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## Effect of the Prospect of Transport on Captive Tiger Behavior and Fecal Cortisol in Naïve and Experienced Tigers

Callan Lichtenwalter  
*University of Arkansas, Fayetteville*

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Effect of the Prospect of Transport on Captive Tiger Behavior and Fecal Cortisol in Naïve and Experienced Tigers

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Animal Science

by

Callan Lichtenwalter  
University of Arkansas  
Bachelor of Science in Agricultural, Food, and Life Science, 2018

July 2020  
University of Arkansas

This thesis is approved for recommendation to the graduate council.

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Beth Kegley, Ph.D.  
Thesis Director

---

Kate Chapman, Ph.D.  
Committee member

---

Michelle Calvo-Lorenzo, Ph.D.  
Committee member

---

Jason Apple, Ph.D.  
Committee member

## ABSTRACT

With close to 5,000 captive tigers in the United States, it is vital to ensure that these tigers are receiving adequate welfare whether they are in an accredited facility, or privately owned. The goal of this study was to assess whether captive tigers at a rescue facility with experience being transported outside of the facility would respond differently to the presentation of their transport vehicle than their naïve counterparts who had only been transported within the facility. The behavior of 5 naïve and 7 experienced tigers ( $n = 12$ ) located at Turpentine Creek Wildlife Refuge in Eureka Springs, Arkansas was monitored an hour before, an hour during, and an hour after a rollcage (their normal transport apparatus) was placed directly in front of their enclosure. Behavior was measured by using instantaneous sampling of focal individuals. Behavior data were converted into a percentage of time the tiger spent doing a behavior during each of the 3 sessions (pre, stress, post). Fecal samples were collected on three days prior to and 3 days after rollcage placement for 1 hour. The samples were analyzed using a radioimmunoassay for corticosterone to determine the concentration of fecal cortisol metabolites present. Both behavioral and fecal cortisol metabolite data were analyzed using the Glimmix procedure of SAS with experience, session, day, and sex as fixed effects, tiger as subject, day as a repeated measure, and a behavior or cortisol concentration as the dependent variable. Each fixed effect had only 1 significant effect or tendency on behavior, with sex affecting sleep ( $P < 0.013$ ), day affecting excretion ( $P < 0.056$ ), session affecting autogrooming ( $P < 0.03$ ), and experience affecting sleep ( $P < 0.063$ ). Several tendencies between the fixed effects occurred in relation to behavior, as well as one for cortisol metabolite concentration ( $P < 0.099$ ), but there was no pattern to suggest that experienced tigers endured more stress with the prospect of transport than naïve tigers. Further study on captive tigers that utilizes a larger sample size and more behavioral and biological samples should be done to confirm these results.

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# **CHAPTER 1**

## **INTRODUCTION**

Tigers have long been captivating to humans because of their combination of beauty, grace, and potentially deadly power. All of this intrigue has made people desire to incorporate tigers into their world by hunting, exhibition, or close contact. Human fascination has led to tigers in captivity reaching far greater numbers than tigers in the wild (Glausiusz, 2008). Not all captivity is detrimental to tiger health and well-being, and many captive tigers receive excellent care by individuals and institutions that support the health and well-being of their tigers. This is not always the case though, and tigers sometime live in poor living conditions in the care of someone who may not have all the information they need to properly care for their tigers.

Stress and stereotypies, a repetitive act that serves no function, are commonly studied in captive animals to determine if adjustments in care are needed. Enriched environments with places to hide seem to allow for felid species to engage in more relaxed and less stereotypical behavior (Lyons et al, 1997; Clubb and Mason, 2007), and creating stable social groups leads tigers to display less aggressive and more affiliative behaviors (Miller and Kuhar, 2008). Transportation is a common source of stress in animals, and it can occur often in captive tigers if they are used in the entertainment industry. Being transported a single time can have a long term impact on the psychological and physiological health of an animal (Cattet et al., 2008), but despite very few studies on transport in captive tigers, there is some evidence that they can habituate to the stresses of travel (Dembiec et al., 2004).

Measuring glucocorticoid concentrations is a reliable way to monitor stress in both captive and wild animals. Glucocorticoid metabolites from feces can be easily and safely collected from tigers and, unlike with blood samples, collection does not create stress in the tiger.

Fecal samples allow for an extended picture of what stress the tiger has experienced in the 24 to 48 hours before excretion, and approximately 80% of glucocorticoid metabolites are excreted through bile that is passed in the feces (Graham and Brown, 1996). Once analyzed, concentrations of fecal glucocorticoid metabolites and behavior data can be combined to create a more complete picture of the stress a tiger incurred over the study (Wielebnowski et al., 2002). These data can be used to routinely monitor stress in captive and wild animals, or it can be used to assess how past experiences affect current stress responses, as in this study.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **History and Current Human Use of Tigers**

Tigers have been extirpated from large swaths of their original ecological range. The use of tigers today for pelts, traditional medicine, decoration, and entertainment continues to drive down their population numbers. A study done to analyze wild tiger numbers and the land that they occupy found that in the late 2000s, tigers only occupied 7% of their historic territory and in just the decade prior to the study, tiger numbers had decreased by 41% (Dinerstein et al., 2007). Estimates of exact numbers indicate that there were approximately 100,000 tigers in Eurasia at the start of the 1900s, with 40,000 in India alone in 1930. Current numbers of tigers in the wild are estimated at just 4,500 (Cohen, 2012b). Wildlife preserves have been established all over the tigers' home range of Eurasia. At first these reserves seemed to be efficient at restoring tiger population numbers, but over time it became apparent that managing reserves and enforcing laws on them was more difficult than previously expected, especially as the value of whole tigers and their products increased on legal and illegal markets. The cost of the efforts to restore tiger populations was estimated to be around \$82 million a year and included law enforcement, management of lands, and monitoring of tigers and their prey. With the demand for and value of tigers as a commodity not expected to decrease in the near future, the cost to keep tigers from ecological extinction is likely to increase steadily over time (Walston et al., 2010).

The numbers of captive tigers in the world are trending in the opposite direction of wild tiger numbers. Captive tiger numbers are estimated to be anywhere between 15,000 to 20,000 globally. Around 4,700 of these are in the United States alone, with only about 1,200 in zoos and the rest in private ownership, entertainment, and sanctuaries that rescue genetically "impure"

individuals from their former locations (Glausiysz, 2008). Tigers are held in many accredited zoos and are often one of the most popular attractions at zoos, however, visitors are often disappointed in the stereotypic actions and lack of naturalistic behavior tigers carry out in captivity. Despite this desire to witness tiger's natural hunting behaviors, zoo guests in one study drew the line at the feeding of live rabbits during display hours (Cottle et al., 2010).

Outside of accredited facilities, tigers are often "tamed" and prompted, as amusement for visitors, to behave in a manner that would not be seen in their wild counterparts. Circus and magic show goers are impressed when a tiger can seemingly be subdued into submission under the control of a trainer (Carmeli, 1999). Tigers are also kept in private facilities that market to visitors by allowing physical contact between tigers and people. Training occurs to teach the tigers to walk with humans, pose for pictures, and allow close human contact. Tigers are often kept from attacking by brutal training by their handler, and perhaps even drugs. This training in submission allows for a veneer of tameness, but underneath the tigers are just as wild and able to attack people (Cohen, 2012a).

Historically, tiger attacks have been common in the areas where tigers and people coexist. Tigers earned the nickname "maneater" in certain parts of Asia because of their reputation to attack and kill people. Because of this reputation, many tigers were killed before, but even more so, after the Indian subcontinent was colonized (Boomgaard, 2001). Tiger attacks still happen in a wild setting, but also in a captive setting, despite the tiger's reputation as a dangerous species and the world's largest large cat. Medical journals have described the severity, and potentially fatality, of tiger attacks on both adults and children that were visitors of establishments with privately-owned animals, and many deem the captivity and forced contact with these animals to be a serious issue of public health (Chapenoire et al., 2001). When the

ratio of fatal attacks to the number of each species present in the United States was compared, tigers were more fatal per year than dogs in the United States at a rate of 360 to 720 times (Nyhus et al., 2003). In the four years between 1998 and 2001, 59 tiger attacks on humans were reported with an average of 15 attacks per year. Of all the incidents reported, 75% occurred in either private ownership or at a non-accredited facility. Handlers and men were killed more often in private facilities, while women were killed more often in zoos. The listed causes of attack in order of relevance were getting too close to the tiger, handling tigers, taking photographs with tigers, feeding tigers, and escaped tigers. Similar data occurred in both the United States and abroad, although less data were available outside of the United States (Nyhus et al., 2003).

Attacks by tigers are likely to cause traumatic injuries because of their size and morphology. The nape of the neck is the most common site of injury, and tigers will orient their jaw in order to bite down between the spinal cord and the vertebrae. Infections can also cause death even if a person survives an initial injury (Oller and Udekwu, 1996). The American Veterinary Medical Association (AVMA), the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), the Centers for Disease Control (CDC), and the American Zoo and Aquarium Association (AZA) are all against the private ownership of tigers for either public health and safety concerns, or because of concern of animal welfare (Nyhus et al., 2003).

### **Legislation and Legal Cases**

The Endangered Species Act was passed in 1973 and prohibited the “import, export, take, and sale or offer for sale in interstate or foreign commerce” of species listed under the act unless authorized by a permit (USFWS, 2016). Permits could be obtained if the purpose was scientific

or for the propagation of the species. Certain species can be exhibited for educational purposes under the act. Further legislation to restrict permits was passed in 1979, and in 1993 exhibition for education could no longer be considered the primary purpose for obtaining a permit. Despite this improved legislation, inter-subspecies tigers were removed from full protections in 1998 since they were seen as non-contributing to species conservation due to their genetic impurity. The Captive Wildlife Safety Act made purchases, transport, and sales of tigers even stricter, but organizations with a license, including entertainment facilities, were exempted from these restrictions (USFWS, 2016).

When a bill was suggested to allow “generic” tigers to be placed back under full protections, the United States Fish and Wildlife Service (USFWS) allowed for a 2 month comment period about the proposed bill. Most of the commenters were in support of the bill, but several wanted stricter regulations that would prevent private ownership and breeding of tigers and the use of tigers in entertainment. The USFWS responded to these comments by saying that under current federal laws, as long as a tiger was purchased legally and the owner obtained a permit, other regulations were not highly feasible. With a permit, an owner can breed and display a tiger however they please as long as they do not sell the animal over state lines or kill the animal. The USFWS does not have the jurisdiction to restrict ownership or create more stringent or frequent inspections of individuals with a legally obtained permit (USFWS, 2016).

Every state has its own rules regarding ownership of large felids. These regulations include a full ban on private ownership outside of an accredited facility, partial bans on certain native species, special licensing, or no ban beyond those set by federal regulations. In 2005, Arkansas passed an act that regulated ownership of lions, tigers, and bears in the state. From the date the law passed, no new ownership permits would be given. Further, facilities housing these

species must meet AZA requirements, spay or neuter all animals unless deemed unsafe by a veterinarian, obtain special permits, and have at least \$100,000 in liability insurance (AGFC, 2011).

Private ownership of wild animals has increased over recent decades, and the internet has made the process of finding an exotic animal much easier. There are strong opinions on the ownership of big cats that divide people into stringent camps on the issue. Private owners claim that it is their constitutional right to have possession of big cat species and believe they help with conservation efforts. Groups like the World Wildlife Fund and the Humane Society of the United States staunchly disagree and believe that tiger ownership has a negative impact on humans, tiger welfare, and the environment. Those that support private ownership, be it in a home or within a large circus, feel that as long as the owner has the resources to take adequate care of their animals, they should have the right to keep the animals and potentially profit off of them. However, private owners with even the best intentions are sometimes not well equipped to maintain big cat species due to lack of funding, experience, or knowledge (Beetz, 2005).

The Lacey Act furthered the wildlife ownership legislation but had a flawed exception. Under the act, big cat owners must be licensed through the Animal and Plant Health Inspection Service (APHIS), which only gives out permits to those who intend to engage in commercial activities such as display and breeding. Thus, many people that want to own tigers as pets breed their animals to qualify for this permit. This permit also allowed big cat owners to display the young animals for photographs and cub petting, which is potentially detrimental to the health of the cubs and the safety of the visiting public. A bill introduced to limit permits that allow this exception, called the Big Cats Public Safety Protection Act, has yet to be passed by the U.S. federal government. The bill would prevent further permits of this type to be issued, while

current permit holders would be allowed to keep their animals. In theory, the number of captive tigers held by private owners in the U.S. under this exception would eventually decrease to zero (Young, 2014).

A horrifying example of what could go wrong when big cats are allowed to be kept by private owners occurred in Zanesville, Ohio in 2011. Terry Thompson had recently been released from federal prison when he committed suicide and released all 56 of his exotic animals including tigers, lions, leopards, wolves, primates, and bears. The release of such dangerous animals caused a major public safety concern, and 50 of the released animals had to be killed by law enforcement in order to protect the public. As a private owner, Thompson had acquired all of the necessary permits to possess the animals, and that still did not stop such a tragic loss of animal life from occurring. Attacks on humans by escaped exotic animals are more common than many in the public are aware. Seventy-seven people died in the United States between 1990 and 2012 by way of escaped exotic animals. Advocates for private ownership assert that the deaths associated with big cats most often fall on owners, exhibitors, and guests that have assumed the risk associated with interacting with such wildlife. Despite this point of view, private ownership can still be a considerable potential risk to public safety if animals escape or become unmanageable during encounters with visitors (Lucca, 2013).

### **Behavior and Physiology**

Ethograms are used to code for behavioral observations in animals. Partial ethograms are often developed for studies to meet the needs of the behavioral questions being asked, and when a full ethogram is developed it is often excluded from publications due to length (Martin and Bateson, 2007). Normal species behaviors must be known before abnormal behavior can be detected, which is why the use of a well-developed ethogram during behavioral observations can be vital for the accuracy of observations. Behaviors commonly observed during observations to



assess welfare are level of activity, movement, patterns of sleep, aggression level, and consumption of food and water (Squires, 2003). Although there are many factors that can indicate poor welfare following a stressful event, such as physiology and pathology, behavior is thought to be an animal's first response to stress and may help alert those that care for them that the animal is in a stressful situation. By regularly monitoring behavior, animal care takers have the ability to provide needed welfare interventions before a stressful situation has an impact on the body of the animal (Dawkins, 1998). Most felids have very similar behavioral repertoires and differ mostly in vocalizations and hunting strategies. Captive animals may differ from their wild counterparts, but captive felid species behave in an analogous manner to one another (Carlstead, 1996).

Owners of tigers used for human interaction and animal performances can claim that their tigers are tame, but even tigers that have been in captivity for several generations have not reached a stage of domestication. Tameness is an ongoing process of experimental learning and training that takes place over the life of an individual animal. Acquired tameness is limited to an individual and is not passed down genetically from a mother to her young. Through habituation and training, wild animals can adapt to human presence in captivity, and this ability to adapt is an important indicator of individual fitness for captive wild animals. Ease of tameness can lead to an ease of domestication, but the process of domestication requires many generations of intensive selective breeding to occur and is not likely to be seen in captive tigers since they have only been in captivity for a short time (Price, 1999).

### *Welfare*

The public's idea of animal welfare is a constantly evolving one, and considerations for wild animals in captivity have recently been added to this growing field of science. With public

opinion being incredibly influential in most animal industries, scientists have worked to create scientific means of addressing welfare without being overshadowed by moral sentiments. Both behavioral and physiological parameters to assess welfare have been developed to objectively measure stress in several animal species. Behavioral indicators of stress include novel motor patterns, apathy, increased aggression and sexual behavior, stereotypies, and excessive grooming along with other subjective measures. Physiological indicators of stress include decreased immune function and reproductive success, increases in cardiovascular output, and changes in endocrine parameters (Jordan, 2005).

Animals can learn that certain experiences are frightening and work to actively avoid those experiences, as in the case of chickens that learned to evade an inflating balloon (Duncan and Filshie, 1980). The cognitive abilities of animals are still not well understood, so it is not known whether animals are able to consider the past or the future, especially if they are experiencing a particularly noxious stimulus in the present. This is an important consideration for animals in acute pain or discomfort because they may not be able to consider a time beyond the present when they will no longer be in pain (Duncan and Pethrick, 1991).

Species and individuals vary in their ability to cope with stressors, and when an animal is no longer able to cope with a severe or prolonged stressor, health issues are likely to occur. An individual's ability to cope can be influenced by their genotype, development, experiences in early life, and social support among other factors (Koolhaas et al., 1999). The different coping styles are often broken into the two components of the fight-or-flight response. Active or fight responses are characterized by aggressive behavior and territorial control, while the flight response is identified by low levels of aggressive behavior and avoidance behavior (Engel and Schmale, 1972). Illegal animal trade, how most tigers outside of accredited facilities end up in

captivity, can potentially be stressful to animals because of frequent transportation and changing environments. Tigers used in illegal trade and in live entertainment sometimes have urine from other tigers sprayed in their faces as a way to force them to be submissive. Hierarchical changes, especially those between humans and animals, can also be a potential source of stress (CWI, 2008).

Assessing welfare in animals can be difficult because of their desire to conceal any behavior that would make them seem vulnerable to predators. Individual differences can also make it difficult to compare changes in welfare between animals based on behavior. It is recommended to assess changes in behavior before and after a stressful event as a way to better analyze how a stressor causes behavioral changes in an animal indicative of decreased welfare (Dawkins, 2001). Maladaptive behaviors can develop in animals that struggle to adapt to an environment and in animals that are unable to fulfill evolutionary behavioral and physiological needs. These maladaptive behaviors can manifest as stereotyping or behavior that causes injury, both of which can further decrease welfare and be very visual indicators of stress in an animal (Broom and Johnson, 2000).

### *Glucocorticoids*

In order to get a more complete picture of the state of an animal's welfare, it is important to obtain both behavioral and physiological data, as they can both potentially be deceiving on their own (Wielebnowski et al., 2002). Behavioral and physiological data can indicate conflicting results (Gusset, 2005), however fecal glucocorticoid metabolite (FGM) concentrations have changed along with behavior in felid species when aspects of their enclosure or management schedule were manipulated (Fanson and Wielebnowski, 2013)

It is ideal to also measure physiological indicators in animals that include heart and respiratory rate, carcass characteristics, neurotransmitters, and adrenal response (Hill and Broom, 2009). A common physiological indicator of stress is a change in hypothalamic-pituitary-adrenal (HPA) axis, often measured through assessment of fecal glucocorticoids or glucocorticoid metabolites. Like behavior, many things can influence the HPA axis, such as reproductive cycles, playing, and novel enrichment. Since behavior and the HPA axis can both be influenced, they are stronger indicators of stress or poor welfare when measured together in the same study (Hill and Broom, 2009). Coping styles are influenced by the HPA axis, and animals that more often exhibit an active response to stress have been shown to have a high reactivity of the HPA axis compared to animals that hide or act submissive in the face of stress. Different levels of HPA axis activation can lead to several health issues if the stress is prolonged or repetitive (Koolhaas et al., 1999).

The endocrine response during a stressful event involves two waves of reactions. The first wave is a sympathetic response that occurs almost immediately and leads to the secretion of catecholamines like epinephrine and norepinephrine. In the hypothalamus, corticotropin releasing hormone (CRH) is released and acts on the pituitary gland to secrete adrenocorticotrophic hormone (ACTH). The ACTH then enters the circulation and acts on the adrenal glands for the release of glucocorticoids, mostly cortisol in mammals. The release of glucocorticoids initiates the second wave of the endocrine response that lasts much longer than the very fast acting first wave that works through second messenger cascades, while the actions of glucocorticoids are carried out mostly through genomic responses that take an hour to days to occur (Sapolsky et al., 2000).

There are various effects that these genomic responses cause in a stressed animal. As a way to ensure the animal has the physical ability to respond to a stressor, energy is diverted to muscles from other areas of the body. At the same time, energy mobilization from stores is enhanced while gluconeogenesis and energy storage is inhibited. Cardiovascular tone is improved so that these mobilized forms of energy move to where they are needed in the muscles as quickly as possible. Functionality of the immune system, digestive system, and the reproductive system are inhibited to focus on the needs of an immediate response. Behaviorally, this translates to an animal that is not interested in or receptive to reproduction, has a decreased appetite, and has a potential increase in risk of illness. Finally, the animal will have increased cognitive and sensory abilities with improved glucose utilization and perfusion rates in the cerebral cortex (Sapolsky et al., 2000). There is also evidence that glucocorticoid responses can help with memory formation, but these memories can be warped based on feelings of fear associated with the event (McEwen and Sapolsky, 1995). Individual differences in life spans of mRNAs and hormone decay rate, as well as the potential for continued stressors in an environment, can lead to a prolonged glucocorticoid response. Over the course of a few days this can be highly beneficial for an animal, however, over a long period of time it can be harmful and perhaps fatal (Akana et al., 1994). Ultimately, it is vital that the animal is able to regain homeostasis within a few days of the stressful stimulus that set off the glucocorticoid response (Windle et al., 1998).

Fecal glucocorticoid metabolites allow for a non-invasive measurement of cortisol in animals. Blood collection to test for changes in cortisol concentrations can themselves be stressful, which can cause any results to be confounded. Serum cortisol concentrations can also only be interpreted as a snapshot of how the animal felt in the moment the sample was taken,

which includes daily and seasonal fluctuations in glucocorticoid concentrations. The FGM analysis allows for a non-invasive way to measure glucocorticoid concentrations over a more stable period of time because after a stressful event, glucocorticoids are metabolized by the liver, move through the digestive tract, and accumulate in the fecal matter (Bayazit, 2009). The delay between the presence of glucocorticoids in the plasma and the feces depends on the passage rate of the bile that contains the metabolized glucocorticoids from the liver to the rectum (Schwarzenberger et al., 1996). Cortisol is heavily metabolized by the liver, so it is necessary to test for metabolites or use a common test that cross-reacts well with the metabolites present in a particular species (Palme et al., 2005).

The popularity of using FGM to measure cortisol concentrations in wildlife and zoo species has increased in recent years because it allows for a hands-off way to measure stress (Lane, 2006) and as a tool for conservation and management of wild species (Brown, 2006). Because of the differences in sampling techniques and analysis methods, different studies can only be compared based on the conclusions of their physiological data, and not by the absolute glucocorticoid concentrations obtained (Schwarzenberger, 2007).

The basic process of analysis is the same across labs and species, however. It is best to collect fresh samples and store them at -20°C as soon as possible to avoid degradation of the steroids of interest by bacteria and other biochemical processes (Wasser et al., 1988). When the samples are ready for analysis, it is necessary to first extract the FGM from the feces. Procedures vary across studies, but most involve the use of 90% ethanol as a solvent. The FGM are collected in a supernatant through either repeated agitation or centrifugation (Möstl and Palme, 2002).

Next, an appropriate immunoassay analysis is performed. Since many steroids are found in the feces in their metabolite form, it is important to choose a test that is either specific for a certain metabolite or that cross-reacts well with many of the metabolites in question. When measuring FGM, using corticosterone antibodies has been shown to be the most effective way to get a full picture of a glucocorticoid response as they cross-reacted well with many metabolites in all but one of the species tested (Wasser et al., 2000).

Both radio-immunoassays (RIA) and enzyme-immunoassays (EIA) can be used to quantify FGM. While EIA tend to be less expensive, RIA are known to have increased precision, specificity, and sensitivity. Once a reliable method of FGM collection and analysis has been established at a facility, regular non-invasive monitoring of stress can take place to ensure the welfare and psychological needs of an animal are being met (Kumar and Umapathy, 2019).

#### *Glucocorticoid Studies in Felids*

Glucocorticoid research in felids started in domestic cats since they are commonly used in the lab setting and serve as a good model for other felids. This work was important because many felid species are threatened in the wild, and glucocorticoid analysis allows researchers to monitor the welfare of these species. Early glucocorticoid work was done on urinary samples and found elevated cortisol concentrations in domestic cats that endured a stressful caretaking routine (Carlstead et al., 1992), but also in nondomestic cats after translocation (Carlstead et al., 1993). Urine is not the best way to study glucocorticoid concentrations for a few reasons; it can be difficult to collect, felids spray urine which would lessen collection quantity, and very few glucocorticoids are excreted in the urine. Most glucocorticoids are eliminated through the bile, which is excreted with feces (Taylor and Scratcherd, 1963). Felid studies have shown that, after

ACTH injection, around 80% of adrenal metabolites are excreted in the feces and can be found in fecal matter between 24 and 48 hours after injection. Results indicated that fecal matter is the preferred biological substance to be used for glucocorticoid analysis in felid species (Graham and Brown, 1996).

Analyses to determine FGM have been conducted on several felid species since members of this family all metabolize glucocorticoids in similar ways. In jaguars an ACTH challenge, administering ACTH intravenously and then checking biological samples for glucocorticoid concentration changes demonstrated that although male jaguars had a greater baseline concentration of FGM, both males and females responded to the ACTH challenge with no significant differences in magnitude between the two groups. When comparing both sex and origin (male/female and captive born/wild born), females that had been born in captivity had the lowest FGM concentration than any other combination of the two groups. During the study, no group was subject to any stimulus or change in routine that would have caused them to have greater FGM concentrations than another group (Conforti et al., 2012).

