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A Multi-Species Study on Several Types of Visitor Effects on the Behavior and Physiology of Animals at the Oregon Zoo

by

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A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
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Abstract

The visitor effect on zoo animals is one of the expanding research topics in zoo animal research. As visitors are a mandatory feature of zoological institutions, understanding their effects on zoo animals is imperative for maximizing zoo animal welfare. Zoo animals are subject to many anthropogenic influences: visual, olfactory, and audible, for example. This dissertation investigates several of the effects of visitor presence and its relative influence on six mammalian species: cheetahs (*Acinonyx jubatus*), African painted dogs (*Lycaon pictus*), Asian elephants (*Elephas maximus indicus* & *Elephas maximus borneensis*), giraffes (*Giraffa camelopardalis reticulata* & *Giraffa camelopardalis tippelskirchi*), ring-tailed lemurs (*Lemur catta*), and red-ruffed lemurs (*Varecia rubra*) at the Oregon Zoo.

After-hour events are a growing attraction amongst zoological institutions as new avenues of community engagement and revenue are needed. However, the effects of such after-hour events on animals have been minimally studied, so far. Therefore, one of the goals of this study was to evaluate the impact of after hour events on several species at the Oregon Zoo. Interestingly, this study found no statistically significant effects of after-hour events on either, fecal glucocorticoid metabolite (fGM) expression (adrenal activity) or behaviors based on event type for any of the species studied. No increase in potentially problematic behaviors was observed, indicating no measurable negative influence of after-hour events on these species.

The onset of the global SARS-CoV-2 (COVID-19) pandemic created a unique opportunity to observe zoo animals without the influence of visitors for a prolonged period of time. An *ad hoc* study was carried out on the effects of two transition periods on a subset of the study species (giraffe and cheetah): 1) The initial closure of the Oregon Zoo (March 2020), and 2) The subsequent reopening (July 2020). In this study, no significant differences in fGM concentrations were observed between the two transition times and times with visitor presence versus visitor absence, but significant differences were found in behaviors. However, these changes were minor and could more likely be attributed to social and medical changes that occurred within the same time period for both species.

Finally, the influence of various types of sounds as a potential source of enrichment was investigated for two species of lemur: ring-tailed lemur and red-ruffed lemur. Three sound types were used as possible enrichment: spoken word, generic rainforest noise, and species-specific lemur call-backs. Behavioral responses and fGM concentrations within each sound type were compared against each other and against periods of silence. There were significant differences in exhibit use based on sound type as well as some minor, yet also significant, differences in behavior responses, but no significant correlation between fGM concentration and sound type. However, variability in weather with cold and rainy days during the study time may have had a significant impact on behavior and/or exhibit use responses. A repetition of this study in warmer weather has been suggested and may provide additional detail to identify a more definitive impact of sound type.

The conclusions of this dissertation highlight the overwhelming individuality of zoo animal responses and the importance of testing any potential effects of visitor presence and effects of sound at the individual level. While these studies found no obvious negative effects on the animals at the Oregon Zoo, each individual responded slightly differently to the various stimuli related to visitor presence or the lack thereof. Other species and other individuals of the same species may respond differently to the same stimuli, and results from this dissertation should be extrapolated with caution to other institutions, events, species, and individual. As more studies like these continue to be conducted across zoological institutions, with a multitude of species, individuals, and visitor access types (e.g., concerts, dinner events, over-night camps), animal response patterns may begin to emerge that can assist in guiding future visitor access decisions in terms of intensity, frequency, and type most conducive to ensuring good welfare of both animals and visitors.

To Buddy

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Glossary

Term	**Definition**
Acoustics	The subdivision of physics that studies the properties of sound
Wavelength	λ; measurable distance between crests of a soundwave
Frequency	*f*; number of wavelengths that pass a specific point within a time period (usually one second)
Pitch	The physiological interpretation of a sound – how high or low a frequency is perceived
Amplitude	*A*; The distance between the equilibrium of a wave and the crest.
Decibel scale	dB; a logarithmic scale used to measure sound intensity
Air conduction	Method of deciphering sound waves through the ear canal to the cochlea as changes in air pressure
Bone conduction	Process of deciphering sound waves as they vibrate the skull rather than through changes in air pressure
Functional head size	Measurable delay in time it takes for sound to travel from the opening of one ear canal to the other (terrestrial organisms) or middle ear to middle ear (aquatic organisms)
Noise	Any unwanted sound
Masking	Overlapping tones that result in the degradation of at least one
Phonation	The production of speech / speech sounds
Threshold Shift	TS; the difference between sound sensitivity in pre-exposure and post-exposure sound exposure
Permanent Threshold Shift`	PTS; when the threshold shift is permanent and the animal cannot recover its hearing – regardless of time past
Temporary Threshold Shift	TTS; the threshold shift associated with increased sound exposure is recoverable over a certain duration of time

Chapter One: Introduction

General

Visitor attendance is a necessity within zoological institutions. Visitors provide revenue and are crucial to most zoo's education-based priorities and conservation projects. However, many researchers and zoo personnel have increased interest in the overarching affect visitors may have on zoo animals due to the introduction of novel olfactory (e.g., Farrand 2007), visual (e.g., Sherwen et al. 2015), and audible (e.g., Edes et al. 2021; Fanning, Larsen, and Taylor 2020; Farrand 2007; Sherwen et al. 2014) stimuli. Specifically, this dissertation analyzed the effect of anthropomorphic sounds and visitor density on four species: giraffe (*Giraffa camelopardalis reticulata* & *Giraffa camelopardalis tippelskirchi*), cheetahs (*Acinonyx jubatus*), African painted dogs (*Lycaon pictus*), and Asian elephants (*Elephas maximus indicus* and *Elephas maximus borneensis*); as well as the potential of sound as environmental enrichment on two additional species: ring-tailed lemur (*Lemur catta*) and red-ruffed lemur (*Varecia rubra*). Zoos have continuously evolved and developed new visitor experiences designed to increase education, awareness, and bring in revenue. These new programs require analysis and monitoring to understand how zoo animals react to them as potential stressors. This dissertation will contribute to the understanding of visitor impacts on zoo animals using three aims:

1) To quantify the effects of large-scale events and associated noise and visitor density on giraffes (*Giraffa camelopardalis reticulata* & *Giraffa camelopardalis tippelskirchi*), cheetahs (*Acinonyx jubatus*), African painted dogs (*Lycaon pictus*), and Asian elephants (*Elephas maximus indicus* & *Elephas maximus borneensis*) housed at the Oregon Zoo. For this aim, I analyzed how both an excess and a deprivation of visitor presence affected the physiology and behavior expression of the previously listed species.
2) I designed an experimental study to examine the use of sound as an enrichment tool for two species of lemur: ring-tailed lemur (*Lemur catta*) and red-ruffed lemur (*Varecia rubra*).
3) Finally, I opportunistically investigated the impact of a major transition in visitor presence (i.e., forced closure and visitor absence due to the COVID-19 pandemic) and the impact on the physiology and behavior of some of the species previously mentioned (cheetah and giraffe).

To meet these aims, I used behavioral observations coupled with fecal glucocorticoid metabolite (fGM) monitoring and environmental observations (e.g., sound monitoring). This combination of methodology provided multi-faceted data to comprehensively evaluate the effect of visitors and their overall influence on zoo animals.

Potential Sources of Eustress and Distress in Zoological Institutions

Life is inherently stressful for animals, whether they are in the wild or zoos. True avoidance of stress is impossible as stressors exist in all environments and animals are continuously adapting to environmental demands (Friend 1980; Selye 1976). Additionally, stress is not necessarily a negative experience and both positive (eustress) and negative (distress) exist. The stress response is an important biological function that is necessary for the survival of an individual (Sibly and Calow 1989), and evolutionary adaptation of a species (Badyaev 2005). Both wild-living and zoo-living animals are exposed to stress. In the wild, animals are subjected to predation, territorial fights, resource gathering, and a multitude of social stressors. While zoos eliminate the threat of predation and provide resources, they do create their own unique stressors. The following section focuses on distress and eustress sources in zoos but is confined to the six species included in this dissertation due to the broad scope of this topic.

Environmental stressors in zoo habitats include various types of anthropogenic effects on all senses: visual, oral, audio, olfactory, and tactile. While this study focuses specifically on visitor effects and the resulting noise changes, it is important to understand the difficulty of isolating visual, audio, and olfactory effects on zoo animals. I will be concentrating on the current research on audio anthropogenic effects and relevant visitor presence effects on the species presented in this study.

Multiple studies have linked negative welfare indicators, such as behavioral changes and physiological changes, to the prevalence of anthropogenic sounds like construction (Jakob-Hoff et al. 2019; Volfova et al. 2020), visitors (O'Donovan et al. 1993; Quadros et al. 2014), and road noise (Evans 1970). The results revealed a variety of responses. For instance, a study of a female cheetah and her cubs showed no behavioral reaction to noisy visitors (O'Donovan et al. 1993), whereas a study of giraffes showed increased levels of stress-based behaviors with increased construction noise (Jakob-Hoff et al. 2019). These findings were also supported by increased fecal glucocorticoid metabolite (fGM) concentrations following construction events in ring-tailed lemurs (Volfova et al. 2020). Asian elephants were found to increase trunk manipulation behaviors (curling and sucking), foot lifting, and alert behaviors when intermittent construction noise was played, albeit the differences were statistically insignificant (Jakob-Hoff et al. 2019).

Visual visitor presence has also been shown to influence both the behavior and the fGM concentrations of some of the focal species. However, it is important to note that very few studies have successfully isolated visual visitor presence from their noise and olfactory effects. The following summary focuses on visitor presence studies that do not specifically specify olfactory or audio manipulation. Szokalski, Foster, and Litchfield (2013) found the addition of a behind-the-scenes tour (increased visitor visibility) increased inactive behaviors and decreased feeding behaviors on tour days compared with non-tour days in cheetahs, indicating an effect of increased visitor presence. Interestingly, visitor presence was shown to have no effect on fGM concentrations in cheetahs (Koester et al. 2015, 2017). For Asian elephants, reduced levels of stereotypic behaviors were correlated with higher visitor numbers (Krishnan and Braude 2014). Additionally, Asian elephant salivary cortisol levels were significantly higher when visitor presence is new (e.g., during an initial zoo opening) (Menargues, Urios, and Mauri 2008). However, this study did not attempt to isolate visual effects from olfactory and auditory effects. While multiple studies have been conducted on the effect of visitors on giraffes, most are constricted to impacts of direct visitor-animal contact (e.g., feeding platforms) due to the popularity of giraffe visitor experiences (Lynn 2018; Normando et al. 2018; Orban, Siegford, and Snider 2016). Additionally, research on zoo-housed African painted dogs has generally not focused directly on the visitor effect. Due to the complicated social dynamics within African painted dog packs, most research has centered on the social conflicts or management, rather than visitor effects (Fernandez and Harvey 2021; O'Malley 2013; Tighe 2013). Unlike the previous species, multiple studies have been conducted comparing visitor presence with changes in lemur behavior expression. For instance, both low and high visitor numbers resulted in increasing rates of stereotypic behaviors (such as hand-chewing) in ring-tailed lemurs, indicating sensitivity to visitor numbers (Shire 2012). Another study found a decrease in overall activity, grooming behaviors, and an increase in agnostic behaviors displayed by ring-tailed lemurs when visitors were present (Chamove, Hosey, and Schaetzel 1988). Interestingly, free-ranging ring-tailed lemurs in an Ireland zoo showed no behavioral effect based on visitor presence (Collins et al. 2017). The differences in these results show ring-tailed lemur response is potentially unique to the individual, locations, husbandry practices, and specific visitor interactions.

Outside of visitor presence, other zoo-specific stressors exist. According to Morgan and Tromborg (2007), confinement-specific stressors are those that are specific to zoo-housing such as, behavioral alteration due to confinement and unnatural social changes. Many of these stressors come in the form of reduced choice and lack of control. For instance, entire habitats – including *Elephant Lands* at the Oregon Zoo (Glaeser et al. 2021) – have been redesigned to all for the expression of natural behavior repertoires and provide more choices for their inhabitants. Naturalistic behavior expression, such as solving natural or abiotic challenges (e.g., cognitive foraging challenges and cooperative hunting techniques), frequently increase eustress and environmental enrichment policies should be designed for their expression (Meehan and Mench 2007).

Physiological Stress Response

Despite the negative connotation of stress, the physiological reaction to both distress and eustress is the same within the body. Stress is considered a complex physiological state that requires both behavioral and physiological processes to address a threat, or perception of a threat, to homeostasis (Ralph and Tilbrook 2016). Animals frequently use both behavioral and physiological adjustments to address and adapt to a stressor (Moberg 1976). Stressors exist everywhere in the environment and require constant adaptation (Friend 1980; Selye 1976). In fact, stressors are so common and varied that true avoidance of stress is, essentially, impossible. Additionally, stress is not necessarily a negative experience. According to Selye (1976) (pg. 137), both “a painful blow and a passionate kiss can be equally stressful" indicating the presence of positive stressors. While there may be no difference in positive or negative stressors on the physiological response within the body, the intensity and duration of a stressful event can dramatically affect the individual (Engelmann, Landgraf, and Wotjak 2004).

There are two different types of stressors: acute and chronic. Acute stress is a fairly brief response to a non-disruptive single stressor, while chronic stress is considered a combination of multiple acute stressors that can force an animal towards a pathological state (Moberg 2000). Individuals are adapted to overcome acute stress without any lasting effects. However, many biological functions, such as ovulation, require precise timing and an ill-timed acute stressor may disrupt that timing and interrupt the animal's standard functioning (Moberg 2000; Roozendaal et al. 1995; Vinícius et al. 2007). Alternatively, chronic stress can cause a plethora of detrimental effects, including suppressed immune system functions (Chrousos 2009).

The detection of a stressor triggers a response in the hypothalamic pituitary adrenal (HPA) axis while also stimulating the sympathetic nervous system (SNS). Both systems harmoniously work to return an individual's internal environment to homeostasis (Sheriff et al. 2011). The SNS is initiated by an increase in catecholamines (dopamine, epinephrine, and norepinephrine) and the activation of the SNS prepares the animal for the well-known "fight or flight" stress response. This "fight or flight" reflex consists of behavioral and physiological responses (Cannon 1915; Koolhaas et al. 1999) that focus on combating a stressor by either removing the individual from the stressful situation or the removal of the stressor itself. Heightened levels of catecholamines are associated with increased blood pressure, heart rate, and vasoconstriction, acting to prepare an individual for a higher metabolic output (Mellor, Cook, and Stafford 2000). The activity of the HPA axis with the perception of a stressor also results in an increase in glucocorticoids, primarily cortisol or corticosterone, and these can be measured in the blood, saliva, urine, and feces of an animal (Blanchard, McKittrick, and Blanchard 2001).

After the stressor has passed, there is an automatic negative-feedback loop in which the high concentrations of glucocorticoids bind with specialized receptors in the hippocampus, hypothalamus, and pituitary, resulting in the suppression of the HPA axis (De Kloet et al. 1998). This process is efficient in response to acute stressors, and the negative-feedback loop causes a rapid return to baseline glucocorticoid concentrations. However, when presented with a chronic stressor, the increased duration creates inefficiencies within the feedback loop and the levels of serum glucocorticoids remain elevated.

While glucocorticoids are associated with a stress response, they continuously exist in the body at low levels in order to provide a buffer to day-to-day stress (Munck, Guyre, and Holbrook 1984). Glucocorticoid levels are not considered elevated until pressure from a stressor exceeds what the basal glucocorticoid levels can accommodate (Munck et al. 1984). When elevated, the excess glucocorticoids may cause anti-inflammation, increased water excretion, prevention of glucose absorption by the peripheral tissues, and immune-system suppression (Munck et al. 1984). The elevation of glucocorticoid concentrations during a stress response is imperative in preventing the body's natural defense mechanisms from overreacting to a stressor and becoming a threat itself as the increased blood pressure, heart rate, and vasoconstriction associated with confrontation of a stressor can be dangerous if elevated for an extended period (Mellor, Cook, and Stafford 2000; Munck, Guyre, and Holbrook 1984).

Chronic elevation of glucocorticoids can have disastrous effects on many physiological responses. A review by Munck et al. (1984) provides a detailed explanation of the effects unchecked glucocorticoids can have in the body. A few examples include: the inhibition of T cell growth factor (TCGF), inhibition of inflammatory effects by serotonin, bradykinin, and histamine, inhibition of insulin secretion, and increased secretion of glucagon (Munck et al. 1984). For animals within captive breeding programs – like many zoo animals – one of the most concerning effects of increased glucocorticoid concentrations is a decrease in normal reproductive function (DeCatanzaro and MacNiven 1992).

Measuring Glucocorticoids

Measuring stress in captive animals has been extensively studied (for example, Carlstead and Shepherdson 2000; Koolhaas et al. 1999; Moberg 2000; Morgan and Tromborg 2007; Sheriff et al. 2011) and changes in glucocorticoid concentrations are accepted as indications of change in animal welfare. However, as glucocorticoids are secreted following a circadian rhythm, thorough understanding of a standard glucocorticoid cycle is imperative prior to inferring what changes in glucocorticoid concentrations may indicate in regard to an animal's welfare (Ralph and Tilbrook 2016; Young et al. 2004). Glucocorticoids exist within the body in two different forms: "free" or bound to proteins (corticosteroid-binding globulin or albumin) (Breuner et al. 2003). Protein-binding protects glucocorticoids as they move through the body and the glucocorticoids will readily unbind when they reach their target sites (Ralph and Tilbrook 2016; Sapolsky, Romero, and Munck 2000). While high levels of glucocorticoids exist throughout body, only the unbound or "free" glucocorticoids cause physiological changes because only "free" glucocorticoids can enter cells and bind to the receptors within the nucleus (Ralph and Tilbrook 2016). Approximately 5-10% of glucocorticoids naturally exist as "free" glucocorticoids (Rosner 1990).

Inferring information about changes in glucocorticoid concentrations is complicated due to the similar glucocorticoid response regardless of the positive or negative attributes of the stressor. Therefore, understanding an individual's baseline and "typical" glucocorticoid responses to different stressors is important (Ralph and Tilbrook 2016). Otherwise, it is difficult to make inferences regarding about which "spikes" or "valleys" may represent abnormal responses to standard stressors.

A variety of matrices can be used to extract glucocorticoid concentrations. There are four prominent ones currently used for wildlife management: serum (blood), saliva, urine, and feces (Palme 2019; Sheriff et al. 2011). This study exclusively used feces in the analyses of glucocorticoids.

Fecal Glucocorticoids

Fecal glucocorticoid measurements are among the most promising methods of measuring the effect of long-term stress in animals (Palme 2019). Like urine samples, the fecal glucocorticoid analyses measure glucocorticoid metabolites as glucocorticoids are metabolized in the liver prior to being secreted into the gut via the bile duct and excreted from the body (Palme 2005; Taylor 1971). There are many advantages to fecal glucocorticoid monitoring. For instance, the collection procedure is usually entirely non-invasive, and the samples can be used for multiple analyses (Huber, Palme, and Arnold 2003; Sheriff et al. 2011). In captivity, fecal collection can be relatively simple, with care staff collecting fecal samples during their day-to-day routine. Additionally, adding identifying agents (such as edible glitter and food-dye) to a specific target animal's diet provides easy identification of the fecal samples after defecation without altering the glucocorticoid concentrations (Fuller, Margulis, and Santymire 2011). Fecal collection of wild animals is also considerable simpler than other substrate collection processes as researchers can simply collect the feces immediately after defecation (Ganswindt et al. 2010).

While arguably the fastest growing collection technique (Palme 2019), multiple caveats still exist. Like urinary glucocorticoid measurements, fecal glucocorticoid analyses measure fecal glucocorticoid metabolites (fGM) only. The proportion of metabolites that are excreted in the feces verses urine changes both between species and between the sexes, complicating extrapolation of results (Sheriff et al. 2010; Touma and Palme 2005). Additionally, diurnal changes in fGM concentrations have been recorded for most vertebrates, particularly in animals that have frequent defecation (e.g., birds or rodents), requiring collection at the same time each day or pooled fecal samples to avoid circadian influences (Touma and Palme 2005; Wielebnowski and Watters 2007). Unfortunately, fecal samples are subject to degradation from microbes and bacteria (Möstl et al. 1999; Möstl and Palme 2002) and must be frozen as quickly as possible following defecation to minimize the rate of degradation.

All studies conducted as part of this dissertation used fecal glucocorticoid metabolites (fGM) monitoring to assist in analyzing the impact of environmental effects, such as zoo events, concerts, visitor density, and enrichment on the physiology of several species commonly housed in many modern zoos.

Sound

Properties of Sound

As each animal is subject to physics for communication and localization, understanding the physical properties of sound is imperative for fully understanding the effects of anthropogenic noise on animals. For instance, research has shown that animals that require communication over long distances use low-frequency calls due to high-frequency degradation during atmospheric transmission (Wiley and Richards 1978). Therefore, anthropogenic noise that is produced within these low frequencies may mask and compete with long-distance communication. Understanding the physics of both the anthropogenic signal and the animal communication can assist in inferring how the anthropogenic signal may affect the animal’s communication. Additionally, ecosystem characteristics affect sound propagation in different ways based on how sound waves interact with various aspects of an ecosystem. For example, a study by Marten and Marler (1977) showed an increase in sound signal attenuation in forested habitats compared to open fields and indicated animals adjust their communication methodologies based on the physical properties of sound.

The Mechanisms of Hearing

I will be focusing only on vertebrate hearing as this dissertation only includes vertebrate species. Vertebrate hearing is based off two different pathways: air conduction and bone conduction. Air conduction, like the name suggests, is the perception of sound that travels as waves through air and involves the outer ear, middle ear, and cochlea, whereas bone conduction is the process of a sound wave vibrating the bones of the skull to stimulate the cochlea (Henry and Letowski 2007). Air conduction is the primary source of sound perception in terrestrial vertebrates, especially mammals, while bone conduction is the primary mechanism used by aquatic animals. While this dissertation and the investigation of sound impacts on zoo animals is primarily focused on sound perceived through air conduction, bone conduction is an important mechanism for Asian elephant hearing and an important consideration when analyzing effects of sound on Asian elephants.

Air Conduction

In air conduction, sound waves are captured by the pinna and external ear. These waves are then funneled through the outer ear canal, becoming more amplified until they strike the tympanic membrane (eardrum). When sound waves hit the tympanic membrane, the vibrations travel through the membrane to the malleus, then the incus, and finally the stapes – collectively known as the auditory ossicles. As each of the ossicles are slightly smaller than the previous, sound waves are amplified up to 17 times greater than their original intensity when detected. The rate of amplification is dependent on both the frequency and intensity of the original sound wave. This amplification process is exceedingly important as it allows for the detection of very quiet sounds. Without it, the human perception of sound would be approximately 60 decibels (dB) less than it is currently (Britannica 2022). For other mammals, a study by Puria and Steele (2010) linked the vibration of the ossicles with the upper-limit of high-frequency hearing. However, this amplification process can potentially cause damage to the inner ear and is mitigated by the scala tympani and the stapedius muscle. These two muscles work in cohesion to prevent the transmission of too-strong sound waves through the ossicles. However, sounds that are short and intense (e.g., gunshots) can still cause considerable damage to the ear due to a delay in the activation of the scala tympani and the stapedius muscles. Additionally, chronic loud noises can also cause substantial damage to the ear because the two muscles are easily fatigued. If these two muscles fail to prevent the intrusion of excessively strong sound waves, it may result in the damage of stereocilia (hair that assists in frequency detection within the organ of Corti and transform the physical sound into neurological signals processed in the cerebral cortex) (Britannica 2022). While indetectable at first, continuous damage to the stereocilia can result in detectable hearing loss. Permanent hearing loss is more common in aquatic mammals due to the intensity of sound in water (Reichmuth et al. 2019; Slabbekoorn, McGee, and Walsh 2018) and permanent hearing loss has been successfully recorded within laboratory housed terrestrial mammals (Clark 1991). For instance, Moody et al. (1978) found both temporary and permanent hearing loss in old-world monkeys (*Macaca nemestrina, Macaca mulatta,* and *Macaca fascicularis*) after exposure to 117-120 dB sounds over an 8-hour period.

As anthropomorphic noises in various environments (e.g., metal gate closures, road traffic, and visitor noises) have been shown to cause a considerable increase in ambient noise level (Buxton et al. 2017; Milligan, Sales, and Khirnykh 1993), it is important to monitor anthropogenic noises in zoo environments in order to prevent overexposure to intense noises that may result in hearing loss or damage in zoo animals.

Bone Conduction

At its core, bone conduction is similar to air conduction in almost all ways (vibrations still stimulate the inner ear and trigger the release of neurons into the cerebral cortex). The main difference is in the sound wave detection mechanism. In air conduction, the sound waves enter the ear, and the waves cause direct vibration of the tympanic membrane. However, in bone conduction, the skull segments vibrate and cause vibrations of the basilar membrane (Dauman 2013). Skull vibrations can occur through two different pathways: compressional and inertial. Compressional bone conduction is used in high-frequency sound detection and is characterized by the individual vibration of each segment of the skull. Inertial bone conduction, however, is used for low-frequency detection and causes the skull to vibrate as a whole around the suspended parts of the inner ear (Britannica 2018).

Although most species in this dissertation primarily use air conduction for sound detection, evidence exists of bone conduction in Asian elephants (O'connell, Hart, and Arnason 1998; Reuter, Nummela, and Hemilä 1998). This is especially important due to the prevalence of seismic vibrations in human-environments and observations of African elephants (*Loxodonta africana*) expressing retreat behaviors when exposed to seismic vibrations from anthropogenic noises (Mortimer et al. 2021).

Sound Use in Terrestrial Vertebrates

Sound is used for direct communication (e.g., Brumm and Slabbekoorn 2005; Michelsen 1992), localization (Heffner and Heffner 2016), and indirect communication (e.g., Magrath et al. 2015; Schmidt and Ostfeld 2008; Webster, McNutt, and McComb 2010). Sound frequency detection varies from species to species and is correlated with skull size and vocalization frequencies. With the evolution of the middle ear, the resulting frequency range and amplitude detection available to the inner ear expanded (Manley 2012). There are some physical attributes that contribute to how well an animal can detect high or low frequencies; one of which is skull size. Species with smaller heads, such as rodents and cats, can detect frequencies much higher than humans, while animals with larger skull sizes are able to detect much lower frequencies (Figure 1; Heffner and Heffner 2008). Due

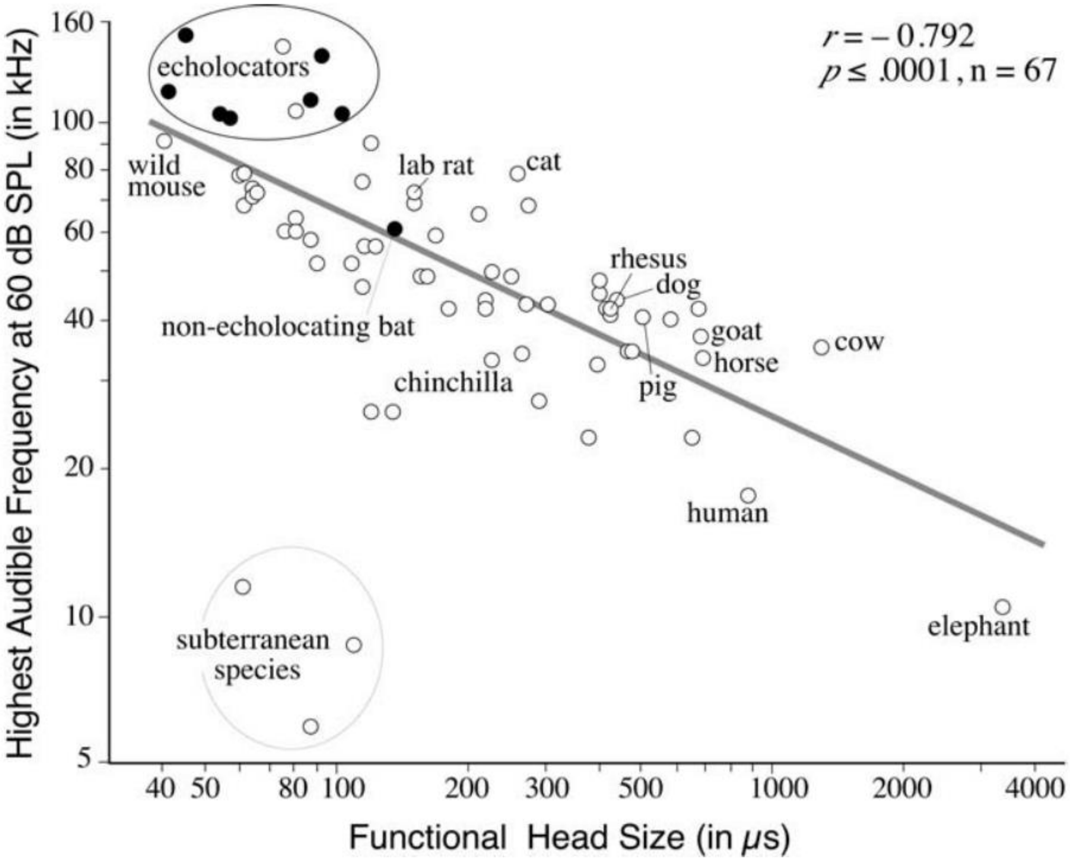


Figure 1: Relation between functional head size and high-frequency hearing (highest frequency audible at 60 dB sound pressure level) for mammals. From Heffner & Heffner (2008).

to the nature of low frequency waves (larger amplitudes and slower oscillations), animals with smaller skulls struggle to impede and capture the information within low frequency waves. To better understand the relationship between head size and hearing frequency thresholds, the "functional head size" was introduced (Heffner and Heffner 2016). Functional head size is the delay in time it takes for one ear to detect noise before the other and, in terrestrial animals it is determined by dividing the distance between one ear canal and the other by the speed of sound in air. It is important to note that functional head size is measured at the species level – a chihuahua does not have a higher frequency threshold than Labrador retriever

(Heffner 1983). This lack of within-species variation in high frequency detection has been hypothesized to be an inherited species-level trait (Heffner 1983). As with many rules in nature, there are exceptions. For instance, bats that use echolocation hear higher frequencies than other mammals with similar head size, including other non-echolocating bat species (Heffner, Koay, and Heffner 2006). This indicates that hearing purpose, not just physical attributes, contribute to hearing ranges.

For this study, functional head size is important to understand as it may explain differences in noise perception. For instance, Asian elephants have significantly larger heads and therefore have better low-frequency hearing than other species included in this study. This may indicate that Asian elephants are more sensitive to anthropogenic noises at the Oregon Zoo than the other species due to this strong affinity for low-frequency detection.

Communication

In the animal kingdom, sound is used for both indirect and direct communication. Vocalizations are directly used to aid mate selection, predator/threat deterrence, and fitness promotion (Larsen and Radford 2018). Additionally, vocalizations have been indirectly used by eavesdropping animals to determine potential dangers, resource availability, and weather patterns (Magrath et al. 2015). In anthropogenic environments, frequencies associated with animal communication can become crowded, resulting in changes in behavior or communication techniques. Specifically, anthropogenic noise affects animal communication through information and energetic masking (Rosa and Koper 2018).

While the species in this study have not been specifically investigated for information or energetic masking from anthropogenic sources, studies have shown this phenomenon in other species. For instance, European robins (*Erithacus rubecula*) have been shown to shift their vocalizations to periods of less urban noise when daytime noise is excessive to avoid competition with anthropogenic noise (Fuller, Warren, and Gaston 2007).

Sound Impacts on Animals

Sound has been shown to impact both wild and captive animals in multiple ways. Anthropogenic noises, specifically, have been the subjects of multiple analyses due to their increased abundance. Increases in road traffic and domestic flights have outpaced population growth since the 1980s (Barber, Crooks, and Fristrup 2010) and have correlated with significant increases in anthropogenic noises in protected natural areas (Buxton et al. 2017). Unfortunately, the effects of these noise changes are notoriously difficult to monitor in wildlife as differing hearing thresholds and evolutionary backgrounds can contribute to multiple reactions across taxa to the same noise source (Brumm and Slabbekoorn 2005). Additionally, anthropogenic noises are difficult to extricate from additional stressors, such as visual and olfactory cues associated with habitat disturbance (Summers, Cunnington, and Fahrig 2011). Despite this difficulty, recent studies have proven the detrimental effect of anthropogenic noise independently of these other stressors (McClure et al. 2013).

Over exposure to noise has been shown to lead to four main effects: permanent hearing loss (permanent threshold shift, PTS), temporary hearing loss (temporary threshold shift, TTS), masking, as well as multiple behavioral and physiological changes. TTS is the most standard form of hearing loss but, as sound exposure increases in intensity, or duration, the amount of time required for full recovery also increases. Eventually the sound intensity or duration reaches a level that is significant enough to permanently alter sound detection resulting in PTS (Saunders and Dooling 2018). Like most animal traits, there are individual differences in the likelihood of experiencing a TTS or a PTS to the same sound exposure level. This variation is potentially linked to genetics (Davis et al. 2001), but is widely uninvestigated.

Impacts of environmental noise can affect almost all aspects of an animal's life. Environmental noise can trigger hormonal changes (Burow, Day, and Campeau 2005; Kaiser et al. 2015; Kleist et al. 2018; Troïanowski et al. 2017), alter reproduction and development rates (Gurule-Small and Tinghitella 2019; Kaiser et al. 2015; Mulholland et al. 2018), disrupt metabolism (Brischoux et al. 2017) and influence many other physiological pathways (see Kight and Swaddle 2011 for a thorough review). This dissertation focuses on the effect of anthropogenic noise on stress hormones and behavior expression on select species of mammals at the Oregon Zoo; specifically how alterations in sound through various zoo events, and changes in visitor density and visitor presence has African painted dogs (*Lycaon pictus*), Asian elephants (*Elephas maximus indicus* & *Elephas maximus borneensis*), giraffes (*Giraffa camelopardalis reticulata* & *Giraffa camelopardalis tippelskirchi*), cheetahs (*Acinonyx jubatus*), ring-tailed lemurs (*Lemur catta*), and red-ruffed lemur (*Varecia rubra*). These species were chosen, in part, due to potential welfare concerns as previous research has indicated potential sensitivity towards stereotypic behavior and health issues within zoo environments (African painted dog: Asa et al. 2014; giraffe: Bashaw et al. 2001; Asian elephant: Greco et al. 2017; ring-tailed lemur: Hosey et al. 2016; red-ruffed lemur: Tarou, Bloomsmith, and Maple 2005; cheetah: Terio, Marker, and Munson 2004). The following sections provide relevant life histories and biological traits for each of the species with special attention to their sensitivity to noise and any previous hearing research.

African Painted Dog (Lycaon pictus)

African painted dogs, (African wild dogs, Cape hunting dog, or African hunting dogs) are canines native to sub-Saharan Africa. Two subpopulations are recognized by the International Union for Conservation of Nature (IUCN): the North African and the West African. These subpopulations are currently being assessed separately by the IUCN due to extreme unlikeliness that they will overcome the large separation distance and

Figure 2: An African painted dog (*L. pictus*). Photo: ©Laurel Fink

ever merge (Woodroffe and Sillero-Zubiri 2012c, 2012b, 2012a). The African painted dog is considered endangered on the IUCN Red List of Threatened Species due to their declining numbers. As of assessment publication in 2020, the declining population includes approximately 6,600 adults, but this number continues to fall due to habitat fragmentation, increasing human-wildlife conflict, and disease (Woodroffe and Sillero-Zubiri 2020).

Taxonomy

First described in 1820 by Coenraad Temminck, the African painted dog was initially mis-classified as a species of hyena (*Hyaena picta*) (Creel and Creel 2002). In 1827, Joshua Brookes renamed the species as *Lycaon tricolor.* With the adaptation of the International Rules on Taxonomic Nomenclature, the African painted dog was finally renamed *Lycaon pictus* (Bothma and Walker 1999a).

Physical Characteristics

African painted dogs are tall (approximately 75 cm at shoulder height), mid-weight (20-25 kg) canines with dappled coats (Frame et al. 1979) (Figure 2). No sexual dimorphism appears in this species. They have large, protruding pinnae which are highly mobile and aid in deciphering complex vocalizations from the environment (Chengetanai et al. 2020).

Social Structure

African painted dogs are social carnivores that live in large (2 - >20 adults) stable packs with a dominance hierarchy (Maddock and Mills 1994). Each pack contains only one dominant breeding pair (although subordinates have been known to breed) and litters are raised cooperatively (Frame et al. 1979). Due to the cooperative nature of pack-rearing, most packs raise a single litter per breeding season (Frame et al. 1979). African painted dogs of the same sex within a pack have a distinctly high level of relatedness in comparison with other African carnivores (Bothma and Walker 1999a). The dominant breeding pair are the only unrelated pack members. All others are either littermates of the dominant pair or their offspring (Bothma and Walker 1999a). This strong sense of social structure leads to limited levels of aggression (Bothma and Walker 1999a). New packs are formed with the intermingling of male or female littermates from different packs. Typically, this occurs when female littermates leave their original pack and join with unrelated males of similar ages and numbers.

Hearing

Little is currently known about the hearing thresholds of African painted dogs. However, the African painted dog exhibits one of the most variable and complex ranges of vocalizations in all canids (Robbins 2000). Therefore, it can be extrapolated that African painted dog hearing must be broad and sensitive enough to decipher both the sounds made by prey and predators as well as communications within the pack members. Despite the common interpretation that African painted dogs have acutely strong hearing, the African painted dog has been shown to have similar systems-level organization of the auditory system and inner organs of the ear not significantly different than those of other mammals (Chengetanai et al. 2020). Until a formal assessment of African painted dog frequency ranges is conducted, the hearing range of domestic dogs is used as a surrogate (67 Hz – 45 kHz at 60 dB SPL) (Heffner 1983).

Vocalizations

Perhaps the most unique characteristic of African painted dogs is their abundance of vocalizations. In addition to typical vocalizations associated with canines (barks, growls, whines), African painted dogs have several unique vocal classes (twitters, rumbles, begging cries) not found in other species (Robbins 2000). While some vocalizations are centered on the individual producing them, others (like greeting ceremonies) cascade into pack-wide vocalizing (Robbins 2000). For African painted dogs, behavior rituals tend to accompany vocalizations. For instance, begging cries are typically produced by whelping females approaching other pack members and initiating muzzle-to-muzzle contact to encourage regurgitation (Robbins 2000). In addition to tone-based vocalizations, research has also revealed the importance of sneezing to assist in decision-making. Walker et al. (2017) discovered a minimum number of nasal exhalations (sneezes) triggers pack movement. This minimum number is lowered whenever the dominant pair initiates the sneeze sequence, providing evidence that vocalization importance varies depending on the hierarchy of the individual.

Asian Elephants (Elephas maximus indicus & Elephas maximus borneensis)

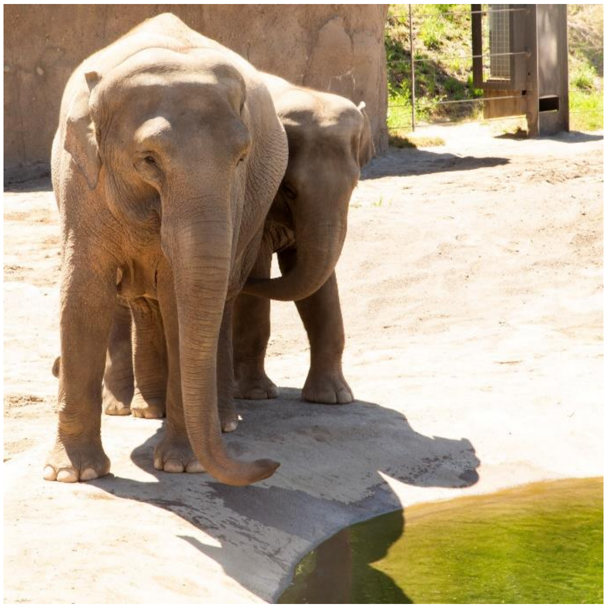


Figure 3: Female Asian elephants (*E. m. indicus*). Photo: © Laurel Fink

The Asian elephant range spans 13 range states: Bangladesh, Bhutan, India, Nepal, Sri Lanka, Cambodia, China, Indonesia, Lao PDR, Malaysia, Myanmar, Thailand, and Viet Nam (Williams et al. 2020). Recent research has shown that the sub-population of Asian elephants found on Borneo (*E. m. borneensis*) are indigenous to the island and not feral descendants of introduced elephants (Fernando et al. 2003). Asian elephants are listed as endangered on the IUCN Red List of Threatened Species due to over a 50% decline in their natural habitat in the last three generations (Williams et al. 2020). As of 2006, there were approximately 38,500 – 52,500 individuals across the thirteen different countries (Sukumar 2006).

Taxonomy

Historically, taxonomists have disagreed on the number of Asian elephant subspecies verses subpopulations. Currently, three subspecies are recognized by the IUCN: *Elephas maximus indicus*, *Elephas maximus maximus,* and *Elephas maximus sumatranus*. These subspecies are located on the Asian mainland, Sri Lanka, and the Indonesian island of Sumatra, respectively. Prior to research by Fernando et al.

(2003), Bornean Asian elephants were included in *E. m. indicus* or *E. m. sumatranus*, however due to morphological and mitochondrial DNA differences, a new subspecies (*Elephas maximus borneensis*) has been proposed. Additionally, two extinct subspecies are proposed (*Elephas maximus asurus* and *Elephas maximus rubridens*) but are currently considered subpopulations (Williams et al. 2020).

Physical Characteristics

Asian elephants are the second largest land mammal behind the African elephant (*Loxodonta africana*) weighing approximately 2,000 – 5,500 kg (4,400 – 12,000 lbs.) (San Deigo Zoo Wildlife Alliance). Unlike African elephants whose tallest point is the shoulder, Asian elephants have maximum height at the head (Shoshani and Eisenberg 1982). The skin of the Asian elephant ranges in texture but is thick and grey with occasional white or pink blotches on the ears, trunk, or neck. This depigmentation has been proposed to be a visual identification aid due to its individuality (Vidya, Prasad, and Ghosh 2014). Again, in contrast with African elephants, only male Asian elephants have tusks, and they are thinner and straighter than their African counterparts. The Asian elephant's trunk is hypothesized to be more extendable than African elephants and culminates in a single "finger" rather than two (Shoshani and Eisenberg 1982) (Figure 3). The Bornean subpopulation (*E. m. borneensis*) is the smallest Asian elephant (Shoshani and Eisenberg 1982); however they have the largest pinnae and longest tail of the Asian elephants (San Deigo Zoo Wildlife Alliance).

Social Structure

Both African and Asian elephants have a fission-fusion social system. In African elephants, this social system minimizes inter-herd food competition during times of limited resources with herds splitting during low resource availability and fusing together during periods of food abundance (Nandini, Keerthipriya, and Vidya 2017; Wittemyer, Douglas-Hamilton, and Getz 2005). However, in Asian elephants, there are minimal seasonal effects on either the group size or social structure, and this may be due to the relatively stable seasonal food abundance and food dispersal in their habitats (Nandini et al. 2017).

The basic social unit of Asian elephants includes an older matriarch and her offspring; however, the small units join to form larger multi-family units. Upon maturation, male Asian elephants disperse and live either alone or in small groups called “bull herds” with weak social bonds and travel between the matriarchal units for reproductive purposes (Sukumar 2006). Bull elephants become dominant when they enter musth. When in musth, the blood testosterone level increases and the bulls express heightened levels of aggression towards other bulls, thus increasing their chance of successfully mating a cycling female (Sukumar 2006).

Hearing

Elephants arguably have the best low-frequency hearing of all terrestrial mammals and among the worst high-frequency hearing (Heffner and Heffner 2008). The Asian elephant hearing threshold was determined to be approximately 17 Hz – 10.5 kHz at 65 dB SPL (Heffner and Heffner 1982). They can detect frequencies up to an octave lower than humans but fall well-short of the average mammalian high-frequency cut-off of 55 kHz. They have been found to localize sound origin more consistently at lower frequencies than at higher frequencies (Heffner and Heffner 1982).

The hearing threshold determined by Heffner and Heffner (1982) was based on air conduction, but more recent studies have indicated that elephants may rely on bone conduction to detect sound sources significantly further away (Reuter et al. 1998). Asian elephant "rumbles" and foot-stomps have been shown to produce correlating seismic signals at near and far distances that are detectable within the Asian elephant's low-frequency hearing threshold (O'Connell-Rodwell, Arnason, and Hart 2000). Elephants may detect and respond to these low-frequency disturbances through bone conduction, vibration-sensitive mechano-receptors, or a combination of the two (O'connell et al. 1998). The vibrations from these low-frequency calls are detected through the feet and travel through the legs and shoulders into the middle-ear cavity (O'Connell-Rodwell 2007). When detecting seismic waves, elephants lean more weight onto their front legs, creating a more direct pathway from foot to middle ear, and orient themselves perpendicular to the source – potentially allowing for more sensitive localization (O'Connell-Rodwell 2007). A study by Bouley et al. (2007) proposed that Asian elephants use specified mechanoreceptors to detect seismic waves through the foot, in addition to bone conduction.

Vocalization

Asian elephants utilize both air-borne sounds and seismic vibrations to communicate (O’Connell-Rodwell et al. 2000). Due to the Asian elephant’s superb low-frequency hearing, it is unsurprising that they communicate using low-frequency tones. In some cases, such as mock-charges which culminate in foot-stomping behavior or rumbles which are produced at such high amplitudes they propagate as Rayleigh waves (O’Connell-Rodwell, Guan, and Puria 2019). The two different acoustic methods propagate at different velocities with the acoustic signals moving faster than the seismic signals. However, seismic waves attenuate less than air-borne signals and transmit information over greater distances (O’Connell-Rodwell et al. 2000).

Giraffe (Giraffa camelopardalis reticulata & Giraffa camelopardalis tippelskirchi)

Giraffes are widespread across southern and eastern Africa and maintain smaller ranges in west and central Africa. They have adapted to successfully inhabit a multitude of different ecosystems, from desert to woodland environments (Muller et al. 2018). Currently, there are nine different subspecies recognized by the IUCN: Angolan (smokey) giraffe (*Giraffa camelopardalis angolensis*), Kordofan giraffe

Figure 4: The giraffe (*G. c. reticulata*). Photo: © Laurel Fink

(*Giraffa camelopardalis antiquorum*), Nubian giraffe (*Giraffa camelopardalis camelopardalis*), West African giraffe (*Giraffa camelopardalis peralta*), reticulated giraffe (*Giraffa camelopardalis reticulata*), Rothschild's giraffe (*Giraffa camelopardalis rothschildi*), Masai giraffe (*Giraffa camelopardalis tippelskirchi*), South African giraffe (*Giraffa camelopardalis giraffa*) and Thornicroft giraffe (*Giraffa camelopardalis thornicrofti*). Giraffes are considered vulnerable on the IUCN Red List of Threatened Species due to the population decline of 36-40% over the last three generations. Best estimates put the overall giraffe population at 97,562 giraffes in 2015 (Muller et al. 2018).

Taxonomy

There is considerable debate among giraffe experts concerning the classification of giraffes. Some experts believe there are species-level differences between some subspecies. Fennessy et al. (2016), for instance, considers that there are four distinct species of giraffe based on multi-locus population genetic analyses. This proposed classification has been met with hesitation (see Bercovitch et al. 2017 and Fennessy et al. 2017 for more details), but if this new classification were to be accepted, the four new species of giraffes would be: Southern giraffe (*G. giraffa* – a combination of Angolan giraffe and South African giraffe), Masai giraffe (*G. tippelskirchi* – combining Masai giraffes and Thornicroft's giraffe), reticulated giraffe (*G. reticulata*), and the Northern giraffe (*G. camelopardalis* – combining Rothschild's giraffe, Numbian giraffe, West African giraffe, and Kordofan giraffe) (Coimbra et al. 2021; Fennessy et al. 2016). I will be focusing the remainder of this section on the reticulated giraffe (*G. c. reticulata*) and Masai giraffe (*G. c. tippelskirchi*) due to their inclusion in my study.

Physical Characteristics

Giraffes are approximately 5-6 meters tall with sexual dimorphism resulting in smaller females than males. Both the legs and the necks of giraffes exceed 1.5 meters in length and small, course hairs are present as a mane along the neck (Dagg 1971). Giraffe males weigh approximately 973-1395 kg (2145-3075 lbs.) while females weigh approximately 703-950 kg (1550-2094 lbs.) (San Deigo Zoo Wildlife Alliance). Both male and female giraffes also have ossicones "horns" which are specific to giraffids and consist of cartilage fused to the skull and covered with skin and hair. Perhaps most unique to giraffes is their extended neck. This neck is the result of elongation of the vertebrae and provides a unique hurdle for the cardiovascular system. In order to circulate blood through the elongated neck, the cardiovascular system maintains a considerably higher blood pressure (Mitchell and Skinner 2009). Giraffe hide has irregular brown blotches that vary in size and intensity depending on size and subspecies (Dagg 1971) (Figure 4).

Social Structure

Historically, giraffes were thought to form loose structured herds with distribution of individuals in the same herd spaced up to a kilometer apart (Foster and Dagg 1972). However, more recent research on giraffe social structure indicate a fission-fusion social dynamic based on choice rather than relatedness or distributional overlap (Carter, Brand, et al. 2013; Carter, Seddon, et al. 2013).

Interestingly, giraffes living in fission-fusion societies fail to exhibit the cooperative behaviors typically associated with herd living (Carter, Seddon, et al. 2013). This lack of cooperative behavior may have contributed to the previous lack of herd recognition. It is only with detailed research on social association and genetic relationships that giraffe herd composition has been understood (Carter, Brand, et al. 2013; Carter, Seddon, et al. 2013). Male giraffes tend to occupy more densely forested regions than females who may require more visual communication due to the presence of offspring and subsequent increased need for predator detection (Foster and Dagg 1972). Similar to elephants, lone giraffes are much more likely to be male than female, but lone females have been spotted just before giving birth (Foster and Dagg 1972).

Hearing and Vocalizations

Surprisingly little is known about giraffe hearing thresholds and what little is known is based on their vocalizations. One of the main requirements of a successful fission-fusion society is an established and well-developed communication system (Baotic, Sicks, and Stoeger 2015), so it is reasonable to hypothesize that giraffes maintain auditory communication. Giraffes have been shown to have well developed larynx and laryngeal nerves (Erdoğan and Pérez 2013), but historical research proposed that giraffes would have difficulty producing enough velocity to cause vibrations of the vocal fold due to their elongated neck (Harrison 1981). Thus, giraffes were considered mute until research began to document instances of vocalizations. Despite the report by Harrison (1981), anecdotal reports of giraffe vocalizations have been recorded for decades. Recently, reports of "hisses" and "snorts" have been analyzed and hypothesized to be short-distance alarm calls as they were directed either at the researchers or when near cheetahs and cubs (Volodina et al. 2018).

One important discovery has been the recognition of infrasound (frequencies lower than human detection) production by giraffes. Von Muggenthaler (2013) demonstrated the presence of Helmholtz resonance (a specific type of resonance that relies on subjecting an enclosed body of air with one pathway in or out to vibration) to produce multiple low-frequency vocalizations. These vocalizations occurred within a frequency of 11Hz (at 60 dB SPL) to 11,200 Hz (at 89 dB SPL) and were always accompanied by either a neck stretch or a head throw (Von Muggenthaler 2013). Additional evidence for the production and use of low-frequency communication is the relatively quiet nature of giraffe habitats within the frequencies giraffe vocalizations are broadcast (10 to 20 Hz) – only a few other species of animal have been shown to communicate in these frequencies (Von Muggenthaler 2013). In addition to the low-frequency tones reported by Von Muggenthaler (2013), nocturnal "humming" was another low-frequency communication analyzed in 2015 at approximately 92.01 (± 25.78) Hz. This humming has been hypothesized to be a supplemental nocturnal communication when visual communication is difficult or absent (Baotic et al. 2015).

Cheetah (Acinonyx jubatus)

Figure 5: Cheetah (*A. jubatus*). Photo: © Laurel Fink

The cheetah (or hunting leopard) is among the smallest of the "big cat" species. Their range stretches throughout Africa and into southern Asia. The largest and most steady populations of cheetah reside in southern and eastern Africa, yet these populations are currently maintained on 22% and 6% of their historical range, respectively (Durant et al. 2015). The entire population of cheetahs resides on approximately 10% of their historical range and exist at extremely low densities (2.3 individuals per 10,000 km2) (Belbachir et al. 2015). Cheetahs are considered vulnerable on the IUCN Red List of Threatened Species due to their continuing population decline. As of 2015, there were estimated to be 6,674 mature individuals (Durant et al. 2015). Among the reasoning for the population decline is the loss of genetic variation due to a severe historical genetic bottleneck or series of demographic reductions (Schmidt-Küntzel et al. 2018).

Taxonomy

First described by J.C.D. von Shreber in 1775 as *Felis jubata* (Bothma and Walker 1999b), there are currently five subspecies of cheetah based on habitat range and recognized by the IUCN: *A. j. hecki*, *A. j. fearsoni*, *A. j. jubatus*, *A. j. soemmerringi*, and *A. j. venaticus* (Krausman and Morales 2005). Cheetahs are the only living representative of its genus (Schmidt-Küntzel et al. 2018).

Physical Characteristics

Cheetahs are tall, lanky cats with spotted coats. Adult males weigh up to 65 kg (143lbs) while females weigh slightly less at up to 55 kg (121lbs). A cheetah's coat is representative of its habitat. Cheetahs who live in the open, sandy desert are more pale while cheetahs in the mountain ranges have darker spots (Bothma and Walker 1999b). A rare coat coloration also occurs in southern Africa and individuals exhibiting it are referred to as "king cheetahs". King cheetah's mid-dorsal spots are merged to form parallel stripes along the back – similar to the serval (*Leptailurus serval*) (Bothma and Walker 1999b) (Figure 5).

Social Structure

Cheetahs have a combination of nomadic and stationary lifestyles. They are also the only large African cat, besides the African lion (*Panthera leo*) to form groups. These groups typically consist of either a female and her cubs or male siblings. Unrelated males have been known to form coalitions to defend or conquer territory, as single cheetahs were significantly less likely to successfully defend or take over territory (Collins and Caro 2010). Adolescent cheetahs had higher rates of survival when they lived in groups of 2-4 (Durant, Kelly, and Caro 2004). Unlike in lion prides, these coalitions do not have a seemingly dominant male and all males participate in mating (Bothma and Walker 1999b). Durant, Kelly, and Caro (2004) recorded a strong correlation between dispersal and sex; approximately 72% of female and only 54% of male cheetahs were observed in their study area from birth.

Hearing

Unfortunately, a formal hearing threshold analysis has not been conducted for cheetahs. However, information from other felids can be extrapolated to provide background information on cheetah hearing thresholds until a formal study is completed. Huang, Rosowski, and Peake (2000) analyzed the hearing thresholds of 11 species of exotic felids and linked increases in low-frequency hearing sensitivity to increased body mass and skull length. Only one behavior-based hearing threshold study has been conducted on a felid, domestic cat (*Felis catus*), (Heffner and Heffner 1985). Domestic cats have a hearing threshold of 48 Hz – 85 kHz (70 dB SPL) (Heffner and Heffner 1985), but their skull length is considerably smaller than the cheetah – approximately 9 cm (Ramos et al. 2021) verses 23 cm (Boast et al. 2013). Therefore, based on estimates by Huang et al. (2000), it can be inferred that cheetah low-frequency hearing extends beyond the domestic cat. How far it extends, however, is undetermined.

Vocalizations

Cheetahs have been shown to have three types of vocalization sounds: tonal, noisy, and sounds with pulsation (Volodina 2000). There are some overall frequency range overlaps within each of these vocalizations. Tonal sounds (meows, chirps, and howls) range from 20 Hz – 8 kHz, noisy sounds (hisses) range from 20 Hz – 7 kHz, and pulsating sounds (purring, growling, gurgling) range from 20 Hz – 7 kHz (Volodina 2000). While most sounds are produced only through expiration, purring vocalizations occur throughout the entire respiratory cycle (Sissom, Rice, and Peters

1991). Different vocalizations are correlated with specific behaviors and emotional states. For instance, gurgling calls occur as greetings between familiar conspecifics in familiar locations, purring is correlated with relaxed, friendly behavior, growling is typically conducted when a confident cheetah is threatening something, while meowing occurs when a less-confident cheetah attempts to appear threatening (Volodina 2000).

Ring-Tailed Lemur (Lemur catta)

The ring-tailed lemur are native to multiple habitats in southern and south-western Madagascar, as well as a humid forest in south-eastern Madagascar (LaFleur and Gould 2020). Unfortunately, climatic models from Brown and Yoder (2015) have predicted a loss of 63% of available ring-tailed lemur habitats. This prediction, paired with confirmed severe habitat fragmentation and exploitation for hunting and the illegal pet trade, justify the endangered status of the ring-tailed lemur on the IUCN Red List of Threatened Species (LaFleur and Gould 2020).

