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NON-INVASIVE MEASUREMENT OF TRANSPORT EFFECTS ON TIGERS

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# NON-INVASIVE MEASUREMENT OF TRANSPORT EFFECTS ON TIGERS

By

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## ABSTRACT

NON-INVASIVE MEASUREMENT OF TRANSPORT EFFECTS ON TIGERS

By

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The transport of tigers between zoos is a common captive management practice that may affect tiger welfare. The objectives here are to validate a simple fecal cortisol extraction protocol for Radioimmunoassay (RIA) analysis, measure baseline effects of transport on tigers during a controlled 30-minute transport, and measure the effects of transport between zoos. Feces were spiked with known amounts of cortisol. After extraction 79.4 % was consistently recovered and measurements paralleled expected recoveries. Five tigers were crated and transported for 30-minutes. Average respiration rates increased during transport and remained elevated 10 minutes afterwards. Average immune-reactive fecal cortisol concentrations (IRFCC) peaked 3-6 days after transport and returned to baseline 9-12 days afterwards. Naïve tigers exhibited a higher average increase in IRFCC and longer recovery times than the experienced tigers. Naïve tigers also performed repertoires with greater intensity. Results suggest that prior exposure to transport may lead to habituation, thus reducing its effects. Fecal samples were collected from four tigers before, during, and after transport between zoos. Using RIA, IRFCC was measured. On average, IRFCC peaked 6-8 days afterwards and remained higher than baseline for 15-17 days. Results indicate that variable crate durations and methods of transport may affect tigers differently.

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# Chapter 1: Introduction to Zoo Animal Welfare

Zoos were established in the late 1800's, but the consideration of animal welfare was initially driven only by economic concerns. For example, zoo managers would improve welfare standards only when their poor practices caused animals to die resulting in economic losses. Since then, the philosophy of human superiority over animals and our concern for animal welfare have evolved. Animals are no longer viewed only in the context of how they are directly useful to humans, and zoos no longer exist for entertainment purposes alone (Mench and Kreger, 1996).

The role of zoos in society has evolved from being purely an institution for entertainment to an institution that supports wildlife conservation, and educates the general public about the importance of wildlife and current conservation issues (Conway, 2000; Margodt, 2000; Mench and Kreger, 1996; Norton, et al., 1995; Shapiro, 2000). As the function of zoos evolve from entertainment to conservation, animal welfare standards evolve as well (Figure 1). If a zoo exists purely for public entertainment, then managers will primarily focus on public needs instead of animal needs. Therefore, a zoo that exists purely to entertain will generally have low animal welfare standards resulting in an increase in poor welfare issues. Conversely, if a zoo exists purely to conserve wildlife and educate the public, then it is necessary for that zoo to provide optimal welfare standards to accomplish these objectives. In general, a zoo that exists purely for conservation and education purposes will have high welfare standards and a low

number of poor welfare issues. This evolution has been perceived and documented (Fisher, 1967; Mench and Kreger, 1996; Norton, et al., 1995; Shapiro, 2000) over time. Past zoos contained poorly enriched enclosures usually deficient in size and content. Over time, zoos have continued to provide more appropriately sized and enriched exhibits (Fisher, 1967; Mench and Kreger, 1996; Norton, et al., 1995; Shapiro, 2000).

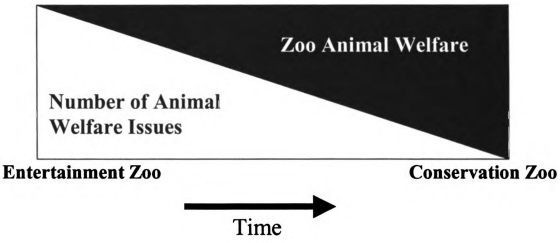



**Figure 1.** General model of the relationship between zoo animal welfare and the number of animal welfare issues that exist over time as the function of zoos has evolved from being an institution committed to public entertainment to an institution committed to wildlife conservation (adapted from: Fisher, 1967; Mench and Kreger, 1996; Norton, et al., 1995; Shapiro, 2000).

If zoos are continually evolving to conserve and educate, then why do welfare issues persist? The goal of conserving animals and their natural biology can only be accomplished if zoo managers provide the most natural environment

possible for each animal, thus reinforcing the animal's natural biology by allowing the animal to live in simulated wild-like conditions. Similarly, the public education goal can only be accomplished by providing the animals with as close to a natural environment as possible. Under these conditions it is hoped that they can perform natural behaviors for the public to see. If zoos are moving towards these two goals, shouldn't poor welfare issues cease to exist?

Although poor welfare issues should be decreasing, they persist in zoos for a number of reasons. One major reason is that although the traditional function of zoos was to entertain, this function still exists because entertaining the public is an important means for generating funds that are needed to support conservation and education programs. At the same time the entertainment function attracts large numbers of people who inadvertently become educated about the biology and ecology of animals and conservation issues. A second major reason is the lack of resources such as space, personnel, and appropriate substrate for enclosures. Often the lack of resources can be linked to a lack of funds to obtain those resources furthering the need to generate revenue by public entertainment.

A third major reason for the existence of animal welfare issues is the lack of information about the natural history of exotic animal species, which prevents zoo managers from providing an optimal captive environment. This lack of knowledge further justifies the need for continual research *in situ* and *ex situ* so that the natural history of exotic species can be better understood and an appropriate captive environment can be provided.

A fourth reason is lack of knowledge of how an animal's biology is affected by captive management practices and how to measure and reduce those effects. In many cases, common practices may alter behavior or physiology. For example, pandas housed in traditional enclosures perform more stereotypic behavior than pandas enclosed in more natural environments (Liu et al., 2003) and free-ranging female elephants above the age of 50 produce calves with greater success than captive female elephants above the age of 50 (Brown, 2000).

Transport of zoo animals from one place to another is a common practice that can affect behavior and physiology. Studies have assessed transport effects on farm animals to help improve agricultural practices (Lambooy, 1988 and Bradshaw et al., 1996). These studies demonstrated that transport might result in body temperature increase (Brundige et al, 1998), an increase in hypothalamic-pituitary-adrenal (HPA)-axis activation (Broom and Johnson, 1993), and a depressed immune response (Baker and Gemmell, 1999; Broom and Johnson, 1993). Zoo animals often appear stressed during transport as well making them susceptible to these negative effects (pers. obs.). Few studies have assessed transport effects on zoo species, and no studies on tigers have been rigorously documented.

The term "stress" has been applied in a wide range of contexts, and disagreement of its meaning among researchers makes it difficult to clearly define (Wielebnowski, 2003). Selye generally defined stress as any stimulus that threatens or appears to threaten the homeostasis of an individual (Selye, 1984).

In the following text, the term "stress" will refer to any homeostatic changes in tiger physiology or behavior that are perceived to occur as a result of the stressor (transport).

Many studies have used the hormone cortisol to measure an animal's stress level during common management procedures (Merlot et al., 2004, Roussel et al., 2004, Braastad et al., 1998). To assess transport effects on tiger stress, a means to measure changes in cortisol production using non-invasive methods must be implemented. The following chapter explores the use of feces (which can be collected non-invasively) as a substrate from which cortisol levels can be measured.

Feces may be the substance of choice to measure hormonal fluctuations in tigers because feces collection is simple, safe, and noninvasive. Feces have been shown to contain cortisol concentrations that can be linked to the amount an animal produces. Based on this information one can learn how the physiology of the animal has been affected by a management procedure, and appropriate management decisions can be made. For example, the measurement of cortisol in cheetah feces has allowed managers to identify increases in stress and its effects on cheetah reproductive cycles so that more effective breeding decisions can be made (Jurke, 1997).

The use of tiger feces to measure cortisol had not been documented, and methods to measure cortisol in feces had not been perfected. Current corticoid extraction methods (Palme et al., 1998; Brown et al., 2001) can be labor-intensive and often alter the sample (Terio et al., 2002) resulting in skewed

measurements. It is necessary to develop other extraction methods because to date the published methods all demonstrate specific problems that either mask or alter the samples. Similarly, variable amounts of water content in feces can affect resulting hormone measurements. To eliminate these effects without altering the sample by drying (Terio et al., 2002) the use of a correction factor was investigated.