Work with cheetahs has shown that even an acute stress response can lead to changes in FGM concentrations on the 2 days following the stressor, with a return to baseline starting on day 3. Similar to the study with jaguars above (Conforti et al., 2012), as well as clouded leopards (Wielebnowski et al., 2002) and tigers (Parnell et al., 2014; Parnell et al., 2015), the cheetahs had great individual variability in FGM concentrations, however, FGM concentrations changed in consistent ways after a stressful event no matter where they started. The female cheetahs in this study with the greatest concentrations of FGM were also the ones that performed the most “nervous” behaviors and the ones that were non-cyclic. The authors suggest that individual differences in cortisol concentrations could have an impact on the viability of that



animal to be used for breeding, and management strategies should be in place to ensure that these individuals are exposed to as little stress as possible (Jurke et al., 1997).

Cheetahs in captivity have been found to contract unusual diseases at a high incidence and also have very low reproductive success. In 3 populations of captive cheetahs, FGM concentrations were consistently greater than those of wild cheetahs in Namibia. The adrenal glands of captive cheetahs were also significantly larger than those of wild cheetahs. From these data, the authors concluded that cheetahs in captivity are under more stress and have a constant physiological response to stress that most likely causes their morbidity and poor reproductive performance (Terio et al., 2004).

Leopards are also commonly found in captivity, and their coping mechanisms have been studied to increase welfare and find best management practices. The Zurich Zoo tried implementing feeding boxes as a way to enrich the process of feed consumption and allow their leopards to attempt at natural foraging behavior associated with food consumption. During defined intervals during the day, the boxes would unlock, and through manual manipulation of the box by the leopard's paws or mouth, the box could be opened, and meat could be recovered. Fecal samples were collected to see if the ability to forage had any impact of FGM concentration. The leopard's FGM concentrations did not decrease, however, the author noted that the small sample size of 2 and the large variability between animals and over each day makes their results less than conclusive. They did suggest that these differences show a range of coping abilities in individuals based on their personal physiology and that the feeding boxes may not have provided the correct stimulation based in the leopard's natural foraging ecology (Burgener et al., 2008).

Margays and tigrinas are two other species of leopard whose behavioral and physiological response to stress have been studied in captivity. Researchers at the Itaipu Binational Wildlife Conservation Center in Brazil studied the effects of moving these species from a well enriched, large enclosure to a small barren enclosure, and then back to an enriched enclosure. Both species, but especially the tigrinas, experienced a large spike in FGM concentrations when they were introduced to the barren environment. This transfer was also associated with stressors of transport and restraint, which likely made the response even greater. Along with the physiological change, the animals showed an increase in agitated behavior that largely consisted of pacing. Once returned to the enriched environment, the animals' FGM concentrations returned to a baseline level, and the stereotypic behavior ceased. The authors concluded that having an environment that allowed felid species to hide and be mentally stimulated is necessary for proper welfare and management (Moreira et al., 2007).

Data on behavior and glucocorticoid changes were assessed in a population of clouded leopards in North America and compiled to understand the relationship between these two factors and how husbandry can impact them both. Contrary to the work in jaguars (Conforti et al., 2012), female clouded leopards consistently had greater concentrations of FGM. The authors theorized that this is an evolutionary adaptation that could improve mothering ability and watchfulness in females (Buirski et al., 1978). Another theory was that males and females have differences in their ability to secrete, metabolize, and excrete steroid hormones (Handa and McGivern, 2000). Similar to Conforti et al. (2012), males and females had a response of the same magnitude to an ACTH challenge, so baseline adrenal secretion varied, but the response to stress did not. When comparing behavior and FGM concentrations, the authors found that an increase in pacing, hiding, sleeping, and self-injurious behavior was associated with increases in

FGM concentrations. They suggested that, paired together, these two factors are reliable indicators of distress in captive felids (Wielebnowski et al., 2002).

Tigers are considered endangered by the International Union for the Conservation of Nature, and their health in both the wild and captivity is important in order to maintain the species (Goodrich et al., 2015). Work with wild tigers has focused largely on the best method of preserving samples while in the field since remote locations do not always allow access to a freezer capable of storing samples at -20°C. Analysis of samples collected at different time delays has shown that FGM concentrations begin to significantly decay at around 48 hours, which makes the collection of fresh samples and access to adequate storage vital in order to obtain the most accurate results (Shutt et al., 2011). A majority of the variation of FGM concentrations among samples was found to be attributed to inter-individual variation, but up to 32% of variation is also related to distribution of FGM being unequal within the fecal sample. This makes thoroughly mixing samples at analysis an important factor for accuracy and consistency (Parnell et al., 2015).

Other studies on wild tigers have looked at how the environment and close proximity to humans' affect FGM concentrations. A study in India collected fecal samples from a population of tigers that frequently came in close contact with human settlements. Similar to studies of tigers in captivity, females had greater FGM concentrations than males. There was also no difference found in FGM concentrations between months or across seasons, implying that changes in weather do not cause a significant stress response. There was a relationship between proximity to human settlements and FGM concentrations, with concentrations decreasing with increasing distance from areas where people were prevalent. The impact that this stress will

have on the wild tiger population is unclear, but it is likely to negatively influence both reproductive behaviors and physiology (Bhattacharjee et al., 2015).

Bengal tigers of India and Amur tigers of Russia have also been compared. They have greatly different habitats, and Bengal tigers are found at a much greater density with more pressure from anthropogenic forces. This is consistent with Bengal tigers having greater FGM concentrations than Amur tigers. Unlike the study by Bhattacharjee (2015) mentioned above with Bengal tigers, Amur tigers exhibited an increase in FGM concentrations during winter, but it was still not greater than the FGM concentrations of Bengal tigers at the same time of year in India that falls within their dry season. The exact cause of this difference between Amur and Bengal tigers is not clear. It could be subspecies differences but is likely influenced by the small space allotted to each individual Bengal tiger in protected reserves that continue to see an increase in human visitors and developments around their perimeter (Naidenko et al., 2019).

The FGM concentrations of wild and captive tigers were compared in Russia and showed that wild tiger FGM concentrations were significantly greater than those of captive tigers during each testing period. For both populations, FGM concentrations were greater in winter. The authors theorized that wild tigers need these greater concentrations to aid with their greater metabolism from hunting and moving across a territory. In the winter, both populations would experience an increase in metabolism to help maintain body heat and increases in FGM concentrations to aid in energy uptake and usage during this time (Naidenko et al., 2011). In contrast to Siberian tigers in Russia, another group of 5 Siberian tigers at a zoo in Minnesota showed no seasonal differences in FGM concentrations (Byers et al., 1990). This conflicting result may be explained by extremes in weather. Perhaps the Russian tigers experienced a greater change or a greater low in temperatures that resulted in an adrenocorticotrophic response

in order to maintain body heat than the tigers in Minnesota. The tigers in Minnesota were all males, and the Russian group consisted of an equal number of males and females. Since females have greater FGM concentrations, especially tied to the reproductive cycle, the seasonal average may have been increased in the mixed gender group of Russian tigers as opposed to the all-male Minnesota tigers. It is also possible that differences in management and FGM analysis were responsible for the discrepancy.

Several studies have been conducted on captive tigers in zoos throughout the world to study the effects that various aspects of captive life can have on the tigers. One such study compared 2 populations of captive tigers at different zoos in Australia. They found that females' FGM concentrations were significantly greater than those of male tigers. As mentioned for other felid species, the authors suggested this discrepancy was likely due to the females' reproductive cycles and differences in reproductive demands. All animals in this study were subject to a blood draw, and all showed a response in FGM concentrations with peaks occurring 1 to 2 days after blood draw and returning to baseline within 5 days of blood draw. No other stressful event occurred during this study, so it was concluded that the blood draw was enough of a stressor to cause a significant increase in FGM concentrations in tiger populations at both zoos studied (Narayan et al., 2013). Because individual variation, and variation among males and females is so common, it is not practical to compare individual animals. Different testing methods used by zoos and research facilities can also make comparisons across studies difficult (Parnell et al., 2014).

More extreme tests of the stress response have also been conducted. In Russia, a comparison was made between 2 captive tigers; 1 received an ACTH challenge, and the other was sedated, transported via helicopter for 3 hours, and placed in a new enclosure at a different

facility. Both animals had increased FGM concentrations, but the tiger that was transported to a new enclosure increased 10 times over baseline, while the FGM concentration of the tiger that received the ACTH challenge increased only 3 times over baseline. It was concluded that the ACTH challenge dose was not as great as the biological maximum, and the level of response is highly dependent on the severity of the stressor experienced (Rozhnov et al., 2010).

### *Behavior of Tigers in the Wild*

Collecting data on the behavior of wild tigers is difficult because of their large but fragmented home ranges, solitary lifestyle, and sensitivity to human presence. Traditionally tigers were tracked by researchers who followed their footprints in the snow and collected appropriate samples when available. This method obviously has several limitations, including available snow cover, so radio collars have become a vital tool for collecting behavioral data on tigers in the wild (Kerley et al., 2002).

Studies using radio telemetry have provided information on a variety of behavior in the elusive tiger. Female tigers with cubs have a vast home range despite the limitations that young can bring. The area these female tigers use tends to increase as the seasons transition from winter into summer and early fall. Maximum area of space used was recorded in June and October. Consistent with these data, tigers daily distance traveled tended to be greater in the period of the year without snow than the period of the year with snow (Rozhnov, 2011).

Females with cubs vary the size of their home range based on the age of their cubs. When cubs were a very young age and still relied entirely on their mother, the mother's home range was decreased. When cubs gained independence and maturity, they were able to follow their mother and learn hunting skills from her. At this time it also takes more food to sustain the growing cubs, so the mother must cover more ground to encounter and successfully obtain enough prey species (Hernandez-Blanco et al., 2015).

When moving, tigers seem to pause every so often and usually only cover a few kilometers a day, however, sometimes they were recorded covering vast distances in short periods of time. Tigers are also most active during the evening period, with decreased activity during the day, and the least activity at night. Overall, tigers were found to have an economical activity budget that aligns with the demand for energy typical of a large apex predator (Rozhnov, 2011).

The size of a tiger's territory affects its sociality and the likelihood of encountering other tigers for either mating or territorial disputes. Territories are demarcated based on sex, and males' larger territories will often overlap with at least one female (Goodrich et al., 2010). Male tiger territoriality can vary, and sometimes their home ranges can overlap at the same rate as male tiger home ranges overlap with female home ranges. While territories may crossover at some points, the core area of a territory usually belongs exclusively to one individual male. Crossover of home ranges may be a product of space reduction for tigers. Goodrich and others (2010) also noted a skewed sex ratio favoring males. Females are more susceptible to poaching as they will defend their cubs before fleeing a poacher. In the population being studied, this seemed to lead to more males in a smaller area that have to settle for a smaller territory in order to accommodate for all of the competition. This conclusion was made by comparing home range size and territoriality in male tigers in a neighboring game reserve. There, the sex ratio was more equal, and males were able to have larger, more defined home ranges (Hernandez-Blanco et al., 2015).

### *Behavior in Captivity*

Although many zoos, particularly accredited ones, have improved animal habitats to make them more natural and enriching, several facilities are now allowing more up close and

personal experiences with their more iconic species, including tigers. When the animals involved respond negatively to the human contact and visitors are harmed there can be negative consequences for the animals including euthanasia (Szokalski et al., 2013). Often times, it can be stressful for animals to be forced to interact with visitors, especially if close contact is involved. There is also evidence that visitor presence can be stimulating for certain species, but most of this research was in primate species and cannot be generalized across the animal kingdom (Davey, 2007).

On a protected contact tour (visitors feed animals through a fence), tigers were noted to have increased activity on tour day that was largely explained by an increase in time spent feeding and pacing. Pacing was not only at a greater rate on tour vs. non-tour days, it was observed at a higher rate during the tour as opposed to before or after and was also greater before the tour than after the tour (Szokalski et al., 2013). Although pacing is often regarded as a stereotypic behavior that may indicate decreased welfare (Mason et al., 2007), it can also be a sign of anticipation, especially if food is involved. Animals that are fed on a predictable schedule will often pace shortly before an expected meal (Bassett and Buchanan-Smith, 2007).

In a hands-on tour in the same facility as the protected contact tour mentioned above, cheetahs also showed an increase in activity on days when visitors were present. Pacing was less common in the cheetahs during tours, and they spent 10% of their time out of sight of the observers. Further, although the cheetahs did spend some of the tour time in close proximity to the visitors without agonistic behavior, they also spent more than half of their time in a distant proximity from the visitors. The authors posit that the familiarity of handlers and ability to escape lessened the likelihood of agonistic behaviors occurring in these cheetahs (Szokalski et al., 2013).



Captivity can have a range of effects on animals. A review of skull sizes of captive and wild animals found that captive lion and tigers tended to have reduced brain size (3.5 to 10.5%) compared to other wild subjects. The authors also hypothesized that captive cat brain size decreases while the animals are in captivity without any changes in genetics (Yamaguchi et al., 2009). It has been recognized that individual animals vary in their personality and response to stress. A study of a group of captive tigers in an Italian zoo showed that overall, the presence of visitors increased affiliative behavior among the tigers, but there was individual variation in the response. Three of the tigers had a strong positive correlation between visitor presence and affiliative behavior, 2 had a moderate positive correlation, and 2 showed a moderate negative correlation. It was suggested in this study that welfare should be customized to the individual when possible, so that the tigers that had a positive reaction to visitors would have access to that stimulus, and those that had a negative reaction to visitors could engage in avoidance behaviors as a way to decrease their stress levels (Pastorino et al., 2017).

Differences in personality can also reflect differences in physiological responses. Zoos often have difficulties breeding their captive animals that cannot be predicted by physiology or genetic studies alone. Cheetahs that were characterized as having a higher level of tense-fearfulness were the least likely to be successful breeders. Personality can have a significant impact on the success of propagation of endangered species in captive environments (Wielebnowski, 1999).

It is likely that experiences early in the life of an individual can have a lasting impact on their personality. There has been evidence of this in many species, including felids, that genes that affect personality are most active during a certain window of early development. This

makes it likely that individual personality will change little after the window of development closes (Lowe and Bradshaw, 2001).

The personality of keepers at facilities where tigers are kept can also have an impact on tiger behavior. Keepers that had a higher level of neuroticism tended to have less physical interaction with the animals, and those that tended to be self-conscious were more likely to be snarled at by the tigers. It is possible that tigers pick up on human behavior that is suggestive of dominant and submissive behavior, and from there they are able to figure out how to interact with a certain individual (Philips and Peck, 2007).

Although activity budgets of tigers in captivity can vary widely based on their individual circumstances, studies in similar conditions have been compiled to estimate the average activity budget of a tiger kept in an accredited facility. Tigers spend 32.64% of their time sleeping, 27.5% resting, and 17.3% walking (Biolatti et al., 2016). These data were consistent with behavior of tigers in the wild. Free-ranging tigers spend a large amount of their time sleeping in order to conserve energy. Outside of resting, they spend a considerable amount of time walking their territory which may present in captive animals as pacing or walking alternating paths across an enclosure (De Rouck et al., 2005).

#### *Sources of Stress and Stress Response in Captivity*

There are many causes of stress for tigers in a captive environment. Sound, restricted space, olfactory cues from predators, unstable social groups, lack of concealment, feeding competition, and forced interactions with visitors can all induce stress and be detrimental to welfare, and often times many are present in concert (McPhee and Carlstead, 2010). In clouded leopards, abnormal behavior and increases in fecal cortisol were found when they were on public

display, in proximity to predators, lacked vertical space in enclosures, and experienced frequent changes in keepers (Wielebnowski et al., 2002).

Although many stereotypies are likely to occur when animals are in captivity, such as self-mutilation, lethargy, and coprophagy, pacing is the most common stereotypic behavior in captive felids, and can occur under circumstances of stress, in enclosures void of external stimuli, and as a coping strategy for an environment that is sub optimal. Pacing is defined as being repetitive and invariant, spatially restricted, and a seemingly functionless behavior (Mason, 1991). Stereotyping during the anticipation of food is also common. As a way to combat this, studies have been conducted to assess novel ways of presenting food to captive felids that requires them to manipulate their environment in some way to receive the food. One such study used a box filled with food rewards that would be available to open at only certain times of the day. During these windows of time the tigers had to manipulate the box in order to slide a door horizontally to obtain the food. When the boxes were present, the two tigers in the study spent less time pacing and more time sleeping. These behavior changes are consistent with improved welfare because of a more naturalistic time budget (Jenny and Schmid, 2002).

In a study of multiple felid species, the edges of enclosures were not used more often than the rest of the enclosure in overall space use, but the edges were used most often for pacing. Movement of these animals was also correlated with enclosure size, with increased movement in larger enclosures. Enclosure views were ranked based on quality, and cats that had the most clear, unobstructed views spent more time on elevated surfaces. These results together suggest that designing an enclosure to fit the species' needs is important so that individuals can carry out natural behaviors (Lyons et al., 1997).

Mice raised in an enriched environment and then moved to a barren cage were more likely to perform stereotypic behavior than mice that were moved from one standard cage to another. The authors used the Frustration Hypothesis to justify this response by suggesting that the stereotypical behavior was a way for the mice to work out their frustration of a barren environment by performing motions similar to the actions taken in an enriched environment (Latham and Mason, 2010). A review that analyzed stereotypic behavior in a range of captive carnivores found that foraging behavior (frequent kills per day, long distance chases, and long distances in between kills) did not correlate with stereotypic behavior. Instead a combination of body weight and home range size were positively correlated with stereotypic behavior in captivity. These two factors were not enough on their own to elicit stereotyping, but when found together were accurate predictors of the behavior (Clubb and Mason, 2007).

Most carnivores tend to have a solitary lifestyle, although captive animals tend to be more flexible in their ability to be housed in different ways than wild animals. Factors that determine an animal's ability to be housed in groups include competition, sex, the role of the captive animal in its facility, and individual personality (Price and Stoinski, 2007). Tigers are often housed in pairs or small groups, and if the animals have been with each other since they were young and have a history of affiliative social interactions, they can continue to be housed together until an issue between them arises. Studies of the behavioral interactions of group housed tigers are important to be able to balance the costs and benefits of shared enclosure space and to be able to understand common affiliative and aggressive behaviors (Tilson et al., 1995).

A group of 6 female tigers at Magic Kingdom in Bay Lake, Florida were separated into 2 groups of 3 during the day when they had access to their enclosure and were allowed both private space and intermingled space with all but 1 tiger with a history of aggression at night.

Keepers wanted to study their behavior to test the efficacy of this housing system. When aggression was specifically analyzed, the most common contact aggressive behavior and more generally the most common aggressive behavior, was a strike with paw. Charging, biting, and pouncing were observed in this contact group and all occurred at a much lower rate. The most common non-contact aggressive behavior was strike at, which increased in frequency over time, followed by charging without contact. Hissing was the most frequent aggressive vocalization, and spraying was common, but decreased over time. Affiliative behavior was measured as the average number of social partners a tiger had over a given time. The tigers had more social pairings in the morning than other times of the day and had more affiliative reactions in the winter than in the summer. Since the tigers were still young when brought to the facility, it was also found that the amount of time they spent together decreased as they got older, and the most solitary tigers tended to exhibit the most non-contact aggression. The viability of this group of tigers living together was overall successful, but the authors warn their pre-mixing familiarity and large, enriched enclosure space may have allowed for their ability to cohabitate (Miller and Kuhar, 2008).

Another study looked at behaviors of single and paired tigers with and without visual access to conspecifics. Paired tigers that were able to observe their tiger neighbors were more likely to pace than paired or single tigers that were not able to see their neighbors. The paired animals without tiger neighbors also performed significantly more allogrooming than paired animals with tiger neighbors. The authors concluded that it was likely the tigers with tiger neighbors had a lower state of welfare because of a higher incidence of pacing. They postulated that seeing their neighbors pace induced other tigers to pace as well. Another observation was that lower levels of pacing corresponded with higher levels of normal walking, suggesting the

pacing was a frustrated behavior used to compensate for a lower level of normal walking. They concluded that overall, housing in pairs could be beneficial if the individual tigers allowed it but being in sight of conspecifics could be a potential source of stress (De Rouck et al., 2005).

A study, on the same group of tigers at Magic Kingdom mentioned above, investigated how aggressive and affiliative behavior would change as a result of increased contact during the renovation of 1 of the 2 enclosures the tigers were housed in. During the renovation period, the tigers spent half of the day in their outdoor enclosure and half of the day in their night house within alternating combinations of animals. During the renovation period, the amount of aggressive behavior decreased, and the amount of affiliative behavior increased. These behaviors are in line with the social avoidance strategy that is often seen when animals are in close proximity to one another and unable to escape. The strategy allows tigers to avoid any potentially dangerous altercations by decreasing overall contact with one another. The effects of this strategy were still seen after the second enclosure was reopened, because once evoked, the behaviors of the social avoidance strategy can have lasting effects on the social structure of a group (Miller et al., 2011).

Another study at Magic Kingdom looked at differences in behavior when tigers were either group housed or singly housed in their overnight stalls. Behavior was not significantly different in the tigers when they were kept in social groups or housed individually, and in both settings, the tigers were observed to be sleeping in about 75% of scans. Sleep position, pacing, aggression upon reuniting with conspecifics, and vocalizations were also unchanged based on housing method. Since sleeping behavior is an indicator of welfare in felids, the lack of change in sleeping behavior in the two housing systems indicates that one is not more stressful than the other, and both can be used to maintain good welfare (Miller et al., 2013).

## **Behavior, Welfare, and Physiology during Transportation**

The movement of animals is often necessary to maintain their health or economic value, but it can be a stressful experience for any animal, and especially animals that travel on a regular basis in less than ideal conditions. An animal's past experiences and individual personality can impact how they will react to travel, and the amount of stress that travel induces. One fearful experience with travel could have a lasting impact on the amount of stress an animal feels due to transportation, especially with species or individuals that have a more excitable temperament or behavioral response. The amygdala is the organ of the brain most heavily involved in fear responses and conditioning an animal to fear a stimulus or situation can be accomplished very quickly but undone very slowly because of the intense responses encoded here. Experiences in early life are also more likely to have a lasting impact on an animal and impact its physiological response to that stressor as it gets older. Because animals often hide stressed behavior, it is important to measure both behavioral and physiological indicators of stress in order to get a complete picture of the stress response in that animal (Grandin, 1997).

Translocation is a strategy used in wildlife management to move problem populations or restore a species in an area where they have been previously extirpated. This strategy can often fail because of the stress experienced by the animal during handling and transportation (Dickens et al., 2010). A single handling event can have long term effects on an animal and cause a myopathy in the ability to mount future appropriate stress responses (Cattet et al., 2008).

Transportation can be particularly stressful because it is a constant stressor that the animal is unable to escape from. Transportation can lead to an increase in the secretion of glucocorticoids and an increase in heart rate. The degree to which the HPA axis is stimulated often depends on the distance an animal travels in confinement and the time in transit (Dickens et

al., 2010). Immediately after a 14-hour journey in poultry cages, chukar birds showed a total inability to mount a stress response (Dickens et al., 2009). This inhibited ability to produce a response of the HPA axis is known as exhaustion of the system. Although an animal may be able to mount a stress response again in the future, an experience with exhaustion can have a long-term impact on how the animal views transportation. Stress can be decreased by designing transportation enclosures suitable for the species being transported, not over-crowding animals, ventilation, and the quality of the roads used for travel (Dickens et al., 2010).

Studies on the impact of translocation in wild tigers have been conducted, but the results of survival and vitality of the animal can often be difficult to discern because of close human contact in their range. Tigers could be dying as a result of a physiological response to stress that induces illness or starvation, or, as is often the case, they could be dying because of a fragmented habitat and a high incidence of poaching at the site of translocation (Goodrich and Miquelle, 2005).

Very few studies have been conducted on the impact that transportation has on captive tigers, and even fewer have been conducted on tigers outside of the entertainment industry. Five tigers at Turpentine Creek Wildlife Refuge were used in one such study. The goal was to monitor the behavior of the tigers an hour before transportation, during transportation in a roll cage around the facility and back to their home cage, and an hour after the transportation. Fecal samples were collected for 6 days before and 12 days after transportation. After transportation, each of the tigers was observed to spend at least 5 minutes laying down and the group spent an average 75% of the hour after transport laying down. There was, however, no significant difference in activity budgets before and after transportation. On average, fecal cortisol concentrations peaked in the block of 3 to 6 days post transportation but ranged from the block



of 0 to 3 days post transportation to the block of 6 to 9 days post transportation. Samples had all returned to a concentration not significantly different from baseline by the block of 9 to 12 days, but one sample returned to baseline as early as the day 3 to 6 block, indicating recovery time varies based on individual and can vary from a few days to over a week (Dembiec et al., 2004).