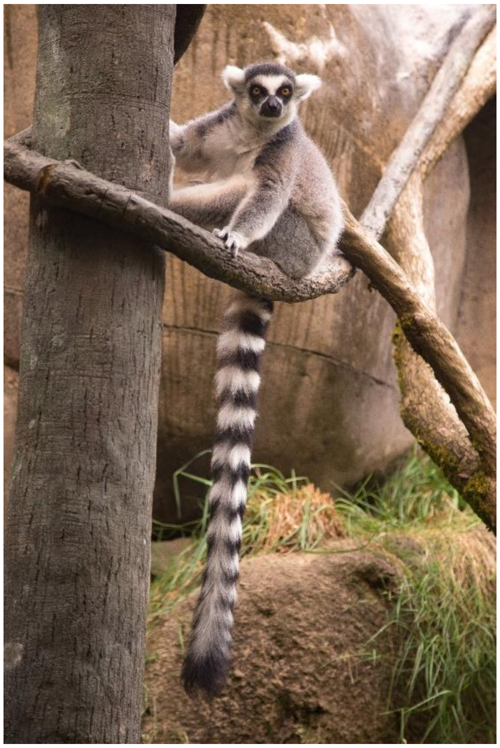


Figure 6: A ring-tailed lemur (*L. catta*). Photo: © Laurel Fink

Taxonomy

Ring-tailed lemurs were first described by Linnaeus (1758). There are no recognized subspecies of ring-tailed lemurs. Therefore, ring-tailed lemurs are members of a monotypic taxon and the only known surviving semiterrestrial, diurnal lemur in Madagascar (Mittermeier et al. 2008).

Physical Characteristics

The ring-tailed lemur is a medium-sized lemur weighing less than 2.2 kg (4.9 lbs.) (Mittermeier et al. 2008). They are among the most easily recognized lemur species due to the black and white bands on their long tails. They have grey to mauve coloration on their backs, limbs, and haunches but their stomachs are white. Their facial coloration is white with black eye-patches and ears. Both males and female ring-tailed lemurs have specialized glands on their wrists used for scent marking (Figure 6).

Social Structure

Ring-tailed lemur troops average approximately 13-15 individuals but can range from 5-27 individuals (Sauther and Sussman 1993). In each breeding season, up to 85% of females give birth to combat the 30% - 50% mortality of offspring within the first year (Mertl-Millhollen et al. 1979; Sauther and Sussman 1993). Female ring-tailed lemurs remain in their natal group while males tend to migrate between 3-to-5 years old to other troops (Sussman 1992). Interestingly, the territoriality of ring-tailed lemur troops has been under debate due to their overlapping home ranges. Sauther and Sussman (1993), using the definition of territoriality as maintaining exclusive use of a home range, hypothesized that ring-tailed lemurs were not territorial as they do not maintain exclusivity of a specific area.

Ring-tailed lemurs are hierarchical within both sexes despite being a female-dominant species. Dominant females tend to dictate the direction of migration and cause troop agitation when they cannot be found by other troop members (Sauther and Sussman 1993). During observations of ring-tailed lemurs in Beza Mahafaly, Sauther and Sussman (1993) identified a group of core males that maintained positive rapport with the troop females. However, one male did appear to monopolize the early breeding opportunities and have the earliest feeding opportunities. The dominance hierarchy is determined by the results of agonistic encounters (Sauther and Sussman 1993). Thus, males who have recently migrated are among the lowest ranked individuals until they participate, and win, enough agonistic encounters.

Hearing

Ring-tailed lemurs have been shown to not be as sensitive to low frequencies as other primates (Mitchell, Vernon, and Herman 1971). At 55 dB SPL, ring-tailed lemurs do not respond to auditory stimuli starting at 25 kHz (Mitchell et al. 1970). The same study also showed ring-tailed lemurs low-frequency hearing is approximately 100 Hz at 55 dB SPL and extrapolated the frequency range of ring-tailed lemur to approximately 100 Hz – 32 kHz at 60 dB SPL (Gillette et al. 1973).

This lower frequency threshold cut-off is at a much higher frequency than other animals reported in this dissertation.

Vocalizations

Macedonia (1993) provided an in-depth review of ring-tailed lemur vocal repertoires. Ring-tailed lemurs have approximately 22 defined vocalizations which fall into three main categories: affiliative, agonistic, and alert calls. Some vocalizations are sex-specific including male-specific “squeals” that are used in male-male interactions to assert dominance (Bolt 2013), while others – like antipredator vocalizations – are conducted by the entire troop (Bolt et al. 2015). The tonal vocalizations range from 192 Hz (“gulp”) to 8.3 kHz (“shriek”) in frequency, while the pulsatile/atonal calls ranged from 40 Hz (“Open-Mouth click series” calls) to 24 kHz (“cackle”) (Macedonia 1993).

Red-Ruffed Lemur (Varecia rubia)

Figure 7: Red-ruffed lemur (*V. rubia*). Photo: © Michael Durham, courtesy of the Oregon Zoo

Red ruffed lemurs are native to the remaining primary forests on the Masoala Peninsula and northern Makira region in northeastern Madagascar (Borgerson et al. 2020). Unfortunately, like all forest-dwelling lemur species of Madagascar, red ruffed lemurs are subject to the significant deforestation occurring in Madagascar. Their current distribution is approximately 6,423 km2 (Borgerson et al. 2020). As of 2014, approximately half of Madagascar's remaining forests are located within 100 meters of the forest's edge, subjecting the ecosystem to considerable edge effects (Vieilledent et al. 2018). The current rate of deforestation coupled with unsustainable hunting has placed the red ruffed lemur into the critically endangered category on the IUCN Red List of Threatened Species (Borgerson et al. 2020).

Taxonomy

Red-ruffed lemurs were first described by Geoffroy (1812). There are no recognized subspecies of red-ruffed lemurs. They are a member of the largest living quadrupedal lemur genus known as the "ruffed lemurs" (Mittermeier et al. 2008).

Physical Characteristics

Red ruffed lemurs are typically a dark, rusted red with black extremities, tail, forehead, and crown, although color variations do exist (Mittermeier et al. 2008). They are approximately 50-55 cm (1.6-1.8 ft) in body length with an additional 60-65 cm (2.0-2.1 ft) long tail and weigh 3.3-3.6 kg (7.3-7.9 lbs.) (San Deigo Zoo Wildlife Alliance). They are amongst the largest living members of the family Lemuridae (Figure 7).

Social Structure

Red ruffed lemurs exhibit a fission-fusion, multi-layered social structure. They have one of the highest reproductive costs due to high litter size, shortest gestation period, and rapidly growing offspring requiring much more milk than infants of other species (Vasey 2006). They are considered territorial with most clashes occurring within the warmer, bountiful months (Vasey 2006). These battles are seemingly led by female troop members with males engaging on the outskirt. While the social structure of red ruffed lemurs is similar to the social structure of the ring-tailed lemurs, their core group members only loosely affiliate with each other and do not form cohesive groups (Vasey 2006). Interestingly, there is a significant difference in ranging patterns based on sex. Male red ruffed lemurs maintain a relatively stable home range with very little overlap, while females shift their ranging patterns based on seasons and reproductive stages (Vasey 2006).

Hearing

Red ruffed lemurs have a high frequency threshold of 59 kHz at 60 dB SPL, which has been hypothesized to correspond to their relatively large social group size (Ramsier et al. 2012). The low frequency threshold at 60 dB SPL was reported at less than 50 Hz.

Vocalizations

No studies have specifically categorized and described the vocal repertoire of red-ruffed lemurs. However, Pereira, Seeligson, and Macedonia (1988) have described vocalizations of black-and-white ruffed lemurs (*Varecia variegata*). These vocalizations can be broken into “high-amplitude”, “moderate-amplitude”, and “low-amplitude” calls (Pereira et al. 1988). High-amplitude calls are designed to capture attention, alert conspecifics to sudden disturbances and predatory threats, and can be interspersed in mating rituals. Moderate amplitude calls signal low-level disturbances, social yielding, or behavioral frustration. Finally, low amplitude calls indicate mild aggravation or calls frequently exchanged between mother and infants

(Pereira et al. 1988). While the context of the calls may be overwhelmingly similar, there may be small species-specific differences that require additional analyses.

Research Objectives

The following chapters integrated audio sensitivity of overall decibel increases, implementation of new audio enrichment, and visitor-based noise levels with behavioral and physiological monitoring. Each chapter analyzed a different noise or visitor environment. Chapter 2 analyzed the effects of large-scale events and their associated noise and visitor density fluctuations on giraffes (*Giraffa camelopardalis reticulata* & *Giraffa camelopardalis tippelskirchi*), cheetahs (*Acinonyx jubatus*), African painted dogs (*Lycaon pictus*), and Asian elephants (*Elephas maximus indicus* & *Elephas maximus borneensis*) housed at the Oregon Zoo. Chapter 3 was an investigation of sound as a novel enrichment tool for ring-tailed lemurs (*Lemur catta*) and red-ruffed lemurs (*Varecia rubra*). Finally, chapter 4 studied the impact of a major transition in visitor presence (i.e., forced closure and visitor absence due to the COVID-19 pandemic) and its effect on the physiology and behavior of some of the species previously mentioned (cheetah and giraffe).

References

Asa, Cheryl S., Karen L. Bauman, Sarah Devery, Martín Zordan, Gerardo R. Camilo, Sally Boutelle, and Anneke Moresco. 2014. "Factors Associated With Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility." *Zoo Biol* 33:8–19. doi: 10.1002/zoo.21069.

Badyaev, Alexander V. 2005. "Role of Stress in Evolution: From Individual Adaptability to Evolutionary Adaptation." Pp. 277–302 in *Variation*, edited by B. Hallgrímsson and B. K. Hall. London, UK: Elsevier Inc.

Baotic, Anton, Florian Sicks, and Angela S. Stoeger. 2015. "Nocturnal 'Humming' Vocalizations: Adding a Piece to the Puzzle of Giraffe Vocal Communication." *BMC Research Notes* 8:425. doi: 10.1186/s13104-015-1394-3.

Barber, Jesse R., Kevin R. Crooks, and Kurt M. Fristrup. 2010. "The Costs of Chronic Noise Exposure for Terrestrial Organisms." *Trends in Ecology & Evolution* 25(3):180–89. doi: 10.1016/j.tree.2009.08.002.

Bashaw, Meredith Joy, Loraine Rybiski Tarou, Todd S. Maki, and Terry L. Maple. 2001. "A Survey Assessment of Variables Related to Stereotypy in Captive Giraffe and Okapi." *Applied Animal Behaviour Science* 73(3):235–47. doi: 10.1016/S0168-1591(01)00137-X.

Belbachir, Farid, Nathalie Pettorelli, Tim Wacher, Amel Belbachir-Bazi, and Sarah M. Durant. 2015. "Monitoring Rarity: The Critically Endangered Saharan Cheetah as a Flagship Species for a Threatened Ecosystem." *PLoS ONE* 10(1):1–15. doi: 10.1371/journal.pone.0115136.

Bercovitch, Fred B., Philip S. M. Berry, Anne Dagg, Francois Deacon, John B. Doherty, Derek E. Lee, Frédéric Mineur, Zoe Muller, Rob Ogden, Russell Seymour, Bryan Shorrocks, and Andy Tutchings. 2017. "How Many Species of Giraffe Are There?" *Current Biology* 27(4):R136–37. doi: 10.1016/j.cub.2016.12.039.

Boast, Lorraine K., Ann Marie Houser, Kyle Good, and Markus Gusset. 2013. "Regional Variation in Body Size of the Cheetah (Acinonyx Jubatus)." *Journal of Mammalogy* 94(6):1293–97. doi: 10.1644/13-MAMM-A-076.1.

Bolt, Laura M. 2013. "Squealing Rate Indicates Dominance Rank in the Male Ring-Tailed Lemur (Lemur Catta)." *American Journal of Primatology* 75(12):1174–84. doi: 10.1002/ajp.22179.

Bolt, Laura M., Michelle L. Sauther, Frank P. Cuozzo, and Ibrahim Antho Youssouf Jacky. 2015. "Antipredator Vocalization Usage in the Male Ring-Tailed Lemur (Lemur Catta)." *Folia Primatologica* 86(1–2):124–33. doi: 10.1159/000369064.

Borgerson, C., T. M. Eppley, E. Patel, S. Johnson, E. E. Louis, and J. Razafindramanana. 2020. "Varecia Rubra." *The IUCN Red List of Threatened Species* e.T22920A1.

Bothma, J. du P., and Clive Walker. 1999a. "The African Wild Dog." Pp. 130–57 in *Larger Carnivores of the African Savannas*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Bothma, J. du P., and Clive Walker. 1999b. "The Cheetah." Pp. 92–115 in *Larger Carnivores of the African Savannas*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Bouley, D. M., C. N. Alarcón, T. Hildebrandt, and C. E. O'Connell-Rodwell. 2007. "The Distribution, Density and Three-Dimensional Histomorphology of Pacinian Corpuscles in the Foot of the Asian Elephant (Elephas Maximus) and Their Potential Role in Seismic Communication." *Journal of Anatomy* 211(4):428–35. doi: 10.1111/j.1469-7580.2007.00792.x.

Brischoux, François, Alizée Meillère, Andréaz Dupoué, Olivier Lourdais, and Frédéric Angelier. 2017. "Traffic Noise Decreases Nestlings' Metabolic Rates in an Urban Exploiter." *Journal of Avian Biology* 48(7):905–9. doi: 10.1111/jav.01139.

Britannica, The Editors of Encyclopaedia. 2018. "Bone Conduction." *Encyclopedia Britannica*.

Britannica, The Editors of Encyclopaedia. 2022. "Transmission of Sound by Air Conduction." *Britannica*.

Brown, Jason L., and Anne D. Yoder. 2015. "Shifting Ranges and Conservation Challenges for Lemurs in the Face of Climate Change." *Ecology and Evolution* 5(6):1131–42. doi: 10.1002/ece3.1418.

Brumm, Henrik, and Hans Slabbekoorn. 2005. "Acoustic Communication in Noise." 35(05):151–209. doi: 10.1016/S0065-3454(05)35004-2.

Burow, Andrew, Heidi E. W. Day, and Serge Campeau. 2005. "A Detailed Characterization of Loud Noise Stress: Intensity Analysis of Hypothalamo-Pituitary-Adrenocortical Axis and Brain Activity." *Brain Research* 1062(1–2):63–73. doi: 10.1016/j.brainres.2010.09.038.Physical.

Buxton, Rachel T., Megan F. McKenna, Daniel Mennitt, Kurt Fristrup, Kevin Crooks, Lisa Angeloni, and George Wittemyer. 2017. "Noise Pollution Is Pervasive in U.S. Protected Areas." *Science* 356(6337):531–33. doi: 10.1126/science.aah4783.

Carter, Kerryn D., Rachel Brand, John K. Carter, Bryan Shorrocks, and Anne W. Goldizen. 2013. "Social Networks, Long-Term Associations and Age-Related Sociability Ofwild Giraffes." *Animal Behaviour* 86(5):901–10. doi: 10.1016/j.anbehav.2013.08.002.

Carter, Kerryn D., Jennifer M. Seddon, Celine H. Frère, John K. Carter, and Anne W. Goldizen. 2013. "Fission-Fusion Dynamics in Wild Giraffes May Be Driven by Kinship, Spatial Overlap and Individual Social Preferences." *Animal Behaviour* 85(2):385–94. doi: 10.1016/j.anbehav.2012.11.011.

Chamove, Arnold S., Geoffrey R. Hosey, and Peter Schaetzel. 1988. "Visitors Excite Primates in Zoos." *Zoo Biology* 7(4):359–69. doi: 10.1002/zoo.1430070407.

Chengetanai, Samson, Adhil Bhagwandin, Mads F. Bertelsen, Therese Hård, Patrick R. Hof, Muhammad A. Spocter, and Paul R. Manger. 2020. "The Brain of the African Wild Dog. III. The Auditory System." *Journal of Comparative Neurology* 528(18):3229–44. doi: 10.1002/cne.24989.

Coimbra, Raphael T. F., Sven Winter, Vikas Kumar, Klaus Peter Koepfli, Rebecca M. Gooley, Pavel Dobrynin, Julian Fennessy, and Axel Janke. 2021. "Whole-Genome Analysis of Giraffe Supports Four Distinct Species." *Current Biology* 31(13):2929-2938.e5. doi: 10.1016/j.cub.2021.04.033.

Collins, Courtney, Ilse Corkery, Amy Haigh, Sean McKeown, Thomas Quirke, and Ruth O'Riordan. 2017. "The Effects of Environmental and Visitor Variables on the Behavior of Free-Ranging Ring-Tailed Lemurs (Lemur Catta) in Captivity." *Zoo Biology* 36(4):250–60. doi: 10.1002/zoo.21370.

Collins, D. .., and T. M. Caro. 2010. "Male Cheetah Social Organization and Territoriality." *Ethology* 74(1):52–64. doi: 10.1111/j.1439-0310.1987.tb00921.x.

Creel, Scott, and Nancy Marusha Creel. 2002. "History and Natural History." Pp. 1–14 in *The African Wild Dog: Behavior, Ecology, and Conservation2*. Princeton University Press.

Dagg, Anne Innis. 1971. "Giraffa Camelopardalis." *Mammalian Species* (5):1–8. doi: 10.2307/3503830.

Dauman, R. 2013. "Bone Conduction: An Explanation for This Phenomenon Comprising Complex Mechanisms." *European Annals of Otorhinolaryngology, Head and Neck Diseases* 130(4):209–13. doi: 10.1016/j.anorl.2012.11.002.

Davis, Rickie R., J. Kelly Newlander, Xiao Bing Ling, Gino A. Cortopassi, Edward F. Krieg, and Lawrence C. Erway. 2001. "Genetic Basis for Susceptibility to Noise-Induced Hearing Loss in Mice." *Hearing Research* 155(1–2):82–90. doi: 10.1016/S0378-5955(01)00250-7.

Durant, S., N. Mitchell, A. Ipavec, and R. Groom. 2015. *Acinonyx Jubatus*.

Durant, Sarah M., Marcella Kelly, and Tim M. Caro. 2004. "Factors Affecting Life and Death in Serengeti Cheetahs: Environment, Age, and Sociality." *Behavioral Ecology* 15(1):11–22. doi: 10.1093/beheco/arg098.

Edes, Ashley N., Eli Baskir, Karen L. Bauman, Nathasha Chandrasekharan, Michael Macek, and Anne Tieber. 2021. "Effects of Crowd Size, Composition, and Noise Level On Pool Use in a Mixed-Species Penguin Colony." *Animal Behavior and Cognition* 8(4):507–20. doi: 10.26451/abc.08.04.05.2021.

Engelmann, Mario, Rainer Landgraf, and Carsten T. Wotjak. 2004. "The Hypothalamic-Neurohypophysial System Regulates the Hypothalamic-Pituitary-Adrenal Axis under Stress: An Old Concept Revisited." *Frontiers in Neuroendocrinology* 25(3–4):132–49. doi: 10.1016/j.yfrne.2004.09.001.

Erdoğan, S., and W. Pérez. 2013. "Anatomical Characteristics of the Larynx in Giraffe (*Giraffa Camelopardalis*)." *Journal of Morphological Sciences* 30(4):266–71.

Evans, E. M. Nesbit. 1970. "The Reaction of a Group of Rothschild'S Giraffe To a New Environment." *African Journal of Ecology* 8(1):53–62. doi: 10.1111/j.1365-2028.1970.tb00830.x.

Fanning, Lara, Hannah Larsen, and Peta S. Taylor. 2020. "A Preliminary Study Investigating the Impact of Musical Concerts on the Behavior of Captive Fiordland Penguins (*Eudyptes Pachyrhynchus*) and Collared Peccaries (*Pecari Tajacu*)." *Animals* 10(11):2035. doi: 10.3390/ani10112035.

Farrand, Alexandra. 2007. "The Effect of Zoo Visitors on the Behaviour and Welfare of Zoo Mammals." University of Stirling.

Fennessy, Julian, Tobias Bidon, Friederike Reuss, Vikas Kumar, Paul Elkan, Maria A. Nilsson, Melita Vamberger, Uwe Fritz, and Axel Janke. 2016. "Multi-Locus Analyses Reveal Four Giraffe Species Instead of One." *Current Biology* 26(18):2543–49. doi: 10.1016/j.cub.2016.07.036.

Fennessy, Julian, Sven Winter, Friederike Reuss, Vikas Kumar, Maria A. Nilsson, Melita Vamberger, Uwe Fritz, and Axel Janke. 2017. “Response to ‘How Many Species of Giraffe Are There?’” *Current Biology* 27(4):R137–38. doi: 10.1016/j.cub.2016.12.045.

Fernandez, Eduardo J., and Emma Harvey. 2021. “Enclosure Use as a Measure of Behavioural Welfare in Three Zoo-Housed African Wild Dogs Lycaon Pictus.” *Journal of Zoo and Aquarium Research* 9(2):88–93. doi: https://doi.org/10.19227/jzar.v9i2.526.

Fernando, Prithiviraj, T. N. C. Vidya, John Payne, Michael Stuewe, Geoffrey Davison, Raymond J. Alfred, Patrick Andau, Edwin Bosi, Annelisa Kilbourn, and Don J. Melnick. 2003. “DNA Analysis Indicates That Asian Elephants Are Native to Borneo and Are Therefore a High Priority for Conservation.” *PLoS Biology* 1(1):110–15. doi: 10.1371/journal.pbio.0000006.

Foster, J. B., and A. I. Dagg. 1972. “Notes on the Biology of the Giraffe.” *East African Wildlife Journal* 10(1):1–16. doi: 10.1111/j.1365-2028.1972.tb00855.x.

Frame, Lory Herbison, James R. Malcolm, George W. Frame, and Hugo Van Lawick. 1979. “Social Organization of African Wild Dogs (*Lycaon Pictus*) on the Serengeti Plains, Tanzania 1967-1978).” *Zeitschrift Für Tierpsychologie* 50:225–2.

Friend, T. 1980. “Stress: What Is It and How Can It Be Quantified?” *International Journal for the Study of Animal Problems* 1(6):366–74.

Fuller, Grace, Susan W. Margulis, and Rachel Santymire. 2011. “The Effectiveness of Indigestible Markers for Identifying Individual Animal Feces and Their Prevalence of Use in North American Zoos.” *Zoo Biology* 30(4):379–98. doi: 10.1002/zoo.20339.

Fuller, Richard A., Philip H. Warren, and Kevin J. Gaston. 2007. “Daytime Noise Predicts Nocturnal Singing in Urban Robins.” *Biology Letters* 3(4):368–70. doi: 10.1098/rsbl.2007.0134.

Gillette, Richard G., Richard Brown, Paul Herman, Steven Vernon, and Jack Vernon. 1973. “The Auditory Sensitivity of the Lemur.” *American Journal of Physical Anthropology* 38(2):365–70. doi: 10.1002/ajpa.1330380234.

Glaeser, Sharon S., David Shepherdson, Karen Lewis, Natalia Prado, Janine L. Brown, and Bob Lee. 2021. “Supporting Zoo Asian Elephant (Elephas Maximus) Welfare and Herd Dynamics with a More Complex and Expanded Habitat.” 1–44.

Greco, Brian J., Cheryl L. Meehan, Jennifer L. Heinsius, and Joy A. Mench. 2017. “Why Pace? The Influence of Social, Housing, Management, Life History, and Demographic Characteristics on Locomotor Stereotypy in Zoo Elephants.” *Applied Animal Behaviour Science* 194:104–11. doi: 10.1016/j.applanim.2017.05.003.

Gurule-Small, Gabrielle A., and Robin M. Tinghitella. 2019. “Life History Consequences of Developing in Anthropogenic Noise.” *Global Change Biology* 25(6):1957–66. doi: 10.1111/gcb.14610.

Harrison, D. F. N. 1981. “Fibre Size Frequency in the Recurrent Laryngeal Nerves of

Man and Giraffe." *Acta Oto-Laryngologica* 91(1–6):383–89. doi: 10.3109/00016488109138519.

Heffner, H. E. 1983. "Hearing in Large and Small Dogs: Absolute Thresholds and Size of the Tympanic Membrane." *Behavioral Neuroscience* 97(2):310–18. doi: 10.1037/0735-7044.97.2.310.

Heffner, H. E., and R. S. Heffner. 2008. "High-Frequency Hearing." *The Senses: A Comprehensive Reference* 3(December 2008):55–60. doi: 10.1016/B978-012370880-9.00004-9.

Heffner, Henry E. 1983. "Hearing in Large and Small Dogs: Absolute Thresholds and Size of the Tympanic Membrane." *Behavioral Neuroscience* 97(2):310–18.

Heffner, Henry E., and Rickye S. Heffner. 2016. "The Evolution of Mammalian Sound Localization." Pp. 20–35 in *Acoustics Today*. Vol. 20.

Heffner, R. S., G. Koay, and H. E. Heffner. 2006. "Hearing in Large (*Eidolon Helvum*) and Small (*Cynopterus Brachyotis*) Non-Echolocating Fruit Bats." *Hearing Research* 221(1–2):17–25. doi: 10.1016/j.heares.2006.06.008.

Heffner, Rickye S., and Henry E. Heffner. 1982. "Hearing in the Elephant (Elephas Maximus): Absolute Sensitivity, Frequency Discrimination, and Sound Localization." *Journal of Comparative and Physiological Psychology* 96(6):926–44.

Heffner, Rickye S., and Henry E. Heffner. 1985. "Hearing Range of the Domestic Cat." *Hearing Research1* 19:85–88.

Henry, Paula, and Tomasz R. Letowski. 2007. *Bone Conduction: Anatomy, Physiology, and Communication*. Aberdeen, MD.

Hosey, Geoff, Vicky Melfi, Isabel Formella, Samantha J. Ward, Marina Tokarski, Dave Brunger, Sara Brice, and Sonya P. Hill. 2016. "Is Wounding Aggression in Zoo-Housed Chimpanzees and Ring-Tailed Lemurs Related to Zoo Visitor Numbers?" *Zoo Biology* 35(3):205–9. doi: 10.1002/zoo.21277.

Huang, G. T., J. J. Rosowski, and W. T. Peake. 2000. "Relating Middle-Ear Acoustic Performance to Body Size in the Cat Family: Measurements and Models." *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology* 186(5):447–65. doi: 10.1007/s003590050444.

Jakob-Hoff, Kingan, Fenemore, Schmid, Cockrem, Crackle, Bemmel, Connor, and Descovich. 2019. "Potential Impact of Construction Noise on Selected Zoo Animals." *Animals* 9(504):1–25. doi: 10.3390/ani9080504.

Kaiser, Kristine, Julia Devito, Caitlin G. Jones, Adam Marentes, Rachel Perez, Lisa Umeh, Regina M. Weickum, Kathryn E. McGovern, Emma H. Wilson, and Wendy Saltzman. 2015. "Effects of Anthropogenic Noise on Endocrine and Reproductive Function in White's Treefrog, Litoria Caerulea." *Conservation Physiology* 3(1):1–8. doi: 10.1093/conphys/cou061.

Kight, Caitlin R., and John P. Swaddle. 2011. "How and Why Environmental Noise Impacts Animals: An Integrative, Mechanistic Review." *Ecology Letters* 14(10):1052–61. doi: 10.1111/j.1461-0248.2011.01664.x.

Kleist, Nathan J., Robert P. Guralnick, Alexander Cruz, Christopher A. Lowry, and Clinton D. Francis. 2018. "Chronic Anthropogenic Noise Disrupts Glucocorticoid

Signaling and Has Multiple Effects on Fitness in an Avian Community.” *Proceedings of the National Academy of Sciences of the United States of America* 115(4):E648–57. doi: 10.1073/pnas.1709200115.

Koester, Diana C., Elizabeth W. Freeman, Janine L. Brown, David E. Wildt, Kimberly A. Terrell, Ashley D. Franklin, and Adrienne E. Crosier. 2015. “Motile Sperm Output by Male Cheetahs (Acinonyx Jubatus) Managed Ex Situ Is Influenced by Public Exposure and Number of Care-Givers.” *PLoS ONE* 10(9):1–21. doi: 10.1371/journal.pone.0135847.

Koester, Diana C., David E. Wildt, Janine L. Brown, Karen Meeks, and Adrienne E. Crosier. 2017. “Public Exposure and Number of Conspecifics Have No Influence on Ovarian and Adrenal Activity in the Cheetah (Acinonyx Jubatus).” *General and Comparative Endocrinology* 243:120–29. doi: 10.1016/j.ygcen.2016.11.010.

Krausman, Paul R., and Susana M. Morales. 2005. “Acinonyx Jubatus.” *Mammalian Species* 771:1–6. doi: 10.1644/771/2600836.

Krishnan, Kavitha, and Stan Braude. 2014. “The Effect of Visitor Group Size on Stereotypic Behaviour and Use of Available Space by Captive Asian Elephants.” *Gajah* 41:3–11.

LaFleur, M., and L. Gould. 2020. “Lemur Catta.” *The IUCN Red List of Threatened Species* e.T11496A1.

Larsen, Ole Næsbye, and Craig Radford. 2018. “Acoustic Conditions Affecting Sound Communication in Air and Underwater.” Pp. 109–44 in *Effects of Anthropogenic Noise on Animals*.

Lynn, Brian Lee. 2018. *Zoo Giraffe Welfare: A Literature Review and the Behavioral Effects of Guest Feeding Programs*. Davis, CA: ProQuest Dissertations Publishing.

Macedonia, Joseph M. 1993. “The Vocal Repertoire of the Ringtailed Lemur (*Lemur CattA*).” *Folia Primatologica* 61(4):186–217. doi: 10.1159/000156749.

Maddock, A. H., and M. G. L. Mills. 1994. “Population Characteristics of African Wild Dogs Lycaon Pictus in the Eastern Transvaal Lowveld, South Africa, as Revealed through Photographic Records.” *Biological Conservation* 67(1):57–62. doi: 10.1016/0006-3207(94)90009-4.

Magrath, Robert D., Tonya M. Haff, Pamela M. Fallow, and Andrew N. Radford. 2015. “Eavesdropping on Heterospecific Alarm Calls: From Mechanisms to Consequences.” *Biological Reviews* 90(2):560–86. doi: 10.1111/brv.12122.

Manley, Geoffrey A. 2012. “Vertebrate Hearing: Origin, Evolution and Functions.” *Sensory Perception: Mind and Matter* 23–40. doi: 10.1007/978-3-211-99751-2_2.

Marten, Ken, and Peter Marler. 1977. “Sound Transmission and Its Significance for Animal Vocalization - I. Temperate Habitats.” *Behavioral Ecology and Sociobiology* 2(3):271–90. doi: 10.1007/BF00299740.

McClure, Christopher J. W., Heidi E. Ware, Jay Carlisle, Gregory Kaltenecker, and Jesse R. Barber. 2013. “An Experimental Investigation into the Effects of Traffic Noise on Distributions of Birds: Avoiding the Phantom Road.” *Proceedings of the Royal Society B: Biological Sciences* 280(1773). doi: 10.1098/rspb.2013.2290.

Meehan, Cheryl L., and Joy A. Mench. 2007. “The Challenge of Challenge: Can

Problem Solving Opportunities Enhance Animal Welfare?” *Applied Animal Behaviour Science* 102(3–4):246–61. doi: 10.1016/j.applanim.2006.05.031.

Mellor, D. J., C. J. Cook, and K. J. Stafford. 2000. “Quantifying Some Responses to Pain as a Stressor.” Pp. 171–98 in *The Biology of animal Stress: basic prinicples and implications for animal welfare*, edited by G. P. Moberg and J. A. Mench. Wallingford: CABI Publishing.

Menargues, A., V. Urios, and M. Mauri. 2008. “Welfare Assessment of Captive Asian Elephants (Elephas Maximus) and Indian Rhinoceros (Rhinoceros Unicornis) Using Salivary Cortisol Measurement.” *Animal Welfare* 17:305–12.

Mertl-Millhollen, Anne S., Herbert L. Gustafson, Norman Budnitz, Kathryn Dainis, and Alison Jolly. 1979. “Population and Territory Stability of the Lemur Catta at Berenty, Madagascar.” *Folia Primatologica* 31(1–2):106–22. doi: 10.1159/000155875.

Michelsen, Axel. 1992. “Hearing and Sound Communication in Small Animals: Evolutionary Adaptations to the Laws of Physics.” Pp. 61–77 in *The Evolutionary Biology of Hearing*, edited by D. B. Webster, A. N. Popper, and R. R. Fay. New York, NY: Springer New York.

Milligan, S. R., G. D. Sales, and K. Khirnykh. 1993. “Sound Levels in Rooms Housing Laboratory Animals: An Uncontrolled Daily Variable.” *Physiology and Behavior* 53(6):1067–76. doi: 10.1016/0031-9384(93)90361-I.

Mitchell, Curt, Richard Gillette, Jack Vernon, Paul Herman, T. Mitchell, Ack Vernon, and Paul Hernan. 1970. “Pure-Tone Auditory Behavioral Thresholds in Three Species of Lemurs Pure-Tone Auditory Behavioral Thresholds in Three Species o f Lemurs.” *Citation: The Journal of the Acoustical Society of America* 48:531. doi: 10.1121/1.1912168.

Mitchell, Curt, Jack Vernon, and Paul Herman. 1971. “What Does the Lemur Really Hear?” *Citation: The Journal of the Acoustical Society of America* 50:710. doi: 10.1121/1.1912687.

Mitchell, G., and J. D. Skinner. 2009. “An Allometric Analysis of the Giraffe Cardiovascular System.” *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 154(4):523–29. doi: 10.1016/j.cbpa.2009.08.013.

Mittermeier, Russell A., Jörg U. Ganzhorn, William R. Konstant, Kenneth Glander, Ian Tattersall, Colin P. Groves, Anthony B. Rylands, Andreas Hapke, Jonah Ratsimbazafy, Mireya I. Mayor, Edward E. Louis, Yves Rumpler, Christoph Schwitzer, and Rodin M. Rasoloarison. 2008. “Lemur Diversity in Madagascar.” *International Journal of Primatology* 29(6):1607–56. doi: 10.1007/s10764-008-9317-y.

Morgan, Kathleen N., and Chris T. Tromborg. 2007. “Sources of Stress in Captivity.” *Applied Animal Behaviour Science* 102(3–4):262–302. doi: 10.1016/j.applanim.2006.05.032.

Mortimer, Beth, James A. Walker, David S. Lolchuragi, Michael Reinwald, and David Daballen. 2021. “Noise Matters: Elephants Show Risk-Avoidance Behaviour in Response to Human-Generated Seismic Cues.” *Proceedings of the Royal Society B: Biological Sciences* 288(1953). doi: 10.1098/rspb.2021.0774.

Von Muggenthaler, Elizabeth. 2013. “Giraffe Helmholtz Resonance.” P. 010012 in *Proceedings of Meetings on Acoustics*. Vol. 19.

Mulholland, Tracy I., Danielle M. Ferraro, Kelley C. Boland, Kathleen N. Ivey, My Lan Le, Carl A. LaRiccia, John M. Vigianelli, and Clinton D. Francis. 2018. “Effects of Experimental Anthropogenic Noise Exposure on the Reproductive Success of Secondary Cavity Nesting Birds.” *Integrative and Comparative Biology* 58(5):967–76. doi: 10.1093/icb/icy079.

Muller, Z., F. Bercovitch, R. Brand, D. Brown, M. Brown, D. Bolger, K. Carter, F. Deacon, J. B. Doherty, J. Fennessy, Fennessy S., A. A. Hussein, D. Lee, A. Marais, Strauss. M., Tutchings. A., and T. Wube. 2018. “Giraffa Camelopardalis, Giraffe (Amended Version).” *The IUCN Red List of Threatened Species* 8235:1–15.

Munck, Allan, Paul M. Guyre, and Nikki J. Holbrook. 1984. “Physiological Functions of Glucocorticoids in Stress and Their Relation to Pharmacological Actions.” *Endocrine Reviews* 5(1):25–44. doi: 10.1210/edrv-5-1-25.

Nandini, S., P. Keerthipriya, and T. N. C. Vidya. 2017. “Seasonal Variation in Female Asian Elephant Social Structure in Nagarahole-Bandipur, Southern India.” *Animal Behaviour* 134:135–45. doi: 10.1016/j.anbehav.2017.10.012.

Normando, Simona, Ilaria Pollastri, Daniela Florio, Linda Ferrante, Elisabetta Macchi, Valentina Isaja, and Barbara de Mori. 2018. “Assessing Animal Welfare in Animal-Visitor Interactions in Zoos and Other Facilities. A Pilot Study Involving Giraffes.” *Animals* 8(9). doi: 10.3390/ani8090153.

O’Connell-Rodwell, C. E., B. T. Arnason, and L. A. Hart. 2000. “ Seismic Properties of Asian Elephant (Elephas Maximus) Vocalizations and Locomotion .” *The Journal of the Acoustical Society of America* 108(6):3066–72. doi: 10.1121/1.1323460.

O’Connell-Rodwell, Caitlin E. 2007. “Keeping an ‘Ear’ to the Ground: Seismic Communication in Elephants.” *Physiology* 22:287–94.

O’Connell-Rodwell, Caitlin, Xiying Guan, and Sunil Puria. 2019. “Vibrational Communication in Elephants: A Case for Bone Conduction.” Pp. 259–76 in *Biotremology: Studying Vibrational Behavior, Animal Signals, and Communication*.

O’connell, Caitlin, Lynette A. Hart, and Byron T. Arnason. 1998. “Comments on "Elephant Hearing" [J. Acoust. Soc. Am. 104, 1122-1123 (1998)].” *The Journal of the Acoustical Society of America* 105:2051–52. doi: 10.1121/1.426748.

O’Donovan, Declan, Joanne E. Hindle, Sean Mckeown, and Sean O’Donovan. 1993. “Effect of Visitors on the Behaviour of Female Cheetahs and Cubs.” *International Zoo Yearbook* 32:238–44.

O’Malley, Carly Irene. 2013. “Individual Behavior Types and Social Dynamics in a Pack of African Painted Dogs (Lycaon Pictus) at Brookfield Zoo.” Western Illinois University.

Orban, David A., Janice M. Siegford, and Richard J. Snider. 2016. “Effects of Guest Feeding Programs on Captive Giraffe Behavior.” *Zoo Biology* 35(2):157–66. doi: 10.1002/zoo.21275.

Palme, Rupert. 2019. "Non-Invasive Measurement of Glucocorticoids: Advances and Problems." *Physiology and Behavior* 199(November 2018):229–43. doi: 10.1016/j.physbeh.2018.11.021.

Pereira, Michael E., Martha L. Seeligson, and Joseph M. Macedonia. 1988. "The Behavioral Repertoire of the Black-and-White Ruffed Lemur, Varecia Variegata Variegata (Primates: Lemuridae)." *Folia Primatologica* 51(1):1–32. doi: 10.1159/000156353.

Puria, Sunil, and Charles Steele. 2010. "Tympanic-Membrane and Malleus-Incus-Complex Co-Adaptations for High-Frequency Hearing in Mammals." *Hearing Research* 263(1–2):183–90. doi: 10.1016/j.heares.2009.10.013.

Quadros, Sandra, Vinicius D. L. Goulart, Luiza Passos, Marco A. M. Vecci, and Robert J. Young. 2014. "Zoo Visitor Effect on Mammal Behaviour: Does Noise Matter?" *Applied Animal Behaviour Science* 156:78–84. doi: 10.1016/j.applanim.2014.04.002.

Ramos, Joana, Inês Viegas, Hugo Pereira, and João Filipe Requicha. 2021. "Morphometrical Study of the European Shorthair Cat Skull Using Computed Tomography." *Veterinary Sciences* 8(8). doi: 10.3390/vetsci8080161.

Ramsier, Marissa A., Andrew J. Cunningham, James J. Finneran, and Nathaniel J. Dominy. 2012. "Social Drive and the Evolution of Primate Hearing." *Philosophical Transactions of the Royal Society B: Biological Sciences* 367(1597):1860–68. doi: 10.1098/rstb.2011.0219.

Reuter, Tom, Sirpa Nummela, and Simo Hemilä. 1998. "Elephant Hearing." *The Journal of the Acoustical Society of America* 104(2):1122–23. doi: 10.1121/1.423341.

Robbins, Robert L. 2000. "Vocal Communication in Free-Ranging African Wild Dogs (Lycaon Pictus)." *Behaviour* 137(10):1271–98.

Rosa, Patricia, and Nicola Koper. 2018. "Integrating Multiple Disciplines to Understand Effects of Anthropogenic Noise on Animal Communication:" *Ecosphere* 9(2). doi: 10.1002/ecs2.2127.

San Deigo Zoo Wildlife Alliance. n.d. "Asian Elephant (*Elephas Maximus*) Fact Sheet." *San Diego Zoo Willdlife Alliance Library*. Retrieved June 1, 2022a (http://ielc.libguides.com/sdzg/factsheets/ asianelephant).

San Deigo Zoo Wildlife Alliance. n.d. "Giraffes (Giraffa Spp.) Fact Sheet." *San Diego Zoo Wildlife Alliance Library*. Retrieved June 1, 2022b (https://ielc.libguides.com/sdzg/factsheets/giraffes/summary).

San Deigo Zoo Wildlife Alliance. n.d. "Red-Ruffed Lemur (*Varecia Rubra*) Fact Sheet." *San Diego Zoo Wildlife Alliance Library*. Retrieved November 1, 2022c (https://ielc.libguides.com/sdzg/factsheets/redruffedlemur).

Saunders, James C., and Robert J. Dooling. 2018. "Characteristics of Temporary and Permanent Threshold Shifts in Vertebrates." Pp. 83–107 in *Effects of Anthropogenic Noise on Animals*, edited by H. Slabbekoorn, R. J. Dooling, A. N. Popper, and R. R. Fay. Springer Science + Business Media, LLC.

Sauther, Michelle L., and Robert W. Sussman. 1993. "A New Interpretation of the Social Organization and Mating System of the Ringtaled Lemur (Lemur Catta)."

Lemur Social Systems and Their Ecological Basis 111–21. doi: 10.1007/978-1-4899-2412-4_8.

Schmidt-Küntzel, Anne, Desiré L. Dalton, Marilyn Menotti-Raymond, Ezequiel Fabiano, Pauline Charruau, Warren E. Johnson, Simone Sommer, Laurie Marker, Antoinette Kotzé, and Stephen J. O'Brien. 2018. *Conservation Genetics of the Cheetah: Genetic History and Implications for Conservation*. Elsevier Inc.

Schmidt, Kenneth A., and Richard S. Ostfeld. 2008. "Eavesdropping Squirrels Reduce Their Future Value of Food under the Perceived Presence of Cache Robbers." *American Naturalist* 171(3):386–93. doi: 10.1086/527497.

Selye, Hans. 1976. "Stress without Distress." Pp. 137–46 in *Psychopathology of Human Adaptation*, edited by G. Serban. New York: Springer Science + Business Media, LLC.

Sheriff, Michael J., Ben Dantzer, Brendan Delehanty, Rupert Palme, and Rudy Boonstra. 2011. "Measuring Stress in Wildlife: Techniques for Quantifying Glucocorticoids." *Oecologia* 166(4):869–87. doi: 10.1007/s00442-011-1943-y.

Sherwen, Sally L., Trista J. Harvey, Michael J. L. Magrath, Kym L. Butler, Kerry V. Fanson, and Paul H. Hemsworth. 2015. "Effects of Visual Contact with Zoo Visitors on Black-Capped Capuchin Welfare." *Applied Animal Behaviour Science* 167:65–73. doi: 10.1016/j.applanim.2015.03.004.

Sherwen, Sally L., Michael J. L. Magrath, Kym L. Butler, Clive J. C. Phillips, and Paul H. Hemsworth. 2014. "A Multi-Enclosure Study Investigating the Behavioural Response of Meerkats to Zoo Visitors." *Applied Animal Behaviour Science* 156:70–77. doi: 10.1016/j.applanim.2014.04.012.

Shire, Taylor. 2012. "Differences in Behavior between Captive and Wild Ring-Tailed Lemur (Lemur Catta) Populations: Implications for Reintroduction and Captive Management." Iowa State University.

Shoshani, Jeheskel, and John F. Eisenberg. 1982. "Elephas Maximus." *Mammalian Species* 182:1–8.

Sibly, R. M., and P. Calow. 1989. "A Life-cycle Theory of Responses to Stress." *Biological Journal of the Linnean Society* 37(1–2):101–16. doi: 10.1111/j.1095-8312.1989.tb02007.x.

Sissom, Dawn E. Fraze., D. A. Rice, and G. Peters. 1991. "How Cats Purr." *Journal of Zoology* 223(1):67–78. doi: 10.1111/j.1469-7998.1991.tb04749.x.

Sukumar, R. 2006. "A Brief Review of the Status, Distribution and Biology of Wild Asian Elephants Elephas Maximus." *International Zoo Yearbook* 40(1):1–8. doi: 10.1111/j.1748-1090.2006.00001.x.

Summers, Patricia D., Glenn M. Cunnington, and Lenore Fahrig. 2011. "Are the Negative Effects of Roads on Breeding Birds Caused by Traffic Noise ?" 1527–34. doi: 10.1111/j.1365-2664.2011.02041.x.

Sussman, Robert W. 1992. "Male Life History and Intergroup Mobility among Ringtailed Lemurs (Lemur Catta)." *International Journal of Primatology* 13(4):395–413. doi: 10.1007/BF02547825.

Szokalski, Monika S., Wendy K. Foster, and Carla A. Litchfield. 2013. "Behavioral Monitoring of Big Cats Involved in 'Behind-the-Scenes' Zoo Visitor Tours."

International Journal of Comparative Psychology 26(1). doi: 10.46867/ijcp.2013.26.01.02.

Tarou, Loraine Rybiski, Mollie A. Bloomsmith, and Terry L. Maple. 2005. "Survey of Stereotypic Behavior in Prosimians." *American Journal of Primatology* 65(2):181–96. doi: 10.1002/ajp.20107.

Terio, Karen A., Laurie Marker, and Linda Munson. 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):259–66. doi: 10.7589/0090-3558-40.2.259.

Tighe, Emily J. 2013. "The Effects of Captivity on Display-Based Communication and Social Interaction in the Captive African Wild Dog (Lycaon Pictus)." University of Canterbury.

Touma, C., and Rupert Palme. 2005. "Measuring Fecal Glucocorticoid Metabolites in Mammals and Birds: The Importance of Validation." *Annals of the New York Academy of Sciences* 1046:54–74.

Troïanowski, Mathieu, Nathalie Mondy, Adeline Dumet, Caroline Arcanjo, and Thierry Lengagne. 2017. "Effects of Traffic Noise on Tree Frog Stress Levels, Immunity, and Color Signaling." *Conservation Biology* 31(5):1132–40. doi: 10.1111/cobi.12893.

Vasey, Natalie. 2006. "Impact of Seasonality and Reproduction on Social Structure, Ranging Patterns, and Fission–Fusion Social Organization in Red Ruffed Lemurs." Pp. 275–304 in *Lemurs*. Boston, MA: Springer US.

Vidya, T. N. C., Deepika Prasad, and Arjun Ghosh. 2014. "Individual Identification in Asian Elephants." *Gajah* 40(January 2014):3–17.

Vieilledent, Ghislain, Clovis Grinand, Fety A. Rakotomalala, Rija Ranaivosoa, Jean Roger Rakotoarijaona, Thomas F. Allnutt, and Frédéric Achard. 2018. "Combining Global Tree Cover Loss Data with Historical National Forest Cover Maps to Look at Six Decades of Deforestation and Forest Fragmentation in Madagascar." *Biological Conservation* 222(March):189–97. doi: 10.1016/j.biocon.2018.04.008.

Volfova, Martina, Zuzana Machovcova, Eva Voslarova, Iveta Bedanova, and Vladimir Vecerek. 2020. "Comparison of the Glucocorticoid Concentrations between Three Species of Lemuridae Kept in a Temporary Housing Facility." *Animals* 10(6):1–19. doi: 10.3390/ani10061013.

Volodina, E. V. 2000. "Vocal Repertoire of the Cheetah Acinonyx Jubatus (Carnivora, Felidae) in Captivity: Sound Structure and Their Potential for Estimating the State of Adult Animals." *Zoologicheskii Zhurnal* 79(7):842–43.

Volodina, Elena V., Ilya A. Volodin, Elena V. Chelysheva, and Roland Frey. 2018. "Hiss and Snort Call Types of Wild-Living Giraffes Giraffa Camelopardalis: Acoustic Structure and Context." *BMC Research Notes* 11(1):1–7. doi: 10.1186/s13104-017-3103-x.

Walker, Reena H., Andrew J. King, J. Weldon McNutt, and Neil R. Jordan. 2017. "Sneeze to Leave: African Wild Dogs (Lycaon Pictus) Use Variable Quorum Thresholds Facilitated by Sneezes in Collective Decisions." *Proceedings of the*

Royal Society B: Biological Sciences 284(1862):20170347. doi: 10.1098/rspb.2017.0347.

Webster, Hugh, John W. McNutt, and Karen McComb. 2010. "Eavesdropping and Risk Assessment between Lions, Spotted Hyenas and African Wild Dogs." *Ethology* 116(3):233–39. doi: 10.1111/j.1439-0310.2009.01729.x.

Wielebnowski, Nadja, and Jason Watters. 2007. "Applying Fecal Endocrine Monitoring to Conservation and Behavior Studies of Wild Mammals: Important Considerations and Preliminary Tests." *Israel Journal of Ecology & Evolution* 53(3–4):439–60. doi: 10.1560/IJEE.53.3.439.

Wiley, R. Haven, and Douglas G. Richards. 1978. "Physical Constraints on Acoustic Communication in the Atmosphere: Implications for the Evolution of Animal Vocalizations." *Behavioral Ecology and Sociobiology* 3(1):69–94. doi: 10.1007/BF00300047.

Williams, C., S. K. Tiwari, V. R. Goswami, S. De Silva, A. Kumar, N. Baskaran, K. Yoganand, and V. &. Menon. 2020. "Elephas Maximus. The IUCN Red List of Threatened Species." *Elephas Maximus* 2020(The IUCN Red List of Threatened Species 2020). doi: https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T7140A45818198.en.

Wittemyer, G., I. Douglas-Hamilton, and W. M. Getz. 2005. "The Socioecology of Elephants: Analysis of the Processes Creating Multitiered Social Structures." *Animal Behaviour* 69(6):1357–71. doi: 10.1016/j.anbehav.2004.08.018.

Woodroffe, R., and C. Sillero-Zubiri. 2012a. "Lycaon Pictus." *The IUCN Red List of Threatened Species* 7.

Woodroffe, R., and C. Sillero-Zubiri. 2012b. "Lycaon Pictus (North Africa Subpopulatio)." *The IUCN Red List of Threatened Species*.

Woodroffe, R., and C. Sillero-Zubiri. 2012c. "Lycaon Pictus (West Africa Subpopulation)." *The IUCN Red List of Threatened Species*.

Woodroffe, R., and C. Sillero-Zubiri. 2020. "Lycaon Pictus (African Wild Dog)." *The IUCN Red List of Threatened Species.* 8235:e.T12436A166502262.

Chapter Two: The Behavioral and Physiological Effects of After-Hour Events on Four Species of Mammals at the Oregon Zoo

Introduction

The use of after-hours events is a popular way for zoos and aquariums to generate additional income and increase attendance. However, few formal studies have observed the effects these events have on the resident zoo animals (Bastian et al. 2020; Fanning, Larsen, and Taylor 2020; Meade, Formella, and Melfi 2017). Typically, studies analyze the effect of visitor presence during day-to-day activities (Davey 2007; Morgan and Tromborg 2007; Woolway and Goodenough 2017). Increased attendance has been shown to increase ambient noise levels in popular exhibits which can lead to increased fear responses, mimicking responses to predators (Frid and Dill 2002; Quadros et al. 2014). However, increasing attendance is a priority of zoological institutions, both for revenue and conservation education goals, and zoos are turning to more creative ways to engage visitors. As of October 2018, nearly all the 233 zoological institutions accredited by the Association of Zoos and Aquariums (AZA) hosted events beyond typical zoo visitation, but only a handful of quantitative studies have been conducted to monitor the effects (Bastian et al. 2020; Fanning et al. 2020; Meade et al. 2017; de Queiroz 2018). After-hour events at zoos can range from small and intimate to large and populous. They can introduce novel sounds, smells, and visual effects that may be considered significant stressors to the animals adjacent to the event locations. With zoos focusing heavily on increasing welfare for their animals, it is imperative that an in-depth investigation of the invasiveness of these after-hour events be conducted. While the increased attendance brings in needed revenue, the increased visitor presence and subsequent noise levels may influence animal behavior and / or physiology.

Sound

As mentioned in the introduction of this dissertation, multiple studies have begun to analyze the effect of sound on zoo animals. These studies range from observing deliberate changes in the soundscape through enrichment (e.g., Hanbury et al. 2009; Kelling et al. 2012; Ogden, Lindburg, and Maple 1994; Piitulainen 2020; Pons, Carter, and Jaen 2016) to monitoring passive noises zoo animals are exposed to every day (e.g., Birke 2002; Farrand 2007; Larsen, Sherwen, and Rault 2014; Powell et al. 2006; Quadros et al. 2014; Queiroz and Young 2018; Wark 2015). Like most studies on animals, the results are dependent on both species and individuals. Multiple factors influence the effect of sound on an individual's welfare. These can include sound intensity, novelty, complexity, or genre. For instance, audio stimuli were shown to be less effective in stimulating a behavioral response as novelty wanes for captive gorillas (*Gorilla gorilla gorilla*) (Robbins and Margulis 2014) and increasing tempo of auditory stimuli significantly altered anxiety levels in the same species (Brooker 2016).

Typically, sounds at the zoo are centered on enhancing the human experience (e.g., artificial lion roars to reflect the habitat ambiance) or increasing attendance (e.g., rock concerts and happy hour events). These sounds are curated to encompass frequencies appropriate to human hearing and are typically analyzed using anthropocentric frequencies, like A-weighted filters (dB(A)). Filters are used to analyze complex, multi-frequency noises for a targeted hearing range. Human hearing is analyzed in A-weighted frequencies which truncates the upper-most and lower-most frequencies while placing further emphasis on mid-range frequencies to reflect the human hearing range (20-20,000 Hz). Discounting the influence of low- and high-frequency tones has been shown to underestimate the perception of loudness (St. Pierre Jr. and Maguire 2004). Despite these limitations, the use of dB(A) has become the standard for noise analyses – regardless of the actual hearing threshold of the target species (Buxton et al. 2017; Quadros et al. 2014; de Queiroz 2018; Wark 2015). In fact, very few studies use a weighting other than A-weighting, but newer studies are analyzing other frequency weightings (Edes et al. 2021; Jakob-Hoff et al. 2019; Orban et al. 2017; Pelletier et al. 2020). Alternate frequency weightings are C-weighting (includes low-frequency sounds outside of traditional human hearing ranges) and Z-weighting (unweighted but shortens the frequency threshold to 8 Hz – 20 kHz to mimic the limits of human hearing). Both alternate weighting methods still use the human audiogram as a template and may be inappropriate depending on the focal species' hearing threshold.

While frequency-weighting can assist in measuring tones that are appropriate for the target species, choosing the incorrect weighting can result in inappropriate interpretations. Specifically, the hearing physiology of the targeted species must be understood as part of the decision-making process. For instance, Asian elephants (*Elephas maximus*) have been shown to be considerably better at detecting and deciphering information from low-frequency tones than other mammals, with a low-hearing threshold of 16 Hz at 65 dB SPL (ref: 20 μPa) (Heffner and Heffner 1982) – almost a full octave lower than humans at 31 Hz at 60 dB SPL (ref: 20 μPa) (Jackson, Heffner, and Heffner 1999). This means that the lowest tones that are detectable by Asian elephants are not detectable by the human ear at the same intensity. However, as Heffner and Heffner (2007) have stated, frequencies lower than 31 Hz are not absolutely undetectable by humans, they just require much higher intensities in order to be detectable. This means that events at zoos that use sound centered on human enjoyment (especially musical concerts) may increase these low frequencies to be detectable by humans; potentially pushing the intensity past what is comfortable for Asian elephants. However, while Asian elephants have strong low-frequency hearing, they lack high frequency detection. In a previous study the maximum high-frequency range for Asian elephants at 60 dB SPL (ref: 20 μPa) was 10.5 kHz (Heffner and Heffner 1982). In comparison, human high-frequency detection at 60 dB SPL (ref: 20 μPa) was 17.6 kHz (Jackson et al. 1999).