To test the validity of tiger feces analysis and to subsequently test transport effects, the following objectives were addressed:

*Objective 1*: Validate a new fecal cortisol extraction protocol for Radioimmunoassay (RIA) analysis

*Objective 2*: Develop and test a correction factor that eliminates water content of feces as a source of error

*Objective 3*: Determine effects of a controlled 30-minute transport on tiger behavior and physiology

*Objective 4*: Measure effects of the common transport procedure from one zoo to another on the HPA-axis of tigers and compare results with the 30-minute controlled transport

# Chapter 2: Measuring Fecal Cortisol in Tiger Feces

## INTRODUCTION

Fecal corticoid levels have been used as an effective welfare indicator for nondomestic felids. Wielebnowski et al. (2002) reported that clouded leopards performing more stress-related behaviors such as pacing and self-injury (i.e., fur plucking or tail chewing) had higher fecal corticoid levels than those that performed less stress-related behaviors. Since increases in cortisol levels were correlated with an increase in stress, and transport is perceived to induce stress (pers. obs.), measuring cortisol in feces is a practical way to identify physiological effects of transportation stress. As shown by Graham and Brown (1996), when cortisol was injected into domestic cats, feces contained 80% of adrenocorticoid metabolites, making the feces a reliable substrate to correlate with cortisol levels in the body. Presence of cortisol in tiger feces has not been documented, and methods used to measure cortisol in feces have not been perfected. Current extraction methods (Palme and Mostl, 1997; Graham and Brown, 1996) are labor-intensive and may alter the sample (Terio et al., 2002), resulting in inaccurate measurements. To counter these problems we devised a simple cortisol extraction protocol that minimizes sample manipulation.

Fecal water content varies considerably between samples due to individual differences such as gut passage time, diet, water intake, and time exposed to air. Water content must be eliminated as a source of error so that

cortisol concentration can accurately be determined. Two possible solutions are to either dry the feces to 0 % water content or determine the percent dry matter from an aliquot of the sample and calculate a correction factor. Since drying an entire sample takes time and some drying techniques may cause the breakdown of glucocorticoids (Terio et al., 2002), normalizing samples for water content may be quicker and yield more accurate results. This study was designed to test the effectiveness of our extraction protocol, and to determine if normalization for water content eliminates the effects of variation in dry matter content on cortisol measurement.

## METHODS

### Validation of a new cortisol extraction protocol

Each entire fecal sample was combined with a 20% methanol, 80% distilled water solution so that they were in a 1:4 proportion (weight by weight). The resulting mixture was homogenized thoroughly using a tissue homogenizer (Brinkmann Instruments, Westbury, NY) and filtered through 7 $mm^2$ gauge wire mesh to eliminate bones and hair. Three aliquots (1.5 mL each) were kept to determine dry matter content. Next, 40 g of solution was transferred to a 50 mL polypropylene tube (USA Scientific Incorporation, Ocala, FL) and centrifuged at 3,000x *g* for 30 min. Then, 1.5 mL supernatant was transferred to a 1.5 mL eppendorf tube, and each tube was microcentrifuged at 13,000x *g* for 15 min (these steps were performed in triplicate). One mL of the supernatant was transferred to an RIA tube ($^{125}$I-cortisol RIA kit; Coat-A-Count; Diagnostic

Products Corporation, Los Angeles, CA), and immediately dried in a vacuum concentrator (Appropriate Technical Resources Inc.; Laurel, MD). Dried supernatant was resuspended in 200 μL distilled water before RIA. Resulting RIA values were corrected for water content as performed by Wilson et al. (2002) using the determined proportion dry matter from the three aliquots taken.

The assay sensitivity of the RIA kit is 0.2 μg/dL. When performing an RIA, test tubes lined with cortisol antibodies are used. To show that these antibodies accurately bind to cortisol the antibody cross-reactivity is as follows: 100 % with cortisol, 11.4 % with 11-deoxycortisol, 1.6 % with betamethasone, 0.98 % with cortisone, 0.94 % with corticosterone, 0.26 % with 11-deoxycorticosterone, 0.34 % with tetrahydrocortisol, and < 0.1 % with testosterone, aldosterone, androsterone, progesterone, estriol, estradiol, and estrone.

This method for extracting cortisol from tiger feces was tested by adding 10 μL of $^{3}$H-cortisol to 6 original samples of 40 g feces and measuring the recovered $^{3}$H-cortisol after extraction. Indicators of accuracy and parallelism were obtained by adding 500 μg of 5 different cortisol concentrations (0, 0.012, 0.062, 0.123, and 0.246 μg/dl) to 40 g of 5 separate aliquots of the same homogenized fecal sample. The 0 μg/dl concentration was used as the control to which all other concentrations were compared.

**Using a mathematical correction factor to eliminate effects of water content**

To measure water content effects on fecal cortisol measurements we added 500 μl of 6 different cortisol concentrations (0, 0.012, 0.062, 0.123, 0.246,

and 0.615 ug/dl) to 40 g of 6 separate aliquots of a large, pooled sample of tiger feces. Next, we added 2 times their weight of a 20% methanol, 80% distilled water solution and homogenized them. Each concentration was then separated into 5 different 5 mL treatments: the control (C), experimental control (EC), experimental low (EL), experimental medium (EM), and experimental high (EH). Then, each EC, EL, EM, and EH were dried completely, and water was added to each to obtain 0, 15, 30, and 60 % water content by weight respectively. The control was frozen throughout the treatment period. Finally, each sample was extracted using the previously described method, and cortisol was measured in triplicate using RIA.

**Correcting for dry matter content and statistical analysis**

Resulting cortisol concentrations were compared using a one-way analysis of variance (SAS Institute Inc., Cary, North Carolina, Copyright © 2001). Then, resulting concentrations were normalized for water content. Once corrected, resulting concentrations were again compared using a one-way analysis of variance.

## RESULTS

**Validation of a new cortisol extraction protocol**

The intra-assay coefficient of variation was 5.1 %. After extraction, the average recovery of $^{3}$H-cortisol was 79.4 ± 0.1 %. When testing for accuracy and parallelism, we recovered 83±0.3, 92±0.1, 91±0.1, 93±0.1% of the different

concentrations (0.012, 0.062, 0.123, and 0.246 ug/dl) of spiked cortisol respectively.

**Using a mathematical correction factor to eliminate effects of water content**

Before mathematically correcting for dry matter content, significant differences in cortisol concentration were found between the control and all experimental treatments and between experimental treatments (ANOVA;$F_{4,25}$=7.84,$p<0.001$). After mathematically correcting for dry matter content, we found no significant differences among any treatments (ANOVA; $F_{4,25}$=1.43;NS).

## DISCUSSION

Based on these results, the extraction protocol measured concentration of cortisol in tiger feces with 79 % accuracy. We also recovered cortisol with great consistency, as the standard error was only 0.1 % between all samples. Similarly, our test for parallelism showed that the amount of cortisol recovered proportionately increased with an increase in cortisol concentration.

Drying is a recurring step in current extraction protocols to avoid skewed measurements caused by a variation in dry matter content. In this study, there were significant differences in recovered cortisol between samples from the same pooled feces, but with different concentrations of dry matter. Normalizing for water content eliminated the significant differences. Fecal glucocorticoids break down as a result of some drying techniques (Terio et al., 2002). To

eliminate this problem we propose that samples not be dried during the extraction protocol, and are instead normalized for water content.

Based on these results this protocol serves as an accurate method to measure cortisol concentration in tiger feces. The next step is to pharmacologically validate this protocol by demonstrating that increases in recovered fecal cortisol indicate an increase in adrenal activity. To do so an increase in measured cortisol must be linked with a stressful event that would stimulate adrenal activity. The study presented in the following chapter attempts to link fecal cortisol increase with transportation stress.

# Chapter 3

Dembiec, Daniel P., Snider, Richard J., and Zanella, Adroaldo J. 2004. The effects of transport stress on tiger physiology and behavior. *Zoo Biology* 23(4): 335-346.

