of a low-density tiger population in Southeast Asia in the context of improved law enforcement. *Conservation Biology*, *30*(3), pp. 639-648.

Duckler, G.L., 1998. An unusual osteological formation in the posterior skulls of captive tigers (*Panthera tigris*). *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *17*(2), pp.135-142.

Dunishenko, Y.M., Smirnov, E.N., Salkina, G.P., Nikolaev, I.G. 1999. Rules on how people should behave and maintain livestock in areas of tiger habitat in Primorsky Krai. Primorsky Primorsky Krai State Committee for Environmental Protection, Vladivostok, Russia.

Erwin, J. and Deni, R. 1979. “Strangers in a strange land: Abnormal behaviors or abnormal environments?”. In *Captivity and behavior: Primates in breeding colonies, laboratories, and zoos*, Edited by: Erwin, J., Maple, T. L. and Mitchell, G. 1–28. New York: Van Nostrand Reinhold.

Fàbregas, M.C., Fosgate, G.T. and Koehler, G.M., 2015. Hunting performance of captive-born South China tigers (*Panthera tigris amoyensis*) on free-ranging prey and implications for their reintroduction. *Biological Conservation*, *192*, pp.57-64.

Fanson, K.V., Wielebnowski, N.C., Shenk, T.M. and Lucas, J.R., 2012. Comparative patterns of adrenal activity in captive and wild Canada lynx (*Lynx canadensis*). *Journal of Comparative Physiology B*, *182*(1), pp. 157-165.

© University of Pretoria

Fieß, M., Heistermann, M. and Hodges, J.K., 1999. Patterns of urinary and fecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). *General and Comparative Endocrinology*, *115*(1), pp. 76-89.

Forthman-Quick, D.L. (1984) An integrative approach to environmental enrichment. *Zoo Biology,* 3, pp.65–77

Frank, L.G., Woodroffe, R.O.S.I.E. and Ogada, M.O., 2005. People and predators in Laikipia district, Kenya. *Conservation Biology Series – Cambridge*, *9*, p.286.

Fureix, C., Benhajali, H., Henry, S., Bruchet, A., Prunier, A., Ezzaouia, M., Coste, C., Hausberger, M., Palme, R. and Jego, P., 2013. Plasma cortisol and faecal cortisol metabolites concentrations in stereotypic and non-stereotypic horses: do stereotypic horses cope better with poor environmental conditions?. *BMC Veterinary Research*, *9*(1), p.3.

Galster, S., Schaedla, W. and Redford, T., 2010. Partnering to stop poaching: Developing cross-sector strategic responses to wildlife poaching. In *Tigers of the World* (pp. 113-124). William Andrew Publishing.

Ganswindt, A., Heistermann, M., Borragan, S. and Hodges, J.K., 2002. Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *21*(1), pp.27-36.

Ganswindt, A., Brown, J.L., Freeman, E.W., Kouba, A.J., Penfold, L.M., Santymire, R.M., Vick, M.M., Wielebnowski, N., Willis, E.L. and Milnes, M.R., 2012. International Society for Wildlife Endocrinology: the future of endocrine measures for reproductive science, animal welfare and conservation biology. *Biology Letters*, *8*(5).

© University of Pretoria

Germano, J.M., Field, K.J., Griffiths, R.A., Clulow, S., Foster, J., Harding, G. and Swaisgood, R.R., 2015. Mitigation-driven translocations: are we moving wildlife in the right direction?.*Frontiers in Ecology and the Environment*, *13*(2), pp.100-105.

Ghate, R., 2005. Relocation versus wildlife preservation. *Economic and Political Weekly*, pp.4807-4809.

Gispen, W.H. and Isaacson, R.L., 1981. ACTH-induced excessive grooming in the rat. *Pharmacology & Therapeutics*, *12*(1), pp.209-246.

Glaser, R. and Kiecolt-Glaser, J.K., 2005. Stress-induced immune dysfunction: implications for health. *Nature Reviews Immunology*, *5*(3), pp. 243-251.

Goodrich, J.M. and Miquelle, D.G., 2005. Translocation of problem Amur tigers *Panthera tigris altaica* to alleviate tiger-human conflicts. *Oryx*, *39*(4), pp.454-457.

Goodrich, J.M., 2010. Human–tiger conflict: a review and call for comprehensive plans. *Integrative Zoology*, *5*(4), pp.300-312

Goodrich, J.M., Miquelle, D.G., Smirnov, E.N., Kerley, L.L., Quigley, H.B. and Hornocker, M.G., 2010. Spatial structure of Amur (Siberian) tigers (*Panthera tigris altaica*) on Sikhote-Alin Biosphere Zapovednik, Russia. *Journal of Mammalogy*, *91*(3), pp.737-748.

Goodrich, J.M., Lynam, A., Miquelle, D.G., Wibisono, H.T., Kawanishi, K., Pattanavibool, A., Htun, S., Tempa, T., Karki, J., Jhala, Y.V., et al. (2015). *Panthera tigris*, Tiger. The IUCN Red List of Threatened Species.

© University of Pretoria

Gopal, R., Qureshi, Q., Bhardwaj, M., Singh, R.J. and Jhala, Y.V., 2010. Evaluating the status of the endangered tiger *Panthera tigris* and its prey in Panna Tiger Reserve, Madhya Pradesh, India. *Oryx*, *44*(3), pp.383-389.

Goymann, W., Möstl, E., Van't Hof, T., East, M.L. and Hofer, H., 1999. Noninvasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta*. *General and Comparative Endocrinology*, *114*(3), pp. 340-348.

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*(4), pp.757-765.

Gratwicke, B., Mills, J., Dutton, A., Gabriel, G., Long, B., Seidensticker, J., Wright, B., You, W. and Zhang, L., 2008. Attitudes toward consumption and conservation of tigers in China. *PloS One*, *3*(7).

Gurung, B., Smith, J.L.D., McDougal, C., Karki, J.B. and Barlow, A., 2008. Factors associated with human-killing tigers in Chitwan National Park, Nepal. *Biological Conservation*, *141*(12), pp.3069-3078.

Hagen, K., Langbein, J., Schmied, C., Lexer, D. and Waiblinger, S., 2005. Heart rate variability in dairy cows—influences of breed and milking system. *Physiology & Behavior*, *85*(2), pp.195-204.

Håkansson, J. and Jensen, P., 2005. Behavioural and morphological variation between captive populations of red junglefowl (*Gallus gallus*) – possible implications for conservation. *Biological Conservation*, *122*(3), pp. 431-439.

© University of Pretoria

Hammond, C.E., 1991. An excursion in tiger lore. *Asia Major*, 4(1), pp.87-100.

Heistermann, M., Palme, R. and Ganswindt, A., 2006. Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *American Journal of Primatology: Official Journal of the American Society of Primatologists*, *68*(3), pp. 257-273.

Hemmer, H., 1981. Die evolution der pantherkatzen modell zur überprüfung der brauchbarkeit der hennigschen prinzipien der phylogenetischen systematic für wirbeltierpaläontologische studien. *Paläontologische Zeitschrift*, *55*(1-2), pp.109-116.

Hodges, K., Brown, J., Heistermann, M., Kleiman, D.G. and Thompson, K.V., 2010. Endocrine monitoring of reproduction and stress. *Wild mammals in captivity: principles and techniques for zoo management*, The University of Chicago Press, USA, pp.447-468.