Endocrinology

In addition to behavior observations, a popular way to monitor animal welfare is through non-invasive hormone monitoring (Palme 2005; Sheriff et al. 2011). Primarily, studies on fecal glucocorticoid metabolite (fGM) concentrations have become a leading methodology in long-term welfare monitoring studies (Palme 2019). Fluctuations of measured steroid metabolites can reflect changes in animal adrenal activity potentially associated with changes in animal welfare status in response to husbandry changes (Fazio et al. 2020; Razal, Bryant, and Miller 2017), changes in enrichment (Rafacz and Santymire 2014), social changes (Loeding et al. 2011), and institutional transfers (Fanson et al. 2013; Ferreira et al. 2020; Glaeser et al. 2021). These fluctuations occur after the activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of steroid hormones (e.g., glucocorticoids). Once the stimuli disappear, the HPA axis self-regulates causing the glucocorticoid concentrations to fade to background levels. However, if the stimuli remain – or the perceptions of the stimuli remain – the HPA axis remains activated and the self-regulation fails to lower the glucocorticoid levels, subsequently causing prolonged glucocorticoid elevations (Sheriff et al. 2011). The prolonged or chronically elevated glucocorticoid concentrations can eventually lead to several negative effects on animal physiology and health. However, it is important to distinguish between positive (eustress) and negative (distress) responses (Rose and Riley 2019; Selye 1976). While some activation of the stress response is healthy and necessary for good health and survival, continuous activation of the stress response due to prolonged and chronic distress can lead to long-term negative effects on welfare and health.

Combining fGM data with behavioral data is one way to help determine whether changes in fGM concentrations are representative of positive, neutral, or negative effects. As increases in glucocorticoid concentrations occur in response to positive or negative stressors (Lay Jr. 2000), it is important to pair them with behavioral observations to further aid interpretation (Wielebnowski and Watters 2007). This study used noise analysis data paired with behavioral observations and fGM concentrations to observe potential changes in welfare associated with after-hour events.

Study Questions and Aims

Based on previous studies and available information, we decided to test the following hypotheses: 1) Due to the increase in visitor presence and likely increase in sound pressure level (SPL) beyond typical zoo opening hours, we predicted significantly higher fGM concentrations following the after-hours events than control nights; and 2) We predicted the significant increase in vigilant or hiding behaviors with increasing SPL and during after-hour events. This study aimed to provide underrepresented data on the effects of popular after-hour events on four charismatic mammal species at the Oregon Zoo. Very few studies have been conducted to quantify the effects of after-hour events on zoo animals, and, of these, only two have included fGM concentrations, while a few others relied entirely on behavioral data analyses (Bastian et al. 2020; de Queiroz 2018).

Methods

General

This study was performed over two summer concert seasons. The initial study took place between June and September 2018 and the second part took place over the same time period of 2019. Behavioral and hormone data was collected on four species over three different treatments: “concert”, “control”, and “other” (Table

1). Three studies were used to address the aims: 1) analysis of behavior rates based on event type; 2) analysis of fGM concentrations based on event type; and, 3) correlations between sound level, behavior rate, fGM concentrations, and other descriptive information regardless of event type.

Table 1: Treatment definitions and quantity of observations. Days of behavior observations (obs.) and fecal glucocorticoid metabolite (fGM) collection days representative of all species.

	Definition	**Behavior Obs. Days**	**fGM Collection Days**
Concert	Large-scale musical performances attended by approximately 3,000 people.	N = 70	N = 118
Control	Instances of typical zoo night-time operations.	N = 44	N = 661
Other	After-hour events that have high attendance rates but include no broadcast music or other noise	N = 36	N = 56

Study Species

Four different species housed at the Oregon Zoo were included in the initial study: giraffe (*Giraffa camelopardalis reticulata* & *Giraffa camelopardalis tippelskirchi*), cheetah (*Acinonyx jubatus*), African painted dog (*Lycaon pictus*), and Asian elephant (*Elephas maximus indicus* & *Elephas maximus borneensis*). Each species was chosen for specific reasons which included general sensitivity to stress observed for the species in the past and exhibit location in relation to visitor events. All animals are housed primarily on-exhibit during the day with access to indoor off-exhibit areas at night. Husbandry practices for all species are in accordance with guidelines presented by the Association of Zoos and Aquariums (AZA). See Appendix A for detailed view of exhibits and exhibit locations.

African Painted Dog (Lycaon pictus)

During the first year of the study there were three adult African painted dogs. Two sisters (B70096 and B70097) and one male (B70167) that had been transferred to the Oregon Zoo in June 2017 and November 2017, respectively, with the hope of creating a successful breeding pack. In November 2018, twelve pups were born. As the behavioral analysis of fifteen painted dogs proved to be impossible for the number of volunteers, behavioral analysis of the African painted dogs was discontinued in 2019. Therefore, only limited amount of data was available for final analyses.

The African painted dogs were housed in a large outdoor habitat with visitor viewing from glass panels and a mesh wall (Appendix B). The habitat contained a water feature with flowing freshwater and multiple enrichment items. Additionally, the African painted dog habitat also had an indoor area without any visitor access. The African painted dogs were granted access to the indoor habitat after the zoo closing hours.

Cheetah (Acinonyx jubatus)

Two female cheetahs were included in this study. The littermates, B70094 and B70095, were born in November 2015 at the San Diego Wild Animal Park (SD-WAP). They arrived at the Oregon Zoo in June 2017. The cheetahs are fed a diet consisting of species-specific meat (Milliken Meat Products, Canada), skinned whole-rabbits, and a variety of meaty bones. Both cheetahs were housed together in an enclosure that included three indoor off-exhibit areas (one 109 ft2 and two 80 ft2 habitats) and a large grassy outdoor exhibit (approximately 19,200 ft2) consisting of visitor viewing glass panels along the northeast side of the enclosure. In 2018, the cheetahs had access to their outdoor enclosure until they were given access to the indoor areas by the night-keeper between 20:00 and 21:00hrs, but in 2019 they were given access to indoor areas earlier at approximately 19:00 due to changes in husbandry practices.

Asian elephant (Elephas maximus indicus & Elephas maximus borneensis)

The Oregon Zoo is home to a small herd of six elephants – five Asian elephants (*E. m. indicus*) and one Borneo elephant (*E. m borneensis*). At the beginning of this study, the matriarchal family unit consisted of three older females: 1963 (36 years old), 94122 (24 years old), and 99270 (25 years old); and two young offspring: A80122 (11 years old) and B20189 (5 years old). Elephant B20189 died of elephant endotheliotropic herpesvirus (EEHV) in November 2018. Early in this study, an older male (B80043, 20 years old) was slowly introduced to the family unit and was managed separately until he was fully integrated with the females. He and A80122 were housed separately for the duration of 2018. The elephants are housed in *Elephant Lands*, an innovative new exhibit that is located next to a large lawn area also used for summer concerts (Appendix A). *Elephant Lands* consists of three outdoor habitats, *South Habitat, North Meadow,* and *Encounters Yard*, and one indoor habitat, *Forest Hall.* Elephants are viewable from the perimeter of the *South Habitat, Encounters Yard,* and *Forest Hall,* but restricted to a small viewing platform on the southern tip of *North Meadow*. The elephants have access to indoor and outdoor facilities throughout the day. In anticipation of noisy events, the main access doors to *Forest Hall* are shut – creating a noticeably quieter indoor environment. Elephants can still access the indoor habitat through smaller side-doors.

Giraffe (Giraffa camelopardalis reticulata & Giraffa camelopardalis tippelskirchi)

Two male giraffes were included in the study. These included one reticulated giraffe (B20186, 8 years old) and one Masai giraffe (B60180, 4 years old). Giraffe B20186 has been a resident at the Oregon Zoo since March 2017 and giraffe B60180 came to the zoo in October 2016. Both giraffes are housed in a multi-species exhibit with two Speke's gazelles (2.0 *Gazella spekei*) and two southern ground hornbills (1.1 *Bocorvus leadbeateri*). The giraffes are fed alfalfa and pellets throughout the day from dispensers around the perimeter of the exhibit and keeper-provided browse from various locations around the exhibit. Water is provided *ad libitum* from drinking fountains. The giraffes are allowed outdoor access whenever the temperature exceeds 50°F (10°C) and there is minimal precipitation. When the temperatures fall below that threshold, the giraffes are kept in the indoor barn where the temperature is constant at 65°F (15.6°C). The giraffe exhibit area is also surrounded by three visitor viewing platforms located on the southern perimeter of the exhibit. Two platforms are raised to provide an aerial view of the giraffes while the third is located at the ground level.

Sound Data Collection

Five SM$_4$ Wildlife Acoustic Song Meters (Wildlife Acoustics Inc. ©, Maynard, MA) were deployed for each monitoring event (Table 1). These sound meters recorded a full-spectrum sound file from 15:00 – 0:00 on each day of monitoring. Each sound meter consisted of two non-directional microphones with sensitivities of -33.5 dB. The sound meters were placed at four locations each night: behind the African carnivore (cheetah and African painted dog) exhibits, the giraffe exhibit, and two were deployed at elephants (Appendix B). In 2018, the sound meters at elephants were rotated between the habitat closest (*South Habitat*) and farthest (*North Meadow*) to the stage to determine the variation in sound levels within the entire exhibit. One sound meter was permanently located in *Forest Hall* as this was considered a respite area for the Asian elephants. An additional sound meter was purchased in 2019 and the addition of this sound meter allowed for simultaneous monitoring of *North Meadow*, *South Habitat*, and *Forest Hall*.

Sound File Processing

The sound files recorded by the sound meters were analyzed using the "Noise Analysis" function within the Wildlife Acoustics Inc. software, "*Kaleidoscope*" (version 5.2, Wildlife Acoustics Inc. ©, Maynard, MA). The files were analyzed as un-weighted (equal emphasis on all frequencies between 10Hz – 192 kHz) to prevent overemphasis of frequencies related to human-hearing rather than the focal species. A 127.5 dB SPL conversion was factored into the software to transform the data to µPa (-94 dB) and account for the sensitivities of the microphones (-33.5 dB). All subsequent mentions of SPL are with reference at 20 μPa. Sound files were analyzed in 20-minute increments that aligned with the start and end of each observation session. As the Asian elephant habitat had three sound meters, sound recordings at the sound meter closest to the focal elephant during the majority of the observation period was indicated for that focal animal. The mean dB SPL values were calculated and used in the following analyses.

Behavioral Data

ZooMonitor (Ross et al. 2016) software was used to collect behavioral data on each of the focal animals. Species specific ethograms (Tables 2 - 5) were used to quantify the behaviors and consisted of interval and all-occurrence data collection.

Table 2: African Painted Dog ethogram. Behavior descriptions adapted from Creel & Creel 2002 and Tighe 2013)

African Painted Dogs		
Interval Behaviors		
Behavior	Modifiers	Description
Not Visible	NA	Individual is out of sight at time of reading. Includes being wholly or partially obstructed to the point of being unable to confidently identify the behavior.
Keeper Present	NA	Keeper is present. Actively interacting with animals or just walking past. Still record location for individual.
Play	NA	Episodes of play can contain any of the following behaviors: mounting (one dog jumps or climbs on back of another individuals – not sex specific and not to be confused with mounting for mating purposes), wresting/pouncing (individuals jump on back legs and paw, box, or grip another individuals face), bowing (front half of an individual is lowered by extending the front legs, rear remains upright), and / or chasing (individuals chase each other around – dog being chased has an exaggerated run posture, tucking its rear under itself and stiffly moving its front legs, tend to look behind itself to tease the pursuer to continue).
Chase	NA	One dog clearly chases another dog around the yard.
Flee	NA	Dogs actively avoiding a pursuant.
Rest	Heap	2+ individuals laying ≤1.5m from each other.

	Individual	A single individual resting outside of a close proximity from other pack members.
Stationary Stand / Sit	NA	Dog is standing still or sitting for at least 10 seconds.
Pacing	NA	Individual repeatedly moves across same pathway without aim. Body posture is neutral and trance-like. Does not seem to have a direct focus.
Begging	NA	Front half of body is lowered toward the ground and accompanied by licking and whining vocalizations. Usually shown by a lower-ranking individual to a higher-ranking individual.
Eating/Drinking	NA	Individual is actively ingesting food or water
Locomotion	NA	Individual is moving from one area to another. Distinguished from "pacing" as it is not repetitive.
All Occurrence Behaviors		
Biting	NA	Targeted to head and neck area. The instigator can hold another individual for a period (can be quite short) and appears to apply pressure with mouth. Typically exhibited around food.
Pseudo-Biting	NA	Biting in a non-aggressive manner. The bite does not pierce the skin and instigator never actively holds onto another individual. Can be seen in play, dominance displays, etc.
Mounting	Dominance	Individual balances forelegs on the hindquarters or head of a submissive individual.
	Mating	Mounting that occurs during reproduction.
Forced Dominance	NA	Higher ranking dog approaches a lower ranking dog and forcefully nuzzles the other dog in the groin or neck region. Dominant dog may push the submissive individual to the ground, or even flip the individual to a submissive posture
Wheelbarrow	NA	Dominant individual puts head under submissive and lifts the back legs up, forcing submission off balance.

Table 3: Pre-established cheetah ethograms (Lewis 2020)

Cheetahs		
Interval Behaviors		
Behavior	Modifiers	Description
Not Visible	NA	Individual is out of sight at time of reading. Includes being wholly or partially obstructed to the point of being unable to confidently identify the behavior.
Keeper Present	NA	Keeper is present. Actively interacting with animals or just walking past. Still record location for individual.
Environmental Interaction	NA	Actively engaged with an element of its environment, e.g., eating, drinking, interacting with the pool, rubbing against, pawing at, scratching, sniffing, etc. Does not

		include interaction with zoo visitors or inactive contact with enrichment, or incidental contact with exhibit furniture
Stereotypy	NA	Walking from one point to another, turning and walking back to the starting point, or walking in a loop or to-and-fro, for 3 or more repetitions without interruption. Also include non-locomotor stereotypy (over-grooming, tail/paw biting, etc.).
Social Interaction	Positive	Any active social interaction with another cheetah, regardless of who initiated it
	Negative	
Locomotion	NA	Any movement that transports the animal more than one body length forward, backward, or sideways at any speed, includes walk, trot, run, or jump.
Groom	NA	Focal animal is engaged in self-grooming; licking, chewing, scratching (self). Grooming others is included in “social interaction”.
Stationary	NA	Not deliberately locomoting for at least 3-seconds. If Stationary for fewer than 3-seconds at the beep, record the behavior occurring immediately prior to becoming Stationary.
All Occurrence Behaviors		
Charge Glass	NA	Charge toward visitor to within 1 body length of the glass, may or may not include a strike or hiss.
Glass Strike	NA	Forceful paw contact with glass typically near a visitor.
Hiss	NA	Hiss directed at visitor at glass

Table 4: Previously established Asian elephant ethograms (Lewis 2020)

Asian Elephants		
Interval Behaviors		
Behavior	Modifiers	Description
Not Visible	NA	Individual is out of sight at time of reading. Includes being wholly or partially obstructed to the point of being unable to confidently identify the behavior.
Keeper Present	NA	Keeper is present. Actively interacting with animals or just walking past. Still record location for individual.
Social Physical Contact	NA	Social contact (positive or negative) with another individual that results in physical touching.
Social No Contact	NA	Social interactions that do not result in physical contact.
Food Object Interaction	NA	Interacting with food object without actively consuming it.
Feeding / Drinking	NA	Focal animal actively ingesting food or drinking.
Enrichment Object Interaction	NA	Interaction with a non-permanent object deliberately left by keepers as enrichment (e.g., hanging tires)
Environmental Interaction	NA	Actively engaged with an element of its environment (e.g., rubbing against, pawing at, scratching, sniffing, etc.) Does

		not include interaction with zoo visitors or inactive contact with enrichment (e.g., laying down), or incidental contact with exhibit furniture.
Stereotypy	NA	Locomotor: Walking from one point to another, turning and walking back to the starting point, or walking in a loop or to-and-fro for more than three repetitions without interruption.
Locomotion	NA	Any movement that transports the animal more than one body length forward, backward, or sideways at any speed, includes walk, trot, run, or jump.
Solitary	NA	Individual conducting solitary behaviors (e.g., masturbation, grooming)
Stationary	NA	Not deliberately locomoting for at least 3-seconds
All Occurrence Behaviors		
Flehmen	NA	Place dorsal trunk finger onto roof of mouth after touching trunk to urine, feces, or another elephant.
Mount	NA	Placing front legs on another individual with rear legs still on the ground. Can be for mating or otherwise.
Strike / Push	NA	Strike: Forceful body contact initiated by one elephant. Includes: head butt, trunk strike, kick. Push: Forceful head-to-head, head-to-body, body-to-body contact that appears to show intent to displace or control.

Table 5: Pre-established giraffe ethograms (Lewis 2020)

Giraffe		
Interval Behaviors		
Behavior	Modifiers	Description
Not Visible	NA	Individual is out of sight at time of reading. Includes being wholly or partially obstructed to the point of being unable to confidently identify the behavior.
Keeper Present	NA	Keeper is present. Actively interacting with animals or just walking past. Still record location for individual.
Eat	Browse	Can either be eating browse off trees or keeper-provided branches, or from hay and pellet feeders across the habitat
	Hay / Pellets	
Environmental Interaction	NA	Actively engaged with an element of its environment (e.g., eating, drinking, rubbing against, pawing at, scratching, sniffing, etc.) Does not include interaction with zoo visitors or inactive contact with enrichment (e.g., laying down), or incidental contact with exhibit furniture
Stereotypy	Pacing	Walking from one point to another, turning and walking back to the starting point, or walking in a loop or to-and-fro for more than three repetitions without interruption
	Tongue Flagging	Individual rotates tongue outside of mouth seemingly without purpose
	Excessive Licking	Individual licks continuously for at least 20 seconds.

Social Interaction	NA	Any active social interaction with another giraffe.
Locomotion	NA	Any movement that transports the animal more than one body length forward, backward, or sideways at any speed. Includes walk, trot, run, or jump.
Grooming	NA	Self-grooming using tongue or hoof.
Vigilant	NA	Standing still with an erect neck and appearing to be actively watching something.
Stationary	NA	Not deliberately locomoting for at least 3-seconds
All Occurrence Behaviors		
Urine Testing	NA	Interaction with freshy excreted or currently being excreted urine from another individual. Typically followed by a flehmen behavior.
Flehmen	NA	Lips pull back and neck erect after interacting with either urine, feces, or genitals of another giraffe.
Interaction with Speke's gazelle	NA	Any interaction with the Speke's gazelles – includes physical and non-physical interactions (chasing).
Interaction with hornbills	NA	Any interaction with the southern ground hornbills – includes physical and non-physical interactions (chasing).
Run	NA	Instances of running. New instances occur after a pause for at least 5 seconds.
Lay Down	NA	Individual is laying on ground. Head and neck can either be elevated, laying along body, or laying on the ground.

Qualified Oregon Zoo volunteers assisted in behavior collection. Each volunteer was required to pass a reliability test (> 80% overlap with observations taken by the primary researcher) (Wark 2021) to ensure behaviors were accurately recorded by each volunteer. One-minute scan sampling occurred over 20-minute observational periods and were repeated three times across an event. Observations were conducted at 18:00, 19:00, and between 20:30 and 21:00 based on light availability and concert start time (observations on August 24, 2018, were at 17:15, 18:45 & 20:00 due to early concert start time). The timings were the same across all treatment types to minimize temporal bias.

Endocrine Data

Fecal Sample Collection

Fecal samples were collected approximately three times per week for giraffes, cheetahs, and African painted dogs and daily for Asian elephants between 0700 and 1000 by animal care staff (see Table 6 for collection start dates & total samples collected per focal animal). Care staff were instructed to avoid samples contaminated by urine or other substances. Samples were labelled with name, species, date, and time collected before being immediately frozen (-4°F / -20°C) until extraction. Food-grade dye was used to identify samples from individuals housed together.

Table 6: Table of sample collection dates, frequencies, and total amount for each species and individual.

Species	**2018 Collection Start Date**	**2018 Collection End Date**	**2019 Collection Start Date**	**2019 Collection End Date**	**Total Samples Collected**
L. pictus	6/2/2018	11/26/2018	6/8/2019	11/26/2019	553
B70096	6/2/2018	11/25/2018	8/6/2019	11/24/2019	170
B70097	6/2/2018	11/26/2018	7/25/2019	11/25/2019	194
B70167	6/2/2018	11/26/2018	6/8/2019	11/26/2019	189
A. jubatus	6/1/2018	10/1/2018	6/1/2019	9/30/2019	272
B70094	6/1/2018	9/30/2018	6/1/2019	9/27/2019	141
B70095	6/1/2018	10/1/2018	6/1/2019	9/30/2019	131
E. maximus	6/1/2018	9/28/2018	6/1/2019	9/30/2019	775
99270	6/1/2018	9/25/2018	6/2/2019	9/30/2019	150
B80043	6/1/2018	9/13/2018	6/1/2019	9/30/2019	166
B20189	6/3/2018	9/28/2018	NA	NA	58
A80122	6/2/2018	9/28/2018	6/1/2019	9/30/2019	152
94122	6/1/2018	9/26/2018	6/1/2019	9/30/2019	147
1963	6/1/2018	9/13/2018	6/2/2019	9/26/2019	102
G. camelopardalis	6/2/2018	9/30/2018	6/1/2019	9/30/2019	296

B20186	6/2/2018	9/30/2018	6/1/2019	9/30/2019	148
B60180	6/2/2018	9/30/2018	6/1/2019	9/30/2019	148

Steroid Extraction

Fecal glucocorticoid metabolites (fGM) were extracted from each species using methods based on (Bryant and Wielebnowski 2018). Briefly, 0.500g (±0.025g) of wet, homogenized fecal matter was mixed with 5mL 80% alcohol (methanol: giraffes, Asian elephants, or ethanol: cheetah, African painted dog), vortexed, and shaken overnight for 17 hours (Fisherbrand™ open air rocking shaker). As Asian elephant fecal samples consist mostly of alfalfa hay, special care was taken to avoid larger pieces of hay that could prevent a proper vortex. Samples were then revortexed and centrifuged (Sorvall™ ST 16) at 2500 rpm for 15 minutes. 3 mL of the resulting 1:1 supernatant was removed and species-specific amounts (cheetah, giraffe, and African painted dogs: 500µL, Asian elephants 750µL) was subsequently dried in a SpeedVac (Savant Speedvac DNA110) before being reconstituted in 500µL (cheetah, giraffe, African painted dog) or 250µL (Asian elephants) Tris HCl assay buffer. Dilutions were then created with Tris assay buffer based on the optimum concentration for the detection of fGMs (Appendix C).

Enzyme Immunoassay (EIA) Analysis

Glucocorticoid metabolites have previously been successfully extracted from fecal samples in each species (cheetah - Uetake et al. 2014, giraffe - Bashaw et al. 2016, African painted dog - Rafacz, Heintz, and Santymire 2016, and Asian elephants - Watson et al. 2013). A double-antibody corticosterone enzyme immunoassay (EIA)

(CJM006, 1:100,000, C. Munro, University of California, Davis) was used for all species. A 96-well microtiter plate was pre-coated with secondary goat anti-rabbit IgG antibody (150µL / well at 0.10 mg/mL, A009, Arbor Assays, Ann Arbor, MI, USA) using the standard Arbor Assay methodology. The inter- and intra- assay coefficients of variation (CV) were maintained below 15% and 10% respectively. The corticosterone antibody cross-reacts at 100% with corticosterone, 14.25% with desoxycorticosterone, 2.65% with progesterone, 0.90% with tetrahydrocorticosterone, 0.64% with testosterone, 0.23% with cortisol, and less than 0.10% for five other steroids tested (C. Munro).

The corticosterone EIA was validated for each species by showing: (1) parallelism between the binding inhibition curves of a corticosterone standard curve and a serially diluted pool of fecal extractions for each species (Appendix C), and (2) biological validation consisting of an increase (>2 standard deviation) in fecal glucocorticoid concentrations immediately following an intense stressor (e.g., institution transfer or veterinary procedures) (Appendix D).

Correlation Analysis

Multiple correlation analyses were conducted to determine if the potential changes in behavior rates and fGM concentration levels were correlated with changes in average dB SPL levels, daily attendance, and temperature (°F) – regardless of event type. To do so, the average dB SPL levels for each observation period were paired with the behavior rates within the corresponding observation period. The attendance numbers were the total number of zoo attendees throughout the entire day. The temperature of each observation period was recorded. Finally, the fGM concentrations were paired with the observation date based on species-specific gut metabolic rates (Table 7).

Statistical Analysis

Behavioral Analyses

Behavioral analyses were conducted using IBM SPSS Statistics for Windows (version 21.0) and R-statistical package (version 4.0.3) using RStudio (version 1.3.1093) (R Core Team 2020). Behavioral analyses were conducted on each individual focal animal. Methods by Powell et al. (2006) and Tarou et al. (2005) were followed. Briefly, behavior observations were converted to percentages of total time observes ("behavior rates"). Then, confidence interval (CI) analyses were conducted on the behavior rates with 95% CI around the mean value for "control" event type as the standard. Mean behavior rates that were not included within the 95% CI window of "control" event types were considered statistically significantly different.

Endocrinology Analyses

Visual evaluations of QQ plots and Shapiro-Wilks tests were used to find the best-fit distributions for each species at the group and individual level (Table 8). Due to the unbalanced and non-normal data distribution, general linear mixed models (GLMMs) were run using the package 'MASS' (Venables and Ripley 2002) in R-statistical package (version 4.0.3)(R Core Team 2020). GLMMs were chosen due to their ability to include non-normal distributions without transformation of the original data. Each GLMM consisted of similar fixed and random effects and was conducted on each species at the individual and grouped level (Table 8).

Previous research has designated species-specific lag times, based on metabolic passage and excretion time, between stressor exposure and relative spikes in fGM concentrations (Table 7). To correctly link fGM concentrations with the correct event, a calculated adjusted date (adj. date) was used in the models in lieu of collection date.

Table 7: Lag time between a stressful event and subsequent metabolite excretion into the feces for the study species.

Species	Lag time	Method	Citation
L. pictus	24 hours	ACTH	(Monfort et al. 1998)
A. jubatus	24 hours	ACTH	(Ludwig et al. 2013)
G. camelopardalis	24-48 hours	Biological Event and ACTH	(Bashaw et al. 2016)
E. maximus	48 hours	Biological Event	(Laws et al. 2007)

The following sections indicate any deviation from the outlined statistical methodology and its justification.

African Painted Dogs

In September 2018, African painted dog B70096 conceived a litter of pups. In response to previous research signifying significantly higher fGM concentrations in pregnant African painted dogs (Van Der Weyde 2013), a comparison of fGM concentrations pre-, during, and post-pregnancy was conducted for African painted dog B70096 (Appendix E). A significant change in fGM concentrations due to her pregnancy was determined and subsequently all of B70096’s fecal samples were removed from the grouped GLMM analysis. With B70096’s samples removed, the analysis of “sex” and “individual” were redundant and only “sex” was included in the model.

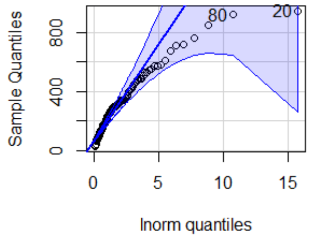
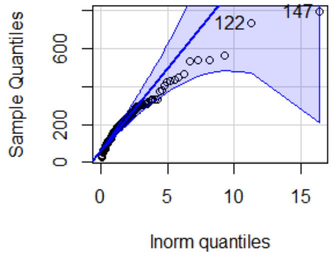
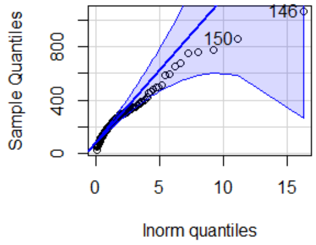
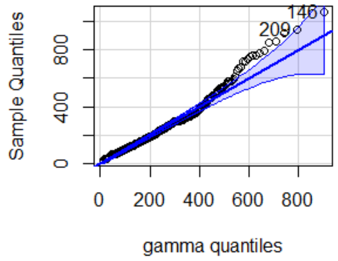
Asian Elephants

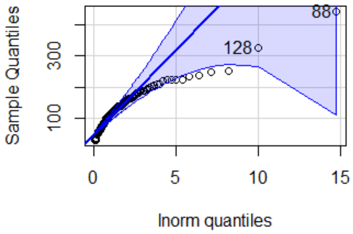
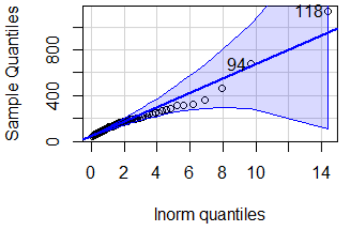
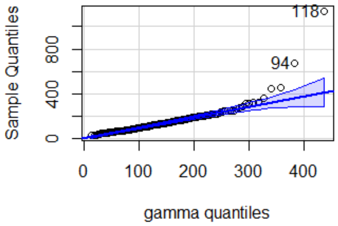
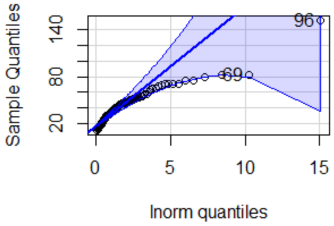
Due to the presence of an Asian elephant subspecies (*E. m. borneensis*), a non-parametric Mann-Whitney U test was used to compare the fGM concentrations of *E. m. borneensis* and *E. maximus* to confirm the similarity in fGM response (Appendix F). As the fGM concentrations were insignificantly different from one another, all Asian elephant data were included in the grouped GLMM. Additionally, the presence of different sexes prompted another Mann-Whitney U test to determine differences in median fGM concentrations based on sex.

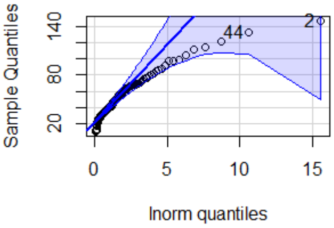
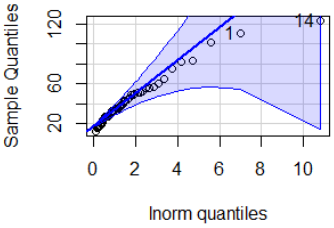
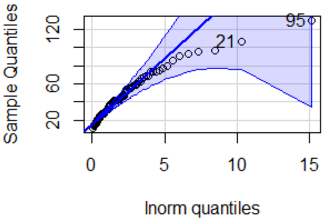
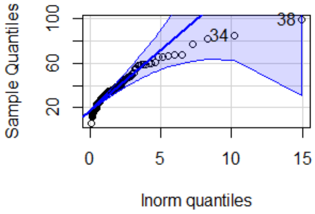
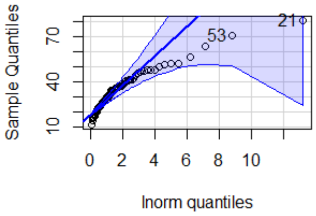
Correlation Analyses

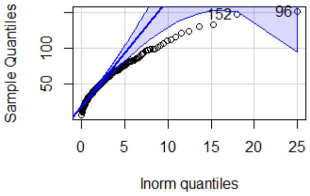
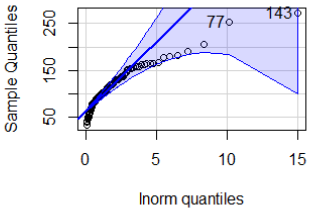
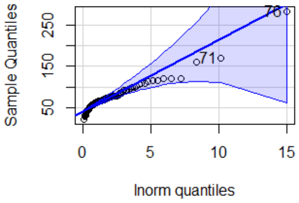
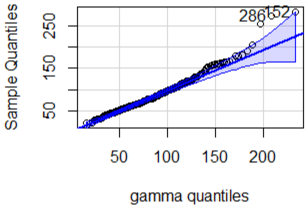
Correlation matrices were created to determine any potential correlations between behavior rate, fGM concentrations, average dB SPL level, temperature (°F), and daily attendance regardless of event classification. All statistics in this section were computed using the base package and ggplot2 (Wickham 2016) in R-statistical package (version 4.0.3) & RStudio (version 1.3.1093) (R Core Team 2020). Multiple Kruskal-Wallis rank sum tests were used to observe the potential correlations. Correlations that were insignificant at p-value < 0.05 were eliminated from the visualization.

Table 8: Distribution information and fixed effects analyzed in GLMMs

Animal ID	Sex	Distribution	QQ plot	Fixed Effects Analyzed in GLMM
African Painted Dog				
B70096	F	Log-normal		Pregnancy, Event
B70097	F	Log-normal		Event
B70167	M	Log-normal		Event
APD Combined	1.2	gamma		Sex, Event
Cheetah				

B70094	F	Log-normal		Event
B70095	F	Log-normal		Event
All Cheetah Combined	0.2	gamma		Event, Individual
Asian Elephant				
99270	F	Log-normal		Event

B80043	M	Log-normal		Event
B20189	F	Log-normal		Event
A80122	M	Log-normal		Event
94122	F	Log-normal		Event
1963	F	Log-normal		Event

All Elephants Combined	2.4	Log-normal		Event, Individual, Sex
B20186	M	Log-normal		Event
B60180	M	Log-normal		Event
All giraffe combined	2.0	Gamma		Event, Individual

Results

Sound Analysis Results

Table 9: Results of Kruskal-Wallis rank sum tests. ^{A} – indicates Bonferroni corrected *p*-values.

	Average dB SPL (unweighted)					
Location	Concert ± CI (n)	Other ± CI (n)	Control ± CI (n)	χ2	df	*p*-value^A
African Painted Dog & Cheetah Habitat	72.7 ± 1.23 (60)	69.4 ± 0.35 (30)	69.2 ± 0.52 (36)	27.474	2	<0.001***

Forest Hall (Asian Elephants)	67.8 ± 1.29 (76)	64.7 ± 1.29 (37)	60.3 ± 1.23 (47)	50.361	2	<0.001***
North Meadow (Asian Elephants)	68.9 ± 2.10 (27)	64.7 ± 0.98 (23)	64.2 ± 5.20 (10)	10.073	2	0.007**
South Habitat (Asian Elephants)	90.7 ± 2.65 (65)	71.2 ± 2.21 (35)	66.8 ± 1.10 (42)	86.577	2	<0.001***
Giraffe Habitat	68.6 ± 0.61 (82)	65.3 ± 0.59 (40)	65.2 ± 0.71 (30)	52.549	2	<0.001***

The average dB SPL (unweighted) was significantly different (Bonferroni *p* - value adjustment method) based on event type as identified by Kruskal-Wallis rank sum tests (Table 9) in every habitat monitored. All data is reported as mean dB SPL ± 95% CI. The African carnivore (cheetah and African painted dog) habitats reported significantly higher average dB SPL during “concert” event types (72.7 ± 1.23 dB SPL) than in both remaining event types (“other” – 69.4 ± 0.35 dB SPL, adj. *p* – value = 0.0032 & “control” – 69.2 ±0.52 dB SPL, adj. *p* – value < 0.001). However,

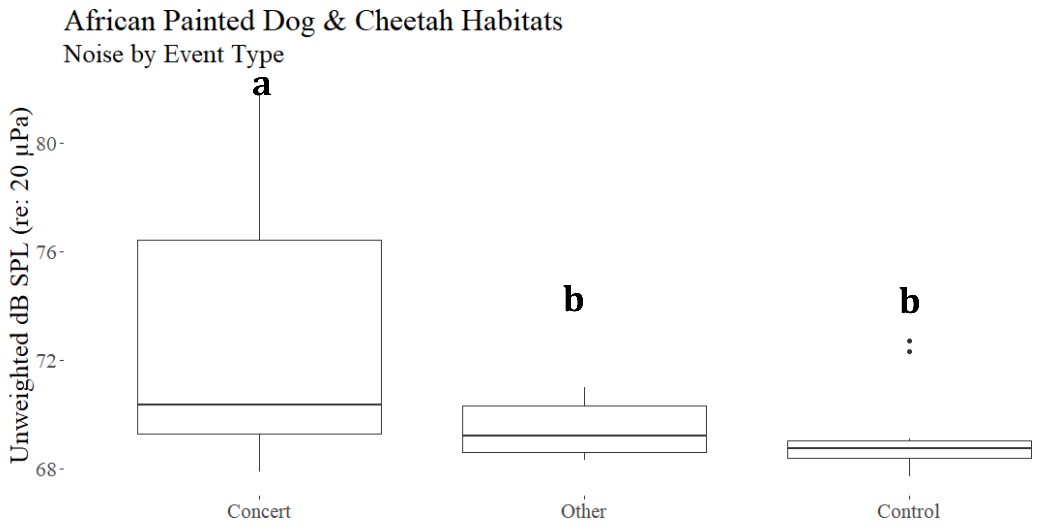


Figure 8: Comparison of average unweighted dB SPL (re: 20 µPa) levels based on event type at the African carnivore habitats. Kruskal-Wallis rank sum test showed significant differences in mean dB levels ($p < 0.001$) based on event type. Pairwise post-hoc (Wilcoxon rank sum) revealed significant differences between “concert” and the remaining event types (adj. *p*-value < 0.05). Significance on graph is shown with differentiating letters.

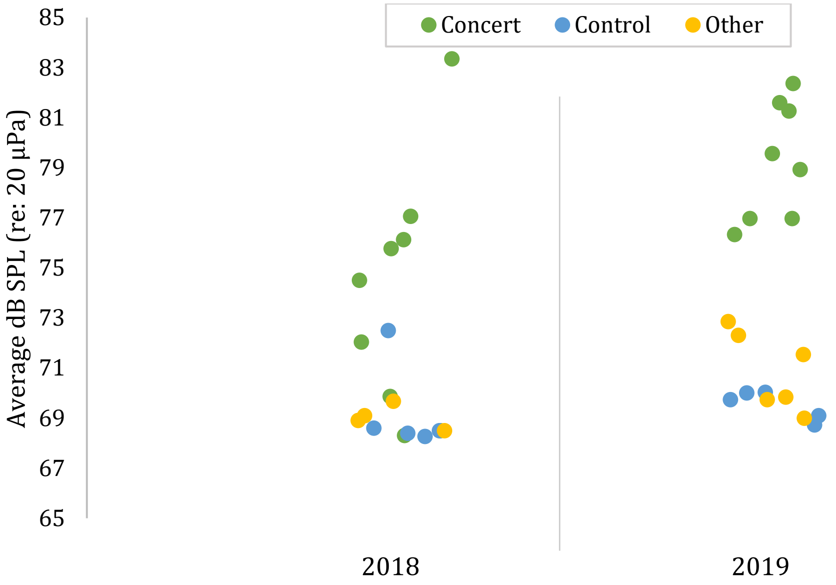


Figure 9: African carnivore habitat (cheetah and African painted dog) average dB SPL by observation period.

there was a statistically insignificant difference between "other" and "control" events within the African carnivore habitats (adj. p – value = 0.147) (Figure 8). Raw dB SPL levels are presented in Figure 9 to show the overlap between event types.

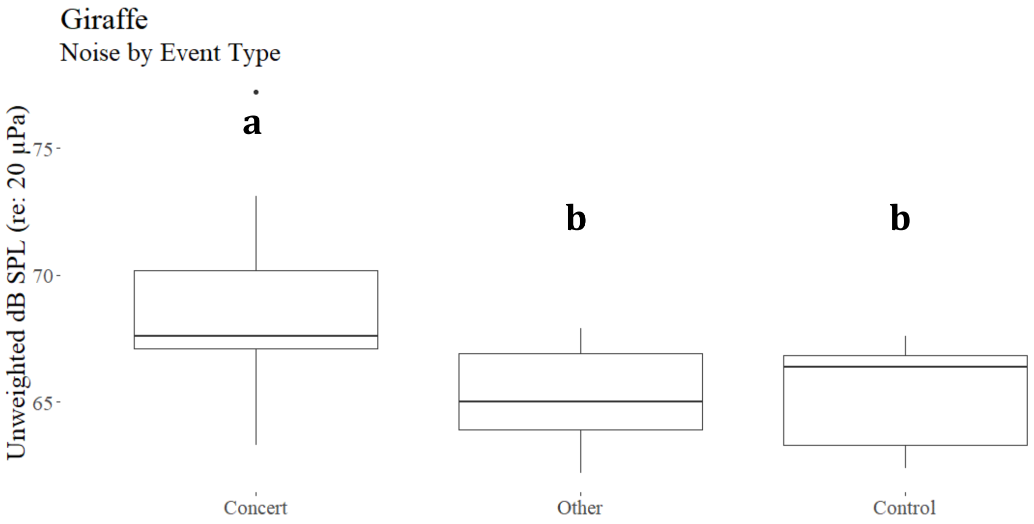


Figure 10: Comparison of average unweighted dB SPL (re: 20 µPa) levels based on event type at the giraffe habitat. Kruskal-Wallis rank sum test showed significant differences in mean dB levels ($p < 0.001$) based on event type. Pairwise post-hoc (Wilcoxon rank sum) revealed significant differences between "concert" and the remaining event types (adj. p-value < 0.001). Significance on graph is shown with differentiating letters.

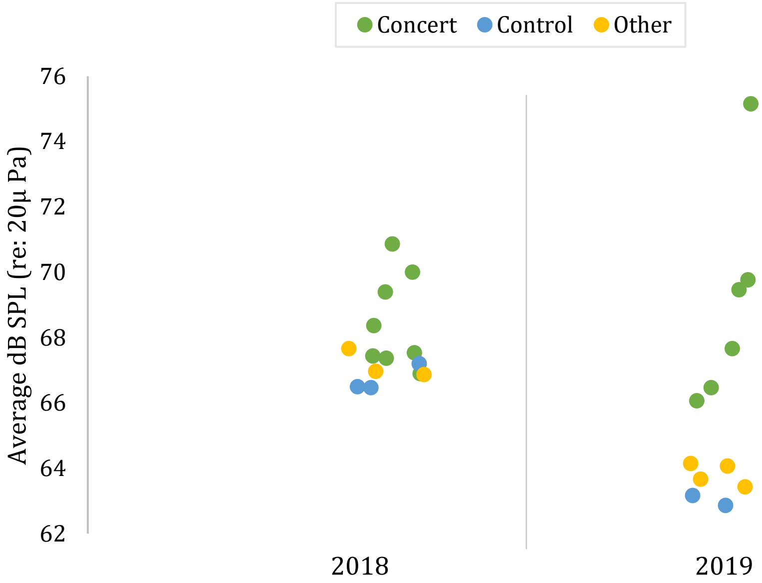


Figure 11: Giraffe average dB SPL by observation period.

The giraffe habitat also recorded significantly higher average dB SPL during "concert" event types (68.6 ± 0.61 dB SPL) than the remaining types ("other" – 65.3 ± 0.59 dB SPL, adj. p – value < 0.001 & "control" – 65.2 ± 5.20 dB SPL, adj. p – value < 0.001) (Figure 10). Like the African carnivore habitats, the giraffe habitat recorded statistically insignificant differences in average dB SPL between "other" and "control" event types (adj. p – value = 1.0). Average dB SPL for each observation period is presented in Figure 11.

Finally, significantly different overall average dB SPL was recorded for the separate Asian elephant habitats (*North Meadow, South Habitat*, and *Forest Hall*) (adj. p – value <0.001) (Figure 12). *South Habitat* (78.8 ± 2.27) recorded the highest dB SPL and was significantly more elevated than either *Forest Hall* (64.9 ± 0.91) (adj. p – value <0.001) and *North Meadow* (66.5 ± 1.35) (adj. p – value < 0.001).

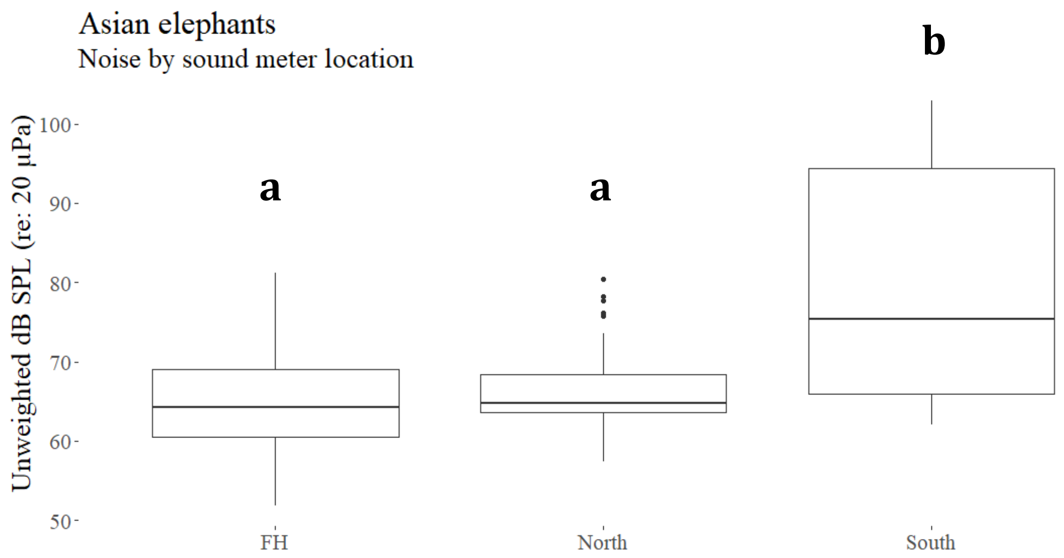


Figure 12: Comparison of average unweighted dB SPL (re: 20 µPa) levels based on sound meter location. Kruskal-Wallis rank sum test showed significant differences in mean dB levels ($p < 0.001$) based on location. Pairwise post-hoc (Wilcoxon rank sum) revealed significant differences between *South Habitat* and both other habitats (adj. p-value < 0.001), but not between *North Meadow* and *South Habitat* (adj. p-value: 0.18). Significance on graph is shown with differentiating letters.

However, *Forest Hall* and *North Meadow* were insignificantly different from one another (adj. p – value = 0.18).

Due to this significance, the average dB SPL per event type was independently analyzed by habitat (Figure 13). *Forest Hall* recorded significantly higher average dB SPL in “concert” event types (67.8 ± 1.29) than the remaining event types (“other” – 64.7 ± 1.29, adj. p – value = 0.015 & “control” – 60.3 ±1.23, adj. p – value < 0.001). *Forest Hall* also recorded a significant difference between “other” and “control” event types (adj. p – value <0.001); a result not seen in other habitats. Even though the average dB SPL at *North Meadow* was less variable than the other Asian elephant habitats, there was still a significant elevation in average dB SPL associated with “concert” events (68.9 ± 2.10) when compared with “control” events

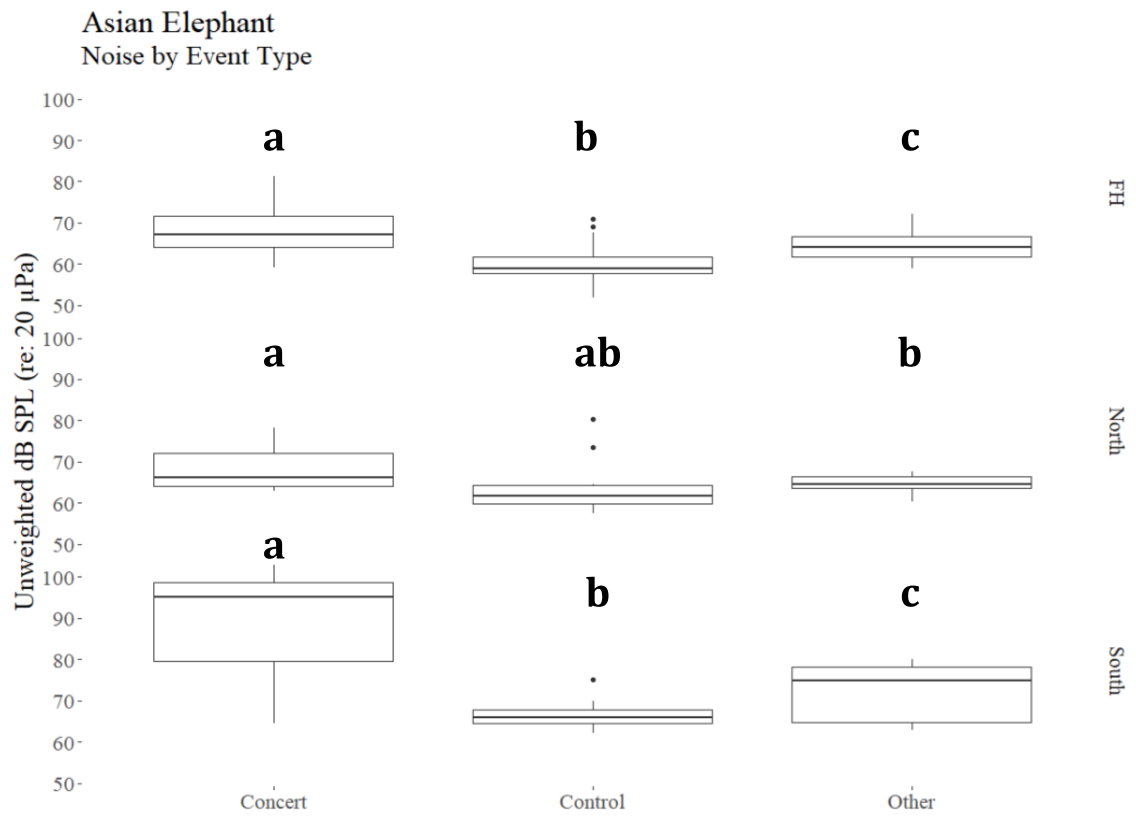


Figure 13: Comparison of average unweighted dB SPL (re: 20 μPa) levels based on event type at the three different Asian elephant habitats. Kruskal-Wallis rank sum test showed significant differences in mean dB levels ($p < 0.001$) based on event type at each habitat. Pairwise post-hoc (Wilcoxon rank sum) revealed significant differences between specific event types. Significance on graph is shown with differentiating letters.

(64.2 ± 5.20) (adj. *p* – value = 0.032). Average dB SPL was insignificantly different between "other" event types (64.7 ± 0.98) and either "control" (adj. *p* – value = 0.287) or "concert" (adj. *p* – value = 0.058). Finally, average dB SPL at *South Habitat* were significantly higher during "concert" events (90.7 ± 2.65) than during "other" event types (71.2 ± 2.21) (adj. *p* – value < 0.001) and "control" event types (66.8 ± 1.10) (adj. *p* – value < 0.001). However, the differences in average dB SPL between "control" event types and "other" event types (adj. *p* - value = 0.053) were statistically insignificant. Average dB SPL by observation period and location is presented in Figure 14.

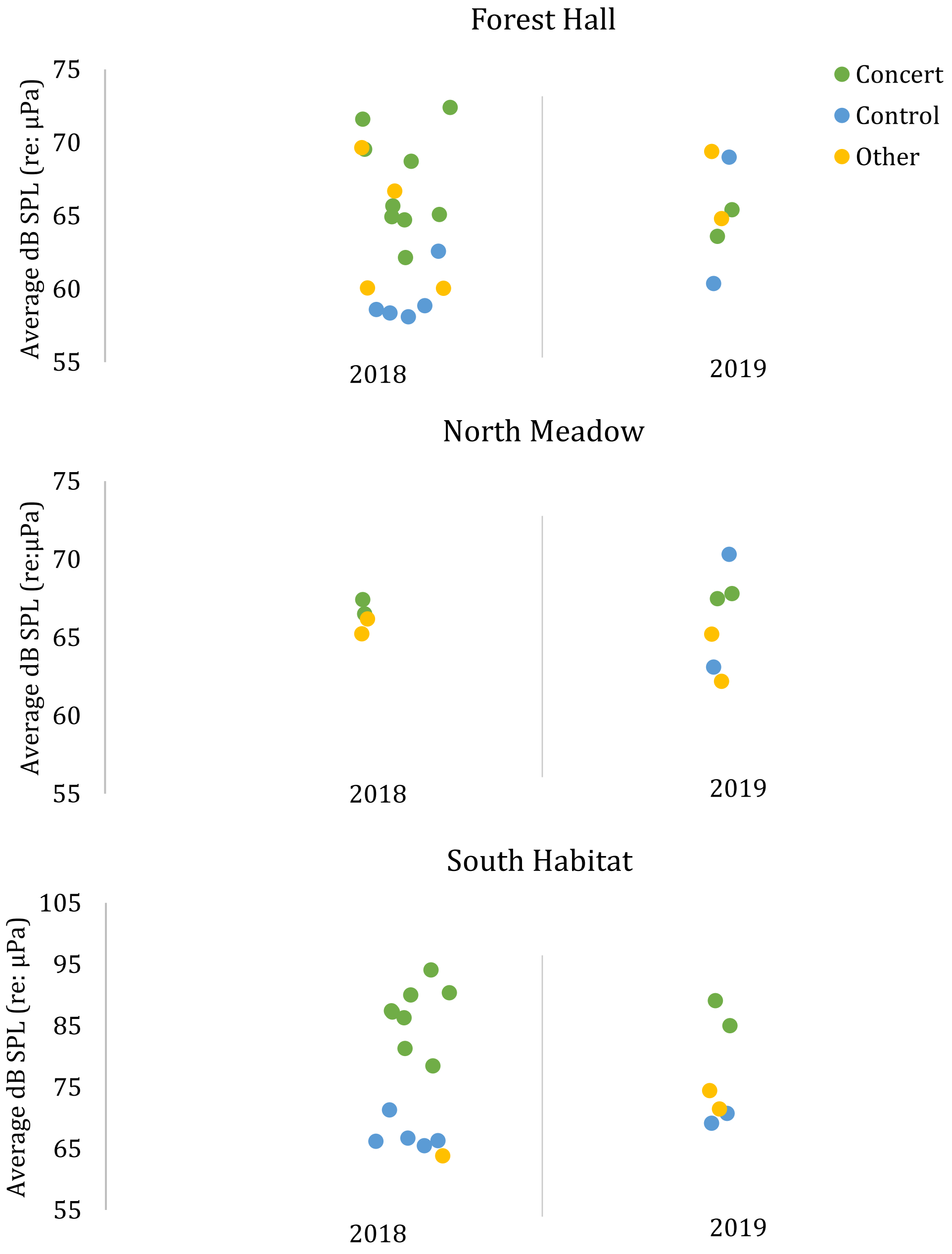


Figure 14: Asian elephant average dB SPL by observation period and location.

Behavior Results

African Painted Dog

Behavior data for African painted dogs were collected solely in 2018 due to the birth of 12 puppies in late 2018. Using behavior rates observed during “control” event types as the standard, multiple changes in behavior rates were noted for each individual (Table 10). B70167 had three behavior rates significantly vary from “control” observations: 1) “chase” behavior was significantly more represented during “concert” events (0.25%) than the “control” (CI = 0.00 – 0.00); 2) “locomotion” was significantly higher during “other” event types (5.4%) than “control” event types (CI = -1.001 – 2.668); and, 3) the average rate of “play” behaviors were significantly higher in both “concert” (0.50%) and “other” (1.10%) event types than “control” (CI = 0.00 – 0.00). Both African painted dog B70096 and B70097 had elevated rates of “play” behavior during “concert” events (B70096: 2.8% & B70097: 3.00%) in comparison to “control” (B70096 & B70097: CI = -0.403 – 2.070) events.

Table 10: African Painted Dog 95% Confidence Interval analysis. Asterisks (*) indicate the means fall outside of the “control” standard and are therefore significantly different.

	B70096			**B70097**		
Behavior	Concert mean (%)	Other mean (%)	Control 95% CI	Concert mean (%)	Other mean (%)	Control 95% CI
Beg	0.00	0.00	-0.500-1.334	0.00	0.00	-5.00-1.334
Chase	0.00	1.10	-0.500-1.334	0.75	0.00	-0.403-2.070
Eat	1.30	0.00	-3.003-8.003	1.00	1.10	-6.005-16.005
Flee	0.75	0.00	-0.403-2.070	0.00	1.10	-0.500-1.334
Locomotion	2.40	1.00	-1.501-4.001	2.45	4.80	-2.502-6.669

Not Visible	63.95	60.60	19.923-80.077	61.05	56.80	20.304-78.530
Pacing	1.30	0.50	-2.002-5.335	7.95	3.60	-2.682-17.682
Play	2.80*	2.60*	-0.403-2.070	3.00*	2.10	-0.403-2.070
Rest	17.45	24.30	6.478-67.689	15.00	24.10	1.095-55.572
Stationary	10.05	10.00	-1.773-11.773	8.50	5.90	-0.672-10.505

	B70167		
Behavior	Concert mean (%)	Other mean (%)	Control 95% CI
Beg	0.00	0.00	0.00–0.00
Chase	0.25*	0.00	0.00–0.00
Eat	1.55	0.60	-7.506–20.006
Flee	0.00	0.00	0.00–0.00
Locomotion	1.15	5.40*	-1.001–2.668
Not Visible	65.35	58.80	20.800–82.537
Pacing	6.65	5.50	-3.503–9.336
Play	0.50*	1.10*	0.00–0.00
Rest	17.90	23.10	3.222–65.111
Stationary	6.35	5.00	-1.051–9.384

Cheetah

There were no significant differences in behavior rates for cheetah B70094, but there were two significant differences in behavior rate for cheetah B70095 (Table 11). Cheetah B70095 had significantly lower average “locomotion” rates during “other” events (1.39%) and significantly higher rates of “stereotypy” during the “concert” events (0.09%) than in “control” events (“locomotion” CI = 2.029 – 9.024 & “stereotypy” CI = 0.00-0.00).

Table 11: Cheetah 95% Confidence Interval analysis. Asterisks (*) indicate the means fall outside of the “Control” standard and are therefore significantly different.