### *The Circus and Transportation for Entertainment*

Animals in the circus are transported in containers called beast wagons that are often small enough to be placed in a trailer and stacked on top of other beast wagons. Even after arrival to a destination, animals may remain confined to their beast wagon or allowed to move to an exercise pen that is attached to their beast wagon. During the off-season animal are moved to private facilities, but there is very little information about these facilities. Animals end up in circuses through legal and illegal breeding and purchasing, and from animals that zoos are not able to care for because they are either at capacity, or the animal is not able to contribute to a conservation program (Iossa et al., 2009). The space allotted for circus animals is usually very limited. On average exercise pens are about 25% of the size of zoo outdoor enclosures, and beast wagons are about 25% of the size of zoo indoor enclosures. Beast wagons for tigers in the United States are only required to be 9 m<sup>2</sup> (Krawczel et al., 2005).

The circus lifestyle can have many negative effects on its animal performers. Tigers have been shown to develop gastroenteritis as a direct result of the stress of recurrent, loud noises (Cociu et al., 1974). An analysis of North American circuses detailed the unrelenting travel schedule endured by the animals. On average a circus spent 4.7 days in one location with 2.6 days resting (45% with no resting days) and 473.7 miles between destinations (Iossa et al., 2009). When 6 circuses in the United States were studied, only 2 had transport environments

with high capacity ventilation and insulated walls, both of which are vital for temperature regulation within a safe range (Toscano et al., 2001).

Even in a well maintained, accredited zoo, there can be welfare concerns and animals can develop stereotypies. The limited research that is conducted on circus animals often occurs in well-financed zoos and is not representative of the many circuses that struggle financially. Regardless, circus animals are rarely, if ever, kept in environments as well curated as zoos, and many fail to provide basic needs for social behavior, space, and nutritional requirements. Animals spend between 1 to 9% of their days performing in a circus, so performance is not seen as an enriching event. The performance itself doesn't seem to have either a negative or positive impact on welfare, but the crowd present at the performance is likely to be a large source of stress for the animal performer (Iosssa et al., 2009).

Since tigers are wide ranging carnivores, they are one of the species least suited for circus life, while at the same time being one of the most popular circus animals. The small enclosure sizes and lack of mental stimulation lead to stereotypic behavior and reduced welfare. Loading and unloading of cages onto transport vehicles can also be a source of stress because of the close human contact and jostled movement of cages, but circus tigers have the ability to habituate to this experience due to its frequency (Kiley-Worthington, 1990).

A few studies on the behavior of tigers during transport have been conducted. During the movement of a circus in Texas, the behavior of 3 groups of circus tigers was observed. In all 3 groups, the tigers paced more as the duration of transportation increased. Most of the time not dedicated to pacing was spent lying down. Tigers that were in their exercise pens or performed 2 hours or less before transport were more likely to lay down for longer periods of time during transportation. The tigers within the 3 groups showed a wide variety of behaviors that made it

difficult to draw overarching conclusions about coping mechanisms of circus tigers during transportation (Nevill and Friend, 2003).

The environment of the transport cages is also important for the welfare of the tigers. A study on tiger body temperature and noxious gasses in transport cages found no significant differences in either measure before, during, and after transportation. Individual tigers showed differences in body temperature at loading, possibly associated with performance and the stress of loading, but body temperatures were all back to baseline the morning following a transport event. Body temperatures were elevated during loading in both hot and cold weather, so it is probable this is a physiological response to movement and stress. The temperatures tigers experienced during transport were also within range of what they would experience in their natural environment, which would allow them the ability to regulate their internal body temperature even as the external temperature exceeded 40° C. It is likely that noxious gas levels were low during this study period because the tigers' cages had been cleaned just before transport, but that is not always the case (Nevill et al., 2004).

Performance itself can also be a stressor for circus animals. Behaviors of performing tigers were observed before and after both a single performance and a set of 3 performances. Before the 1 hour performance, pacing increased every hour to a rate of about 50% in the hour just before the show. After the performance, little if any pacing was observed in the tigers. Similar results were seen on the days with 3 performances, except pacing peaked 2 hours before the show and decreased just slightly in the last hour before a performance. There were no differences in behavior in the 15 hours after a performance between the group that performed once and the group that performed 3 times. Pacing also increased during an open house in which the circus attendees were allowed to view the animals in close proximity. In most instances of

pacing, tigers would walk along the edges of the cage and were oriented to look outside of their cage. The authors concluded that the increase in pacing seen before performances was likely due to a combination of anticipatory activity and increased close contact with people (Krawczel et al., 2005).

While exercise pens can be beneficial in allowing more movement and species typical behavior, one study found that the amount of time a tiger spent in an exercise pen did not have an impact on the time the tiger spent pacing, lying down, standing, or walking when moved back into its cage. There was a comparable amount of pacing that occurred in a small set of cages and a large set of cages. As with other circus tiger behavior, there was a considerable amount of variation among individuals and pacing behavior. When placed in the exercise pens, most of the movement was seen during the first half of the allotted time, but a majority of tigers showed active behavior until their exercise period was completed. Tigers that traversed a greater distance while in their exercise pen tended to pace less when back in their cage. All together this study shows that exercise can have an impact on pacing, but further study of length of time and stimulation in the exercise pen will need to be conducted to get a clearer picture of the impact these exercise pens have on the welfare of circus tigers (Nevill and Friend, 2006).

## **Conclusion**

To the author's knowledge, there are currently no studies that investigated what lasting impact a history of transport can have on the stress response of a tiger that is faced with the prospect of being transported. Many tigers involved in the entertainment industry have had an experience of continued travel, and several of the tigers at Turpentine Creek Wildlife Refuge (TCWR) were rescued from destinations that required a long trip back to the facility. It is not known if the tigers keep a negative association of travel with them that induces a stress response

when exposed to stimuli associated with travel later in life. Anecdotally, many tigers that have experienced travel frequently or at a great distance seem to shy away from the transport vehicle used to move them around TCWR.

In this study, we investigated if tigers that have more experience with transport outside of TCWR have the same behavioral and physiological response to the anticipation of travel as the tigers that have little to no experience with transport outside of TCWR. We monitored tiger behavior for the 60 minutes before, during, and after a rollcage was placed at the entrance of each tiger's enclosure. Fecal samples were collected from 3 days before and 3 days after this potentially stressful event occurred. When assessed together, these data can help TCWR determine if their tigers have a stress response to anticipated transportation, and if the tigers with more experience demonstrated a stress response indicative of a greater magnitude. This will allow them to make management decisions that benefit the welfare of their tigers.

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## **CHAPTER 3**

### **Effect of the Prospect of Transport on Captive Tiger Behavior and Fecal Cortisol in Naïve and Experienced Tigers**

#### **INTRODUCTION**

There are estimated to be more captive tigers in the United States than in all of their wild range. With this great number, approaching 5,000 captive tigers, comes the responsibility that tigers are provided with the best possible care with regular analysis of their welfare. With only about a quarter of these tigers in accredited zoos, it becomes difficult to know that each tiger is receiving an acceptable standard of care (Glausiuz, 2008). The recent Netflix documentary *Tiger King* showed the appeal and power that owning tigers can bring to an individual. Unfortunately, it also showed that even when private tiger owners have the best intentions in mind when deciding to purchase a tiger, they are often not able to appropriately keep up with the needs of that tiger.

Tigers in the entertainment industry and private zoos are often encouraged into situations and close encounters that deviate from their natural behavior, and this can potentially cause repeated stressful responses. A study of circus tigers found that pacing increased to 50% greater than the baseline level in the hours leading up to a performance, but pacing observations after the performance were negligible (Krawczel et al., 2005). Performance in circus tigers was not found to have a positive or negative effect on welfare when stereotypies were used as the indicators. It is possible that the performance itself is stimulating, but it accounts for such a small portion of the tiger's activity budgets that it is unlikely this is enough enrichment for the tiger. While the performance was not stressful, large crowds and people viewing animals before a show were seen to increase pacing in tigers (Kiley-Worthington, 1990).

When tigers are involved in entertainment, they are often exposed to frequent transportation, and for all animals, transport tends to be a stressful event. In the wild, tigers that need to be translocated are difficult to study because of several environmental factors, but there is an increased rate of death among these tigers compared to tigers that have not been translocated (Goodrich and Miquelle, 2005). Tigers from a circus in Texas paced more the longer they were in a transport vehicle, however, the authors noticed a wide variety of behaviors among animals (Nevill and Friend, 2003). A study on tigers at Turpentine Creek Wildlife Refuge (TCWR) seemed to show that tigers with experience with travel in the facility were habituated to the stressful event, while tigers that were not experienced showed a behavioral and physiological response more indicative of a stressful response (Dembiec et al., 2004).

One way to measure stress physiology in tigers and other wild animals is by assessing fecal glucocorticoid metabolites (FGM) because feces can be collected without further stressing the animals, and around 80% of these stress metabolites are excreted with the bile in the feces. Fecal samples also provide a more complete picture of day to day stress because they have an accumulation of metabolites from 24 to 48 hours prior, while a serum sample would just account for the stress response the moment the sample was taken (Graham and Brown, 1996). The FGM have been analyzed in several species starting with domestic cats (Carlstead et al., 1992) and extending to various wild cats such as jaguars (Conforti et al., 2012) and leopards (Buirski et al., 1978).

Very few studies on the transportation of tigers, other than the ones mentioned above, have been conducted, and there is little to no evidence suggesting what the long-term impact of frequent or long-lasting travel can have on tigers. It is unclear whether travel experiences would cause a tiger to habituate as mentioned by Dembiec and others (2004), or if experience would



cause a predictable, stressful response to travel. When behavioral data and FGM data are pooled together, they can be used to create a more complete picture of what a tiger is experiencing when exposed to travel stimuli, such as seeing a rollcage, a mobile enclosure used for transportation, in the case of this study (Wielebnowski et al., 2002). With the tigers at TCWR we wanted to see if only presenting the rollcage at the door of their enclosure would be enough to elicit a behavioral or physiological response in two groups of tigers. One group consisted of tigers that had little to no experience with transport outside the facility, and the other group contained tigers with extensive experience with travel outside the facility. Fecal samples were collected 3 days before and 3 days after exposure, and behavioral data were recorded for an hour before, an hour during, and an hour after exposure. Any differences found would be important for the staff of TCWR to know so that best management practices can be implemented such as limiting exposure to rollcages or different husbandry methods applied to animals with different backgrounds and sexes. Differences would also shed a light on any long-term effects of tiger response to travel based on a history of travel in their past.

## **MATERIALS AND METHODS**

### **Animals, Care, and Location of Observations**

All tigers were housed at Turpentine Creek Wildlife Refuge, a non-profit 501(c)(3) facility located in Eureka Springs, Arkansas. Turpentine Creek Wildlife Refuge does not breed their animals and either separates male-female pairs or performs a sterilization procedure on the male animals. No direct human-animal interaction is permitted by the public, and direct contact only occurs with staff when the animal is sedated for veterinary procedures. Animals at TCWR are never bought or sold but are rescued from other facilities and individuals when deemed appropriate. No animals are ever exhibited outside the facility, and within each enclosure

animals have access to shelter, shade, and clean drinking water. At TCWR all animals are cared for by animal care staff and interns whose daily responsibilities include cleaning animal enclosures and feeding animals to fit their individual dietary needs. Behavioral management (operant conditioning with positive reinforcement) is used to train animals to comply with hands-off health checks and TCWR enriches animals on a 5-day rotation program (new enrichment every 5 days). Turpentine Creek Wildlife Refuge is accredited by the Global Federation of Animal Sanctuaries, whose purpose is to ensure the welfare of animals in captivity, and by the Big Cat Sanctuary Alliance that works to end the private ownership and improve the welfare of big cats (TCWR, 2019).

Twelve tigers were selected for observation based on their histories with transportation and divided into a naïve ( $n = 5$ ) and experienced ( $n = 7$ ) group. Transportation experience was determined by facility staff who were familiar with the tigers' backgrounds and were able to determine if the tigers had enough exposure to transportation outside of TCWR to be deemed experienced (Table 1). In the naïve group, there were two sets of pair-housed tigers (one set related and one set unrelated) and one individually housed tiger. In the experienced group, there was one set of 3 individual tigers housed in a group of 4 total, unrelated tigers (the individual was not included in the study because he was a Ti-Liger), one pair of related tigers that shared their enclosure with an additional related tiger every other day, and two individually housed tigers (Table 2). Of the 12 tigers, 8 were female and 4 were male. Age at the time of study ranged from 3 years to 13 years. Time spent at TCWR at the time of study ranged from 1 year to 10 years (Table 3).

### **Behavioral Data Collection**

Anecdotally, TCWR staff have observed that tigers act visibly stressed when a rollcage, the device used to transport tigers for regular veterinary procedures and for relocation, is wheeled by their enclosure. To more closely examine how the tigers are impacted by the anticipation of inevitable transportation, each enclosure had a rollcage placed at its entrance which was left for 1 hour so behavioral observations could occur. An hour of pre-stress behavior and an hour of post-stress behavior were also recorded directly before and after the rollcage was introduced, respectively. All behavioral observations were recorded over the course of 3 Saturdays, 22 February through 7 March, 2020, and 1 Sunday, 15 March, 2020. After behavioral observations occurred in groups of 6 animals a day over the first two Saturdays, the procedure was repeated over a second Saturday and Sunday with the same groups in the same order, with the second observation of group 2 occurring one day later than previous observations due to dangerous weather conditions.

The 12 tigers were split into 2 groups of 6 individuals of mixed naïve and experienced backgrounds. The first group included AU, JO, KH, KI, TA, and TH, and the second group included AT, CH, RO, SN, SH, TO. The 2 groups were decided based on location and visibility of other tiger enclosures included in the study. Individuals in group 1 could not easily see the enclosures of group 2 and thus would not be affected by introduction of the roll cage when it was not their week for exposure.

Behavioral observations were recorded using instantaneous sampling of focal individuals during the pre-stress, stress, and post-stress period. A data sheet (Appendix) was created to record behavior for the first 10 seconds of every minute for 60 minutes in each of the 3 observation periods. This sheet also included information on the weather, if other tigers were present in the enclosure, if the tiger was in its den or out of sight, if visitors were present, and an

additional notes section. An ethogram (Appendix) created for previous studies at TCWR was used for this study. In each 10 second window of observation, all behaviors seen were coded on the data sheet. Data were collected by the author and research assistants. All research assistants were trained to use the ethogram to code behavior and to record data using the sheets provided. After all observation periods, particular behaviors and groups of behaviors that were common or of interest were selected for analysis (Appendix).

### **Fecal Sample Collection and Storage**

Fecal samples were collected to examine any changes in cortisol from the days before and after the rollcage was presented. Samples were collected on day -4, -2, 0, 1, 3, and 5 in relation to when the tigers were exposed to the rollcage. The samples from the second repetition of group 2 were collected on day -5, -3, -1, 1, 2, and 4 in relation to when the tigers were exposed to the rollcage to account for the one-day delay in exposure (Table 4). Regardless, the first 3 days of fecal sample collection represent baseline fecal cortisol concentrations for each tiger, and the last 3 days of fecal sample collection represent the post transport stress exposure fecal cortisol concentrations for each tiger. Each tiger had a full 2 weeks between exposures to the rollcage, so fecal cortisol metabolite concentrations had ample time to return to baseline. The animals in this study were not exposed to any other known distressing stimuli throughout this time, and other than the introduction of the rollcage outside their enclosure twice for 1 hour 2 weeks apart, no part of their daily routine was altered.

To identify individual's fecal samples when more than one tiger was housed in an enclosure, non-toxic glitter was added to size 00 gelatin capsules inside a meat ball and fed to each animal separately. The staff at TCWR use this method of no contact individual feeding when giving medication, so the tigers were familiar with the concept and readily consumed the

color of glitter assigned to them. Glitter was fed daily beginning 2 days before the first sample was to be collected throughout the last collection day to ensure its presence in the fecal matter. Each tiger received the same color of glitter throughout the study period, and the 3 colors used (blue, hot pink, and gold) were easily distinguishable in the feces.

All samples were collected by a TCWR staff member in pre-marked seal-topped plastic bags shortly before pick-up and left cool, dry container before being transported to the University of Arkansas Department of Animal Science laboratories for storage. Occasionally, a sample was not able to be collected on the assigned day due to individual tiger physiology. When this occurred, a sample was retrieved as soon as possible and included in the next pickup day. Enclosures were cleaned daily, so each sample collected would correspond to the most recent stool voided by the tiger. When the samples arrived in the lab air was removed from the bags, and they were stored in a freezer at  $-20^{\circ}\text{C}$  until ready to be used for analysis.

### **Fecal Hormone Extraction**

Fecal samples were thawed at room temperature and thoroughly mixed within their individual bags. Approximately 20 g of fecal material from each sample were placed in individual beakers with care to avoid adding bones, fur, forage, and debris. These subsamples were dried in a lyophilizer for 5 days at which point they were deemed fully dry. Each dried fecal sample was pulverized in a coffee grinder until the consistency reached that of a fine power. Any remaining fur or debris was sifted from samples and the powdered feces were stored in individual bags at  $-20^{\circ}\text{C}$  until further extraction could occur.

At time of extraction, approximately 0.2 g of powdered fecal material (exact weight was recorded) was added to a 16 x 125 mm Pyrex screw top test tube. Each of the 144 samples were extracted in duplicate for a total of 288 extracted samples. After samples were added, 5 mL of

90% aqueous ethanol was added to each test tube and the tube was thoroughly vortexed. A mark was placed as each 2.5 mL of ethanol were added so ethanol levels could be checked during boiling. 48 samples at a time were placed in a test tube rack and boiled in a hot water bath at 100° C for 20 minutes. Tubes were checked every 5 minutes to ascertain if too much ethanol had evaporated, and if so 2.5 to 5 mL of ethanol were added before the samples were placed back in the water bath.

After boiling, the samples were centrifuged at  $1,500 \times g$  for 20 minutes at 22° C. The supernatant that formed after centrifugation was poured into another test tube of varying larger sizes. Another 5 mL of 90% aqueous ethanol was added to the remaining fecal pellet and vortexed thoroughly. The samples were once again centrifuged with the same settings. The second supernatant was added to the test tube containing the corresponding first supernatant. From there the combined supernatants were taken to dryness in a fume hood.

### **Cortisol Metabolite Analysis**

Once samples were completely dry, they were reconstituted in their test tube by adding 5 mL of pure, aqueous methanol and vortexing thoroughly to ensure as much of the dried hormone extracted was brought into solution as possible. The methanol-fecal extract solution was diluted 20:1 with phosphate-buffered saline (PBS) (800 mL of distilled water, 8 g of NaCl, 0.2 g of KCl, 1.44 g of  $\text{Na}_2\text{HPO}_4$ , 0.24 g of  $\text{KH}_2\text{PO}_4$ , pH adjusted to 7.4 with NaOH, distilled water added so total volume was 1 L) using a Hamilton 500 series automated pipetter (Hamilton Company, Reno, Nevada).

A double-antibody corticosterone  $^{125}\text{I}$  radioimmunoassay (RIA; MP Biomedicals, Inc., Costa Mesa, CA) that had been previously validated for the domestic cat (Graham and Brown, 1996), cheetah (Terio et al., 1999), clouded leopard (Wielebnowski et al., 2002), and tigrina and

margay (Moreira et al., 2007) was used to analyze fecal cortisol concentrations from the extracted samples. Cortisol is largely broken down in the body before excretion, so an antibody for cortisol would not be effective. An antibody for corticosterone was used because it had exhibited consistent cross-reaction with metabolites of cortisol in a previous analysis (Wasser et al., 2000). Sensitivity was 7.7 ng/mL and all samples were extracted and run in duplicate.

#### *Extraction and Assay Validation*

Several validation steps were performed to ensure the accuracy of the results. Before the unknown samples were extracted and validated, the entire process was run on samples from 2 tigers not included on the study. One tiger was suspected to have a greater concentration of cortisol than the other because she had just arrived at TCWR a few weeks prior and was still adjusting, while the other tiger was established and not on display. To make sure that 2 centrifugation steps would be sufficient in extracting cortisol from the fecal samples, a third centrifugation step was conducted twice on duplicates of both samples and analyzed separately from previous supernatants. The concentration of cortisol in the supernatant of the third centrifugation step was much less than the combined first and second centrifugation step supernatants, and thus deemed negligible.

The extra samples were also used to find the best dilution rate. Previous literature (Wielebnowski et al., 2002; Terio et al., 1999) diluted extracted supernatants with PBS and a rate of 1:10. Dilution rates of 1:2, 1:5, and 1:10 were all tried in duplicate in 4 pellets from each of the 2 additional samples. Even when diluted at a rate of 1:10, after RIA analysis, some cortisol metabolite concentrations were greater than the upper limit the kit could detect. For this reason, the final dilution rate was set at 1:20.

In total, 4 RIA kits were used to analyze cortisol metabolite concentrations in all samples. Interassay CV was 5.22%, while intraassay CV was 4.21%. Linearity was determined by taking the 2 PBS diluted samples with the greatest cortisol metabolite concentrations from the first 3 kits and performing an 8-fold serial dilution. Linearity was consistent for 3 to 4 dilutions, at which point the cortisol concentrations were below a reliable range of detection for the kit used. Recovery was determined by spiking a sample with a lesser, intermediate, and greater concentration with the highest standard. The standard was diluted 0 times, 2.5 times, 5 times, and 10 times with each of the chosen samples in duplicate. Recovery averaged 105.6%, which is in the acceptable range of 70% to 120%.

## **Statistical Analysis**

### *Behavioral Data*

Behavioral data were analyzed using the glimmix procedure of SAS (SAS 9.4, Cary, NC). Session, day, experience, and sex were set as fixed effects, and day was included as a repeated measure. Tiger was the subject, and dependent variables were individual behaviors of interest that included pacing, vigilance, autogrooming, excretion, being in the den, being still, being in motion, sleeping, sniffing, and being out of sight.

### *Cortisol Metabolite Data*

Cortisol metabolite concentrations were analyzed using the glimmix procedure of SAS. Session, day, experience, and sex were set as fixed effects, and day was included as a repeated measure. Values for session were averaged for the 3 collection days in each session for each animal. Tiger was the subject, and the dependent variable was cortisol concentration.

Cortisol metabolite concentrations were also analyzed by finding the difference between the post session value for days 1, 2, 3, and the average of days 1 and 2 and the average of



corresponding pre session for each tiger on each day. These differences were analyzed as dependent variables in the Glimmix procedure of SAS with experience, sex, and day as fixed effects, day as a repeated measure, and tiger as the subject.

## RESULTS

### Cortisol Metabolite Concentrations

#### *Pre and Post Session Averages*

There were no effects of experience, session, sex, or day on the fecal cortisol metabolite concentration (Table 11). There was a tendency for an interaction of session, sex, and day ( $P < 0.099$ ; Table 12). Males in the pre session of day 1 tended to have a greater concentration of cortisol metabolites than males in the post session of day 1 ( $P < 0.0510$ ), females in the post session on day 1 ( $P < 0.0547$ ), and the females of the pre session on day 1 ( $P < 0.0831$ ; Graph 16).

#### *Differences in Post Sessions and Pre Session Averages*

There was a tendency for an effect of day on the difference in fecal cortisol metabolite concentrations between the first post session and the average of the pre session ( $P < 0.0859$ ; Table 13). Tigers on day 2 tended to have a more positive difference in fecal cortisol metabolite concentration than tigers on day 1 (Graph 17). There was an interaction of sex and day on the difference in the difference in fecal cortisol metabolite concentrations between the first post session and the average of the pre session ( $P < 0.0479$ ; Graph 18). Males on day 1 had a more negative difference in fecal cortisol metabolite concentrations than males on day 1 ( $P < 0.0233$ ). There was a tendency for an interaction of experience and day on the difference in fecal cortisol metabolite concentrations between the first post session and the average of the pre session ( $P < 0.0821$ ; Graph 19). Naïve tigers on day 1 had a more negative difference in fecal cortisol

metabolite concentrations than experienced tigers on day 1 ( $P < 0.0517$ ), experienced tigers on day 2 ( $P < 0.0534$ ), and naïve tigers on day 2 ( $P < 0.0330$ ).

There was a tendency for an effect of experience on the difference in fecal cortisol metabolite concentrations between the second post session and the average of the pre sessions ( $P < 0.0867$ ; Graph 20). Naïve tigers tended to have a more negative difference fecal cortisol metabolite concentrations than experienced animals.

There was a tendency for a sex by day interaction on the difference in fecal cortisol metabolite concentrations between the third post session and the average of the pre sessions ( $P < 0.0658$ ; Graph 21). Females on day 1 had a more positive difference in fecal cortisol metabolite concentration than males on day 1 ( $P < 0.0204$ ) and tended to have a more positive difference in fecal cortisol metabolite concentration than females on day 2 ( $P < 0.0532$ ).