Hooijer, D.A. and Granger, W., 1947. Pleistocene remains of *Panthera tigris* (Linnaeus) subspecies from Wanhsien, Szechwan, China, compared with fossil and Recent tigers from other localities. American Museum novitates; no. 1346.

Hulsman, A.H., 2010. Non-invasive hormone monitoring in brown hyaenas (*Hyaena brunnea*). Research Project.

Hunt, K.E. and Wasser, S.K., 2003. Effect of long-term preservation methods on fecal glucocorticoid concentrations of grizzly bear and African elephant. *Physiological and Biochemical Zoology*, *76*(6), pp.918-928.

© University of Pretoria

Ivanov, E.A., Rozhnov, V.V. and Naidenko, S.V., 2017. The effect of ambient temperature on glucocorticoid level in the Amur tiger (*Panthera tigris altaica*). *Russian Journal of Ecology*, *48*(3), p .294.

Jackson, P. and Kemf, E., 1994. Wanted alive. *Tigers in the Wild. A WWF Status report. Gland, Switzerland: World Wide Fund for Nature*.

Johnson, W.E., Eizirik, E., Pecon-Slattery, J., Murphy, W.J., Antunes, A., Teeling, E. and O'Brien, S.J., 2006. The late Miocene radiation of modern Felidae: a genetic assessment. *Science*, *311*(5757), pp.73-77.

Jones, M.A., Mason, G. and Pillay, N., 2010. Early social experience influences the development of stereotypic behaviour in captive-born striped mice *Rhabdomys*. *Applied Animal Behaviour Science*, *123*(1-2), pp.70-75.

Kalman, B., 2004. *Endangered tigers*. Crabtree Publishing Company, New York, USA.

Kalsbeek, A., Van der Spek, R., Lei, J., Endert, E., Buijs, R.M. and Fliers, E., 2012. Circadian rhythms in the hypothalamo–pituitary–adrenal (HPA) axis. *Molecular and cellular Endocrinology*, *349*(1), pp.20-29.

Karanth, K.U., Madhusudan, M.D. 2002. Mitigating human– wildlife conflicts in Southeast Asia. In: Terborgh, J. ed. Making Parks Work: Strategies for Preserving Tropical Nature. Island Press, Washington, DC, pp. 250–63.

Karanth, K.U. and Gopal, R., 2005. An ecology-based policy framework for human-tiger coexistence in India. *Conservation Biology Series*, *9*, p.373.

© University of Pretoria

Karanth, K.U., 2006. *A view from the Machan: how science can save the fragile predator*. Permanent Black, Delhi, India.

Keeling, L. and Jensen, P., 2002. Behavioural disturbances, stress and welfare. *The Ethology of Domestic Animals: an Introductory Text, 3rd Edition*. CABI International, UK. pp. 79-98.

Kelt, D. A. and Van Vuren, D. H. 2001. The ecology and macroecology of mammalian home range area. *The American Naturalist*, *157*(6), pp. 637-645.

Kenney, J., Allendorf, F.W., McDougal, C. and Smith, J.L., 2014. How much gene flow is needed to avoid inbreeding depression in wild tiger populations?. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1789), pp. 20133337.

Khansari, D.N., Murgo, A.J. and Faith, R.E., 1990. Effects of stress on the immune system. *Immunology today*, *11*, pp.170-175.

Kinoshita, K., Inada, S., Seki, K., Sasaki, A., Hama, N. and Kusunoki, H., 2011. Long-term monitoring of fecal steroid hormones in female snow leopards (*Panthera uncia*) during pregnancy or pseudopregnancy. *PLoS One*, *6*(5), p.e19314.

Kirkpatrick, R.C. and Emerton, L., 2010. Killing tigers to save them: fallacies of the farming argument. *Conservation Biology*, *24*(3), pp.655-659.

Kitchener, A.C., Dugmore, A.J. 2000. Biogeographical change in the tiger, *Panthera tigris*. *Animal Conservation*, 3, 113–124.

© University of Pretoria

Kitchener, A.C., and Yamaguchi, N. (2010). What Is a Tiger? Biogeography, Morphology, and Taxonomy. In Tigers of the World: The Science, Politics and Conservation of *Panthera tigris*, Second Edition, R. Tilson, and P. Nyhus, eds. (Elsevier), pp. 53–84.

Kitchener, A.C., Breitenmoser, C., Eizirik, E., Gentry, A., Werdelin, L., Wilting, A., Yamaguch, N., Abramov, A.V., Christiansen, P., Driscoll, C.A., et al. (2017). A Revised Taxonomy of the Felidae. The final report of the cat classification task force of the IUCN/SSC Cat Specialist Group. CATnews *11*, 1–79.

Kitpipit, T., Tobe, S.S., Kitchener, A.C., Gill, P. and Linacre, A., 2012. The development and validation of a single SNaPshot multiplex for tiger species and subspecies identification—Implications for forensic purposes. *Forensic Science International: Genetics*, *6*(2), pp.250-257.

Kolmstetter, C., Munson, L. and Ramsay, E.C., 2000. Degenerative spinal disease in large felids. *Journal of Zoo and Wildlife Medicine*, *31*(1), pp.15-19.

Kolter, L. (1995) Control of behaviour and the development of disturbed behaviour patterns. In: Ganslober, U., Hodges, J.K. and Kaumanns, W. (eds) Research and Captive Propagation. Finlander Verlag, Furth, pp. 248–255.

Kovács, L., Jurkovich, V., Bakony, M., Szenci, O., Póti, P. and Tőzsér, J., 2014. Welfare implication of measuring heart rate and heart rate variability in dairy cattle: literature review and conclusions for future research. *Animal*, *8*(2), pp.316-330.

Kreger, M.D., Hutchins, M. and Fascione, N. (1998) Context, ethics, and environmental enrichment in zoos and aquariums. In: Shepherdson, D.J., Mellen, J.D. and Hutchins, M. (eds)

© University of Pretoria

Second Nature: Environmental Enrichment for Captive Mammals. Smithsonian Institution Press, Washington, DC, pp. 59–82.

Kudielka, B.M. and Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. *Biological Psychology*, *69*(1), pp. 113-132.

Laidler, L. and Laidler, K., 1996. *China's threatened wildlife*. Blandford, London.

Langbein, J., Nürnberg, G. and Manteuffel, G., 2004. Visual discrimination learning in dwarf goats and associated changes in heart rate and heart rate variability. *Physiology & Behavior*, *82*(4), pp.601-609.

Lasgorceix, A. and Kothari, A., 2009. Displacement and relocation of protected areas: a synthesis and analysis of case studies. *Economic and political weekly*, pp.37-47.

Latham, N.R. and Mason, G.J., 2008. Maternal deprivation and the development of stereotypic behaviour. *Applied Animal Behaviour Science, 110*(1-2), pp. 84-108.

Lattin, C.R., Reed, J.M., DesRochers, D.W. and Romero, L.M., 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. *Journal of Avian Biology*, *42*(3), pp. 247-252.

Laver, P.N., Ganswindt, A., Ganswindt, S.B. and Alexander, K.A., 2012. Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges. *General and Comparative Endocrinology*, *179*(2), pp. 178-183.

© University of Pretoria

Lei, W.E.I., XiaoBing, W., Zhu, L. and Jiang, Z., 2011. Mitogenomic analysis of the genus *Panthera*. *Science China Life Sciences*, *54*(10), pp.917-930.