## INTRODUCTION

The transport of animals from one place to another is a common practice that can affect their behavior and physiology. Many studies have assessed transport effects on farm animals to help improve agricultural practices. Lambooy (1988) showed that pig activity levels decrease during transport, while Bradshaw et al. (1996) reported that transport increased cortisol levels in pig and sheep saliva. Few studies have assessed transport effects on the physiology and behavior of zoo species, and no studies on tigers have been rigorously documented. An increase in body temperature of pigs (Brundige et al., 1998) an increase in hypothalamic-pituitary-adrenal (HPA)-axis activation (Broom and Johnson, 1993), and a depressed immune response in brushtail possums (Baker and Gemmell, 1999; Broom and Johnson, 1993) have been identified as effects of transportation stress. While a depressed immune response may be viewed as a negative effect, short-term activation of the HPA-axis may be beneficial to adaptive processes. For example, it has been shown that HPA-axis activation in some cases enhances learning in some mammals and humans (Wolf, 2003).

It is estimated that only between 5,000 and 7,500 tigers remain in the wild (Seidensticker, 1999). Zoos are currently playing a large role in preventing extinction of endangered tiger subspecies through captive breeding. Transport under these conditions is often necessary to introduce animals of the opposite sex for mating purposes. Tigers are also transported simply to be added to a zoo's collection to help educate and entertain the public. Their coloration and behavior make them effective animals to help illustrate important ecological

concepts such as adaptive coloration and the ethology of forest predators, and their size (Ward et al., 1998) and beauty make them popular with the public. Therefore, tigers are often transported between zoos for breeding purposes and to enhance the quality of the zoo following the recommended terms in the American Zoo and Aquarium Association's (AZA) Tiger Species Survival Plan (SSP).

Conservation efforts are often successfully supported by *in-situ* studies of animals in captivity. As wild populations continue to decline, the information we obtain from captive animals about their basic biology becomes increasingly important. In addition, captive animals can serve as a resource for reestablishing endangered populations and maintaining genetic diversity. Captive breeding programs have been very effective in some cases, but in others animals have failed to breed for unknown reasons. For example, the giant panda is well-known for reproducing poorly in captivity (McGeehan, et al., 2002).

When the natural biology of zoo animals is altered, they serve a less effective role in educating the public. For example, Baxter and Plowman (2001) showed that the lack of browsing opportunity for giraffes in captivity caused an increase in stereotypic behavior, and Mallapur and Chellam (2002) reported that Indian leopard activity was influenced by feeding time and differed from activity levels in the wild. To effectively utilize zoos as ex-situ conservation and public education facilities, any effects captive management may have on the behavior and physiology of zoo animals must be investigated and understood. Transportation effects is one management practice that has not been intensively

studied. The better understanding of zoo animal behavior and physiology coupled with understanding of techniques used to measure these indicators of well-being enable us to identify threats to animal welfare.

Fear may inhibit exploratory behavior. On the other hand, fear may also stimulate flight or hiding behavior. Space limitations in a transfer cage prevent such responses to fear, which often results in the performance of stereotypies such as rapid circling or pacing (Manser, 1992). Stereotypies may also result from conditions of conflict or lack of control (Dantzer, 1986; Mason, 1991).

Facial expressions may serve an important role in assessing an animal's well-being. Ears positioned back and low in domestic cats has been used in past studies to identify a defensive or fearful response to stimuli (Yoshinobu et al., 2001; Levine et al., 1990; Johansson et al., 1979). In general, ears positioned back and low have been associated with defensive behavior, fear, and confusion while ears perked up and forward are associated with relaxation, alertness, and inquisitiveness (Thorne, 1992).

In feline species, cortisol has been identified as the most important naturally occurring glucocorticoid (Goossens et al., 1995) and is a product of the HPA-axis. Immune-reactive (IR) cortisol can then be extracted from feces (Schatz and Palme, 2001; Graham and Brown, 1997) and used as an effective indicator of levels in the body (Mostl and Palme, 2002). In tigers, the time between cortisol production and excretion in feces is unknown.

This study was performed to quantitatively and qualitatively evaluate how transportation affects the behavior and physiology of tigers. Grandin (1997)

stated that previous experience may influence the way an animal reacts to transport. Therefore, a comparison between effects on experienced and inexperienced tigers was included in this study.

## METHODS

### Animals and Study Site

The Michigan State University All-University Committee on Animal Use and Care approved this study as did the AZA's Tiger SSP. This study was performed during the summer of 2001 at Turpentine Creek Wildlife Refuge (TCWR) in Eureka Springs, Arkansas. Turpentine Creek Wildlife Refuge is a non-profit wildlife refuge that provides a home to over 100 large carnivores confiscated or otherwise received from private ownership. Five tigers were chosen for the study (Table 1). Selected tigers were similar in age, housed individually, and acclimated to their enclosure. Two were female, and three were male. Tigers were also chosen based on their experience with the transport procedure. Two tigers had experienced the transport procedure at least two times before our study began (experienced group). Both tigers had been transported previously to be introduced to a new enclosure. Three tigers had never experienced this transport procedure (naïve group).

**Table 1.** Characteristics of each tiger subjected to transportation. Experience refers to whether the tiger has experienced the transportation procedure previous to the study.

| Tiger | Sub-species | Age (years) | Gender | Experience |
|---|---|---|---|---|
| 1 | Bengal/Siberian hybrid | 7 | Female | Yes |
| 2 | Bengal | 7 | Male | Yes |
| 3 | Corbetti | 6 | Male | No |
| 4 | Siberian | 6 | Female | No |
| 5 | Bengal/Siberian hybrid | 8 | Male | No |

All tigers were exposed to the same environmental conditions and daily diet. They were enclosed individually outdoors in wire mesh cages with concrete flooring. Each cage contained a small concrete den for shelter and was partially covered by a tarpaulin cover for additional shelter from the summer sun.

**Transport Procedure, Sample Collection, and Behavioral Observation**

The transport procedure was consistent with the recommended transport procedure outlined by the AZA's Tiger SSP (Tiger Information Center, 2003). To establish baseline cortisol levels, entire stools were collected from each tiger for 6 days prior to transport and immediately frozen. On day 6 each tiger was led into a 2.7 x 1.2 x 1.2 m wire mesh cage. The cage was already mounted on a flat trailer attached to a 4-wheel drive vehicle. All tigers readily entered the cage when the door was opened within 5 min of having access to it. The tiger was driven over gravel and dirt roads to a shaded area outside of the refuge walls approximately 0.4 km from the original enclosure. The relocation site was not in close proximity to any other animals. Following transport, each tiger was

released back into its original enclosure. Each transport episode lasted 30 min from the time the cage door was locked to the time the tiger was released back into the original enclosure, but the average time in transit was 8.5 min. Voided feces during transport were collected (if necessary) afterwards. Finally, feces was collected continuously during the 12 d following transport so that IR cortisol concentration could be determined.

Tigers were videotaped in their home enclosure for 1 h prior to transport, during transport, and for 1 h following release from the transport cage. Behavior was subsequently analyzed for all 5 tigers using behavioral observation recording software (Observer Base Package for Windows Version 3.0, Noldus Information Technology, 1996). The behavioral analysis included activity level, time spent pacing, pace rate (turns per min), time spent investigating (sniffing transfer cage), and ear position.

Tiger respiration rate (breaths per min) was estimated by counting rib cage flex at 15 s intervals. The only common time respiration rate could be observed for all tigers was approximately 10 min before, during, and approximately 10 min after transport. For one tiger rib cage flex could not be seen so respiration rates could only be counted for four tigers.

**IR Cortisol Extraction and Radioimmunoassay**

The entire fecal sample was combined with a 20% methanol, 80% distilled water solution so that they were in a 1:4 proportion (weight by weight). The resulting mixture was homogenized thoroughly using a tissue homogenizer

(Brinkmann Instruments, Westbury, NY) and filtered through 7 $mm^2$ gauge wire mesh to eliminate bones and hair. Three aliquots (1.5 mL each) were kept to determine dry matter content. Next, 40 g of solution was transferred to a 50 mL polypropylene tube (USA Scientific Incorporation, Ocala, FL) and centrifuged at 3,000x *g* for 30 min. Then, 1.5 mL supernatant was transferred to a 1.5 mL eppendorf tube, and each tube was microcentrifuged at 13,000x *g* for 15 min (these steps were performed in triplicate). One mL of the supernatant was transferred to an RIA tube ($^{125}$I-cortisol RIA kit; Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA), and immediately dried in a vacuum concentrator (Appropriate Technical Resources Inc.; Laurel, MD). Dried supernatant was resuspended in 200 μL distilled water before RIA. Resulting RIA values were corrected for water content as performed by Wilson et al. (2002) using the determined proportion dry matter from the three aliquots taken.