There was a tendency for an effect of day on the difference in fecal cortisol metabolite concentrations between the average of the first and second post session and the average of the pre sessions ( $P < 0.0994$ ; Graph 22). Tigers on day 2 had a more positive difference in fecal cortisol metabolite concentrations than tigers on day 1. There was a tendency for a sex by day interaction on the difference in fecal cortisol metabolite concentrations ( $P < 0.0637$ ; Graph 23). Males on day 1 had a more negative difference in fecal cortisol concentrations than males on day 2 ( $P < 0.0308$ ) and tended to have a more negative difference in fecal cortisol concentrations than females on day 1 ( $P < 0.0927$ ). There was a tendency for an experience by day interaction on the difference in fecal cortisol metabolite concentrations ( $P < 0.0756$ , Graph 24). Naïve tigers on day 1 had a more negative difference in fecal cortisol metabolite concentrations than experienced tigers on day 1 ( $P < 0.0278$ ), experienced tigers on day 2 ( $P < 0.0338$ ), and naïve tigers on day 2 ( $P < 0.0344$ ).

## Behavioral Data

### *Pacing*

There was no main effect of experience, session, sex, or day on the amount of time tigers spent pacing. There was a tendency for an interaction between experience and sex ( $P < 0.056$ ; Table 5). Naïve females tended to pace more than experienced females ( $P < 0.086$ ) and naïve females tended to pace more than naïve males ( $P < 0.060$ ; Graph 1). Pacing also showed a tendency for an interaction between experience and day ( $P < 0.092$ ; Table 6). The naïve tigers tended to pace less on day 1 than on day 2 ( $P < 0.051$ ; Graph 2). There was also a tendency for a three-way interaction between experience, sex, and day ( $P < 0.087$ ; Graph 3). Naïve females on day 2 paced more than naïve females on day 1 ( $P < 0.006$ ), more than experienced females on day 1 ( $P < 0.013$ ) and on day 2 ( $P < 0.008$ ), more than naïve males on day 1 ( $P < 0.013$ ) and day 2 ( $P < 0.012$ ), more than experienced males on day 1 ( $P < 0.034$ ) and on day 2 ( $P < 0.033$ ).

### *Autogrooming*

There was no main effect of experience, sex, or day on the amount of time the tigers spent autogrooming. Session had a significant effect on autogrooming [ $(P < 0.030)$  (Table 7)]. More autogrooming occurred during the pre session than either the stress session ( $P < 0.026$ ) or the post session ( $P < 0.016$ ; Graph 4). There was a tendency for an experience by day interaction ( $P < 0.078$ ; Table 6). Experienced tigers on day 1 tended to pace more than naïve tigers on day 1 ( $P < .077$ ; Graph 5). There was also a tendency for a session by experience by sex interaction ( $P < 0.075$ ). Pre-naïve females performed autogrooming more than post-naïve females ( $P < 0.032$ ) and post experienced males ( $P < 0.040$ ) and tended to autogroom more than stress-experienced males ( $P < 0.069$ ) and females ( $P < 0.075$ ). Pre-experienced males autogroomed more than post-experienced males ( $P < 0.009$ ), post-naïve females ( $P < 0.008$ ) and males ( $P < 0.041$ ), pre-naïve

males ( $P < 0.031$ ), stress-experienced males ( $P < 0.018$ ) and females ( $P < 0.017$ ), and stress-naïve females ( $P < 0.043$ ) and males ( $P < 0.031$ ). Pre-experienced males also tended to autogroom more than post-experienced females ( $P < 0.052$ ; Graph 6).

### *Excretion*

There was no main effect of experience, session or sex on the amount of time the tigers spent performing excretory behaviors. There was a tendency for an effect of day on excretion ( $P < 0.056$ ). Day 2 showed more excretion behavior than day 1 (Table 8; Graph 7).

### *In Den*

There was no main effect of experience, session, sex, day on the amount of time the tigers spent in their dens. There was an interaction between experience and sex on how long the tigers were in their den ( $P < 0.038$ ). Naïve males were in their den for a greater period of time than experienced males ( $P < 0.0239$ ) and they tended to be in their den for a greater period of time than naïve females ( $P < 0.080$ ; Table 5; Graph 8).

### *Still*

There was no main effect of experience, session, sex, day on the amount of time the tigers spent still. There was an interaction between experience and sex ( $P < 0.049$ ; Table 5). Naïve males tended to be still more than naïve females ( $P < 0.072$ ; Graph 9). There was also an interaction between experience and day ( $P < 0.047$ ; Table 6). More naïve tigers were still on day 1 than day 2 ( $P < 0.032$ ; Graph 10). A tendency for an interaction of session, sex, and day on stillness also occurred ( $P < 0.094$ ). Females in the post session of day 1 tended to be more still than females in the pre session of day 1 ( $P < 0.085$ ). Males in the pre session of day 1 were more still than females in the pre session of day 1 ( $P < 0.033$ ), males in the stress session of day 2 ( $P < 0.046$ ), and females in the post session of day 2 ( $P < 0.043$ ) and tended to be more still than

females in the pre session of day 2 ( $P < 0.083$ ) and females in the stress session of day 2 ( $P < 0.064$ ; Graph 11).

### *In Motion*

There was no main effect of experience, session, sex or day on the amount of time the tigers spent in motion. There was an interaction between experience and sex ( $P < 0.049$ ; Table 5). Naïve females tended to be in motion more than naïve males ( $P < 0.072$ ). There was also an interaction between experience and day ( $P < 0.047$ ; Table 6). More naïve tigers were in motion on day 2 than day 1 ( $P < 0.032$ ). A tendency for an interaction of session, sex, and day on motion also occurred ( $P < 0.094$ ). Females in the post session of day 1 tended to be in motion less than females in the pre session of day 1 ( $P < 0.085$ ). Males in the pre session of day 1 were in motion less than females in the pre session of day 1 ( $P < 0.033$ ), males in the stress session of day 2 ( $P < 0.046$ ), and females in the post session of day 2 ( $P < 0.043$ ) and tended to be in motion less than females in the pre session of day 2 ( $P < 0.083$ ) and females in the stress session of day 2 ( $P < 0.064$ ).

### *Sleep*

There was no main effect of session or day on the amount of time the tigers spent asleep. Sex had a significant effect on sleep ( $P < 0.013$ ; Table 9). Males slept more than females (Graph 12). There was a tendency for experience to have an effect on sleep ( $P < 0.063$ ; Table 10). Naïve animals tended to sleep more than experienced animals (Graph 13). There was also a tendency for an interaction of experience and sex ( $P < 0.094$ ). Naïve males slept more than naïve females ( $P < 0.011$ ), experienced females ( $P < 0.006$ ), and experienced males ( $P < 0.030$ ; Graph 14).

### *Out of Sight*

There was no main effect of experience, session, sex, or day on the amount of time a tiger spent out of sight. There was a tendency for an interaction between sex and day on if the tigers were out of sight of the observers ( $P < 0.059$ ). Females on day 2 were out of sight more than females on day 1 ( $P < 0.025$ ) and tended to be out of sight more than males on day 2 ( $P < 0.098$ ; Graph 15).

#### *Vigilance and Sniffing*

There were no main effects or interactions of experience, session, sex, or day on vigilance or sniffing behavior.

### **DISCUSSION**

The objective of this study was to determine if previous experience with transport outside of routine handling would affect the behavioral and fecal cortisol response of captive tigers when exposed to a rollcage at the entrance of their enclosures. We hypothesized that experienced tigers and all tigers in the stress and post session would indicate more signs of stress than naïve tigers and tigers in the pre session. The behavior and cortisol metabolite data both revealed a great deal of variation among and within the tigers. These data are consistent with previous work investigating differences in personality (Pastorino et al., 2017) and fecal cortisol metabolite concentrations (Conforti et al, 2012) in tigers.

The analysis of average pre and post session fecal cortisol metabolites did not reveal any main effects of experience, session, sex or day. There was vast variety in the concentration of metabolites among and between animals. This has been previously reported in several studies (Conforti et al., 2012; Buirski et al., 1978; Parnell et al., 2014; Parnell et al., 2015), and this variation likely made it difficult to find differences due to the effects considered. There was a tendency for males in the pre session of day 1 to have a greater concentration of metabolites than

males in the post session of day 1 and females in the pre session of day 1. Some studies show that males have a greater concentration of fecal cortisol metabolites (Conforti et al., 2012), while others indicate that females have the greater metabolite concentration (Buirski et al., 1978; Bhattacharjee et al., 2015). Given the lack of control of the environment, there could have also been a number of factors that influenced fecal cortisol metabolites that we were unaware of and unable to account for.

Analysis of the differences in post session and averaged pre session fecal cortisol metabolite concentrations showed more consistent effects than when both pre and post session concentrations were averaged. Day effected or tended to affect every session analyzed. The differences between the first post session and the averaged pre session fecal cortisol metabolite concentrations showed values that were negative during the first day, and then positive on the second day. Males on day 2 and naïve animals on day 2 both showed an increase in the difference of fecal cortisol metabolite concentration as well. There could have been a stressful event that occurred in one or both groups of animals at this time that staff at TCWR were unaware of. Males and naïve tigers increasing in metabolite concentration from day 1 to day 2 could mean that the rollcage was more of a stressor the second time it was presented to these groups than the first. Since experienced tigers had positive differences in concentration for both days, the rollcage may have been a minor stressor for them on both occasions. The naïve tigers and male tigers could also have more variant cortisol metabolite secretion. There was a mix of positive and negative differences for the first post session. Since the sample was collected just 48 hours after rollcage placement, this may not have been enough time to elicit a measurable physiological response as digesta passage time in tigers ranges from 24-48 hours (Graham and Brown, 1996).

The difference between the second post session and the averaged pre session fecal cortisol metabolite concentrations indicated that naïve tigers decreased in cortisol metabolite concentration after rollcage placement significantly more than experienced tigers. The decrease in post session differences could be due to conditions in the pre session that led to the release of cortisol in the tigers. If this occurred it would be difficult to see any clear effects of rollcage placement. The results might also indicate a recovery in cortisol concentrations that can begin to occur as soon as 3 days after a stressful stimulus (Dembiec et al., 2004).

The difference between the third post session and the averaged pre session fecal cortisol metabolite concentrations revealed that female tigers on day 1 had an increase in cortisol metabolite concentration, while females on day 2 and males on both days had a significant decrease in cortisol metabolite concentrations. Females on day 1 may have still been recovering from any effects of rollcage placement. The literature on tiger fecal cortisol metabolite concentrations is unclear regarding differences in males and females and the rate at which they recover from stress, but females either recovered more slowly from stress related to rollcage placement or had another stressor occur around this time that caused an increase in metabolite concentration.

The difference between the averaged first and second post session and the averaged pre session fecal cortisol metabolite concentrations showed an increase in value from negative to positive between day 1 and 2. Males on day 2 and naïve animals on day 2 also had greater metabolite concentrations. These differences exhibit a similar pattern to those shown in session 1. The average of both sessions still shows an increase in in concentration on day 2 and a decrease on day 1. This phenomenon could again be explained by additional stressors during the second day, or a greater impact of the rollcage the second time it was presented. Naïve animals



showed a drastic change in concentration difference from day 1 to day 2, while the experienced group was largely stable. The naïve animals could have been more effected by the rollcage or outside stressors the second time the rollcage was placed, or the experienced group may have a more predictable response to the transportation stressor because they are more familiar with it. Like in post session 1, males showed the greatest change in concentration difference with a large negative value on day 1 and a positive value on day 2. Male fecal cortisol metabolite concentrations may be more erratic than female concentrations. This has not been addressed in the literature, but it could explain why the difference in concentration in males changed so drastically from day 1 to day 2.

Naïve females and females on day 2 tended to pace more. One female naïve tiger was suggested to be interested in a male neighbor on day 2, but this cannot be confirmed because it is only anecdotal evidence. In the wild, tigers roam vast ranges and can travel great distances looking for mates (Goodrich et al., 2010). If she was interested in her male neighbor, it is possible that she was pacing as a way to cope with her frustration about not being able to reach him. If this anecdotal evidence is not accurate, it is also possible that the naïve female group used pacing more often when stereotypic behavior was induced by stressful or anticipatory stimuli.

Surprisingly, vigilance showed no differences in any effect or interaction. This was a common behavior among all groups of tigers, so one explanation for a lack of differences might be that all of the tigers became alert when the rollcage was introduced and when they became aware of something new in their environment. Vigilance is also a less objective behavior than the other behaviors included in this study, so different interpretations of what vigilance looked like in tigers may have occurred. Sniffing also showed no differences in behavior. Olfaction is a

large component of communication, sociality, and food procurement in felids (Ellis and Wells, 2010), so it is apparent that all groups of tigers utilized this sense to investigate the rollcage and their environment during the observation periods.

Felids spend around 50% of their waking hours grooming (Eckstein and Hart, 2000), and grooming can be used as a self-soothing behavior (Seskel and Lindeman, 1998). We thought that the tigers would autogroom the most during either the stress or post session as a way to cope with the stress of the rollcage being present. Tigers actually autogroomed the most in the pre session, particularly experienced males. Instead of using grooming as a self-soothing behavior the tigers may have groomed when they were more relaxed. After the rollcage was introduced, more of the tiger's time may have been used to investigate the rollcage and readjust after its removal. The positions a tiger assumes when grooming make it more vulnerable, so they may have limited this behavior to be more alert of their surroundings after the introduction of the rollcage.

Excretion tended to be greater on day 2 than on day 1. No excretory behaviors were common among the tigers, so it is possible that these behaviors were not likely to happen during the 3-hour window of observation. Felids in the wild will spray to communicate with other solitary conspecifics (Harmsen et al., 2016), but with the close range of conspecifics at TCWR, spraying may not be as necessary a behavior. The tigers may have been excreting more to communicate about the presence of the rollcage, or there could have also been an environmental effect that we were unaware of on day 2 that caused a greater rate of excretion.

Hiding in a den would allow the tigers to escape loud noises and visitors at TCWR. Construction was occurring near the enclosures being observed during this study, and related noise may have had an impact on how often the tigers chose to enter their dens. Naïve males

spent more time in their dens than experienced males and tended to spend more time in their dens than naïve females. Since there was no effect of session, it is not likely that the introduction of the rollcage caused the tigers to hide in their dens more, although some tigers hid behind trees and other elements in their enclosures. The experience by sex interaction is likely due to personality differences among tigers. Females on day 2 tended to be out of sight more than females on day 1 or males on day 2. Because of the layout of the enclosures and the vantage point of the observations, tigers that were out of sight were often closer to the rollcage. We are unable to know what other behaviors they were performing, but they were either investigating the rollcage, or attempting to get away from visitors and observers on the opposite end of their enclosure.

The tigers spent most of their time during observations resting. Tigers in the wild also spend large amounts of time resting to conserve energy (Rozhnov et al., 2011). The introduction of the rollcage did not seem to affect this, and tigers would often lay down in front of the rollcage after it was placed outside of their enclosure. There were a few interactions between experience, session, day, and sex, and naïve males in the pre session of day 1 tended to be still the most. Males also slept more than females, and naïve animals tended to sleep more than experienced animals. This could again be influenced by personality, physiology, or evolution for females to be more alert for danger when rearing cubs (Hernandez-Blanco et al., 2015). Age could have possibly had an effect on activity level as the naïve animals were younger than the experienced animals, average 5.8 years and 7.5 years respectively, but this was not accounted for statistically.

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## **CHAPTER 4**

### **CONCLUSION**

It is not clear that a tiger's previous history with transportation impacts their current response to transportation. None of the fixed effects consistently impacted behavior or average cortisol metabolite concentrations. Although several interactions occurred, none of them showed a pattern of differences among sex, experience, session, or day. In the data depicting the difference in post session and averaged pre session fecal cortisol metabolites, day emerged as a consistently significant fixed effect or as part of an interaction. This could be due to cycling in cortisol in individual tigers, and if more collection days occurred, this effect could be studied further. Cortisol changes have been seen in some tigers in the wild, but not enough evidence is available to definitively define a season or seasons in which cortisol is greatest (Naidenko et al., 2011). Cortisol concentrations also vary throughout the day, and the time at which the tiger defecated could have an impact on the amount of cortisol metabolites that were excreted with the feces (Edwards et al., 2001)

Previous studies have indicated that tigers can habituate to transport (Dembiec et al., 2004; Nevill and Friend, 2003). The experienced tigers at TCWR may have habituated to transport before their arrival or may have grown used to transport within the facility. If they had associated travel with discomfort in the past, their experiences at TCWR may have changed their associations of travel to something more positive or at least less stressful. Naïve tigers may have experienced very few if any negative experiences with transportation, so when the rollcage was presented they were not negatively affected by it. Changes in metabolite concentrations seen in certain groups could be indicative of greater arousal when the rollcage was presented,

particularly on the second day, or could have been influenced by other stimuli that elicit cortisol secretion.

It is very possible that just presenting the rollcage outside of the enclosure without attempting to move the tigers inside of it was not enough stimulation to elicit a behavioral or physiological stress response. The tigers see the rollcages move around the facility but are not often subjected to transportation. Mere presentation may have been enough to pique their curiosity, but not enough to cause the animals to become fearful or stressed.

There are many other factors outside of experimental control at a rescue facility including visitors, weather, keeper interactions, and individual personality and physiology. All of these factors have the potential to influence the tigers' stress responses outside of the 1-hour window every other week that the rollcage was placed in front of their enclosure.

The study of the impact that past transport has on captive tigers in the present is extremely limited, and more research is warranted. We had a relatively small sample size of 12, so using a greater number of tigers of varying age, sex, and experience could shed light on any differences that individuals have. Loading tigers on to the rollcage or going through the preparatory steps for loading could also elicit a greater stress response and weed out any differences with experience. Videotaping the tigers and collecting more samples could also enhance the data. Video footage could be used to see days' worth of baseline and post transport stressor exposure behavior to more closely look for differences in behavior. Taking more fecal samples could also help establish a more consistent baseline and measure of cortisol metabolite concentrations. More samples could reduce the variability among individuals and help to better examine trends in fecal cortisol metabolite changes. Further study of fecal cortisol metabolites



in tigers could also elucidate differences in males and females and uncover if they experience seasonal changes in cortisol while in captivity.

Although this study did not conclusively reveal that tigers with a past experience of transport outside of TCWR showed a greater stress response when exposed to rollcages, it is important to monitor how routine handling affects captive tigers. If some aspect of husbandry has a negative impact on the stress response of that tiger, it should be altered to better the health and welfare of the tiger.

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## TABLES AND FIGURES

**Table 1. Tiger Experience**

<b>Name</b>	<b>Abbreviation</b>	<b>Naïve or Experienced</b>	<b>History</b>
Athena	AT	Experienced	Rescued from close encounter facility
Aurora	AU	Experienced	Rescued from cub petting facility
Chuff	CH	Experienced	Rescued from close encounter facility
Joey	JO	Experienced	Rescued from cub petting facility
Khaleesi	KH	Experienced	Rescued from cub petting facility
Kizmin	KI	Naïve	Born shortly before rescue from cub petting facility
Robbie	RO	Naïve	Rescued from private owner
Shasta	SH	Naïve	Rescued from private owner
Snowball	SN	Experienced	Rescued from cub petting facility
Tanya	TA	Naïve	Born shortly before rescue from cub petting facility
Thurston	TH	Experienced	Rescued from cub petting facility, history in magic shows
Tommie	TO	Naïve	Rescued from private owner

**Table 2. Housing**

<b>Name</b>	<b>Housing</b>
AT	Shares enclosure with CH (sibling), time share with other tiger (sibling) not on study
AU	Shares enclosure with JO, KH, and Ti-Liger not on study (all unrelated)
CH	Shares enclosure with AT (sibling), time share with other tiger (sibling) not on study
JO	Shares enclosure with AU, KH, and Ti-Liger not on study (all unrelated)
KH	Shares enclosure with AU, JO, and Ti-Liger not on study (all unrelated)
KI	Shares enclosure with TA (sibling)
RO	Shares enclosure with TO (unrelated)
SH	Individually housed
SN	Individually housed
TA	Shares enclosure with KI (sibling)
TH	Individually housed
TO	Shares enclosure with RO (unrelated)

**Table 3. Age and Arrival at TCWR**

<b>Name</b>	<b>Sex</b>	<b>Approx. DOB</b>	<b>Arrival at TCWR</b>
AT	F	16-Mar-2007	16-Mar-2010
AU	F	30-May-2015	30-Sep-2016
CH	F	16-Mar-2007	16-Mar-2010
JO	F	23-Jul-2015	30-Sep-2016
KH	F	4-Aug-2015	30-Sep-2016
KI	F	18-Sep-2016	11-Oct-2016
RO	M	17-Jan-2015	17-Jan-2019
SH	F	14-Feb-2007	14-Sep-2012
SN	M	27-Sep-2015	30-Sep-2015
TA	F	18-Sep-2016	11-Oct-2016
TH	M	16-Oct-2007	6-Oct-2016
TO	M	17-Jan-2016	17-Jan-2019

**Table 4. Experiment Schedule**

Repetition	Name	BL 1	BL 2	BL 3	Exposure Day	Post 1	Post 2	Post 3
1	AT	25-Feb-2020	27-Feb-2020	29-Feb-2020	29-Feb-2020	1-Mar-2020	3-Mar-2020	5-Mar-2020
1	AU	18-Feb-2020	20-Feb-2020	22-Feb-2020	22-Feb-2020	23-Feb-2020	25-Feb-2020	27-Feb-2020
1	CH	25-Feb-2020	27-Feb-2020	29-Feb-2020	29-Feb-2020	1-Mar-2020	3-Mar-2020	5-Mar-2020
1	JO	18-Feb-2020	20-Feb-2020	22-Feb-2020	22-Feb-2020	23-Feb-2020	25-Feb-2020	27-Feb-2020
1	KH	18-Feb-2020	20-Feb-2020	22-Feb-2020	22-Feb-2020	23-Feb-2020	25-Feb-2020	27-Feb-2020
1	KI	18-Feb-2020	20-Feb-2020	22-Feb-2020	22-Feb-2020	23-Feb-2020	25-Feb-2020	27-Feb-2020
1	RO	25-Feb-2020	27-Feb-2020	29-Feb-2020	29-Feb-2020	1-Mar-2020	3-Mar-2020	5-Mar-2020
1	SH	25-Feb-2020	27-Feb-2020	29-Feb-2020	29-Feb-2020	1-Mar-2020	3-Mar-2020	5-Mar-2020
1	SN	25-Feb-2020	27-Feb-2020	29-Feb-2020	29-Feb-2020	1-Mar-2020	3-Mar-2020	5-Mar-2020
1	TA	18-Feb-2020	20-Feb-2020	22-Feb-2020	22-Feb-2020	23-Feb-2020	25-Feb-2020	27-Feb-2020
1	TH	18-Feb-2020	20-Feb-2020	22-Feb-2020	22-Feb-2020	23-Feb-2020	25-Feb-2020	27-Feb-2020
1	TO	25-Feb-2020	27-Feb-2020	29-Feb-2020	29-Feb-2020	1-Mar-2020	3-Mar-2020	5-Mar-2020
2	AT	10-Mar-2020	12-Mar-2020	14-Mar-2020	15-Mar-2020	16-Mar-2020	17-Mar-2020	19-Mar-2020
2	AU	3-Mar-2020	5-Mar-2020	7-Mar-2020	7-Mar-2020	8-Mar-2020	10-Mar-2020	12-Mar-2020
2	CH	10-Mar-2020	12-Mar-2020	14-Mar-2020	15-Mar-2020	16-Mar-2020	17-Mar-2020	19-Mar-2020
2	JO	3-Mar-2020	5-Mar-2020	7-Mar-2020	7-Mar-2020	8-Mar-2020	10-Mar-2020	12-Mar-2020
2	KH	3-Mar-2020	5-Mar-2020	7-Mar-2020	7-Mar-2020	8-Mar-2020	10-Mar-2020	12-Mar-2020
2	KI	3-Mar-2020	5-Mar-2020	7-Mar-2020	7-Mar-2020	8-Mar-2020	10-Mar-2020	12-Mar-2020
2	RO	10-Mar-2020	12-Mar-2020	14-Mar-2020	15-Mar-2020	16-Mar-2020	17-Mar-2020	19-Mar-2020
2	SH	10-Mar-2020	12-Mar-2020	14-Mar-2020	15-Mar-2020	16-Mar-2020	17-Mar-2020	19-Mar-2020
2	SN	10-Mar-2020	12-Mar-2020	14-Mar-2020	15-Mar-2020	16-Mar-2020	17-Mar-2020	19-Mar-2020

2	TA	3-Mar-2020	5-Mar-2020	7-Mar-2020	7-Mar-2020	8-Mar-2020	10-Mar-2020	12-Mar-2020
2	TH	3-Mar-2020	5-Mar-2020	7-Mar-2020	7-Mar-2020	8-Mar-2020	10-Mar-2020	12-Mar-2020
2	TO	10-Mar-2020	12-Mar-2020	14-Mar-2020	15-Mar-2020	16-Mar-2020	17-Mar-2020	19-Mar-2020

Table 5. Effect of Interaction of Experience and Sex on Mean Percentage of Time Spent Performing Selected Behaviors in Tigers