Lexen, E., El-Bahr, S.M., Sommerfeld-Stur, I., Palme, R. and Mostl, E., 2008. Monitoring the adrenocortical response to disturbances in sheep by measuring glucocorticoid metabolites in the faeces. *Wiener Tierarztliche Monatsschrift*, *95*(3/4), pp. 64.

Li, B., Zhang, E., Liu, Z. 2009. Livestock depredation by Amur tigers in Hunchun Nature Reserve, Jilin, China. *Acta Theriologica Sinica,* 29, 231–8.

Linkie, M., Martyr, D.J., Harihar, A., Risdianto, D., Nugraha, R.T., Leader-Williams, N. and Wong, W.M., 2015. Editor's choice: Safeguarding Sumatran tigers: evaluating effectiveness of law enforcement patrols and local informant networks. *Journal of Applied Ecology*, *52*(4), pp.851-860.

Liu, Y.C., Sun, X., Driscoll, C., Miquelle, D.G., Xu, X., Martelli, P., Uphyrkina, O., Smith, J.L., O'Brien, S.J. and Luo, S.J., 2018. Genome-wide evolutionary analysis of natural history and adaptation in the world's tigers. *Current Biology*, *28*(23), pp.3840-3849.

Luo, S.J., Kim, J.H., Johnson, W.E., van der Walt, J., Martenson, J., Yuhki, N., Miquelle, D.G., Uphyrkina, O., Goodrich, J.M., Quigley, H.B., et al. 2004. Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *PLoS Biology* 2, e442.

Luo, S.J., Johnson, W.E., Martenson, J., Antunes, A., Martelli, P., Uphyrkina, O., Traylor-Holzer, K., Smith, J.L. and O'Brien, S.J., 2008. Subspecies genetic assignments of worldwide captive tigers increase conservation value of captive populations. *Current Biology*, *18*(8), pp.592-596.

© University of Pretoria

Lupien, S.J., McEwen, B.S., Gunnar, M.R. and Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), p.434.

Lyons, J., Young, R.J. and Deag, J.M., 1997. The effects of physical characteristics of the environment and feeding regime on the behavior of captive felids. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *16*(1), pp.71-83.

Madhusudan, M.D. and Karanth, K.U., 2002. Local hunting and the conservation of large mammals in India. *Ambio*, 31(1), pp.49-54.

Mainka, S.A. and Mills, J.A., 1995. Wildlife and traditional Chinese medicine: supply and demand for wildlife species. *Journal of Zoo and Wildlife Medicine*, 26(2), pp.193-200.

Majelantle, T.L., McIntyre, T. and Ganswindt, A., 2020. Monitoring the effects of land transformation on African clawless otters (Aonyx capensis) using fecal glucocorticoid metabolite concentrations as a measure of stress. *Integrative Zoology*.

Malviya, M., Kumar, V., Mandal, D., Sarkar, M.S., Nigam, P., Gopal, R., Sankar, K., Umapathy, G. and Ramesh, K., 2018. Correlates of physiological stress and habitat factors in reintroduction-based recovery of tiger (*Panthera tigris*) populations. *Hystrix, the Italian Journal of Mammalogy,* 29(2), pp.195-201.

Mardiastuti, A., 2018. Lessons learned from the tiger translocation and release in Tambling, Lampung, Indonesia. *Journal of Indonesian Natural History*, *6*(1), pp.42-47.

© University of Pretoria

Martinez, A., Schoenig, S., Andresen, D. and Warren, S., 2006, August. Ingestible pill for heart rate and core temperature measurement in cattle. In *2006 International Conference of the IEEE Engineering in Medicine and Biology Society* (pp. 3190-3193). IEEE.

Mason, G.J. (1991) Stereotypies and suffering. *Behavioural Processes,* 25, 103–115.

Mason, G.J., 2010. Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology & Evolution*, *25*(12), pp. 713-721.

Mason, G., Burn, C.C., Dallaire, J.A., Kroshko, J., Kinkaid, H.M. and Jeschke, J.M., 2013. Plastic animals in cages: behavioural flexibility and responses to captivity. *Animal Behaviour*, *85*(5), pp. 1113-1126.

Massei, G., Quy, R.J., Gurney, J. and Cowan, D.P., 2010. Can translocations be used to mitigate human–wildlife conflicts? *Wildlife Research*, *37*(5), pp.428-439.

Mazák, V. (1981). *Panthera tigris*. *Mammalian Species 152*, 1–8.

Mazák VJ. Der Tiger. Magdeburg: Westarp Wissenschaften (reprint of 1983 edition, 1996).

Mazák, J.H. (2010). Craniometric variation in the tiger (*Panthera tigris*): Implications for patterns of diversity, taxonomy and conservation. *Mammalian Biology 75*, 45–68.

McDougal, C., 1987. The man-eating tiger in geographical and historical perspective. *Tigers of the world: the biology, biopolitics, management, and conservation of an endangered species. New Jersey*, p.435.

© University of Pretoria

McKinney, M.L., 2002. Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *Bioscience*, *52*(10), pp.883-890.

Mesa Cruz, J.B., 2014. Non-invasive assessment of stress hormones, parasites, and diet, using scat of five felid species in Belize, Central America (Doctoral dissertation, Virginia Tech).

Meyer-Holzapfel, M. (1968) Abnormal behaviour in zoo animals. In: Fox, M.W. (ed.) Abnormal Behaviour in Animals. Saunders, London, pp. 476–503.

Miller, C.S., Petrunenko, Y.K., Goodrich, J.M., Hebblewhite, M.A.R.K., Seryodkin, I.V. and Miquelle, D.G., 2011. Translocation a success, but poaching remains a problem for Amur tigers. *Cat News*, *55*, pp.22-25.

Miller, L.J., Vicino, G.A., Sheftel, J. and Lauderdale, L.K., 2020. Behavioral diversity as a potential indicator of positive animal welfare. *Animals*, *10*(7), p.1211.

Mills, J.A. and Jackson, P., 1994. *Killed for a cure: a review of the worldwide trade in tiger bone*. Cambridge: Traffic International.

Miquelle, D.G., Smirnov, E.N., Merrill, T.W., Myslenkov, A.E., Quigley, H.B., Hornocker, M.G. and Schleyer, B., 1999. Hierarchical spatial analysis of Amur tiger relationships to habitat and prey. *Riding the tiger: tiger conservation in human-dominated landscapes. Cambridge University Press, Cambridge*, pp.71-99.

Miquelle, D.G., Goodrich, J.M., Smirnov, E.N., Stephens, P.A., Zaumyslova, O.Y., Chapron, G., Kerley, L., Murzin, A.A., Hornocker, M.G. and Quigley, H.B., 2010. The Amur tiger: a case

© University of Pretoria

study of living on the edge. *Biology and conservation of wild felids. Oxford University Press, Oxford, UK*, pp.325-339.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D.H. and Tabira, T., 2001. Chronic stress differentially regulates glucocorticoid negative feedback response in rats. *Psychoneuroendocrinology*, *26*(5), pp.443-459.

Mizoguchi, K., Ishige, A., Aburada, M. and Tabira, T., 2003. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. *Neuroscience*, *119*(3), pp.887-897.

Moberg, G.P., Mench, J.A., 2000. The biology of animal stress: basic principles and implications for animal welfare. CABI Publishing, United Kingdom.

MoEF. 2008. Minutes of the 11th Meeting of the Standing Committee of National Board for Wildlife (NBWL). 22 May, Ministry of Environment and Forests, http://envfor. nic. in/divisions/wildlife/Minutes_Standing_Committee.pdf.