This method for extracting glucocorticoid metabolites from tiger feces was biochemically validated by adding 10 μL of $^{3}$H-cortisol to 6 original samples of 40 g feces. Cross-reactivity of the antibody was as follows: 100 % with cortisol, 11.4 % with 11-deoxycortisol, 1.6 % with betamethasone, 0.98 % with cortisone, 0.94 % with corticosterone, 0.26 % with 11-deoxycorticosterone, 0.34 % with tetrahydrocortisol, and < 0.1 % with testosterone, aldosterone, androsterone, progesterone, estriol, estradiol, and estrone. The assay sensitivity of the RIA kit is 0.2 μg/dL. The intra-assay coefficient of variation was 5.1 %. After extraction, the average recovery of $^{3}$H-cortisol was 79.4 ± 0.1 %.

Indicators of accuracy and parallelism were obtained by adding 500 μg of 5 different cortisol concentrations (0, 0.012, 0.062, 0.123, and 0.246 ug/dl) to 40 g of 5 separate aliquots of the same homogenized fecal sample. After extraction, we recovered 81±0.2, 83±0.3, 92±0.1, 91±0.1, 93±0.1% of cortisol respectively. Although this protocol has not been pharmacologicaly validated through conducting an ACTH challenge, the biological response associated with transportation stress indicates that changes in IR fecal cortisol serve as an index of adrenal activity. Moreover, the magnitude of the response among tigers may reflect individual differences in HPA-axis activity.

**Statistical Analysis**

We used a repeated measures design to compare differences in cortisol concentration between a 3 d block before transport (-3 to 0) with each 3 d block after transport representing time frames 0 to 3, 3 to 6, 6 to 9, and 9 to 12 d after transport. The RIA-determined IR fecal cortisol concentration from each defecation was averaged (Mean ± SE) within 3 d blocks due to irregularity in defecation patterns between tigers. This was to ensure that we had analogous data for each tiger at each corresponding time block and to avoid potential effects of circadian patterns on hormonal production. Missing data for tiger 5 for one of the blocks resulted in this subject being omitted from the analysis. Differences between experienced and naïve tiger behavior and respiration rate were determined using a one-way analysis of variance (SAS Institute Inc., Cary, North Carolina, Copyright © 2001).

## RESULTS

### Behavioral Effects

Response to transport varied between tigers. Time budgets for all tigers before and after transport are given in Table 2. There were no significant differences between any of the five tigers' behavioral time budgets before and after transport. All tigers spent at least 5 min lying down immediately after transport, and tigers spent an average (Mean ± SE) of 75 ± 13 % of the hour following transport lying.

**Table 2.** Activity budget of each tiger one hour before and one hour after transport in percentage of time spent performing each state

| Tiger | Lay | | Stand | | Walk | | Pace | | Sit | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After | Before | After |
| 1* | 83.0 | 99.0 | 1.5 | 0.5 | 4.6 | 0.6 | 10.0 | 0.0 | 0.0 | 0.0 |
| 2* | 100.0 | 84.0 | 0.0 | 4.6 | 0.0 | 5.9 | 0.0 | 5.5 | 0.0 | 0.0 |
| 3 | 25.2 | 24.6 | 6.1 | 5.3 | 4.5 | 2.8 | 62.5 | 67.3 | 1.7 | 0.0 |
| 4 | 72.7 | 87.9 | 7.2 | 2.2 | 6.1 | 4.6 | 8.7 | 5.3 | 5.4 | 0.0 |
| 5 | 100.0 | 80.3 | 0.0 | 6.1 | 0.0 | 13.6 | 0.0 | 0.0 | 0.0 | 0.0 |

* denotes experienced tiger

Time budgets for all tigers during transport are given in Table 3. One experienced tiger spent almost the entire time upright (standing, slow pacing, or propping on the side of the cage) during transport. When this tiger paced, the average rate was 17 turns per min. The other experienced individual alternated between standing, walking, and laying. One naïve tiger paced rapidly the majority of the time (63 %) with an average of 35 turns per min. The second

naïve tiger sat the majority of the time (73 %), and the third laid the majority of the time (63 %).

**Table 3.** Percent of time tigers spent performing each behavior during transport.

| Tiger | Lay | Stand | Walk | Sit | Prop | Pace | Investigate | Ears Back |
|---|---|---|---|---|---|---|---|---|
| 1* | 47.5 | 20.4 | 30.8 | 0.0 | 1.2 | 0.0 | 11.0 | 8.6 |
| 2* | 0.5 | 26.0 | 43.1 | 0.0 | 30.5 | 34.0 | 3.9 | 3.9 |
| 3 | 10.4 | 11.6 | 4.5 | 7305 | 0.0 | 0.0 | 2.6 | 78.7 |
| 4 | 12.7 | 11.6 | 73.9 | 0.0 | 0.6 | 73.9 | 0.6 | 92.5 |
| 5 | 63.4 | 11.4 | 19.5 | 5.7 | 0.0 | 2.3 | 0.3 | 74.5 |

* denotes experienced tiger

Naïve tigers had their ears oriented back and low (82 ± 5.4 %) for a significantly greater percentage of time (P = 0.01) than experienced tigers (6 ± 2.7 %). Experienced tigers spent an average of 7.5 ± 3.6 % of the time investigating the transport cage, while naïve tigers spent an average of 1.4 ± 1.0 %.

**Physiological Effects**

For one tiger (tiger 3) rib cage flex could not be seen. Therefore, analysis was performed for 4 out of the 5 tigers. Baseline respiration rates varied between tigers (Figure 2). The average (Mean ± SE) respiration rate of all tigers 10 min before transport was 56.1 ± 17.9 breaths/min. During transport the average respiration rate was 94.6 ± 19.3 breaths/min (~169 % above baseline), and 10 min after transport it was 132.3 ± 9.5 breaths/min (~236 % above baseline).

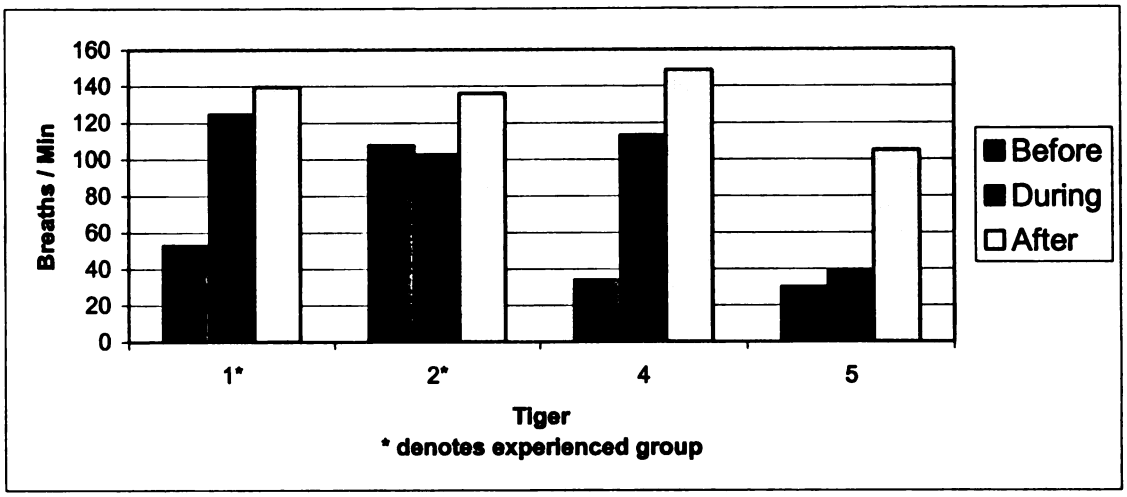



**Figure 2.** Respiration rates for 4 tigers 10 min before, during, and 10 min after transfer.

The average respiration rate of the experienced group was 166 ± 57 % above baseline during transport and 195 ± 56 % above baseline after transport (Figure 3). The average respiration rate of the naïve group increased to 230 ± 84 % above baseline during transport and 394 ± 36 % above baseline after transport. The differences between the experienced and naïve groups were not significant during ($P = 0.66$) or after ($P = 0.14$) transport.