	LSM				SEM	<i>P</i> -value
	Experi/F <sup>a</sup>	Experi/M	Naïve/F	Naïve/M		
Pacing	0.034	0.069	0.114	0.007	0.032	0.056
Vigilance	0.253	0.189	0.109	0.175	0.056	0.282
Autogrooming	0.029	0.032	0.029	0.014	0.008	0.399
Excretion	0.005	0.002	0.012	0.004	0.005	0.617
In Den	0.183	0.045	0.132	0.361	0.073	0.038
Still	0.806	0.737	0.704	0.853	0.047	0.049
In Motion	0.194	0.263	0.297	0.147	0.047	0.049
Sniff	0.018	0.048	0.018	0.016	0.012	0.752
Sleep	0.107	0.182	0.122	0.414	0.057	0.094
Out of Sight	0.125	0.089	0.133	0.114	0.061	0.489

<sup>a</sup> Experi = Experienced, F = Female, M = Male



Table 6. Effect of Interaction of Experience and Day on Mean Percentage of Time Spent Performing Selected Behaviors in Tigers

	LSM				SEM	<i>P</i> -value
	Experi/1 <sup>a</sup>	Experi/2	Naïve/1	Naïve/2		
Pacing	0.056	0.046	0.004	0.116	0.032	0.092
Vigilance	0.219	0.222	0.156	0.129	0.056	0.794
Autogrooming	0.041	0.017	0.011	0.032	0.010	0.078
Excretion	0.001	0.006	0.000	0.016	0.005	0.287
In Den	0.048	0.181	0.180	0.314	0.074	0.371
Still	0.755	0.788	0.872	0.685	0.047	0.047
In Motion	0.245	0.212	0.128	0.315	0.047	0.047
Sniff	0.041	0.026	0.006	0.029	0.012	0.166
Sleep	0.103	0.186	0.332	0.204	0.057	0.103
Out of Sight	0.056	0.157	0.095	0.155	0.062	0.761

<sup>a</sup> Experi = experienced, 1 = Day 1, 2 = Day 2

Table 7. Effect of Session on Mean Percentage of Time Spent Performing Selected Behaviors in Tigers

	LSM			SEM	P-value
	Pre	Stress	Post		
Pacing	0.059	0.062	0.046	0.028	0.908
Vigilance	0.149	0.261	0.134	0.049	0.164
Autogrooming	0.048	0.016	0.014	0.009	0.030
Excretion	0.003	0.006	0.004	0.004	0.752
In Den	0.151	0.233	0.158	0.064	0.618
Still	0.773	0.755	0.797	0.041	0.768
In Motion	0.227	0.245	0.203	0.041	0.768
Sniff	0.024	0.032	0.020	0.011	0.752
Sleep	0.241	0.157	0.221	0.050	0.479
Out of Sight	0.063	0.133	0.150	0.054	0.489

Table 8. Effect of Day on Mean Percentage of Time Spent Performing Selected Behaviors in Tigers

	LSM		SEM	<i>P</i> -value
	Day 1	Day 2		
Pacing	0.030	0.081	0.023	0.146
Vigilance	0.187	0.176	0.040	0.838
Autogrooming	0.026	0.026	0.007	0.981
Excretion	0.000	0.011	0.003	0.056
In Den	0.114	0.247	0.052	0.109
Still	0.814	0.736	0.033	0.139
In Motion	0.187	0.264	0.033	0.139
Sniff	0.023	0.027	0.009	0.745
Sleep	0.218	0.195	0.041	0.697
Out of Sight	0.075	0.156	0.044	0.224

Table 9. Effect of Sex on Mean Percentage of Time Spent Performing Selected Behaviors in Tigers

	LSM		SEM	P-value
	Female	Male		
Pacing	0.074	0.038	0.022	0.295
Vigilance	0.181	0.182	0.040	0.986
Autogrooming	0.029	0.023	0.007	0.591
Excretion	0.009	0.003	0.003	0.286
In Den	0.158	0.203	0.052	0.557
Still	0.759	0.795	0.033	0.419
In Motion	0.241	0.205	0.033	0.419
Sniff	0.018	0.032	0.009	0.295
Sleep	0.115	0.298	0.040	0.013
Out of Sight	0.129	0.101	0.044	0.665

Table 10. Effect of Experience on Mean Percentage of Time Spent Performing Selected Behaviors in Tigers

	LSM		SEM	P-value
	Naïve	Experienced		
Pacing	0.060	0.051	0.022	0.146
Vigilance	0.142	0.221	0.040	0.202
Autogrooming	0.021	0.030	0.007	0.422
Excretion	0.008	0.003	0.003	0.348
In Den	0.247	0.114	0.052	0.111
Still	0.778	0.772	0.033	0.895
In Motion	0.222	0.228	0.033	0.895
Sniff	0.017	0.033	0.009	0.745
Sleep	0.144	0.268	0.040	0.063
Out of Sight	0.124	0.107	0.044	0.224

Table 11. Effect of Day, Experience, Session, and Sex on Tiger Fecal Cortisol Metabolite Concentration (ng/g of feces)

	LSM	SEM	<i>P</i> -value
Day 1	65078		
Day 2	59253	10072	0.693
Naïve	74290		
Experienced	50041	10032.575	0.127
Pre	71980		
Post	52351	10072	0.206
Female	56110		
Male	68221	10032.575	0.420

Table 12. Effect of Interaction of Session, Sex, and Day on Tiger Fecal Cortisol Metabolite Concentration

	LSM								SEM	<i>P</i> -value
	Pre/F/1	Pre/F/2	Pre/M/1	Pre/M/2	Post/F/1	Post/F/2	Post/M/1	Post/M/2		
ng/g feces	52316	66050	108718	60834	61420	44652	37858	65476	20065	0.0999

Table 13. Differences in Tiger Fecal Cortisol Metabolite Concentrations in Pre and Post Sessions

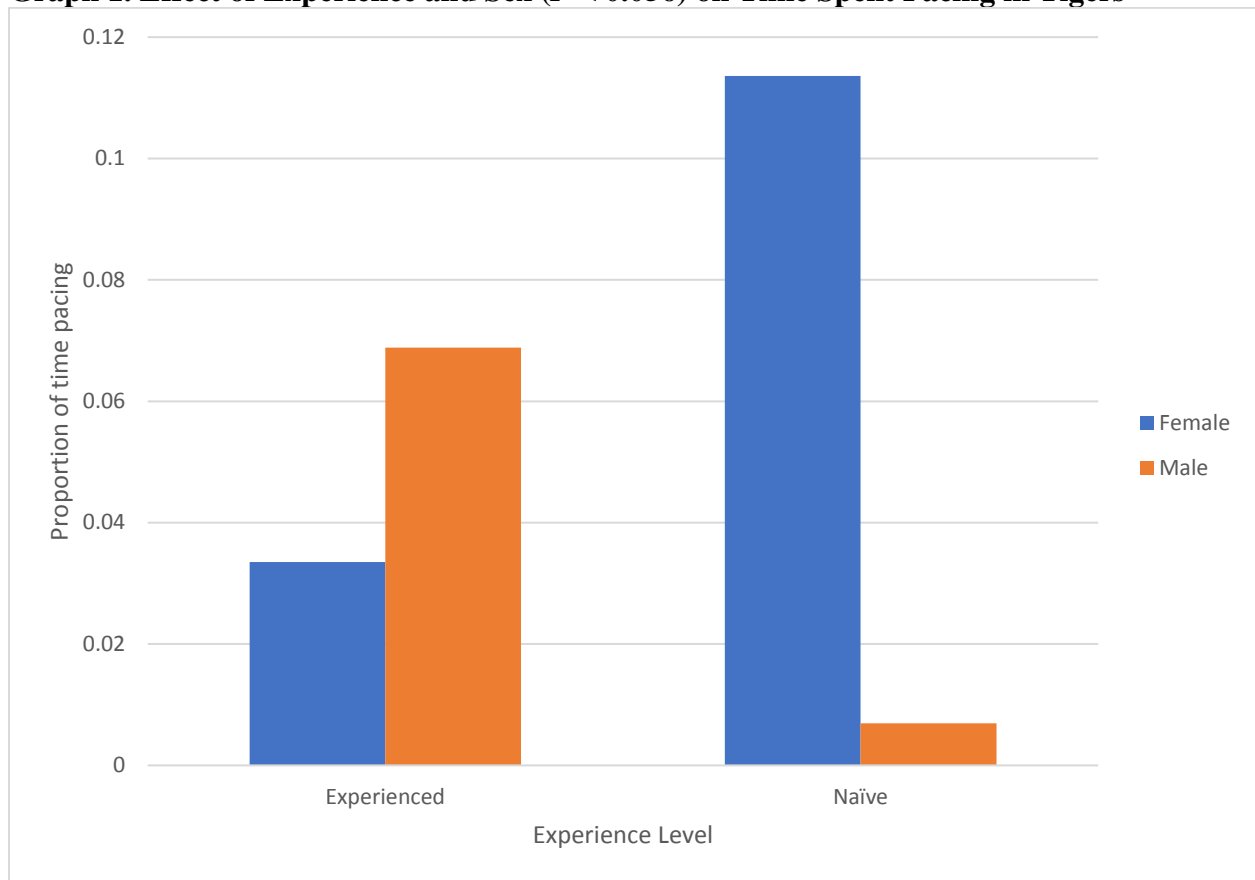
Tiger	Day	Experience	Sex	PreAVG <sup>a</sup>	Po1-Pr <sup>b</sup>	Po2-Pr <sup>c</sup>	Po3-Pr <sup>d</sup>	Po1&2-Pr <sup>e</sup>
Athena	1	Experienced	F	52359	-20930	-16683	17962	-18807
Athena	2	Experienced	F	45198	-11998	-11583	-17248	-11791
Aurora	1	Experienced	F	41671	19057	-17860	-26151	598
Aurora	2	Experienced	F	60129	-23886	-5774	-42395	-14830
Chuff	1	Experienced	M	62669	-33553	5068	-28165	-14242
Chuff	2	Experienced	M	46226	-6886	-6486	-12863	-6686
Joey	1	Experienced	F	25193	19195	62460	89900	40827
Joey	2	Experienced	F	67738	-8315	-32205	-37372	-20260
Khaleesi	1	Experienced	F	31427	68210	21706	134281	44958
Khaleesi	2	Experienced	F	58410	21775	43466	15595	32621
Kizmin	1	Naïve	F	33251	-11974	-8412	27891	-10193
Kizmin	2	Naïve	F	19040	8429	21817	-649	15123
Robbie	1	Naïve	M	38711	-8226	-16740	-13272	-12483
Robbie	2	Naïve	M	32588	2428	44548	-5502	23488
Shasta	1	Naïve	F	103790	-80657	-33959	100935	-57308
Shasta	2	Naïve	F	189042	-149936	-88280	-120309	-119108
Snowball	1	Experienced	M	42704	-11872	-21359	-10154	-16616
Snowball	2	Experienced	M	48157	-6998	-6849	-16742	-6923
Tanya	1	Naïve	F	63866	-43808	-41284	-8215	-42546
Tanya	2	Naïve	F	14615	22059	9925	-5765	15992
Thurston	1	Experienced	M	39282	1013	-9402	50894	-4195



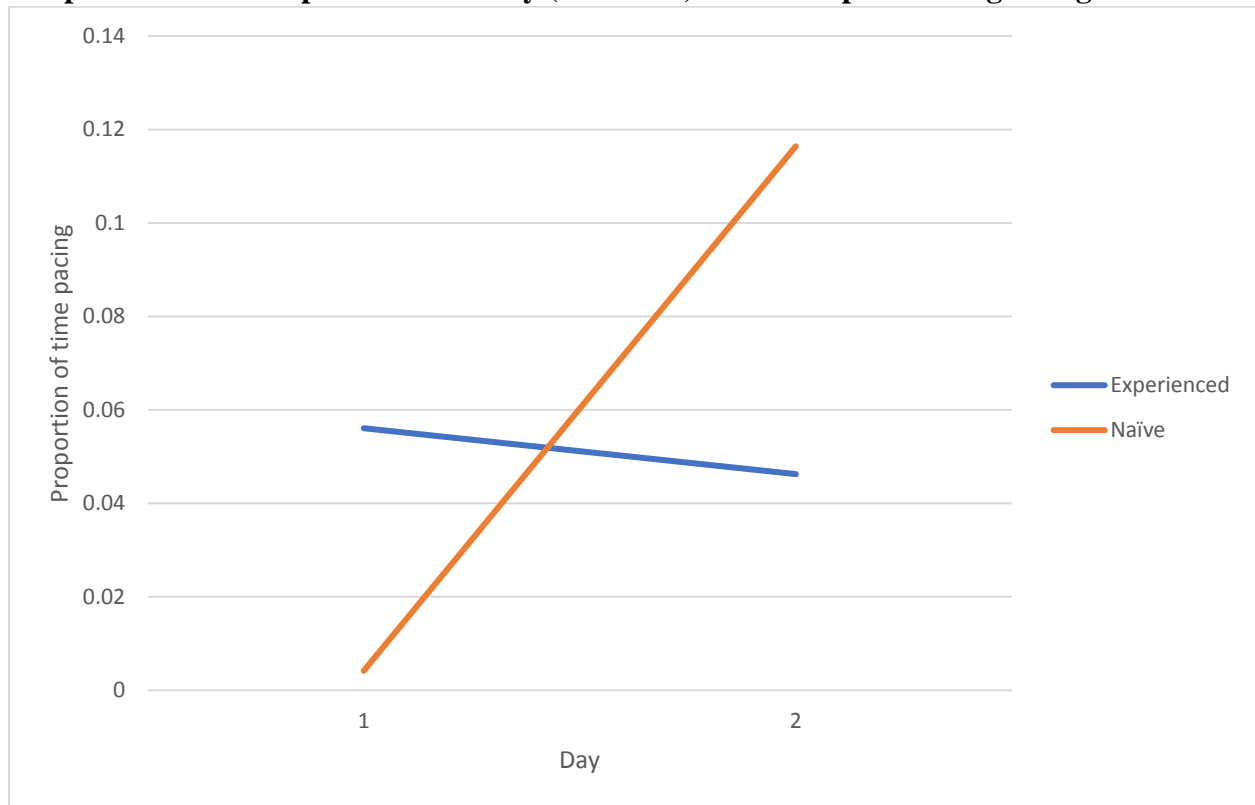
Thurston	2	Experienced	M	60831	45537	-14297	-13626	15620
Tommie	1	Naïve	M	299725	-254755	-246368	-246368	-250561
Tommie	2	Naïve	M	107271	171587	-59997	-71223	55795

- a. PreAVG = The average of pre sessions for that tiger on that day
- b. Po1-Pr = Post session 1 minus the average of the pre session for that tiger on that day
- c. Po2-Pr = Post session 2 minus the average of the pre session for that tiger on that day
- d. Po3-Pr = Post session 3 minus the average of the pre session for that tiger on that day
- e. Po1&2-Pr = Average of post sessions 1 and 2 minus the average of the pre session for that tiger on that day

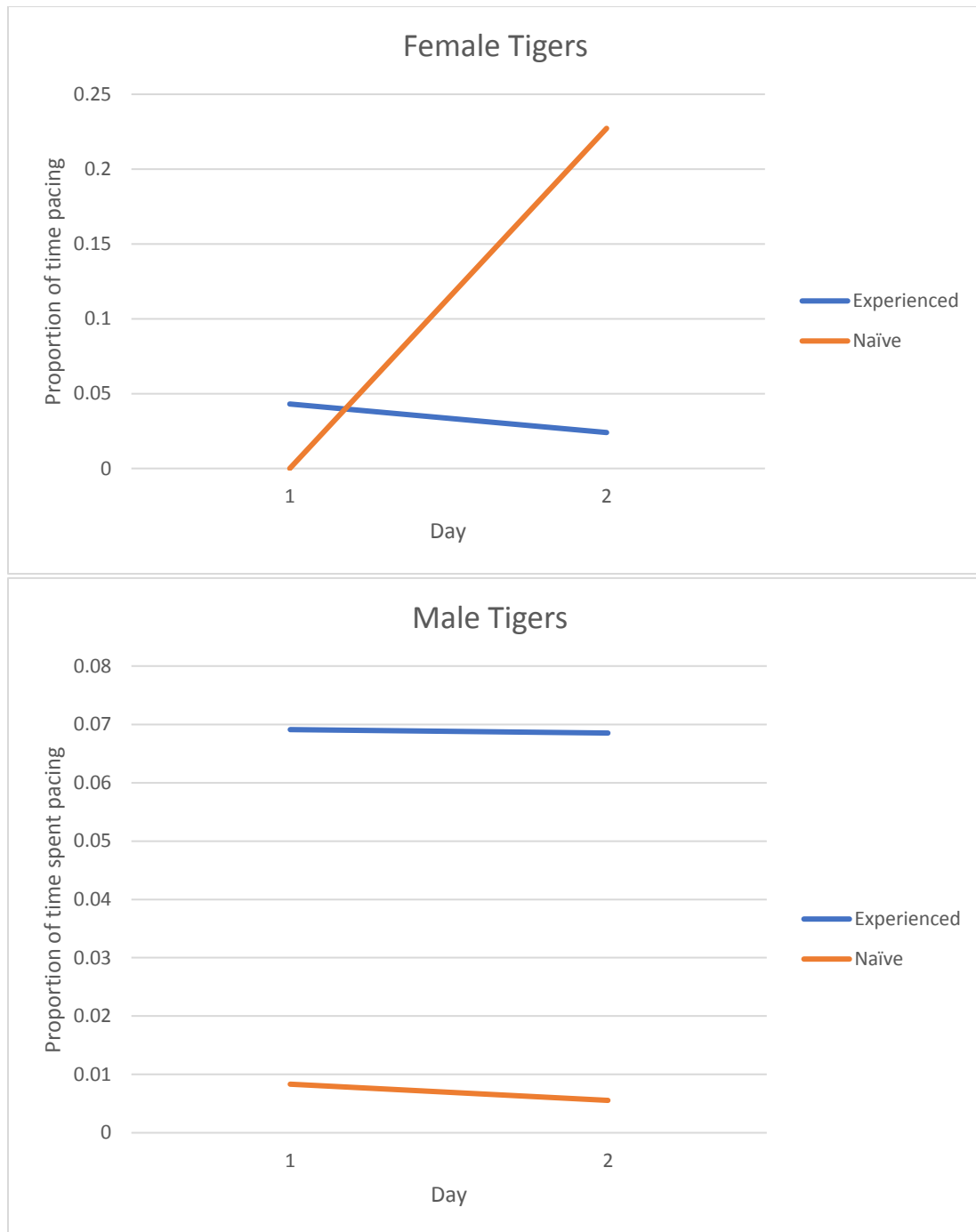
**Graph 1. Effect of Experience and Sex ( $P < 0.056$ ) on Time Spent Pacing in Tigers**



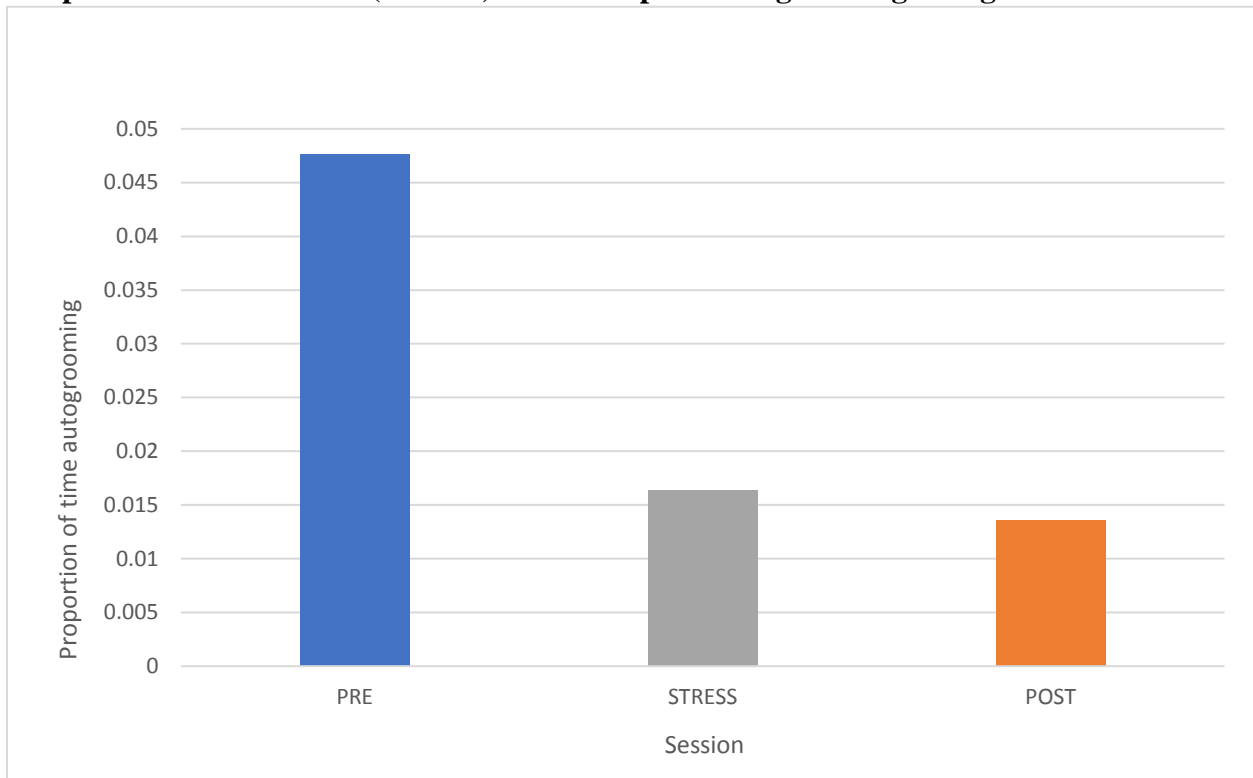
**Graph 2. Effect of Experience and Day ( $P < 0.092$ ) on Time Spent Pacing in Tigers**



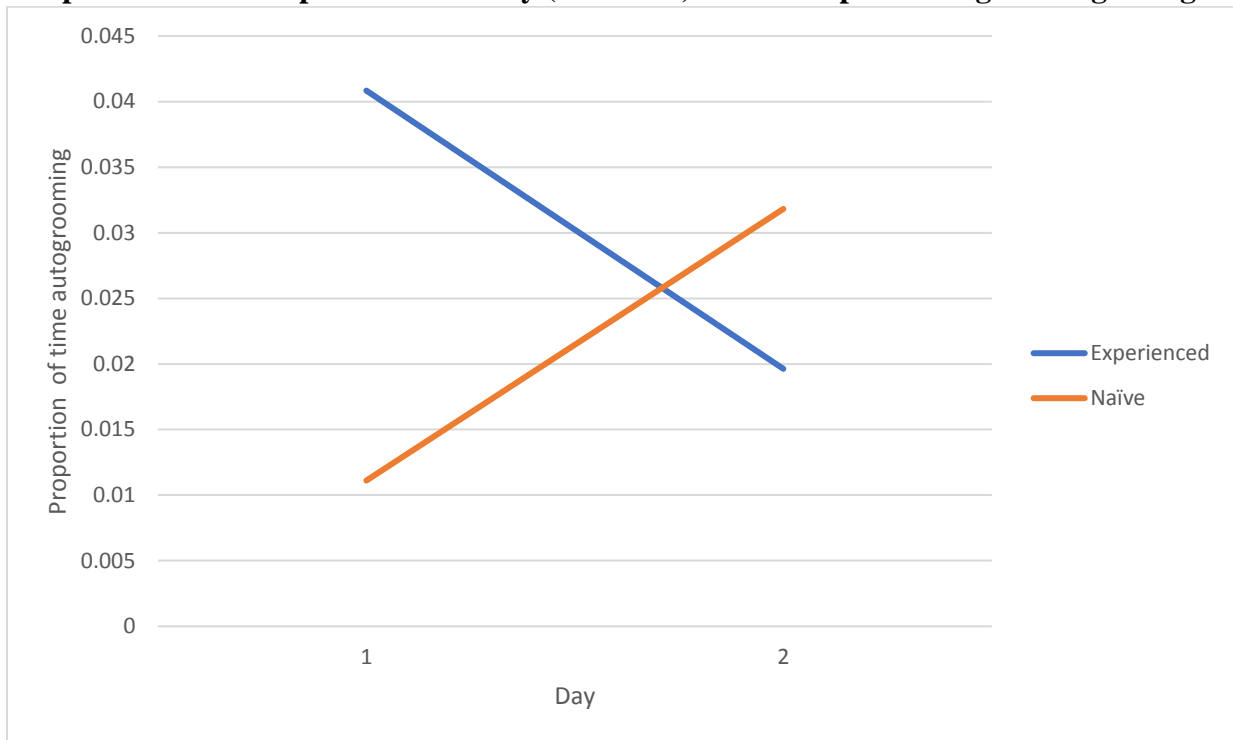
**Graph 3. Effect of Experience and Day on Pacing in Female and Male Tigers ( $P < 0.087$ )**