Mondol, S., Bruford, M.W. and Ramakrishnan, U., 2013. Demographic loss, genetic structure and the conservation implications for Indian tigers. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1762), p.20130496.

Moreira, N., Brown, J.L., Moraes, W., Swanson, W.F. and Monteiro-Filho, E.L.A., 2007. Effect of housing and environmental enrichment on adrenocortical activity, behavior and reproductive cyclicity in the female tigrina (*Leopardus tigrinus*) and margay (*Leopardus wiedii*). *Zoo Biology*, *26*(6), pp.441-460.

© University of Pretoria

Morris, D. (1964) The response of animals to a restricted environment. Symposium of the Zoological Society, London 13, 99–120.

Möstl, E., Maggs, J.L., Schrötter, G., Besenfelder, U. and Palme, R., 2002. Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications*, *26*(2), pp. 127-139.

Möstl, E. and Palme, R., 2002. Hormones as indicators of stress. *Domestic animal endocrinology*, *23*(1-2), pp.67-74.

Naidenko, S.V., Ivanov, E.A., Lukarevskii, V.S., Hernandez-Blanco, J.A., Sorokin, P.A., Litvinov, M.N., Kotlyar, A.K. and Rozhnov, V.V., 2011. Activity of the hypothalamo-pituitary-adrenals axis in the Siberian tiger (*Panthera tigris altaica*) in captivity and in the wild, and its dynamics throughout the year. *Biology Bulletin*, *38*(3), pp. 301-305.

Naidenko, S.V., Berezhnoi, M.A., Kumar, V. and Umapathy, G., 2019. Comparison of tigers' fecal glucocorticoids level in two extreme habitats. *Plos One*, *14*(4), p.e0214447.

Narayan, E.J., Parnell, T., Clark, G., Martin-Vegue, P., Mucci, A. and Hero, J.M., 2013. Faecal cortisol metabolites in Bengal (*Panthera tigris tigris*) and Sumatran tigers (*Panthera tigris sumatrae*). *General and Comparative Endocrinology*, *194*, pp. 318-325.

Ning, Y., Qi, J., Dobbins, M.T., Liang, X., Wang, J., Chen, S., Ma, J. and Jiang, G., 2020. Comparative Analysis of Microbial Community Structure and Function in the Gut of Wild and Captive Amur Tiger. *Frontiers in Microbiology*, *11*, p.1665.

Norris, D.O., Carr, J.A., 2013. Vertebrate Endocrinology. Academic Press, Cambridge, USA.

© University of Pretoria

Nowell, K. and Ling, X., 2007. Lifting China's tiger trade ban would be a catastrophe for conservation. *Cat News*, *46*, pp.28-29.

Nugraha, R.T. and Sugardjito, J., 2009. Assessment and management options of human-tiger conflicts in Kerinci Seblat National Park, Sumatra, Indonesia. *Mammal Study*, *34*(3), pp.141-154.

Nyhus, P.J., Osofsky, S.A., Ferraro, P., Madden, F., Fischer, F. 2005. Bearing the cost of human–wildlife conflict: The challenges of compensation schemes. In: Woodroffe, R., Thirgood, S., Rabinowitz, A., eds. People and Wildlife: Conflict or Coexistence? Cambridge University Press, Cambridge, UK, pp.107–121.

Nyhus, P.J. and Tilson, R., 2010. *Panthera tigris vs Homo sapiens*: conflict, coexistence, or extinction. In *Tigers of the World* (pp. 125-141). William Andrew Publishing.

Nyhus, P.J., Caitlin, E.D., Ambrogi, M.C., Hart, S.E., Caroll, C., and Tilson, R., 2010. Human–tiger conflict over time. In: Tilson R, Nyhus PJ, eds. Tigers of the World: The Science, Politics, and Conservation of *Panthera tigris*, 2nd edn. Academic Press, Burlington, MA, USA, pp.132–135.

Nyhus, P.J., Tilson, R. and Hutchins, M., 2010. Thirteen thousand and counting: how growing captive tiger populations threaten wild tigers. In *Tigers of the World* (pp. 223-238). William Andrew Publishing.

Ormrod, S.A. (1987) Standards for modern captive animal management. In: Gibson, T.E. (ed.) Proceedings of the Animal Welfare Foundation's Fourth Symposium: The Welfare of Animals in Captivity. BVA Animal Welfare Foundation, pp. 22–27.

© University of Pretoria

Ortolani A, Caro TM. The adaptive significance of colour patterns in carnivores. In: Gittleman J, ed. Ithaca: Cornell University Press; 1996: 132–188. Carnivore Behaviour, Ecology ad Evolution; vol. 2.

Pagett, M., 2007. *What shat that?: the pocket guide to poop identity*. Random House Digital, Inc. New York, USA.

Palme, R. and Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Zeitschrift fuer Saeugetierkunde (Germany), 62*(2), pp. 192-197.

Palme, R., 2005. Measuring fecal steroids: guidelines for practical application. *Annals of the New York Academy of Sciences*, *1046*(1), pp.75-80.

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M. and Möstl, E., 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Annals of the New York Academy of Sciences*, *1040*(1), pp. 162-171.

Palme, R., 2012. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. *Animal Welfare-The UFAW Journal*, *21*(3), p.331.

Palme, R., Touma, C., Arias, N., Dominchin, M.F. and Lepschy, M., 2013. Steroid extraction: get the best out of faecal samples. *Wien Tierarztl Monatsschr*, *100*(9-10), pp. 238-46.

Palme, R., 2019. Non-invasive measurement of glucocorticoids: advances and problems. *Physiology & Behavior*, *199*, pp.229-243.

© University of Pretoria

Panwar, H.S., 1978. Decline and restoration success of the Central Indian barasinga (*Cervus duvauceli branderi).* In *IUCN Working Meeting of the Deer Specialist Group, Longview,* Washington. (USA), 26 Sep 1977. IUCN.

Parnell, T., Narayan, E.J., Magrath, M.J., Roe, S., Clark, G., Nicolson, V., Martin-Vegue, P., Mucci, A. and Hero, J.M., 2014. Evaluating physiological stress in Sumatran tigers (*Panthera tigris* ssp. *sumatrae*) managed in Australian zoos. *Conservation Physiology*, *2*(1).

Parnell, T., Narayan, E.J., Nicolson, V., Martin-Vegue, P., Mucci, A. and Hero, J.M., 2015. Maximizing the reliability of non-invasive endocrine sampling in the tiger (*Panthera tigris*): environmental decay and intra-sample variation in faecal glucocorticoid metabolites. *Conservation physiology*, *3*(1).

Pitsko, L.E., 2003. Wild tigers in captivity: a study of the effects of the captive environment on tiger behavior (Doctoral dissertation, Virginia Tech).

Pocock, R.I., 1929. Tigers. *Journal of the Bombay Natural History Society*, *33*, pp.505-541.

Pongsiri, M.J., Roman, J., Ezenwa, V.O., Goldberg, T.L., Koren, H.S., Newbold, S.C., Ostfeld, R.S., Pattanayak, S.K. and Salkeld, D.J., 2009. Biodiversity loss affects global disease ecology. *Bioscience*, *59*(11), pp. 945-954.

Pottinger, T.G., 1999. The impact of stress on animal reproductive activities. In: Balm, Paul H.M., (ed.) *Stress physiology in animals.* Sheffield, UK, Sheffield Academic Press, 130-177.