The time block in which IR fecal cortisol concentration peaked varied between the 4 tigers analyzed for IR fecal cortisol (Table 4). Peaks ranged from the 0 to 3 d block to the 6 to 9 d block. After averaging all tigers' IR fecal cortisol concentrations within each block (Figure 3), IR cortisol concentrations peaked after transport during the 3 to 6 d block. At peak levels the IR cortisol concentration increased to 239 % above baseline concentration. Recovery time also varied between tigers. The 0 to 3 d, 3 to 6 d, and 6 to 9 d blocks were all

significantly higher than baseline (P = 0.02, P < 0.01, and P = 0.01 respectively). On average, IR cortisol concentration returned to levels comparable to baseline during the 9 to 12 d block. Samples collected during this time block did not differ significantly from baseline (P = 0.15).

**Table 4.** Immune reactive fecal cortisol concentrations (ng/g) for each time block of 4 tigers. Time 0 represents time of transport. Baseline refers to three days prior to transport.

| Tiger | Baseline | 0 to 3 Days | 3 to 6 Days | 6 to 9 Days | 9 to 12 Days |
|---|---|---|---|---|---|
| 1* | 35. 0 ± 0.4 | 42.2 ± 1.4<br>P = 0.086 | 79.3 ± 1.3+<br>P = 0.002 | 52.4 ± 4.1+<br>P = 0.003 | 47.4 ± 1.1+<br>P = 0.025 |
| 2* | 88.1 ± 1.2 | 171.4 ± 4.0+<br>P < 0.001 | 137.8 ± 1.9+<br>P = 0.044 | 116.0 ± 2.0<br>P = 0.271 | 82.7 ± 2.1<br>P = 0.834 |
| 3 | 58.3 ± 9.6 | 61.8 ± 1.1<br>P = 0.241 | 56.9 ± 5.5<br>P = 0.226 | 241.2 ± 8.8+<br>P = 0.034 | 54.9 ± 1.5<br>P = 0.901 |
| 4 | 29.4 ± 6.1 | 134.0 ± 2.0+ | 365.9 ± 6.1+<br>P < 0.001 | 110.3 ± 6.9+<br>P < 0.001 | 34.0 ± 2.1+<br>P = 0.02 |
| Ave. | 65.2 ± 1.4 | 85.8 ± 1.4+<br>P = 0.025 | 155.5 ± 4.6<br>P < 0.001 | 112.5 ± 4.1+<br>P = 0.011 | 57.5 ± 2.4<br>P = 0.152 |

* denotes experienced tiger
+ denotes significantly higher than baseline concentration

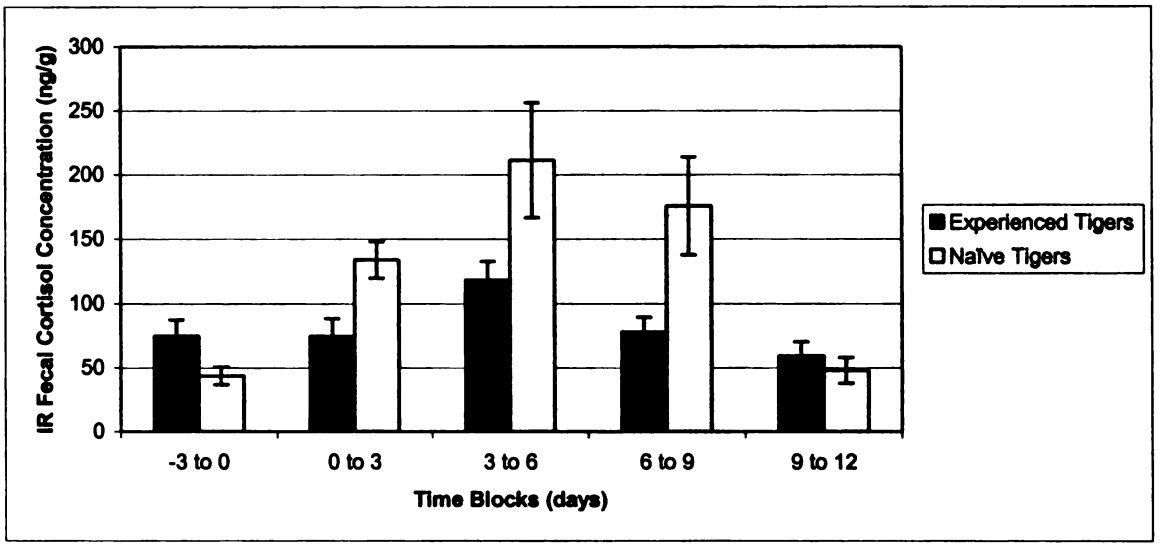



**Figure 3.** Average immune reactive fecal cortisol concentrations of experienced (N=2) and naive tigers (N=2). Day 0 represents time of transport.

Both average experienced and naïve tiger IR cortisol concentrations peaked during the 3 to 6 d time block (Figure 3). For experienced tigers the 3 to 6 d time block was the only one significantly ($P < 0.05$) higher than baseline. For naïve tigers IR cortisol concentrations were significantly higher than baseline ($P < 0.05$) for all blocks until the 9 to 12 d block ($P = 0.74$) when concentrations returned to baseline. Average IR cortisol concentration for experienced tigers during the peak interval was 158 % above average baseline concentration. Average IR cortisol concentration for naïve tigers during the peak interval was 482 % above average baseline concentration.

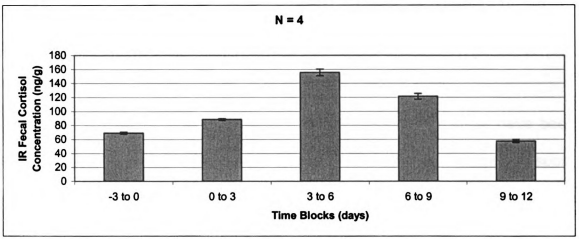



**Figure 4.** Average immune reactive fecal cortisol concentrations of experienced and naive tigers combined. Day 0 represents time of transport

## DISCUSSION

As we continue to find new measures to assess zoo animal welfare, we must use them to better understand how common management practices may affect their behavior and physiology, ensuring that the effects of these practices do not impede an animal's effectiveness as a conservation and educational animal. This is especially important with felid species because very few studies have identified behavioral or physiological effects of management practices. Observed behavioral responses to transport may be indicative of negative physiological responses.

The data collected on the IR cortisol concentrations in fecal samples provides novel information on the HPA-axis activity of tigers, which has not previously been documented. We found that baseline fecal glucocorticoid concentrations in tigers appear to vary greatly between individuals as is true in

cheetahs (Jurke et al., 1997) and clouded leopards (Wielebnowski et al., 2002). This may be due to individual variation in past experience, response to past experience, metabolic functioning, or to subspecies in this study.

This is also the first time the effects of transportation stress have been quantified in tigers. Although respiration rate increased overall during transport, it increased further during the 10 min following transport, indicating that the effects of the stressor may continue after the physical challenge ceases. All tigers rested in a shaded area immediately upon release into their respective enclosures after transport. The increase in respiration rate and immediate resting response suggests that transport can be an exhausting experience for tigers although high summer temperatures may have contributed.

The percent increase in cortisol levels following transport varied between individuals, indicating that individuals respond to stress differently. However, it may also reflect differences due to age, sex, or subspecies. The timing of the observed peaks in IR cortisol concentrations also varied between individuals, which may indicate differences in gut passage time. Finally, behavioral responses varied between individuals. Two tigers reacted to the stressor actively by pacing or walking the majority of time during transport; two tigers were inactive by laying and sitting in the same spot with little movement the majority of the time; and one tiger shifted between positions. This indicates tigers may exhibit either flight or hiding behavior, which often results in pacing when subjected to a restrictive environment, or they may exhibit an inhibition of exploratory behavior or activity during transport. Therefore, activity level alone

without accompanying type of behaviors performed may not serve as a sufficient indicator of tiger well-being.