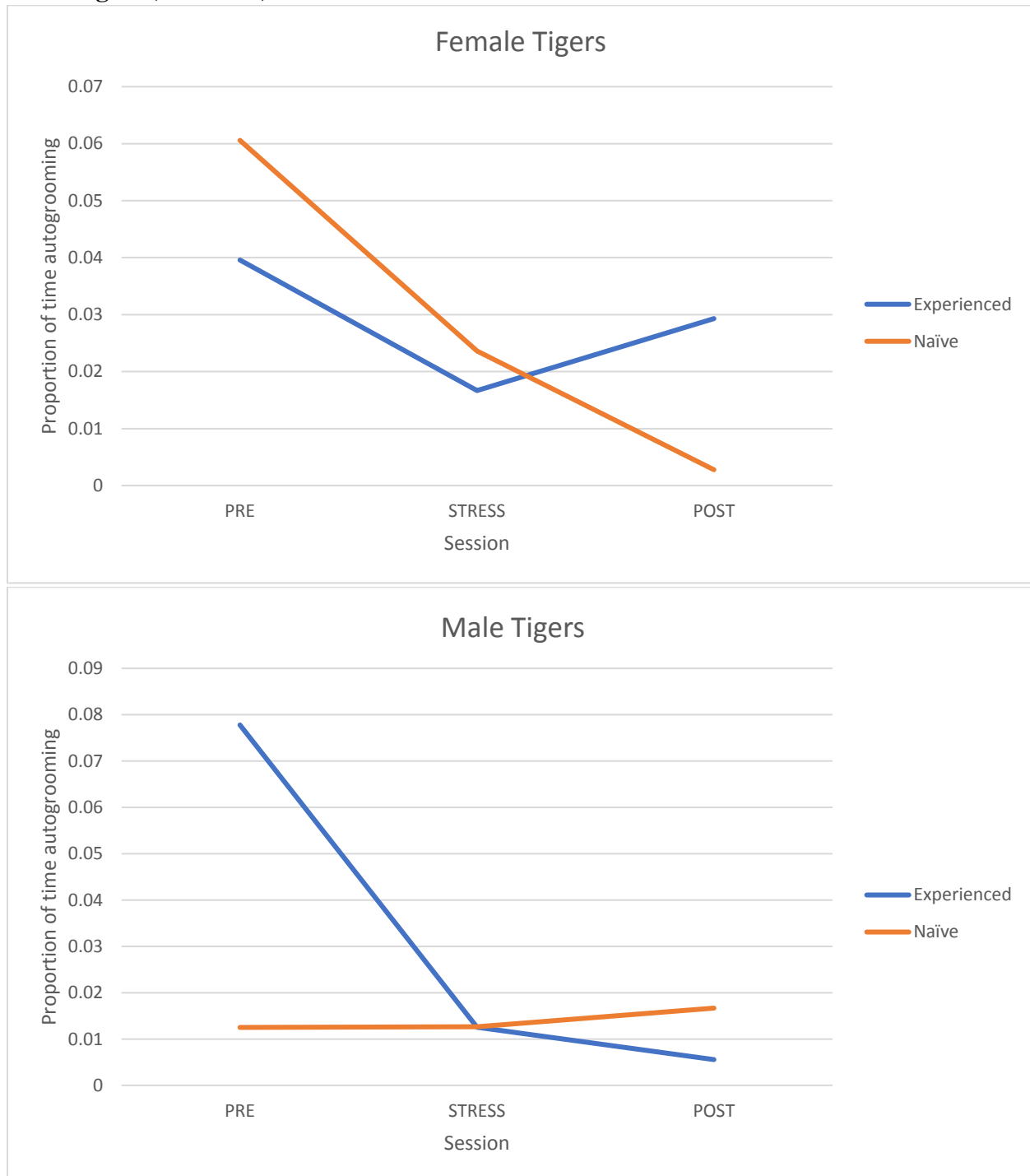
**Graph 4. Effect of Session ( $P < 0.03$ ) on Time Spent Autogrooming in Tigers**



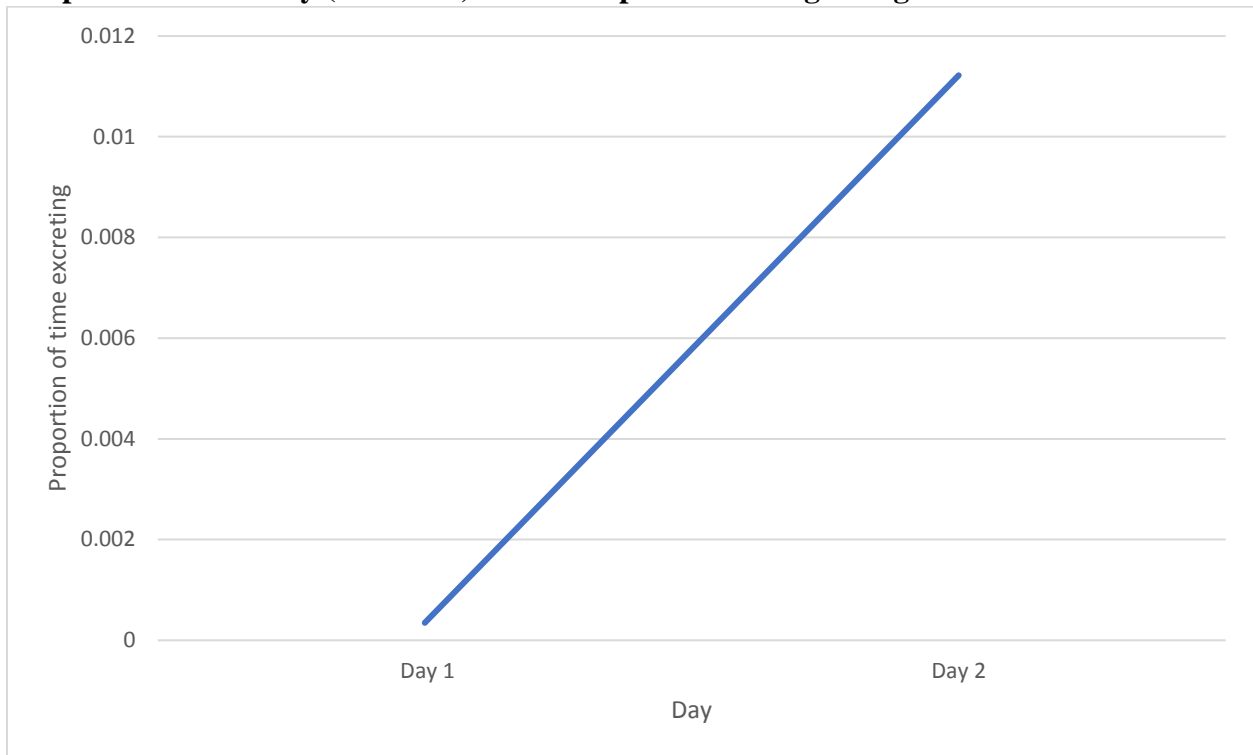
**Graph 5. Effect of Experience and Day ( $P < 0.078$ ) on Time Spent Autogrooming in Tigers**



**Graph 6. Effect of Experience and Session on Time Spent Autogrooming in Female and Male Tigers ( $P < 0.075$ )**

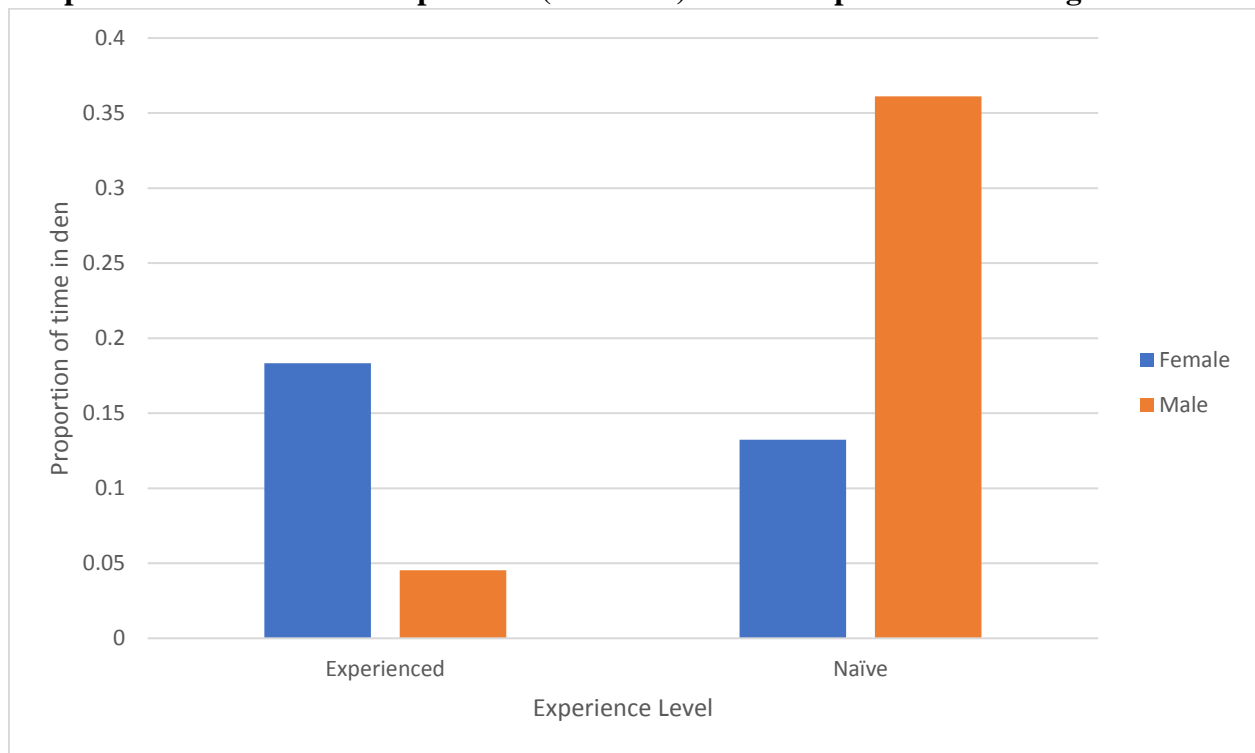


**Graph 7. Effect of Day ( $P < 0.056$ ) on Time Spent Excreting in Tigers**

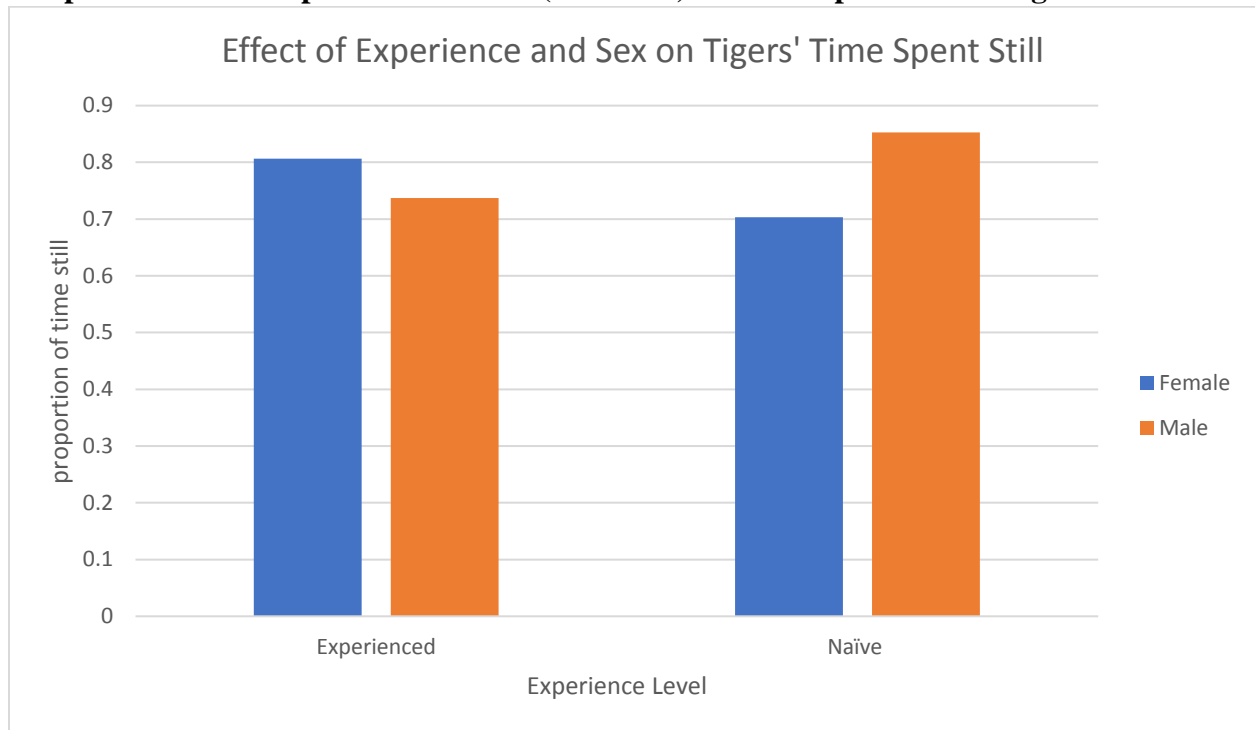




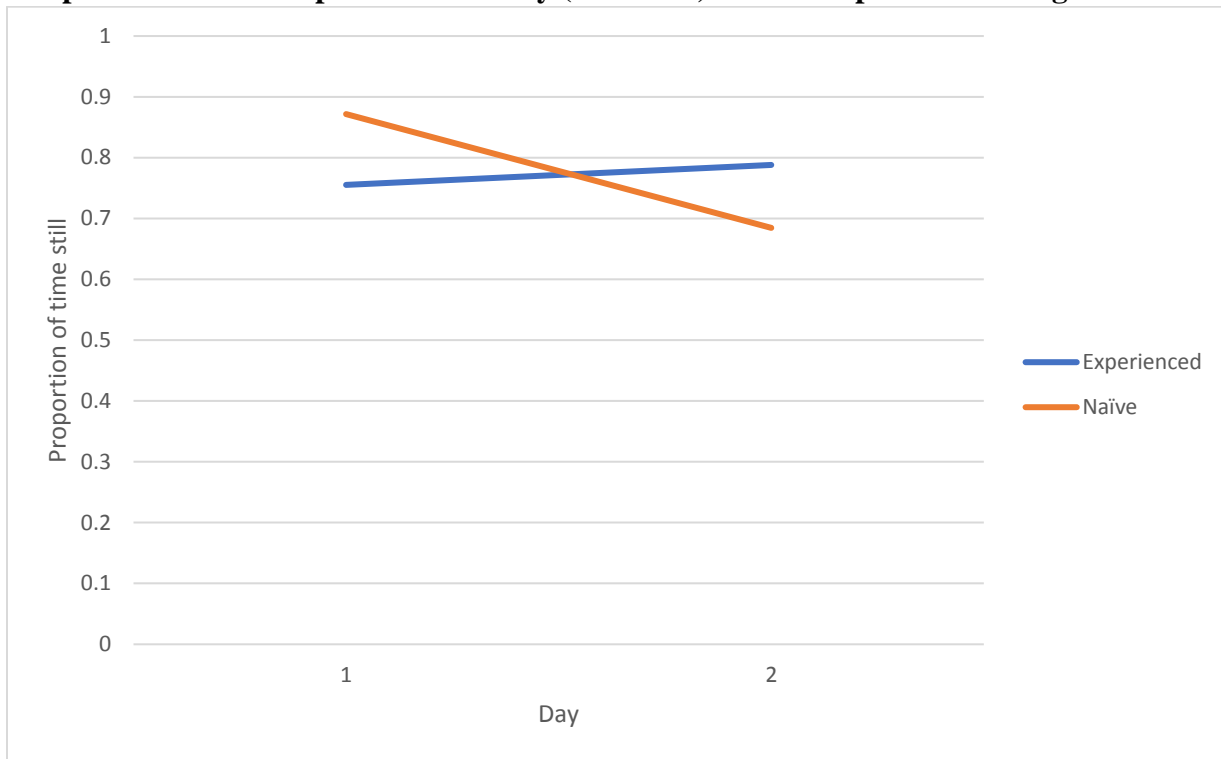
**Graph 8. Effect of Sex and Experience ( $P < 0.038$ ) on Time Spent in Den in Tigers**



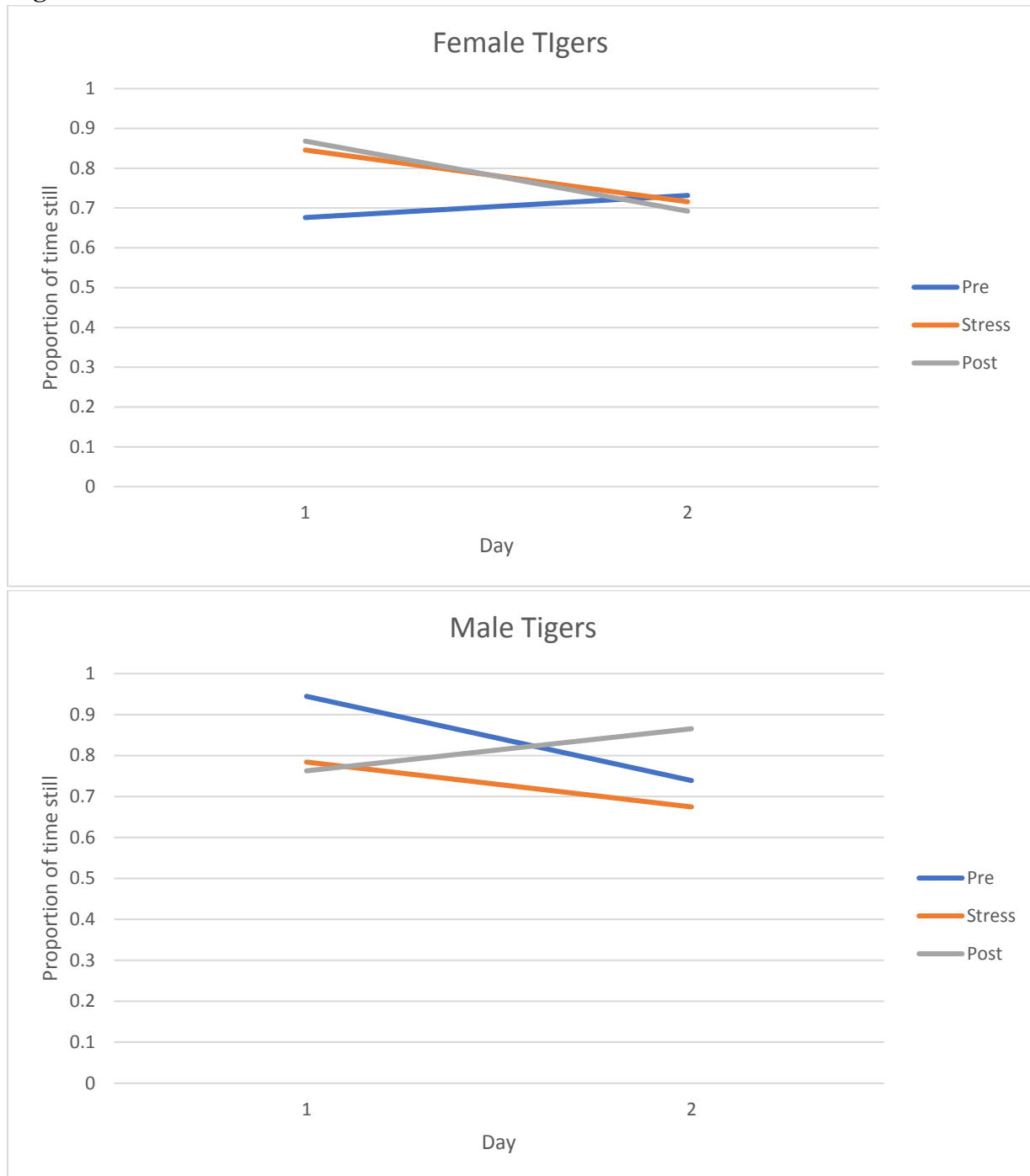
**Graph 9. Effect of Experience and Sex ( $P < 0.049$ ) on Time Spent Still in Tigers**



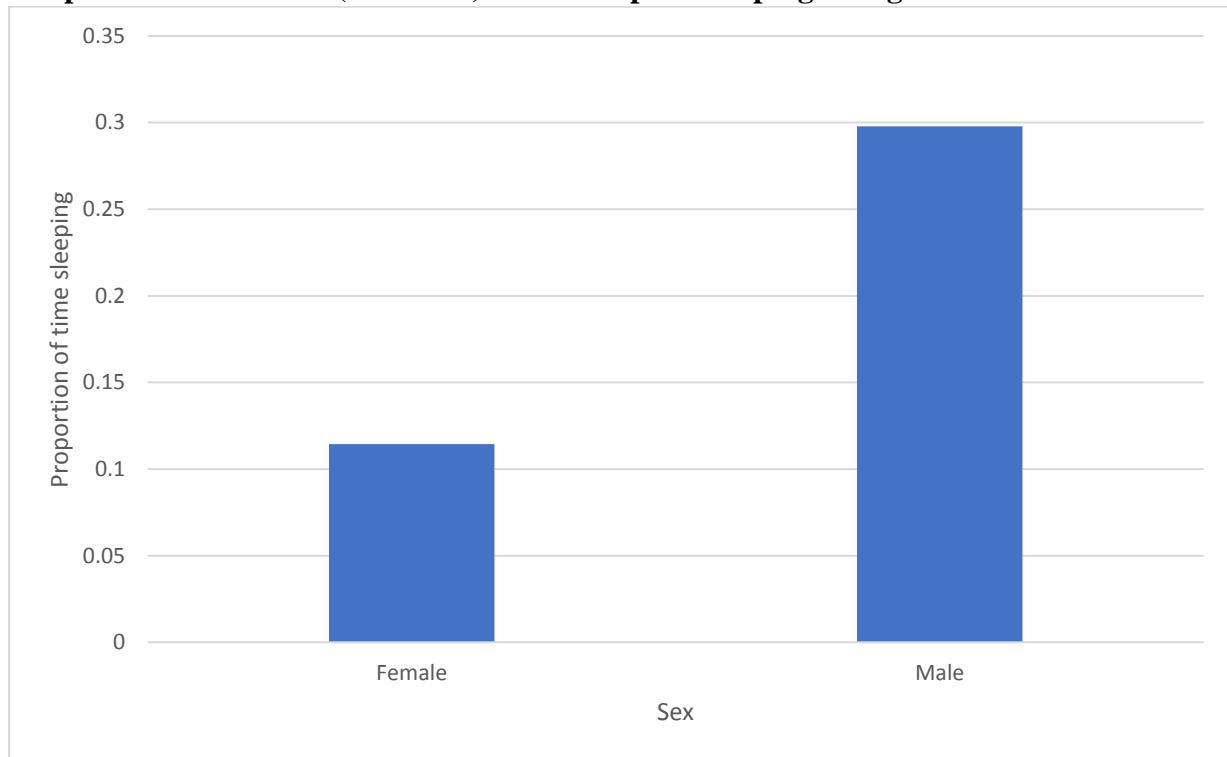
**Graph 10. Effect of Experience and Day ( $P < 0.047$ ) on Time Spent Still in Tigers**



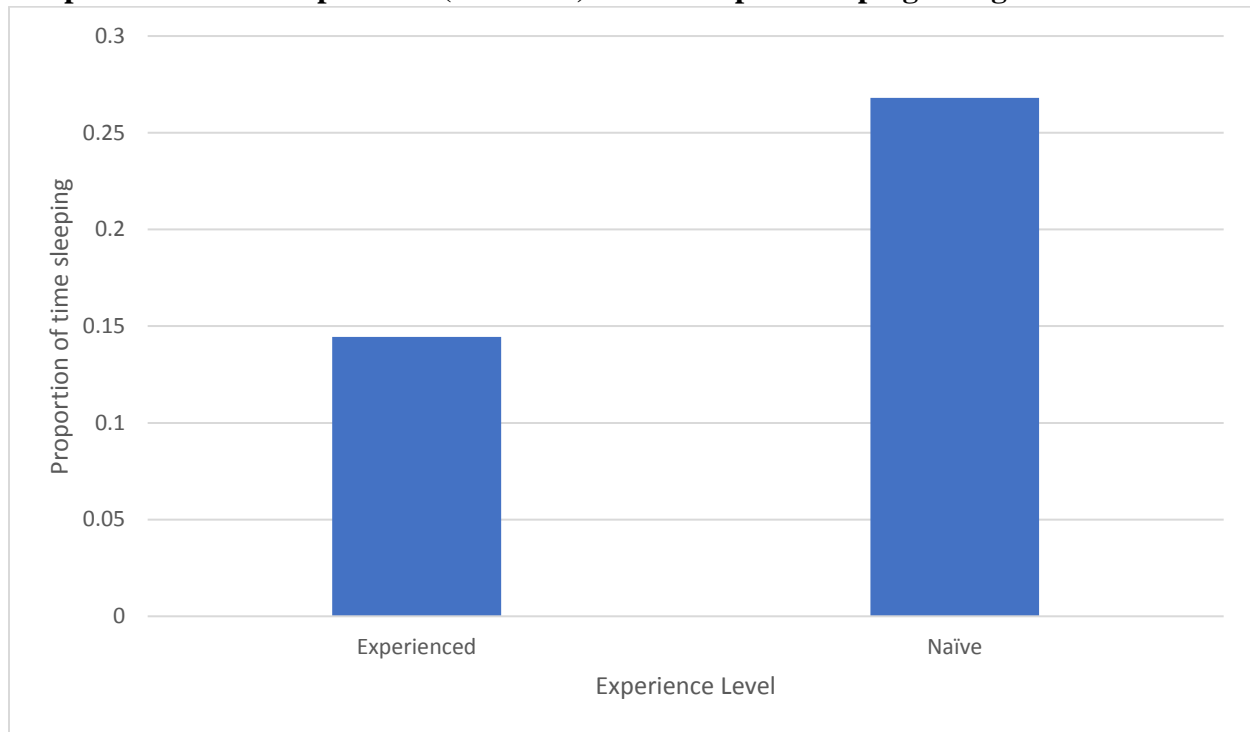
**Graph 11. Effect of Session and Day ( $P < 0.094$ ) on Time Spent Still in Female and Male Tigers**