Priatna, D., Santosa, Y., Prasetyo, L.B. and Kartono, A.P., 2012. Home range and movements of male translocated problem tigers in Sumatra. *Asian Journal of Conservation Biology*, *1*(1), pp.20-30.

© University of Pretoria

Qin, Y., Nyhus, P.J., Larson, C.L., Carroll, C.J., Muntifering, J., Dahmer, T.D., Jun, L. and Tilson, R.L., 2015. An assessment of south China tiger reintroduction potential in Hupingshan and Houhe National Nature Reserves, China. *Biological Conservation*, *182*, pp.72-86.

Quigley, K.S., Evermann, J.F., Leathers, C.W., Armstrong, D.L., Goodrich, J., Duncan, N.M. and Miquelle, D.G., 2010. Morbillivirus infection in a wild Siberian tiger in the Russian Far East. *Journal of wildlife diseases*, *46*(4), pp.1252-1256.

Quirke, T., O'Riordan, R.M. and Zuur, A., 2012. Factors influencing the prevalence of stereotypical behaviour in captive cheetahs (*Acinonyx jubatus*). *Applied Animal Behaviour Science*, *142*(3-4), pp. 189-197.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rabinowitz, A.R., 1986. Jaguar predation on domestic livestock in Belize. *Wildlife Society Bulletin (1973-2006)*, *14*(2), pp.170-174.

Reeder, D.M., Kramer, K.M., 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. *Journal of Mammalogy,* 86(2), pp.225-235.

Ripple, W.J. and Beschta, R.L., 2003. Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park. *Forest Ecology and Management*, *184*(1-3), pp.299-313.

© University of Pretoria

Ripple, W.J., Estes, J.A., Schmitz, O.J., Constant, V., Kaylor, M.J., Lenz, A., Motley, J.L., Self, K.E., Taylor, D.S. and Wolf, C., 2016. What is a trophic cascade? *Trends in Ecology & Evolution*, *31*(11), pp.842-849.

Risdianto, D., Martyr, D.J., Nugraha, R.T., Harihar, A., Wibisono, H.T., Haidir, I.A., Macdonald, D.W., D'Cruze, N. and Linkie, M., 2016. Examining the shifting patterns of poaching from a long-term law enforcement intervention in Sumatra. *Biological Conservation*, *204*, pp.306-312.

Ritchie, E.G., Elmhagen, B., Glen, A.S., Letnic, M., Ludwig, G. and McDonald, R.A., 2012. Ecosystem restoration with teeth: what role for predators? *Trends in Ecology & Evolution*, *27*(5), pp.265-271.

Robinson, R., 1969. The white tigers of Rewa and gene homology in the Felidae. *Genetica*, *40*(1), pp.198-200.

Robinson, R., 1990. Homologous genetic variation in the Felidae. *Genetica*, *46*(1), pp.1-31.

Rose, P.E., Nash, S.M. and Riley, L.M., 2017. To pace or not to pace? A review of what abnormal repetitive behavior tells us about zoo animal management. *Journal of Veterinary Behavior*, *20*, pp.11-21.

Sadler, R.A., Ramsay, E., McAloose, D., Rush, R. and Wilkes, R.P., 2016. Evaluation of two canine distemper virus vaccines in captive tigers (*Panthera tigris*). *Journal of Zoo and Wildlife Medicine*, *47*(2), pp.558-563.

Sahasrabudhe, S. and Motter, A.E., 2011. Rescuing ecosystems from extinction cascades through compensatory perturbations. *Nature Communications*, *2*(1), pp.1-8.

© University of Pretoria

Sajjad, S., Farooq, U., Anwar, M., Khurshid, A. and Bukhari, S.A., 2011. Effect of captive environment on plasma cortisol level and behavioral pattern of Bengal tigers (*Panthera tigris tigris*). *Pakistan Veterinary Journal*, *31*(3), pp.195-198.

Sanderson, E.W., Forrest, J., Loucks, C., Ginsberg, J., Dinerstein, E., Seidensticker, J., Leimgruber, P., Songer, M., Heydlauff, A., O'Brien, T. and Bryja, G., 2010. Setting priorities for tiger conservation: 2005–2015. In *Tigers of the World* (pp. 143-161). William Andrew Publishing, New York, USA.

Sangay, T. and Vernes, K., 2008. Human–wildlife conflict in the Kingdom of Bhutan: Patterns of livestock depredation by large mammalian carnivores. *Biological Conservation*, *141*(5), pp.1272-1282.

Sankar, K., Qureshi, Q., Nigam, P., Malik, P.K., Sinha, P.R., Mehrotra, R.N., Gopal, R., Bhattacharjee, S., Mondal, K. and Gupta, S., 2010. Monitoring of reintroduced tigers in Sariska Tiger Reserve, Western India: preliminary findings on home range, prey selection and food habits. *Tropical Conservation Science*, *3*(3), pp.301-318.

Sapolsky, R.M., 1990. Adrenocortical function, social rank, and personality among wild baboons. *Biological Psychiatry*, *28*(10), pp.862-878.

Sarkar, M.S., Ramesh, K., Johnson, J.A., Sen, S., Nigam, P., Gupta, S.K., Murthy, R.S. and Saha, G.K., 2016. Movement and home range characteristics of reintroduced tiger (*Panthera tigris*) population in Panna Tiger Reserve, central India. *European Journal of Wildlife Research*, *62*(5), pp.537-547.

© University of Pretoria

Schaller George, B., 1967. The Deer and the Tiger. A study of wildlife in India. University of Chicago Press, USA.

Schatz, S. and Palme, R., 2001. Measurement of faecal cortisol metabolites in cats and dogs: a non-invasive method for evaluating adrenocortical function. *Veterinary Research Communications*, *25*(4), pp.271-287.

Scheun, J., Tordiffe, A.S., Wimberger, K. and Ganswindt, A., 2020. Validating a non-invasive technique for monitoring physiological stress in the samango monkey. *Onderstepoort Journal of Veterinary Research*, *87*(1), pp. 1-8.

Seidensticker, J., 1976. On the ecological separation between tigers and leopards. *Biotropica*, 8(4), pp.225-234.

Seidensticker, J., 1996. *Tigers*. Vancouver: Voyageur Press, Minnesota, USA.

Seimon, T.A., Miquelle, D.G., Chang, T.Y., Newton, A.L., Korotkova, I., Ivanchuk, G., Lyubchenko, E., Tupikov, A., Slabe, E. and McAloose, D., 2013. Canine distemper virus: an emerging disease in wild endangered Amur tigers (*Panthera tigris altaica*). *MBio*, *4*(4), pp.e00410-13.

Sekhar, N.U., 2003. Local people's attitudes towards conservation and wildlife tourism around Sariska Tiger Reserve, India. *Journal of environmental Management*, *69*(4), pp.339-347.

Selye, H. (1936). A syndrome produced by diverse nocuous agents. Nature, *138*, pp. 32.

© University of Pretoria

Sharma, R., Stuckas, H., Bhaskar, R., Rajput, S., Khan, I., Goyal, S.P. and Tiedemann, R., 2009. mtDNA indicates profound population structure in Indian tiger (*Panthera tigris tigris*). *Conservation Genetics*, *10*(4), pp.909-914.

Sharma, K., Wright, B., Joseph, T. and Desai, N., 2014. Tiger poaching and trafficking in India: Estimating rates of occurrence and detection over four decades. *Biological Conservation*, *179*, pp.33-39.