Mammals respond differently to handling and transport procedures depending on their past experience (Grandin, 1997). In both the physiological and behavioral assessments, naïve tigers seemed to respond more strongly to the transport procedure than experienced tigers. Naïve tiger respiration rates increased by a greater percentage both during and after transport, although the differences were not significant. The average IR cortisol concentration of experienced tigers increased after transport but was significantly higher than baseline only during the peak block (3 to 6 d), whereas the average IR cortisol concentration of naïve tigers increased to levels significantly higher than baseline in the block immediately following transport and for every block up to the 9 to 12 d block. The peak fecal corticoid concentration of naïve tigers was also greater in comparison to baseline levels than the peak fecal corticoid concentration of experienced tigers. In spite of the low replication, our data support the hypothesis that transport caused the naïve tigers' IR cortisol concentration to be higher than the experienced tigers', and that naïve tigers were affected longer.

Although our physiological data do support the hypotheses that transport caused an increase in cortisol production and resulted in a greater response by naïve tigers, one must be critical when just looking at glucocorticoid response. An increase in glucocorticoid production also can occur as a result of positive stimuli. For example, De Jong et al. (1998) showed that pigs housed in a more enriched environment had greater levels of salivary cortisol than did pigs housed

in a less enriched environment. It has also been shown that cortisol production in horses increases during mate introduction (Colborn et al., 1991). Therefore, behavioral data should be coupled with glucocorticoid data before determining whether a stimulus can be considered "good" or "bad" stress.

The observed physiological effects correspond to similar behavioral trends. In both the experienced and the naïve group at least one tiger was predominantly active during transport and at least one tiger was predominantly inactive. The major difference between the naïve and experienced tiger groups was the intensity of the behaviors performed. Naïve tigers tended to respond with greater intensity than experienced tigers. The naïve tiger that paced the majority of time did so almost continuously (stopping only once to rest) and at a much faster rate than the experienced tiger that paced. Conversely, the experienced tiger paced more slowly and stopped pacing more often. This stereotypy (pacing) may have been a result of the tiger's inability to express flight behavior. The more intense performance of pacing may be indicative of a greater motivation to perform the behavior. Similarly, naïve tigers that were inactive (sitting or lying the majority of time) simply remained in one spot while the experienced tigers alternated between states. This possibly indicates a greater suppression of exploratory behavior and activity due to higher levels of fear.

Finally, we looked at ear position and investigative behavior. In our study, experienced tigers had their ears positioned back and low approximately 6 % of the time, while naïve tigers did approximately 82 % of the time. This suggests

that the naïve tigers were experiencing fear or were defensive the majority of the time, and experienced tigers may have been either relaxed or even interested. Naïve tigers also performed less investigative behavior than experienced tigers. This is expected if naïve tigers were experiencing more fear as fear may inhibit exploratory behavior (Manser, 1992).

Novelty can be both a strong stressor (Dantzer and Mormede, 1983), or it can be stimulating (Grandin, 1997), depending on how it is presented. If the novel item or situation is threatening in any way, or if the animal has no control over the situation, it is likely to react adversely to it. Negative past experiences during a procedure will result in a negative association with that procedure (Mendl et al. 2001). This will further result in either a lack of cooperation by the animal or an increase in the adverse effects of the stressor. Therefore, it must be ensured that experience with a stressor is not negative so that adverse effects can be eliminated.

Animals have been able to habituate to many common procedures. Wienker (1986) showed that giraffes could be trained to voluntarily enter a squeeze cage. To motivate animals to cooperate, one must first present the novel situation in a way that will not be threatening. Positive stimuli should be provided to counteract any negative stimuli that may result from a procedure. For example, Grandin et al. (1995) showed that nyala antelope could be trained for blood sampling by using a food reward as positive reinforcement. In the case of potentially stressful situations like transport, it is the manager's job to make the procedure as positive as possible. Currently, it is common practice for zoos to

crate train animals before transport. This study further supports the importance of properly acclimating animals to transport elements through procedures such as crate training to reduce stress. Through positive reinforcement, it may even be possible to stimulate a positive association with transport. If this is the case, animals may even await transport, and the negative stress previously experienced will transform into positive stress.

This study demonstrated that stress from transport even as short as 30 min can affect a tiger physiologically for up to 9 to 12 d. We suggest that the duration of the challenge is not the only factor contributing to the physiological response. Other factors that may have contributed to increased release of IR fecal glucocorticoids include confinement, forced movement, cage motion, handling by humans as perceived by the animal, or novel sensory stimuli experienced during transport.

We anticipate that during transport from one zoo to another, there are even more factors that may play a role. In this study, tigers were re-released into their original, familiar enclosure. When transported to another zoo, an animal must withstand much longer durations of confinement in the transfer cage, become accustomed to an unfamiliar environment, and adjust to new people, management procedures, and sensory stimuli. It is conceivable that such extended transport could result in more extreme effects. Therefore, future studies should focus on one of the individual factors suggested to better understand the extent to which each factor affects tigers.

## CONCLUSIONS

1. IR cortisol concentrations can be measured in tiger feces and may be used as an indicator of glucocorticoid production in response to stressful stimuli.

2. Even short-term transportation procedures can cause significant increases in IR cortisol concentration of tigers.

3. Response repertoires vary from active to inactive between individual tigers during transport.

4. Previous exposure to transport correlated with less adverse behaviors. Naïve tigers were more extreme in their activity level time budget. They also investigated less and had their ears oriented back for a much greater percentage of time. Such repertoires may be indicative of poor tiger welfare.

5. Effects of transport on glucocorticoid production may be reduced by increasing tiger experience with elements of the management procedure.

# Chapter 4: The effects of transport from one zoo to another on the HPA-axis of tigers

## INTRODUCTION

An entire transport procedure can be divided into three major stages each of which may induce a stress response in tigers (Kranz, 1996). The first stage is preparation for transport. This stage includes all novel stimuli a tiger may experience before transport such as crate training or changes in daily husbandry. The second stage is the transportation method. This stage includes the method used to crate the tiger, the method used to move the crate, the stimuli the tiger experiences while in the crate, and the method used to release the tiger. The third stage is the introduction into a new enclosure. Included in this stage are all stimuli the tiger experiences after it is released into its new enclosure such as the physical structure of a novel environment and novel management practices.

The 30-min controlled study in the previous chapter was designed to test only the effects of two factors common to all transport procedures, crating a tiger and relocating it. Since these factors are always a part of transport procedures, the effects measured in that study could be considered the minimum or baseline effect of the transport procedure. When tigers are transported between zoos, many more factors contribute to transportation stress. For example, the duration the tiger is crated is always longer than 30 min. Depending on the distance between facilities, tigers may be crated for as little as three hours or as much as one week according to Felid Taxon Advisory Group (TAG) standards

(Shoemaker, 2003). The effect crate duration has on the stress axis of tigers is currently unknown.

The method each zoo uses to crate a tiger may vary. Sometimes a tiger must be anesthetized because either the facility's structure does not allow for simple transfer into a crate or because the tiger is uncooperative, while other times facilities have the structure and are able to crate train, making the use of anesthesia unnecessary. Similarly, the method of transport once the tiger is crated may vary. If the distance between institutions can be driven, usually a specialized van or truck will be used. For long journeys, or journeys over-seas, a tiger may spend time in an airplane or a vessel. The effect that different transport methods have on tigers has not yet been quantified.

Finally, factors exist that may affect a tiger after arrival at a new facility. First, the tiger is exposed to a completely new environment. In the previous study, tigers were released into the same enclosure where they originally dwelled, therefore eliminating a novel environment as a factor that contributed to the stress response. In AZA-accredited zoos, tigers are required to be transported to a quarantine holding area for 30 days before being allowed on exhibit (Shoemaker, 2004). As a result, tigers are often being moved from a familiar, large, and highly enriched area to an unfamiliar, small, and poorly enriched area. Holding areas seldom provide for adequate flight or hiding behaviors and because individuals may respond to novelty with fear, stereotypic behaviors may be performed (Manser, 1992).