	B70094			**B70095**		
Behavior	Concert mean (%)	Other mean (%)	Control CI	Concert mean (%)	Other mean (%)	Control CI
Environmental Interaction	0.26	0.72	0.225 – 2.650	2.47	0.35	0.010 – 2.622
Grooming	0.90	0.69	-0.563 – 2.142	1.05	0.95	-0.050 – 2.419
Locomotion	4.80	3.55	1.887 – 10.523	6.54	1.39*	2.029 – 9.024
Not Visible	56.13	43.92	35.757 – 63.038	50.46	42.47	34.636 – 62.732
Social Interaction	0.90	0.58	-0.294 – 3.216	0.79	0.00	-0.300 – 3.195
Stationary	37.01	49.51	27.139 – 54.260	38.52	54.84	28.255 – 55.429
Stereotypy	0.00	0.00	0.00	0.09*	0.00	0.00 – 0.00

Asian Elephants

Behavior data was collected in both 2018 and 2019 for all Asian elephants except B20189 who died in late 2018. Multiple behavior rates during “concert” and “other” event types were significantly different from “control” event types (Table 12). Elephant 99270 had significantly higher rates of “feeding / drinking” during “concert” event types than in “control” event types, as well as higher “social (physical contact)” rates and “solitary” rates but lower rates of “food object interaction” and “stereotypy” during “other” event types. Elephant 94122 exhibited significantly lower rates of “food object interaction” during “concert” events but significantly higher rates of “feeding / drinking” and “social (physical contact)” during both “other” and “control” events. Elephant B80043 showed significantly higher rates of “enrichment object interaction” during the “concert” event types, significantly higher rates of “solitary” and “environmental interaction” and lower rates of “food object interaction” during “other” event types, and significantly higher rates of “feeding / drinking”, significantly lower rates of “social (physical contact)” and “stationary” behaviors during both “concert” and “other” event types. Elephant A80122 showed significantly lower rates of “environmental interaction” and higher rates of “locomotion” and “stereotypy” during “concert” event types, but significantly higher rates of “enrichment object interaction” and “social (no contact)” as well as significantly lower rates of “food object interaction” and “feeding / drinking” during “other” event types. Finally, Elephant 1963 exhibited significantly higher rates of “enrichment object interaction” during “concert” event types, significantly higher rate of “feeding / drinking” and “stereotypy” during “other” events, and significantly higher rates of “solitary” behaviors during both “concert” and “other” event types.

Table 12: Asian Elephants 95% Confidence Interval analysis. Asterisks (*) indicate the means fall outside of the “Control” standard and are therefore significantly different.

	99270			**94122**		
Behavior	Concert mean (%)	Other mean (%)	Control 95% CI	Concert mean (%)	Other mean (%)	Control 95% CI
Enrichment Object Interaction	0.28	0.00	-0.331 – 2.989	0.81	0.54	-0.291 – 5.726
Environmental Interaction	3.44	2.96	1.025 – 8.443	5.88	3.57	1.971 – 5.984
Feeding/Drinking	17.65*	16.83	5.396 – 17.616	7.97*	13.36*	2.141 – 6.586
Food Object Interaction	12.15	14.12	3.720 – 14.913	18.37*	21.29	20.203 – 34.574
Locomotion	24.30	18.72	17.776 – 33.810	16.58	18.87	11.649 – 20.453
Social (No Contact)	1.89	8.30	-0.469 – 1.346	18.81	16.71	9.661 – 22.591

Social (Physical Contact)	1.25	11.91*	0.118 – 6.219	8.41*	7.99*	1.545 – 7.656
Solitary	0.29	1.07*	-0.179 – 1.013	0.22	0.16	-0.101 – 0.876
Stationary	5.83	7.07	1.647 – 8.872	5.34	8.34	5.115 – 16.723
Stereotypy	20.78	1.14*	5.805 – 29.777	0.10	0.00	-0.116 – 0.343

	B80043			**A80122**		
Behavior	Concert mean (%)	Other mean (%)	Control 95% CI	Concert mean (%)	Other mean (%)	Control 95% CI
Enrichment Object Interaction	1.46*	0.00	-0.181 – 1.010	4.73	5.57*	1.344 – 5.193
Environmental Interaction	3.96	5.01*	0.989 – 4.263	5.13*	6.62	6.090 – 13.890
Feeding/Drinking	10.48*	15.21*	2.344 – 8.715	6.68*	13.88	9.927 – 23.227
Food Object Interaction	31.65	22.03*	22.381 – 42.677	19.88	15.06*	16.162 – 32.834
Locomotion	9.55	17.68*	4.906 – 13.501	20.82*	13.41	12.614 – 19.744
Social (No Contact)	14.66	8.75	5.475 – 19.745	4.20	9.26*	1.071 – 7.974
Social (Physical Contact)	9.47*	8.74*	1.076 – 7.454	2.22	3.11	1.588 – 7.737
Solitary	0.47	1.68*	-0.066 – 1.020	0.86	0.78	0.420 – 2.724
Stationary	7.76*	5.62*	8.557 – 23.143	9.90	8.82	5.548 – 14.311
Stereotypy	0.00	0.00	-0.812 – 2.375	0.08*	0.00	0.00 – 0.00

		1963	
Behavior	Concert mean (%)	Other mean (%)	Control 95% CI
Enrichment Object Interaction	0.59*	0.29	-0.116 – 0.342
Environmental Interaction	5.50	7.58	3.581 – 12.038
Feeding/Drinking	8.90	11.26*	4.445 – 10.973
Food Object Interaction	17.68	15.27	12.668 – 22.848
Locomotion	15.95	16.84	15.352 – 30.040
Social (No Contact)	11.92	15.37	7.711 – 21.274

Social (Physical Contact)	5.35	4.18	3.579 – 10.265
Solitary	0.82*	3.23*	-0.049 – 0.754
Stationary	11.63	9.73	6.062 – 16.485
Stereotypy	0.74	7.94*	0.113 – 4.444

Giraffe

Giraffe B60180 exhibited significantly lower rates of "vigilance" behaviors during "other" (1.96%) events compared with "control" events (CI = 1.191 – 4.672), while giraffe B20186 had significantly higher rates of "grooming" during "other" (0.78%) events and significantly higher rates "social interaction" (0.90%) during "concert" event types (Table 13) when compared with "control" ("grooming" CI: -0.143-0.421 & "social interaction" CI: -0.058-0.891).

Table 13: Giraffe 95% Confidence Interval analysis. Asterisks (*) indicate the means fall outside of the "Control" standard and are therefore significantly different.

	B60180			B20186		
Behavior	Concert mean	Other mean	Control CI	Concert mean	Other mean	Control CI
Eat	42.56	37.15	36.888 – 56.416	51.67	45.95	41.070 – 58.389
Environmental Interaction	12.59	16.62	9.974 – 23.271	8.25	8.63	5.907 – 14.868
Grooming	0.18	0.32	-0.115 – 0.671	0.27	0.78*	-0.143 – 0.421
Locomotion	11.96	13.43	6.183 – 14.538	14.01	15.96	10.269 – 18.264
Social Interaction	0.72	0.21	-0.058 – 0.891	0.90*	0.37	-0.058 – 0.891
Stationary	10.13	7.01	2.542 – 13.535	10.53	8.53	6.157 – 16.450
Stereotypy	15.40	15.57	4.328 – 15.774	6.41	6.75	0.879 – 11.899
Vigilance	1.96	1.09*	1.191 – 4.672	1.71	2.22	0.588 – 3.871

Endocrinology Results

African Painted Dogs

GLMM results are in Table 14. There were no significant differences in fGM concentrations based solely on event type for the African painted dogs in either individual or grouped analyses ($p > 0.05$ for both). However, when “sex” was included as a fixed effect, the effect of event type on fGM concentrations approached significance (Table 14; Figure 15).

Table 14: African painted dog results of GLMM tests. Asterisks (*) indicate statistical significance (p-value < 0.05). Tests are run at the individual and group level.

Event	**Estimate**	**Standard Error**	**t-value**	***p*-value**
B70096				
Intercept (Concert)	5.183	0.260	19.941	<0.001
Control	0.398	0.265	1.499	0.136
Other	0.352	0.381	0.923	0.357
*Pregnancy	0.296	0.102	2.887	0.004*
B70097				
Intercept (Concert)	5.211	0.137	38.037	<0.001
Control	0.012	0.146	0.084	0.933
Other	0.067	0.238	0.284	0.777
B70167				
Intercept (Concert)	5.471	0.117	46.875	<0.001
Control	-0.143	0.125	-1.139	0.256
Other	-0.231	0.182	-1.273	0.205
Grouped				
Intercept (Concert)	0.005	0.0005	10.579	<0.001
Control	-0.0001	0.0005	-0.025	0.980
Other	0.0004	0.0007	0.539	0.591
Sex	-0.0006	0.0003	-1.908	0.058

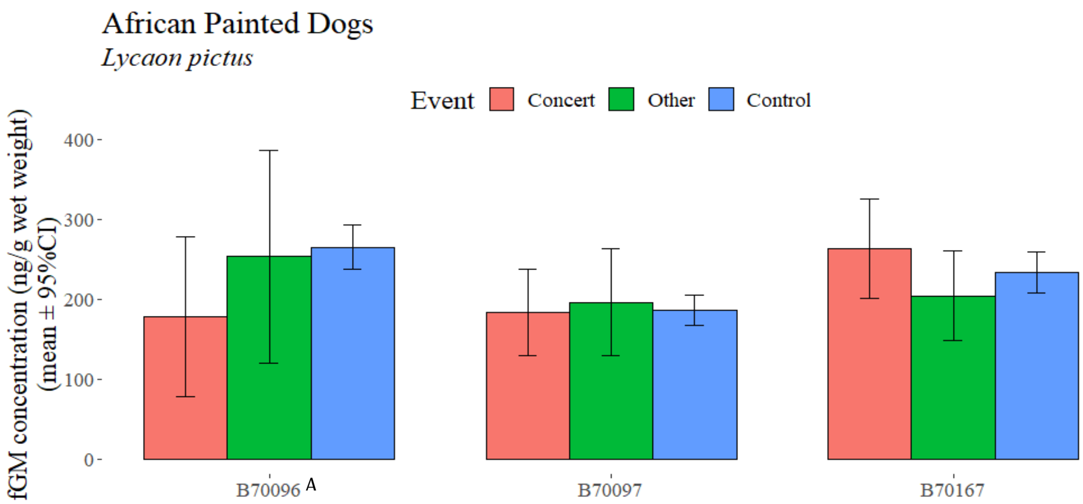


Figure 15: Average fGM concentrations (ng/g wet weight) of African painted dogs by event and individual. Data are presented as mean ± 95% confidence interval. No significant (*p*-value> 0.05) differences occurred. ^{A} indicates data from 2019 only.

Cheetahs

There were no significant differences in average fGM concentrations based on event type for individual cheetahs or cheetahs as a group (Figure 16). Average fGM concentrations based on event type was close to significance when "individual" was included as a fixed effect (Table 15).

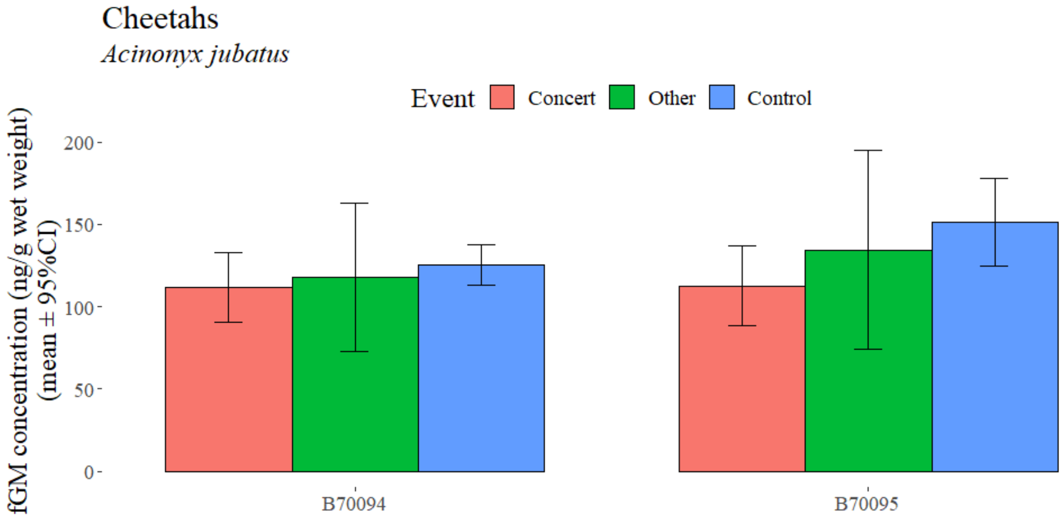


Figure 16: Average fGM concentrations (ng/g wet weight) of cheetahs by event and individual. Data are presented as mean ± 95% confidence interval. No significant (*p*-value> 0.05) differences occurred.

Table 15: Cheetah results of GLMM tests. Asterisks (*) indicate statistical significance (*p*-value < 0.05). Tests are run at the individual and group level.

Event	Estimate	Standard Error	t-value	*p*-value
B70094				
Intercept (Concert)	4.649	0.088	52.833	<0.001
Control	0.106	0.098	1.079	0.282
Other	0.050	0.173	0.291	0.771
B70095				
Intercept (Concert)	4.626	0.115	40.252	<0.001
Control	0.195	0.127	1.532	0.128
Other	0.176	0.238	0.741	0.460
All Combined				
Intercept (Concert)	4.632	0.132	35.199	<0.001
Control	0.212	0.131	1.613	0.110
Other	0.121	0.227	0.533	0.595
Individual	0.163	0.088	1.860	0.066

Asian Elephants

GLMMs revealed no significant differences in average fGM concentrations by event type for any Asian elephants individually or as a group (Figure 17). The group GLMMs revealed significant differences in fGM concentrations per event type when grouped by sex (Table 16). Comparison of distributions revealed no significant difference, allowing for comparisons of medians. Subsequent Mann-Whitney U tests showed significant differences between median fGM concentrations of female (32.15) and male (37.04) Asian elephants, $U = 86080$, $z = 4.377$, $p < 0.001$ (Figure 18). Additionally, age had an insignificant effect on average fGM concentrations (Figure 19).

Table 16: Asian elephants results of GLMM tests. Asterisks (*) indicate statistical significance (*p*-value < 0.05). Tests are run at the individual and group level.

Event	Estimate	Standard Error	t-value	*p*-value
99270				
Intercept (Concert)	3.676	0.081	45.143	<0.001
Control	-0.066	0.092	-0.717	0.475
Other	-0.180	0.173	-1.041	0.300
B80043				
Intercept (Concert)	3.894	0.091	42.760	<0.001
Control	-0.049	0.101	-0.480	0.632
Other	0.088	0.167	0.530	0.598

B20189				
Intercept (Concert)	3.361	0.166	20.296	<0.001
Control	0.253	0.182	1.393	0.169
Other	0.283	0.277	1.023	0.311
A80122				
Intercept (Concert)	3.597	0.140	25.340	<0.001
Control	0.027	0.149	0.182	0.856
Other	0.069	0.215	0.322	0.748
94122				
Intercept (Concert)	3.507	0.0874	40.109	<0.001
Control	-0.042	0.097	-0.436	0.664
Other	0.105	0.155	0.679	0.498
1963				
Intercept (Concert)	3.382	0.081	41.557	<0.001
Control	0.025	0.092	0.266	0.791
Other	0.146	0.170	0.857	0.394
All Combined				
Intercept (Concert)	3.599	0.061	59.252	<0.001
Control	-0.030	0.066	-0.455	0.649
Other	-0.002	0.111	-0.020	0.984
Sex*	0.182	0.031	5.931	<0.001*
Age	0.001	0.002	0.283	0.778

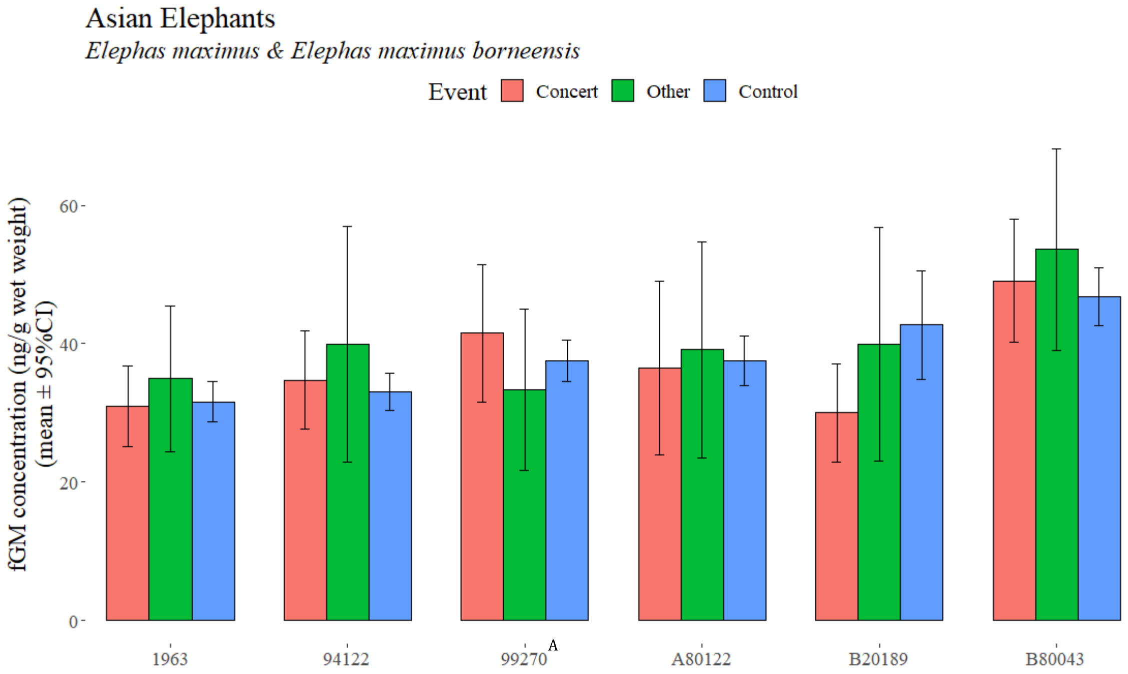


Figure 17: Average fGM concentration (ng/g wet weight) of Asian elephants by event and individual. Data are presented as mean ±95% confidence interval. No significant (*p*-value > 0.05) differences occurred. ^{A} indicates *E. m. borneensis.*

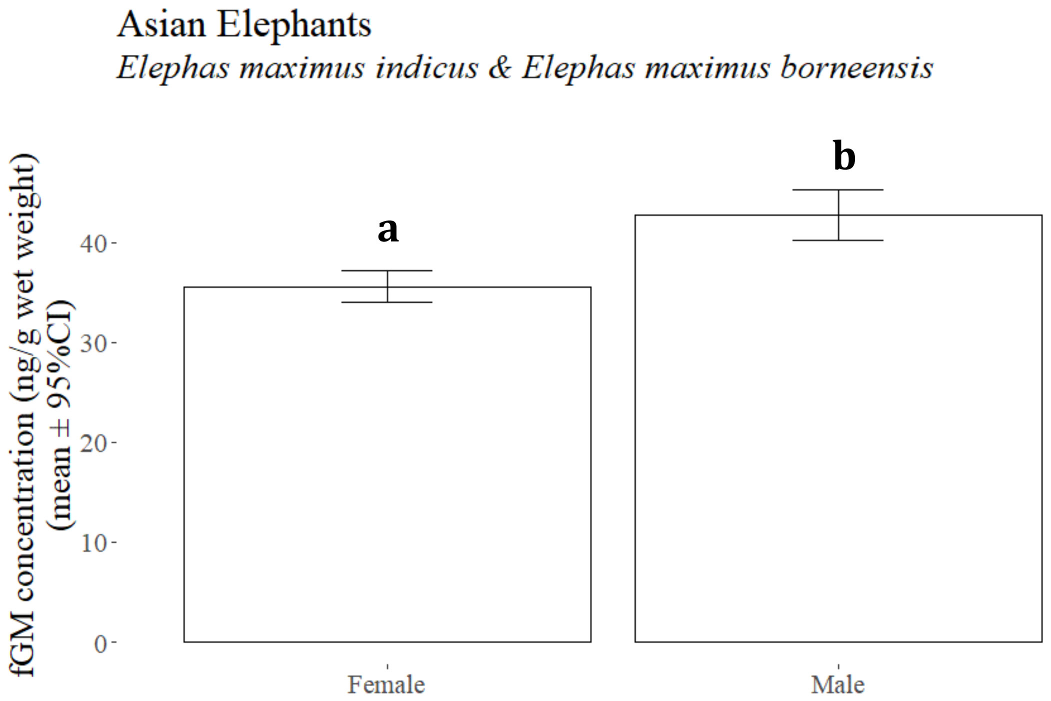


Figure 18: Average fGM concentrations (ng/g wet weight) of Asian elephants are significantly (*p*-value < 0.05) different when compared by sex. Data are presented as mean ± 95% confidence interval and significance is indicated by differing letters.

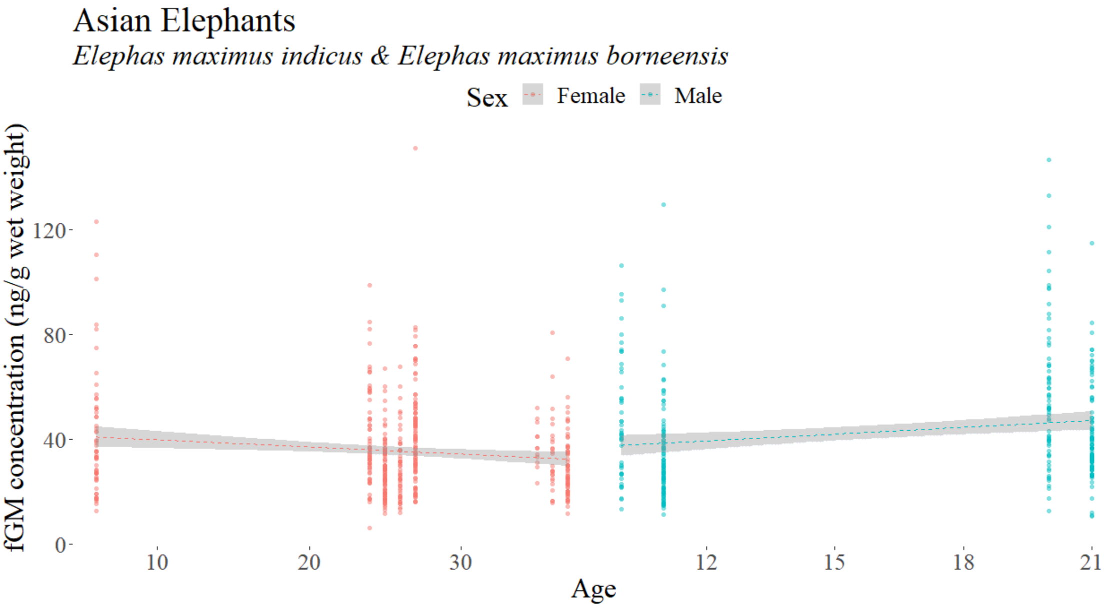


Figure 19: Asian elephant fGM concentrations (ng/g wet weight) by age and grouped by sex. Trendlines are superseded on top of the data to highlight trending changes in fGM concentration by age. Age had no significant (*p*-value > 0.05) effect on fGM concentrations..

Giraffes

Table 17: Giraffe results of GLMM tests. Asterisks (*) indicate statistical significance (p-value < 0.05). Tests are run at the individual and group level.

Event	Estimate	Standard Error	t-value	*p*-value
B20186				
Intercept (Concert)	4.571	0.086	53.394	<0.001
Control	0.011	0.091	0.115	0.909
Other	0.066	0.151	0.436	0.664
B60180				
Intercept (Concert)	4.049	0.089	45.712	<0.001
Control	0.099	0.095	1.042	0.299
Other	-0.010	0.151	-0.069	0.945
All Combined (gamma distribution)				
Intercept (Concert)	0.010	0.001	10.335	<0.001
Control	-0.001	0.001	-0.511	0.610
Other	-0.0002	0.002	-0.119	0.906
Individual *	0.006	0.001	13.500	<0.001*

GLMMs revealed no significant differences in average fGM concentrations by event type for any giraffes individually or as a group (Figure 20). However, there was a significant difference in fGM concentrations between the two individuals (Table 17). Subsequent Mann-Whitney U tests also showed median fGM

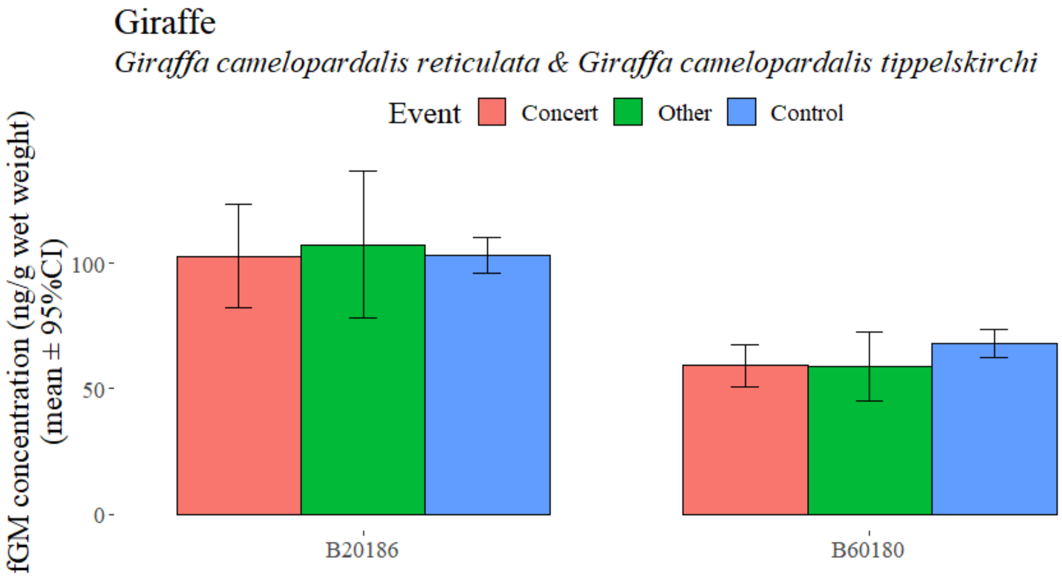


Figure 20: Average fGM concentration (ng/g wet weight) of giraffes by event and individual. Data are presented as mean ± 95% confidence interval. No significant (p-value > 0.05) differences occurred for either individual.

concentrations for B20186 (96.23) were significantly higher than B60180 (63.68), $U = 4061$, $z = -9.359$, $p < 0.001$ (Figure 20).

Correlation Analysis Results

African Painted Dogs

Correlations between average dB SPL data per observation period, fGM data, attendance, temperature (F) and average behavior rates are presented in Figure 21**.** There were three significant correlations between mean dB and behavior rates (stationary: tau = 0.211, $p = 0.027$, pacing: tau = 0.0.200, $p = 0.045$ & not visible: tau = -0.227, $p = 0.013$). There were no significant correlations between fGM

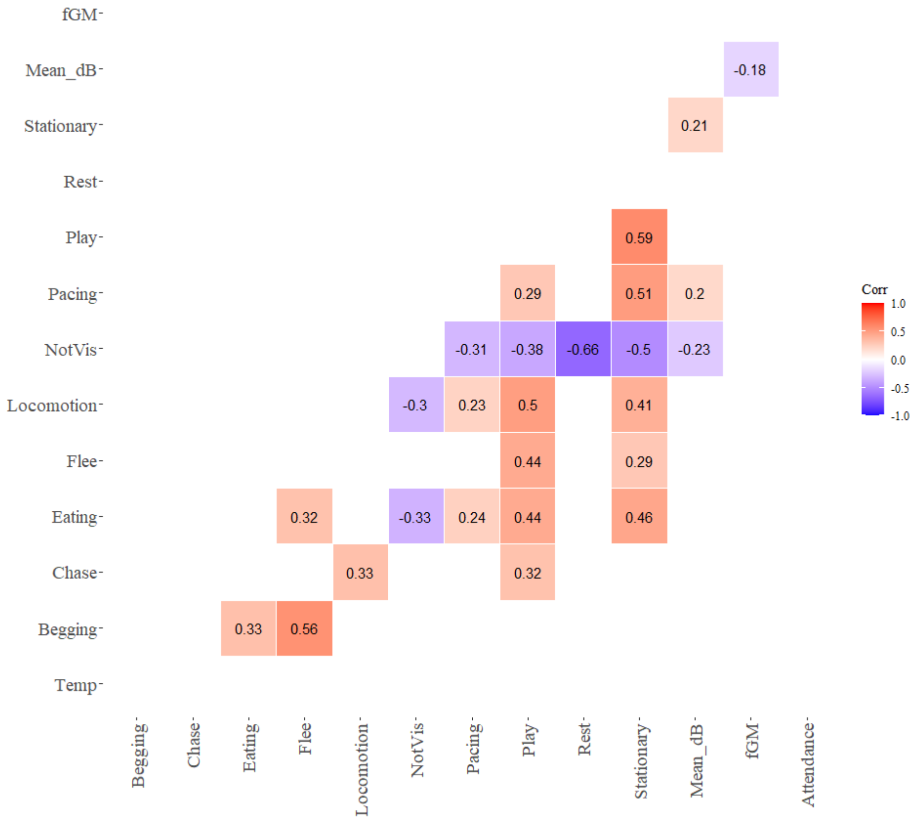


Figure 21: African Painted Dog Correlation Matrix – Kendall's Tau calculations for African painted dogs. Shown data are significant correlations (*p*-value < 0.05). Correlations values of -1 are all discordant, values of 1 indicate concordance and a value of 0 indicates no relationship at all.

concentrations and behaviors; nor were there significant correlations between attendance and any parameters. Finally, there was a significant inverse correlation between mean dB and fGM concentrations (tau = -0.177, p = 0.040).

Cheetahs

Correlations between average dB SPL data per observation period, fGM data, attendance, temperature (F) and average behavior rates are presented in Figure 22. There was one significant correlation between mean dB data and behavior rates (social interaction: tau = -0.229, p = 0.002) and between mean dB and temperature

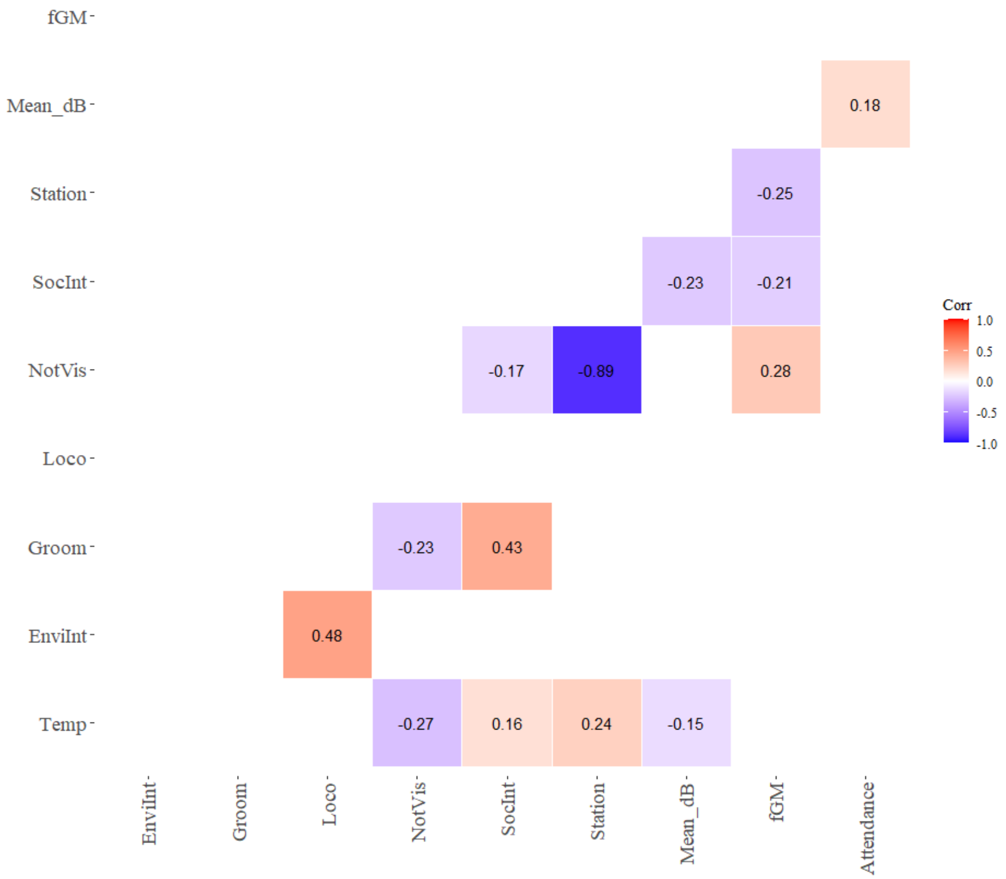


Figure 22: Cheetah Correlation Matrix – Kendall's Tau calculations for cheetahs. Shown data are significant correlations (p-value < 0.05). Correlation values of -1 are all discordant, values of 1 indicate concordance and a value of 0 indicates no relationship at all.

(tau = -0.160, $p = 0.013$). There were multiple significant correlations between fGM data and behavior rates (not visible: tau = 0.278, $p < 0.001$; social interaction: tau = -0.208, $p = 0.006$; & stationary: tau = -0.253, $p = 0.0002$). Finally, there were no significant correlations between behavior rates and attendance.

Asian Elephants

Correlations between average dB SPL per observation period data, fGM data, attendance, temperature, and average behavior rates are presented in Figure 23. There were multiple significant correlations between mean dB and behavior (food object interaction: tau = -0.129, $p = 0.006$; locomotion: tau = 0.120, $p = 0.009$; &

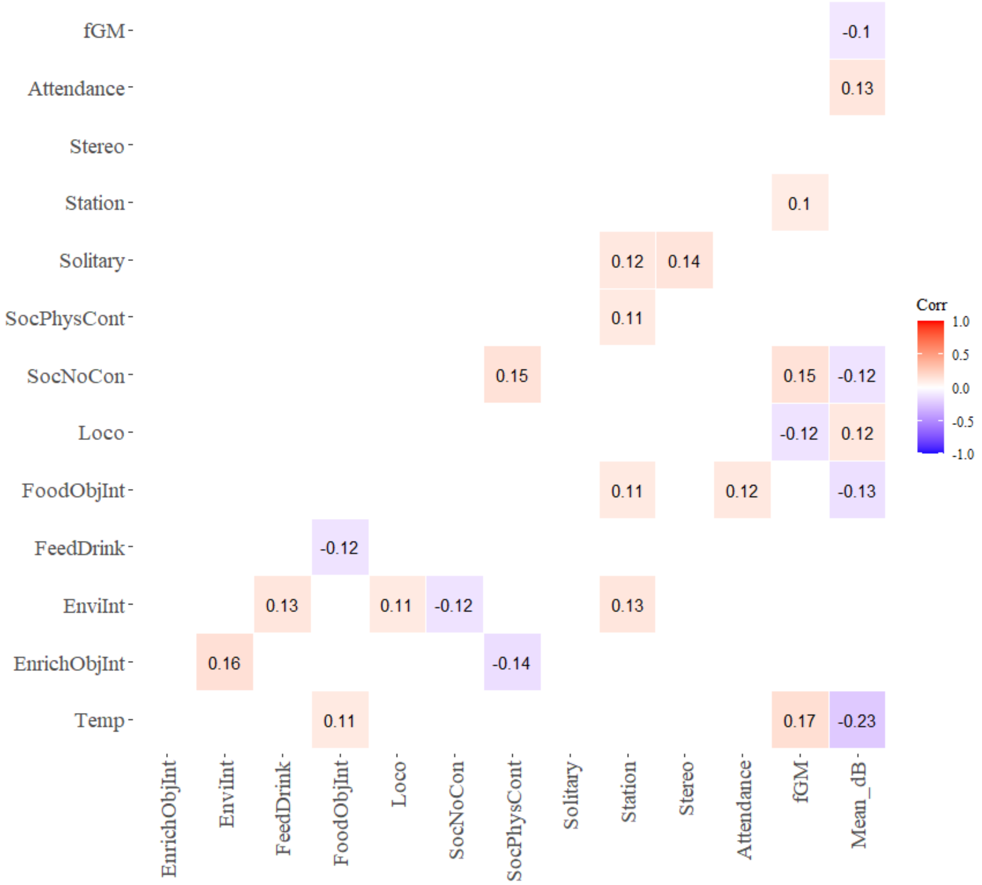


Figure 23: Kendall's Tau calculations for Asian elephant. Shown data are significant correlations (*p*-value < 0.05). Correlation values of -1 are all discordant, values of 1 indicate concordance and a value of 0 indicates no relationship at all

social – no contact: tau = -0.117, $p = 0.016$) as well as between mean dB and temperature (tau = -0.228, $p < 0.001$), and mean dB and fGM (tau = -0.100, $p = 0.028$). There were also significant correlations between fGM concentrations and multiple behaviors (locomotion: tau = -0.121, $p = 0.009$; social – no contact: tau = 0.147, $p = 0.002$; & stationary: tau = 0.102, $p = 0.037$) as well as between fGM concentration and temperature (tau = 0.170, $p = 0.0002$). Finally, there was a significant correlation between attendance and food object interaction (tau = 0.127, $p = 0.007$) as well as mean dB data (tau = 0.129, $p = 0.005$).

Giraffes

Correlations between average dB SPL per observation period, fGM data, attendance, temperature (F) and average behavior rates are presented in Figure 24. There were no significant correlations between noise levels and behaviors. Additionally, there were no significant correlations between fGM data and any behavior rates. However, there were two significant correlations between attendance and behavior rates (environmental interaction: tau = -0.161, $p = 0.042$ & locomotion: tau = 0.165, $p = 0.034$) as well as temperature (tau = -0.506, $p < 0.001$).

Discussion

This study aimed to provide necessary information on the possible behavioral and endocrine effects associated with large-scale after-hour events at the Oregon Zoo. As with most behavioral and hormonal studies, the results I found varied by species and individual. The hypotheses were: 1) significantly increased fGM concentrations following after-hour events, in particular following "concert"

Figure 24: Giraffe Correlation Matrix - Kendall's Tau calculations for giraffes. Shown data are significant correlations (*p*-value < 0.05). Correlation values of -1 are all discordant, values of 1 indicate concordance and a value of 0 indicates no relationship at all.

event types due to the additional noise; and 2) significantly increased vigilant, avoidance, or abnormal behaviors during after-hour events.

Endocrinology

The fGM concentrations of all species were insignificantly different based on the event type. For all species, fGM concentrations associated with "other" event types were more variable than the other event types. Research by Edwards et al. (2019) linked increased fGM variability in Asian and African elephants with increased instances of foot lesions indicating a relationship between fGM variability and stress. The rapid activation and suppression of the HPA axis associated with multiple stressors can result in more variability and higher concentrations of fGMs. One explanation by Edwards et al. (2019) was that fGM variability was likely associated with acute stressors while overall elevation was associated with chronic stressors, although this specific hypothesis has not be tested. This variability may indicate that while "concert" and "control" event types were not on average statistically different from "other" event types, "other" event types were associated with an increased number of acute stressors that the individuals were able to adjust to quickly. "Other" event types allowed extended visitor access to all habitats while both "control" and "concert" event types prevented visitor access after a certain time (19:00 for "concert" events and 18:00 for "control" events). However, average dB SPL was loudest during "concert" events. This juxtaposition of increased average dB SPL but less variable fGM concentrations may indicate that the visibility of visitors was more influential to changes in physiology than auditory perception of visitors. This effect of "crowd density" has been shown to correspond with significantly increased corticosterone / cortisol concentrations in other species (Indian blackbuck, *Antilope cervicapra L.*- Rajagopal et al. 2011, & spider monkeys, *Ateles geoffroyi rufiventris* - Davis et al. 2005).

The lack of change in fGM concentrations associated with different events was supported by results in multiple other species. For instance, a study on gorillas found no significant difference in fGM concentrations in fecal samples collected pre-

ZooLights, ZooLights, and post-ZooLights periods (Bastian et al. 2020). An additional study on the effects of a summer concert series on orangutan (*Pongo pygmaeus*) behavior and fGM concentrations also found insignificant differences in fGM concentrations relative to event type, however this study was unable to provide adequate control comparisons due to visitor presence year-round (de Queiroz 2018). One study on Asian elephants did show a 2-to-10 fold increase in fGM concentrations over baseline levels following an event in one of their Asian elephants who was frequently used for public processions (Kumar et al. 2014). While this is interesting, the differences in husbandry practices, event types, and lack of social housing in the Asian elephant described in the study by Kumar et al. (2014) are more likely the cause of such dramatic differences in fGM concentration responses.

Similar to Asian elephants, the cheetahs did not show a significant difference in fGM concentrations based on a specific event type, indicating additional stressors may be more influential on fGM concentrations than event type. This finding was in-line with another, later study conducted in 2020 as part of this dissertation (chapter 4, page x, Fink et al. 2021). Although a different pair of cheetahs was observed for the study in 2020 (the females had been sent out for breeding and a pair of males arrived in early 2020), no significant changes in fGM concentrations based on visitor presence was also reported.

The relationship between fGM concentrations and event type remained insignificant when compared at the individual level. However, the variability in fGM responses by individuals should be noted. It is common for individuals in the same species to have drastically different reactions to stressors and for these stressors to cause different physiological responses. This can happen for many different reasons including personality, age, sex, and social status. For instance, all three African painted dogs varied in which "event" treatment elicited the highest fGM concentrations – however, none of these differences were significant. For African painted dogs, these differences could be due to status within the pack. It had previously been found that alpha female and male African painted dogs had significantly higher fGM concentrations in comparison to the other subordinates in the pack (Creel 2005; Creel et al. 1997). This was supported by our data. While the Oregon Zoo only had three African painted dogs in 2018, painted dog B70096 was the alpha female and, by default, painted dog B70167 was the alpha male. The single subordinate female, painted dog B70097, showed lower fGM concentrations than her pack members – although these trends were insignificant. While there were an additional twelve African painted dogs born in winter 2018, African painted dogs do not reach sexual maturity until two years of age (Van Der Weyde 2013) and, therefore probably minimally affected the social status of the alpha male and female during the 2019 observation period.

Finally, while there were no significant changes based specifically on event type, it is important to mention other stressors that may have overshadowed the influence of event type on fGM concentrations. For all zoo animals, there are multiple stressors that may cause increased arousal and, subsequently, increased fGM concentrations. For instance, a new male breeding-age Asian elephant (Asian elephant B80043) was introduced to the females in the herd on May 11, 2018. Social introductions have been shown to correlate with increased fGM concentrations in multiple species – including Asian elephants (Laws et al. 2007). Fanson et al. (2013) also found significantly elevated fGM concentrations in Asian elephants up to 28 weeks after transfer and introduction to the herd. Additionally, Asian elephant 94122 was actively weaning Asian elephant B20189 during the 2018 observation period. Lactation has been correlated with significantly higher fGM concentrations in Asian elephants (POkharel 2017). Finally, Asian elephant B20189 died due to elephant endotheliotropic herpesvirus (EEHV) in November 2018. While a study by Boonprasert et al. (2021) noted significantly increased fGM concentrations in a calf that developed EEHV, further research is required to conclusively link elevated fGM concentrations and EEHV.

Contrary to the Asian elephants, the cheetahs had relatively few reported external stressors during the observational periods. The most notable were some changes in medications (discontinuation of Omeprazole for gastritis in August 2018) and the addition of a new shelter in June 2019. For giraffes, very few stressors were linked with changes in fGM concentrations during the observation period. Personality traits, however, may provide explanation for the significant difference in individual fGM concentrations in the giraffes. Giraffe B20186 had significantly higher fGM concentrations than giraffe B60180 during this study and has previously been described as skittish and nervous in relation to the installation of a feeding platform in July 2019 (keeper observations). a previous study on capuchin monkeys (*Sapajus libidinosus*) linked sociable and active individuals with less variable fGM concentrations (Ferreira et al. 2020). This link between fearfulness or shy individuals and higher fGM concentrations has also been shown in clouded leopards (*Neofelis nebulosa*, Wielebnowski et al. 2002), silver foxes (*Vulpes vulpes*, Hovland et al. 2017), and cheetahs (Baird 2018). It was possible that these personality differences may also have been present in the giraffes included in this study and caused changes in fGM concentrations; however, specific personality traits were not monitored, and additional research is required to concrete these links. It must be noted that both giraffes were members of different subspecies (Masai giraffe, *G. c. tippelskirchi* & reticulated giraffe, *G. c. reticulata*), which may have contributed to the variation in fGM concentrations.

Interestingly, when fGM concentrations were compared to behavior, sound data, and other descriptive information regardless of event type, distinct correlations were discovered. Specifically, the African painted dogs showed an inverse correlation between dB SPL levels and fGM concentrations. This was the opposite relationship than predicted. It was assumed that increasing average dB SPL levels would correlate with a positive increase in fGM concentrations. However, it was possible that the average dB SPL did not approach a stressful limit and therefore, did not correlate with a subsequent increase in fGM concentrations. This negative correlation was also present in Asian elephants. Additionally, it is important to reiterate that while correlations can be a useful way of describing data, they do not directly point to causation and require further analyses to conclusively link the dB SPL levels and fGM concentrations.

Additionally, fGM concentrations were also correlated with multiple changes in behavior rates. For instance, average fGM concentrations were positively correlated with increasing rates of visitor viewing area ("not visible") behaviors in cheetahs. It is possible that the cheetahs are removing themselves from areas of visibility to mitigate increased stress. Additionally, there was a negative correlation between fGM concentrations and "locomotion" rates for Asian elephants. This may have indicated the Asian elephants successfully used "locomotive" behaviors to mitigate increased fGM concentrations. There was also a significant positive correlation between "social (no contact)" and fGM concentrations, which may indicate that social interactions between Asian elephants correlated with an increased stress response. However, due to the design of the ethogram, positive and negative social interactions were recorded in the same category and additional observations would be required to indicate if the social interactions were positive or negative in nature.

Finally, there was also a significantly positive correlation between fGM concentrations and recorded temperature for Asian elephants. This result is contradictory to results presented by Dal Porto (2007) which showed no significant correlation between serum glucocorticoid levels and daily temperature in African elephants. However, as behavior was only monitored during a subset of the day, the correlations between behavior rates and fGM concentrations may not be accurate reflections of the relationship between behavior rates and fGM concentrations. Conducting behavior analyses during multiple parts of the day, rather than specific events, and correlating the overall average behavior rate with daily fGM concentrations would provide more conclusive evidence for a relationship between the two.

While none of our fGM analyses revealed significant differences in concentrations based on event types, they did highlight the individuality between species and within species. Of the 13 focal animals studied, only four individuals had the highest fGM concentrations within the control period (Giraffe B60180, Asian elephant B20189, and both cheetahs B70094 / B70095). This indicates a possibility that while large-scale after-hour events may not elicit a significant difference in fGM concentrations, the nevertheless somewhat increased fGM concentrations of nine focal animals that were correlated with both “concert” and “other” event types may indicate a somewhat increased arousal that might become significant if either the intensity, duration, or frequency of events increased.

Behavior

Our second hypothesis of increased vigilance or avoidance behaviors during after-hour events was only supported in two elephants and one cheetah. However, other interesting differences in behavior rate based on event type were noted within each species. Asian elephants 1963 and A80122, showed an increased rate of stereotypic behaviors during “other” and “concert” event types, respectively, and cheetah B70095 also showed significantly higher stereotypic behaviors during "concert" event types. While these increases are technically significant, they were very small differences since overall frequency of such behaviors was small and thus closely resembled "presence/absence" data. While the increased stereotypic behaviors are important to note, there may be causes beyond after-hours events that contribute to their increased prevalence. For instance, Asian elephant A80122 was housed solitarily in the *North Meadow* (the Asian elephant habitat located farthest from the concert stage) multiple times either when he was in musth or when his female relatives were in heat. When appropriate, Asian elephants 1963 and 99270 were housed with Asian elephant A80122 in the *North Meadow* to minimize solitary time. Solitary housing has been shown to increase stereotypic behaviors in Asian elephants due to their inability to engage in social behaviors (Greco et al. 2017). It must be reiterated that the increase in stereotypic expression was minimal, albeit significant, for Asian elephant A80122. In wild Asian elephant families, males disperse their familial herd between 10 and 15 years old (Vidya and Sukumar 2005) and the time A80122 spent in *North Meadow* may have mimicked the natural dispersal pattern of wild Asian elephants, resulting in only a minor increase in stereotypic behavior rate despite the increased time spent separated from the females in the herd. Directly monitoring the social compositions of the Asian elephant herd at the Oregon Zoo and their subsequent responses to stressors is a potential future project. Another potential cause of the increased stereotypic behavior rate in A80122 was active musth cycles. Male elephants in musth have been shown to exhibit more frequent locomotive stereotypies (Kurt and Garai

2001). Data collected by the Asian elephant care staff for a previous project (Glaeser 2020) indicated Asian elephant A80122 entered a period of musth in late-summer 2018 (musth was not tracked in the summer of 2019). This period of musth may have also contributed to the subsequent increase rate of stereotypic behavior.

Outside of behavior rates associated with the second hypothesis, the Asian elephants had multiple significant behavioral differences dependent on event type. All Asian elephants, except Asian elephant A80122, had significantly higher rates of “feeding/drinking” or “food-object interactions” during “concert” or “other” event types in comparison to “control” days. This may be due to differences in husbandry tactics rather than as a response to the event itself. As mentioned previously, Asian elephant A80122 was frequently housed in *North Meadow*, which is subject to different food-drop times. Food-troughs surrounded all three Asian elephant habitats and food is provided on a timed schedule to encourage walking and activity levels (Glaeser et al. 2021). Additionally, all Asian elephants except 1963 and A80122 showed increased “social (physical contact)” behaviors during both “other” and “concert” events. A previous study on the Oregon Zoo Asian elephants indicated that social, both physical and no contact, behaviors were exhibited while eating and foraging (Glaeser et al. 2021). This was fully supported by our data as we observed most social interactions when the Asian elephants were engaging with their feeders, either actively eating or simply interacting with the feeder. Finally, the significant increase in “solitary” behavior rates by Asian elephant 1963 was most likely due to her frequent housing in *North Meadow* with Asian elephant A80122 during after-hour events. In all, there were no reported shifts in behavior rates that were conclusively linked to event type in any of the Asian elephants. In fact, social make up and food availability timing had the strongest effect on changes in behavioral rates.

For cheetahs, a significant increase in rates of "stereotypy" expressed by cheetah B70095 during the "concert" event types did support the second hypothesis. However, this was a singular instance of locomotive stereotypy during one concert on August 26, 2018, and appeared to be an isolated event, possibly indicating a weak link between stereotypy and event type. While many behavioral studies have previously removed instances of rare behaviors, such as this example of stereotypy, integrating rare behaviors is important to understand the full behavioral representation (Cook et al. 2021). Outside of the increased rate of stereotypy associated with the second hypothesis, cheetah B70095 exhibited significantly lower rates of locomotion during "other" event types. Instead of locomoting, the cheetah was more likely to be stationary or "not visible". This may be due to the extended visitor presence associated with the "other" events. This reaction is similar to another study that showed increased rates of "not visible" behavior in cheetahs at the Oregon Zoo during period with visitor access (Fink et al. 2021, chapter 4, page X), however the data collection by Fink et al (2021) occurred after the conclusion of this study.

Unlike cheetahs and Asian elephants, African painted dogs did not exhibit any significant difference in behavior rates associated with the second hypothesis.

However, other interesting changes in behavior rates were observed. For instance, the significant increase in the rate of "play" behavior during "concert" event types was suggestive of increased arousal during these event types. However, the fact that the arousal manifested as "play" behaviors rather than antagonistic behaviors provided evidence that the arousal was associated with eustress (positive) rather than distress (negative). Vocalizations that accompany play behavior have previously been categorized as "high arousal" and indicated affiliative social situations (Robbins 2000). A previous study also reported an increase in social behaviors during and after the use of enrichment by African painted dogs (Packard, Turner, and Shepard 2010), which is similar to the results presented here. The "concert" event type may provide auditory enrichment for the African painted dogs, increasing their arousal and subsequent play bouts. Additionally, there were significantly higher rates of locomotion in African painted dog B70167 during "other" event types that appeared to be anticipatory in nature. African painted dog B70167 historically increases his locomotive rates in anticipation of indoor habitat access granted by the night-keeper. Anticipatory behaviors indicate excitement for a positive reward and can be considered positive in nature (Watters 2014). It is important to note that the behavioral observation of the African painted dogs only occurred over a single summer concert series due to the birth of a dozen pups in late 2018.

Finally, neither giraffe had many behavioral changes, and no changes supported the second hypothesis. Interestingly, while there were no significant differences in rates of “vigilance” behaviors between “concert” and “control” event types, giraffe B60180 had significantly lower rates of vigilance during the “other” event types. Like the other habitats, visitor presence at the giraffe exhibit was significantly extended during “other” event types compared to “control” and “concert” events. It has previously been reported that the giraffes at the Oregon Zoo exhibit higher rates of vigilance when there are fewer visitors (Fink et al. 2021). This may be due to the giraffe’s ability to single out a single person more conclusively during periods of low visitor attendance. Previous research has shown significantly increased rates of vigilance in ungulates with decreasing rates of human contact (Brown et al. 2012); conclusions which are supported by our data.

In summary, while there were some behavioral differences within each event type, they were more associated with unassociated stressors than the events. For instance, the Asian elephants appeared to react more strongly to social-group composition and feeding schedule than the event type. The only behaviors that appeared to be associated with event type were increased play behavior by the African painted dogs in “concert” events, lower rates of locomotion in cheetahs during “other” events, and decreased vigilance in giraffes during “other” events.

Sound

While the differences in fGM concentrations and behavior rates have been previously discussed based on event types, it is important to also analyze how the sound levels affect behavior independent of event type. As expected, the average dB levels were the highest during the “concert” event types, however there was considerable overlap in the average dB SPL in the other two event types in all habitats except *Forest Hall*. Understanding the relationship between dB SPL regardless of event type may reveal behavioral and physiological trends that would be otherwise masked by grouping the data as event type. Different intensities of music produce varying dB levels and different genres have been shown to affect animals in different ways. For instance, chimpanzees have been shown to have a trending increase in aggression when exposed to pop/rock genres (Wallace et al. 2017), whereas classical music statistically lowered anxious behaviors in shelter dogs (Bowman et al. 2015).

The increased dB SPL levels ungrouped by event type correlated with multiple behavioral and hormonal changes within each species. The African painted dogs had three behaviors that correlated with average dB SPL levels. There were direct correlations between “pacing”, “stationary”, and dB SPL levels and an indirect correlation between “not visible” and mean dB levels. The correlation between high dB SPL levels and increased instances of “pacing” may be of concern as pacing is a known stereotypy in African painted dogs (Shyne and Block 2010) and could indicate heightened arousal associated with increased noise levels. These correlations between heightened dB SPL levels and behavior rates were masked when the data were grouped by event type, reiterating the importance of analyzing the factors both within event categories and independently. Additionally, there was an inverse correlation between fGM concentrations and average dB SPL levels, which means the increased rate of “pacing” may have acted as an appropriate coping technique for any added stress the associated with increased dB SPL levels. According to Weschler (1995), coping is a behavioral reaction to an adverse situation that, otherwise, would have caused a physiological stress response. While it requires further testing, it appeared that the pacing behavior successfully acted as a coping mechanism to prevent an increase in fGM concentrations. However, if noise levels were permitted to increase in duration, frequency, or intensity, they may provide too much stimulation and pacing may no longer provide adequate mitigation and fGM concentrations may increase.

For cheetahs, there were significant inverse correlations between average dB SPL levels, temperature and "social interaction" behaviors and a significant direct correlation between average dB SPL levels and attendance. The inverse relationship between temperature and average dB SPL levels may be explained by the physics of sound. Most sound waves do not travel as far and are perceived as quieter during cooler temperatures due to the temperature gradient of the air fluctuating just above the ground (Rasmussen 1986). Interestingly, this pattern is not as clear with low-frequency tones. Low-frequency tones are amplified when the ground is cooler than the air; the soundwaves bounce back towards the earth by the higher temperatures in the air, causing little attenuation (Garstang 2004). Additionally, the average dB SPL levels were positively correlated with attendance, an unsurprising result. Interestingly, this pattern was not seen when analyzed within the African painted dog matrix (sound data was only analyzed in 2018), indicating a strong influence of sound data collected in 2019. The inverse correlation between "social interaction” behavior rates and noise levels requires further investigation. Very few studies have reported direct correlations between dB SPL levels and behavioral changes (Cronin et al. 2018; Quadros et al. 2014), and no studies have been linked average dB SPL levels and cheetah behavior. If increased noise levels are perceived as threatening, then the behaviors they trigger should be similar to anti-predator responses. As cheetahs are more skittish than other big cats, their anti-predator behaviors tend to be avoidance and vigilance based, rather than social behaviors (Caro 1987; Hunter, Durant, and Caro 2007). Therefore, the decrease in social interactions may indicate a perceived threat associated with higher mean dB SPL levels. As this significant decrease in “social interactions” was unsubstantial when analyzed by event type, it provides evidence that cheetahs may be more sensitive to the noise level in general and not the type of event. This is an important distinction as research typically centers on zoo-animal reactions to specific events rather than the factors associated within them and some animals may require mitigation even if an event is not occurring.