Shepherdson, D.J., Mellen, J.D. and Hutchins, M. (eds) (1998) Second Nature: Environmental Enrichment for Captive Mammals. Smithsonian Institution Press, Washington, DC.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R. and Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia*, *166*(4), pp.869-887.

Simcharoen, A., Savini, T., Gale, G.A., Simcharoen, S., Duangchantrasiri, S., Pakpien, S. Smith, J.L., 2014. Female tiger *Panthera tigris* home range size and prey abundance: important metrics for management. *Oryx*, *48*(3), pp.370-377.

Singer, D.H., 1995. Changes in heart rate variability associated with sudden cardiac death. *In Heart Rate Variability*, pp.429-448.

Singh, R., Krausman, P.R., Pandey, P., Qureshi, Q., Sankar, K., Goyal, S.P. and Tripathi, A., 2014. First parturition of tigers in a semi-arid habitat, western India. *European Journal of Wildlife Research*, *60*(2), pp.383-386.

Smith, J.L.D., McDougal, C., Gurung, B., Shrestha, N., Shrestha, M., Allendorf, T., Joshi, A. and Dhakal, N., 2010. Securing the future for Nepal's tigers: lessons from the past and

© University of Pretoria

present. In *Tigers of the World: The Science, Politics, and Conservation of* Panthera tigris, 2nd edn. (pp. 331-344). Academic Press, Burlington, MA, USA, pp. 331-344.

Springer, M.S., Burk-Herrick, A., Meredith, R., Eizirik, E., Teeling, E., O'Brien, S.J., and Murphy, W.J. 2007. The adequacy of morphology for reconstructing the early history of placental mammals. *Systematic Biology 56*, 673–684.

Stevenson, M. F. 1983. "The captive environment: Its effect on exploratory and related behavioural responses in wild animals". In *Exploration in animals and humans*, Edited by: Archer, J. and Birke, L. I. A. 176–197. Berkshire, UK.

Still, J., 2003. Use of animal products in traditional Chinese medicine: environmental impact and health hazards. *Complementary Therapies in Medicine*, *11*(2), pp.118-122.

Stratakis, C.A. and Chrousos, G.P., 1995. Neuroendocrinology and pathophysiology of the stress system. *Annals of the New York Academy of Sciences*, *771*(1), pp. 1-18.

Stubsjøen, S.M., Bohlin, J., Dahl, E., Knappe-Poindecker, M., Fjeldaas, T., Lepschy, M., Palme, R., Langbein, J. and Ropstad, E., 2015. Assessment of chronic stress in sheep (part I): The use of cortisol and cortisone in hair as non-invasive biological markers. *Small Ruminant Research*, *132*, pp.25-31.

Szokalski, M.S., Litchfield, C.A. and Foster, W.K., 2012. Enrichment for captive tigers (*Panthera tigris*): Current knowledge and future directions. *Applied Animal Behaviour Science*, *139*(1-2), pp. 1-9.

© University of Pretoria

Terio, K.A., Marker, L. and Munson, L., 2004. Evidence for chronic stress in captive but not free-ranging cheetahs (*Acinonyx jubatus*) based on adrenal morphology and function. *Journal of Wildlife Diseases*, *40*(2), pp.259-266.

Thapar, V., 1996. The tiger—road to extinction. In *The exploitation of mammal populations* (pp. 292-301). Springer, Dordrecht.

Thornton, I.W., Yeung, K.K., and Sankhala, K.S., 1967. The genetics of the white tigers of Rewa. *Journal of Zoology*, *152*(1), pp.127-135.

Thornton, I.W.B., 1978. White tiger genetics–further evidence. *Journal of Zoology*, *185*(3), pp.389-394.

Tidière, M., Gaillard, J.M., Berger, V., Müller, D.W., Lackey, L.B., Gimenez, O., Clauss, M. and Lemaître, J.F., 2016. Comparative analyses of longevity and senescence reveal variable survival benefits of living in zoos across mammals. *Scientific reports*, *6*, p.36361.

Tilbrook, A.J., Turner, A.I. and Clarke, I.J., 2000. Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. *Reviews of Reproduction*, *5*(2), pp. 105-113.

Tilson, R., Defu, H., Muntifering, J. and Nyhus, P.J., 2004. Dramatic decline of wild South China tigers *Panthera tigris amoyensis*: field survey of priority tiger reserves. *Oryx*, *38*(1), pp.40-47.

Tilson, R., and Nyhus, P. (2010). Tigers of the World: The Science, Politics and Conservation of *Panthera tigris*, Second Edition (Elsevier).

© University of Pretoria

Ting, N.T., 1978. A type index of Chinese folktales in the oral tradition and major works of non-religious classical literature. *FF Communications Turku*, 223, pp.7-294.

Touma, C., Sachser, N., Möstl, E. and Palme, R., 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *General and Comparative Endocrinology*, *130*(3), pp. 267-278.

Touma, C. and Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Annals of the New York Academy of Sciences*, *1046*(1), pp.54-74.

Treves, A. and Karanth, K.U., 2003. Human-carnivore conflict and perspectives on carnivore management worldwide. *Conservation Biology*, *17*(6), pp.1491-1499.

Tyagi, A., Kumar, V., Kittur, S., Reddy, M., Naidenko, S., Ganswindt, A. and Umapathy, G., 2019. Physiological stress responses of tigers due to anthropogenic disturbance especially tourism in two central Indian tiger reserves. *Conservation Physiology*, *7*(1), p.coz045.

Urrutia, A., Martínez-Byer, S., Szenczi, P., Hudson, R. and Bánszegi, O., 2019. Stable individual differences in vocalisation and motor activity during acute stress in the domestic cat. *Behavioural processes*, *165*, pp.58-65.

Vaz, J., Narayan, E.J., Dileep Kumar, R., Thenmozhi, K., Thiyagesan, K. and Baskaran, N., 2017. Prevalence and determinants of stereotypic behaviours and physiological stress among tigers and leopards in Indian zoos. *PloS one*, *12*(4), p.e0174711.

Vester, B.M., Burke, S.L., Dikeman, C.L., Simmons, L.G. and Swanson, K.S., 2008. Nutrient digestibility and fecal characteristics are different among captive exotic felids fed a beef-based

© University of Pretoria

raw diet. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *27*(2), pp.126-136.

Vick, M.M., Wildt, D.E., Turner, J.B., Palme, R., Wolfe, B.A. and Pukazhenthi, B.S., 2012. Glucocorticoid response to changes in enclosure size and human proximity in the Persian onager (*Equus hemionus onager*). *Stress*, *15*(1), pp.52-61.

Vickery, S.S. and Mason, G.J. (2003b) Behavioural persistence in captive bears: implications for reintroduction. *Ursus* 14, 35–43.

Vingerhoets AJ, Perski A., 2000. The psychobiology of stress. *Psychology in Medicine*. Bohn Stafleu Van Loghum, Houten/Diegem. pp. 34-49.

Vlaming, Z., 2013. Factors influencing pacing in the South China tiger *(Pt amoyensis)* (Master's thesis).

Von Borell, E., Langbein, J., Després, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A. and Valance, D., 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—A review. *Physiology & Behavior*, *92*(3), pp.293-316.

Von Borell, E., Bünger, B., Schmidt, T. and Horn, T., 2009. Vocal-type classification as a tool to identify stress in piglets under on-farm conditions. *Anim Welfare*, *18*, pp.407-416.