Included in a new environment are novel sensory stimuli. For example, a tiger may experience for the first time loud noise from construction that is being performed near its new enclosure, or it may experience novel smells caused by different surrounding plants. How an individual responds to novelty can vary both behaviorally and hormonally (Birke, 1979) and can often be influenced by past experience (Joseph and Gallagher, 1980) as was seen in the 30-minute controlled study.

Similarly, the tiger is exposed to novel management procedures. Due to the dynamic differences between each zoo, no two zoos manage an animal in exactly the same way. For example, the zoo that the tiger arrived at may feed at a different time of day or may feed a different type of diet than the zoo from which the tiger came. A tiger also must adjust to being managed by new individuals. Often animals may react more adversely to unfamiliar individuals. Boivin, et al. (1998) reported that calves were more likely to allow a familiar person to touch them more quickly than an unfamiliar person.

The ultimate goal of understanding the effects of transportation stress on tigers is to understand how each one of these factors contributes to stimulating the stress response. While the previous chapter dealt with understanding the baseline response to transport, one goal of this chapter is to assess the effects of common transport procedures between zoos on the hypothalamus-pituitary-adrenal (HPA) axis of 4 different tigers during 4 different transportation episodes. Since there were differences between the tigers and the transportation episodes, another goal of this study is to compare the effect different factors involved with

different transportation procedures have on tigers. A common transport procedure includes all factors previously discussed and therefore, can be described as the common response to a transport procedure. The final goal of this study is to compare the baseline response with the common response.

## METHODS

The Michigan State University All-University Committee on Animal Use and Care approved this study as did the AZA's Tiger SSP. Included in this study were 4 tigers. Characteristics of each tiger are given in Table 5. All tigers were housed individually during the study, fed a consistent daily diet, and housed on exhibit for a month before transport. All tiger were healthy for the month before transport. After transport no medical problems or major behavioral problems were observed. The tigers were again fed a consistent diet and were housed individually in quarantine for the 30 days after transport. Tiger 4 was exposed to construction noise after transport and zookeepers observed an increase in pacing while noise from construction was loudest.

**Table 5**. Characteristics of each tiger transported between zoos.

| Tiger | Sub-species | Age (yrs) | Gender | Duration Crated (hr) | Transport Method | Transport Experience |
|---|---|---|---|---|---|---|
| 1 | Amur | 9 | Male | 14 | Van, Anesthetized to be crated | Twice previously |
| 2 | Amur | 3 | Female | 9 | Van | None |
| 3 | Sumatran | 12 | Male | 4 | Van, Anesthetized to be crated | Three times previously |
| 4 | Corbetti | 3 | Female | ~12 | Airplane, Anesthetized for release | Once previously |

The transport procedures were consistent with the recommended transport procedures outlined by the AZA's Tiger SSP [Tiger Information Center, 2003]. Entire stools were collected from each tiger for 6 days prior to transport and immediately frozen. Voided feces were collected from each tiger during transport (if necessary) and continuously for 28 days following transport so that Immune Reactive (IR) cortisol concentration could be determined. Occasionally, fecal samples could not be collected daily because the tiger was not being cooperative (especially immediately after transport) or because the tiger did not defecate. For comparison, 8 mo after transport feces was collected every third day from Tiger 1 (control) for one month to ensure that there was not a monthly spike in cortisol that coincided with spikes assumed to result from transport. Cortisol was extracted using the procedure outlined in Chapter 2 and measured using Radioimmunoassay (RIA).

**Statistical analysis**

Resulting IR fecal cortisol concentrations from samples collected before transport were averaged for each tiger individually and for all tigers collectively. All IR fecal cortisol concentrations derived from samples after transport were averaged into 3-day blocks. Then, a repeated measures test was used to compare the average IR fecal cortisol concentration before transport with each average concentration within each 3-day block after transport for each tiger individually and for all tigers collectively.

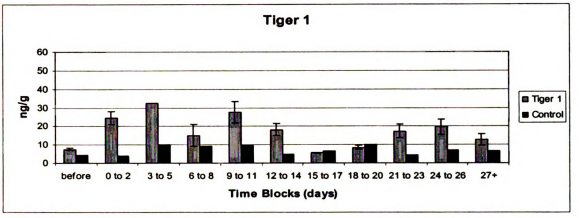
## RESULTS

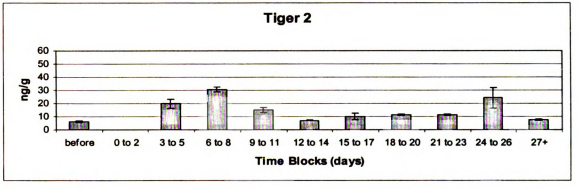
Each tiger showed a significant spike in cortisol following transport (Table 6, Figure 5.). Tiger 1's cortisol level peaked during the 3 to 5-day block to 434 % greater than the baseline value. The IR fecal cortisol concentration of this tiger did not return to baseline until the 15 to 17-day block. The IR fecal cortisol level of Tiger 2 reached a maximum during the 6 to 8-day block to 494 % greater than baseline. Her cortisol level did not return to baseline until the 12 to 14-day block. Tiger 3's cortisol level peaked on the 0 to 2-day block to 516 % above baseline. His cortisol level did not return to baseline until the 6 to 8-day block. Finally, Tiger 4's cortisol level peaked during the 6 to 8-day block to 341 % above baseline. Although Tiger 4's cortisol levels never significantly returned to baseline, it did level off during the 15 to 17-day block.

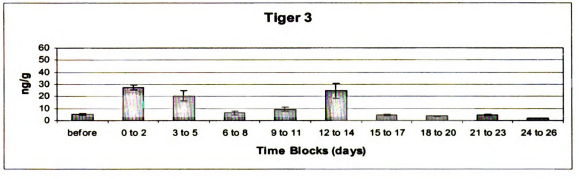
**Table 6.** Mean ± SE of immune reactive fecal cortisol concentrations (ng/g) for each time block of 4 tigers. Time 0 represents time of transport. Baseline refers to the average fecal cortisol concentration for 6 days prior to transport. N is variable from 2 to 3 for individual tigers and variable from 8 to 12 for all tigers.

| Time Block | Tiger 1 | Tiger 2 | Tiger 3 | Tiger 4 | All Tigers |
|---|---|---|---|---|---|
| Baseline | 7.5 ± 0,7 | 6.2 ± 0,5 | 5.3 ± 0.5 | 16.1 ± 1.0 | 8.9 ± 0.6 |
| 0 to 2 Days | 24.3 ± 3.5* P < 0.001 | N/A | 27.5 ± 2.1* P < 0.001 | 38.0 ± 1.2* P < 0.001 | 29.6 ± 2.5* P < 0.001 |
| 3 to 5 Days | 32.6 ± 19.8* P = 0.014 | 19.8 ± 3.5* P < 0.001 | 20.4 ± 4.3* P < 0.001 | 50.4 ± 7.8* P < 0.001 | 26.9 ± 3.6* P = 0.014 |
| 6 to 8 Days | 15.0 ± 5.8 P = 0.206 | 30.9 ± 1.8* P < 0.001 | 6.3 ± 1.8 P = 0.596 | 54.9 ± 2.5* P < 0.001 | 28.7 ± 3.7 P = 0.206 |
| 9 to 11 Days | 27.6 ± 5.8* P = 0.001 | 15.3 ± 1.9 P = 0.214 | 9.4 ± 1.5* P = 0.011 | 40.0 ± 3.0* P < 0.001 | 21.0 ± 2.6* P = 0.001 |
| 12 to 14 Days | 18.0 ± 3.2* P = 0.002 | 7.0 ± 0.4 P = 0.128 | 24.7 ± 6.2* P = 0.003 | 35.3 ± 0.8* P < 0.001 | 21.6 ± 2.8* P = 0.002 |
| 15 to 17 Days | 5.5 ± 0.3* P = 0.013 | 10.0 ± 2.4* P < 0.001 | 4.6 ± 0.9 P = 0.459 | 30.3 ± 1.6* P < 0.001 | 12.4 ± 2.4* P = 0.014 |
| 18 to 20 Days | 8.4 ± 1.2 P = 0.579 | 11.3 ± 0.4* P < 0.001 | 3.7 ± 0.3* P = 0.010 | 32.6 ± 1.5* P < 0.001 | 15.2 ± 2.2 P = 0.579 |
| 21 to 23 Days | 17.2 ± 3.7* P = 0.013 | 11.3 ± 0.8* P = 0.030 | 4.8 ± 0.6 P = 0.458 | 49.5 ± 3.0* P < 0.001 | 15.1 ± 3.1* P = 0.013 |
| 24 to 26 Days | 19.5 ± 4.2* P = 0.006 | 24.1 ± 8.0 P = 0.200 | 2.11 ± 0.1* P < 0.001 | 53.2 ± 0.1* P < 0.001 | 23.2 ± 4.2* P = 0.006 |
| > 27 Days | 12.6 ± 3.1 P = 0.115 | 7.5 ± 0.8 P = 0.085 | N/A | N/A | N/A |