For Asian elephants, multiple behavioral changes, as well as fGM concentrations, were correlated with mean dB levels. Increasing mean dB SPL levels were significantly correlated with less “social (no contact)” and “food object interactions” but increased rates of “locomotion”. As previously mentioned, feeding schedules were variable to prevent anticipatory behaviors and increase locomotive behaviors (Glaeser et al. 2021), which may explain the decreased rate of “food object interaction” behaviors. The increased rate of locomotive behaviors with increasing mean dB SPL levels could have indicated agitation as explained in a previous study (Jakob-Hoff et al. 2019). This same study used location data to reveal that the Asian elephants were moving to quieter parts of their exhibits when exposed to continuous construction noise. Significant differences in average dB SPL levels were reported in three different places in the Asian elephant exhibit; however, as we did not record direction of movement it was difficult to ascertain if the focal animals involved in our study were actively moving away from the noise sources. In fact, anecdotal reports by care-staff and researchers reported the Asian elephants actively moving toward the noise source. Interestingly, increased dB SPL levels were shown to significantly correlate with decreased fGM concentrations. As increased fGM concentrations correlate with arousal in Asian elephants (Fanson et al. 2013), this decrease may indicate that increased mean dB SPL levels did not increase arousal enough to cause a subsequent increase in fGM levels.

Finally, giraffes showed no significant correlations between mean dB SPL levels and any behaviors. This does not support findings by Jakob-Hoff et al. (2019) who found increased rates of social behaviors in response to elevated noise. This may largely be due to the location of the giraffe habitat. The habitat was the farthest away from the concert stage and the stage orientation promotes soundwave propagation away from the giraffe habitat. In order to fully understand how the giraffes react to average dB SPL levels, sound origin must be closer to the habitat.

Conclusions

When analyzed within the confines of event type, average dB SPL levels insignificantly affected many aspects of the behavioral and physiological health of animals at the Oregon Zoo. However, when sound levels and other descriptive information was analyzed independently of event type, they revealed some important correlations that provide evidence of sensitivity to noise and visitor presence in some of the species monitored. Each event type combined different aspects of visitor presence: 1) “concert” event types linked high daily attendance, high average dB SPL levels, and limited visual visitor contact with all species except Asian elephants (visual visitor contact was present through all “concert” event types for Asian elephants); 2) “other” event types had extremely high visitor visual contact and slightly higher average dB SPL levels; and, 3) “control” event types had the lowest dB SPL levels and no visitor visibility. Only analyzing behavioral and physiological changes within specific event types may limit the usefulness of determining overall effects of visitor presence and mask important correlations that may otherwise be overshadowed.

In conclusion, there is little evidence that large, after-hour events have negatively impacted the adrenal activity of any of the study species at the Oregon Zoo. However, fGM concentrations were inversely correlated with noise levels in Asian elephants and African painted dogs and uncorrelated in giraffes and cheetahs providing evidence that noise level was insufficient in activating the adrenal stress response in any species. However, this may be due to an increase in coping behaviors. The presence of increased locomotive behaviors in Asian elephants, decreased social interactions in cheetahs, and increased pacing during more intense average dB SPL levels all may indicate increased arousal that is being addressed by changes in behavior rates. While there were relatively few significant differences in behavior rates dependent on event type, the significant correlations between average dB SPL, fGM concentrations and multiple behaviors may indicate potential harmful effects of factors associated with events (such as overall noise levels) that could become problematic if they are allowed to increase in duration, frequency, or intensity.

Future studies are required to solidify the correlation between average dB SPL levels, adrenal responses, and shifts in behavior rates. These studies should use different types of noise to determine if the style of noise influences the behavior and physiology of zoo animals. Additionally, research investigating the relationship between animal personality and visitor presence could be extended to determine how different personality types react to specific factors (visual and auditory) of visitor presence. Finally, this project should be conducted at other institutions and on other species prior to generalizing the results for animals in other institutions.

References

Baird, Bonnie A. 2018. “Ambassador Animal Welfare: Using Behavioral and Physiological Indicators to Assess the Well-Being of Animals Used for Education Programs in Zoos.” Case Western Reserve University.

Bashaw, Meredith J., Florian Sicks, Rupert Palme, Franz Schwarzenberger, Adrian S. W. Tordiffe, and Andre Ganswindt. 2016. “Non-Invasive Assessment of Adrenocortical Activity as a Measure of Stress in Giraffe (*Giraffa Camelopardalis*).” *BMC Veterinary Research* 12(1):235. doi: 10.1186/s12917-016-0864-8.

Bastian, Meredith L., David R. Glendinning, Janine L. Brown, Nicole P. Boisseau, and Katie L. Edwards. 2020. “Effects of a Recurring Late-night Event on the Behavior and Welfare of a Population of Zoo-housed Gorillas.” *Zoo Biology* 39(4):217–29. doi: 10.1002/zoo.21553.

Birke, Lynda. 2002. “Effects of Browse, Human Visitors and Noise on the Behaviour of Captive Orang Utans.” *Animal Welfare* 11(2):189–202.

Boonprasert, Khajohnpat, Yaoprapa Yun, Worapong Kosaruk, Patcharapa Towiboon, Pallop Tankaew, Veerasak Punyapornwithaya, Thittaya Janyamathakul, Panida Muanghong, Janine L. Brown, Chatchote Thitaram, and Chaleamchat Somgird. 2021. “A Longitudinal Study of Hematology and Stress Biomarker Profiles in Young Asian Elephants (Elephas Maximus) in Relation to Elephant Endotheliotropic Herpesvirus (Eehv) in Thailand.” *Animals* 11(9). doi: 10.3390/ani11092530.

Bowman, A., S. P. C. A. Scottish, F. J. Dowell, and N. P. Evans. 2015. “‘Four Seasons’ in an Animal Rescue Centre; Classical Music Reduces Environmental Stress in Kennelled Dogs.” *Physiology and Behavior* 143:70–82. doi: 10.1016/j.physbeh.2015.02.035.

Brooker, Jake S. 2016. “An Investigation of the Auditory Perception of Western Lowland Gorillas in an Enrichment Study.” *Zoo Biology* 35(5):398–408. doi: 10.1002/zoo.21312.

Brown, Casey L., Amanda R. Hardy, Jesse R. Barber, Kurt M. Fristrup, Kevin R. Crooks, and Lisa M. Angeloni. 2012. “The Effect of Human Activities and Their Associated Noise on Ungulate Behavior.” *PLoS ONE* 7(7). doi: 10.1371/journal.pone.0040505.

Bryant, Jocelyn, and Nadja C. Wielebnowski. 2018. “Environmental Impact on Activity Level and Fecal Glucocorticoid Metabolite Concentration of African Elephants and Black Rhinoceros at Brookfield Zoo.” *International International Journal of Avian & Wildlife Biology* 3(2):101–7. doi: 10.15406/ijawb.2018.03.00064.

Buxton, Rachel T., Reina Galban, Megan F. McKenna, Cecilia L. White, and Victoria Seher. 2017. "Visitor Noise at a Nesting Colony Alters the Behavior of a Coastal Seabird." *Marine Ecology Progress Series* 570:233–46. doi: 10.3354/meps12073.

Caro, T. M. 1987. "Cheetah Mothers' Vigilance: Looking out for Prey or for Predators?" *Behavioral Ecology and Sociobiology* 20(5):351–61. doi: 10.1007/BF00300681.

Cook, Chelsea N., Angela R. Freeman, James C. Liao, and Lisa A. Mangiamele. 2021. "The Philosophy of Outliers: Reintegrating Rare Events Into Biological Science." *Integrative and Comparative Biology* 1–15. doi: 10.1093/icb/icab166.

Creel, Scott. 2005. "Dominance, Aggression, and Glucocorticoid Levels in Social Carnivores." *Journal of Mammalogy* 86(2):255–64. doi: 10.1644/bhe-002.1.

Creel, Scott, Nancy Marusha Creel, Michael G. L. Mills, and Steven L. Monfort. 1997. "Rank and Reproduction in Cooperatively Breeding African Wild Dogs: Behavioral and Endocrine Correlates." *Behavioral Ecology* 8(3):298–306. doi: 10.1093/beheco/8.3.298.

Cronin, Katherine A., Emily J. Bethell, Sarah L. Jacobson, Crystal Egelkamp, and Lydia M. Hopper. 2018. "Evaluating Mood Changes in Response to Anthropogenic Noise with a Response-Slowing Task in Three Species of Zoo-Housed Primates." *Animal Behavior and Cognition* 5(2):209–21. doi: 10.26451/abc.05.02.03.2018.

Dal Porto, Dessa Marie. 2007. "Relationship of Airborne Particulate Matter and Daily Temperature to Serum Glucocorticoid Concentrations and Cyclicity Status in Zoo Elephants." Georgetown University.

Davey, Gareth. 2007. "Visitors' Effects on the Welfare of Animals in the Zoo: A Review." *Journal of Applied Animal Welfare Science* 10(2):169–83. doi: 10.1080/10888700701313595.

Davis, Nick, Colleen M. Schaffner, and Tessa E. Smith. 2005. "Evidence That Zoo Visitors Influence HPA Activity in Spider Monkeys (Ateles Geoffroyii Rufiventris)." *Applied Animal Behaviour Science* 90(2):131–41. doi: 10.1016/j.applanim.2004.08.020.

Edes, Ashley N., Eli Baskir, Karen L. Bauman, Nathasha Chandrasekharan, Michael Macek, and Anne Tieber. 2021. "Effects of Crowd Size, Composition, and Noise Level On Pool Use in a Mixed-Species Penguin Colony." *Animal Behavior and Cognition* 8(4):507–20. doi: 10.26451/abc.08.04.05.2021.

Edwards, Katie L., Michele A. Miller, Kathy Carlstead, and Janine L. Brown. 2019. "Relationships between Housing and Management Factors and Clinical Health Events in Elephants in North American Zoos." *PLoS ONE* 14(6):1–29. doi: 10.1371/journal.pone.0217774.

Fanning, Lara, Hannah Larsen, and Peta S. Taylor. 2020. "A Preliminary Study

Investigating the Impact of Musical Concerts on the Behavior of Captive Fiordland Penguins (*Eudyptes Pachyrhynchus*) and Collared Peccaries (*Pecari Tajacu*).” *Animals* 10(11):2035. doi: 10.3390/ani10112035.

Fanson, Kerry V., Michael Lynch, Larry Vogelnest, Gary Miller, and Tamara Keeley. 2013. “Response to Long-Distance Relocation in Asian Elephants (*Elephas Maximus*): Monitoring Adrenocortical Activity via Serum, Urine, and Feces.” *European Journal of Wildlife Research* 59(5):655–64. doi: 10.1007/s10344-013-0718-7.

Farrand, Alexandra. 2007. “The Effect of Zoo Visitors on the Behaviour and Welfare of Zoo Mammals.” University of Stirling.

Fazio, Jilian M., Elizabeth W. Freeman, Erika Bauer, Larry Rockwood, Janine L. Brown, Katharine Hope, Jessica Siegal-Willott, and E. C. M. Parsons. 2020. “Longitudinal Fecal Hormone Monitoring of Adrenocortical Function in Zoo Housed Fishing Cats (*Prionailurus Viverrinus*) during Institutional Transfers and Breeding Introductions” edited by G. Umapathy. *PLOS ONE* 15(3):e0230239. doi: 10.1371/journal.pone.0230239.

Ferreira, Vitor Hugo Bessa, Elanne De Paiva Fonseca, Ana Cecilia Correia Santos Das Chagas, Luiz Guilherme Mesquita Pinheiro, Maria Bernardete Cordeiro de Sousa, Hélderes Peregrino Alves da Silva, Nicole Leite Galvão-Coelho, and Renata Gonçalves Ferreira. 2020. “Personality Traits Modulate Stress Responses after Enclosure Change of Captive Capuchin Monkeys (Sapajus Libidinosus).” *Applied Animal Behaviour Science* 232(August). doi: 10.1016/j.applanim.2020.105111.

Fink, Laurel B., Candace D. Scarlata, Becca VanBeek, Todd E. Bodner, and Nadja C. Wielebnowski. 2021. “Applying Behavioral and Physiological Measures to Assess the Relative Impact of the Prolonged COVID-19 Pandemic Closure on Two Mammal Species at the Oregon Zoo: Cheetah (A. Jubatus) and Giraffe (G. c. Reticulata and G. c. Tippelskirchi).” *Animals* 11(12):3526. doi: 10.3390/ani11123526.

Frid, Alejandro, and Lawrence Dill. 2002. “Human-Caused Disturbance Stimuli as a Form of Predation Risk.” *Ecology and Society* 6(1). doi: 10.5751/es-00404-060111.

Garstang, Michael. 2004. “Long-Distance, Low-Frequency Elephant Communication.” *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 190(10):791–805. doi: 10.1007/s00359-004-0553-0.

Glaeser, Sharon. 2020. *Assessing Well-Being of Asian Elephants (Elephas Maximus) Through Major Events and Transitions, with Development and Aging, and Husbandry Changes*. Portland, OR.

Glaeser, Sharon S., David Shepherdson, Karen Lewis, Natalia Prado, Janine L. Brown, and Bob Lee. 2021. “Supporting Zoo Asian Elephant (Elephas Maximus)

Welfare and Herd Dynamics with a More Complex and Expanded Habitat." 1–44.

Greco, Brian J., Cheryl L. Meehan, Jennifer L. Heinsius, and Joy A. Mench. 2017. "Why Pace? The Influence of Social, Housing, Management, Life History, and Demographic Characteristics on Locomotor Stereotypy in Zoo Elephants." *Applied Animal Behaviour Science* 194:104–11. doi: 10.1016/j.applanim.2017.05.003.

Hanbury, David B., M. Babette Fontenot, Lauren E. Highfill, Willie Bingham, David Bunch, and Sheree L. Watson. 2009. "Efficacy of Auditory Enrichment in a Prosimian Primate (Otolemur Garnettii)." *Lab Animal* 38(4):122–25. doi: 10.1038/laban0409-122.

Heffner, Henry E., and Rickye S. Heffner. 2007. "Hearing Ranges of Laboratory Animals." *Journal of the American Association for Laboratory Animal Science* 46(1):20–22.

Heffner, Rickye S., and Henry E. Heffner. 1982. "Hearing in the Elephant (Elephas Maximus): Absolute Sensitivity, Frequency Discrimination, and Sound Localization." *Journal of Comparative and Physiological Psychology* 96(6):926–44.

Hovland, Anne Lene, Anne Marit S. Rød, Marit Skog Eriksen, Rupert Palme, Janicke Nordgreen, and Georgia J. Mason. 2017. "Faecal Cortisol Metabolites as an Indicator of Adrenocortical Activity in Farmed Silver Foxes (Vulpes Vulpes)." *Applied Animal Behaviour Science* 197:75–80. doi: 10.1016/j.applanim.2017.08.009.

Hunter, J. S., S. M. Durant, and T. M. Caro. 2007. "To Flee or Not to Flee: Predator Avoidance by Cheetahs at Kills." *Behavioral Ecology and Sociobiology* 61(7):1033–42. doi: 10.1007/s00265-006-0336-4.

Jackson, Laura L., Rickye S. Heffner, and Henry E. Heffner. 1999. "Free-Field Audiogram of the Japanese Macaque (Macaca Fuscata)." *The Journal of the Acoustical Society of America* 106(5):3017–23. doi: 10.1121/1.428121.

Jakob-Hoff, Kingan, Fenemore, Schmid, Cockrem, Crackle, Bemmel, Connor, and Descovich. 2019. "Potential Impact of Construction Noise on Selected Zoo Animals." *Animals* 9(504):1–25. doi: 10.3390/ani9080504.

Kelling, Angela S., Stephanie M. Allard, Nicholas J. Kelling, Estelle A. Sandhaus, and Terry L. Maple. 2012. "Lion, Ungulate, and Visitor Reactions to Playbacks of Lion Roars at Zoo Atlanta." *Journal of Applied Animal Welfare Science* 15(4):313–28. doi: 10.1080/10888705.2012.709116.

Kumar, Vinod, Vivekananda Palugulla Reddy, Adiseshu Kokkiligadda, Sisinthy Shivaji, and Govindhaswamy Umapathy. 2014. "Non-Invasive Assessment of Reproductive Status and Stress in Captive Asian Elephants in Three South

Indian Zoos." *General and Comparative Endocrinology* 201:37–44. doi: 10.1016/j.ygcen.2014.03.024.

Kurt, Fred, and Marion Garai. 2001. "Stereotypies in Captive Asian Elephants-A Symptom of Social Isolation." Pp. 57–63 in *Scientific Progress Reports*. Vienna.

Larsen, Megan J., Sally L. Sherwen, and Jean Loup Rault. 2014. "Number of Nearby Visitors and Noise Level Affect Vigilance in Captive Koalas." *Applied Animal Behaviour Science* 154:76–82. doi: 10.1016/j.applanim.2014.02.005.

Laws, Nicole, Andre Ganswindt, Michael Heistermann, Moira Harris, Stephen Harris, and Chris Sherwin. 2007. "A Case Study: Fecal Corticosteroid and Behavior as Indicators of Welfare during Relocation of an Asian Elephant." *Journal of Applied Animal Welfare Science* 10(4):349–58. doi: 10.1080/10888700701555600.

Lay Jr., D. C. 2000. "Consequences of Stress During Development." Pp. 243–68 in *The Biology of Animal Stress: Basic Prinicples and Implications for Animal Welfare*, edited by G. P. Moberg and J. A. Mench. CABI Publishing.

Loeding, Erin, Jeanette Thomas, Dave Bernier, and Rachel Santymire. 2011. "Using Fecal Hormonal and Behavioral Analyses to Evaluate the Introduction of Two Sable Antelope at Lincoln Park Zoo." *Journal of Applied Animal Welfare Science* 14(3):220–46. doi: 10.1080/10888705.2011.576968.

Ludwig, C., B. Wachter, S. Silinski-Mehr, A. Ganswindt, H. Bertschinger, H. Hofer, and M. Dehnhard. 2013. "Characterisation and Validation of an Enzyme-Immunoassay for the Non-Invasive Assessment of Faecal Glucocorticoid Metabolites in Cheetahs (Acinonyx Jubatus)." *General and Comparative Endocrinology* 180(1):15–23. doi: 10.1016/j.ygcen.2012.10.005.

Meade, J., I. Formella, and V. Melfi. 2017. "A Note on the Effect of Concerts on the Behaviour of Domestic Dogs Canis Lupus Familiaris at Taronga Zoo, Sydney." *International Zoo Yearbook* 51(1):225–31. doi: 10.1111/izy.12141.

Monfort, S. L., K. L. Mashburn, B. A. Brewer, and S. R. Creel. 1998. *Evaluating Adrenal Activity in African Wild Dogs (Lycaon Pictus) by Fecal Corticosteroid*. Vol. 29.

Morgan, Kathleen N., and Chris T. Tromborg. 2007. "Sources of Stress in Captivity." *Applied Animal Behaviour Science* 102(3–4):262–302. doi: 10.1016/j.applanim.2006.05.032.

Ogden, Jacqueline J., Donald G. Lindburg, and Terry L. Maple. 1994. "A Preliminary Study of the Effects of Ecologically Relevant Sounds on the Behaviour of Captive Lowland Gorillas." *Applied Animal Behaviour Science* 39(2):163–76. doi: 10.1016/0168-1591(94)90136-8.

Orban, David A., Joseph Soltis, Lori Perkins, and Jill D. Mellen. 2017. "Sound at the Zoo: Using Animal Monitoring, Sound Measurement, and Noise Reduction in Zoo Animal Management." *Zoo Biology* 36(3):231–36. doi: 10.1002/zoo.21366.

Packard, Jane M., Stephanie J. Turner, and Susan Shepard. 2010. "Behavioral Enrichment for African Wild Dogs (Lycaon Pictus): Response to Stimuli Used in an Ongoing Program at the Houston Zoo." *Biodiversity Stewardship Report No. BS10-2 Biodiversity Stewardship Lab, TAMUS, College Station, Texas 2258.* 1–9.

Palme, Rupert. 2005. "Measuring Fecal Steroids: Guidelines for Practical Application." *Annals of the New York Academy of Sciences* 1046:75–80.

Palme, Rupert. 2019. "Non-Invasive Measurement of Glucocorticoids: Advances and Problems." *Physiology and Behavior* 199(November 2018):229–43. doi: 10.1016/j.physbeh.2018.11.021.

Pelletier, Catherine, Robert B. Weladji, Louis Lazure, and Patrick Paré. 2020. "Zoo Soundscape: Daily Variation of Low-to-High-Frequency Sounds." *Zoo Biology* 39(6):374–81. doi: 10.1002/zoo.21560.

St. Pierre Jr., Richard L., and Daniel J. Maguire. 2004. "The Impact of A-Weighting Sound Pressure Level Measurements during the Evaluation of Noise Exposure." Pp. 702–8 in *NOISE-CON - The National Conference on Noise Control Engineering*. Baltimore, Maryland.

Piitulainen, Roosa. 2020. "Designing Auditory Enrichment for White-Faced Saki Monkeys." Aalto University.

POkharel, Sanjeeta Sharma. 2017. "Stress Physiology of Free-Ranging Asian Elephants (Elephas Maximas): Influence of Ecological and Anthropogenic Stressors." Indian Institute of Science.

Pons, Patricia, Marcus Carter, and Javier Jaen. 2016. "Sound to Your Objects." Pp. 1–5 in *Proceedings of the Third International Conference on Animal-Computer Interaction*. Vols. 15-17-Nove. New York, NY, USA: ACM.

Powell, David M., Kathy Carlstead, Loraine R. Tarou, Janine L. Brown, and Steven L. Monfort. 2006. "Effects of Construction Noise on Behavior and Cortisol Levels in a Pair of Captive Giant Pandas (Ailuropoda Melanoleuca)." *Zoo Biology* 25(5):391–408. doi: 10.1002/zoo.20098.

Quadros, Sandra, Vinicius D. L. Goulart, Luiza Passos, Marco A. M. Vecci, and Robert J. Young. 2014. "Zoo Visitor Effect on Mammal Behaviour: Does Noise Matter?" *Applied Animal Behaviour Science* 156:78–84. doi: 10.1016/j.applanim.2014.04.002.

Queiroz, Marina B., and Robert J. Young. 2018. "The Different Physical and Behavioural Characteristics of Zoo Mammals That Influence Their Response to Visitors." *Animals* 8(8). doi: 10.3390/ani8080139.

de Queiroz, Marina Bonde. 2018. "How Does the Zoo Soundscape Affect the Zoo Experience for Animals and Visitors?" University of Salford.

R Core Team. 2020. "R: A Language and Environment for Statistical Computing."

Rafacz, Michelle L., Matthew R. Heintz, and Rachel M. Santymire. 2016. "Hormonal and Behavioral Responses to Odor Cues in Zoo-Housed African Painted Dogs (*Lycaon Pictus*)." Pp. 391–99 in *Chemical Signals in Vertebrates 13*, edited by B. A. Schulte, T. E. Goodwin, and M. H. Ferkin. Cham: Springer International Publishing.

Rafacz, Michelle L., and Rachel M. Santymire. 2014. "Using Odor Cues to Elicit a Behavioral and Hormonal Response in Zoo-Housed African Wild Dogs." *Zoo Biology* 33(2):144–49. doi: 10.1002/zoo.21107.

Rajagopal, Thangavel, Govindaraju Archunan, and Mahadevan Sekar. 2011. "Impact of Zoo Visitors on the Fecal Cortisol Levels and Behavior of an Endangered Species: Indian Blackbuck (Antelope Cervicapra L.)." *Journal of Applied Animal Welfare Science* 14(1):18–32. doi: 10.1080/10888705.2011.527598.

Rasmussen, K. B. 1986. "Outdoor Sound Propagation under the Influence of Wind and Temperature Gradients." *Journal of Sound and Vibration* 104(2):321–35. doi: 10.1016/0022-460X(86)90271-3.

Razal, Catherine, Jocelyn Bryant, and Lance Miller. 2017. "Monitoring the Behavioral and Adrenal Activity of Giraffe (*Giraffa Camelopardalis*) to Assess Welfare during Seasonal Housing Changes." *Animal Behavior and Cognition* 4(2):154–64. doi: 10.12966/abc.03.05.2017.

Robbins, Lindsey, and Susan W. Margulis. 2014. "The Effects of Auditory Enrichment on Gorillas." *Zoo Biology* 33(3):197–203. doi: 10.1002/zoo.21127.

Robbins, Robert L. 2000. "Vocal Communication in Free-Ranging African Wild Dogs (Lycaon Pictus)." *Behaviour* 137(10):1271–98. doi: 10.1163/156853900501926.

Rose, Paul, and Lisa M. Riley. 2019. "The Use of Qualitative Behavioural Assessment in Zoo Welfare Measurement and Animal Husbandry Change Enclosure Design and Husbandry Influence Breeding Success in Flamingos View Project Repetitive Behaviour-Zoo Animals View Project SEE PROFILE." *Journal of Zoo and Aquarium Research* 7(4):150–61.

Selye, Hans. 1976. "Forty Years of Stress Research: Principal Remaining Problems and Misconceptions." *Canadian Medical Association Journal* 115(1):53–56.

Sheriff, Michael J., Ben Dantzer, Brendan Delehanty, Rupert Palme, and Rudy Boonstra. 2011. "Measuring Stress in Wildlife: Techniques for Quantifying Glucocorticoids." *Oecologia* 166(4):869–87. doi: 10.1007/s00442-011-1943-y.

Shyne, Amanda, and Martin Block. 2010. "The Effects of Husbandry Training on Stereotypic Pacing in Captive African Wild Dogs (Lycaon Pictus)." *Journal of Applied Animal Welfare Science* 13(1):56–65. doi: 10.1080/10888700903372069.

Tarou, Loraine Rybiski, Jessamine Williams, David M. Powell, Raquel Tabet, and

Mary Allen. 2005. “Behavioral Preferences for Bamboo in a Pair of Captive Giant Pandas (Ailuropoda Melanoleuca).” *Zoo Biology* 24(2):177–83. doi: 10.1002/zoo.20038.

Uetake, Katsuji, Yumi Une, Shu Ito, Marino Yamabe, Hideto Toyoda, and Toshio Tanaka. 2014. “Relationship of Climatic Conditions to Fecal Corticosterone Levels of Captive Cheetahs Reared in Japan.” *Animal Science Journal* 85(10):915–18. doi: 10.1111/asj.12226.

Venables, W. N., and B. D. Ripley. 2002. *Modern Applied Statistics with S.* Fourth. New York: Springer.

Vidya, T. N. C., and Raman Sukumar. 2005. “Social and Reproductive Behaviour in Elephants.” *Current Science* 89(7):1200–1207.

Wallace, Emma K., Drew Altschul, Karoline Korfer, Benjamin Benti, Amanda Kaeser, Susan Lambeth, Bridget M. Waller, and Katie E. Slocombe. 2017. “Is Music Enriching for Group-Housed Captive Chimpanzees (Pan Troglodytes)?” *PLoS ONE* 12(3):1–27. doi: 10.1371/journal.pone.0172672.

Wark, Jason D. 2015. “The Influence of the Sound Environment on the Welfare of Zoo-Housed Callitrichine Monkeys.” Case Western Reserve University.

Watson, Rebecca, Coralie Munro, Katie L. Edwards, Vicki Norton, Janine L. Brown, and Susan L. Walker. 2013. “Development of a Versatile Enzyme Immunoassay for Non-Invasive Assessment of Glucocorticoid Metabolites in a Diversity of Taxonomic Species.” *General and Comparative Endocrinology* 186:16–24. doi: 10.1016/j.ygcen.2013.02.001.

Watters, Jason V. 2014. “Searching for Behavioral Indicators of Welfare in Zoos: Uncovering Anticipatory Behavior.” *Zoo Biology* 33(4):251–56. doi: 10.1002/zoo.21144.

Weschler, Beat. 1995. “Coping and Coping Strategies: A Behavioural View.” *Applied Animal Behaviour Science* 43:123–34.

Van Der Weyde, Leanne K. 2013. “Reproductive Biology of the Endangered African Wild Dog (Lycaon Pictus) in Captive and Free-Ranging Populations: An Endocrine, Behavioural and Demographic Approach.” University of Western Australia.

Wickham, Hadley. 2016. *Ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.

Wielebnowski, Nadja C., Karen Ziegler, David E. Wildt, John Lukas, and Janine L. Brown. 2002. “Impact of Social Management on Reproductive, Adrenal and Behavioural Activity in the Cheetah (Acinonyx Jubatus).” *Animal Conservation* 5(4):291–301. doi: 10.1017/S1367943002004043.

Wielebnowski, Nadja, and Jason Watters. 2007. “Applying Fecal Endocrine

Monitoring to Conservation and Behavior Studies of Wild Mammals: Important Considerations and Preliminary Tests." *Israel Journal of Ecology & Evolution* 53(3–4):439–60. doi: 10.1560/IJEE.53.3.439.

Woolway, Eleanor E., and Anne E. Goodenough. 2017. "Effects of Visitor Numbers on Captive European Red Squirrels (Sciurus Vulgaris) and Impacts on Visitor Experience." *Zoo Biology* 36(2):112–19. doi: 10.1002/zoo.21357.

Chapter Three: Understanding Sound Preference Based on Behavioral and Physiological Changes of Two Species of Lemur (*Varecia rubra* & *Lemur catta*) – a Pilot Study

Introduction

With approximately 800,000 animals in Association of Zoos and Aquariums (AZA) accredited zoos (Marcy 2021), there is a great need for positively enriching stimuli to maximize animal welfare. Enrichment (or environmental enrichment) is a medley of processes that aim to provide the optimum environment for a captive animal's psychological and physiological well-being (Shepherdson 1998). While the overall goals of enrichment have remained constant, there are two main pathways to achieving positive welfare benefits: a naturalistic approach and behavioral engineering approach (for a thorough review see Young 2003). Naturalistic approaches to enrichment focus on recreating various aspects of the wild environment to promote natural behaviors specific to the species within the exhibit, while behavioral engineering aims to increase stimulation by providing tools and technology that animals can manipulate for a reward (Young 2003). For instance, under the naturalistic approach, enrichment was used to mitigate and mask the plethora of unnatural stimuli found in a zoo environment, while behavioral engineering made use of unnatural stimuli to create an engaging environment for an animal. In 1984, a narrative by Forthman Quick highlighted the importance and potential of integrating both approaches to increase the overall benefit of enrichment (Forthman Quick 1984).

Many animals in captivity are exposed to significantly different environments than their wild counterparts. Zoos, in particular, thrive on housing exotic species in all types of environments. Meaning, zoo animals are subject to olfactory, visual, and auditory stimuli that can be both unspecific to their native habitats and / or anthropogenically sourced. These stimuli can either be intentional (e.g., purposefully placed enrichment items) or passive (e.g., ambient noise). Gaining a better understanding of how these various stimuli may affect the animals housed in zoos is imperative to maximizing positive animal welfare.

In 2011, a complex review by Kight and Swaddle (2011) highlighted the potential dangers of unnatural or intense environmental noise on animals. They concluded that unmitigated environmental noise could alter neural, developmental, immunological, and physiological functions, ultimately negatively affecting the overall welfare of animals (Kight and Swaddle 2011b). Typical zoo auditory environments often include mechanical noises from exhibits, visitor / staff communication, radios, and automobile traffic. The evaluation of the impact of non-natural noises (such as construction) on animals has been correlated with an increase in fecal glucocorticoid metabolite (fGM) concentrations (Chosy, Wilson, and Santymire 2014), an increase in detrimental behaviors (Owen et al. 2004), and a decrease in reproductive efficacy (Rasmussen et al. 2009). Specifically in the zoo environment increased visitor noise has been shown to increase vigilant behavior (Larsen et al. 2014) and negatively correlate with viewing potential (Woolway and Goodenough 2017).

Recently, zoo researchers have begun to evaluate the effects of altering the sound environment in a way that positively influences animal welfare. Multiple responses to acoustic enrichment have been observed. Positive responses include benefits from masking unnatural / mechanical noises (Ogden et al. 1994), increasing curious or affiliative behaviors (Snowdon and Teie 2010; Snowdon, Teie, and Savage 2015), and decreasing abnormal behaviors (Wells and Irwin 2008). Negative responses include increased aggression (McCraty et al. 1996), increased vigilance and use of off-exhibit areas when exposed to waterfall white-noise (Wark 2015), and potential fear responses (Wells, Coleman, and Challis 2006). These varying responses indicate the importance of understanding species specific and individual specific preferences with regards to auditory enrichments.

Most auditory enrichment studies highlight the importance of determining which sound type is most beneficial to each species. The main types of auditory enrichments are white noise, human-centric music, habitat-based nature sounds, and even "music" specifically composed using species-specific qualifications. White noise has typically been used to mask potentially stressful ambient sounds (e.g., visitor conversations or construction) and has been both positively (Carlson et al. 1997) and negatively (Wark 2015) shown to influence behavior. Human-centric music is one of the most common types of passive auditory enrichment. Many animal care staff play music when conducting routine keeping duties (anecdotal observations) and many zoos play music through speaker systems in various locations and at various times around zoo grounds to elevate the zoo-experience for humans. However, the style of music played may alter zoo animal behavior. Chimpanzees (*Pan troglodytes*), for instance, showed increased activity levels (a behavioral indicator of potential agitation) when exposed to high-tempo music but reduced agitation when exposed to general radio (Wallace et al. 2017). Similarly, many studies have highlighted the calming effects of classical music on kenneled dogs (*Canine lupus familiaris*) (Bowman et al. 2015; Kogan, Schoenfeld-Tacher, and Simon 2012). However, Hanbury et al. (2009) found that stereotypic behaviors were unchanged when Garnett's bushbabies (*Otolemur garnettii*) were exposed to classical music. Interestingly, responses to habitat-based nature sounds have been extremely variable. For instance, gorillas (*Gorilla gorilla gorilla*) showed a correlation between natural sounds and decreased stereotypy levels compared to background noise levels (Robbins and Susan W Margulis 2014), but nature sounds correlated with a decrease in calm vocalizations in parrots (*Amazona oratrix, Psittacus erithacus, Anodorhynchus hyacinthinus, Ara macao, Ara militaris,* and *Ara ararauna*) (Williams, Hoppitt, and Grant 2017). More recently, focus has shifted to tailored auditory enrichment that mimics natural vocalizations to elicit a specific behavior response. For instance, curating vocalization playbacks that include reassuring affiliative vocalization patterns to stimulate calm behaviors. In 2010, Snowdon and Teie developed music specific for tamarins (*Saguinus oedipus*) that included both fear/threat music and affiliation-based music. Playback of the fear/threat music correlated with increased movement and anxious social behaviors in the time period immediately following the music exposure and affiliation-based music caused decreased movement and increased overall calming behaviors (Snowdon and Teie 2010). This preference for species-specific sound curation has been seen in different species (*Macaca mulatta* - Graves 2011; *Felis catus* - Snowdon et al. 2015); however, variable responses by individual focal animals for all types of sound manipulation indicated the need for further investigation into appropriate sound manipulation prior to full implementation as enrichment.

This pilot study determined if auditory enrichment had any effect on the behavior and fecal glucocorticoid metabolite (fGM) concentrations of red-ruffed lemurs (*Varecia rubra*) and ring-tailed lemurs (*Lemur catta*) housed in a multi-species exhibit at the Oregon Zoo. Auditory stimuli included broadcasted human speech, ambient rainforest noises, and red-ruffed and ring-tailed lemur callbacks. Due to the specificity of some of the auditory enrichment, my prediction was that an increase in exploratory behaviors (locomotion and environmental interaction) and vocalizations would occur during the lemur-specific callbacks but little to no difference in behavior rate would happen during the other auditory enrichment sound types. Additionally, I hypothesized that an increase in fGM concentrations may occur as a response to the lemur-specific callbacks due to increased arousal. As increased fGM concentrations can occur following both positive (eustress) and negative (distress) arousal, behavioral analyses were also used to determine if the lemur-specific callbacks were sources of eustress or distress.

Methods

Study Animals

The multi-species lemur exhibit at the Oregon Zoo is located within the *Predators of the Serengeti* zone (see map in Appendix A). The exhibit houses two female red-ruffed lemurs, “B80159” and “B80158” (4 years old) and three female ring-tailed lemurs, “B80084” (12 years), “B80085” (6 years), and “B80086” (5 years). None of the lemurs were born at the Oregon Zoo. Both red-ruffed lemurs were born at the Nashville Zoo. Ring-tailed lemur B80084 was born at the Indianapolis Zoo, and both lemur B80085 and B80086 were born at the Lincoln Children’s Zoo. All ring-tailed lemurs were moved to the Oregon Zoo in June 2018, while the red-ruffed lemurs were moved in October 2018. The exhibit is an open-air design with four visitor viewing possibilities: two with glass barriers and two with mesh barriers (Figure 25). In addition, the lemurs also have an indoor, off-exhibit area where animal care staff can interact with individuals for enrichment and training purposes. The lemurs have access to this area during times of inclement weather and overnight. Across from the lemur exhibit are three other animal habitats housing African lions (*Panthera leo*), cheetahs (*Acinonyx jubatus*), and African painted dogs (*Lycaon pictus*). Both species of lemur are cared for based on husbandry recommendations by the Association of Zoos and Aquariums (AZA).

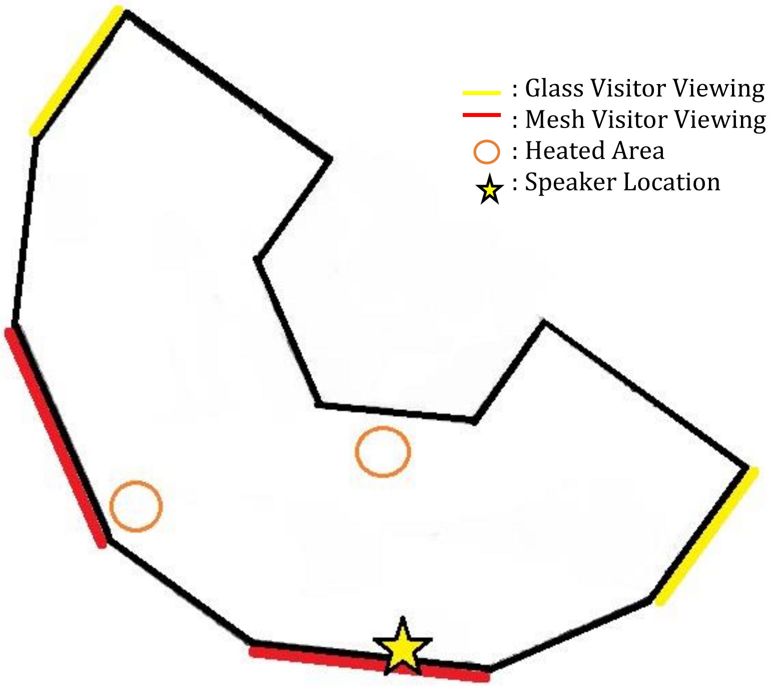


Figure 25: Lemur exhibit map

Endocrinology

Fecal samples were collected approximately 3-times per week in the four weeks prior to the start of the audio testing period. This helped to establish a baseline fecal glucocorticoid metabolite (fGM) measurement for comparison with samples obtained during the auditory manipulation period. Throughout the testing period and the month following, fecal samples were collected daily (Table 18). Consumption-safe food dye (AmeriColor Soft Gel Paste Food Color) was used to differentiate the ring-tailed lemur samples by individual. However, for red-ruffed lemurs, fecal samples were pooled for analyses due to the problematic logistics of discerning the feces of these two individuals from one another. Additionally, multiple red-ruffed lemur samples from the lemur callback period were excluded from analysis due to contamination based on storage equipment failure.

Table 18: Fecal Sample Collection Schedule

Collection type	**Date Range**	**Frequency**
Pre-baseline	9/20/2020 – 10/20/2020	3-4 x per week
Testing period	10/20/2020 – 11/30/2020	Daily
Post-baseline	11/30/2020 – 1/9/2020	Daily

Fecal Metabolite Extraction

Two different fecal glucocorticoid metabolite (fGM) extraction methods were used. Just like Starling et al. (2010), prior comparisons (Appendix C) showed successful fGM corticosterone parallelisms in fecal samples for ring-tailed lemurs and red-ruffed lemurs. To maximize the extraction success for the red-ruffed lemurs and account for the inconsistent water content within fecal samples, methodology similar to Starling et al. (2010) was used. Briefly, fecal samples were dried in an oven at 60°C overnight, crushed into a fine powder, and sifted through a sieve to separate out non-fecal matter. 0.2 grams (± 0.05g) of dried fecal matter was measured and combined with 5.0mL of 80% ethanol. Samples were then vortexed and shaken overnight (17 hours) at 50rpms. Additionally, a strong social dispute between the ring-tailed lemurs was used as a biological validation and showed significantly elevated (> 2 standard deviations above baseline levels) fGM concentrations three days after the altercation (Appendix D).

The ring-tailed lemur fecal steroid metabolite extraction method mirrors the red ruffed lemurs' extraction method except for the fecal condition. Unlike red-ruffed lemur fecal samples, ring-tailed lemurs had consistent fecal water content, allowing for wet fecal analysis. Therefore, the ring-tailed lemur feces did not have to be dried prior to extraction and 0.5g (± 0.05g) of homogenized wet fecal samples were combined with 5mL 80% ethanol. Individual samples were differentiated based on food-safe dye added to individual diets and the subsequent excretion of the dye in feces.

Enzyme Immunoassay (EIA) Analysis

A double-antibody corticosterone enzyme immunoassay (EIA) was used for the quantification of fGM concentrations. This EIA assay used a polyclonal antibody produced against corticosterone-3-CMO-BSA (CJM006: 1:100,000; C. Munro, University of California, Davis), a horseradish-peroxidase conjugated corticosterone label (1:100,000), and corticosterone standards (0.078-20.0 ng/mL; Arbor Assay). Multiple 96-well microtiter plates were pre-coated with secondary goat anti-rabbit IgG antibody (150 µl / well at 0.10 mg/mL, A009, Arbor Assays, Ann Arbor, MI) using a process developed by Arbor Assays (ISWE Plate Coating Protocol). The sensitivity of the assay was 0.100 ng/mL. The inter-and intra-assay coefficients of variation (CVs) were below 15% and 10% respectively. Corticosterone antibody cross-reactivities were as follows: corticosterone – 100%, desoxycorticosterone - 14.25%, progesterone – 2.65%, tetrahydrocorticosterone – 0.90%, testosterone – 0.64%, cortisol – 0.23% and <10% for 5 other steroids tested (C.Munro).

Behavior

Ethogram

Ethograms were developed based on Shire (2012) and preliminary observations (Table 19). Both affiliative and agonistic behaviors were included and used as indicators of welfare, following similar protocols for other primate species

(Barlow, Caldwell, and Lee 2006; Browning-Jones and Moro 2006; Goodenough et al. 2019; Rich and Romero 2005; Shire 2012; Wark 2015).

Table 19: Lemur behavioral ethogram – adapted from Shire (2012) and preliminary observations.

Interval Behaviors

Behavior	**Modifiers**	**Description**
Not Visible	NA	Individual is out of sight enough to not allow for distinguishing behaviors.
Keeper Present	NA	Keeper is present. Actively interacting with animals or just walking past. Still record location for individual.
Environmental Interaction	NA	Individual is engaged with the environment. This includes foraging (does not include actual food consumption) and interaction with any permanent features in the exhibit.
Feeding / Drinking	NA	Actively consuming food or water.
Enrichment Interaction	NA	Individual is interacting with non-permanent aspects of the environment such as toys, food enrichment, blankets, etc.
Stationary	Sit	Individual has not moved for longer than 20 seconds and is in a seated position (head may be down or raised)
	Stand / Crouch	Individual has not moved for longer than 20 seconds and is still in standing or crouched position (head may be moving and ears twitching but no locomotive activity)
	Rest	Individual has not moved for longer than 20 seconds and is lying prone.
Locomotion	Climb	Individual is actively climbing ropes, cliffs, or vegetation within the exhibit. IF the animal is climbing on a non-permanent enrichment item, it is NOT locomotion and IS enrichment item interaction.
	Walk / Run	Individual is actively moving along the flat ground
Self-Groom	NA	Individual is actively engaged with grooming themselves. Includes grooming with hands, feet, or mouth. If individual is using the environment to groom (or scratch) themselves, it is NOT Self-Groom and IS Environmental Interaction.
Groom - Other	B80084	Individual is actively engaged with grooming another lemur. Receiving lemur does NOT have to reciprocate. If receiving lemur does NOT reciprocate, they will be categorized as Positive Social Interaction.
	B80085	
	B80086	
	Red-Ruffed	
Social Interaction	Positive	Individual is engaged with a positive or neutral social interaction that does not fall under previously described behaviors. E.g., individuals being groomed or engaging in play
	Negative	Individual is actively engaged with a negative social interaction. Includes fighting, posturing, etc.

Stereotypy	Locomotive	Pacing the same pathway (or few steps) repeatedly without purpose.
	Non-Locomotive	Overgrooming, self-harm, or tremors.
Huddle	Fear	Individuals are huddled together as a response to a stimulus
	Other	Individuals clustered together for non-fear related reasons (e.g., cold)
All Occurrence Behaviors		
Vocalization - Red Ruffed	NA	All occurrences at the START of a vocalization bout from the red-ruffed lemurs. New vocalizations occur after silence for 20 seconds.
Vocalization – Ring-tailed	NA	All occurrences at the START of a vocalization bout from the ring-tailed lemurs. New vocalizations occur after silence for 20 seconds.
Bite	NA	Individuals use their teeth against another in an aggressive manner
Cuff	NA	Individual hits another using their hands in an aggressive manner - does not include play behavior
Scentmark	NA	Individual uses glands (under tail, arms, or wrist) to mark surfaces
Yawn	NA	Individual stretches mouth

Observations

Scan sampling was conducted at 1-minute intervals for 20-minute observation periods before, during, and after the audio manipulation period. The study was conducted four times per week (randomized to prevent anticipatory behaviors) between 8:00am and 9:30am. Behavior observed and the corresponding exhibit location at each 1-minute interval were recorded using the ZooMonitor app (Ross et al. 2016). In addition to scan sampling, every observed instance of other rare, yet possibly important behaviors (vocalizations, bite, scentmark, cuff, yawn, and displacement) were recorded.

Audio Manipulation

Four different audio manipulations were conducted in the lemur habitat (Table 20). Sounds were broadcast from a Bluetooth speaker (FX100, Treblab) attached to the mesh enclosure (Figure 26). Audio manipulation followed the following pattern: silence (20 minutes), audio manipulation (20 minutes), silence (20 minutes) and occurred once per observation day. A random-number generator was used to randomly select when observations occurred within the week. To avoid influence of visitor presence, all observations began between 8:00am and 8:30am. The ring-tailed lemurs and red-ruffed lemurs were released into their exhibit less than ten minutes prior to the start of the observations. This was done to minimize the exposure of the lemurs to cold days based on welfare recommendations. Additionally, care staff was instructed to remain off-exhibit during the monitoring period. If a keeper was present, “keeper present” was recorded and that observation was removed from the overall analysis. Only one audio modification was used per-week to observe potential habituation patterns.

Table 20: Audio Descriptions

Sound Condition	**Description**
No Audio	No broadcasted audio. Individuals are subjected to standard day-to-day noise
Lemur-Specific Audio Playback	A compilation of vocalizations from red-ruffed lemurs and ring-tailed lemurs, including fear and territorial calls. Provided by Chris Mercer, University of California, Santa Cruz.
Rainforest Noises	Spotify playlist: “”*<-=Mammals=->*””, shuffled.
Spoken Word	National Public Radio (NPR) Radio Lab Podcast

Data Analysis

All statistical analyses were conducted in SPSS (version 27 – IBM). Wilcoxon signed-rank tests were conducted on the percentage of total time spent exhibiting individual behaviors (“behavior rate”) per individual ring-tailed lemur. Heat maps were compared to observe differences in habitat use based on differing soundscapes. Prior to analysis, a Shapiro-Wilk’s test revealed a non-normal distribution ($p < 0.05$) of fGM concentrations in both individual ring-tailed lemurs and group red-ruffed lemur samples. Data were log-10 transformed and the results then followed a normal distribution (Shapiro-Wilk's test: $p > 0.05$). A repeated measures linear mixed effect model (LMM) was used to analyze the effects of sound type and focal animal on fGM concentrations. Sound type, focal animal, and their interaction were analyzed as fixed effects.

Results

Endocrinology

Results showed a significant difference between individual lemur fGM concentrations, yet no significant effect of sound type on fGM concentrations (Figure 26, Table 21). Ring-tailed lemur "B80085" and the red-ruffed lemur group samples

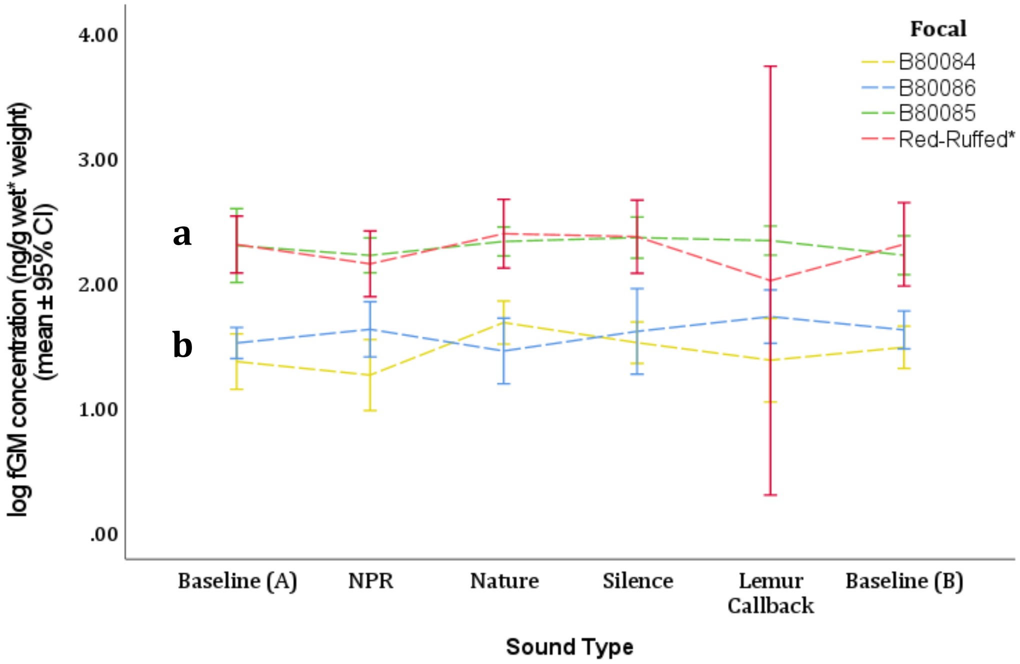


Figure 26: Ring-tailed lemur and red-ruffed lemur fGM concentrations by sound type. Asterisk (*) indicates dry fecal sample analysis. Lemurs in group "a" had significantly (p-value < 0.05) different average fGM concentrations per event type than those in group "b".

were significantly more elevated than the other ring-tailed lemurs. The interaction between sound type and focal animal was non-significant and was excluded from the final model.

Table 21: Estimated model fixed effects. [a]: significant (p-value < 0.05). [b]: Parameter is redundant.

	Effect	**Estimate**	**Std. Error**	**t**	**95% CI**	
	Intercept	2.284	0.063	35.903	2.157	2.410
Sound Type	Baseline (A)	-0.036	0.063	-0.565	-0.161	0.090
	NPR	-0.094	0.063	-1.480	-0.220	0.032
	Nature Sounds	0.056	0.063	0.886	-0.069	0.182
	Silence	0.054	0.066	0.829	-0.075	0.184
	Lemur Callbacks	0.008	0.067	0.115	-0.125	0.141
	Baseline (B)[b]					
Focal Animal	B80084[a]	-0.840	0.069	-12.262	-0.981	-0.700
	B80086[a]	-0.700	0.069	-10.153	-0.837	-0.555
	B80085	0.010	0.069	0.081	-0.135	0.146
	Red-Ruffed[b]					

Behavior

B80084

Kruskal-Wallis analyses revealed no significant differences in any behavior rates dependent on sound type for B80084 (Table 22**,** Figure 27).

Table 22: Results of Kruskal-Wallis tests for ring-tailed lemur B80084. No significant (p-value > 0.05) results occurred.

Behavior	**Sig. (*p*)**	**Test Statistic**
Enrichment Interaction	NA	NA
Environmental Interaction	0.202	7.258
Feeding / Drinking	0.541	4.058
Groom Other	0.416	5.000
Huddle	0.089	9.541
Locomotion	0.970	0.903
Not Visible	0.107	9.055
Self-Groom	0.686	3.088
Social Interaction	0.416	5.000
Stationary	0.085	9.659

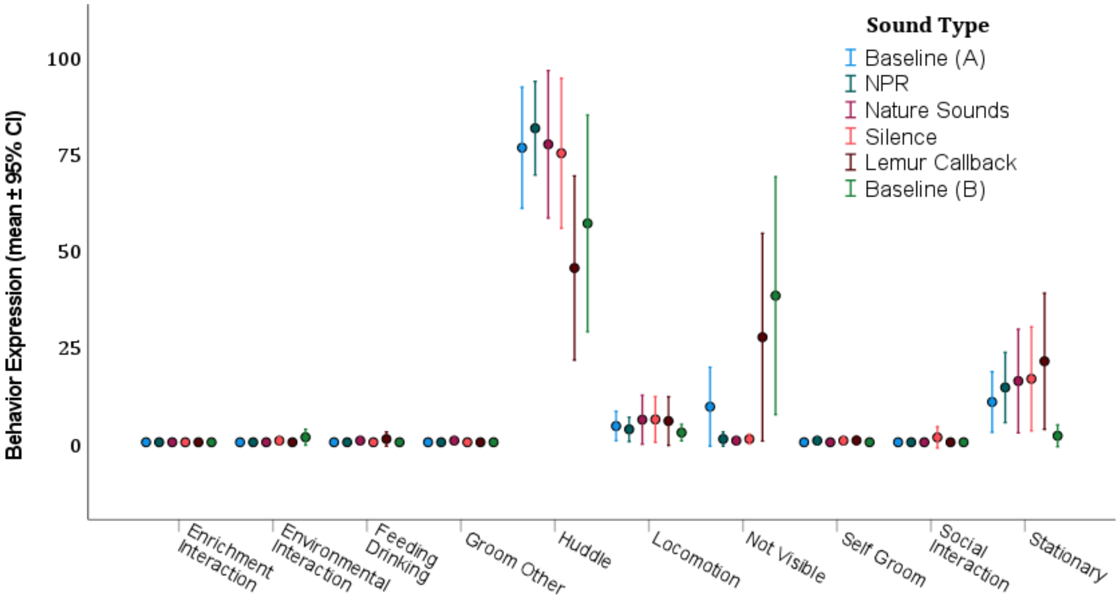


Figure 27: Average behavior rate by sound type for ring-tailed lemur B80084. No significant (*p*-value > 0.05) differences occurred.

B80085

B80085 exhibited no significant differences in behavior rate expression by sound playback type based on a Kruskal-Wallis Test (Table 23, Figure 28).

Table 23: Results of Kruskal-Wallis tests for ring-tailed lemur B80085. ^{a} indicates statistical significance (*p*-value < 0.05).

Behavior	**Sig. (*p*)**	**Kruskal-Wallis (*H*)**
Enrichment Interaction	NA	NA
Environmental Interaction	0.686	3.088
Feeding / Drinking	0.541	4.058
Groom Other	0.416	5.000
Huddle	0.246	6.676
Locomotion	0.810	2.276
Not Visible	0.277	6.317
Self-Groom	0.541	4.058
Social Interaction	0.541	4.058
Stationary	0.265	6.446

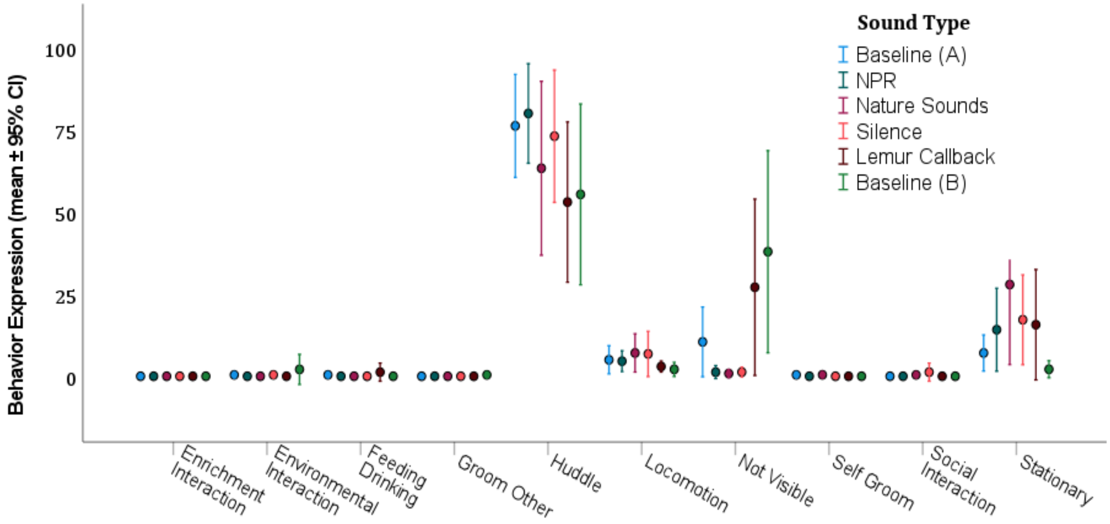


Figure 28: Average behavior expression rate by sound type for ring-tailed lemur B80085. No significant (*p*-value > 0.05) differences occurred.