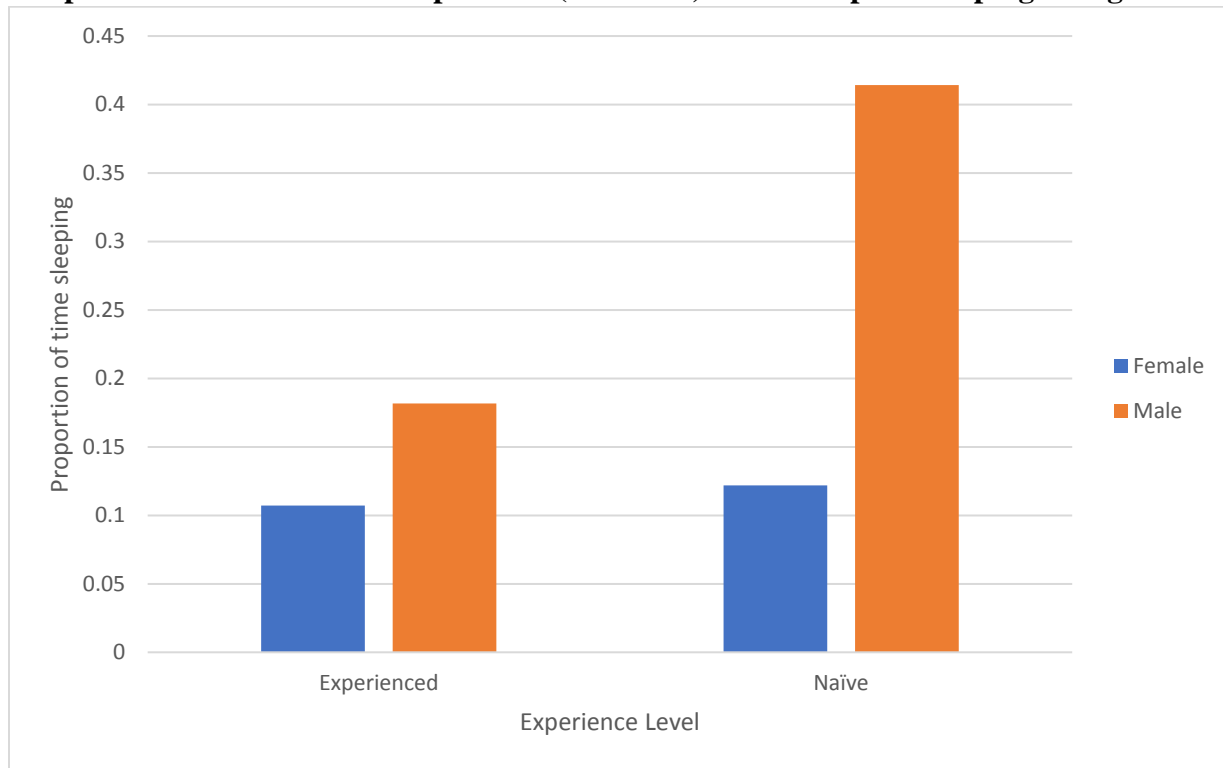
**Graph 12. Effect of Sex ( $P < 0.013$ ) on Time Spent Sleeping in Tigers**



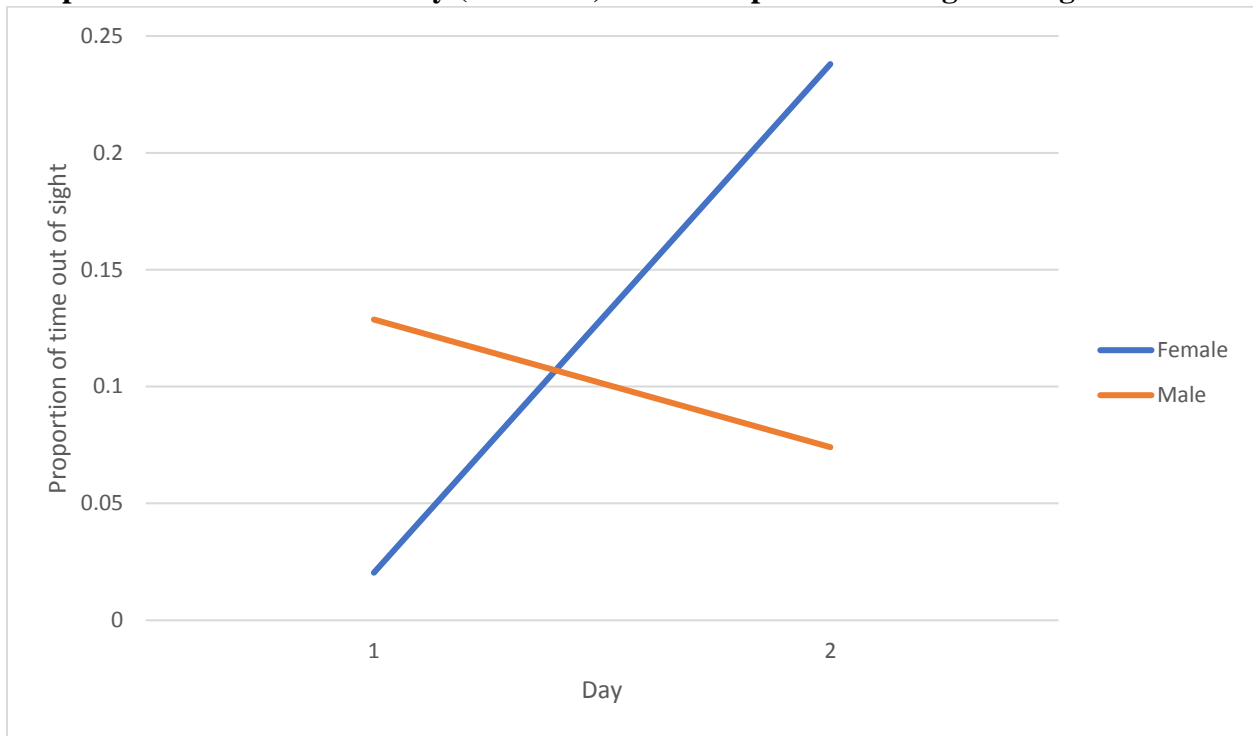
**Graph 13. Effect of Experience ( $P < 0.063$ ) on Time Spent Sleeping in Tigers**



**Graph 14. Effect of Sex and Experience ( $P < 0.094$ ) on Time Spent Sleeping in Tigers**

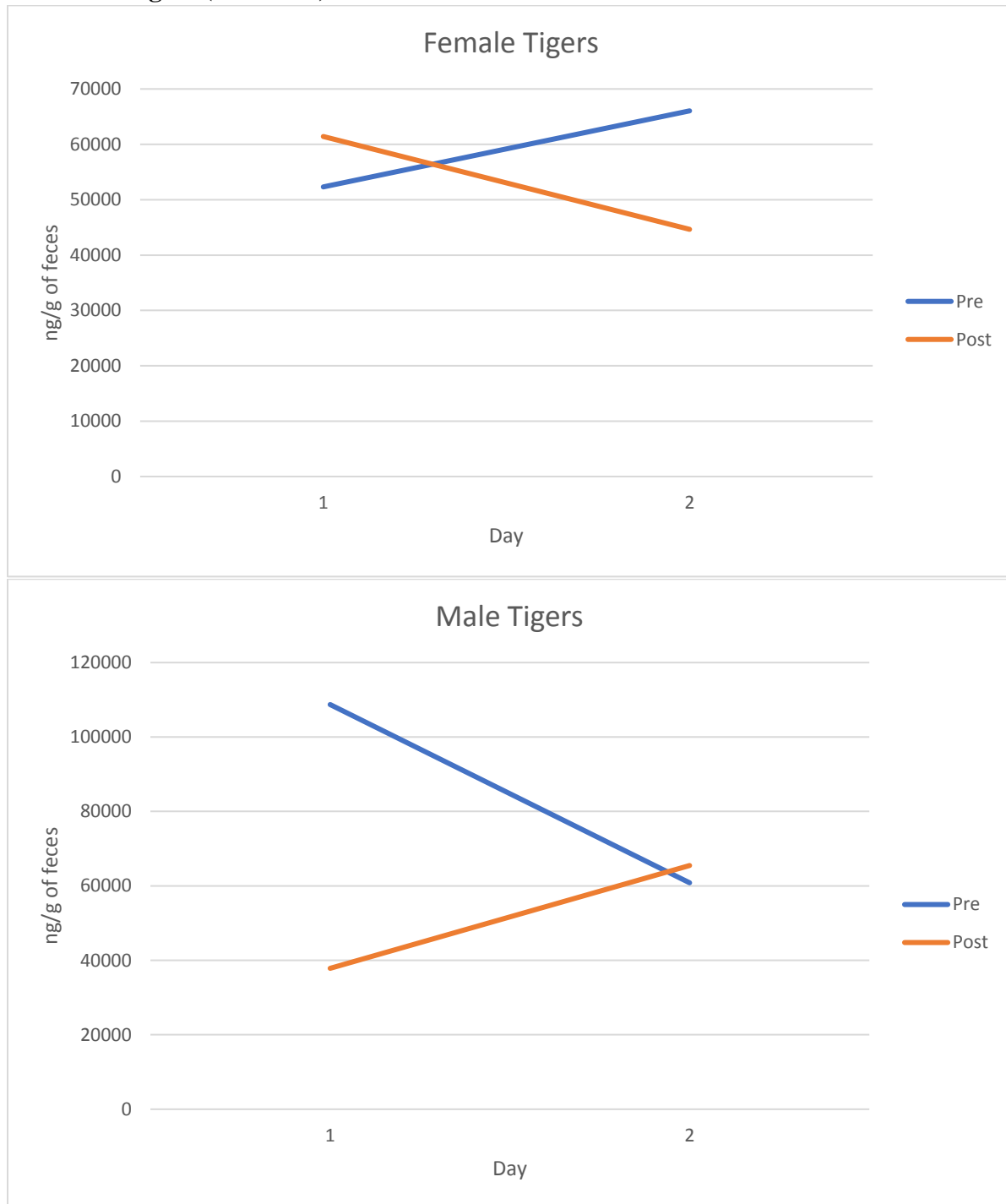


**Graph 15. Effect of Sex and Day ( $P < 0.059$ ) on Time Spent Out of Sight in Tigers**

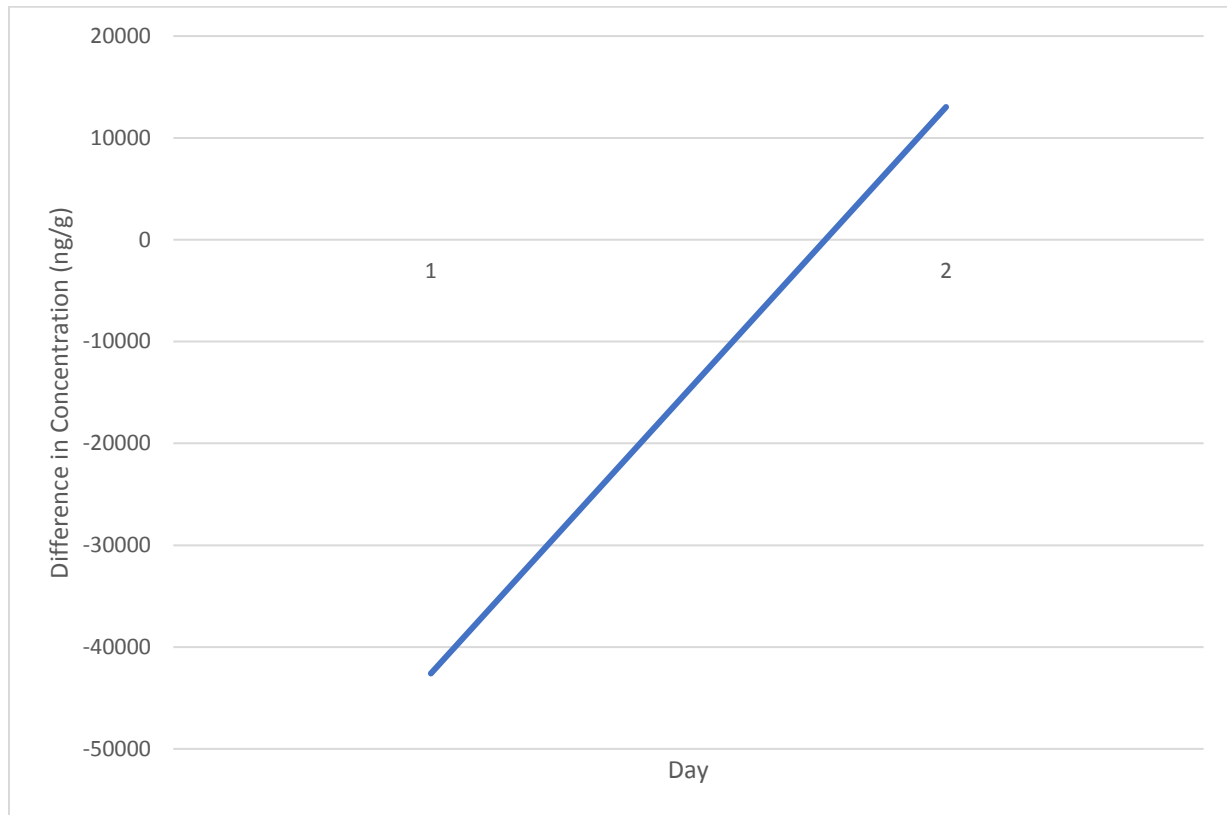




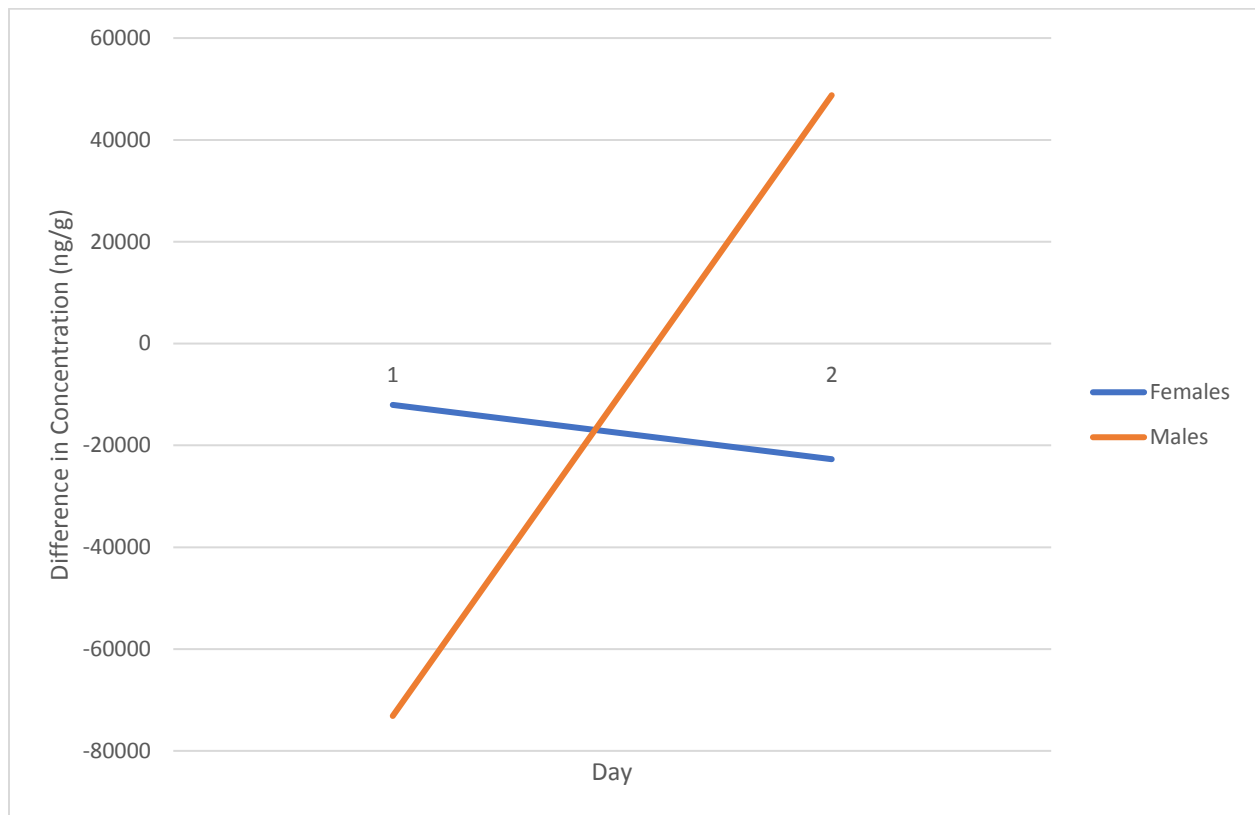
**Graph 16. Effect of Session and Day on Fecal Cortisol Metabolite Concentration of Female and Male Tigers ( $P < 0.099$ )**



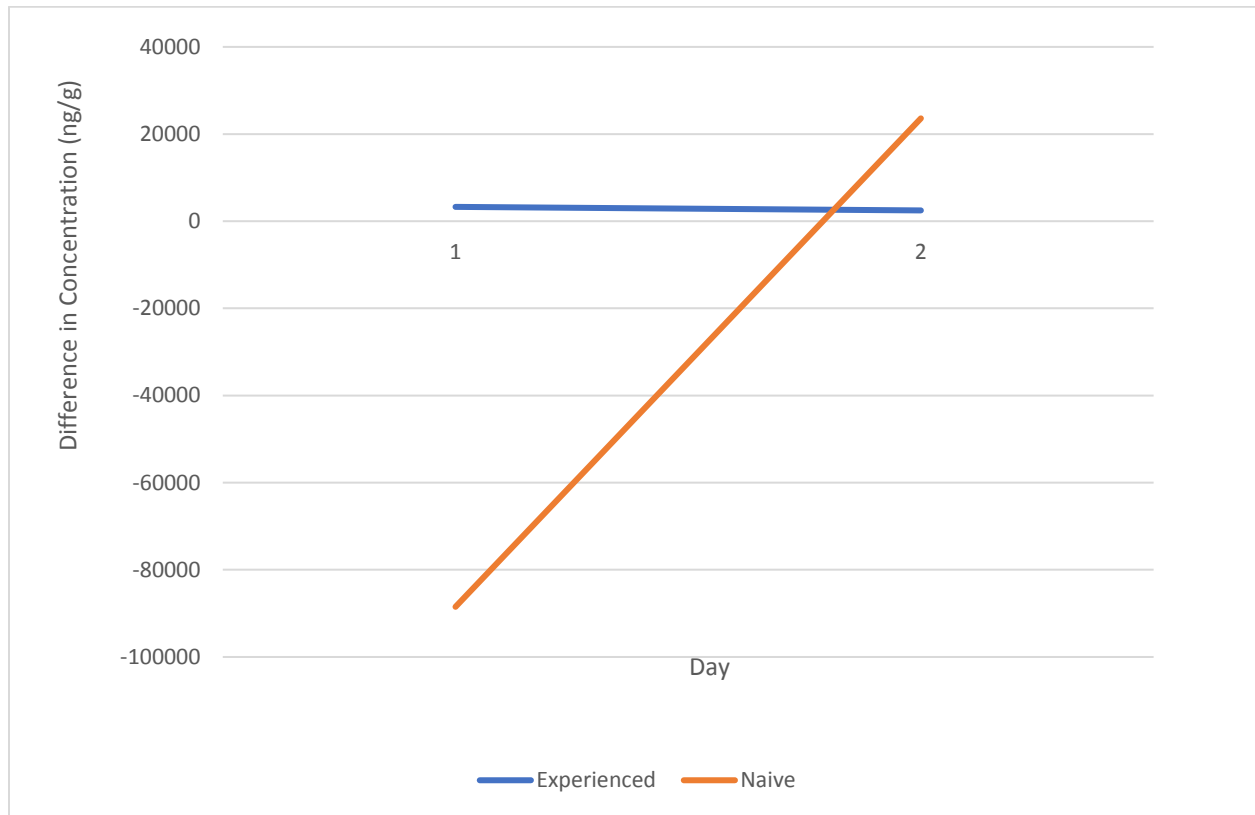
**Graph 17. Effect of Day ( $P < 0.0859$ ) on Difference in Post Session 1 and Average Pre Session Tiger Fecal Cortisol Metabolite Concentration**



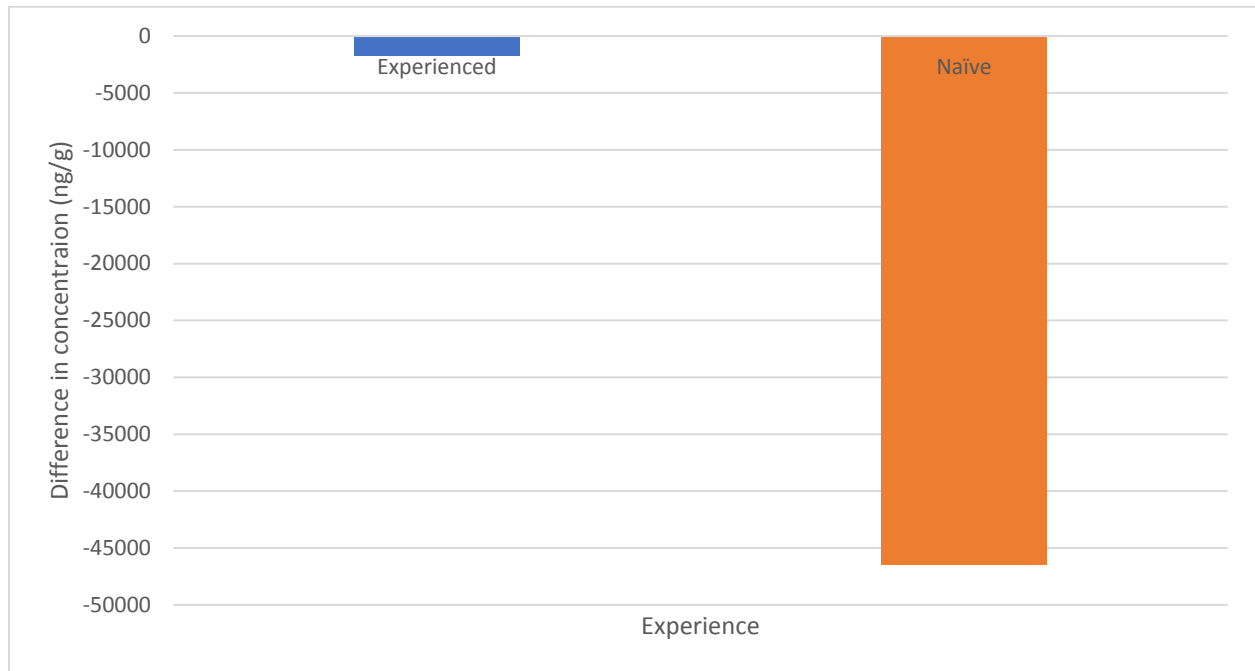
**Graph 18. Interaction of Sex and Day ( $P < 0.0479$ ) on Difference in Post Session 1 and Average Pre Session Tiger Fecal Cortisol Metabolite Concentration**