Wallach, A.D., Johnson, C.N., Ritchie, E.G. and O'Neill, A.J., 2010. Predator control promotes invasive dominated ecological states. *Ecology Letters*, *13*(8), pp.1008-1018.

© University of Pretoria

Wallach, A.D., Ripple, W.J. and Carroll, S.P., 2015. Novel trophic cascades: apex predators enable coexistence. *Trends in Ecology & Evolution*, *30*(3), pp. 146-153.

Walston, J., Karanth, K.U. and Stokes, E., 2010. Avoiding the unthinkable: What will it cost to prevent tigers becoming extinct in the wild. *Wildlife Conservation Society, New York*.

Wang, S.W. and Macdonald, D.W., 2006. Livestock predation by carnivores in Jigme Singye Wangchuck National Park, Bhutan. *Biological Conservation*, *129*(4), pp.558-565.

Wani, M. and Kothari, A., 2006. Conservation and People's Livelihood Rights in India: Final Report of a Research Project Conducted under the UNESCO Small Grants Programme. *Pune: Kalpavriksh*.

Warrick, D.M., 2010. Inbreeding depression in captive white tigers: methods for purifying tiger lineages. *Zoo's Print*, *25*(10), pp.07-15.

Wayne, R.K., Benveniste, R.E., Janczewski, D.N. and O'brien, S.J., 1989. Molecular and biochemical evolution of the Carnivora. In *Carnivore behavior, ecology, and evolution* (pp.465-494). Springer, Boston, MA.

Webster, A.B., Burroughs, R.E., Laver, P. and Ganswindt, A., 2018. Non-invasive assessment of adrenocortical activity as a measure of stress in leopards *Panthera pardus*. *African Zoology*, *53*(2), pp. 53-60.

Weingrill, T., Gray, D.A., Barrett, L. and Henzi, S.P., 2004. Fecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. *Hormones and Behavior*, *45*(4), pp. 259-269.

© University of Pretoria

Whirledge, S. and Cidlowski, J.A., 2010. Glucocorticoids, stress, and fertility. *Minerva Endocrinologica*, *35*(2), pp.109.

Whitham, J.C. and Miller, L.J., 2016. Using technology to monitor and improve zoo animal welfare. *Animal Welfare*, *25*(4), pp.395-409.

Wielebnowski, N.C., Fletchall, N., Carlstead, K., Busso, J.M. and Brown, J.L., 2002. Noninvasive assessment of adrenal activity associated with husbandry and behavioral factors in the North American clouded leopard population. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *21*(1), pp. 77-98.

Wildt, D.E. and Roth, T.L., 1997. Assisted reproduction for managing and conserving threatened felids. *International Zoo Yearbook*, *35*(1), pp.164-172.

Willemse, T. and Spruijt, B.M., 1995. Preliminary evidence for dopaminergic involvement in stress-induced excessive grooming in cats. *Neuroscience Research Communications*, *17*(3), pp.203-208.

Williamson, D.F., Henry, L.A. and America, T.N., 2008. Paper Tigers?. *The Role of the US Captive Tiger Population in the Trade in Tiger Parts. TRAFFIC North America, Washington DC: World Wildlife Fund*.

Wilmers, C.C., Post, E., Peterson, R.O. and Vucetich, J.A., 2006. Predator disease out-break modulates top-down, bottom-up and climatic effects on herbivore population dynamics. *Ecology Letters*, *9*(4), pp.383-389.

Wilting, A., Courtiol, A., Christiansen, P., Niedballa, J., Scharf, A.K., Orlando, L., Balkenhol, N., Hofer, H., Kramer-Schadt, S., Fickel, J., and Kitchener, A.C. (2015). Planning tiger

© University of Pretoria

recovery: Understanding intraspecific variation for effective conservation. *Science Advances, 1*, e1400175.

Wolf, C. and Ripple, W.J., 2017. Range contractions of the world's large carnivores. *Royal Society Open Science*, *4*(7), p.170052.

Woodroffe, R., 2000. Predators and people: using human densities to interpret declines of large carnivores. In *Animal Conservation Forum* (Vol. 3, No. 2, pp. 165-173). Cambridge University Press.

Xu, X., Dong, G.X., Hu, X.S., Miao, L., Zhang, X.L., Zhang, D.L., Yang, H.D., Zhang, T.Y., Zou, Z.T., Zhang, T.T. and Zhuang, Y., 2013. The genetic basis of white tigers. *Current Biology*, *23*(11), pp.1031-1035.

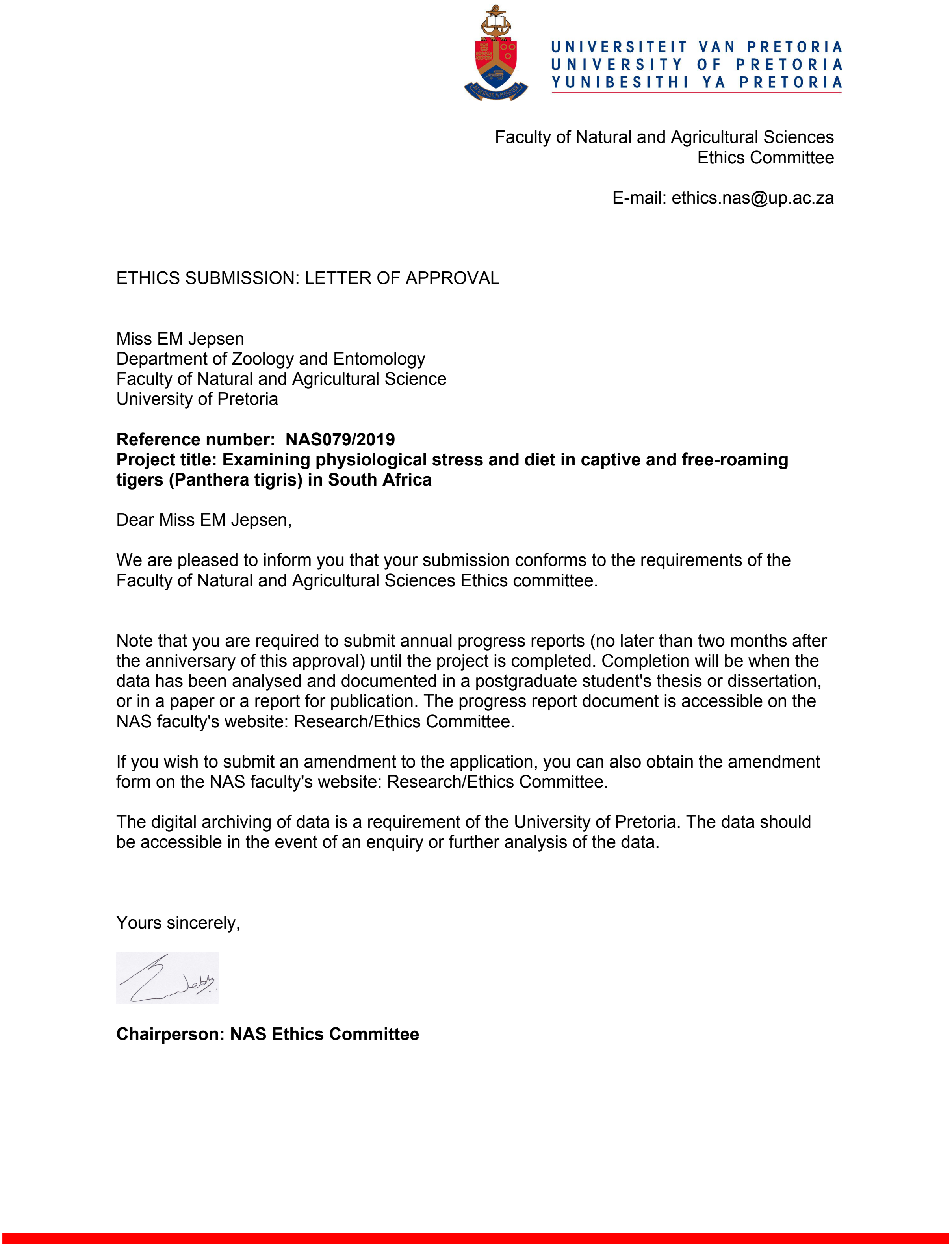
Xue, H.R., Yamaguchi, N., Driscoll, C.A., Han, Y., Bar-Gal, G.K., Zhuang, Y., Mazak, J.H., Macdonald, D.W., O'Brien, S.J., and Luo, S.J. (2015). Genetic ancestry of the extinct Javan and Bali tigers. *Journal of Heredity, 106*, 247–257