B80086

Kruskal-Wallis rank test showed two behaviors rates were expressed significantly differently depending on sound playback type (Table 24**,** Figure 29). B80086 expressed feeding/drinking significantly more during the nature sounds and lemur-specific callback sound playbacks and was more socially interactive during silence than in any of the other sound playbacks. B80086 was recorded feeding and drinking only during the nature-sounds (mean rank = 37.46) and lemur-specific (mean rank = 43.54) sound playback types. Additionally, she only socially interacted with the other lemurs during silence (mean rank = 46.50) (Figure 29).

Table 24: Results of Kruskal-Wallis tests for ring-tailed lemur B80086. [a] indicates statistical significance (*p*-value < 0.05).

Behavior	**Sig. (*p*)**	**Kruskal-Wallis (*H*)**
Enrichment Interaction	0.416	5.000
Environmental Interaction	0.416	5.000
Feeding / Drinking [a]	0.041	11.566
Groom Other	NA	NA
Huddle	0.273	6.352

Locomotion	0.778	2.489
Not Visible	0.150	8.106
Self-Groom	0.687	3.087
Social Interaction [a]	<0.001	20.863
Stationary	0.418	4.984

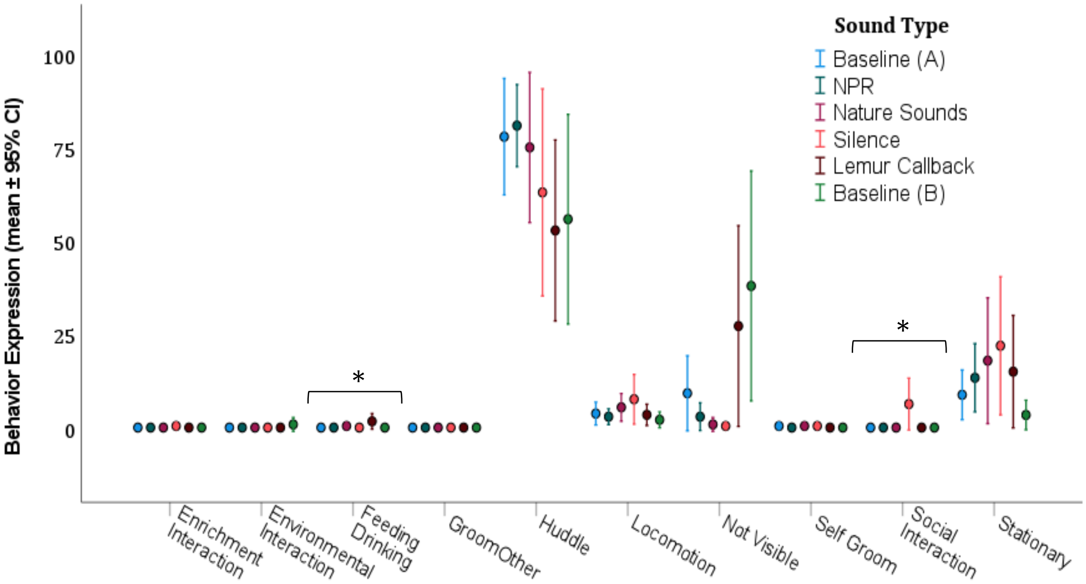


Figure 29: Average behavior expression rate by sound type for ring-tailed lemur B80086. Asterisks (*) indicate statistical significance (*p*-value < 0.05).

All Occurrence Behaviors

All occurrence behavior frequencies are presented in Figure 30. There were significantly more red-ruffed vocalizations during the nature audio manipulation than during any other sound treatment. Additionally, there were more displacement of the ring-tailed lemurs during the “silence” treatment than during any other manipulation. Interestingly, this increase in displacement activities occurred in all three observation types (pre, during, and post). However, since no auditory manipulation occurred (the treatment was silence) there may have been additional social conflicts occurring between the red-ruffed lemurs and the ring-tailed lemurs.

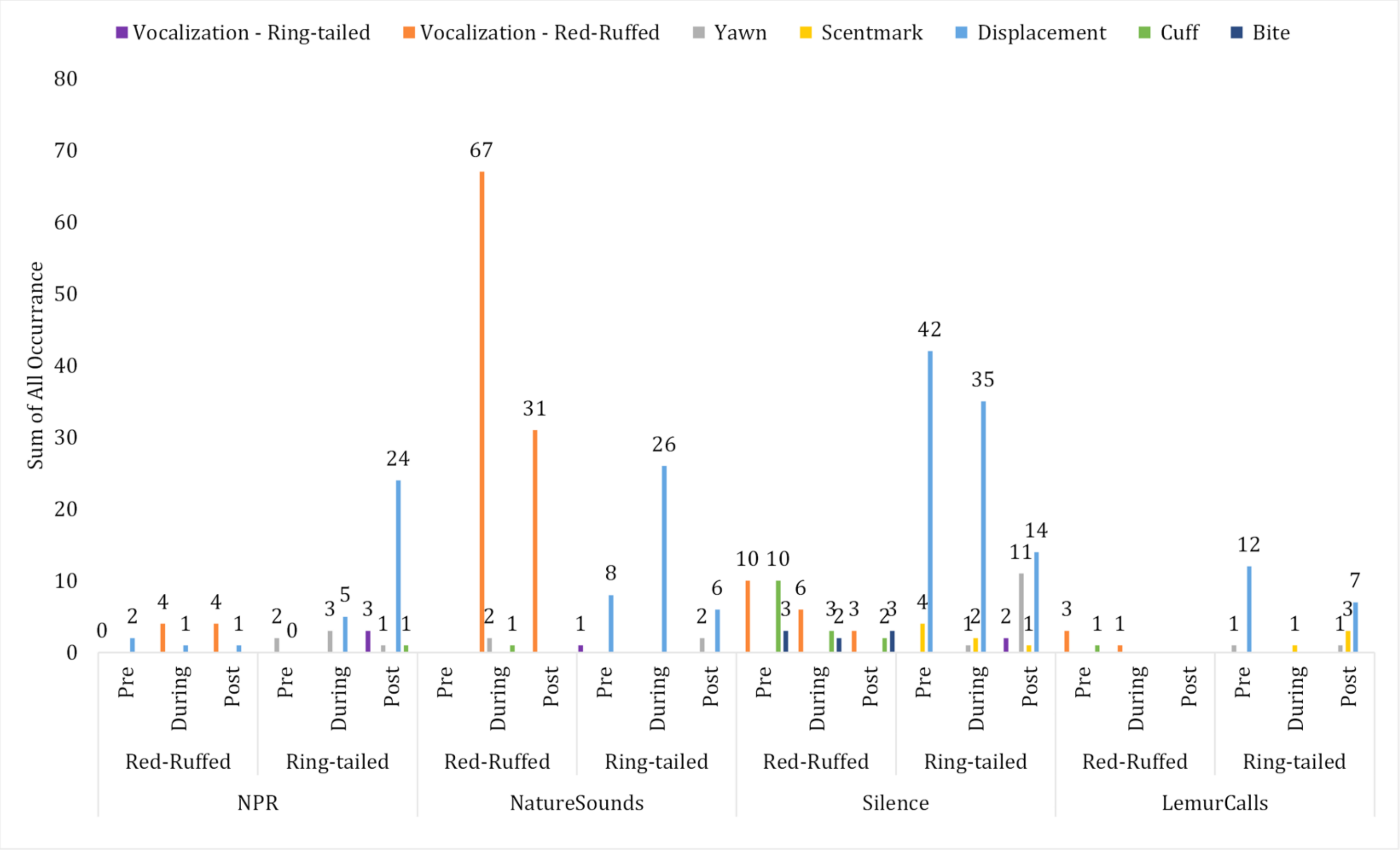


Figure 30: Count of total “All Occurrence” data of each audio manipulation.

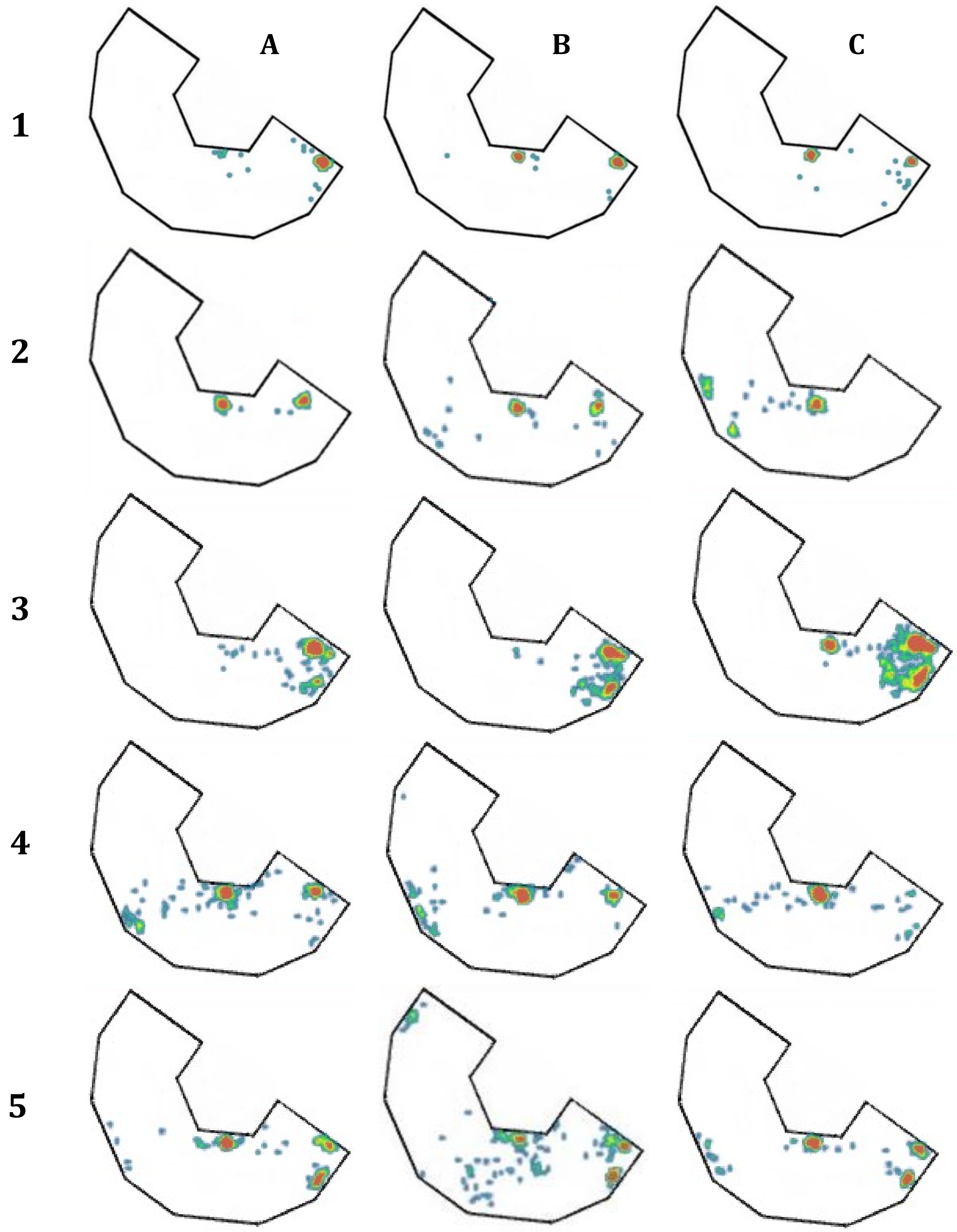


Figure 31: Ring-tailed lemur space use map matrix. 1) Baseline 2) NPR; 3) Nature Sounds; 4) Silence; 5) Lemur Callback. A) Pre; B) During; C) Post.

Space Use

Heat-maps indicated differences in space use based on sound playback. Specifically, the maps illustrate an increased use exhibit areas farthest away from the speaker system (see Figure 26 for exhibit map and speaker location) during lemur call backs. Typically, the ring-tailed lemurs congregated at two heated mats (Figure 31) in their exhibit while the red-ruffed lemurs dominated the use of the

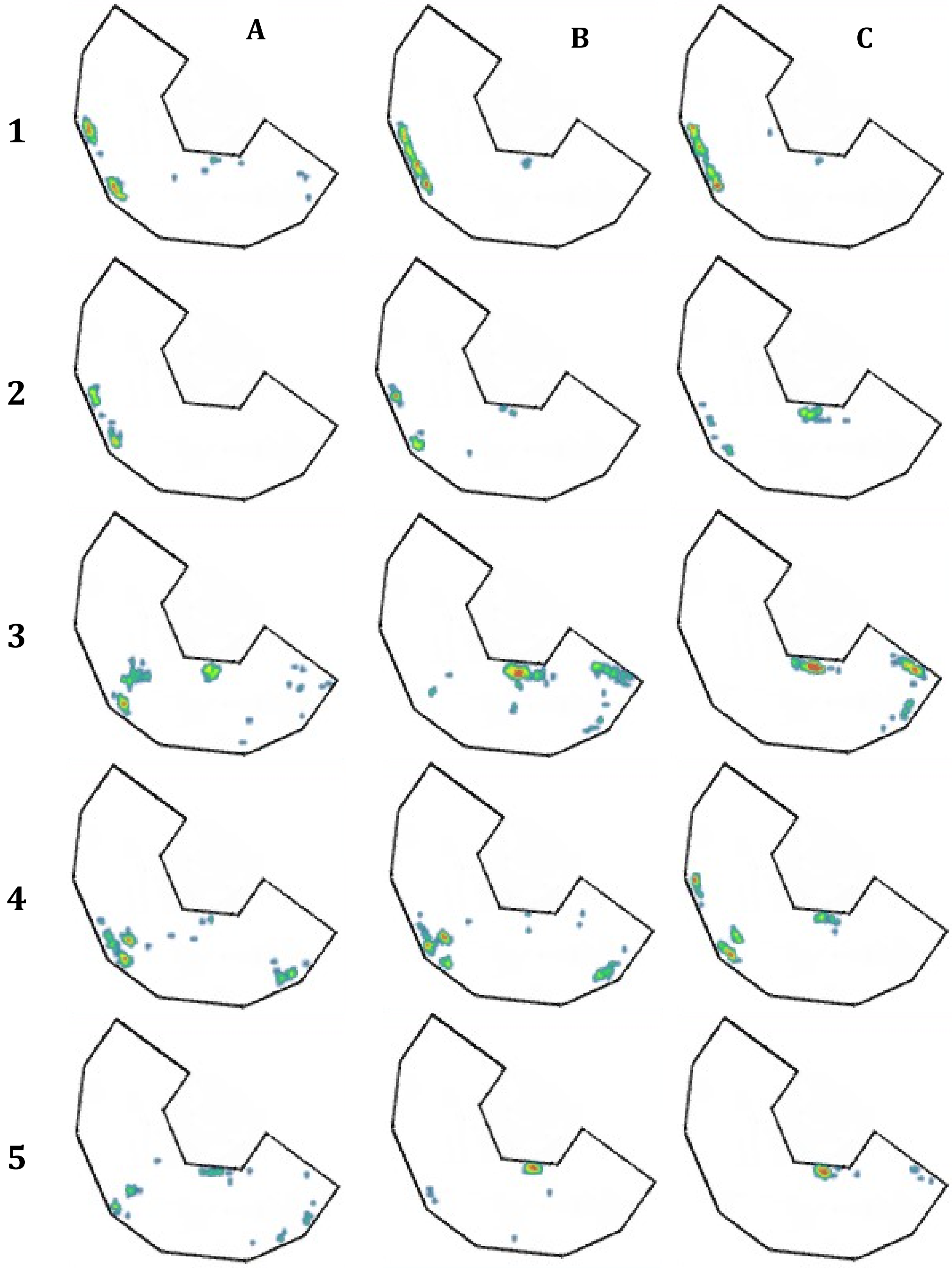


Figure 32: Red-ruffed lemur space use map matrix. 1) Baseline (A+B); 2) NPR; 3) Nature Sounds; 4) Silence; 5) Lemur callback. A) Pre; B) During; C) Post.

heated hammocks at the edge of the exhibit and occasionally congregated at the center heated mat (Figure 32). However, during the “Nature Sounds” audio manipulation, the ring-tailed lemurs chose to congregate away from the heated mat. This may have been due to the presence of the red-ruffed lemurs at the central heated mat (Figure 32), or it may have indicated a reluctance to approach the central heated mat due to its proximity to the speaker system.

Additionally, it is important to note the increased distribution of space use by the ring-tailed lemurs during the lemur callbacks. This audio manipulation resulted in novel use (within this experiment) of the exhibit area farthest away from the speaker system by the ring-tailed lemurs. This change in space use provides direct evidence of an influence of lemur callbacks on the ring-tailed lemur space use.

Discussion

There were no significant differences or correlations between fecal glucocorticoid metabolite (fGM) concentrations and projected sound type for any lemur. While the red-ruffed lemurs appeared to have a dramatic response in fGM concentrations during the lemur callback audio manipulation, the loss of fecal samples from this period limited the accuracy of this variability. However, there were significant differences between individual lemurs – indicating unique responses for each lemur. The individual differences were not indicative of age as the oldest and the youngest ring-tailed lemur showed the lowest average fGM concentrations of the group. Interestingly, the average fGM concentrations may be correlated with behavior traits and/or dominance. Of the ring-tailed lemurs, B80085 had significantly higher overall fGM concentrations than the other two ring-tailed lemurs. According to the lemur care staff at the Oregon Zoo, B80085 is considered the boldest ring-tailed lemur in the group. She is also the most active and explorative ring-tailed lemur, which correlates with findings reported by Hugo Bessa Ferreira et al. (2018) that shows higher concentrations of fGM with increasing activity levels in captive brown capuchin monkeys (*Sapajus libidinosus*). Research that correlates fGM concentrations with personality traits is still in its infancy – more formal research in this category is necessary to determine if these personality trends can be applied to ring-tailed lemurs.

Additionally, we reported species-level differences in average fGM concentrations. These differences may be aligned with differing extraction methods (dry fecal samples vs. wet fecal samples) but are not unusual (Sheriff et al. 2011). Also, the extreme variability within the red-ruffed lemur fGM concentrations reported during the lemur callback audio manipulation is likely due to the limited samples analyzed during that period. Due to bacterial contamination of the fecal samples due to prolonged exposure outside acceptable storage temperature prior to processing, multiple samples were omitted. In order to accurately analyze effect of lemur callbacks on red-ruffed lemur fGM concentrations, the experiment must be repeated. However, adrenal responses in the ring-tailed lemurs indicated that no audio manipulation type was arousing enough to alter the fGM concentrations in a significant way. This was also true of the red-ruffed lemurs for all manipulations besides lemur callback (which should be re-analyzed).

When the behavior rates were analyzed based on audio manipulation, very few significant differences were noted. Only one ring-tailed lemur (B80086) expressed significant behavioral differences. While she showed a significant increase in feeding / drinking during nature sounds and lemur-specific callback sounds, this was most likely due to the presence of food than in response to the audio changes. While care was taken to make each observation period identical – priority was given to animal care, and occasionally the ring-tailed lemurs were provided with snacks to assist in shifting them to their habitat. B80086 was also observed to socially interact with other lemurs (typically the red-ruffed lemurs) during the silent sound type. This could have been a response to the lack of audio manipulation but was most likely due to the red-ruffed lemurs hoarding the heated spaces in the exhibit.

In addition to the significant changes observed in B80086 (feeding / drinking and social interaction behaviors), all the ring-tailed lemurs tended to behave in similar ways during the audio manipulation. The most frequently observed behaviors for all ring-tailed lemurs were 'huddle' and 'stationery', followed by 'locomotion' and 'not visible'. Huddle behaviors are a common way ring-tailed lemurs have been observed to thermoregulate in the absence of sunshine (Kelley et al. 2016). This experiment occurred during the late fall of 2019 when temperatures averaged 41°F (5°C) and wind-chill was prevalent. The abundance of 'not visible' behaviors were similar to those reported in Collins et al. (2017) who found increased rate of 'not visible'. However, that same study reported decreased rates of ‘locomotion’ in periods of adverse weather, while this study found increased rates of ‘locomotion’ during nature sounds, silence, and lemur callbacks. This difference in locomotive rates may be indicative of a response to auditory manipulation, but until this study is repeated in warmer weather, it is impossible to discern if the changes in behavior rates were not associated with temperature fluctuations. Repetition of this experiment during the summer months is planned and will assist in illuminating if behavior rate changes are due to temperature fluctuations or auditory manipulations.

Some interesting results came from the all-occurrence data. Specifically, the increase in vocalizations by the red-ruffed lemurs during the nature sounds audio manipulation was unexpected. These vocalizations most resembled the contagious “roar/shriek chorus” described in black-and-white ruffed lemurs that appear to spontaneously erupt within a group of free-ranging lemurs (Pereira et al. 1988). Previous research found that there were no consistent triggers causing the roar/shriek chorus to begin and that this call type was most likely emitted to help maintain spacing between lemur groups. As this is a spontaneous type of call, it may have been coincidentally emitted by the red ruffed lemurs during the nature sound audio manipulation, but it could also have been causal in nature. It is therefore important to conduct further experimental sessions to help identify whether nature sounds and/or lemur calls resulted in increased vocalizations by some of the lemurs and also whether these reactions were indicative of positive, neutral, or negative experiences for the lemurs. Additionally, there was an increase in displacement of the ring-tailed lemurs during the silent audio manipulation. During this time, the red-ruffed lemurs were frequently chasing and harassing the ring-tailed lemur troop. While there is no evidence that this is related to the silence, it is an important behavior to monitor if deciding to use audio manipulation as enrichment.

In addition to behavior, it is important to observe how the habitat use varies under each condition. During 'silence' and 'lemur callbacks', the ring-tailed lemurs showed differing habitat use when compared to the other three treatments (baselines, NPR, and nature sounds). Specifically, during 'lemur callbacks', B80086 initially went to the perimeter of the exhibit, as far away from the speaker as possible, before cautiously returning to the heated areas. On the other hand, lemur B80084 moved much closer to the speaker while lemur B80085 remained in the heated areas. This response seems to be in accordance with the personality differences that primate care staff have reported. B80085, being the boldest ring-tailed lemur, appeared not affected by the lemur call backs, while B80084 and B80086 were obviously reacting to the sounds. Surprisingly, B80084 – who was reported to be the shyest ring-tailed lemur was the individual who approached the speaker while B80086 was the individual that fled to the back of the exhibit. The red-ruffed lemurs varied slightly in their habitat use, as well. During Baseline (1) they were found almost exclusively in hanging heated hammocks along the perimeter of the exhibit, while during nature sounds and lemur callbacks they were observed on a heated rock in the center of the exhibit or on the highest rock point along the right side of the exhibit. Throughout almost every treatment, the red-ruffed lemurs would chase and displace the ring-tailed lemurs from the heated rocks. These changes – specifically those reported during lemur callbacks – are similar to the behavioral changes reported by Browning et al. (2006), however our study recorded no bouts of aggression during the callbacks. Browning et al. (2006), discontinued their use of lemur callbacks due to the historical aggression seen in their lemur troop. They shifted to nature sounds and classical music which resulted in similar habitat use patterns and behavioral changes when compared to our study.

Conclusions

There were no clear indications that audio manipulation is an inappropriate enrichment source for ring-tailed and red-ruffed lemurs. While interesting changes in behavior rates between the treatment types were noted, further research is required to conclusively link the changes with the auditory manipulation. Currently, we plan to repeat this study during the warmer months to clarify if the behavior rates expressed were due to the cold ambient temperature or the treatment types. The fGM analyses did not reveal potential signs of distress or a prolonged elevated stress response in response to any of the treatments. However, repeated or more prolonged treatments could possibly elicit physiological responses. Future experiments should also examine the duration and intensity of the treatments and possible influences of time of day and surrounding activities in order to look at the possible use of any of the treatments for enrichment. While further research is clearly needed to better understand the impact of the examined audio manipulation treatments and their potential use as enrichment, some of the treatments (nature sounds and lemur callbacks) may be an appropriate way to facilitate increased space use and provide novel environments to lemurs at zoological facilities.

References

Barlow, Catriona J. C., Christine A. Caldwell, and Phyllis C. Lee. 2006. “Individual Differences and Response to Visitors in Zoo-Housed Diana Monkeys (Cercopithecus Diana Diana).” *8th Annual Symposium on Zoo Research Colchester Zoo* (July):129–42.

Bowman, A., S. P. C. A. Scottish, F. J. Dowell, and N. P. Evans. 2015. “‘Four Seasons’ in an Animal Rescue Centre; Classical Music Reduces Environmental Stress in Kennelled Dogs.” *Physiology and Behavior* 143:70–82. doi: 10.1016/j.physbeh.2015.02.035.

Browning-Jones, Heather, and Lisa Moro. 2006. “A Multi-Sensory Enrichment Program for Ring-Tailed Lemurs (Lemur Catta) at Auckland Zoo, Including a Novel Feeding Device.” *Regional Environmental Enrichment*.

Carlson, Synnöve, Pia Rämä, Denis Artchakov, and Ilkka Linnankoski. 1997. “Effects of Music and White Noise on Working Memory Performance in Monkeys.” *NeuroReport* 8(13):2853–56. doi: 10.1097/00001756-199709080-00010.

Chosy, Julia, Megan Wilson, and Rachel Santymire. 2014. “Behavioral and Physiological Responses in Felids to Exhibit Construction.” *Zoo Biology* 33(4):267–74. doi: 10.1002/zoo.21142.

Collins, Courtney, Ilse Corkery, Amy Haigh, Sean McKeown, Thomas Quirke, and Ruth O’Riordan. 2017. “The Effects of Environmental and Visitor Variables on the Behavior of Free-Ranging Ring-Tailed Lemurs (Lemur Catta) in Captivity.” *Zoo Biology* 36(4):250–60. doi: 10.1002/zoo.21370.

Ferreira, Vitor Hugo Bessa, Carolina Pereira Cadório Da Silva, Elanne De Paiva Fonseca, Ana Cecilia Correia Santos Das Chagas, Luiz Guilherme Mesquita Pinheiro, Raissa Nobrega De Almeida, Maria Bernardete Cordeiro de Sousa, Hélderes Peregrino Alves Da Silva, Nicole Leite Galvão-Coelho, and Renata Gonçalves Ferreira. 2018. “Hormonal Correlates of Behavioural Profiles and Coping Strategies in Captive Capuchin Monkeys (Sapajus Libidinosus).” *Applied Animal Behaviour Science* 207:108–15. doi: 10.1016/j.applanim.2018.07.002.

Forthman Quick, Debra L. 1984. “An Integrative Approach to Environmental Engineering in Zoos.” *Zoo Biology* 3(1):65–77. doi: 10.1002/zoo.1430030107.

Goodenough, Anne E., Katie Mcdonald, Kayleigh Moody, and Clare Wheeler. 2019. “Are ‘Visitor Effects’ Overestimated? Behaviour in Captive Lemurs Is Mainly Driven by Co-Variation with Time and Weather.” *Journal of Zoo and Aquarium Research* 7(2):59–66.

Graves, Laura M. 2011. “The Effect of Auditory Enrichment on Abnormal, Affiliative, and Aggressive Behaviors in Laboratory- Housed Rhesus Macaques (Macaca Mulatta).” Texas State University - San Marcos, San Marcos.

Hanbury, David B., M. Babette Fontenot, Lauren E. Highfill, Willie Bingham, David Bunch, and Sheree L. Watson. 2009. “Efficacy of Auditory Enrichment in a Prosimian Primate (Otolemur Garnettii).” *Lab Animal* 38(4):122–25. doi: 10.1038/laban0409-122.

Kelley, Elizabeth A., Nina G. Jablonski, George Chaplin, Robert W. Sussman, and Jason

M. Kamilar. 2016. “Behavioral Thermoregulation in Lemur Catta : The Significance of Sunning and Huddling Behaviors.” *American Journal of Primatology* 78(7):745–54. doi: 10.1002/ajp.22538.

Kight, Caitlin R., and John P. Swaddle. 2011. “How and Why Environmental Noise Impacts Animals: An Integrative, Mechanistic Review.” *Ecology Letters* 14(10):1052–61. doi: 10.1111/j.1461-0248.2011.01664.x.

Kogan, Lori R., Regina Schoenfeld-Tacher, and Allen A. Simon. 2012. “Behavioral Effects of Auditory Stimulation on Kenneled Dogs.” *Journal of Veterinary Behavior* 7:268–75. doi: 10.1016/j.jveb.2011.11.002.

Larsen, Megan J., Sally L. Sherwen, and Jean Loup Rault. 2014. “Number of Nearby Visitors and Noise Level Affect Vigilance in Captive Koalas.” *Applied Animal Behaviour Science* 154:76–82. doi: 10.1016/j.applanim.2014.02.005.

Marcy, Karlyn. 2021. “Interesting Zoo and Aquarium Statistics.” Retrieved August 11, 2021 (https://www.aza.org/connect-stories/stories/interesting-zoo-aquarium-statistics?locale=en).

McCraty, Rollin, Mike Atkinson, Glen Rein, and Alan D. Watkins. 1996. “Music Enhances the Effect of Positive Emotional States on Salivary IgA.” *Stress Medicine* 12:167–75.

Ogden, Jacqueline J., Donald G. Lindburg, and Terry L. Maple. 1994. “A Preliminary Study of the Effects of Ecologically Relevant Sounds on the Behaviour of Captive Lowland Gorillas.” *Applied Animal Behaviour Science* 39(2):163–76. doi: 10.1016/0168-1591(94)90136-8.

Owen, Megan A., Ronald R. Swaisgood, Nancy M. Czekala, Karen Steinman, and Donald G. Lindburg. 2004. “Monitoring Stress in Captive Giant Pandas (Ailuropoda Melanoleuca): Behavioral and Hormonal Responses to Ambient Noise.” *Zoo Biology* 23(2):147–64. doi: 10.1002/zoo.10124.

Pereira, Michael E., Martha L. Seeligson, and Joseph M. Macedonia. 1988. “The Behavioral Repertoire of the Black-and-White Ruffed Lemur, Varecia Variegata Variegata (Primates: Lemuridae).” *Folia Primatologica* 51(1):1–32. doi: 10.1159/000156353.

Rasmussen, Skye, Gary Glickman, Rada Norinsky, Fred W. Quimby, and Ravi J. Tolwani. 2009. “Construction Noise Decreases Reproductive Efficiency in Mice.” *Journal of the American Association for Laboratory Animal Science* 48(4):363–70.

Rich, Erin L., and L. Michael Romero. 2005. “Exposure to Chronic Stress Downregulates Corticosterone Responses to Acute Stressors.” *Am J Physiol Regul Integr Comp Physiol* 288:1628–36. doi: 10.1152/ajpregu.00484.2004.-We.

Robbins, Lindsey, and Susan W. Margulis. 2014. “The Effects of Auditory Enrichment on Gorillas.” *Zoo Biology* 9999:1–7. doi: 10.1002/zoo.21127.

Ross, M. R., T. Niemann, J. D. Wark, M. R. Heintz, A. Horrigan, K. A. Cronin, M. A. Shender, and K. Gillespie. 2016. “ZooMonitor.”

Shepherdson, David J. 1998. “Introduction: Tracing the Path of Environmental Enrichment in Zoos.” Pp. 1–14 in *Second Nature: Environmental Enrichment for*

Captive Animals, edited by D. J. Shepherdson, J. D. Mellen, and M. Hutchins. Smithsonian Books.

Sheriff, Michael J., Ben Dantzer, Brendan Delehanty, Rupert Palme, and Rudy Boonstra. 2011. "Measuring Stress in Wildlife: Techniques for Quantifying Glucocorticoids." *Oecologia* 166(4):869–87. doi: 10.1007/s00442-011-1943-y.

Shire, Taylor. 2012. "Differences in Behavior between Captive and Wild Ring-Tailed Lemur (Lemur Catta) Populations: Implications for Reintroduction and Captive Management." Iowa State University.

Snowdon, Charles T., and David Teie. 2010. "Affective Responses in Tamarins Elicited by Species-Specific Music." *Biology Letters* 6(1):30–32. doi: 10.1098/rsbl.2009.0593.

Snowdon, Charles T., David Teie, and Megan Savage. 2015. "Cats Prefer Species-Appropriate Music." *Applied Animal Behaviour Science* 166:106–11. doi: 10.1016/j.applanim.2015.02.012.

Starling, Anne P., Marie J. E. Charpentier, Courtney Fitzpatrick, Elizabeth S. Scordato, and Christine M. Drea. 2010. "Seasonality, Sociality, and Reproduction: Long-Term Stressors of Ring-Tailed Lemurs (Lemur Catta)." *Hormones and Behavior* 57(1):76–85. doi: 10.1016/j.yhbeh.2009.09.016.

Wallace, Emma K., Drew Altschul, Karoline Korfer, Benjamin Benti, Amanda Kaeser, Susan Lambeth, Bridget M. Waller, and Katie E. Slocombe. 2017. "Is Music Enriching for Group-Housed Captive Chimpanzees (Pan Troglodytes)?" *PLoS ONE* 12(3):1–27. doi: 10.1371/journal.pone.0172672.

Wark, Jason D. 2015. "The Influence of the Sound Environment on the Welfare of Zoo-Housed Callitrichine Monkeys." Case Western Reserve University.

Wells, D. L., and R. M. Irwin. 2008. "Auditory Stimulation as Enrichment for Zoo-Housed Asian Elephants (Elephas Maximus)." *Animal Welfare* 17:335–40.

Wells, Deborah L., Dwyer Coleman, and Mark G. Challis. 2006. "A Note on the Effect of Auditory Stimulation on the Behaviour and Welfare of Zoo-Housed Gorillas." *Applied Animal Behaviour Science* 100(3–4):327–32. doi: 10.1016/j.applanim.2005.12.003.

Williams, Isabelle, Will Hoppitt, and Rachel Grant. 2017. "The Effect of Auditory Enrichment, Rearing Method and Social Environment on the Behavior of Zoo-Housed Psittacines (Aves: Psittaciformes); Implications for Welfare." *Applied Animal Behaviour Science* 186:85–92. doi: 10.1016/j.applanim.2016.10.013.

Woolway, Eleanor E., and Anne E. Goodenough. 2017. "Effects of Visitor Numbers on Captive European Red Squirrels (Sciurus Vulgaris) and Impacts on Visitor Experience." *Zoo Biology* 36(2):112–19. doi: 10.1002/zoo.21357.

Young, Robert J. 2003. *Environmental Enrichment for Captive Animals*. Oxford, UK: Blackwell Science Ltd.

Chapter Four: Applying Behavioral and Physiological Measures to Assess the Relative Impact of the Prolonged COVID-19 Pandemic Closure on Two Mammal Species at the Oregon Zoo: Cheetah (*A. jubatus*) and Giraffe (*G. c. reticulata* and *G. c. tippelskirchi*)

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Introduction

A longstanding interest in zoo researchers has been how the presence or absence of visitors affects the behavior and physiology of zoo animals. In 2000, Hosey formally introduced the term 'visitor effect' (Hosey 2000). Since then, this phenomenon has been studied in a wide variety of species. Typically, these studies have reported varying responses to visitor presence depending on individual and species-specific characteristics. For instance, increased visitor presence has been correlated with 'less time visible to the public' and increased vigilance (both frequently used to indicate a certain level of discomfort with visitor presence and, thus, possible reduced levels of well-being) for multiple species (orangutans—*Pongo pygmaeus* and *Pongo abelii* Choo, Todd, and Li 2011, koalas—*Phascolarctos cinereus* Larsen et al. 2014, ocelots—*Leopardus pardalis* and bobcats—*Lynx rufus* Suárez, Recuerda, and Arias-De-Reyna 2017). However, other studies have shown ambivalence or no reaction to visitor presence (see Davey 2007 for a comprehensive review). Like humans, individuals within the same species can show different behavioral (Polgár, Wood, and Haskell 2017) and adrenal (Wolf et al. 2018) responses to the same event. For instance, Razal et al. (2017) reported significantly different mean response values for individual reticulated giraffe (*Giraffa camelopardalis reticulata*) behaviors between the same seasonal stressors and Polgár et al. (2017) found differing behavioral responses to visitors by captive spider monkeys (*Saimiri sciureus*) based on personality traits. There are several different hypotheses as to why there are such variable responses to visitor presence, both between and within species, including differing individual traits (life histories, genetics, and temperaments) and life experiences as well as evolutionary background (see Sherwen and Hemsworth 2019 for a review).

Visitors may impose three main influences on animals: visual, audible, and olfactory (Robert J. Young 2003). Recent studies have emphasized the importance of olfactory (Farrand 2007) and auditory (Brown et al. 2012; Larsen et al. 2014; Quadros et al. 2014; Robbins and Margulis 2014) stressors when comparing visitor effects. Monitoring these stimuli can be complicated, with the two influences being almost impossible to isolate. One way to monitor the overall impact of the presence of visitors is to entirely eliminate them from the zoo during regular visitor hours and compare the results to normal periods. This is rarely possible due to the need for visitor associated revenue. Thus, a limitation of many visitor-effect studies to date has been the lack of data from times without any visitors. Such periods of complete visitor absence tend to be opportunistic and brief. For instance, Mallapur et al. (2005) reported that captive lion-tailed macaques (*Macaca silenus*) showed a 20% decrease in short-term abnormal behaviors and a 30% decrease in long-term abnormal behaviors when visitors were absent. However, for the short-term study, the visitor absence period was limited to one day whereas the long-term study relied on a comparison between on-exhibit and off-exhibit behaviors. In other studies (e.g., Chiew et al. 2021), access to an exhibit was simply blocked off for a set period to reduce visitor presence. This strategy only highlights different behaviors related to visibility because the focal animals were still exposed to auditory and olfactory changes associated with nearby visitors.

Studies on physiological stress responses are now commonly conducted in combination with behavioral monitoring. Here, we define a stressor as any event that elicits an adrenal response, whether positive or negative. Many nondomestic animals may “hide” signs of stress, making it more difficult to recognize behavioral responses to stressors; yet such physiological reactions can be used as internal indicators of positive or negative stress responses when combined with other external indicators such as behaviors and/or animal health measures.

The activation of the hypothalamic-pituitary-adrenal (HPA) axis is one of the most consistent physiological responses to stimulation across different species and is involved regardless of whether the stressor is considered positive (e.g., mating) or negative (e.g., pain). One result of the HPA-axis stimulation is the eventual excretion of glucocorticoid metabolites, including cortisol and corticosterone, into the feces and/or urine, which can then be monitored to determine the intensity of the stressor (see O'Connor, O'Halloran, and Shanahan 2000 for a review of the HPA-axis). While it is possible to measure the concentration of intact glucocorticoids in plasma, the collection of plasma samples is inherently stressful and can influence the concentration of glucocorticoids in less than three minutes (Romero and Reed 2005). However, a substantial number of studies have shown that glucocorticoids, after being metabolized, get excreted in feces and urine, and the adrenal response to an intense stressor observed in serum samples can be measured in fecal or urine samples noninvasively (Möstl and Palme 2002; Sheriff et al. 2011; Michael J Sheriff, Krebs, and Boonstra 2010; Stead, Meltzer, and Palme 2012; Chadi Touma and Palme 2005).

Among available non-invasive techniques, fecal glucocorticoid metabolite (fGM) monitoring is the most popular for several reasons (see Palme 2019 for a recent review). Fecal samples: (1) are most consistently available for noninvasive collection on wild and captive animals; (2) represent a pooled concentration of adrenal hormone levels over time (depending on species-specific metabolic and excretion patterns); and (3) may be less sensitive to diurnal fluctuations and short-term secretion of adrenal hormones into the bloodstream when compared to serum glucocorticoids (Wielebnowski and Watters 2007). As mentioned previously, the excretion of glucocorticoid metabolites occurs with differing instances of arousal and requires further interpretation (such as behavioral and/or animal health analyses) to determine if a given stressor is positively or negatively affecting an individual (Wielebnowski 2003).

In 2020, the global SARS-CoV-2 (COVID-19) pandemic resulted in the temporary closure of most zoological institutions for periods ranging from weeks to months. On 15 March 2020, amid rapidly increasing positive COVID-19 cases, Oregon's governor ordered the immediate closure of all large recreational facilities in the state. The subsequent closure of the Oregon Zoo resulted in nearly four consecutive months (until 12 July 2020) without visitors and with substantially reduced staffing. This extended closure provided researchers with the opportunity to quantify the potential behavioral changes expressed by some zoo animals that are typically subjected to high visitor attendance. Due to its urban location, the Oregon Zoo receives nearly 1.7 million people per year, with the most heavily attended months occurring in the summer (Anon n.d.). Recent studies have quantified the effects of these closures in other institutions, primarily for mammals (Williams, Carter, Rendle, and Ward 2021), but with at least one reptile study (Riley et al. 2021). Williams et al. (2021) showed a variety of behavioral changes in eight monitored species. Among these were an increase in 'comfort' behaviors (such as self-grooming or self-maintenance behaviors), closer presence to areas where visitors were usually located, and more environmental interactions (investigation or interaction of non-food items in the environment) during zoo closure periods. In contrast, Riley et al. (2021) found significant differences in Nile crocodile (*Crocodylus niloticus*) behavior that more directly related to time of day, temperature, and month rather than the absence of visitors.

In addition to formal research, anecdotal stories reported by zoo staff indicated a range of different responses to the extended absence of visitors. For instance, Daniel Ashe (CEO of the Association of Zoos and Aquariums, AZA – Silver Spring, MD, USA), based on information received from a variety of AZA institutions and their animal care staff, reported "general boredom" across several species (Wright 2020). The same article reported that without visitors, normally "aloof" individuals such as gorillas (*Gorilla gorilla gorilla*) and camels (*Camelus bactrianus*) at the Calgary Zoo approached staff members more frequently for interaction. At the Phoenix Zoo, care staff used their breaks to interact with the petting zoo goats (*Capra hircus*) to counteract the dramatic decrease in human interaction to which goats were previously habituated. Even with such mitigation, the goats received less interaction than they were accustomed to during visitor times (Frank 2020).

Here we report on a study aimed at quantifying how transitions in visitor attendance affected the physiology and behavior of animals at the Oregon Zoo. We tracked behavioral and adrenal responses to two transition periods in visitor absence (the initial closure and the reopening four months later), in giraffes (*Giraffa camelopardalis reticulata* and *Giraffa camelopardalis tippelskirchi*) and cheetahs

(*Acinonyx jubatus*). We chose to focus our analyses on two transition periods, since they were deemed the times of most intense change. For each transition, we aimed to obtain comparable before and after data (equal numbers of observations and fecal samples).

We hypothesized that changes in fGM concentrations and behavior expression would be minimal during the first transition period and more variable during the second transition period, due to potential gradual acclimation to visitor absence over the months of closure and the sudden return of visitors. For behavior analyses, we hypothesized a transition to more exploratory activities and more ‘time spent visible’ without visitors, similarly to previously reported analyses (Choo et al. 2011; Larsen et al. 2014; Suárez et al. 2017).

We hypothesized that the effects of the initial shutdown would be less than the reopening transition because both species had previously experienced short-term periods without visitors. For both cheetahs and giraffes, these included inclement weather closures. Additionally, both cheetahs in this study had been recently housed in entirely “off-exhibit” habitats at their previous institution. As they only arrived in January 2020, they may have still been acclimated to limited visitor access. At the Oregon Zoo, both study species are popular with visitors, and their habitats are located along the main visitor pathway, providing a stark difference in visitor presence during the open and closed time periods. While previous studies have observed the visitor effect on both giraffes (Normando et al. 2018) and cheetahs (O’Donovan et al. 1993), most giraffe behavioral studies have mainly centered around direct human-animal interactions, such as feeding platforms, rather than indirect visitor presence (Normando et al. 2018; Orban, Siegford, and Snider 2016). Many cheetah studies have focused on a single behavior (e.g., stereotypy, Quirke, O'riordan, and Zuur 2012) or a specific relationship (e.g., mother-cub relationship, O'Donovan et al. 1993).

Since this study focused specifically on the transition periods in visitor attendance, it may provide valuable information about the effect of visitor presence on both species. If analyses reveal an increase or decrease in potential positive or negative indicators of stress, more in-depth studies would be needed to identify how the effects of visitor presence or absence can be mitigated appropriately.

Methods

All research was reviewed and approved by the Oregon Zoo Research Review Committee (OZRRC).

General

The Oregon Zoo is located just outside the downtown area of Portland, Oregon, USA. We collected data on two visitor presence transition periods. Each transition period consisted of two month-long treatments, one with visitors and one without. Transition Period 1 included the initial opening period of full visitor access ("Open") and the first period of complete visitor absence ("Closed A"). Transition Period 2 included the end of the visitor absence period ("Closed B") and the beginning of visitor access period ("Reopen"). However, visitor access was somewhat variable between the two transition periods. During "Open" there were no restrictions on visitor numbers, in both “Closed (A)” and “Closed (B)” visitors were unable to attend the Zoo, and in “Reopen” there was a 50% visitor capacity cap. Data collection specific to this study took place between mid-March 2020 and August 2020; however, since both species were part of the Zoo’s ongoing welfare monitoring program (including behavioral and physiological monitoring), some of the previously collected data between January 2020 and early March 2020 were included in the analyses. We decided to focus on the data directly associated with the two main transition periods to provide comparable ‘before and after’ data for statistical analyses as well as to isolate specific changes associated with the transition times.

Focal Animals

Cheetah (Acinonyx jubatus)

We monitored two 10-year-old male cheetahs (“B10183” and “B10184”) that had recently (January 2020) returned to the Oregon Zoo after spending three years at the San Diego Wild Animal Park (SD-WAP). Both cheetahs were provided a diet consisting of feline-specific chow (Milliken Carnivore) and game carcasses. Fresh water was available *ad libitum*. The cheetah habitat included three indoor off-exhibit areas (one 109 ft^2 and two 80 ft^2 habitats) and a large grassy outdoor exhibit (approximately 19,200 ft^2) viewable by the public. The outdoor cheetah yard consisted of a small shelter with heated flooring on the western side, a small pond on the eastern, and a large grassy knoll in the middle which prevents visitor viewing to the back of the exhibit. The cheetahs participated in voluntary training sessions four to five times per week (average duration 5 min). The training durations and frequencies remained consistent over the course of the shutdown. Both cheetahs had pre-existing health issues prior to arriving at the Oregon Zoo, and several medical procedures were conducted between 6 April 2020 and 6 July 2020.

Giraffe (Giraffa camelopardalis reticulata & Giraffa camelopardalis tippelskirchi)

For this study, we observed two male giraffes, a reticulated giraffe (“B20186”, 9 years old, *G. c. reticulata*) and a Masai giraffe (“B60180”, 5 years old, *G. c. tippelskirchi*). In late May 2020 a young female Masai giraffe (“C00028”, 2 years old) was introduced to the herd but was not included in the study. According to fecal progesterone metabolite tracking, the giraffe C00028 was not cycling throughout the duration of this study. Additionally, there was minimal difference in analyzed fGM concentrations following giraffe C00028′s introduction between the two males, despite differences in fecundity (giraffe B20186 was castrated in 2012), which indicated no significant changes in fGM concentrations based on androgen production. The two males had arrived at the Oregon Zoo in 2012 and 2016, respectively. All giraffes shared a multi-species exhibit along the main Zoo pathway with three southern ground hornbills (*Bucorvus leadbeateri*) and one Speke′s gazelle (*Gazella spekei*). All giraffes were fed a combination of alfalfa and pellets (Mazuri Wild Herbivore Hi-Fiber Cube 5V05) daily. Browse was hung around the exhibit while carrot and primate L/S biscuits (cinnamon, Mazuri 5M1S) were supplemented in training sessions when available. Fresh water was available *ad libitum.* The giraffe habitat included an indoor barn for protection in inclement weather and two large outdoor areas (combined approximate area is 20,000 ft^2) which could be separated by a fence, if necessary. The outdoor areas were covered with dirt, decomposed granite substrate, and grass. There were multiple logs (enrichment items for the Speke's gazelle and the southern ground hornbills), a large pond, and multiple large trees scattered throughout the environment which provided shade, browse, and enrichment. The indoor barn consisted of multiple stalls with a rubber-coated concrete flooring lightly covered in wood shavings and was maintained at 65°F (18.3 °C). Visitor viewing access was provided through raised walkways along the southern edge of the outdoor exhibit. The giraffe exhibit also included a feeding platform; however, it was not yet in regular use at the time the COVID-19 closure began and remained closed to the public.

During the day, the giraffes were housed solely indoors when temperatures fell below 40 °F (4.44 °C) but they were allowed outdoors for a maximum of four hours if temperatures were between 40 °F and 50 °F (10 °C) with no precipitation. If temperatures were >50 °F, the giraffes were given continuous access to the outdoor area (except when the outdoor exhibit was actively being maintained by care staff). To maintain a safe environment and minimize slipping hazards, the giraffes were housed indoors whenever there was ice or snow present on exhibit, or if there was significant rainfall. The temperatures remained adequate for outdoor housing throughout the entire study time and all behavior observations occurred outdoors.

Endocrinology

Sample Collection

As previous research has indicated, fGM concentrations change significantly following transfers (Fanson et al. 2013; Grandin 1997; Volfová et al. 2019), introductions (Fazio et al. 2020; Loeding et al. 2011), and veterinary procedures (Rothschild et al. 2008). Sample dates were chosen to minimize the effects of these additional stressors while still providing meaningful data on transition periods. Fecal samples were collected three times per week between 07:00 and 10:00 by animal care staff during the two transition periods (Table 25). Care staff were instructed to avoid samples contaminated by urine or other substances. Samples were labelled with name, species, date, and time collected and immediately frozen (−4 °F/−20 °C) until analysis. Food-grade dye was used to identify samples from individuals housed together.

Table 25: List of different treatment periods, their corresponding dates and the number of fecal samples collected within the time. Samples analyzed within Open and Closed (A) are part of Transition Period 1 while samples analyzed in Closed (B) and Reopen are part of Transition Period 2.

Cheetah				
	Category	Sample Dates	Sample Count (B10183)	Sample Count (B10184)
Transition Period 1	Open	2/20/2020–3/15/2020	15	15
	Closed (A)	3/16/2020–4/4/2020	15	15
Transition Period 2	Closed (B)	6/16/2020–7/12/2020	15	15
	Reopen	7/13/2020–8/7/2020	15	15
Giraffe				
	Category	Sample Dates	Sample Count (B20186)	Sample Count (B60180)
Transition Period 1	Open	2/13/2020–3/15/2020	15	15
	Closed (A)	3/16/2020–4/12/2020	15	15
Transition Period 2	Closed (B)	6/9/2020–7/12/2020	15	15
	Reopen	7/13/2020–8/8/2020	15	15

Steroid Extraction

Fecal glucocorticoid metabolites (fGM) were extracted using an adaptation of methods documented by Bryant and Wielebnowski (Bryant and Wielebnowski 2018). Briefly, 0.500 g (±0.025 g) of wet, homogenized fecal matter was mixed with 5 mL 80% alcohol (giraffe: methanol; cheetah: ethanol), vortexed, and shaken overnight for 17 h (Fisherbrand™ open air rocking shaker). Then, the samples were vortexed again and centrifuged (Sorvall™ ST 16) at 2500 rpm for 15 min. Three milliliters of the resulting 1:1 supernatant was removed and 500 μL was subsequently desiccated in a SpeedVac (Savant Speedvac DNA110) before being reconstituted in 500 μL Tris HCl assay buffer. Using the results of in-house parallelism analyses, dilutions were then created with Tris assay buffer based on optimum concentrations for the detection of fGMs.

Enzyme Immunoassay (EIA) Analysis

It has been previously determined that glucocorticoid metabolites can be reliably extracted from fecal samples in both target species (giraffe - Bashaw et al. 2016, cheetah - Uetake et al. 2014). A double-antibody corticosterone enzyme immunoassay (EIA) (CJM006, 1:100,000, C. Munro, University of California, Davis) was used for both species. A 96-well microtiter plate was pre-coated with secondary goat anti-rabbit IgG antibody (150 μL/well at 0/10 mg/mL, A009, Arbor Assays, Ann Arbor, MI, USA) using the standard Arbor Assay methodology. The inter- and intra-assay coefficients of variation (CV) were maintained below 15% and 10%, respectively. The corticosterone antibody cross-reacts at 100% with corticosterone, 14.25% with desoxycorticosterone, 2.65% with progesterone, 0.90% with tetrahydrocorticosterone, 0.64% with testosterone, 0.23% with cortisol, and less than 0.10% for five other steroids tested (C. Munro).

The corticosterone EIA was validated by showing: (1) parallelism between binding inhibition curves of a corticosterone standard curve and a serially diluted pool of fecal extractions for each species, (2) a biological validation for each species due to a significant increase (less than two standard deviations over baseline) in fGM concentrations within 48 h of an invasive veterinary procedure (cheetahs) or an exhibit transfer (giraffes) (Appendix D), and (3) no significant cross-reactivity with fGM concentrations and androgen concentrations.

Data Analysis

Analyses were conducted using IBM SPSS Statistics for Windows (version 21.0). A repeated measure mixed two-way ANOVA model was used to determine potential differences in average fGM concentrations for each treatment. Shapiro–Wilks tests of normality revealed non-normal datasets for both the cheetah and giraffe fGM data. Once log-transformed, all data were normally distributed except for data included in B10184: Reopen ($p = 0.037$). However, due to the small sample size and the robustness of the ANOVA tests, this break in assumption was ignored. Comparison of studentized residuals indicated no extreme outliers (no studentized residuals exceeded ± 3) for either species. Levene's test of homogeneity ($p > 0.05$) and Box's M test ($p > 0.001$) revealed homogenous variances and covariances, respectively. For cheetahs, Mauchly's test of Sphericity indicated a violation, $\chi^2 =$

14.253, $p = 0.014$, and the Huynh–Feldt epsilon adjustment was used to circumnavigate this violation. Neither cheetahs, ($F_{(2.584, 72.349)} = 9.937$, $p = 0.136$, partial $\eta^2 = 0.065$, $\varepsilon = 0.861$, Huynh-Feldt adjustment), nor giraffes, ($F_{(3,84)} = 0.934$, partial $\eta^2 = 0.032$, $p = 0.428$) had significant differences between individuals and were, therefore, analyzed within species-specific groups. All data is reported after having been back-transformed, unless otherwise stated.

Behavior

Previously developed ethograms used at the Oregon Zoo for ongoing welfare monitoring were replicated for this project (Lewis 2020: Tables 27 and 28). One-minute scan sampling occurred over 20-min observation periods twice per day (1000 and 1430) two days a week (see Table 26 for observation dates). Due to the strong likelihood of care staff presence influencing behavior, any observation during which a member of the care staff was present was omitted from the analysis.

Table 26: List of treatments and their corresponding dates and observation counts. Uneven observation counts are due to restrictions from COVID-19 safety protocols. Asterisks (*) indicate observations from general monitoring (60 min observations instead of 20 min observations) and conducted by volunteers. ^{A}: indicates variable observation dates due to the lack of exhibit access because of routine habitat maintenance.