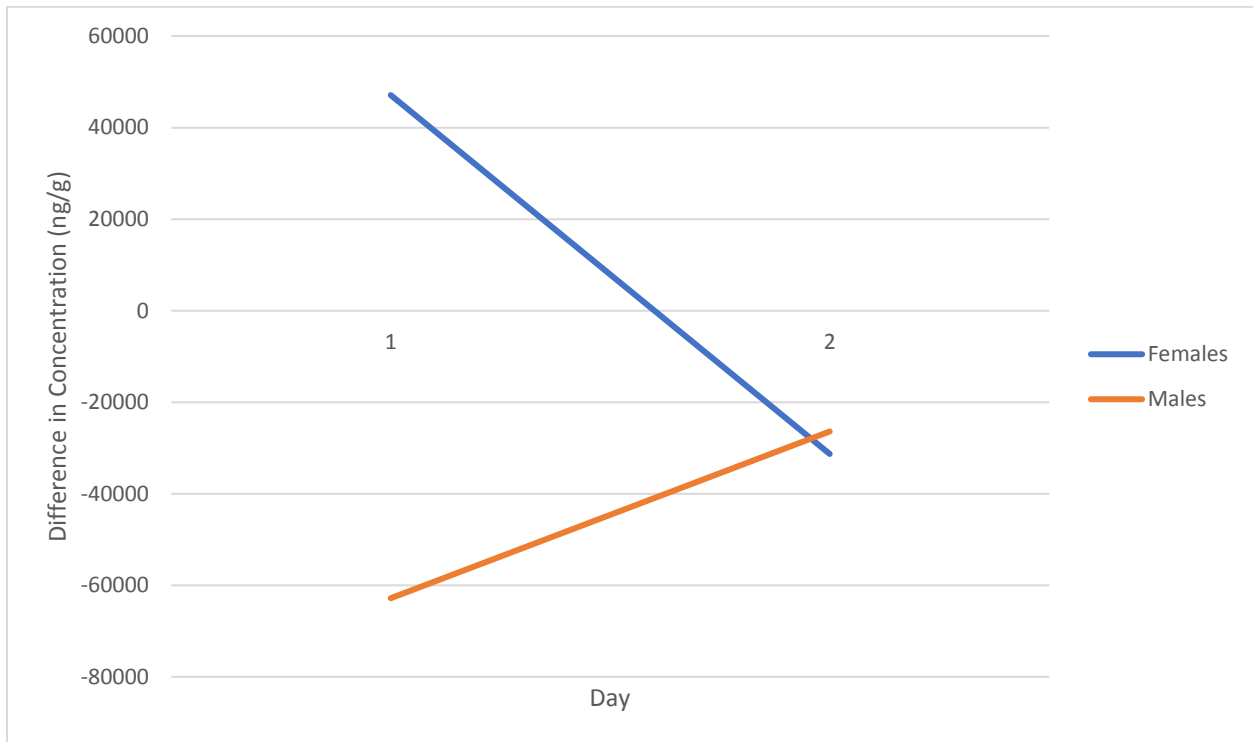
**Graph 19. Interaction of Experience and Day ( $P < 0.0821$ ) on Difference in Post Session 1 and Average Pre Session Tiger Fecal Cortisol Metabolite Concentration**



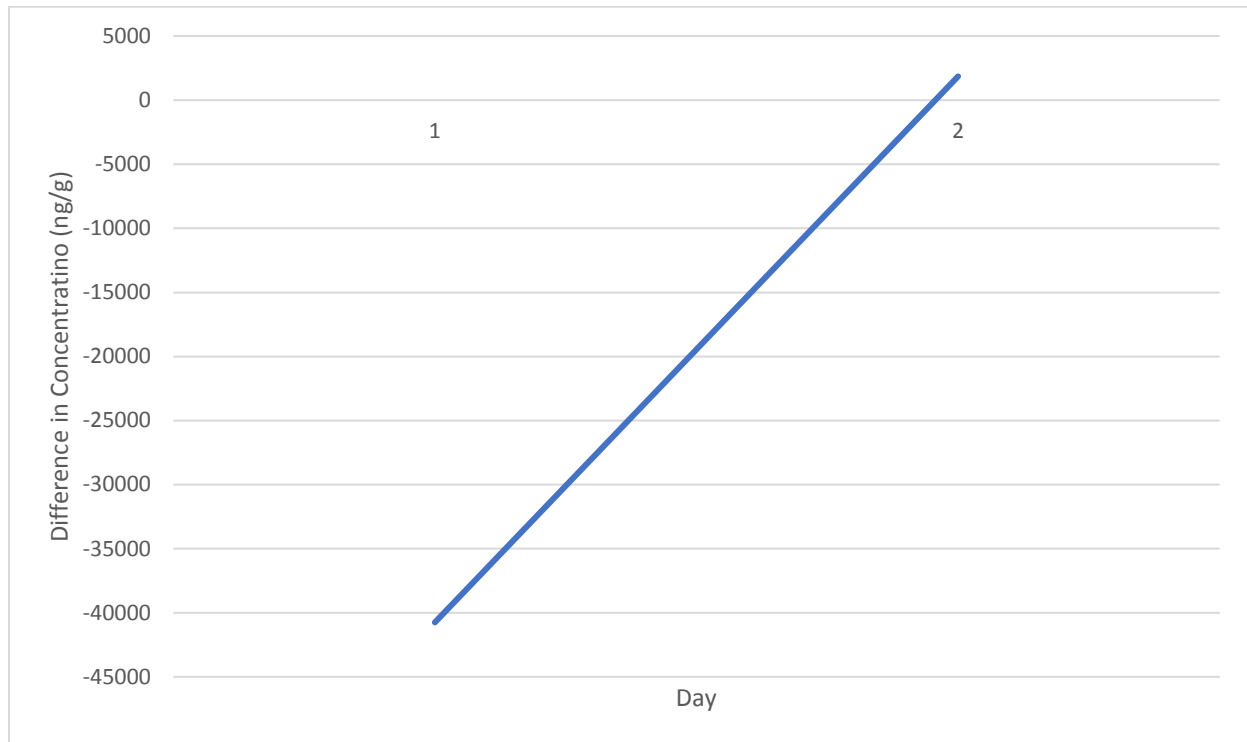
**Graph 20. Effect of experience ( $P < 0.0867$ ) on Difference in Post Session 2 and Average Pre Session Tiger Fecal Cortisol Metabolite Concentration**



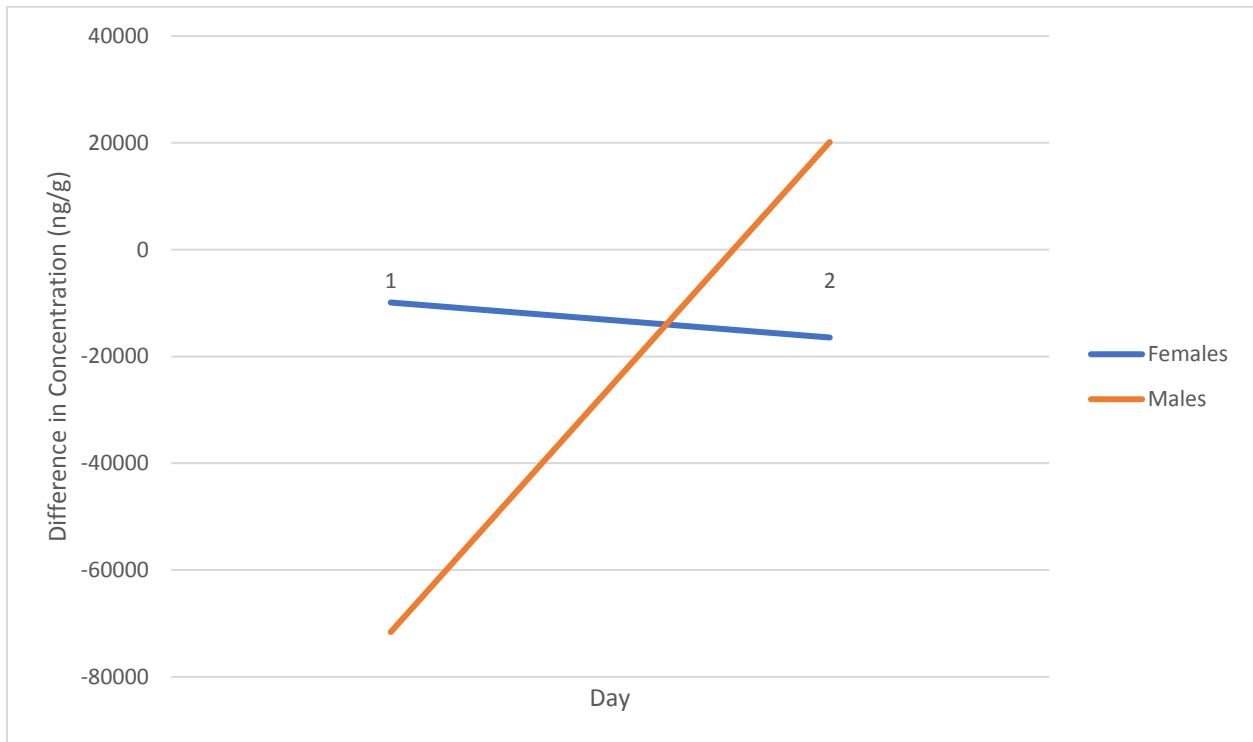
**Graph 21. Interaction of Sex and Day ( $P < 0.0658$ ) on Difference in Post Session 3 and Average Pre Session Tiger Fecal Cortisol Metabolites**



**Graph 22. Effect of Day ( $P < 0.0994$ ) on Difference in Average of Post Session 1 and 2 and Average of Pre Session Tiger Fecal Cortisol Metabolite Concentration**

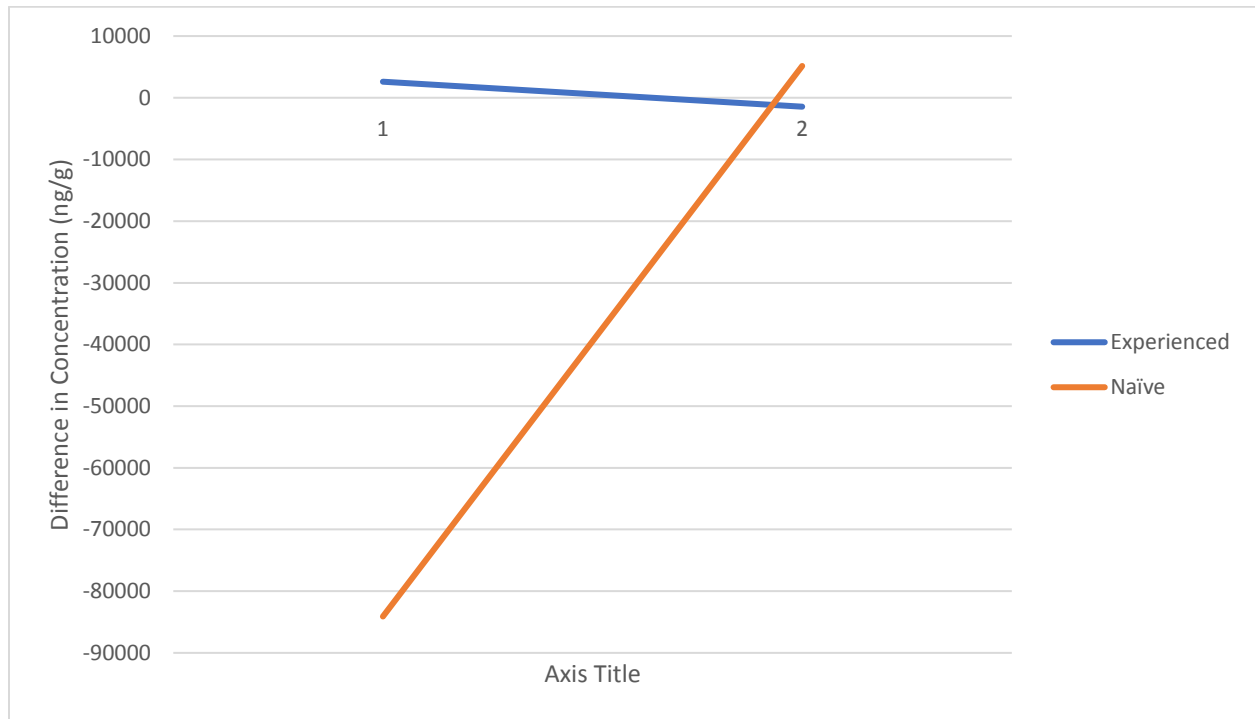


**Graph 23. Interaction of Sex and Day ( $P < 0.0637$ ) on Difference in Average Post Session 1 and 2 and Average Pre Session Tiger Fecal Cortisol Metabolite Concentration**





**Graph 24. Interaction of Experience and Day ( $P < 0.0756$ ) on Difference in Average Post Session 1 and 2 and Average Pre Session Tiger Fecal Cortisol Metabolite Concentration**



## APPENDIX

### 1. IACUC Approval



UNIVERSITY OF  
ARKANSAS

Office of Research Compliance

To: Kate Chapman  
From: Jeff Wolchok  
Date: January 10, 2020  
Subject: IACUC Approval  
Expiration Date: August 31, 2020

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol #20051, *Effect of visual transport stressors on captive tiger behavior and fecal cortisol*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond August 31, 2020 you may submit a modification to extend the project up to three years, or submit a new protocol. The IACUC may not approve a study for more than three years at a time.

The following individuals are approved to work on this study: Kate Chapman, Beth Kegley, and Callan Lichtenwalter. Please submit personnel additions to this protocol via the modification form prior to their starting work.

The IACUC appreciates your cooperation in complying with University and federal guidelines involving the care and use of animals.

JCW/jgr

1424 W. Martin Luther King, Jr. • Fayetteville, AR 72701  
Voice (479) 575-4572 • Fax (479) 575-6527

*The University of Arkansas is an equal opportunity/affirmative action institution.*

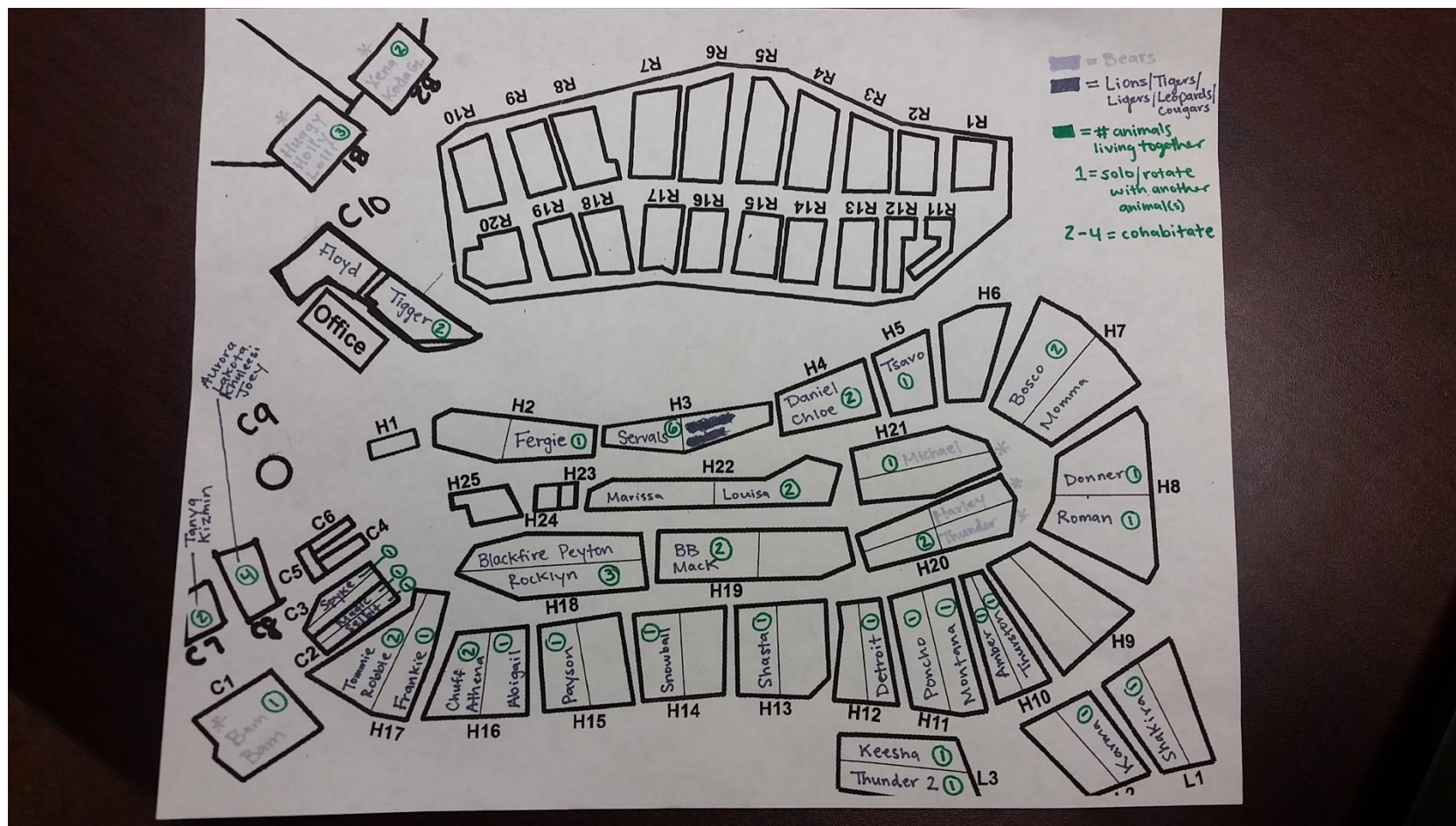
## 2. Ethogram Used

Behavior	Species	Operational Definition	Code
Sigh/Huff	ALL	Audible exhalation of air through the mouth, noise accompanies air flow, sounds like "huff" or "sigh"	HF
Yawn	ALL	Extended opening of jaws, deep breath in and out of mouth, often tongue out and teeth exposed. If vocalization accompanies open mouth, it does not fit one described below.	Y
Stretch	ALL	Move hind legs straight behind, or front legs straight in front, with opposite legs motionless under body	S
Sit*	ALL	Relaxed, calm sitting position; rear limbs and hindquarters on the ground.	SIT
Lay Down	ALL	Relaxed, calm, laying position. Eyes open or closed.	LD
Sleep	ALL	Laying down with eyes closed; more than 5 consecutive minutes	ZZ
Co-Rest	ALL	Focal tiger sitting or lying with another tiger in the same exhibit, body must be in physical contact with other tiger. May look like spooning.	COAR
Vigilant	ALL	Alert, attentive, standing or sitting; head moving or still.	V
Allogroom	ALL	Licking/grooming any part of a conspecific's body	AL
Autogroom	ALL	Licking/grooming any part of one's own body	AT
Stand*	ALL	For felines, all four limbs placed straight on ground, body supported. For bears, standing can occur with all four limbs on the ground or rear two limbs or group, body supported.	STD
Run	ALL	Quicker ambulatory movement in any direction	R
Walk	ALL	Ambulatory movement in any direction	W
Jump	ALL	Ambulating from a lower point to a higher point or vice versa	J
Pounce	ALL	Jumping onto an enrichment object or conspecific's body	P
Roll	ALL	Body on back or on side, moving side to side in a rolling manner. Back or side making contact with ground or with an object.	RL
Stalk	FELIDS	Slow, precise, often low walking movement; all legs slightly bent and underside close to the ground; eyes focused on an item or apparent goal	ST
Guard*	FELIDS	Crouch position; back half of body higher and front part of body lowered to the ground. Body positioned between another animal and a toy or food item. Ears may be back and animal may move in circles with back to item or may lunge at another animal. Growl may accompany this behavior.	GD
Cheek Rub	FELIDS	Moving head back and forth with cheeks and face making contact with object or conspecific's body	CR
Body Rub	ALL	While standing, move body repeatedly against object or conspecific's body (distinct from cheek rub)	BR
Self-Play	ALL	Manipulate own body (paws, limbs, tail) with mouth and/or paws	SLP
Manual Manipulate	ALL	Pawing at/playing with an object or conspecific with paws. Moving an object with paws, swiping at object.	MM
Anchor*	ALL	Using paw(s) to holding an object still, against a surface (ground, bench, etc.)	ANC
Scratch	ALL	Raking claws across surface of object or part of enclosure; marks may be left behind. Usually front paws. (does not include scratching self- that is autogroom)	SC
Carry	ALL	Pick an item off of substrate and move to a new location with mouth (felids/bears) or forelimbs (bears)	CY
Drag	ALL	Move a portion of an item with mouth from one location to another without lifting it off the ground	DG
Lift*	ALL	Move an item off of a substrate into the air with mouth or paws.	LFT
Pull*	ALL	While paw(s) anchor an item, mouth closes on part of object and head is pulled backward away from the substrate and item (generally removing flesh from bone)	PUL
Head Shake*	ALL	While standing, sitting or laying down, head of animal moves rapidly from side to side. Can occur while food or object is in mouth and while paws anchor object. Can also occur when object is in mouth and not anchored to a substrate. Can occur without anything in mouth.	SHK
Sniff	ALL	Low head/face toward an object, or up into the air. Quick inhalation with nostrils, can be repeated. Audible noise may accompany sniffing action.	SN
Flehmen Response	ALL	Curl back upper lip, open mouth, exposed teeth, tongue out inhales with nostrils. Nose appears wrinkled and head often pulled back. Holds position for a few seconds. "Stinky face"	FR
Lick	ALL	Stroke inanimate object with tongue	L

Lick Chops*	ALL	Tongue stroking over ones own lips, teeth, chin, or whiskers	LCH
Bite	ALL	Place mouth around an item and exerting force, usually a single action rather than repetitive. Uses whole mouth or front teeth (rather than side of mouth).	B
Gnaw*	ALL	Repetitive, persistent chewing on an object (bone or toy) with the side of the mouth, generally molars. Distinct from biting.	GN
Eat	ALL	Consume food or grass with mouth and tongue	E
Drink	ALL	Consume water with mouth and tongue	DK
Vomit	ALL	Regurgitation of vomit, grass, food, treats, bile, etc. through mouth (throw up)	VOM
Urinate	ALL	Urination from genital area; typical/short stream	U
Spray	FELIDS	Urination from genital area with tail up; long distance stream, often directed at object or human	SP
Defecate	ALL	Release of feces from anal area; feces must be observed.	DF
Mount/Hump	ALL	Raises front half of body on an object or other animal (or may remain on the ground), legs and hips are used to thrust pelvis one or more times towards object or animal. Implied sexual or dominance behavior.	HU
Splash	TIGERS/ URSIDS	Movement of head, face, upper limbs and/or paws that results in movement and sloshing of water. Tiger can be inside swimming pool or positioned outside of pool during this behavior.	SPL
Swim	TIGERS/ URSIDS	Movement of body's trunk, head, and/or limbs while inside the swimming pool or tub. Full body does not have to be submerged.	SWM
Submerge	TIGERS/ URSIDS	Head, face, head and face, or head and body move completely under the water's surface in the swimming pool or tub full of water. Does not count as submerge if body is underwater but head is above water.	SBM
Climb	URSIDS/ SMALL FELIDS	Vertical upward or downward movement of body on a substrate (tree, structure, fence) using forelimbs and backlimbs. Distinct from jump or pounce in that limbs must be used on substrate in a pattern of multiple contacts.	CLM
Chuff	TIGERS	Friendly vocalization; quiet, soft brrrr sound	CH
Moan	ALL	Soft roar vocalization, often during walking with head towards ground	M
Carol	LIONS, TIGERS, LIGER/ TILIGERS	Roaring/Moaning in response to animals in refuge making vocalizations. Often in response to Lions. Learned behavior.	CL
Roar	URSIDS, LARGE FELIDS	Loud vocalization, audible at 1+ mile distances	RR
Growl	ALL	Low, guttural vocalization directed at an animal or person. Typically accompanied by narrowing eyes, retracted lips, and lowering body in a crouch or pounce position.	GR
Chirp*	SMALL FELIDS	High-pitched squeaking vocalization that may occur with open mouth and retracted lips. Typically directed at an animal or person. Most common in cougars.	CRP
Purr*	SMALL FELIDS	A low tonal, continuous vibratory (fluttering) sound. Mouth generally closed.	PUR
Pace	ALL	Repetitive walking with no apparent goal. Wait 2 rotations of pattern before marking as pacing	PC
Pluck	ALL	Excessive grooming/ chewing of one area on own body, if auto grooming in same body location for more than 1 consecutive minute, first instance is AG, second is Pluck. Any that follow are called plucking.	PL
Head-Twist	ALL	Head moves or turns quickly and in a rotating manner, either occurs repeatedly or occurs with pacing between each turn. Distinct from head-shake, no object in mouth.	HT

*Ethogram Developed by Kate Chapman (2015-2018) with modifications of previous ethograms used by Resende et al., '2011) and McPhee (2002).*

### 3. Layout of Turpentine Creek Wildlife Rescue



#### 4. Data Sheets Used

[illegible]

## 5. Behaviors and Groups of Behaviors Analyzed after Observation

1	Pacing	Walking in a repetitive manner with no apparent goal
2	Vigilance	Alertness and attentiveness. Body facing stimuli of interest
3	Autogrooming	Grooming of one's self
4	Excretion	When the tiger either defecated, urinated, or sprayed during the observation period
5	In Den	When the tiger was in its den either visible or out of sight
6	Still	Inactive behaviors such as laying down, sitting, or sleeping
7	In Motion	When the tiger was moving or engaged in a behavior that required movement
8	Asleep	Laying down with eyes closed for more than 5 minutes
9	Sniffing	Lowered head and face, or nose raised in the air. Quick inhalation through nostrils
10	Out of Sight	When the tiger was not visible to the observer during the observation period