© University of Pretoria

Appendix 1 Ethics Approval

© University of Pretoria

© University of Pretoria



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Natural and Agricultural Sciences
Ethics Committee

E-mail: ethics.nas@up.ac.za

ETHICS SUBMISSION: LETTER OF APPROVAL

Miss EM Jepsen
Department of Zoology and Entomology
Faculty of Natural and Agricultural Science
University of Pretoria

Reference number: NAS079/2019
Project title: Examining physiological stress and diet in captive and free-roaming tigers (Panthera tigris) in South Africa

Dear Miss EM Jepsen,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Natural and Agricultural Sciences Ethics committee.

Note that you are required to submit annual progress reports (no later than two months after the anniversary of this approval) until the project is completed. Completion will be when the data has been analysed and documented in a postgraduate student's thesis or dissertation, or in a paper or a report for publication. The progress report document is accessible on the NAS faculty's website: Research/Ethics Committee.

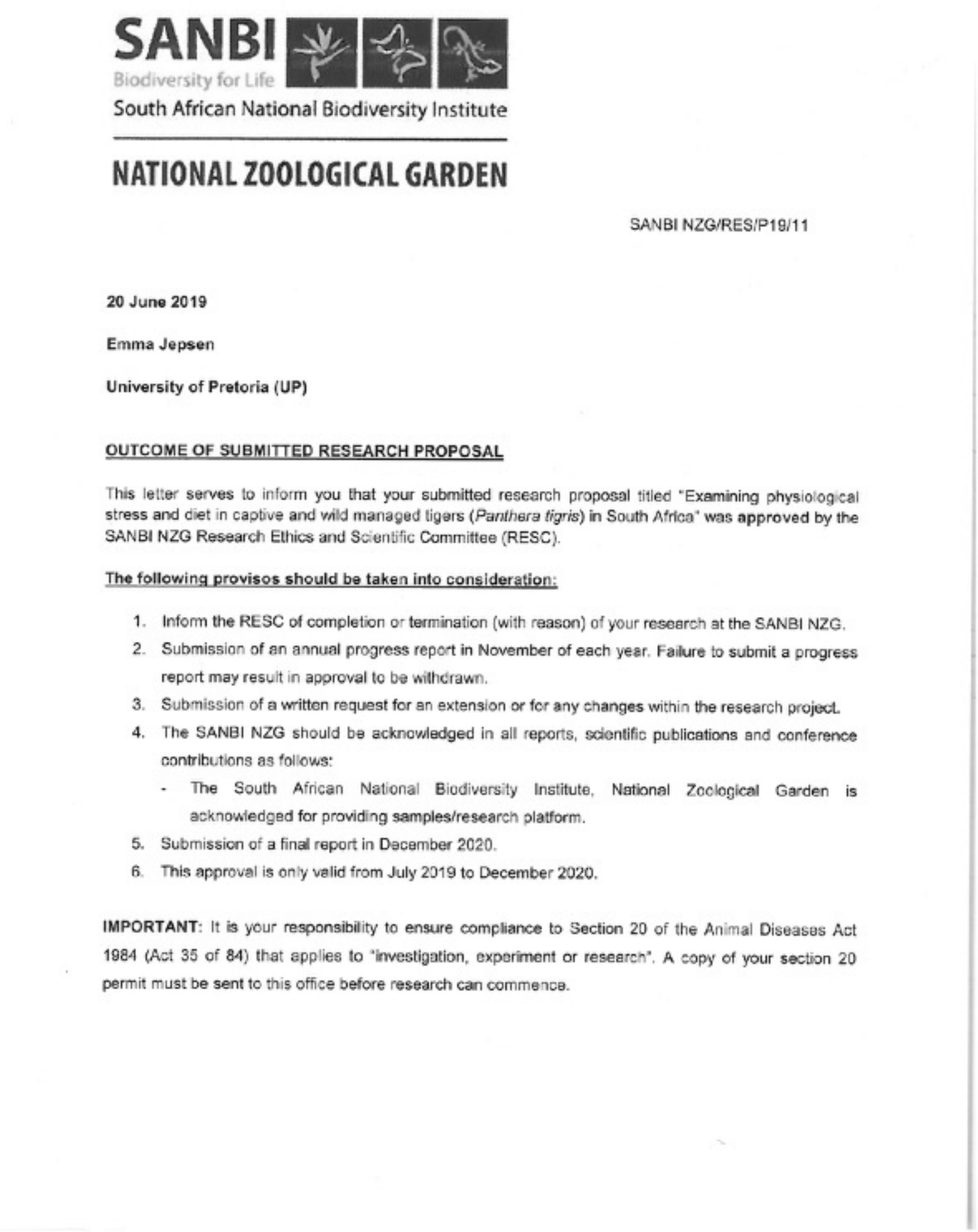
If you wish to submit an amendment to the application, you can also obtain the amendment form on the NAS faculty's website: Research/Ethics Committee.

The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Yours sincerely,

Chairperson: NAS Ethics Committee

© University of Pretoria



SANBI
Biodiversity for Life
South African National Biodiversity Institute

NATIONAL ZOOLOGICAL GARDEN

SANBI NZG/RES/P19/11

20 June 2019

Emma Jepsen

University of Pretoria (UP)

OUTCOME OF SUBMITTED RESEARCH PROPOSAL

This letter serves to inform you that your submitted research proposal titled "Examining physiological stress and diet in captive and wild managed tigers (*Panthera tigris*) in South Africa" was approved by the SANBI NZG Research Ethics and Scientific Committee (RESC).

The following provisos should be taken into consideration:

1. Inform the RESC of completion or termination (with reason) of your research at the SANBI NZG.
2. Submission of an annual progress report in November of each year. Failure to submit a progress report may result in approval to be withdrawn.
3. Submission of a written request for an extension or for any changes within the research project.
4. The SANBI NZG should be acknowledged in all reports, scientific publications and conference contributions as follows:
    - The South African National Biodiversity Institute, National Zoological Garden is acknowledged for providing samples/research platform.
5. Submission of a final report in December 2020.
6. This approval is only valid from July 2019 to December 2020.

IMPORTANT: It is your responsibility to ensure compliance to Section 20 of the Animal Diseases Act 1984 (Act 35 of 84) that applies to "investigation, experiment or research". A copy of your section 20 permit must be sent to this office before research can commence.

© University of Pretoria