Cheetah				
	Category	Sample Dates	Observation Count (B10183)	Observation Count (B10184)
Transition Period 1	Open *	2/11/2020–3/15/2020	3	2
	Closed (A)	3/16/2020–4/4/2020 ^{A}	8	8
Transition Period 2	Closed (B)	6/16/2020–7/12/2020 ^{A}	14	14
	Reopen	7/13/2020–8/8/2020	13	13
Giraffe				
	Category	Sample Dates	Observation Count (B20186)	Observation Count (B60180)
Transition Period 1	Open *	1/18/2020–3/15/2020	4	4
	Closed (A)	3/16/2020–4/12/2020 ^{A}	8	8
Transition Period 2	Closed (B)	6/9/2020–7/12/2020 ^{A}	16	16
	Reopen	7/13/2020–8/8/2020	12	12

Each species was observed from the general visitor viewing areas. Due to restrictions from COVID-19 safety protocols, uneven observation sampling was inevitable. Behavioral observations for the category “Open” were conducted through the general welfare monitoring program at the Oregon Zoo prior to the commencement of the study and were 60-min observation periods, rather than 20-min observation periods, conducted by the first author for the rest of the study. It is important to note that the 60-min observations were conducted by volunteers, rather than the main researcher. However, each volunteer was required to pass a reliability test in order to collect behavior data (behavior observation reliability >80% compared with the author’s observations using procedures from Wark et al. (2021). We therefore felt that these observations could be included in the overall analyses. To make the data comparable over the different observation durations, all count data were converted into percent of behavioral expression using the following equation:

$$\%\ Behavior\ Expression = \frac{Sum\ of\ specific\ behavior\ count\ data}{Sum\ of\ cumulative\ observation\ period} \times 100$$

Table 27: Pre-established cheetah ethogram (Lewis 2020)

All-Occurrence Behavior	**Description**
charge glass	Charge towards glass ending within one body length of the glass; may or may not include a strike or hiss
glass strike	Forceful paw contact with glass
hiss	Lips pulled back to bare teeth and emit sound
Interval Behaviors	**Description**
not visible	Individual out of sight or unable to determine behavior at interval
keeper visible	Keeper is present—can be actively interacting with focal animal or just walking past
environmental interaction	Individual is actively engaged with an element of its environment; does not include interaction with zoo visitors or inactive contact with environment (e.g., laying down on rocks) or incidental contact with exhibit furniture

stereotypy	Locomotor stereotypy: walking from one point to another, turning and walking back to the starting point, or walking in a loop/to-and-fro, for more than three repetitions without interruption.
social interaction	Any active social interaction with another cheetah, regardless of who instigated it
locomotion	Any movement that transports the animal more than one body length forward, backward, or sideways at any speed; includes walk, trot, run or jump
groom	Focal animal is engaged in self-grooming, licking, chewing, scratching (self)
stationary	Not deliberately exhibiting locomotion behaviors for at least three seconds; can be alert (head up, eyes open) and resting (head down or head up with closed eyes)

Table 28: Pre-established giraffe ethogram (Lewis 2020)

All-Occurrence Behavior	**Description**
urine testing	Using flehmen reaction specifically at urine
flehmen	Upper lip curled back and inhalation
interaction with Speke's gazelle	Any interaction between giraffes and resident Speke's gazelle
interaction with hornbills	Any interactions between giraffes and resident hornbills
run	Cantering or sprinting
lay down	Any instance when the giraffe has its stomach on the ground
Interval Behaviors	Description
not visible	Individual out of sight or unable to determine behavior at interval
keeper visible	Keeper is present—can be actively interacting with focal animal or just walking past
eat	Individual is actively eating from designated food stations or keeper-provided browse elements
environmental interaction	Individual is actively engaged with an element of its environment; does not include interaction with zoo visitors or inactive contact with the environment (e.g., laying down on rocks) or incidental contact with exhibit furniture.
stereotypy	Locomotor stereotypy: walking from one point to another, turning and walking back to the starting point, or walking in a loop/to-and-fro, for more than three repetitions without interruption; non-locomotor stereotypy: repetitive licking/tongue flagging
social interaction	Any active social interaction with another giraffe, regardless of who instigated it
locomotion	Any movement that transports the animal more than one body length forward, backward, or sideways at any speed; includes walk, trot, run or jump
groom	Focal animal is engaged in self-grooming, licking, chewing, scratching (self)
vigilant	Standing still with an erect neck and actively observing (rather than scanning) the environment (similar to that defined by Cameron and du Toit (Cameron and du Toit 2005))

stationary	Not deliberately exhibiting locomotion behaviors for at least three seconds; can be alert (head up, eyes open) and resting (head down or head up with closed eyes)

Data Analysis

Analyses were conducted using IBM SPSS Statistics for Windows (version 21.0). Two behavior analyses were conducted per focal animal, comparisons of percent of time spent engaging in each behavior across zoo opening status and time of day. For both species, Shapiro–Wilks tests and visual observations of QQ plots revealed non-normal ($p < 0.05$) data despite transformations. Therefore, non-parametric Kruskal–Wallis tests were conducted on the original, untransformed data. For both species, distributions of behavior proportions were not similarly shaped across each opening status or time of day, as assessed by visual inspections of boxplots. Data are reported as differences in mean ranks of behavior proportions by zoo opening status or time of day. Each individual focal animal was analyzed separately.

Results

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Cheetah

There was a significant increase in fGM concentrations between Transition Period 1 and Transition Period 2. There was no significant difference between the samples collected within each transition period. The repeated measures mixed two-way ANOVA model indicated that these differences in fGM concentrations were statistically significant based on zoo opening status, ($F_{(2.584,\ 72.349)} = 9.937$, $p < 0.001$, partial $\eta^2 = 0.262$, Huynh-Feldt adjustment) (Table 29, Figure 33A).

Giraffe

There were statistically significant differences in fGM concentrations between the different treatments, ($F_{(3,84)} = 4.154$, $p = 0.009$, partial $\eta^2 = 0.129$). Similar to cheetahs, there was no statistically significant difference between the samples collected within each treatment period. However, there was a statistically significant increase in average fGM concentration of 22.7 ng/g ($p = 0.027$) between samples collected during “Closed (A)” and during “Reopen”. This indicates a

Table 29: Results of cheetah and giraffe fGM concentration analysis. Repeated measures two-way mixed ANOVA model comparing different zoo opening statuses. The mean difference results compare fGM concentrations in (I) trials with those in (II) trials. Asterisks (*) indicate significant ($p < 0.05$) differences between trial (I) and trial (II). ^{a} Bonferroni correction for multiple comparisons.

	(I) Trial	(II) Trial	Mean Difference (A-B) (ng/g)	Sig^a
		Cheetah		
Transition Period 1	Open	Closed (A)	−6.06	1.00
		Closed (B) *	−87.46 *	0.008 *
		Reopen *	−79.63 *	<0.001 *
	Closed (A)	Open	6.06	1.00
		Closed (B) *	−81.40 *	<0.001 *
		Reopen *	−73.56 *	0.002 *
Transition Period 2	Closed (B)	Open *	87.46 *	0.008 *
		Closed (A) *	81.40 *	<0.001 *
		Reopen	7.83	1.00
	Reopen	Open *	79.63 *	<0.001 *
		Closed (A) *	73.56 *	0.002 *
		Closed (B)	−7.83	1.00
		Giraffe		
Transition Period 1	Open	Closed (A)	11.13	0.053
		Closed (B)	0.00	1.000
		Reopen	−11.57	1.000
	Closed (A)	Open	−11.13	0.053
		Closed (B)	−11.13	0.262
		Reopen	−22.70 *	0.016 *
Transition Period 2	Closed (B)	Open	0.00	1.000
		Closed (A)	11.13	0.262
		Reopen	−11.57	1.000
	Reopen	Open	11.57	1.000
		Closed (A)	22.70 *	0.016 *
		Closed (B)	11.57	1.000

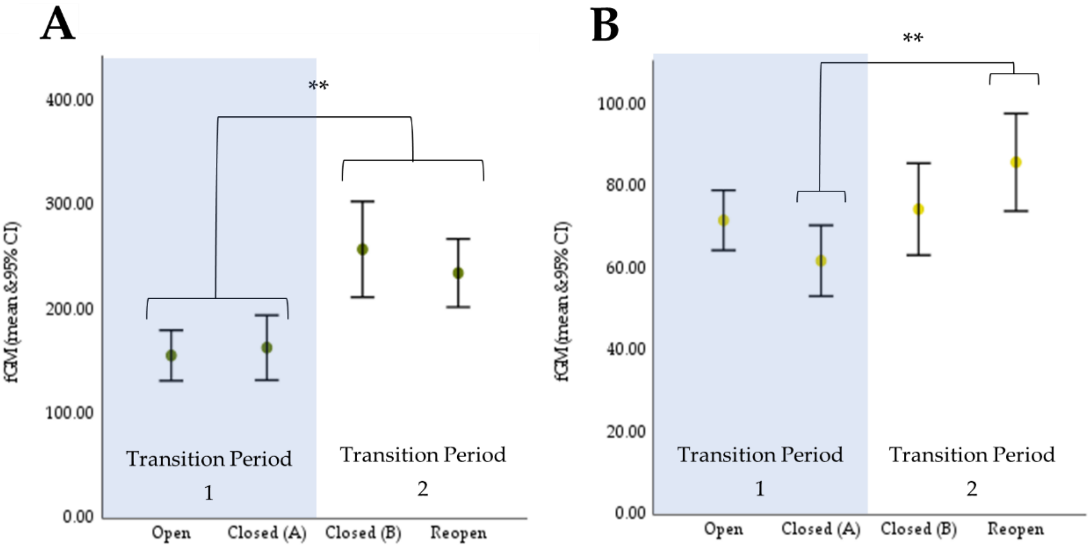


Figure 33: Results from repeated measures mixed 2-way ANOVA models. Asterisks (**) indicate statistical significance (*p*-value < 0.01). Data are presented back transformed from log-transformed data. Error bars are 95% CI. A) Average cheetah fGM concentrations. B) Average giraffe fGM concentrations.

significant difference in average fGM concentration between the end of Transition Period 1 and the end of Transition Period 2 (Table 29, Figure 33B).

Behavior

Cheetah

Table 30: Significant (*p*-value < 0.05) results of the behavior engagement analysis for cheetah based on the Zoo's opening status. Pairwise comparisons (Dunn 1984) were conducted on significant results from Kruskal–Wallis (H) tests. Asterisks (*) indicate adjusted p-values.

Cheetah B10183						
Behavior	Treatment (I)	Treatment (II)	Mean Rank (I)	Mean Rank (II)	Direction of Change	Adj. p-Value *
not visible	Open	Reopen	10.00	28.27	↑	0.034
	Closed (A)	Reopen	15.25	28.27	↑	0.030
	Closed (B)	Reopen	15.82	28.27	↑	0.010
stationary	Open	Reopen	30.50	12.31	↓	0.043

The Kruskal–Wallis (H) tests revealed two significant changes in behavior frequencies for cheetah B10183 and no significant differences in behavior frequencies for cheetah B10184, based on the Zoo's opening status (Figure 34). The

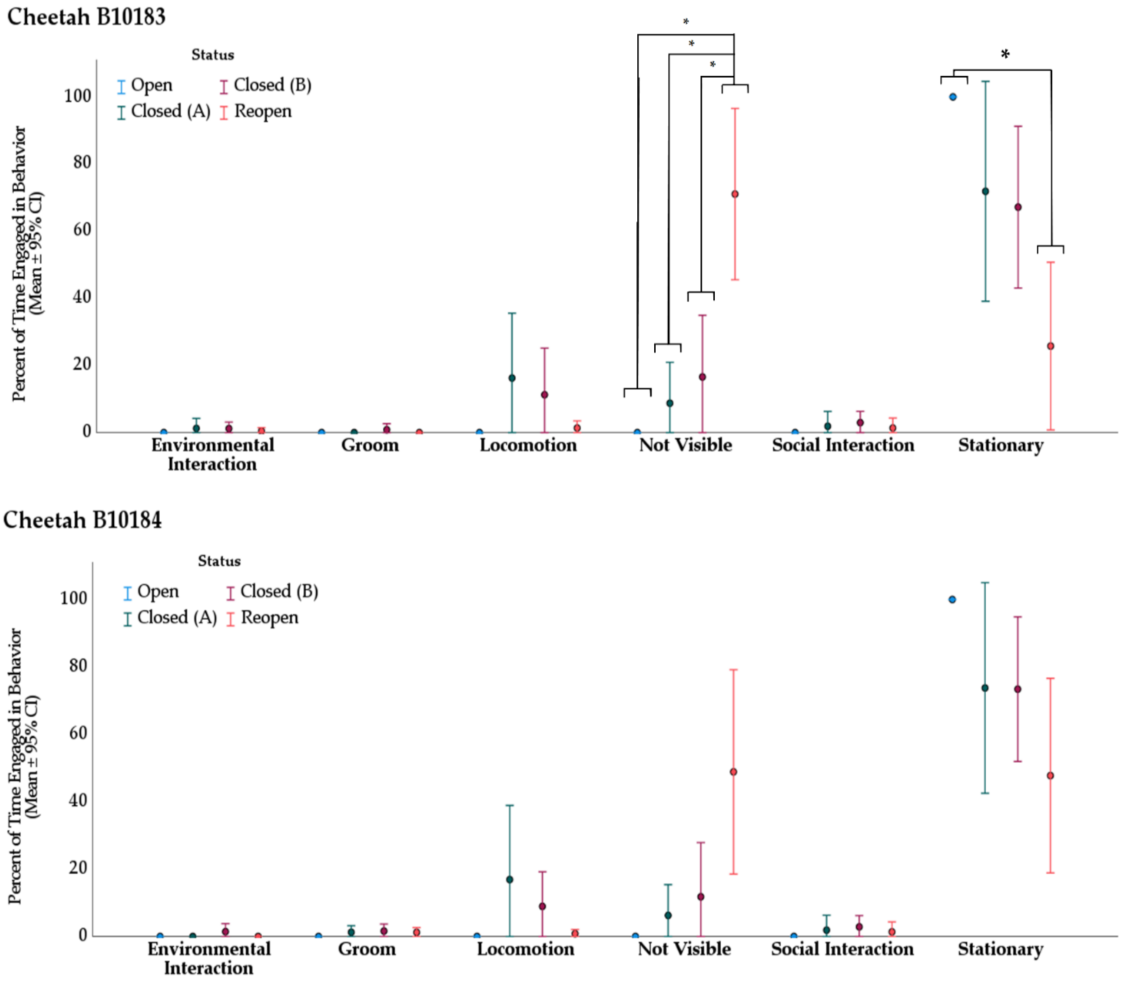


Figure 34: Cheetah average behavior expression proportion (%). Proportion indicates percent of total observation time conducting the behavior. Error bars are 95% confidence intervals. Asterisks (*): statistical significance (*p*-value < 0.05). Observations in category "Open" were 60-minutes and conducted by volunteers. All others were 20-minutes and conducted by the first author.

mean ranks of cheetah B10183′s behavior frequencies were statistically different between the Zoo's opening status for "not visible", $\chi^2(3) = 15.07$, $p = 0.002$, and "stationary", $\chi^2(3) = 10.81$, $p = 0.013$. Subsequently, pairwise comparisons (Dunn 1964, Bonferroni correction for multiple comparisons) were conducted (Table 30).

Giraffe

Kruskal–Wallis (H) tests revealed one difference in behavior frequency for giraffe B20186 and three significant changes in behavior frequency for giraffe

B60180 based on zoo opening status (Figure 35). For giraffe B20186, there was a significant difference in behavior frequency for “vigilant”, $\chi^2(3) = 8.378$, $p = 0.039$. For giraffe B60180 significant differences in behavior frequencies were present for “environmental interaction”, $\chi^2(3) = 10.688$, $p = 0.014$; “stationary”, $\chi^2(3) = 12.761$, $p = 0.005$; and “vigilant”, $\chi^2(3) = 17.826$, $p < 0.001$).

Table 31: Significant (*p*-value < 0.05) results of the behavior engagement analysis for giraffe based on zoo opening status. Pairwise comparisons (Dunn 1984) were conducted on significant results from Kruskal–Wallis (H) tests. Asterisks (*) indicate adjusted *p*-values (Bonferroni correction for multiple comparisons).

Giraffe B20186						
Behavior	Treatment (I)	Treatment (II)	Mean Rank (I)	Mean Rank (II)	Direction of Change	Adj. p-Value *
vigilant	Closed (A)	Reopen	29.63	15.79	↓	0.039
Giraffe B60180						
environmental interaction	Closed (A)	Closed (B)	8.81	23.97	↑	0.013
	Closed (A)	Reopen	8.81	23.42	↑	0.030
stationary	Open	Closed (B)	34.88	17.81	↓	0.016
	Open	Reopen	34.88	16.29	↓	0.009
vigilant	Closed (A)	Closed (B)	32.81	17.59	↓	0.003
	Closed (A)	Reopen	32.81	14.67	↓	0.001

Subsequent pairwise comparisons (Dunn 1964) were conducted on each of the significant results. *p*-values are reported with Bonferroni corrections to account for multiple comparisons (Table 31).

Discussion

This study took advantage of a unique opportunity of prolonged closure of the Oregon Zoo due to the COVID-19 pandemic to provide a novel contribution to the body of research on the impact of visitor presence on two sensitive yet popular species. Our initial hypotheses were: (1) fGM concentrations would decrease in periods without visitors, but this effect would be smaller in the first transition period than the second one, due to potential acclimation to a lack of visitors over

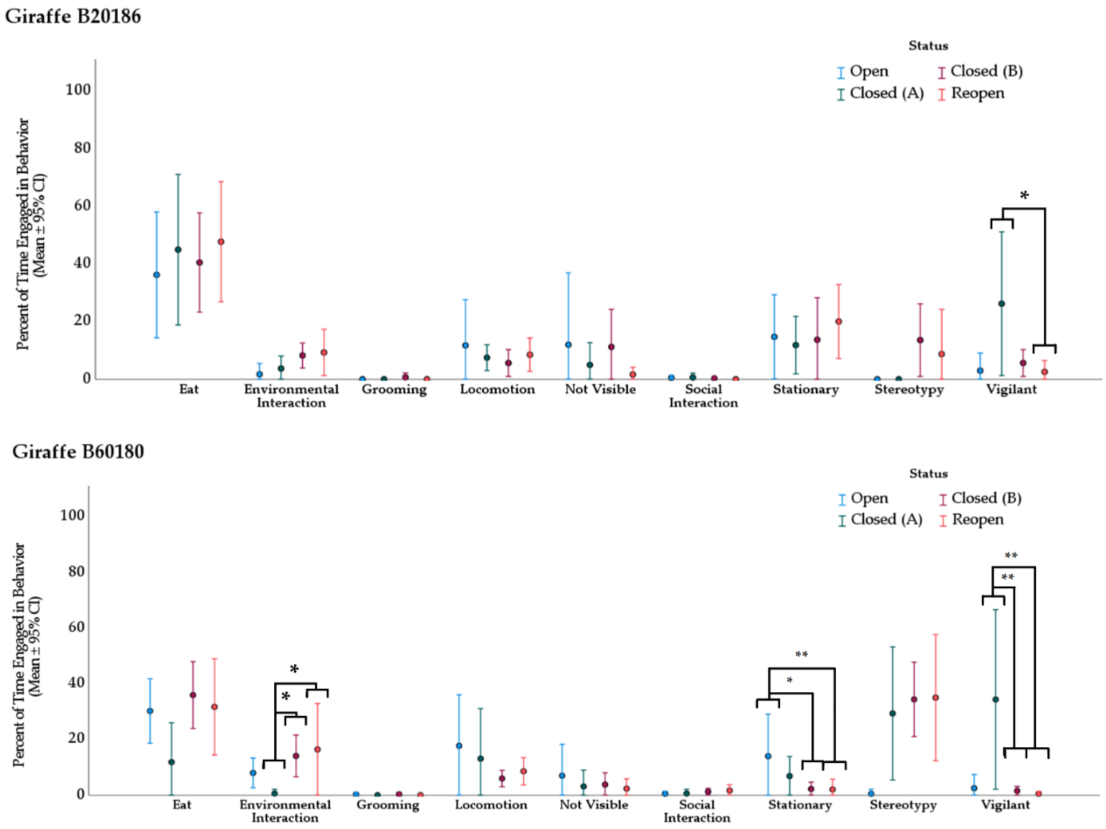


Figure 35: Giraffe average behavior expression proportion (%). Proportion indicates percent of total observation time conducting the behavior. Error bars are 95% confidence intervals. Asterisks (*): significance at *p*-value < 0.05, (**): significance at *p*-value < 0.01. Observations in category "Open" were 60-minutes and conducted by volunteers. All others were 20-minutes and conducted by the first author.

four months of closure; and (2) there would be a transition to more exploratory behaviors and less time spent hidden without visitors. We found that the first hypothesis was partially supported by giraffe fGM concentrations, and the second hypothesis was partially supported by the behavior data obtained for both species. The results were more complex than expected.

For the two male cheetahs, significant increases in average fGM concentrations were observed between the two transition periods, but not within each transition period. The fGM concentrations of treatments "Open" and "Closed (A)" were both significantly lower than the fGM concentrations of treatments

“Closed (B)” and “Reopen”, While the overall average fGM concentration increased, the differences within the actual transitions were minimal. This indicates that there may have been some different factors (aside from visitor presence or absence) that were underlying the observed significant increases in fGM concentrations. A study by Uetake et al. (2014) revealed a trending decrease in cheetah fGM concentrations within the summer months and high fGM concentrations on the day after a strong decrease in air temperature—hypothesizing sensitivity to cold temperatures. This is contradictory to that revealed in our study—indicating that it may not be simply seasonal fluctuations causing the increased fGM concentrations in cheetahs at the end of the summer. However, both cheetahs were subject to more frequent veterinary exams and treatments than usual during the study period. Research has shown that increased veterinary care and repeated veterinary procedures can lead to increased glucocorticoid secretion, in spite of the overall beneficial effects for the individual (Vonderen, Kooistra, and Rijnberk 1998). While we attempted to minimize the effect of veterinary exams on the study by choosing data samples that avoided major procedures, previous injuries and some dental issues observed in the cheetahs resulted in three veterinary procedures for cheetah B10184 and one for cheetah B10183 between April 2020 and August 2020. The effects of these exams may have had a substantial and lingering effect on fGM concentrations during the study period. These medical procedures were, most likely, influential factors on the significant difference in fGM concentrations between the two transition periods and may have overshadowed differences in fGM concentrations based on visitor presence. Without these medical procedures, it is possible that the cheetahs would have better followed patterns found by previous researchers that indicate significant increases in fGM concentrations in cheetahs housed on-exhibit verses off-exhibit (Baird 2018). Several of the procedures were considered to be relatively minor in terms of invasiveness of medical procedures, yet stress responses are individualistic in nature and depend on the individual animal's history and perception of the event. However, there was one surgical procedure for cheetah B10184 on 14 June 2020. In addition, both cheetahs historically had issues with mutual reintroductions after veterinary procedures and this also may have affected their respective fGM concentrations—regardless of which cheetah experienced the veterinary procedure.

Similarly, the samples collected for the giraffe analyses also showed minimal differences within transition periods, but significant differences between the different transition periods. While periods without visitors were found to be lower in fGM concentrations than the samples associated with visitor presence, these differences were statistically insignificant. However, the trending increase in fGM concentrations within the reopening transition period was similar to other visitor presence studies on ungulates (Indian blackbuck—*Antilope cervicapra*, Rajagopal, Archunan, and Sekar 2011, chamois—*Rupicapra rupicara*, Zwijacz-Kozica et al. 2012). Again, the sample dates were chosen to avoid intense stressors that may obscure a possible effect of the visitor transition periods. In May 2020, a two-year-old female Masai giraffe (C00028) was added to the herd. While giraffe C00028 did not show active progesterone cycles during the study, the addition of a female to our all-male herd caused social changes that may have influenced the fGM concentrations of both male giraffes during and at the end of the summer close to the second transition period. Additionally, research has shown an increase in adrenal hormone concentrations for wild African ungulates during the dry season (Cizauskas et al. 2015), similar to the patterns of fGM concentration changes observed during this study. In Portland, Oregon, the dry season stretches from mid-May to mid-October (Anon n.d.). Our male giraffes had significantly higher fGM concentrations in the samples collected in July than March, which followed the seasonality patterns seen in other ungulates.

Individuals of both species experienced significant changes that were social in nature for the giraffes and medical for the cheetahs between the Zoo's closure and reopening periods that may explain at least some portion of the increasing fGM concentrations for the second transition period. However, the Zoo's opening status may still have added to the overall effect of increased fGM concentrations observed after reopening the Zoo. It is possible that the absence of visitors temporarily prevented a larger compounding effect of visitor presence and social/health impacts in each species.

Nevertheless, comparisons of individual behavior frequencies resulted in multiple significant differences for each species; however, none of these significant differences occurred within a transition period. For instance, the giraffes showed significant increases in vigilance, environmental interaction, and significant decreases in stationary behaviors, while one cheetah showed a significant increase in time spent ‘not visible’ and decreased stationary behaviors.

The giraffes were found to be more vigilant when the Zoo was initially closed, however, this difference was statistically trending. This slight change may have been due to the sudden lack of foot traffic associated with the closure between “Open” and “Closed (A)”. The results indicated possible acclimation to the lack of visitors, with both giraffe B60180 and giraffe B20186 showing less vigilant behaviors between “Closed (A)” and “Closed (B)”. When the Zoo is open, visitors tend to move quickly and consistently through the area, possibly causing something akin to a ‘white noise’ effect, and individual spectators may not be noticed much by the giraffes. However, when the Zoo is closed, the giraffes may pay more attention to single individuals (such as care staff or security) on the observation decks. Previous research supports this hypothesis by showing an increased rate of vigilant behaviors in ungulates with decreasing human activity (Brown et al. 2012). Additionally, research conducted on various zebra exhibits found that alert (in some papers, alert and vigilance share the same definition) and locomotive behaviors tended to decrease as visitor numbers increased (Conte 2014). Interestingly, a recent study showed a positive correlation in the time the giraffes spent ‘observing the observer’ (author definition of vigilance behavior, Scheijen et al. 2021, page six) and number of observers present. However, this study had a maximum of three observers—drastically less than the typical visitor numbers experienced in a zoo setting—and the quantity of observers may not have reached the ‘white noise’ effect. Another potential explanation for the increased vigilance behavior is the possibility of food begging. Prior to the closure, both male giraffes were being conditioned to hand feeding from visitors located on a feeding platform. It is possible that the giraffes were looking for the observer to provide food. This follows the pattern discussed by Lynn (2018), who theorized that the presence of visitors on a platform may increase attentiveness (similar behavior to 'vigilance' in our study) of giraffes due to the giraffes' association of visitor presence and food. However, our study varies from this previous research in that vigilance increased with decreasing visitor presence. This indicates that the difference in vigilance we observed may be a combination of food-begging and the ability for the giraffes to focus on a single visitor rather than the 'white noise' of the popular visitation periods. There were other significant differences in behavior for each giraffe, but they were also between different transitional periods and most likely related to other stressors outside visitor presence. For instance, the significant increases between environmental interactions in "Closed (A)" and both "Closed (B)" and "Reopen" for giraffe B60180 may be partially explained by the changes in season. The earliest leaf budding (lilacs and honeysuckle) was reported in early March 2020 (USA National Phenology Network 2018) and the giraffes may have been searching for new buds. As our ethogram only categorizes eating behavior as observable feeding, foraging behaviors that did not result in successful eating were considered as environmental interactions. Nevertheless, the changes are most likely associated with the significant increase in vigilant behaviors. As we only score one behavior per scan, if an individual is expressing one behavior it means they are not expressing another. For “Closed (A)”, there was a dramatic increase in vigilant behaviors that directly resulted in a loss of expression of all other behaviors. This is common when using scan-sampling techniques and necessitates the conversion of behavior occurrences into proportions prior to data analyses (Martin and Bateson 2007). Additionally, there were differences in overnight housing due to low temperatures in the first transition period. While all observations were conducted when the giraffes were in their outdoor habitat, it is possible that the changes in overnight housing may have affected the behavior frequencies and require further investigation.

For the cheetahs, the increased time spent ‘not visible’ was the most notable response to the effect of visitor absence. Unlike the differences in the giraffes' behavior expressions, there was a significant increase in time spent ‘not visible’ within the second transitional period. This indicates that visitor presence may have a stronger influence on this behavioral difference than other stressors. Frequently, an animal’s increased avoidance of visitor viewing areas is used as an indication of negative visitor effects (Ross et al. 2009; Sherwen, Harvey, et al. 2015; Sherwen, Magrath, et al. 2015). For the cheetahs at the Oregon Zoo, observing such a strong increase in time spent ‘not visible’ may indicate they had acclimated to the prolonged time of visitor absence. We note that there was not a decrease in time spent ‘not visible’ when the Zoo was first closed, which would have been a strong indication that visitor presence directly influenced cheetah exhibit use. However, this may be attributed to the presence of a heated area located at the front of the exhibit in which the cheetahs frequently nap during the colder months. This explanation is also supported by the inverse relationship between time spent 'not visible' and stationary behaviors with the least time spent 'not visible' corresponding with the most stationary behaviors. These findings support the hypothesis that the cheetahs at the Oregon Zoo acclimated to the long period without visitors, but it also indicates that the time of year and various environmental factors may have a strong influence on behavior responses.

Additionally, evolutionary backgrounds of either species may have contributed to the differing behavior expressions. A comprehensive study by Queiroz and Young (2018) provided empirical evidence for behavioral and evolutionary traits that could predict which species may be the most sensitive to visitor presence. The researchers found that herbivorous, terrestrial, diurnal species from historically closed habitats would likely be the most vulnerable to visitor presence (Queiroz and Young 2018). Giraffes are an herbivorous, terrestrial species that follows a diurnal activity pattern, albeit mostly found in more open habitat. According to their evolutionary history, giraffes may, therefore, be somewhat more susceptible to behavior changes based on visitor presence compared with other non-herbivorous species. Cheetahs, however, are crepuscular or diurnal terrestrial predators. Based on Queiroz and Young's predictive traits, the cheetahs should be less susceptible to visitor presence than giraffes. However, among medium-to-large predators, cheetahs are known to be more timid and to usually avoid confrontation.

From an evolutionary perspective, cheetahs mostly need to outrun their predators and competitors, making them also potentially more vulnerable to visitor impact than other same-size or larger predators. Previous research has also shown that wild cheetahs choose environments with greater numbers of large predators (e.g., lions and leopards) over environments with human settlements and may view humans as a larger threat (Klaassen and Broekhuis 2018). Additional research conducted by Terio et al. (2004), indicating significantly higher fGM concentrations in captive cheetahs than free-ranging cheetahs, supports this idea. In many zoos, including the Oregon Zoo, cheetahs are housed in exhibits with combined 'off-exhibit' (no visitor viewing) and 'on-exhibit' (visitor viewing) access. However, to maximize the visitor viewing experience, many times cheetahs are only allowed access to their off-exhibit areas at night. This can result in limited retreat options for cheetahs and force cheetahs to remain in an environment that prevents the expression of their natural hiding and escape behaviors, possibly affecting changes in behavior expression (e.g., more hiding, less restful behaviors) and increased fGM concentrations. This was reported by Baird (2018), who found significantly higher fGM concentrations in cheetahs housed on-exhibit than those housed off-exhibit. In summary, it is essential to fully understand a species' natural history and behaviors when designing visitor accessibility to minimize the potential negative effects of visitor attendance.

While this study revealed some significant changes in behavior and minimal changes in fGM concentrations associated with transitions in visitor attendance, we acknowledge the limitations of the study. Since behavioral data for the "Open" observation period were collected through general welfare monitoring sessions (prior to the official start of the study) rather than in conjecture with a formal study, observations did not occur as frequently as for the other categories. These data, while limited in frequency, were important to include as they do represent behaviors observed for both species within the time frame for baseline data observation, while visitors were present under pre-COVID conditions. Second, only two individuals per species were analyzed, since these represented the available study population for the species at Oregon Zoo at the time when the study started. Third, behavior expression and fGM concentration reactions are unique to both species and to the individuals within each species. Multiple individuals of the same species can be exposed to the exact same stressor but show differing physiological and behavioral responses, depending on genetics, life experience, gender, and age.

Finally, the effect of each transition period appeared to be relatively minor for each of the species and individuals. This study highlights that visitor presence is less influential than other factors, such as veterinary procedures, social changes, and seasonal fluctuations. This is an important conclusion; visitor presence is a factor in almost all zoo animals' environment. While visitor presence may be a low-grade chronic stressor, it is not as influential as other factors the animals experience. It is, therefore, of major importance to carefully document and consider all life events possibly impacting an individual's experience when analyzing data obtained for the purpose of visitor presence studies.

Conclusions

While the global COVID-19 pandemic has had many negative impacts on zoos and aquaria, especially from a financial perspective, it did allow for an unusual study opportunity of zoo animals without any visitors present. This has allowed us to gain more insight into which behaviors cheetahs and giraffes at the Oregon Zoo may exhibit when only in contact with care staff. The current study was an opportunistic attempt to document behavior and hormonal changes observed during periods of transition in visitor access in two mammal species we had been already monitoring.

The results of this study indicate two important conclusions: (1) there were significant differences in the adrenal activity of giraffes and cheetahs at the Oregon Zoo between transition periods but not within them and (2) there was only one significant difference in behavior frequency (cheetah: ‘not visible’) that occurred within a transition period. All other significant behavioral changes were between the two different visitor transition periods. This indicates visitor presence may less prominently affect behavior an fGM concentrations on zoo-housed cheetahs and giraffes than other factors, such as seasonal fluctuations, social changes, or medical procedures.

While we note the limitations of this study (small sample size, uneven observation counts, and possible compounding effects), we believe that some of the results may warrant further examination. The finding of an increased amount of time spent ‘not visible’ for cheetahs when the Oregon Zoo reopened may indicate a need for additional retreat areas to provide choices for cheetah to move further away from the public and have a comfortable space to do so, when needed. For giraffe, the increased vigilance behavior may indicate a heightened sensitivity to small amounts of visitors or small groups rather than large consistently moving groups and this phenomenon might benefit from further studies, to look at the impact of small visitor group access afterhours or for giraffe feeding.

Finally, we reiterate the individualistic nature of both behavioral and adrenal responses to stressors and to emphasize the difficulty in crediting these changes to a single cause given the various changes that occurred concomitantly with changes in visitor presence.

References

Anon. n.d. “About the Oregon Zoo.” Retrieved January 1, 2021a (https://www.oregonzoo.org/about/about-oregon-zoo).

Anon. n.d. “Average Weather in Portland, Oregon, United States, Year Round - Weather Spark.” *Cedar Lake Ventures, Inc.* Retrieved July 6, 2021b (https://weatherspark.com/y/757/Average-Weather-in-Portland-Oregon-United-States-Year-Round).

Baird, Bonnie A. 2018. “Ambassador Animal Welfare: Using Behavioral and Physiological Indicators to Assess the Well-Being of Animals Used for Education Programs in Zoos.” Case Western Reserve University.

Bashaw, Meredith J., Florian Sicks, Rupert Palme, Franz Schwarzenberger, Adrian S. W. Tordiffe, and Andre Ganswindt. 2016. “Non-Invasive Assessment of Adrenocortical Activity as a Measure of Stress in Giraffe (*Giraffa Camelopardalis*).” *BMC Veterinary Research* 12(1):235. doi: 10.1186/s12917-016-0864-8.

Brown, Casey L., Amanda R. Hardy, Jesse R. Barber, Kurt M. Fristrup, Kevin R. Crooks, and Lisa M. Angeloni. 2012. “The Effect of Human Activities and Their Associated Noise on Ungulate Behavior.” *PLoS ONE* 7(7). doi: 10.1371/journal.pone.0040505.

Bryant, Jocelyn, and Nadja C. Wielebnowski. 2018. “Environmental Impact on Activity Level and Fecal Glucocorticoid Metabolite Concentration of African Elephants and Black Rhinoceros at Brookfield Zoo.” *International International Journal of Avian & Wildlife Biology* 3(2):101–7. doi: 10.15406/ijawb.2018.03.00064.

Cameron, Elissa Z., and Johan T. du Toit. 2005. “Social Influences on Vigilance Behaviour in Giraffes, *Giraffa Camelopardalis*.” *Animal Behaviour* 69(6):1337–44. doi: 10.1016/j.anbehav.2004.08.015.

Chiew, Samantha J., Kym L. Butler, Kerry V. Fanson, Simon Eyre, Grahame J. Coleman, Sally L. Sherwen, Vicky Melfi, and Paul H. Hemsworth. 2021. “Effects of the Presence of Zoo Visitors on Zoo-Housed Little Penguins (*Eudyptula Minor*).” *New Zealand Journal of Zoology* 0(0):1–22. doi: 10.1080/03014223.2021.1896560.

Choo, Yuanting, Peter Alan Todd, and Daiqin Li. 2011. “Visitor Effects on Zoo Orangutans in Two Novel, Naturalistic Enclosures.” *Applied Animal Behaviour Science* 133(1–2):78–86. doi: 10.1016/j.applanim.2011.05.007.

Cizauskas, Carrie A., Wendy C. Turner, Neville Pitts, and Wayne M. Getz. 2015. “Seasonal Patterns of Hormones, Macroparasites, and Microparasites in Wild African Ungulates: The Interplay among Stress, Reproduction, and Disease” edited by S. Sreevatsan. *PLOS ONE* 10(4):e0120800. doi: 10.1371/journal.pone.0120800.

Conte, Christine. 2014. “Do Visitors Affect Zebra Behavior in Zoos?” *Biological Sciences* 3(2):1–30.

Davey, Gareth. 2007. “Visitors’ Effects on the Welfare of Animals in the Zoo: A

Review." *Journal of Applied Animal Welfare Science* 10(2):169–83.

Fanson, Kerry V., Michael Lynch, Larry Vogelnest, Gary Miller, and Tamara Keeley. 2013. "Response to Long-Distance Relocation in Asian Elephants (*Elephas Maximus*): Monitoring Adrenocortical Activity via Serum, Urine, and Feces." *European Journal of Wildlife Research* 59(5):655–64. doi: 10.1007/s10344-013-0718-7.

Farrand, Alexandra. 2007. "The Effect of Zoo Visitors on the Behaviour and Welfare of Zoo Mammals." University of Stirling.

Fazio, Jilian M., Elizabeth W. Freeman, Erika Bauer, Larry Rockwood, Janine L. Brown, Katharine Hope, Jessica Siegal-Willott, and E. C. M. Parsons. 2020. "Longitudinal Fecal Hormone Monitoring of Adrenocortical Function in Zoo Housed Fishing Cats (*Prionailurus Viverrinus*) during Institutional Transfers and Breeding Introductions" edited by G. Umapathy. *PLOS ONE* 15(3):e0230239. doi: 10.1371/journal.pone.0230239.

Frank, BrieAnna J. 2020. "Phoenix Zoo Working to Combat Loneliness Felt by Animals." *Arizona Republic*. Retrieved February 19, 2021 (https://www.azcentral.com/story/news/local/phoenix/2020/04/04/phoenix-zoo-working-combat-loneliness-felt-animals-during-coronavirus/2948477001/).

Grandin, T. 1997. "Assessment of Stress during Handling and Transport." *Journal of Animal Science* 75(1):249–57. doi: 10.2527/1997.751249x.

Hosey, G., R. 2000. "Zoo Animals and Their Human Audiences : What Is the Visitor Effect?" *Animal Welfare* 9:343–57.

Klaassen, Britt, and Femke Broekhuis. 2018. "Living on the Edge: Multiscale Habitat Selection by Cheetahs in a Human-Wildlife Landscape." *Ecology and Evolution* 8(15):7611–23. doi: 10.1002/ece3.4269.

Larsen, Megan J., Sally L. Sherwen, and Jean Loup Rault. 2014. "Number of Nearby Visitors and Noise Level Affect Vigilance in Captive Koalas." *Applied Animal Behaviour Science* 154:76–82. doi: 10.1016/j.applanim.2014.02.005.

Lewis, Karen. 2020. "Oregon Zoo General Monitoring Ethogram - Giraffe."

Lewis, Karen (Oregon Zoo). 2020. "Oregon Zoo General Monitoring Ethogram - Cheetah."

Loeding, Erin, Jeanette Thomas, Dave Bernier, and Rachel Santymire. 2011. "Using Fecal Hormonal and Behavioral Analyses to Evaluate the Introduction of Two Sable Antelope at Lincoln Park Zoo." *Journal of Applied Animal Welfare Science* 14(3):220–46. doi: 10.1080/10888705.2011.576968.

Lynn, Brian Lee. 2018. *Zoo Giraffe Welfare: A Literature Review and the Behavioral Effects of Guest Feeding Programs*. Davis, CA: ProQuest Dissertations Publishing.

Mallapur, Avanti, Anindya Sinha, and Natalie Waran. 2005. "Influence of Visitor Presence on the Behaviour of Captive Lion-Tailed Macaques (*Macaca Silenus*) Housed in Indian Zoos." *Applied Animal Behaviour Science* 94(3–4):341–52. doi: 10.1016/j.applanim.2005.02.012.

Martin, Paul, and Patrick Bateson. 2007. *Measuring Behavior: An Introductory Guide*. 3rd ed. Cambridge: Cambridge Univ. Press.

Möstl, E., and Rupert Palme. 2002. “Hormones as Indicators of Stress.” *Domestic Animal Endocrinology* 23:67–74.

Normando, Simona, Ilaria Pollastri, Daniela Florio, Linda Ferrante, Elisabetta Macchi, Valentina Isaja, and Barbara de Mori. 2018. “Assessing Animal Welfare in Animal-Visitor Interactions in Zoos and Other Facilities. A Pilot Study Involving Giraffes.” *Animals* 8(9). doi: 10.3390/ani8090153.

O’Connor, T. M., D. J. O’Halloran, and F. Shanahan. 2000. “The Stress Response and the Hypothalamic-Pituitary-Adrenal Axis: From Molecule to Melancholia.” *QJM - Monthly Journal of the Association of Physicians* 93(6):323–33. doi: 10.1093/qjmed/93.6.323.

O’Donovan, Declan, Joanne E. Hindle, Sean Mckeown, and Sean O’Donovan. 1993. “Effect of Visitors on the Behaviour of Female Cheetahs and Cubs.” *International Zoo Yearbook* 32:238–44.

Orban, David A., Janice M. Siegford, and Richard J. Snider. 2016. “Effects of Guest Feeding Programs on Captive Giraffe Behavior.” *Zoo Biology* 35(2):157–66. doi: 10.1002/zoo.21275.

Palme, Rupert. 2019. “Non-Invasive Measurement of Glucocorticoids: Advances and Problems.” *Physiology & Behavior* 199:229–43.

Polgár, Zita, Lara Wood, and Marie J. Haskell. 2017. “Individual Differences in Zoo-Housed Squirrel Monkeys’ (*Saimiri Sciureus*) Reactions to Visitors, Research Participation, and Personality Ratings.” *American Journal of Primatology* 79(5):1–10. doi: 10.1002/ajp.22639.

Quadros, Sandra, Vinicius D. L. Goulart, Luiza Passos, Marco A. M. Vecci, and Robert J. Young. 2014. “Zoo Visitor Effect on Mammal Behaviour: Does Noise Matter?” *Applied Animal Behaviour Science* 156:78–84. doi: 10.1016/j.applanim.2014.04.002.

Queiroz, Marina B., and Robert J. Young. 2018. “The Different Physical and Behavioural Characteristics of Zoo Mammals That Influence Their Response to Visitors.” *Animals* 8(8). doi: 10.3390/ani8080139.

Quirke, Thomas, Ruth M. O’riordan, and Alain Zuur. 2012. “Factors Influencing the Prevalence of Stereotypical Behaviour in Captive Cheetahs (Acinonyx Jubatus).” *Applied Animal Behaviour Science* 142:189–97. doi: 10.1016/j.applanim.2012.09.007.

Rajagopal, Thangavel, Govindaraju Archunan, and Mahadevan Sekar. 2011. “Impact of Zoo Visitors on the Fecal Cortisol Levels and Behavior of an Endangered Species: Indian Blackbuck (Antelope Cervicapra L.).” *Journal of Applied Animal Welfare Science* 14(1):18–32. doi: 10.1080/10888705.2011.527598.

Razal, Catherine, Jocelyn Bryant, and Lance Miller. 2017. “Monitoring the Behavioral and Adrenal Activity of Giraffe (*Giraffa Camelopardalis*) to Assess Welfare during Seasonal Housing Changes.” *Animal Behavior and Cognition* 4(2):154–64. doi: 10.12966/abc.03.05.2017.

Riley, Alex, Megan Terry, Hani Freeman, Andrew C. Alba, Joseph Soltis, and Austin Leeds. 2021. “Evaluating the Effect of Visitor Presence on Nile Crocodile (*Crocodylus Niloticus*) Behavior.” *Journal of Zoological and Botanical Gardens*

2(1):115–29. doi: 10.3390/jzbg2010009.

Robbins, Lindsey, and Susan W. Margulis. 2014. "The Effects of Auditory Enrichment on Gorillas." *Zoo Biology* 33(3):197–203. doi: 10.1002/zoo.21127.

Romero, L. Michael, and J. Michael Reed. 2005. "Collecting Baseline Corticosterone Samples in the Field: Is under 3 Min Good Enough?" *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 140(1):73–79. doi: 10.1016/j.cbpb.2004.11.004.

Ross, Stephen R., Steven J. Schapiro, Jann Hau, and Kristen E. Lukas. 2009. "Space Use as an Indicator of Enclosure Appropriateness: A Novel Measure of Captive Animal Welfare." *Applied Animal Behaviour Science* 121(1):42–50. doi: 10.1016/j.applanim.2009.08.007.

Rothschild, Devon M., Thomas L. Serfass, William L. Seddon, Laxman Hegde, and R. Scott Fritz. 2008. "Using Fecal Glucocorticoids to Assess Stress Levels in Captive River Otters." *Journal of Wildlife Management* 72(1):138–42. doi: 10.2193/2005-700.

Scheijen, Ciska P. J., Sean van der Merwe, Andre Ganswindt, and Francois Deacon. 2021. "Anthropogenic Influences on Distance Traveled and Vigilance Behavior and Stress-Related Endocrine Correlates in Free-Roaming Giraffes." *Animals* 11(5). doi: 10.3390/ani11051239.

Sheriff, Michael J., Ben Dantzer, Brendan Delehanty, Rupert Palme, and Rudy Boonstra. 2011. "Measuring Stress in Wildlife: Techniques for Quantifying Glucocorticoids." *Oecologia* 166(4):869–87. doi: 10.1007/s00442-011-1943-y.

Sheriff, Michael J., Charles J. Krebs, and Rudy Boonstra. 2010. "Assessing Stress in Animal Populations: Do Fecal and Plasma Glucocorticoids Tell the Same Story?" *General and Comparative Endocrinology* 166:614–19. doi: 10.1016/j.ygcen.2009.12.017.

Sherwen, Sally L., Trista J. Harvey, Michael J. L. Magrath, Kym L. Butler, Kerry V. Fanson, and Paul H. Hemsworth. 2015. "Effects of Visual Contact with Zoo Visitors on Black-Capped Capuchin Welfare." *Applied Animal Behaviour Science* 167:65–73. doi: 10.1016/j.applanim.2015.03.004.

Sherwen, Sally L., and Paul H. Hemsworth. 2019. "The Visitor Effect on Zoo Animals: Implications and Opportunities for Zoo Animal Welfare." *Animals* 9(6):366. doi: 10.3390/ani9060366.

Sherwen, Sally L., Michael J. L. Magrath, Kym L. Butler, and Paul H. Hemsworth. 2015. "Little Penguins, *Eudyptula Minor*, Show Increased Avoidance, Aggression and Vigilance in Response to Zoo Visitors." *Applied Animal Behaviour Science* 168:71–76. doi: 10.1016/j.applanim.2015.04.007.

Stead, S. K., D. G. A. Meltzer, and Rupert Palme. 2012. "The Measurement of Glucocorticoid Concentrations in the Serum and Faeces of Captive African Elephants (Loxodonta Africana) after ACTH Stimulation : Research Communication." *Journal of the South African Veterinary Association* 71(3):192–96. doi: 10.4102/jsava.v71i3.712.

Suárez, P., P. Recuerda, and L. Arias-De-Reyna. 2017. "Behaviour and Welfare: The Visitor Effect in Captive Felids." *Animal Welfare* 26(1):25–34. doi:

10.7120/09627286.26.1.025.

Terio, Karen A., Laurie Marker, and Linda Munson. 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):259–66. doi: 10.7589/0090-3558-40.2.259.

Touma, Chadi, and Rupert Palme. 2005. "Measuring Fecal Glucocorticoid Metabolites in Mammals and Birds: The Importance of Validation." *Ann. N.Y. Acad. Sci* 1046:54–74. doi: 10.1196/annals.1343.006.

Uetake, Katsuji, Yumi Une, Shu Ito, Marino Yamabe, Hideto Toyoda, and Toshio Tanaka. 2014. "Relationship of Climatic Conditions to Fecal Corticosterone Levels of Captive Cheetahs Reared in Japan." *Animal Science Journal* 85(10):915–18. doi: 10.1111/asj.12226.

USA National Phenology Network. 2018. "Spring Indices, Historical Annual (1981-Previous Year) - First Leaf - Spring Index Years 1981-2015. Region: 49.9375,-66.4791667,24.0625,-125.0208333." Retrieved October 31, 2021 (https://data.usanpn.org/geoserver-request-builder?service=wms&layer=si-x:average_leaf_ncep_historic&year=2020&format=image/jpeg&projection=4269&width=1700&height=800&state_border=1 . USA-NPN, Tucson, Arizona, USA. Data set accessed 2021-10-31. http://dx.d).

Volfová, Martina, Zuzana Machovcová, Franz Schwarzenberger, Eva Voslářová, Iveta Bedáňová, and Vladimír Večerek. 2019. "The Effects of Transport Stress on the Behaviour and Adrenocortical Activity of the Black-and-White Ruffed Lemur (*Varecia Variegata*)." *Acta Veterinaria Brno* 88(1):85–92. doi: 10.2754/avb201988010085.

Vonderen, Ilse K., Hans S. Kooistra, and Ad Rijnberk. 1998. "Influence of Veterinary Care on the Urinary Corticoid: Creatinine Ratio in Dogs." *Journal of Veterinary Internal Medicine* 12(6):431–35. doi: 10.1111/j.1939-1676.1998.tb02146.x.

Wark, Jason D., Natasha K. Wierzal, and Katherine A. Cronin. 2021. "Gaps in Live Inter-Observer Reliability Testing of Animal Behavior: A Retrospective Analysis and Path Forward." *Journal of Zoological and Botanical Gardens* 2(2):207–21. doi: 10.3390/jzbg2020014.

Wielebnowski, Nadja. 2003. "Stress and Distress: Evaluating Their Impact for the Well-Being of Zoo Animals." *Journal of the American Veterinary Medical Association* 223(7):973–77. doi: 10.2460/javma.2003.223.973.

Wielebnowski, Nadja, and Jason Watters. 2007. "Applying Fecal Endocrine Monitoring to Conservation and Behavior Studies of Wild Mammals: Important Considerations and Preliminary Tests." *Israel Journal of Ecology & Evolution* 53(3–4):439–60. doi: 10.1560/IJEE.53.3.439.

Williams, Ellen, Anne Carter, Jessica Rendle, and Samantha J. Ward. 2021. "Impacts of COVID-19 on Animals in Zoos: A Longitudinal Multi-Species Analysis." *Journal of Zoological and Botanical Gardens* 2(2):130–45. doi: 10.3390/jzbg2020010.

Williams, Ellen, Anne Carter, Jessica Rendle, and Samantha J Ward. 2021. "Understanding Impacts of Zoo Visitors: Quantifying Behavioural Changes of

Two Popular Zoo Species during COVID-19 Closures.” *Applied Animal Behaviour Science* 236:1–8. doi: 10.1016/j.applanim.2021.105253.

Wolf, T. E., N. C. Bennett, R. Burroughs, and A. Ganswindt. 2018. “The Impact of Age-Class and Social Context on Fecal Glucocorticoid Metabolite Levels in Free-Ranging Male Giraffes.” *General and Comparative Endocrinology* 255:26–31. doi: 10.1016/j.ygcen.2017.09.022.

Wright, Robin. 2020. “Some Zoos, and Some of Their Animals, May Not Survive the Pandemic.” *The New Yorker* 1–8. Retrieved January 1, 2021 (https://www.newyorker.com/news/our-columnists/some-zoos-and-some-of-their-animals-may-not-survive-the-pandemic).

Young, Robert J. 2003. “Housing.” Pp. 122–41 in *Environmental Enrichment for Captive Animals*. Oxford, UK: Blackwell Science Ltd.

Zwijacz-Kozica, Tomasz, Nuria Selva, Isabel Barja, Gema Silván, Leticia Martínez-Fernández, Juan Carlos Illera, and Miłosz Jodłowski. 2012. “Concentration of Fecal Cortisol Metabolites in Chamois in Relation to Tourist Pressure in Tatra National Park (South Poland).” *Acta Theriologica* 58(2):215–22. doi: 10.1007/s13364-012-0108-7.

Chapter Five: Conclusions

The combined results provided by the three studies detailed in this dissertation contribute new and valuable findings that increased our understanding of the impact of visitors on several commonly exhibited animal species in modern zoological institutions. Zoos and aquaria must balance the needs of the animals in their care and the experiences of the visitors attending them. Modern accredited zoos are innovative, both in their animal enrichment efforts and their visitor experiences. Balancing the relationship between visitor experience and animal welfare requires constant adjustments based on the newest available scientific evidence and welfare evaluations. Zoo animal welfare is a growing field of research expanding as technology improves and knowledge increases. This dissertation focused on the importance of understanding a variety of potential impacts visitors may have on zoo animals, with a special emphasis on noise and sound.

The study results presented in each of the three chapters especially highlight the individuality of animal reactions to visitor presence and the importance of monitoring multiple factors and each individual animal's reactions. The after-hour event study showed no obvious physiological evidence, as measured through adrenal activity and fGM monitoring, of any sustained negative impact based on the presence of large scale, after-hour events – with or without broadcasted music. However, the fGM concentrations following each type of event were variable by both species and individual. This highlights the necessity of monitoring and determining the individual animal's potential reactions to individual types of visitor events when planning and implementing new events. The behavior expression within different event types was also highly individualistic. For instance, the single cheetah who exhibited significantly more stereotypic behaviors during "concert" event types while her exhibit-mate did not, further illustrate the need for an individual based approach in addition to a species-by-species approach for an impact assessment. Finally, the presence of multiple significant correlations between noise levels and both fGM concentrations and behavior rates independent of event type, indicate the importance of monitoring individual facets of an event in addition to the event as a whole.

While focused on the use of sound as potential animal enrichment rather than any visitor impact related to sound, the lemur study also exemplified the importance of individual differences in responses within the same species, both in terms of fGM concentrations and behavior rates in response to sound type. In this study, one of the most interesting responses was the variation in exhibit use by the two different species of lemurs. This highlights the significance of monitoring both the behavior expressed and where in the exhibit the behavior was performed. The types of behavior expressed during each treatment was similar; however, the locations varied from treatment to treatment. If this study only recorded the behavior type, valuable information about the exhibit use would have been lost.

These individual responses continued even when visitors were entirely absent from the zoo. During the 2020 COVID-19 pandemic, both cheetahs and giraffes showed significant differences in behavior rates at the individual level. For instance, while both giraffes had significantly less rates of vigilant behavior when visitors were readmitted to the zoo, only one giraffe had a simultaneous increase in environmental interaction and stationary behavior rates. Understanding how each individual within an exhibit reacts to the same stimuli is imperative when creating management plans for a species or exhibit. If managers look solely at amalgamated behavior data, single individuals who react slightly differently to the average may be overshadowed and managerial decisions may fail to address these differences. Admittedly, both cheetahs and giraffes were also exposed to a variety of other important external factors (e.g., medical procedures and social changes) during the study and detangling these stimuli from the presence or absence of visitors was difficult.

While individual evaluations can be tedious and complicated in their interpretation, they should not be dismissed. Many researchers have begun to characterize individual focal animals by personality type to help predict how a large event or change may affect them (de Azevedo and Young 2021; Powell and Gartner 2011; Tetley and O'Hara 2012; Wielebnowski 1999; Wilson et al. 2019). While much of this research has focused on the impacts of personality on reproductive success (Fox and Millam 2014; Martin-Wintle et al. 2017; Razal, Pisacane, and Miller 2016; Wielebnowski 1999), these studies can also be useful in predicting which individuals may struggle to adapt to changes in environment. For instance, capuchin monkeys (*Sapajus libidinosus*) that were deemed more "sociable" and "active" did not show a spike in fGM concentrations experienced by the other “less sociable and active” monkeys following an exhibit change (Ferreira et al. 2020). Further studies on the impact of sound in relation to animal personality would be helpful for future event and enrichment evaluations.

While this dissertation helped to further our knowledge of how visitors affect some commonly exhibited zoo animals, it also highlighted important avenues for further research. One important overarching limitation of the three studies was a potential effect of seasonality. Due to the nature of the experimental designs, the studies presented here were confined to a single season (chapter 2 and 4: summers, chapter 3: late fall). As seasonal differences in both behavior rates and fGM concentrations are extremely common, replicating these studies during opposite seasons may provide more wholistic results. Another important future study surrounds the use of sound as enrichment. While our study on the success of auditory enrichment for two species of lemur was inconclusive, understanding how changes in the soundscape affect individuals is imperative to maximizing both overall welfare and enrichment opportunities. Additionally, many zoos have implemented sound features to heighten the visitor experience (e.g., interactive platforms to listen to animal vocalizations, pre-recorded natural sounds, or ambient music) but few quantitative studies exist on the effect of these noises on the individual animals. It is crucial that quantitative studies are conducted of all novel elements added to animal environments in order to achieve high levels of animal welfare while providing an enriching visitor experience.