* denotes significantly higher than baseline concentration



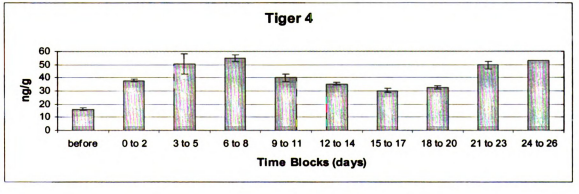




**Figure 5.** IR fecal cortisol concentrations of Tigers 1-4 for each 3-day time block.

All tigers appeared to have a second spike in IR fecal cortisol concentration at least one week after levels returned to baseline. Each one of these spikes was significantly higher than baseline, but not as high as the initial spike. Finally, after averaging all tigers' IR fecal cortisol concentrations into 3-day blocks (Figure 6.), a peak occurred during the 6 to 8-day block to 333 % above baseline and remained elevated until the 15 to 17-day block. The second spike occurred during the 24 to 26-day block.

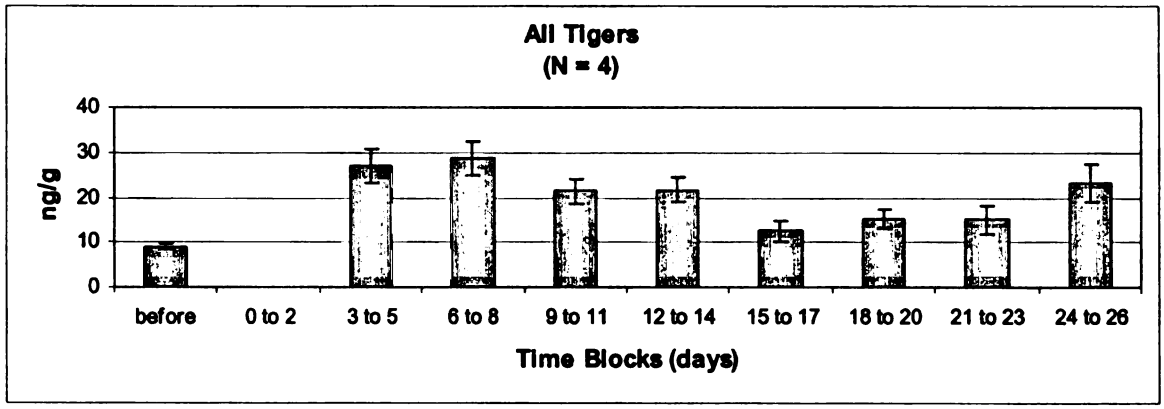



**Figure 6.** Average IR fecal cortisol concentrations of all tigers.

## DISCUSSION

Based on the 30-minute controlled experiment, simply crating a tiger for ½ hour caused IR fecal cortisol levels to increase to 239 % above baseline on average. It also caused cortisol levels to remain elevated for an average of 9 to 12 days. Since a greater number of stressful factors (longer duration crated, introduction into a new environment, etc.) are experienced during all common transport procedures between zoos, it is expected that during transport the average tiger's

cortisol level will increase by at least this baseline factor of 2.39, and tigers will be affected for at least 9 days. In this study, the average spike in IR fecal cortisol was 333 % above baseline, and levels returned to baseline during the 15 to 17-day block showing that tigers experienced a higher spike as well as a longer duration of increased IR fecal cortisol concentration when transported between zoos.

With the knowledge that simply crating and moving each tiger stimulates a 239 % spike in IR fecal cortisol concentration and it takes 9 to 12 days to recover, how do the other factors of transport duration, method of transport, and introduction into a novel environment contribute to the remaining increase in cortisol production and recovery time? For example, how does anesthetizing a tiger for crating or release affect cortisol production? Is stress influenced by a novel environment responsible for the prolonged recovery time, or does the duration of transport have a greater effect on recovery time?

Although the low sample size in this study does not allow definitive statements about how these factors contribute to transportation stress, trends can be identified. For example, the tiger with the most transport experience and the shortest crate duration (Tiger 3) recovered more quickly than tigers with less experience and longer crate durations. These results parallel results from the study in Chapter 3 in which individuals with transport experience recover more quickly than individuals with no transport experience. Braastad et al. (1998) reported that short stressors such as loud noise and short-term human exposure (20 seconds) did not affect silver fox vixen (*Vulpes vulpes*) body temperature and

activity, while long-term stressors such as human handling for 5 minutes or human presence for 90 minutes caused significant increases in both body temperature and activity. Therefore, shorter crate durations may reduce recovery time.

It is recommended that every mammal be housed under conditions that allow shifting and crating without the use of anesthesia (Bush, 1996). Unfortunately, all facilities are not yet appropriately structured for simple shifting into a crate, and sometimes tigers are difficult to crate train. In this study, three tigers (Tiger 1, Tiger 3, and Tiger 4) were anesthetized either during crating or while being removed from the crate. Anesthesia has been shown to reduce cortisol production during surgical procedures (Graf and Senn, 1999, and Dalin et al., 1993). Dalin et al. (1993) also showed that significant increases in cortisol production were delayed until recovery from anesthesia. Both anesthetized tigers experienced lower spikes in cortisol, but it took them longer to recover from transport than the tigers not anesthetized. A delay in recovery may be due to an anesthesia-induced delay in initial increased adrenal activity.

Just as the 30-minute controlled study provided data quantifying a baseline stress response to transport, this study provided data quantifying the average response of tigers to transport between zoos. This study along with the previous 30-minute controlled study lay the foundation for future studies to tease apart the effects each potential stressor (crate duration, transport method used, and introduction into a new environment) has on tiger behavior and physiology. This study also lays the foundation for other future studies. For example, all

tigers in this study experienced a second spike in cortisol 6 to 12 days after recovery. The long-term effects of transport on cortisol production and behavior should also be explored as additional spikes in cortisol after transport may affect tiger behavior and ultimately tiger welfare.

# Chapter 5. Conclusions

To continue improving welfare standards, we must expand our understanding of the effects captive management practices have on zoo animals by devising ways to measure these effects. The fecal cortisol extraction protocol used in the previous studies effectively measures long-term effects of stressful events on the tiger HPA-axis. Fecal cortisol may also be used to measure short-term effects grouped into 3-day blocks. Before fecal cortisol can be used to assess fluxes in adrenal activity during time periods shorter than three days, we must improve our understanding of the lag time between when cortisol is produced and when it shows up in feces. The data presented here suggest that lag time may vary between individuals.

In tigers, behavioral indicators of stress before, during, or after transport may not be obvious. Therefore, physiological indicators may be more important in detecting stress-related effects. Tiger IR fecal cortisol concentrations increase as a result of transport. To reduce stress, steps may be taken such as crate training and introduction to transport components and other novelties a tiger may encounter. During transport between zoos the method used and the duration crated may have a significant impact on tiger stress levels. Further studies should explore the effects of increased crate duration and different transport methods.

Although tigers displayed a second spike in IR fecal cortisol concentration after transport, signifying that there is a longer-term effect than just one spike in cortisol production, chronic effects were not observed. Therefore, crating or even

transport may serve as an effective means to stimulate a short-term stress response. Stimulating short-term stress may be an effective way to exercise natural behaviors in tigers such as mating or flight behavior. The implications of utilizing stress as a means to exercise natural behavior should be explored further.

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