Finally, each dissertation chapter has highlighted the importance of monitoring all extraneous factors in addition to visitor effects. In all three studies, unexpected social changes, medical, or environmental changes appeared to have had a greater impact on the physiological and behavioral expression of the focal individuals than potential visitor effects. This is an important discovery as it increases our confidence that visitor presence does not inherently decrease the welfare of certain species and other factors such as social changes, health issues, personality may be more important to consider either by themselves and/or in relation to potential visitor effects, especially when introducing new types of events or enrichment. Few findings can be generalized across species, exhibits and facilities and each group and individual need to be considered when assessing long-term welfare impact on exhibit animals. This dissertation is unique to the animals housed at the Oregon Zoo and while the Association of Zoos and Aquariums (AZA) provides guidelines for many aspects of animal husbandry, animals at different institutions are exposed to significantly different environmental and social effects. Overall, the information presented supports some of the conclusions of previous studies and also highlights some additional and new avenues for further research directions.

To disseminate the results of this dissertation for possible application, the outcomes have been circulated among the animal care staff at the Oregon Zoo but are also written up for publication in peer-reviewed journals accessed by the zoo community in general. Chapter Four has recently been published in the open-access journal ‘Animals’. Results from Chapter Two have been presented at AZA conferences and the paper will be submitted for publication soon, while the chapter on lemur sound enrichment will potentially be expanded later in the year adding another experimental trial to the study to gain further inside into the use of sound treatments as enrichment for these common zoo-held species. Publications of this chapter will occur once the additional trials have been completed.

The standards for animal welfare in AZA accredited zoos such as the Oregon Zoo continue to increase with an emphasis on animal welfare focused research and the application of new technology, such as high-quality sound measurement equipment and special sound analyses software. Research projects, such as this one, can serve as significant "steppingstones" and catalysts for further investigation while also helping to increase our understanding and provide information to assist in managerial decisions to maximize positive welfare in zoological institutions.

References

de Azevedo, C. S., & Young, R. J. (2021). Animal personality and conservation: Basics for inspiring new research. In *Animals* (Vol. 11, Issue 4, p. 1019). https://doi.org/10.3390/ani11041019

Ferreira, V. H. B., Fonseca, E. D. P., Chagas, A. C. C. S. Das, Pinheiro, L. G. M., Sousa, M. B. C. de, Silva, H. P. A. da, Galvão-Coelho, N. L., & Ferreira, R. G. (2020). Personality traits modulate stress responses after enclosure change of captive capuchin monkeys (Sapajus libidinosus). *Applied Animal Behaviour Science*, *232*(August). https://doi.org/10.1016/j.applanim.2020.105111

Fox, R. A., & Millam, J. R. (2014). *Personality Traits of Pair Members Predict Pair Compatibility and Reproductive Success in a Socially Monogamous Parrot Breeding in Captivity*. *8*(January), 1–8. https://doi.org/10.1002/zoo.21121

Martin-wintle, M. S., Shepherdson, D., Zhang, G., Huang, Y., Luo, B., & Swaisgood, R. R. (2017). Do opposites attract ? Effects of personality matching in breeding pairs of captive giant pandas on reproductive success. *Biological Conservation*, *207*, 27–37. https://doi.org/10.1016/j.biocon.2017.01.010

Powell, D. M., & Gartner, M. C. (2011). Applications of Personality to the Management and Conservation of Nonhuman Animals. In M. Inoue-Murayama, S. Kawamura, & A. Weiss (Eds.), *From Genes to Animal Behavior* (1st ed., pp. 185–199). Springer Japan. https://doi.org/10.1007/978-4-431-53892-9_8

Razal, C. B., Pisacane, C. B., & Miller, L. J. (2016). Multifaceted Approach to Personality Assessment in Cheetahs (Acynonyx jubatus). *Animal Behavior and Cognition*, *3*(1), 22–31. https://doi.org/10.12966/abc.02.02.2016

Tetley, C., & O'Hara, S. (2012). Ratings of animal personality as a tool for improving the breeding, management and welfare of zoo mammals. *Animal Welfare*, *21*(4), 463–476. https://doi.org/10.7120/09627286.21.4.463

Wielebnowski, N. C. (1999). Behavioral Differences as Predictors of Breeding Status in Captive Cheetahs. In *Zoo Biology* (Vol. 18).

Wilson, V., Guenther, A., Øverli, Ø., Seltmann, M. W., & Altschul, D. (2019). Future Directions for Personality Research: Contributing New Insights to the Understanding of Animal Behavior. *Animals*, *9*(5), 240. https://doi.org/10.3390/ani9050240

Appendices

Appendix A: Exhibit Location

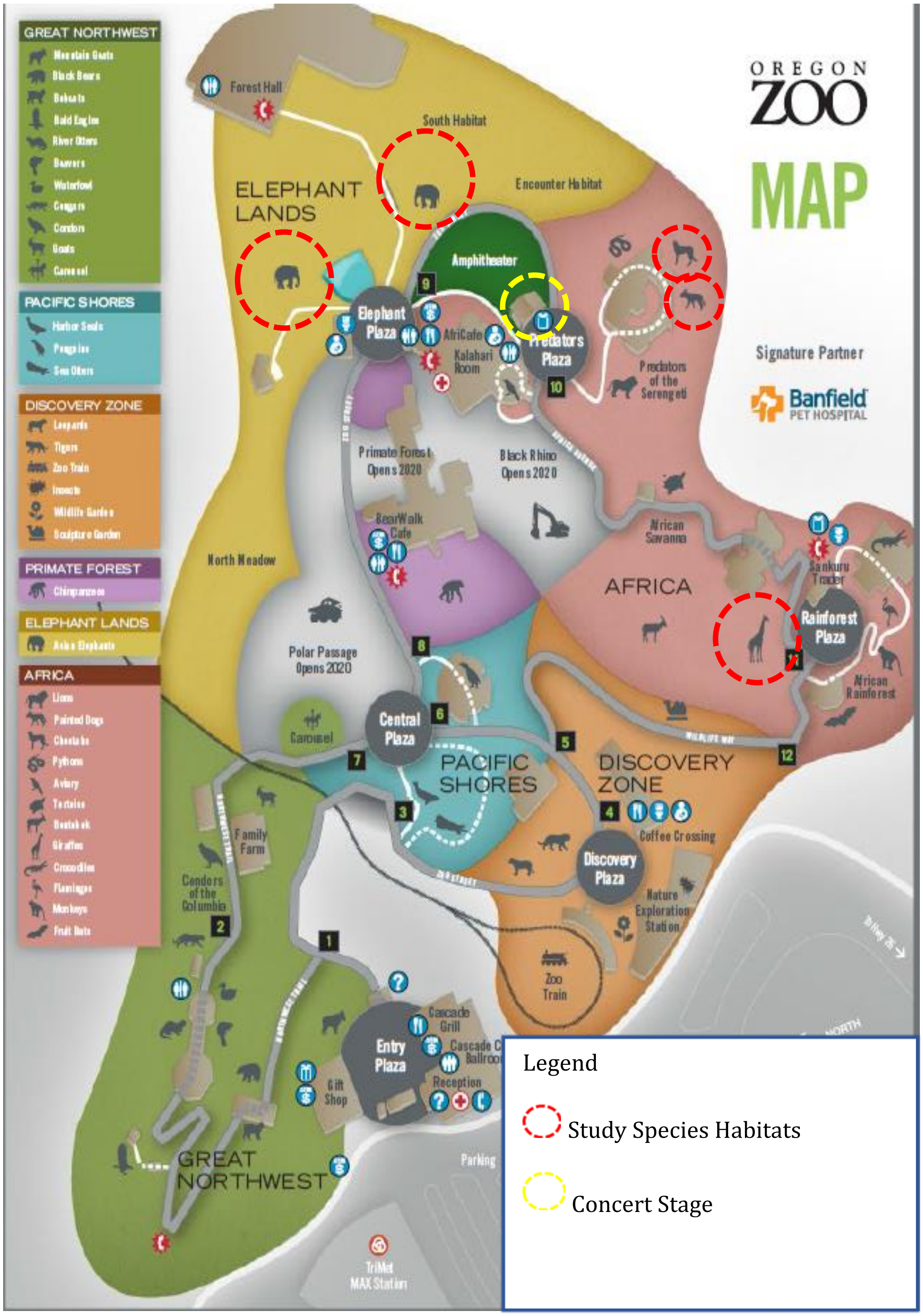


Figure 36: Zoo map (summer 2018)

Appendix B: Sound meter location and visitor viewing locations

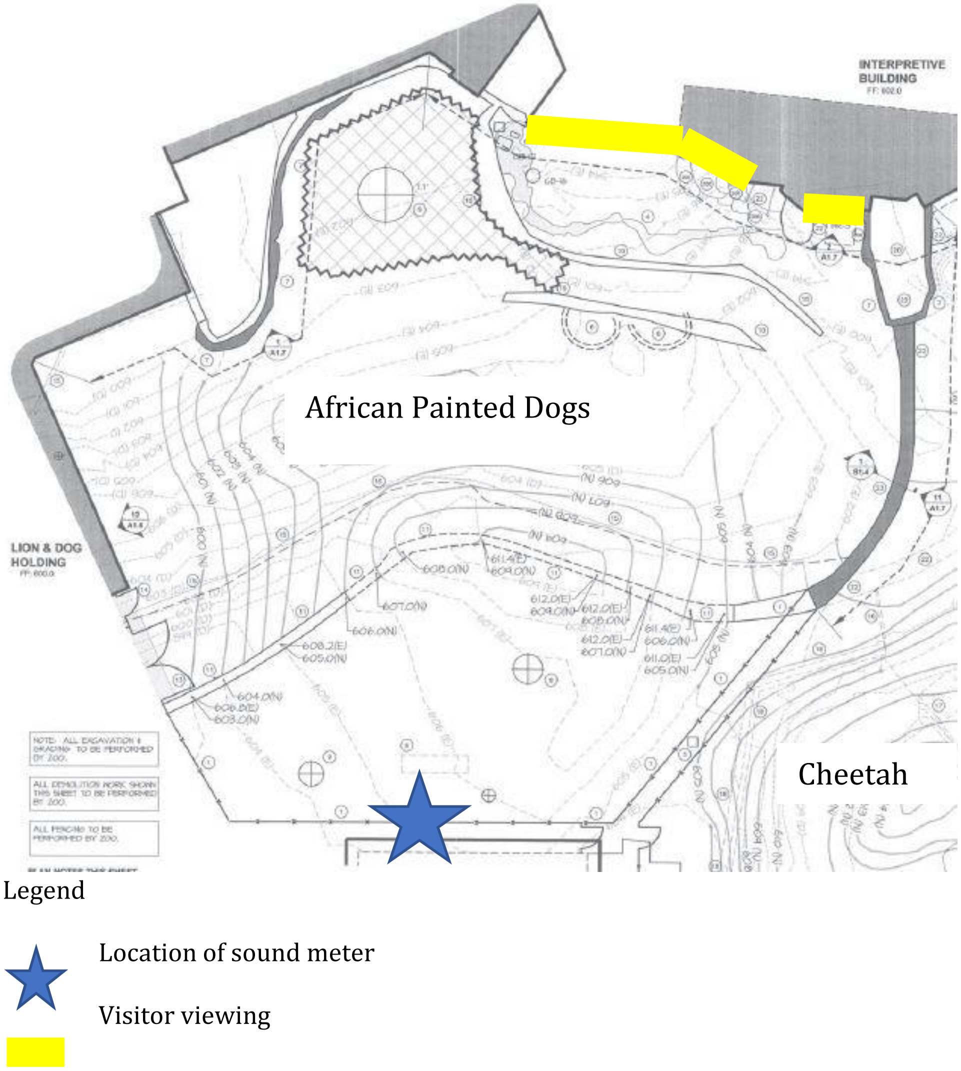
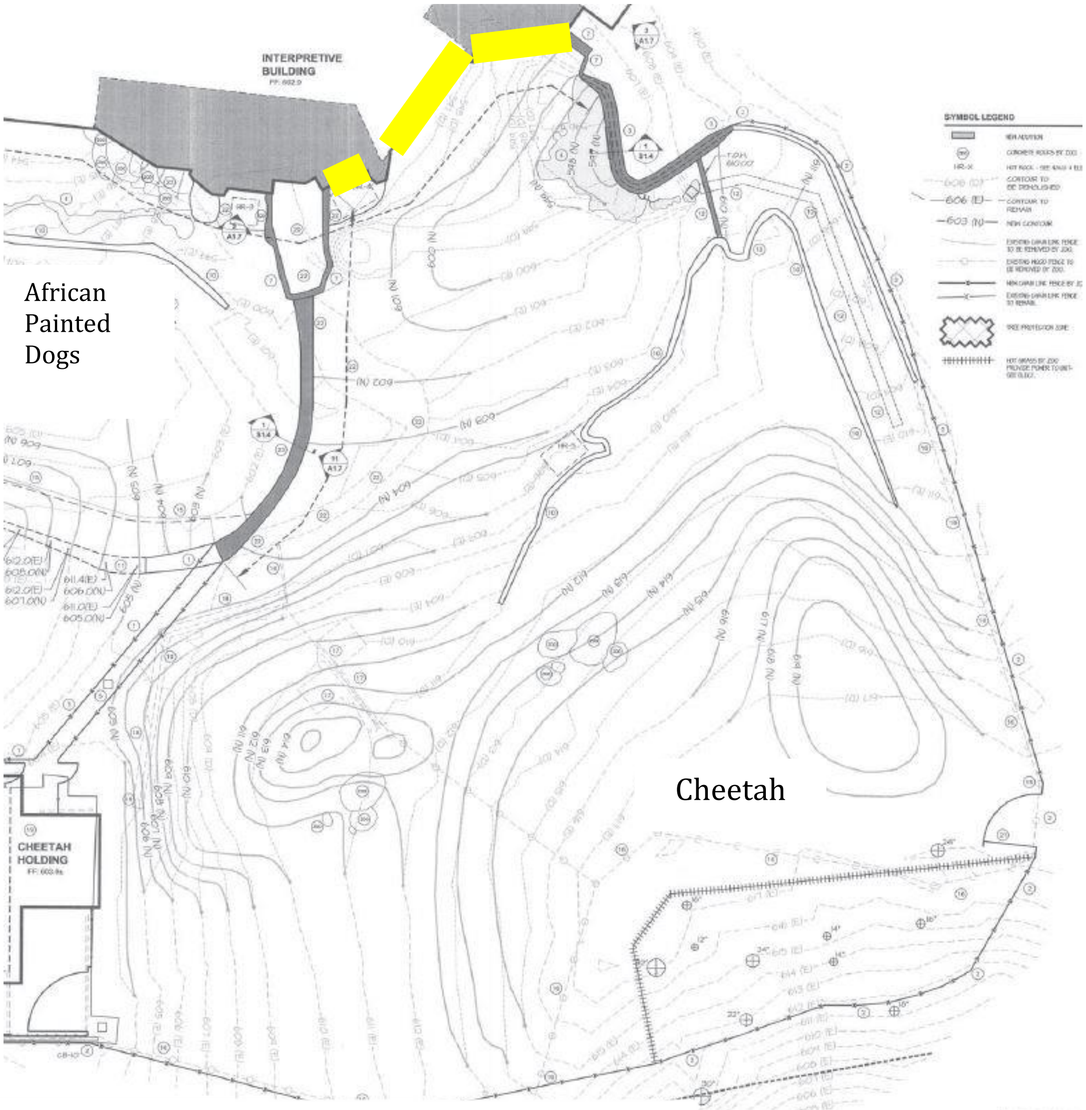


Figure 37: African painted dogs sound meter location and visitor viewing

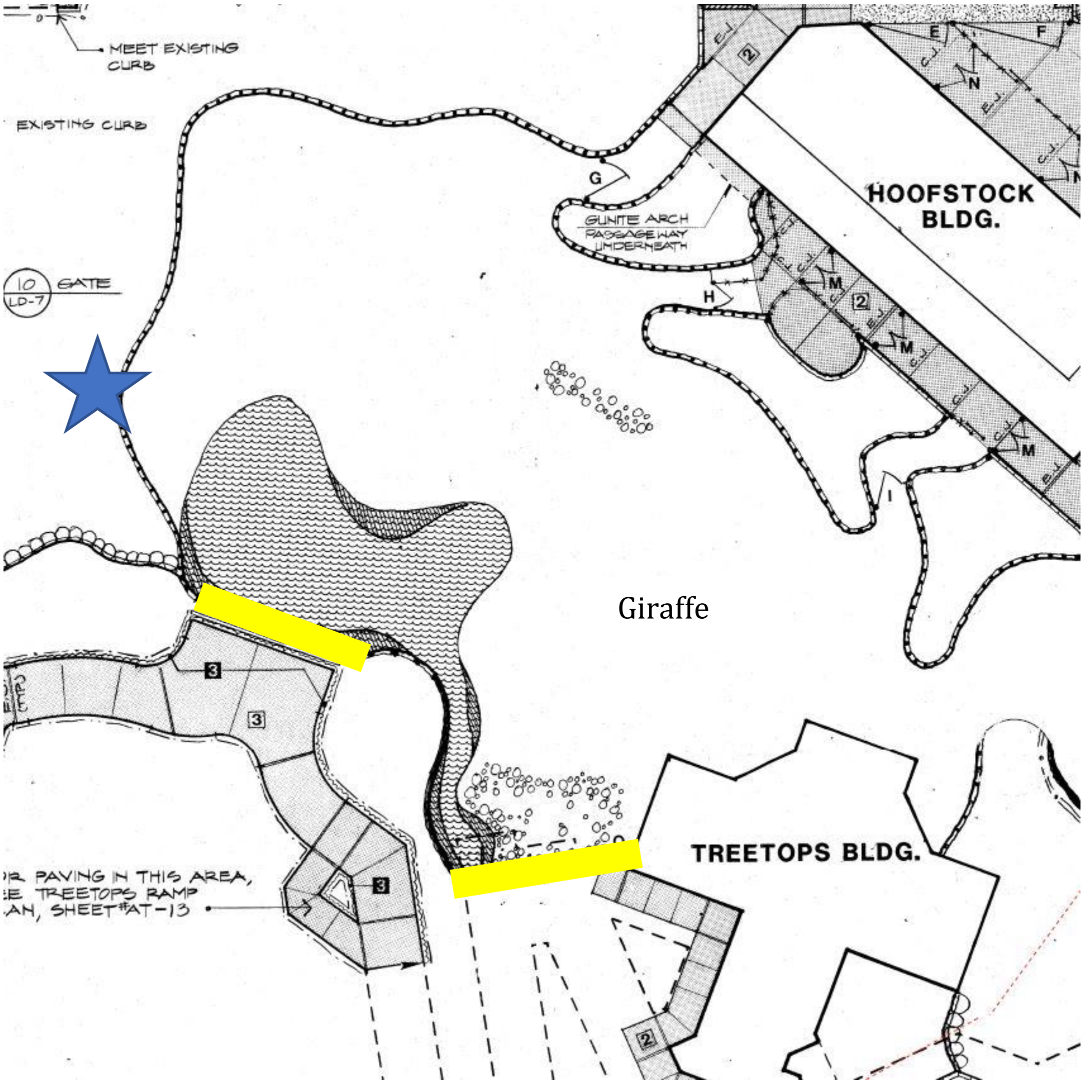


Legend

🟨 Visitor viewing

No sound meter at cheetah due to the close proximity to African painted dog. Sound readings for cheetah taken from sound meter at African painted dogs.

Figure 38: Cheetah visitor viewing locations



Legend

★ Location of sound meter

🟨 Visitor viewing

Figure 39: Giraffe sound meter location and visitor viewing platforms

Legend

★ Location of permanent sound meter

★ Alternating sound meter location in 2018, permanent sound meter locations in 2019

Visitor viewing of elephants during concerts is from all pathways surrounding the habitat

Figure 40: Elephant sound meter locations

Appendix C: Biochemical Validation of Fecal Hormone Monitoring

African Painted Dog (*L. pictus*)

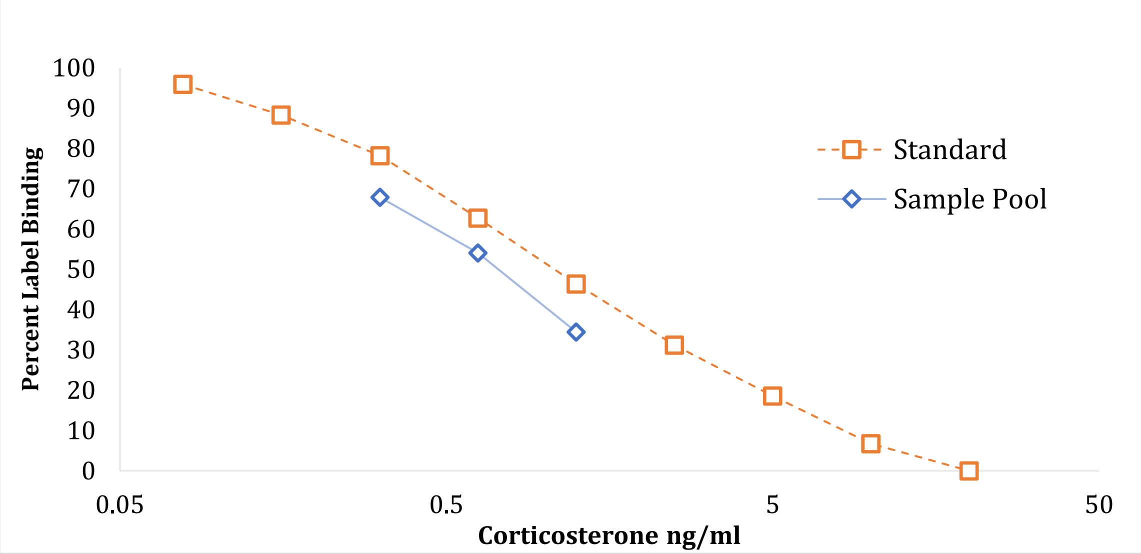


Figure 41: Parallelism between serially diluted corticosterone (ng/g) standard curve (CJM006) and a serially diluted sample pool of African painted dog fecal sample extracts (wet). Only pooled samples between 20% and 80% binding included to comply with quality control practices in the assay protocol.

Cheetah (*A. jubatus*)

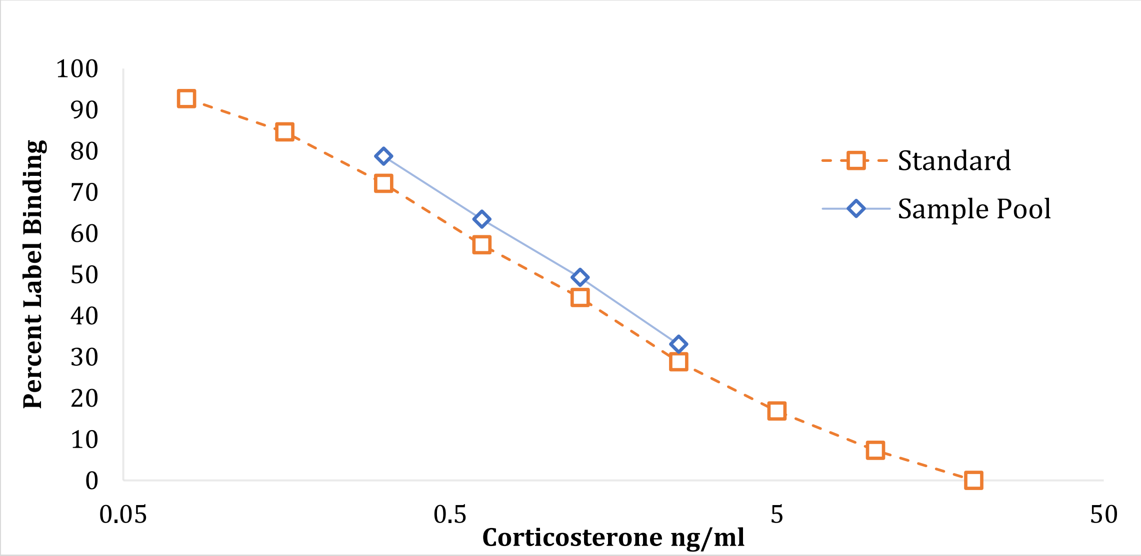


Figure 42: Parallelism between serially diluted corticosterone (ng/g) standard curve (CJM006) and a serially diluted sample pool of cheetah fecal sample extracts (wet). Only pooled samples between 20% and 80% binding included to comply with quality control practices in the assay protocol.

Giraffe (*G. c. reticulata & G. c. tippelskirchi*)

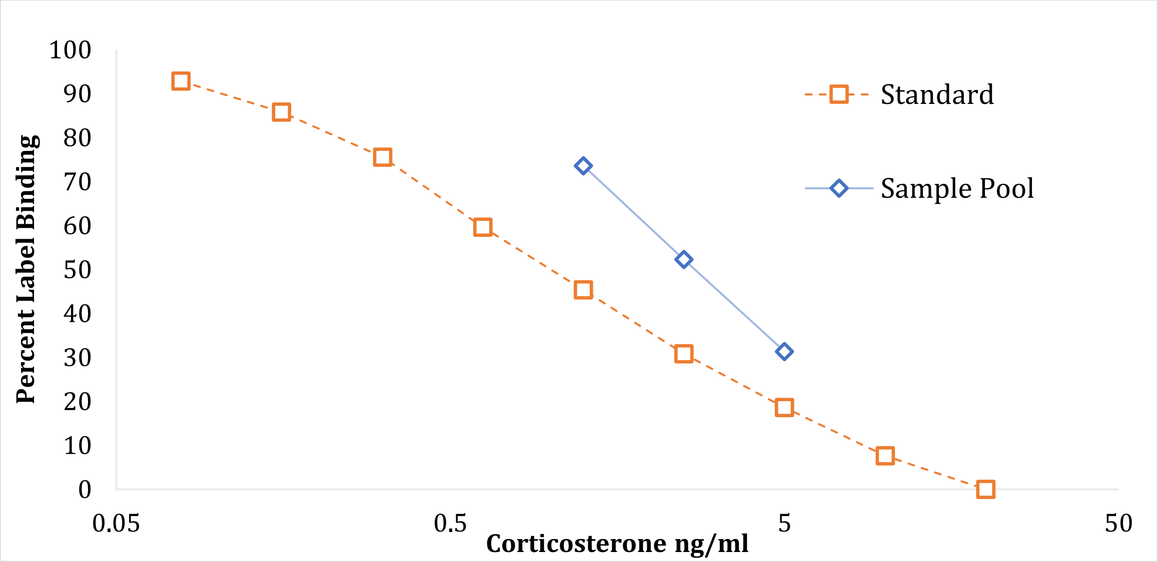


Figure 43: Parallelism between serially diluted corticosterone (ng/g) standard curve (CJM006) and a serially diluted sample pool of giraffe fecal sample extracts (wet). Only pooled samples between 20% and 80% binding included to comply with quality control practices in the assay protocol.

Asian Elephant (*E. m. indicus & E. m. borneensis*)

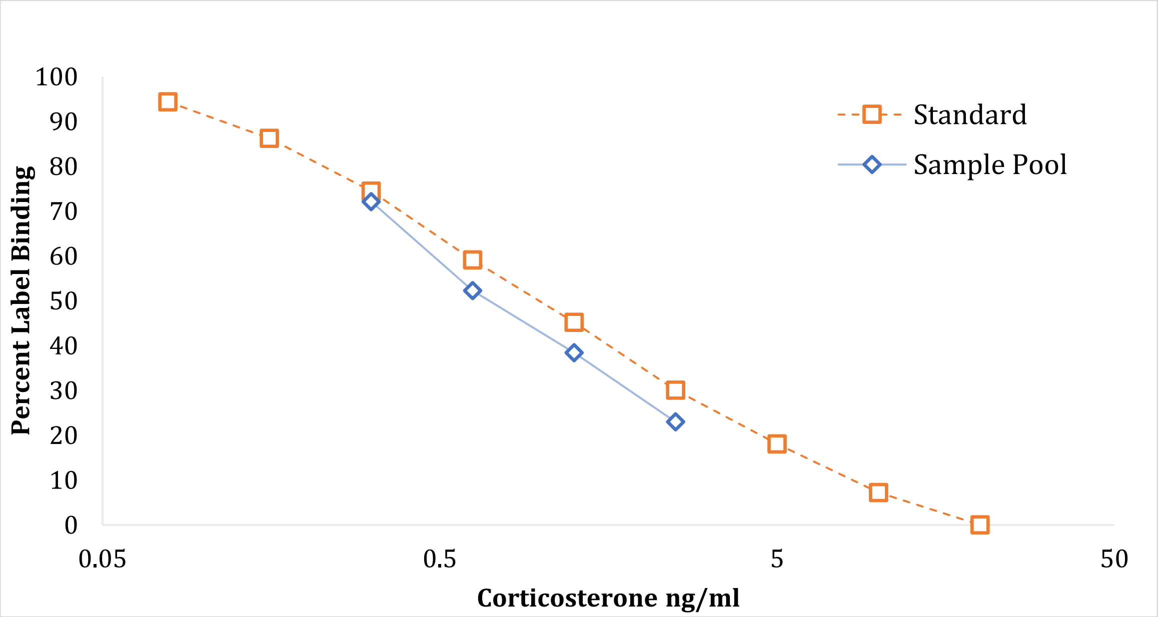


Figure 44: Parallelism between serially diluted corticosterone (ng/g) standard curve (CJM006) and a serially diluted sample of Asian elephant fecal sample extracts (wet). Only pooled samples between 20% and 80% binding included to comply with quality control practices in the assay protocol.

Ring-tailed Lemur (*L. catta*)

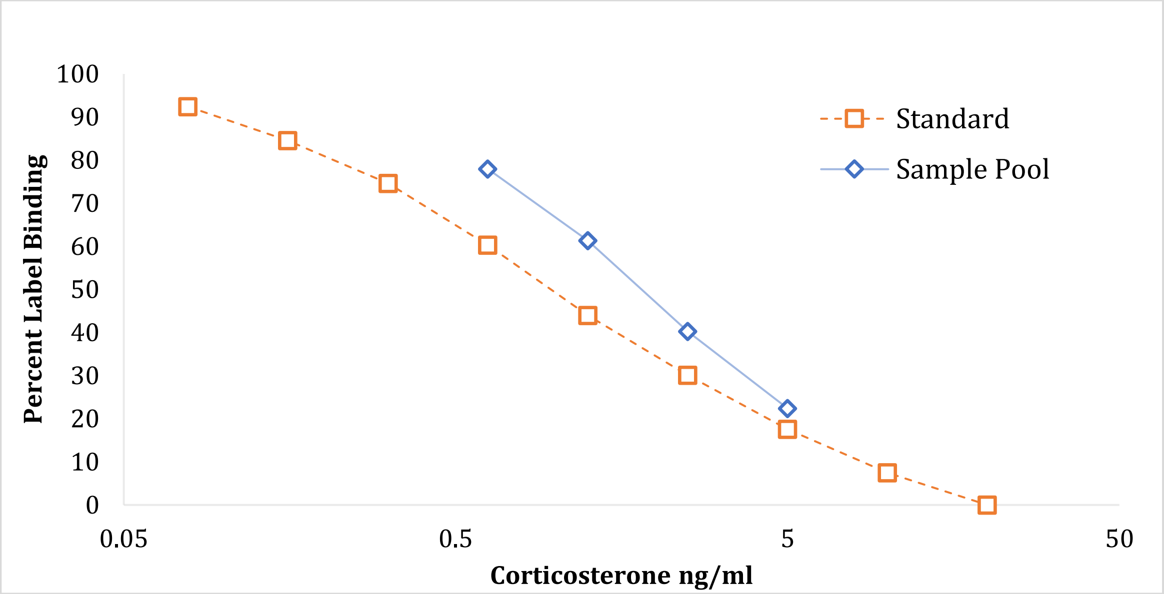


Figure 45: Parallelism between serially diluted corticosterone (ng/g) standard curve (CJM006) and a serially diluted sample of ring-tailed lemur fecal sample extracts (wet). Only pooled samples between 20% and 80% binding included to comply with quality control practices in the assay protocol.

Red-Ruffed Lemur (*V. rubra*)

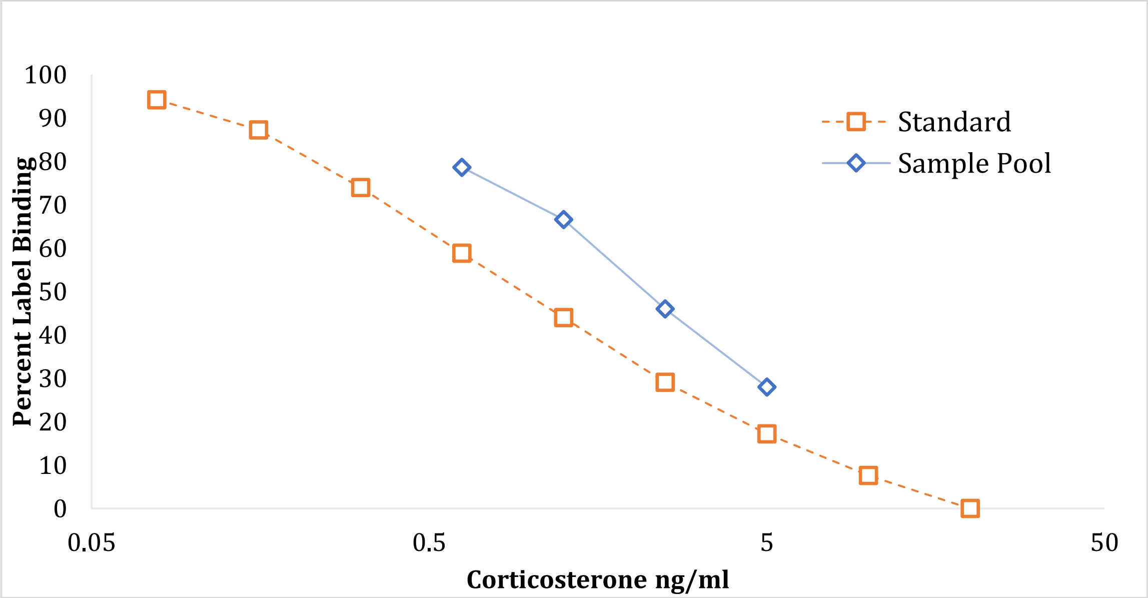


Figure 46: Parallelism between serially diluted corticosterone (ng/g) standard curve (CJM006) and a serially diluted sample of red-ruffed lemur fecal sample extracts (dry). Only pooled samples between 20% and 80% binding included to comply with quality control practices

Appendix D: Biological Validation of Fecal Hormone Monitoring

African Painted Dog (*L. pictus*)

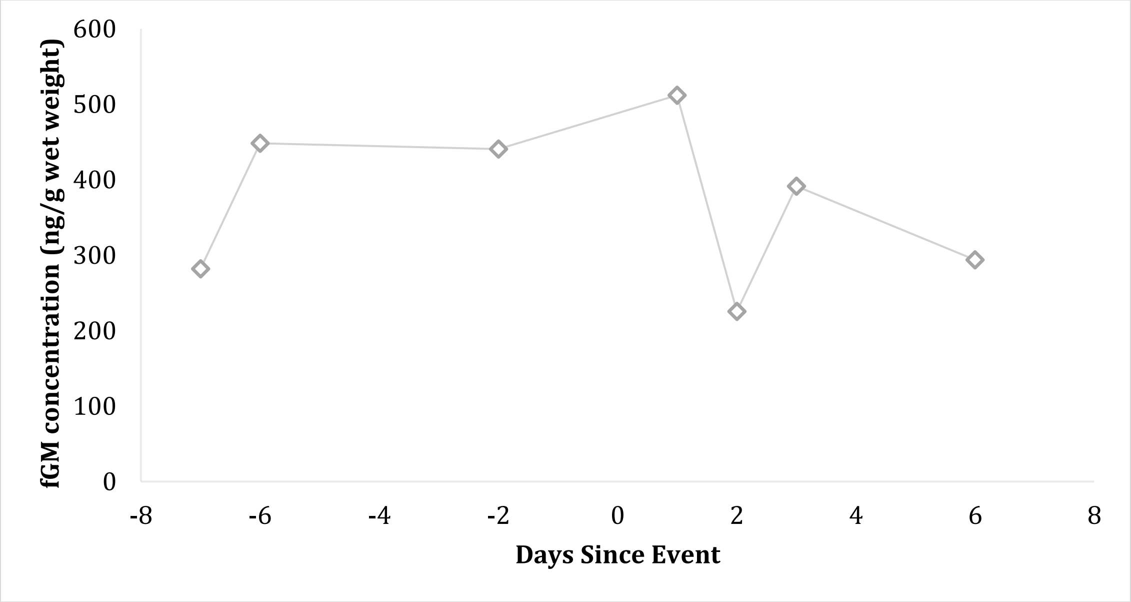


Figure 47: Biological validation of African painted dogs using the corticosterone (CJM006) EIA assay. Showed elevated sample one day after ovariectomy.

Cheetah (*A. jubatus*)

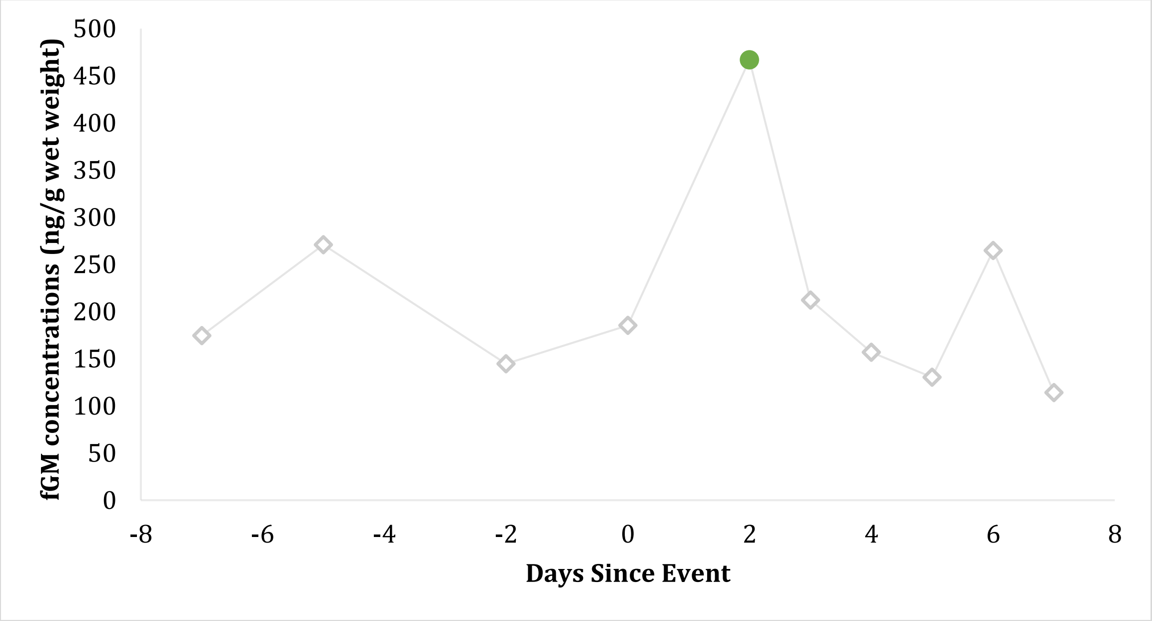


Figure 48: Biological validation of cheetahs using the corticosterone (CJM006) EIA assay. Highlighted (●) samples indicate samples > 2 standard deviations above the baseline values. Significant stressor was invasive surgery.

Giraffe (*G. c. reticulata & G. c. tippelskirchi*)

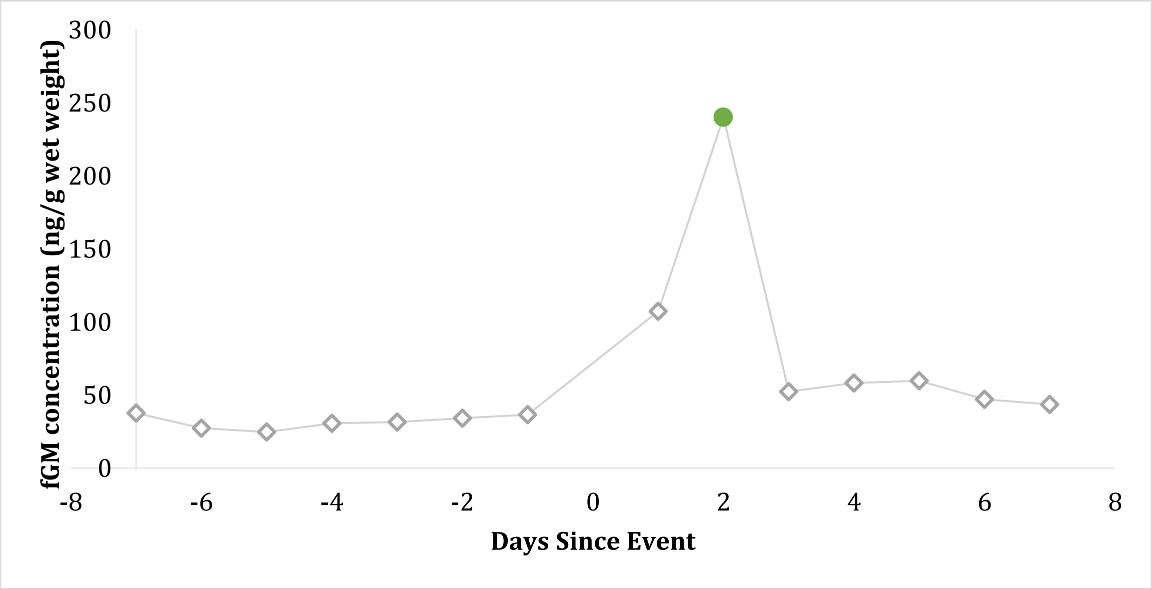


Figure 49: Biological validation of giraffes using the corticosterone (CJM006) EIA assay. Highlighted (●) samples indicate samples > 2 standard deviations above the baseline values. Significant stressor was an exhibit transfer.

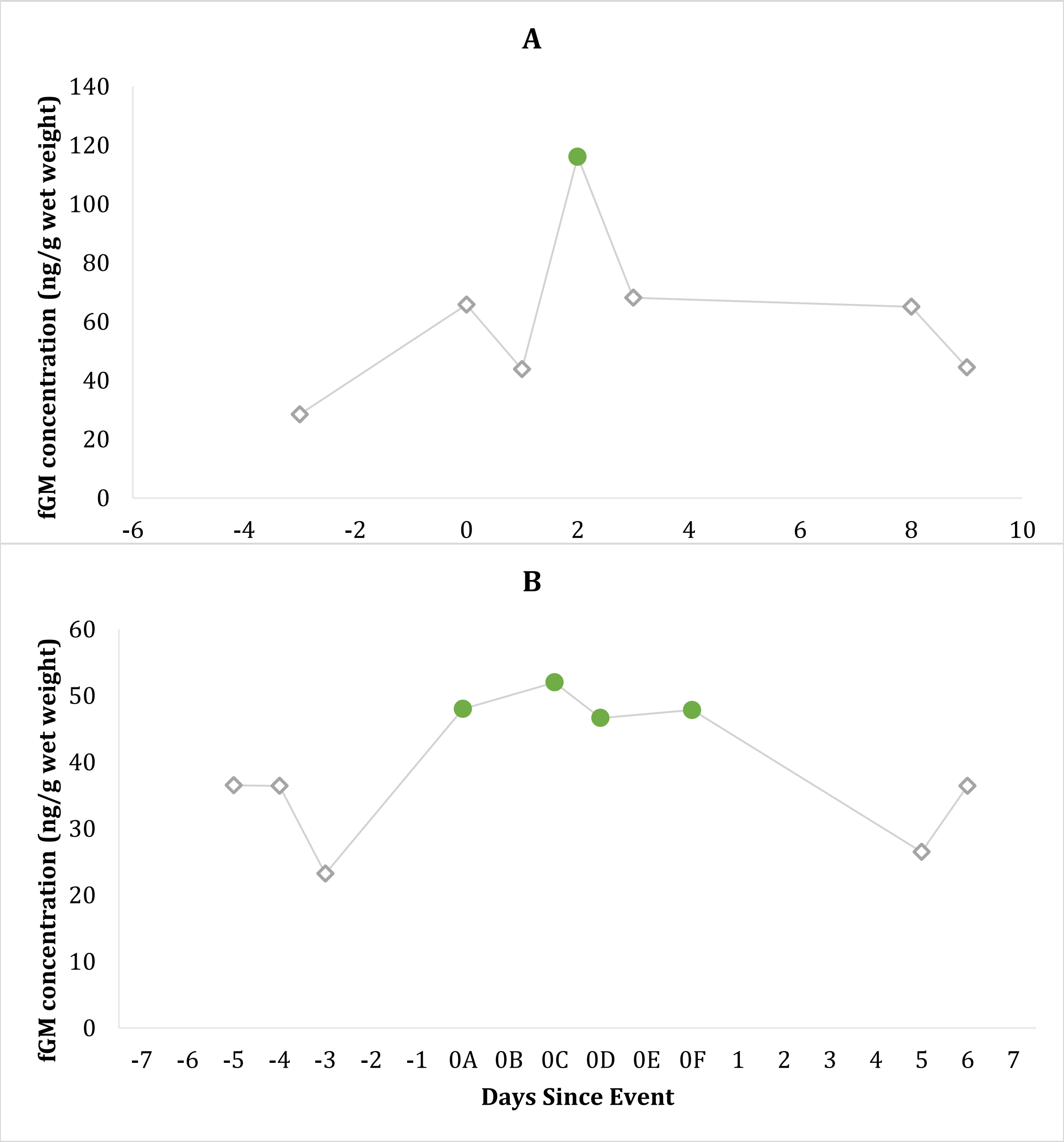


Figure 50: Biological validation of corticosterone metabolite detection using a corticosterone (CJM006) EIA analysis. Highlighted (●) samples indicate > 2 standard deviations above the mean. Both A and B use an elephant introduction as the significant stressor.

Ring-tailed Lemur (*L. catta*)

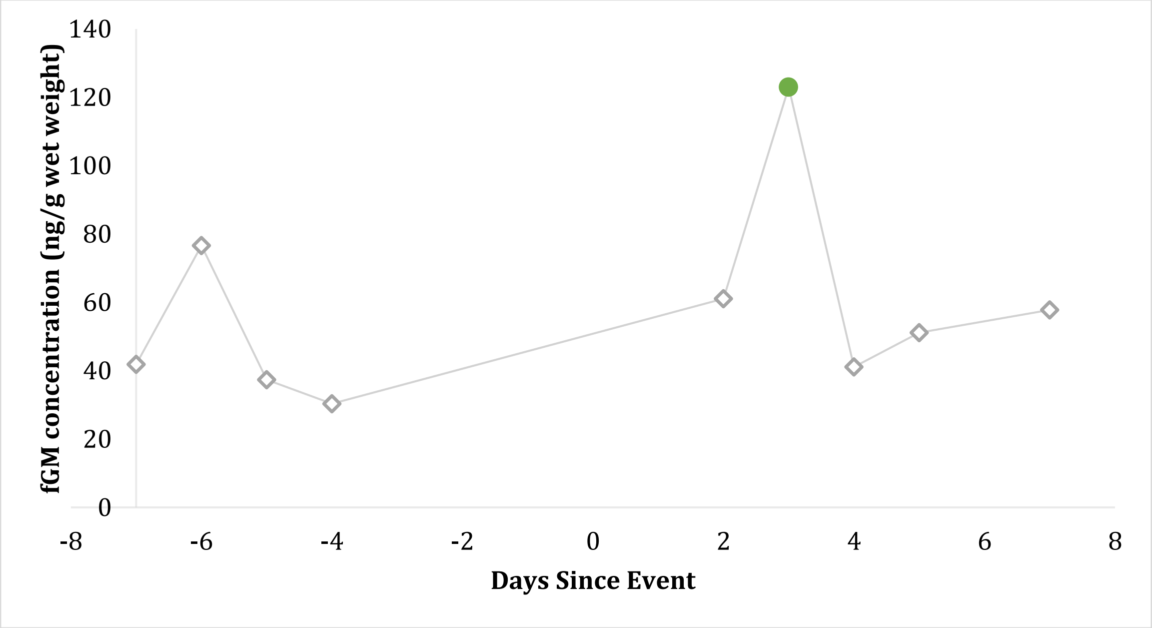


Figure 51: Biological validation of corticosterone metabolite detection using a corticosterone (CJM006) EIA analysis. Highlighted (●) samples indicate > 2 standard deviations above the mean. Significant stressor was a finger amputation.51

Appendix E: Changes in fGM concentrations based on estrus cycle in African painted dogs (Lycaon pictus)

Methods

A one-way ANOVA was run on fecal samples for painted dog B70096 to determine if her pregnancy affected her fecal glucocorticoid metabolite (fGM) concentrations. Statistics were run using IBM SPSS version 21. Data were split into three categories: pre-pregnancy (n = 40), pregnant (n = 46), and post-pregnancy (n=9). Results of a Shapiro Wilks test revealed non-normality of the fGM concentrations for two of the three treatments (pre-pregnancy: $p < 0.001$, pregnant: $p < 0.001$, post-pregnancy: $p = 0.143$). Data were subsequently log-10 transformed and Shapiro-Wilks tests revealed normal data distribution following the transformation (pre-pregnancy: $p = 0.284$, pregnant: $p = 0.433$, post-pregnancy: $p = 0.200$). Visual inspection of a boxplot revealed no extreme outliers on the transformed data. Homogeneity of variances was assumed as shown by Levene's test for equality of variances ($p = 0.170$).

Results

FGM concentrations were statistically significantly different based on the estrus cycle, $F_{(2,92)} = 13.229$, $p < 0.001$. Tukey-Kramer post-hoc tests showed a significant increase from pre-pregnancy fGM concentrations ($p = 0.017$) and a significant decrease from pregnancy to post-pregnancy fGM concentrations ($p < 0.001$). Finally, there was a significant drop in fGM concentrations between pre-pregnancy and post-pregnancy fGM concentrations ($p = 0.005$).

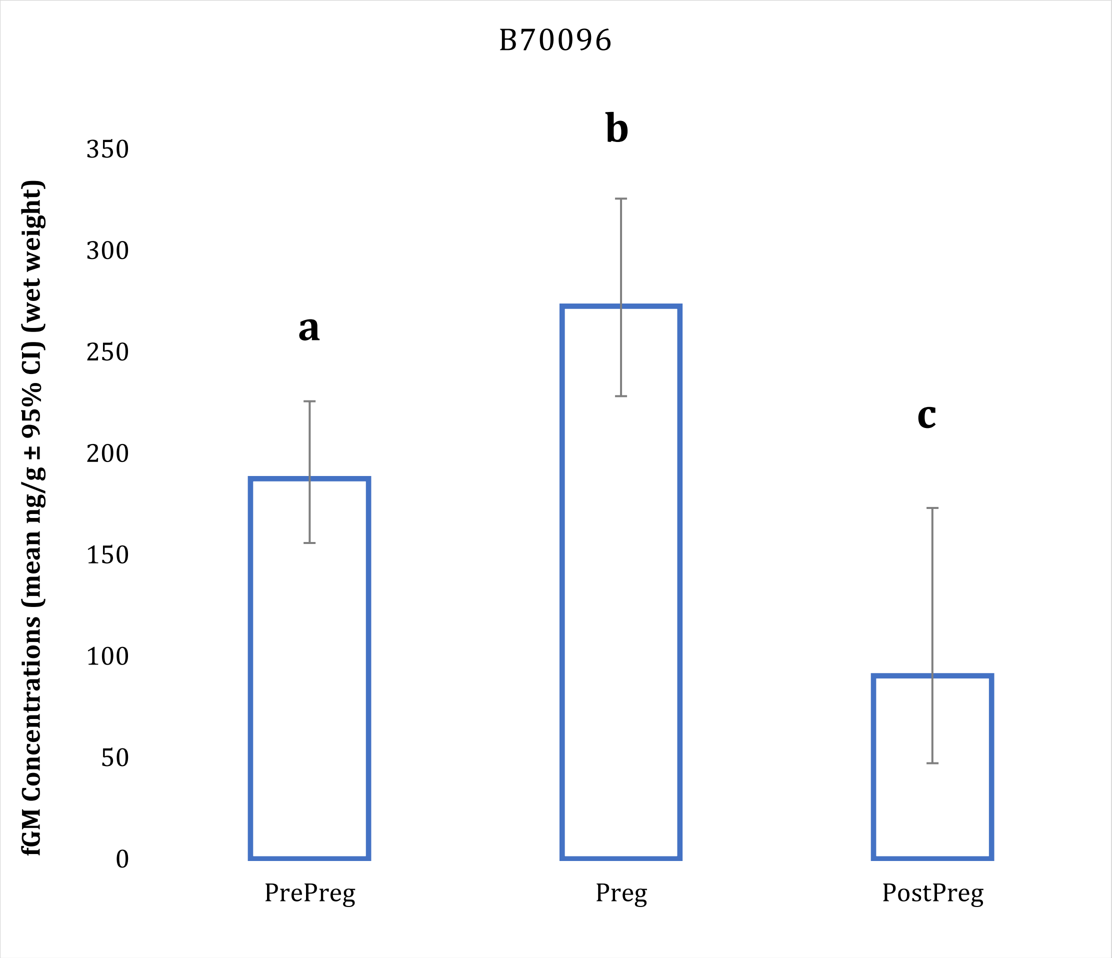


Figure 52: Effect of estrus cycle on African painted dog fGM concentrations. Data are presented back-transformed from log-transformed means ± 95% CI. Different labels (a, b, or c) indicate statistical significance (p-value < 0.05).

Appendix F: Asian elephant subspecies (E. m. indicus & E. m. borneensis) fGM comparison

Methods

A t-test was run on fecal samples from all Asian elephants to determine if a difference exists between the two different subspecies: *E. m. indicus* & *E. m. borneensis*. Shapiro-Wilks test revealed non-normality (*p*-value < 0.05) for both subspecies and a boxplot showed multiple outliers. As these outliers are important to the dataset, they were not removed from the dataset. Instead, a non-parametric Mann-Whitney U test was chosen for analysis. Comparison of histograms revealed similar distributions so comparing medians with a Mann-Whitney U test was appropriate.

Results

A Mann-Whitney U test showed median fGM concentrations for *E. m. indicus* *(*33.53) and *E. m. borneensis* (34.82) were not statistically significantly different, $U = 46037.5$, $z = -0.340$, $p = 0.734$. Average fGM concentrations are presented in Figure 50.

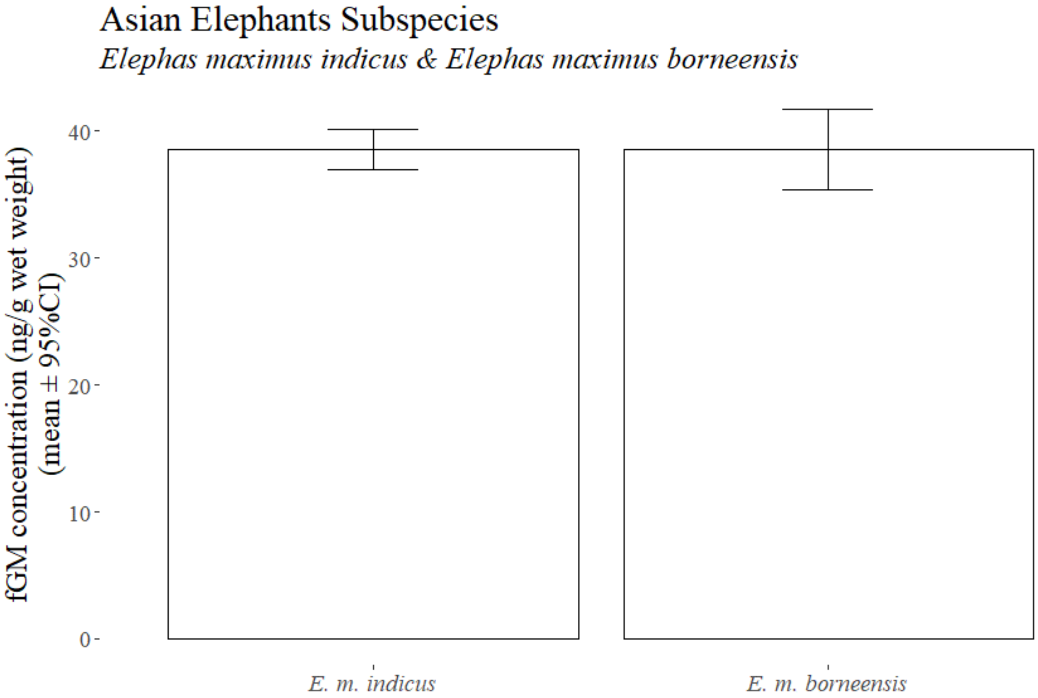


Figure 53: Comparison of average fGM concentrations of both subspecies of Asian elephant. Mann-Whitney U tests revealed no significant ($p > 0.05$) difference in median fGM concentrations between *E. m. indicus* & *E. m. borneensis*.